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Antimicrobial Resistant *Escherichia coli*

Clinical, Epidemiological and Molecular Characteristics in the Australian Region

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ABSTRACT

Background

Escherichia coli is the most common gram-negative bacteria to cause human infection. The pathological manifestations range from minor disease to severe life threatening sepsis. Urinary tract infection, most often caused by *E. coli*, is also the most common bacterial infection in humans. Since the inception of antimicrobial therapy in the early 20th century, *E. coli* has systematically developed resistance to almost all known antimicrobials, posing a challenge for the treatment of such infections.

From a global perspective, the first decade of the 21st century heralded a change in the epidemiology and tempo of resistance amongst *E. coli*. Previously, resistance to third generation cephalosporins (3GC) was primarily associated with current or previous healthcare exposure. In the past decade however, expanded-spectrum cephalosporin resistant *E. coli* (ESC-R-EC), usually mediated by Extended Spectrum beta-lactamase (ESBL) genes has spread widely within the communities of many regions, independent of healthcare associated acquisition.

The latter half of this decade has led to the delineation of two further challenges amongst resistant *E. coli*. The first is the identification of Sequence Type 131 *E. coli* (ST131), a global 'pandemic' clone fine-tuned for resistance and virulence. This clone is now implicated in a significant proportion of community ESBL *E. coli* infections globally. The second challenge is the emergence of *E. coli* harbouring carbapenemase genes, conferring resistance to carbapenem antimicrobials used to treat severe ESBL producing *E. coli* infection.

Methods

Through several studies we have aimed to better define global and local aspects of antimicrobial resistant *E. coli*, in particular ESC-R-EC. The studies have included clinical and laboratory based research.

Clinical research included a multi-centre case-control study of community onset ESC-R-EC infection in Australia and New Zealand, and a national survey of health services' infection control practices pertaining to multi-resistant gram-negative bacilli and patients at risk of harbouring these.

Laboratory research included molecular epidemiological investigation of *E. coli* from two sources. The first was isolates from the 182 participants in the case-control study, comprising a broad sample of community onset 3GC resistant and susceptible *E. coli* from Australia and New Zealand. The second was a collection of isolates from a previously conducted study on carriage of resistant *E. coli* in overseas travellers returning to Australia.

Results 182 patients (91 cases and 91 controls) were recruited across six tertiary hospitals in Australia and New Zealand for the case-control study. Multivariate logistic regression identified risk factors for 3GCR-EC

including birth on the Indian subcontinent (OR=11.13, 2.17-56.98, p=0.003), urinary tract infection in the past year (per infection OR=1.430, 1.13-1.82, p=0.003), travel to South East Asia, China, Indian subcontinent, Africa and the Middle East (OR=3.089, 1.29-7.38, p=0.011), prior exposure to trimethoprim+/-sulfamethoxazole &/or extended spectrum cephalosporins (OR=3.665, 1.30-10.35, p=0.014) and healthcare exposure in the previous six months (OR=3.16, 1.54-6.46, p=0.02).

Molecular epidemiological analysis of isolates demonstrated a predominance of CTX-M type ESBL's, as now reported in most other regions of the world. From a global perspective, a unique distribution of ST131 *E. coli* was demonstrated, with a very low prevalence of ST131 amongst 3GC susceptible isolates compared with resistant isolates (7% vs. 45%). Susceptible isolates showed diversity with six MLST defined clusters of isolates. Amongst 3GCR isolates, ST131 dominated, comprising 40/89 isolates, with 88% (35/40) of ST131 being of the recently defined *fim*H 30 sub-clone variant. Whilst patients with ST131 were significantly more likely to have an upper rather than lower urinary tract infection (relative risk 1.8, p=0.040), they were otherwise relatively epidemiologically homogenous with other 3GCR-EC.

Analysis of isolates from returned travellers gave insight into the dynamics of carriage of antimicrobial resistant *E. coli* in the bowel flora and supported a number of findings from the case control study. The risk of prolonged carriage after travel was lower for 3GC-resistant than ciprofloxacin or gentamicin resistant isolates and the duration of carriage was also longer for the latter resistance phenotypes (75th quartile 8 vs. 62 and 63 days respectively). In multivariate analysis, risks of prolonged carriage included antimicrobial use whilst travelling (RR 3.3, 1.3–8.4) and phylogenetic group B2 (RR 9.3, 3.4–25.6) and D (RR 3.8, 1.6–8.8). Clonality amongst longitudinal isolates from the same participant was demonstrated in 92% of participants and most marked amongst 3GC resistant isolates. ST131 was surprisingly infrequent amongst participants (3% of participants).

Conclusion

Within this thesis, study of a variety of aspects of antimicrobial resistant *E. coli* in Australia and New Zealand has revealed unique insights into this pathogen locally and globally. These insights include the delineation of risks within the community, temporality of risk and a unique molecular epidemiology. Furthermore studies completed within this thesis highlight several key future directions of research including clinical studies to investigate risk-factor modification and optimal therapy, economic impact analyses of resistant *E. coli* infection, and further in-depth genetic studies.

DECLARATION BY AUTHOR

This thesis **is composed of my original work, and contains** no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted **to qualify for the award of any** other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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1) Rogers BA, Aminzadeh Z, Hayashi Y, Paterson DL. Country-to-country transfer of patients and the risk of multi-resistant bacterial infection. Clin Infect Dis. 2011

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1) Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011

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Chapter 3.

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CONTRIBUTIONS BY OTHERS TO THE THESIS

In addition to the contributions outlined above, Laurie Beechy assisted with data entry for the case control study. Ms Tiffany Brown assisted with data collection for the case control study. Wan Keat Yam and Dr Anna Sartor assisted with laboratory work, including genotypic and phenotypic characterisation presented in chapters 3, 4 and 5. Diane Josey (English Language Business) and Sarah Rogers assisted with proof reading of the final version of this thesis.

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KEYWORDS

gram-negative bacteria, *Escherichia coli*, antibiotic resistance, extended spectrum beta-lactamase, urinary tract infection, community onset, hospital acquired, travel.

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LIST OF ABBREVIATIONS USED IN THE THESIS

Abbreviation	Term
3GC	Third generation cephalosporin antimicrobial (Ceftrixaone, Cefotaxime, Ceftazidime)
3GCR-EC	Third generation cephalosporin resistant *E. coli*
AGAR	Australian Group on Antimicrobial Resistance
AIHW	Australian Institute of Health and Welfare
BSI	Blood stream infection
CDC	Centres for Disease Control
CGA	Clonal group A *E. coli*
Cip-R	Ciprofloxacin resistant isolate
CLSI	Clinical and laboratory standards institute
CO-ESCR-EC	Community-onset expanded (or extended)-spectrum cephalosporin resistant *E. coli*
COOEE	Community onset ESBL and AmpC *E. coli* study
CP	Contact precautions
CRE	Carbapenem resistant Enterobacteriaceae
CRO-R	Ceftriaxone resistant isolate
DST	Direct antimicrobial susceptibility testing
EARS-NET	European Antimicrobial Resistance Surveillance Network
ECDC	European Centres for Disease Prevention and Control
ED	Emergency department
ESBL-EC	Extended spectrum beta-lactamase harbouring *E. coli*
ESC-R-EC	Expanded (or extended)-spectrum cephalosporin resistant *E. coli*
ExPEC	Extra intestinal pathogenic *E. coli*
Gent-R	Gentamic resistant isolate
GIT	Gastrointestinal tract
H30	FimH 30 subclone of ST131 *E. coli*
HA	Healthcare associated
ICU	Intensive Care Unit
ID	Infectious disease
IQR	Inter qurartile range
IT-patient	Patient who has undergone an international transfer
KPC	*Klebsiella pneumoniae* carbapenemase
LTCF	Long Term Care Facility (High-level Care Nursing Home)
MBL	Metallo beta-lactamase
MIC	Minimum inhibitory concentration
MLST	Multi-locus sequence typing
MRGNB	Multi-resistant gram-negative bacilli
MRO	Multi-resistant organism
NDM	New Delhi metallo beta-lactamase
NHMRC	National Health and Medical Research Council
non-HA	Non-healthcare associated
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis

rep-PCR	Repetitive extragenic palindromic sequence polymerase chain reaction
RR	Relative risk
SE Asia	South East Asia
ST	Sequence type
SXT	Trimethoprim + sulfamethoxazole
TRUS biopsy	Trans-rectal ultrasound guided prostate biopsy
UK	United Kingdom
UTI	Urinary tract infection

LIST OF FIGURES & TABLE

Figures

Tables

TABLE OF CONTENTS

CHAPTER 1. *ESCHERICHIA COLI* AND ANTIMICROBIAL RESISTANCE

INTRODUCTION

Humans and the Enterobacteriaceae *E. coli* have an intimate relationship. The gastrointestinal tract (GIT) harbours vast numbers of commensal or non-pathogenic *E. coli*. They comprise the most common facultative anaerobe in the human GIT.[1] In contrast, pathogenic *E. coli* strains are able to cause a broad range of human disease. The spectrum of pathology ranges from minor to life threatening. It is the most common cause of community onset bacteraemia and urinary tract infections (UTI) in adults and children.[2-4] In addition, it causes a range of infections including diarrhoeal disease, meningitis and health care associated infection, in critically unwell patients and those with immunocompromise. Infections have been classically divided into three broad syndromes: urinary tract infection, meningitis & sepsis, and diarrhoeal disease.[5] This distinction was made on the basis of differences in susceptible population groups and differing 'pathotypes' of *E. coli* causing such infections, although further molecular insight into bacteria has led to a simpler two-group stratification.

E. coli causing extra-intestinal infection have been broadly described as 'ExPEC' or extra-intestinal pathogenic *E. coli*. This descriptive group evolved from the recognition that phenotypic and genotypic distinction between the *E. coli* causing a variety of extra-intestinal infections, is not exact.[6] Infections include UTI, neonatal meningitis, nosocomial pneumonia, GIT and biliary infections, osteomyelitis and septic arthritis.[1] A simplified schema of infection requires colonisation of non-sterile sites such as the GIT or genital tract, then invasion of a sterile site. Colonisation without infection is also a frequent occurrence with up to 20% people harbouring ExPEC strains as their predominant GIT coloniser.[7] In addition, non-pathogenic commensal strains of *E. coli* are implicated in infection where regular host defences are compromised, such as catheter associated UTIs, peritonitis or host immunocompromise.[5, 6]

In contrast to commensals and ExPEC strains, *E. coli* causing gastrointestinal infection are described as obligate pathogens. They are rarely found in the gastrointestinal tract of the healthy host.[6] Six pathotypes have been described and members of these groups are diverse. They may be divided by genetic and phenotypic characteristics but are ultimately defined by their mechanism of interaction with eukaryotic cells (invasion, toxicity, aggregation etc.).[5] These *E. coli* are relatively genetically and epidemiologically distinct from those causing extra-intestinal infection and will not be explored further in this research.[6]

The clinical manifestations and environmental niches of *E. coli* are protean and thus its genome is highly diverse. Each organism carries approximately 5000 genes, with only 2200 estimated to be common amongst all *E. coli*. The *E. coli* pan-genome may contain up to 13,000 different genes shared across the whole species.[8] Such diversity has most likely driven the marked mobility of the *E. coli* genome. Frequent recombination by conjugation with exchange of genetic material between bacteria, described by some authors as 'bacterial sex', is a common occurrence.[9]

At a more immediate level, the genes encoding much of the emerging antimicrobial resistance amongst *E. coli* do not reside within the *E. coli* genome. Instead, they reside on highly mobile, independently replicating, circular plasmids within the bacteria. Such plasmids have constituted one of the foci of attempts at understanding the spread of resistance through bacterial species.[10]

EXTRA-INTESTINAL *E. COLI* INFECTION

Urinary tract infection and bacteraemia are the two most common presentations of extra-intestinal *E. coli* infection.

URINARY TRACT INFECTION

UTI is one of the most common human bacterial infections, with *E. coli* the most frequent pathogen.[11] *E. coli* causes 85-95% of all uncomplicated cystitis and 75-95% of pyelonephritis.[12, 13]

Incidence studies in many population groups confirm that UTI is a frequent occurrence. In a study, the self-reported incidence amongst females >18 years was 10.8% in the previous 12 months.[14] In sexually active young females, the incidence of symptomatic UTI has been more rigorously estimated at 0.5-0.7/person year.[15] A population based study of pyelonephritis suggested that the highest incidence was in women aged 15-34 at an annual rate of approximately 20 per 10,000 with approximately 1 in 6 patients requiring hospitalisation.[16]

In 2003 the economic burden of community acquired urinary tract infection in North America was estimated with direct costs of over 1 billion United States dollars per year and with significantly higher secondary costs due to lost productivity.[12] More recent data arising from the use of a different methodology places the costs even higher at 2.14 billion USD per year for acute pyelonephritis alone.[17]

Morbidity and mortality from urinary tract infection vary greatly depending upon the clinical setting. Few studies of uncomplicated cystitis and pyelonephritis in adults demonstrate mortality or morbidity. Whilst there is a presumption of minimal long-term consequences, few longitudinal studies have been conducted to confirm this.[17]

Increasing age and male gender were identified as risk factors for mortality in a large study of data from North America of in-patients with *E. coli* pyelonephritis. In this group, mortality was not insignificant at 7.3/1000 and 16.5/1000 hospitalised patients for females and males respectively.[18] Predictors of poor outcome in other studies of *E. coli* UTI have included bedridden status of patients before infection, previous antibiotic therapy and septic shock at presentation.[19]

BACTERAEMIA

In large bacteraemia series, *E. coli* accounts for typically 20-30% of all episodes, with the majority community acquired and of community onset. Frequently, the only comparable organism, by incidence, is the gram-positive *Staphylococcus aureus*.[2-4, 20, 21] Two recent studies have suggested an overall annual population incidence of *E. coli* bacteraemia approximately 30/100,000 people/year.[22, 23]

Clinical and demographic features of *E. coli* bacteraemia are relatively homogenous across several contemporary studies.[22, 24, 25] Cheong, in a large study of community onset *E. coli* bacteraemia found a mean age of 62 and a female predominance (62.4%). A urinary source was most common (47%) followed by hepatobiliary origin (27%). A minority of infections (23.8%) were healthcare associated.[24] These findings are also reflected in recent data from the European Antimicrobial Resistance Surveillance Network (EARS-NET), showing 64% of patients with invasive *E. coli* infection were 65 years or older.[26]

Whilst the mortality from uncomplicated UTI is relatively low, patients with *E. coli* bacteraemia incur significant morbidity and mortality. Historical studies indicate a mortality of up to 30% with this presentation.[20] More recently, mortality in two contemporary studies is lower, although still significant (11% and 13.6%).[22, 24] Predictors of mortality included increased age and co-morbidities and increased severity of the bacteraemic illness. A non-urinary source of infection has also been shown to have a poorer outcome.[22, 24] The healthcare burden of bacteraemia is high, with a mean hospital length-of-stay in the range of 7-12 days.[25, 27]

ANTIMICROBIAL RESISTANT *E. COLI*

Since the mid-20th century, the emergence and spread of *E. coli* resistance to single antimicrobial classes or agents has been repeatedly noted. This includes sulphur-based agents, fluoroquinolones and narrow-spectrum beta-lactams.[28, 29] A number of outbreaks of antimicrobial resistance have been associated with clonal strains.[30]

THE EMERGENCE OF COMMUNITY ONSET ESBL *E. COLI*

The emergence of multi-drug resistant ESBL harbouring *E. coli* (ESBL-EC) as a community pathogen has led to a marked change in the epidemiology of multi-resistant gram-negative bacteria

in the past two decades. Before this time, multi-resistant gram-negative infection was almost entirely nosocomial. Although the organisms implicated included *E. coli*, other species such as *Klebsiella pneumoniae* and *Enterobacter cloacae* dominated. The ESBL genes involved were usually from the bla_{TEM} or bla_{SHV} classes.[31, 32]

In a comprehensive review of the topic, Pitout *et al* identified a 1998 publication describing an elderly patient from Ireland, as the first described case of community acquired ESBL-EC infection.[33, 34] In the following years, a number of publications reported low but increasing prevalence of community onset ESBL infection in European countries, including Spain and France. These infections consisted of a mix of healthcare associated and truly community acquired infection.[35-37]

By 2005 the literature contained a variety of publications, originating from multiple continents describing community onset, community acquired ESBL-EC.[34] Noteworthy amongst these were two articles: one providing the description of an *E. coli* 'epidemic clone,' harbouring the previously infrequent bla_{CTX-M} beta-lactamase, spread throughout many regions of the UK;[38] and the second, providing Canadian data demonstrating bla_{CTX-M} as the predominant ESBL in the Calgary region.[39]

NOMENCLATURE USED TO DESCRIBE ESBL-EC WITHIN THIS THESIS

Within literature on ESBL-EC nomenclature can be somewhat confusing as descriptors may refer to the bacterial strain and/or the resistance genes harboured within this strain.

Nomenclature referring to bacterial strains

Sequence Type (ST) is used to describe bacterial strains classified by multi-locus sequence typing (MLST) of seven conserved housekeeping genes spread across the bacterial chromosome. It is based on a standardised schema so globally comparable.[9]

*fim*H typing is another form of genetic sequence based typing. It classifies bacteria based on polymorphisms in the *fim*H gene. Unlike the housekeeping genes of MLST, the *fim*H gene is a functional component of bacterial virulence. It is also based on a standardised schema.[40]

Nomenclature referring to resistance mechanisms

Beta-lactamase resistance mechanisms are described with a standardised scientific nomenclature. The terminology varies depending on whether the description is of the gene or the enzyme. In description of a gene e.g. $bla_{CTX-M-15}$, the prefix of '*bla'* refers to the gene function (beta-lactamase). The following sub-text e.g. $_{'CTX-M-15'}$ refers to the sub-family 'CTX-M' and the specific variant '15'. Where the beta-lactamase is referred to as simply 'CTX-M-15' this is a reference to the enzyme rather than the gene.

The sub-family naming comes from various original scientific descriptions. Within beta-lactamases it is a common practice to designate the sub-family based on the hydrolysis substrate of the enzyme and the geographical location where the gene was first identified. For instance CTX-M is a 'cefotaximase' from 'Munich'. Newly identified variants of the gene are submitted to a centralised repository for numerical classification e.g CTX-M-13, CTX-M-14 or CTX-M-15.[41]

EXTENDED SPECTRUM BETA-LACTAMASE HARBOURING *E. COLI IN* AUSTRALIAN AND NEW ZEALAND

The Australian and New Zealand epidemiology of ESBL-EC is beginning to be characterised. Whilst there is no unified national surveillance program, the Australian Group on Antimicrobial Resistance has undertaken three point-prevalence surveys amongst hospital-based laboratories over the last decade. In a 2004 nationwide survey, the rate of ceftriaxone resistance amongst 596 *E. coli* isolates ranged by region from 1.1 to 2%. Most isolates were from blood and originated from a mix of in-patients and outpatients.[42] A follow-up 2009 survey of 1746 *E. coli* isolates from hospitalised patients (92% urinary isolates) showed a national rate of 7.7% third-generation cephalosporin (3GC) resistance amongst *E. coli.*[43] The 2010 survey of 2092 isolates originating from out-patient urine samples from the same group of institutions, showed a lower rate of 3GC resistance amongst *E. coli* at 3.4%. This ranged by region from 0.7% (Tasmania) to 5.5% (Victoria).[44]

A number of publications have also reported rates and trends from specific locations and population groups. In a study of *E. coli* bacteraemia during 2000-2004, in Canberra, ACT, Australia a 0.4% rate of cefotaxime resistance was identified.[23] In a survey indicative of current trends, over the years 2003-2007, a Melbourne hospital identified a statistically significant rise in the rates of ESBL harbouring isolates amongst urine *E. coli* (0.4 to 1.8%). Furthermore, by 2007, 63% of infections were community acquired.[45] In a 2009-2011 study of a selected population (women of child-bearing age, with UTI), in a single region of NSW, Australia, a rate of 5% and 9% ESBL expression was identified amongst *E. coli* causing cystitis and pyelonephritis respectively.[46]

Rates of gastrointestinal carriage of ESBL *E. coli*, have been less frequently studied in the Asia Pacific region. A survey of travellers departing from Canberra between 2008-2009, showed a 2% ESBL *E. coli* rate pre-travel.[47] A similarly low rate (1%) was demonstrated in NSW, amongst healthy family members of those individuals who took part in the study above mentioned study.[46] In contrast to these low rates, a 2010 point-prevalence survey of selected long term care facilities (LTCFs) in Melbourne, Victoria, detected ESBL-EC GIT carriage in 12% of residents, although this was highly variable between LTCFs ranging from 0% to 27% across facilities.[48]

As in overseas reports *bla*$_{\text{CTX-M}}$ ESBLs are now the dominant mechanism of multi-resistance in Australia.[44, 49] No contemporary Australian studies have examined local risk factors for ESBL-EC infection.

In New Zealand data from a number of research studies show close similarity to Australian data. In a point prevalence survey in 2006, ESBLs accounted for 0.7% (55/8707) of *E. coli* from urinary tract infections across the nation.[50] Although low, this was a considerably higher rate than identified in a 2000 survey, which reported a prevalence of 0.1%.[51] Isolates in the later study were almost exclusively *bla*$_{\text{CTX-M}}$ ESBLs. A limited 2009-2010 sample of 20 isolates from the industry-sponsored 'SMART' surveillance program showed a higher rate, at 5% ESBL production amongst 'upper' UTI in New Zealand, although these isolates were not stratified by community and hospital onset.[52]

Risk factors for ESBL-EC have been investigated in several small New Zealand studies. Older data identified residence in long-term care and airways disease (potentially representing frequent antimicrobial exposure) as risk factors for ESBL infection.[53] More recent data has identified a strong correlation between overseas travel and community acquired ESBL *E. coli* infection.[54] Further analysis of this patient group also revealed that the sub-type of *bla*$_{\text{CTX-M}}$ ESBL enzyme detected, was highly associated with the ethnicity of the patient.[55] A case-control study of 21 patients with ESBL-EC bacteraemia between 2003-2007, identified fluoroquinolone exposure, first generation cephalosporin exposure, and previously known colonisation with ESBL-EC, as independent risks for bacteraemia.[56] A 2009 survey of stool samples in a single region identified a 4.2% ESBL-EC carriage rate and an overall 5.1% carriage rate for ESBL harbouring Enterobacteriaceae, amongst 1691 patients. A limited multivariate analysis identified male sex and residence in a long term care facility as independent risks for carriage.[57]

Carbapenem resistant Enterobacteriaceae in Australia and New Zealand

Carbapenem resistant *E. coli*, and more generally carbapenem resistant Enterobacteriaceae (CRE), pose an emerging threat on a global scale. Although they comprise only a small subset of 3GC resistant *E. coli* in our region, they pose a disproportionate threat with regard to the impact of possible dissemination.

At present, with the exception of endemic *bla*$_{\text{IMP-4}}$ producing isolates, the majority of carbapenem resistant *E. coli* reported are associated with direct importation from overseas. However, one recent report has described local spread of an imported carbapenemase[58], and unpublished reports suggest possible broader local transmission of other carbapenemases, as outlined below.

Enterobacteriaceae harbouring the *bla*$_{\text{IMP-4}}$ metallo-beta-lactamase (MBL) gene were first identified in Australia in 2004.[59, 60] Since this time they have been implicated in a number of outbreaks of CRE[61, 62]. Currently they are considered to have low-level endemicity in a number of Intensive Care Units (ICUs) along the east coast of Australia.[63] Fortunately, this resistance mechanism has not been reported outside of healthcare settings in Australia.

Newer carbapenemase mechanisms are increasingly being reported in Australia and New Zealand in a variety of settings. Importation of the New Delhi MBL into Australia was identified very soon after initial reports of its dissemination in India and the United Kingdom.[64, 65] Since this time, a number of other importations in a variety of Enterobacteriaceae have been reported in the literature, associated with healthcare contact or community acquisition on the Indian subcontinent.[66-69] Thus far, local acquisition and transmission have not been described.

The *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{OXA-48-like}}$ Ambler class D carbapenemase have also been reported in Australia and New Zealand in patients with overseas healthcare contact. Thus far, they have been exclusively described in *Klebsiella pneumoniae*.[58, 70, 71] In one reported importation, secondary spread within a NSW hospital resulted in four new acquisitions and three deaths were directly attributed to the pathogen.[58]

E. coli and *K. pneumoniae* harbouring the Ambler class A *Klebsiella pneumoniae* carbapenemase (KPC) have been imported into the region in a similar manner to other classes.[72, 73] Of concern, a number of groups have informally reported isolates harbouring KPC's from patients without overseas travel, possibly indicating local transmission. (Unpublished - Ingram PI and Iredell J, reported on OZBUG, Australian infectious diseases email bulletin, 2012)

IMPACT OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance, such as 3GCR-EC, can impact on many facets of patient care. These include increased morbidity, mortality and cost of care. In addition, changes in pathogenicity of resistant bacteria have also been speculated.

Inadequate therapy and increased morbidity and mortality

The presence of unsuspected antimicrobial resistance in bacterial infection may render antimicrobial therapy ineffective. This risk of inappropriate therapy is dependent on two variables: the background incidence of resistant bacteria and the choice of empirical antimicrobial therapy. Marschall *et al*, in a large study examining all non-ICU gram-negative bacteraemia at a single site, identified a rate of 32% inadequate empirical therapy. However, in this institution the relatively low rates of antimicrobial resistant *E. coli* led to a statistically significant lower rate of inadequate empirical therapy (12.7%) in *E. coli* compared to other organisms.[74] In contrast, when a cohort of

patients with 3GC resistant isolates is studied, rates of inadequate therapy are frequently higher than those for susceptible isolates. In a meta-analysis of the impact of bacterial infection when Enterobacteriaceae harbour ESBL enzymes, an odds ratio (OR) of 5.56 for delay in effective therapy was identified.[75] Another comparison of ESBL and non-ESBL *E. coli* infections demonstrated 78% compared with 18%, rates of 'one or more day delay' in appropriate therapy.[25]

A significant delay in effective therapy, for any reason, is strongly associated with increased mortality in sepsis presentations.[76] Several studies have demonstrated this effect specifically with antimicrobial resistant *E. coli*.[24, 25, 77] Inappropriate empirical therapy was a significant predictor of mortality (OR 4.2) in community associate *E. coli* bacteraemia on multivariate analysis by Cheong et al.[24] Another study identified an OR of 3.6 for mortality with ESBL-EC infection, which the authors attributed to delayed therapy.[25]

Recent modelling of population estimates from the American Centres for Disease Control (CDC) provides insight into the scale of morbidity. For healthcare associated disease, in North America, ESBL producing *E. coli* lead to 9000 infections and 600 deaths per year. Estimates for carbapenem resistant *E. coli* indicate 1400 infections and 90 deaths per year.[78]

Economic cost

The economic cost of antimicrobial resistance is high. A recent study of ESBL *E. coli* blood stream infection (BSI) directly calculated a significantly higher cost of care per patient (€5026.00 increase/1.5 fold increase) when compared to susceptible isolates causing BSI. This burden increased further if inadequate empirical therapy was used due to a prolonged of length of stay and other complications.[79] Quite a number of older studies on a variety of ESBL infections have drawn similar conclusions through varied methodology.[80] Recent modelling by the CDC of cost of infection indicates an additional $40,000 USD per infection for healthcare associated ESBL producing Enterobacteriaceae infection.[78]

The economic impact of community ESBL-EC urinary tract infection compared to susceptible infection is yet to be rigorously compared. One small observational study (n=47), which may give some indication of relevant comparisons, analyses the differing cost of two treatment strategies for ESBL-EC. In this study the cost of treatment with oral fosfomycin therapy was considerably lower (less than $1/50^{th}$) of the cost of parenteral carbapenem therapy, whilst outcomes were equivalent between the groups.[81]

Virulence

The issue of potentially increased virulence of antimicrobial resistant *E. coli* has been examined from a clinical perspective by several authors.[46, 82] To date, studies looking at differences in

survival have been hampered by the difficulty in adjusting for differences in other characteristics and treatment of the two groups involved.[75] Other authors have studied virulence from a laboratory perspective and found little difference between *bla*$_{CTX-M}$ producing *E. coli* compared with non-ESBLs and other groups, including older ESBL mechanisms.[25] Once recent development has been the indication of increased 'clinical' virulence amongst ST131 *E. coli*, when compared with other strains. This has been demonstrated through a propensity for the clone to cause upper-tract infections relative to other isolates.[46, 83] Somewhat paradoxically, in-vitro studies have frequently failed to show significantly heightened virulence of this clone, possibly indicating limitations of the in-vitro techniques available.[84, 85]

INFECTION CONTROL MANAGEMENT OF ANTIMICROBIAL RESISTANT *E. COLI*

Contemporary infection control guidelines offer recommendations on the management of patients with multi-resistant *E. coli* and other antimicrobial resistant Enterobacteriaceae. They frequently pertain to bacteria harbouring an ESBL gene and/or those with carbapenem resistance. The Australian NHMRC guidelines and the American CDC guidelines recommend the use of transmission-based precautions for ESBL-EC.[86, 87] Measures recommended include the use of a single room, gowning and gloving when in contact with the patient.

However, due to the relatively recent epidemiological expansion of 3GCR-EC and CRE, many of the recommendations in these guidelines have been derived from research based on other pathogens, including Acinetobacter species and gram-positives such as MRSA. They were also formulated at a time when these pathogens had a lower prevalence and a more traditional healthcare-associated epidemiology. Furthermore, there are considerable differences in the physical characteristics of Enterobacteriaceae, particularly *E. coli,* which may reduce its persistence on hospital surfaces and the likelihood patient-to patient transmission compared with other organisms.[88]

Recently, a number of publications have offered further insight into the infection control requirements for antimicrobial resistant Enterobacteriacea. They have used contemporary molecular epidemiology to investigate transmission within hospital settings in a quantitative manner. The first of these studies was a 3-year observational study of ESBL-EC transmission amongst patients in an intensive care unit. Over this time only three episodes, most likely representing direct transmission of ESBL-EC between patients, were identified from the 74 admissions with known ESBL *E. coli* colonization, and 23 new hospital acquisitions.[89] In contrast, a parallel on *K. pneumoniae* identified 27 acquisitions with over half (52%) matched by pulse-field gel electrophoresis (PFGE) and clinical epidemiology.[90]

Two other studies from the same time period provide data on the transmission of antimicrobial resistant Enterobacteriaceae within the hospital system. Over an 18-month period, a prospective study at a large Dutch hospital characterised all 'highly resistant' gram-negatives (those harbouring an ESBL gene, or resistant to ciprofloxacin and gentamicin) within the hospital.. Transmission based precautions were used for all patients with this resistance. Over the study three epidemiologically linked clusters, totalling four transmission events, were identified amongst 96 *E. coli* isolates meeting the resistance definition.[91] In a follow-up study, over six months at 18 different hospital sites, the authors were able to determine a 'transmission index' for highly resistant gram-negatives, greater than 50% of which were *E. coli.* They identified 22 linked clusters of up to six people. Half of these involved ICUs. In total, approximately 7% of isolates were thought to be due to secondary transmission.[92]

Whilst the above studies demonstrated relatively low transmission with the use of transmission based precautions, more recent studies have provided data without these potentially burdensome measures. Two recent Swiss studies investigated transmission of ESBL Enterobacteriaceae in clinical settings without transmission based precautions. In one study of ESBL harbouring Enterobacteriaceae (75% *E. coli*), investigators studied patients who were roomed with an ESBL Enterobacteriaceae harbouring index patient before contact precautions were instituted. From 220 contacts originating from 93 index patients, only two episodes of transmission were identified (1.5%).[93]

A second study looked at transmission of ESBL harbouring Enterobacteriacea within hospital and also within households after discharge from care. Only 22% of the patients harbouring *E. coli* were managed with transmission based precautions in hospital compared with 75% of patients with *K. pneumoniae*. For *E. coli,* within hospital transmission was detected in 4 of 88 patients exposed to 40 index patients (overall 5.6 new cases per 1000 exposure days). For *K. pneumoniae* the rate was significantly higher at 13.8 cases per 1000 exposure days.[94]

The investigation of subsequent household contacts identified transmission in almost one quarter of this group.[94] Further support for the higher incidence of transmission in a setting of prolonged co-habitation comes from a recent study of a rehabilitation ward in Israel. In the rehabilitation ward, transmission was responsible for 54% of new ESBL-EC acquisitions.[95]

GLOBAL MOVEMENT AND THE SPREAD OF ANTIMICROBIAL RESISTANCE

The literature on CRE in Australia and New Zealand highlights the spread of new mechanisms of antimicrobial resistance, or highly pathogenic clones, by direct movement of patients. In the published paper 'Country-to-Country Transfer of Patients and the risk of multi-resistant bacterial

infection' this paradigm is reviewed.[96] This paper outlines demographics, characteristics and clinical features of patients who may move country. An extensive literature review searched for examples of multi-resistant gram-negative bacteria spread in this manner, and expanded this review to illustrative examples from gram-positive bacteria. In addition the publication provides some recommendations for mitigating this risk when managing patients with recent overseas healthcare contact in a clinical setting.

RESEARCH PROGRAM WITHIN THIS THESIS

Dissemination of antimicrobial resistant *E. coli* and the infections it causes encompass broad ranging issues. Considerations span from the intricate genetics of *E. coli* and its resistance elements to the sociological aspects of global population movements. Likewise, the management of patients with infection caused by such resistant bacteria is multifaceted. In addition to determining effective antimicrobial therapy other considerations include infection control management of patients within hospital and community settings.

The research program presented in this thesis aims to explore various interlinking aspects of antimicrobial resistant *E. coli* focusing on the Australian region. Chapter 2 presents a detailed review of ST131 *E. coli,* a newly described world-wide pandemic clone, which is currently one of the greatest global drivers of fluoroquinolone and ESC resistance spread. This review was a spring-board for the molecular epidemiological research in Chapter 4 and offers a global context in which to assess findings from our region presented in Chapters 3 and 4.

Chapter 3 presents an observational epidemiological study of risks for carriage of ESC-R-EC. This 'classical' research study identifies risk factors for harbouring resistant isolates, relevant to the clinician. It provides pragmatic data for healthcare workers and offers a foundation on which to build more in-depth research. The in-depth molecular epidemiological study in Chapter 4 builds on the observational study (Chapter 3) from which the bacterial isolates were derived. It provides some validation to the risks identified in the observational study, allows comparison with global data on molecular epidemiology and demonstrates findings unique to our region.

The investigation of carriage of resistance in returned travelers (Chapter 5) offers in-depth exploration of a particular aspect of introduction of ESC-R-EC delineated in Chapters 3 and 4, enhancing our understanding of this phenomenon and how it translates to the risk identified in the preceding chapters The survey of infection control practice presented in Chapter 6 investigates the

in-hospital management of patients with antimicrobial resistant *E. coli*. This research was undertaken in response to data collected as part of the observational study in Chapter 3. It informs an often over-looked aspect of patient care.

PUBLICATION: COUNTRY-TO-COUNTRY TRANSFER OF PATIENTS AND THE RISK OF MULTI-RESISTANT BACTERIAL INFECTION (CLINICAL INFECTIOUS DISEASES, VOLUME 53, JULY 2011)

See Appendix A for published version

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ABSTRACT

Management of patients with a history of health-care contact in multiple countries is now a reality for many clinicians. Leisure tourism, the burgeoning industry of medical tourism, military conflict, disasters and changing patterns of human migration may all contribute to this emerging epidemiological trend. Such individuals may be both a vector and victim of healthcare-associated infection with multi-resistant bacteria.

Current literature describes inter-country transfer of multi-resistant Acinetobacter spp. and *Klebsiella pneumoniae* (including *Klebsiella pneumonia*e carbapenemase and New Delhi beta-lactamase producing strains), methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci, and hyper-virulent *Clostridium difficile*. Introduction of such organisms to new locations has led to their dissemination within hospitals.

Healthcare institutions should have sound infection prevention strategies to mitigate the risk of dissemination of multi-resistant organisms from patients who have been admitted to hospitals in other countries. Clinicians may also need to individualize empiric prescribing patterns to reflect the risk of multi-resistant organisms in these patients.

INTRODUCTION

Although the world may not be truly getting smaller, the increasing use of air transport could give this impression. The exponential growth of international air travel means almost 1 billion passengers are projected to take an international flight during 2011[97]. An intercontinental journey now takes a matter of hours, rather than the weeks or months of old. Any medical practitioner could be faced with a person who may have been in hospital in any part of the world in preceding days. Confounding this, practitioners are increasingly managing patients who have travelled vast distances primarily for the purpose of seeking medical or surgical treatment for an illness. This emerging and diverse category of patient has previously been described in various relevant contexts[98-101]. Collectively we term members of this group the 'inter-country patient'. It includes the military and civilian aeromedical evacuee, the 'medical tourist' who travels specifically to seek medical treatment internationally and a larger, less well defined group of informal medical tourists; those whose medical care is divided between countries for a variety of social, familial or financial reasons.

Diseases such as malaria and arbovirus infection are classically described in returned travellers. The acquisition of blood borne viruses such as HIV, Hepatitis B & C has been associated with medical care in some developing health care systems[102]. The inter-country patient is also at high risk of the more prosaic, however. Health care associated multi-resistant bacterial infection is greatly heterogeneous, and not necessarily divided along lines of economic development and industrialisation. Even ubiquitous nosocomial pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae* will harbour vastly differing antimicrobial resistance patterns depending on the location of acquisition[103, 104]. Unsuspected resistance has implications on many levels. At an individual level, inadequate empirical antimicrobial therapy of severe bacterial infection, is now clearly linked to decreased survival.[76] At an institutional level, such patients may be the index case for nosocomial outbreaks of new pathogens. At a community level, inadvertent human carriage has been associated with the global spread of antimicrobial resistance in community pathogens[101].

This review categorises patients at risk of transferring multi-resistant bacterial pathogens from healthcare facilities in one country to another. We outline known examples of country to country transfer of specific multi-resistant bacterial pathogens of emerging and contemporary importance. Data was identified from a systematic search of the Medline and Ovid databases, the reference lists of published works and web-based resources identified in these works.

PATIENTS AT RISK

Aeromedical evacuation of civilians

Aeromedical evacuation, defined as international patient transfer to a medical facility by long distance air flight, is increasingly common. It is noteworthy that people with an increasing burden of co-morbid disease are now travelling internationally and may have been hospitalized overseas[105]. Collated statistics on civilian aeromedical evacuation are not readily available, although anecdotal reports indicate rising numbers[106]. A French insurer reported over 400 evacuations and repatriations in a single year[107]. The indications for transfer from a foreign hospital include a broad spectrum of medical and surgical conditions, occurring in both the military and civilian domains. Recently, mass mobilisation of civilian evacuation services has been utilised in the settings of a natural disaster such as the South East Asian Tsunami in 2004 and of an unnatural disaster such as the 'Bali Bombing' terrorist attacks in 2002[108, 109]. In both disasters, there were reports of transfer of multi-resistant gram-negative bacilli to institutions with low background rates of these organisms[100, 110].

There are factors in the dynamics of aeromedical evacuation that may increase the risk that such patients harbour multi-resistant bacterial pathogens (Table 1). Two European studies have investigated rates of carriage of multi-resistant organisms (MROs) in patients repatriated via air-transfer. Although one study showed rates of MRSA and multi-resistant gram-negative bacilli colonization in patients undergoing aeromedical evacuation to be similar to rates at their receiving institutions[111], a second study showed far higher rates[112]. The risk of MRSA was found to be highest in those with a prolonged ICU stay prior to transfer[111] while risk of multi-resistant gram-negative bacilli was found to be highest in patients transferred from Asia and Eastern Europe[112].

Aeromedical evacuation of military personnel

Military patient movements are frequent, with the United States Air Force Aeromedical evacuation system reporting over 40,000 patient movements globally during an 18 month period[113]. Recent military operations reveal a contemporary view of evacuees from the theatre of war. High rates of infection caused by multi-resistant gram-negative bacilli have been reported in injured military personnel evacuated from Iraq and Afghanistan[114, 115]. Some studies estimate bacterial infection complicating 15 to 25% of admissions[116, 117]. Etiological investigation of infections has implicated both environmental contamination of field hospitals and frequent nosocomial transmission within the military health system[118, 119]. Risk factors for infection during evacuation included abdominal injuries, soft tissue injuries and a high overall injury severity score. Additionally, the occurrence of such infection increased the likelihood of the evacuee requiring intensive care unit management[117]. At receiving institutions in North America, increasing MRO

infection has led to a marked escalation in the use of broader spectrum and higher cost antimicrobials in the military healthcare system[114].

Medical Tourists

Medical tourism has been defined as "organised travel outside one's natural health care jurisdiction for the enhancement or restoration of the individual's health through medical intervention"[120]. With increasing globalisation, such travel is now increasingly common[121]. For many years, patients have travelled internationally to access new and advanced treatment, unobtainable in their home country. Patients are now increasingly travelling outbound from developed countries to centres in South & Central America, South Africa and Asia where treatment may be obtained at a lower cost, without the delay incurred by publicly funded health systems in their home location; or with a greater privacy for cosmetic and other procedures[122, 123]. Destinations for medical tourists now encompass most corners of the globe. Patients travel internationally for procedures ranging from cosmetic surgery through to fertility treatment, major joint replacement and even lifesaving cardiac surgery and organ transplant. The utilisation of overseas medical care in order to defray cost is now employed by some health insurers in North America[121]. The American Medical Association and the American College of Surgeons have recently issued position statements pertaining to medical tourism[124, 125]. The exact numbers of 'medical tourists' of all types has not been documented. Recent estimates suggest that by 2012, 1.6 million patients per year will travel 'outbound' from North America to receive health care in another country[121-123]. Thailand, Hungary, India and Singapore are all expected to have one million or more in-bound medical tourists by 2012[126].

Certain aspects of medical tourism may increase the risk of acquisition and complicate the management of MRO infection (Table 1). There have been no prospective studies of the infections associated with medical tourism, most likely due to the difficulty in prospectively capturing this group. Available data comes from retrospective case series and surveys of patient and physician experience[127]. The largest published experiences arise from solid organ transplantation owing to the obligate need for medical contact in the recipient's home country[128-131]. Illustrating the difficulty of such studies, a single centre experience from North America found fewer bacterial infections in those who received transplants overseas when compared to local recipients. The authors felt this was significantly confounded by the inability to measure the incidence of early transplant infection in the overseas recipients[128]. Few series have specified the infecting bacterial pathogens. A Canadian experience reported eight of 20 patients with bacterial infection after renal transplant overseas, although the location of the transplant procedure was not specified. Four of

these patients suffered infection with bacteria likely producing an extended spectrum ß-lactamase (ESBL)[131].

Care Shared Across Countries

Similar in nature to the above groups are a broader group of informal 'medical tourists'; patients for whom care of an acute or chronic condition is spread across multiple nations. Factors influencing the country of care may include the proximity to friends and family, financial factors and access to advanced facilities. The term "diaspora" has been used in reference to large permanent expatriate populations from many nations. Recently this term as been applied to thriving expatriate Indian and Pakistani communities, which likely number more than 24 million and 7 million individuals, respectively.[132, 133] A nation's "diaspora" may maintain strong familial and cultural links to their nation of origin, including frequent return travel and potentially medical treatment for acute and chronic conditions spread across multiple nations.[65, 67, 134] This group is likely more numerous than that of medical tourists or that of aeromedical evacuees.

INFECTIONS OF CURRENT CONCERN

Gram-Negative Bacilli

ACINETOBACTER SPECIES

In a large outbreak of carbapenem resistant *A. baumannii* in a Belgian hospital, the two index patients were evacuated from a Greek ICU after road trauma. Despite increased infection prevention precautions, 17 subsequent cases of a clonal isolate were identified over the next six months[135]. Also secondary to evacuees from Greece, a smaller outbreak was also described in northern Italy[136].

A widespread carbapenem-resistant Acinetobacter outbreak in medical facilities involved in the treatment of aeromedical evacuees from military operations in Iraq and Afghanistan has been reported[115, 137]. Over 100 cases of bacteraemia over an 18 month period occurred within military hospitals in the United States and Germany[137]. Although Acinetobacter is associated with traumatic injury in many settings, molecular and clinical studies demonstrate the majority of infection in this outbreak was due to nosocomial acquisition[118, 119]. The UK has also reported the introduction of new strains of Acinetobacter from evacuees from Iraq[115]. In the setting of traumatic burns and blast injuries after the 2002 "Bali bombing" terrorist attacks, frequent Acinetobacter infection was noted in patients evacuated to Australia. Subsequent nosocomial spread in receiving hospitals was again reported[110].

Bacteria harbouring KPC & NDM Carbapenem Resistance Genes

Epidemiological investigation suggests that introduction of the KPC gene into several regions has been due to carriage by the inter-country patient. Israel was the first nation outside of the United States to report a large outbreak of KPC-harbouring *K. pneumoniae*. Widespread healthcare associated transmission occurred of a strain identified as of North American origin[138]. Greece has identified widespread clonal KPC producing *K. pneumoniae* indistinguishable from contemporary Israeli clones[139]. In neither case was a single point of introduction identified. The likely index case in a single centre outbreak in Germany was a patient who had previously been hospitalised in Greece[140]. Many further countries, including the United Kingdom and France, have reported episodes of colonisation or infection of patients transferred from endemic countries (Figure 1)[141, 142].

The New Delhi metallo-ß-lactamase gene (NDM-1) also confers almost complete ß-lactam resistance. NDM-1 has been identified in a broad range of gram-negative bacteria including *K. pneumoniae, E. coli* and *Citrobacter freundii*. Almost all isolates are also resistant to aminoglycosides, fluoroquinolones and other classes of antimicrobials. Of concern, some isolates have exhibited resistance to the agents of last resort, tigecycline and colistin[64]. The NDM-1 gene was first described in Sweden[143] and the United Kingdom[144], and was strongly associated with health care received on the Indian Sub-continent. In the UK, 9 of 19 patients had had recent hospitalisation in India or Pakistan for treatment ranging from solid organ transplantation to plastic surgery. Subsequently, imported cases associated with healthcare contact in India and Bangladesh have been reported in other regions, including the United States, Australia, Canada, Japan and several Europe nations[65, 145, 146]. Cases have also been identified amongst patients repatriated to locations in Western Europe from hospitals in Balkan nations and a cluster of cases identified in Kenya[147, 148]. These epidemiological observations require further elucidation (Figure 1).

Bacteria Harbouring ESBL Enzymes

Carriage of bacteria harbouring ESBL enzymes by the inter-country patient is long standing, and still remains a significant risk[149]. Early reports include intercontinental transfer of common nosocomial ESBL-producing bacteria such as *K. pneumoniae*[150]. Current literature reflects the emergence of *E. coli* harbouring CTX-M ESBLs, with healthcare associated acquisition responsible for approximately 15% of travel related infections due to ESBL producers in some studies.[54, 151]

Gram-Positive Organisms

Methicillin-Resistant Staphylococcus aureus

Almost fifty years after its emergence, the spread of MRSA by the inter-country patient still poses a threat to institutions that have maintained low MRSA prevalence. The prevalence amongst hospital acquired *S. aureus* isolates in the Netherlands and Scandinavia remains <1%, contrasting with levels in other European nations and North America (6-63%)[103]. Two outbreaks in the Netherlands were directly linked to the transfer of patients from institutions in France and Turkey where MRSA is endemic[152]. A study in Sweden demonstrated one quarter of 1,733 MRSA cases reported between 2000 and 2003 were likely acquired abroad; over half of these were healthcare associated[153]. The potential inter-country spread of MRSA via healthcare workers, rather than patients, is illustrated by the report of a Swiss physician found to have new nasopharyngeal colonisation with a North American clone of MRSA after returning from a clinical fellowship in North America[154].

Vancomycin Resistant Enterococci

Reports of inter-country spread of VRE come primarily from molecular epidemiologic assessments. VRE clonal complex-17 (CC-17), a group consisting of a number of closely related VRE sequence types, has been responsible for VRE dissemination in countries including the UK, Australia and North America[155]. Investigators linked a sharp rise in the rate of VRE in south-west Germany to the likely importation of CC-17 VRE to their hospital system[156]. An outbreak due to CC-17 has also been reported in Turkey[157]. Neither report identified a single point of introduction.

Clinical reports of VRE transfer between nations have been prevalent in Europe.[158] Low incidence Nordic countries (where VRE accounts for <1% of enterococcal isolates) have had sporadic importation and outbreaks from other nations since the early 1990s[158]. Molecular studies were strongly suggestive of inter-country spread of a distinctive VRE clone from North America to Norway and also to Ireland[159]. In a prospective study from the Netherlands, VRE was identified in approximately 3% of patients repatriated from a number of countries, with Asian origin being a significant risk[112].

Hyper virulent Clostridium difficile

Since the initial description of hypervirulent ribotype O27, there have been repeated descriptions of transfer of the strain with the inter-country patient[160, 161]. A retrospective study in 2007 identified the transfer of a patient infected with the hypervirulent strain from the UK to Ireland very soon after the initial descriptions in 2005; fortunately no outbreak occurred[160]. Introduction of the strain into France in 2006, which now has sustained transmission, was speculated to be due to

transfer with patients from neighbouring Belgium[162]. Australia has reported a single case of importation in a patient recently hospitalized in North America[161].

APPROACH TO THE PATIENT

All hospitals should have a pre-defined approach to management of the patients transferred from other institutions, including those in other countries (Table 2). Pre-emptive contact isolation may be considered when there is a risk of introduction of an MRO not currently found in the institution receiving the patient. In institutions with few or no endemic MROs, there will likely be a greater willingness to institute pre-emptive contact isolation. In some institutions, there may already be a high prevalence of MROs and it may seem to matter little that a patient has come from another institution which also has endemic MROs. However, introduction of new mechanisms of antibiotic resistance or new "hospital adapted" bacterial strains may pose risks of amplifying antibiotic resistance. An example may be the receipt of a patient in a hospital in the United States with endemic KPC producers of a patient from a hospital in India or Pakistan where NDM producers are endemic.

In the setting of management of an individual patient with suspected bacterial infection, the authors suggest a considered approach to the use of empirical therapy (table 3).

Healthcare staff must adopt a pragmatic and non-judgemental approach to the management of the inter-country patient who has acquired an MRO infection. This may be challenging in the setting of a patient who has sought a healthcare intervention believed inappropriate or unethical by the home treating clinician, such as commercial organ transplantation, experimental or cosmetic procedures. This attitude is crucial in order to avoid the patient feeling stigmatised and to facilitate open communication of information between the patient, family and clinician. International institutions may operate with constraints and resource limitations that are not present in the patient's home-nation. Furthermore, the patient may have felt they had no option but to utilize the facilities available in a foreign country due to the urgency of care required or the financial cost of care at home.

Local, national and international regulations may pertain to the notification, transit and control of patients harbouring MROs[163]. This is a complex and evolving area that varies between jurisdictions.

Conclusion

Management of patients transferred from other institutions is a daily reality for almost all health care practitioners. The patient with international healthcare contact may present to healthcare institutions in a variety of forms, ranging from the overt (e.g. aeromedical evacuee) to the unsuspected (e.g. elective surgical day case). In some settings, ready identification of a patient's origin in overseas hospitals is difficult and requires specific questioning. Similarly, a multitude of communication barriers may lead to difficulty obtaining information pertaining to a patient's medical care in another country.

Contemporary molecular epidemiological techniques have allowed us considerable insight into the origins and movement of healthcare-associated MROs. The range of potential MROs acquired by the inter-country patient is broad. The authors have outlined a small number with current significance. A key concept is the dynamic nature of such outbreaks. These may emerge and disseminate before reaching the general medical literature. At times, outbreaks may go undetected in their country of origin until exported with the inter-country patient[64, 139].

Furthermore, emerging data now suggests that the risk of acquisition of some MROs may extend to those without healthcare contact during travel to countries of high endemicity[47, 164]. Given the very large pool of international travellers, this area requires further exploration to better define risk factors and the potential magnitude of this problem.

For an individual patient, the significance of an MRO infection will largely depend on their current medical condition and may range from an incidental finding to a life threatening infection. For an institution, the significance of importation of MROs depends on the pre-existing milieu of MROs and the likelihood of spread, determined by infection prevention practices.

In conclusion, there are many factors that may complicate the identification and management of infections with MROs in the inter-country patient. Clinical vigilance in the form of sensitive and thorough questioning coupled with a high standard of baseline infection prevention practice in all patients must be the first line of defence.

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FIGURES AND TABLES

Table 1

Factors predisposing to increased risk of infection and carriage of multi-resistant bacterial organisms in aeromedical evacuees and medical tourists

FACILITIES
Hospital accreditation varies vastly between nations, providing variable levels of oversight for institutional infection control and antimicrobial use.
Medical tourists may have procedures undertaken in unlicensed settings occasionally using unproven & experimental techniques.
Evacuees may transit through multiple health facilities in a short space of time during the process of evacuation. For example, contemporary US military evacuees averaged 4 facilities in 7 days[118].
Confined spaces and limited facilities of transport vehicles used for evacuation may make some regular infection control practices impossible.
Barriers including language and differing clinical practice may limit the scope of information transferred with a patient.
PATIENTS
Common scenarios for evacuation such as road trauma & combat injuries have high background rates of secondary infection[117].
High acuity of illness in transferred patients means they may be transferred directly from Intensive care units which traditionally have high rates of MROs.
Medical tourists undergoing solid organ transplant or cancer therapy acquire the additional risk factor of immunosuppression whilst overseas.
Medical tourism packages are frequently combined with a vacation which risks exposure to a broader range of community pathogens.

Table 2
Recommendations for the management of patients who have been hospitalized internationally

Maintain vigilance.
Ask specifically about healthcare contact whenever a patient reports international travel within the previous 12 months.
Pre-emptive isolation and screening should be used in patients with a history of hospitalization internationally and who have a high risk of carriage of multiresistant organisms
Isolate patients who have had direct hospital to hospital transfer or recent international hospitalization involving prolonged hospital stay, intensive care or critical care admission, major trauma, burns or receipt of chemotherapy or immunosuppression e.g. solid organ or stem-cell transplant
Screening needs to be customized to the receiving institution: Focus screening on organisms that are not already endemic at your site.
Basic screening may include axillary, inguinal and nose/throat swabs PLUS rectal swab or stool sample PLUS clinical specimens including catheter urine, surgical drain or wound discharges –Screen for MRSA, VRE & ESBL producing or carbapenem resistant GNBs.
Only screen for *C. difficile* if diarrhoea is present
Receive transferred patients in an area of the hospital equipped to manage isolation for MROs
Patients may initially require management in an area of higher acuity than required for their medical care e.g. patients for rehabilitation may need to go to an acute ward until screened
All receiving institutions should have a readily accessible infection prevention policy defining at-risk patients, screening procedures and pre-emptive isolation criteria.
If your institution frequently receives patients from a particular location, a customised protocol should be developed and maintained for this location: Including an outline of the current pathogens of concern and empirical therapy recommended in the case of

infection.

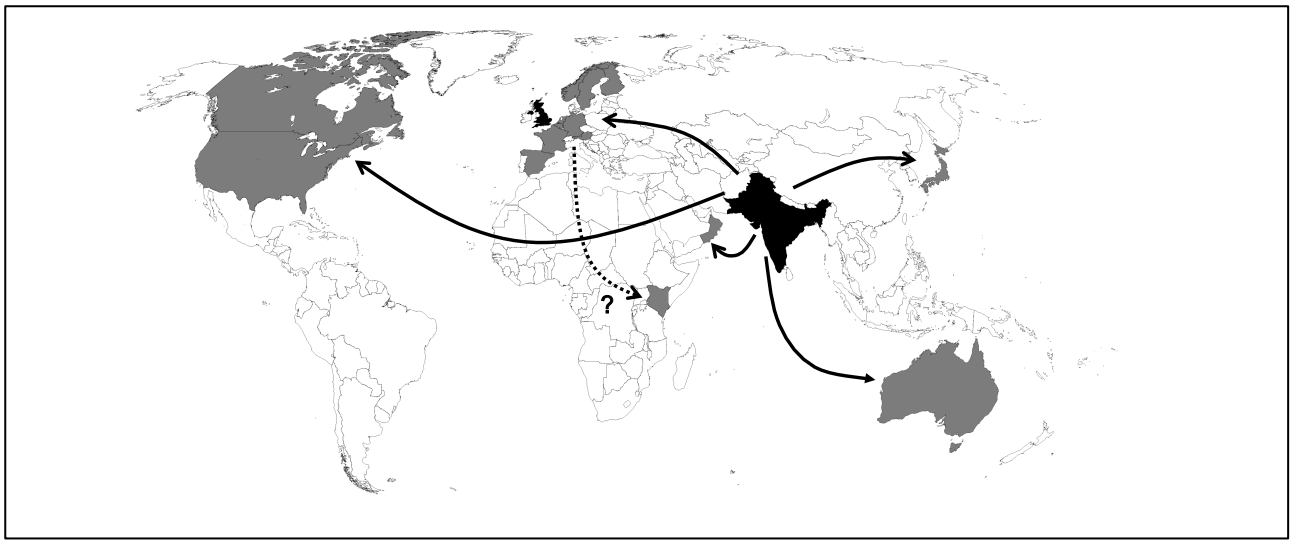
Table 3
Approach to suspected bacterial sepsis in the patient previously hospitalized in another country.

Ensure appropriate microbiology samples for the clinical presentation e.g. blood cultures, urine culture, respiratory tract cultures if required
Notify the microbiology laboratory of the patient origin. They may broaden their testing beyond their normal scope, e.g., detection of NDM-1 beta-lactamase, *C. difficile* ribotype etc
If screening has identified MROs: These bacteria must be targeted in empirical therapy.
If susceptibilities are available, use these to guide antimicrobial selection. If susceptibilities are not available, empirical therapy may include agents such as Linezolid or Daptomycin (for VRE and MRSA) and Polymyxin B, Colistin or Amikacin for MDR gram negative bacilli. If available, consultation with an Infectious Disease physician or clinical microbiologist may be helpful in selecting the optimal agent for identified pathogens.
If no screening results are available: Therapy must target the prevalent pathogens at the transferring institution.
When possible, ascertain these by direct discussion with this institution as recent outbreaks may not be publicized. See suggestions above for empirical therapy.
If screening does not detect an MRO: Treat as per local guidelines. However, screening is not 100% sensitive.
If the patient fails to improve on empirical therapy, then re-assess for occult sites of infection and re-culture as extensively as possible. Consider empirical therapy for organisms prevalent at the transferring institution as above.

MDR=multidrug resistant

Figure 1.
Schematic representation of epicentres (black) and reported/potential importations of NDM-1 (panel A) and KPC (panel B) ß-lactamase producing organisms (grey).[65, 138-142, 145-148]

Panel A – NDM-1



Panel B - KPC

CHAPTER 2. THE *ESCHERICHIA COLI* ST131 PANDEMIC CLONE

INTRODUCTION

The recently emerged worldwide pandemic *E. coli* clone, multi-locus sequence type (MLST) 131 is a significant global actor in the spread of 3GC and fluoroquinolone resistant *E. coli*. Aspects of this clone, including its origins, epidemiology and antimicrobial resistance were extensively reviewed in the 2010 published paper 'Escherichia coli O25b-ST131: a pandemic, multi-resistant, community-associated strain', summarised in the abstract below.[165]

Escherichia coli sequence type 131 (ST131) is a worldwide pandemic clone of E. coli, causing predominantly community-onset antimicrobial-resistant infection. Its pandemic spread was identified in 2008 by utilising multi-locus sequence typing (MLST) of CTX-M-15 extended-spectrum beta-lactamase (ESBL)-producing E. coli from three continents. Subsequent research has confirmed the worldwide prevalence of ST131 harbouring a broad range of virulence and resistance genes on a transferable plasmid. A high prevalence of the clone (approximately 30-60%) has been identified amongst fluoroquinolone-resistant E. coli. In addition, it potentially harbours a variety of beta-lactamase genes, including most often CTX-M family beta-lactamases, and less frequently TEM, SHV and CMY beta-lactamases. Our knowledge of ST131's geographical distribution is incomplete. A broad distribution has been demonstrated amongst antimicrobial-resistant E. coli from human infection in Europe (particularly the UK), North America, Canada, Japan and Korea. High rates are suggested from limited data in Asia, the Middle East and Africa. The clone has also been detected in companion animals, non-companion animals and foods. The clinical spectrum of disease described is similar to other E. coli, with urinary tract infection predominant. This can range from cystitis to life threatening sepsis. Infection occurs in humans of all ages. Therapy must be tailored to the antimicrobial resistance phenotype of the infecting isolate and the site of infection. Phenotypic detection of the ST131 clone is not possible and DNA-based techniques including MLST and PCR to identify known single nucleotide polymorphisms and repetitive sequence PCR are described.

Since 2010 a number of publications have further delineated and tracked changes in the epidemiology of ST131. Many of these studies suggest increasing rates of ST131 amongst fluoroquinolone resistant and/or ESC-R-EC, including a number of regions now reporting that more than half of all ESBL-EC are ST131. Additionally, ST131 has now been found to carry carbapenemases encoding genes, a finding which multiplies the risk created by this successful clone

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INTRODUCTION

Escherichia coli is a finely tuned, ubiquitous human pathogen. It is a common cause of urinary tract infection (UTI) and bacteraemia in humans of all ages. In addition, it is a frequent cause of varied organ infections ranging from the biliary system to the central nervous system. The spectrum of pathology can range from a spontaneously resolving cystitis to life threatening sepsis syndrome.[1] Not confined to the community, *E. coli* infection is also a common hospital acquired pathogen.[166]

Over the past five decades the medical community has witnessed increasing antimicrobial resistance in *E. coli* in the community setting. Initially, resistance was described to particular agents, such as ampicillin, trimethoprim, sulphur-based antimicrobials or tetracyclines.[28] More recently the horizon of resistance has broadened with the emergence of broad resistance to large families of agents. In particular, plasmid-mediated extended-spectrum beta-lactamases (ESBLs) have become prominent in community-onset *E. coli* infection.[31, 34] In addition to the resulting resistance to most beta-lactam antibiotics, ESBL producers are frequently also resistant to aminoglycosides and fluoroquinolones.

There are a variety of reasons for the increase in prevalence of antibiotic-resistant *E. coli. E. coli* is an organism known for its mobile genome and propensity to exchange genetic material.[9] However, the dissemination of 'clonal' organisms harbouring resistance is also well documented. Clonal outbreaks of *E. coli* clinical infection previously described include 'Clonal Group A' (CGA) in North America[7] and O15:K52:H1 in multiple nations.[30, 167] It is estimated that 10-20% of all *E. coli* UTIs may be caused by a small set of clonal groups.[168] In 2008, two research groups analysing the population biology of ESBL-producing *E. coli*, almost simultaneously described 'serogroup O25b, sequence type 131 (ST131)' occurring in multiple countries on three continents. This previously unremarkable molecular clone harboured a CTX-M ESBL gene and larger armamentarium of virulence genes.[169, 170] Since this discovery in 2008, research has retrospectively documented a 'pandemic' emergence amongst ESBL-producing and other antimicrobial-resistant clinical isolates in the middle of this decade. Previous to this, only sporadic isolates of this clone can be identified in multi-locus sequence typing (MLST) databases and published series. The rapid and apparently boundless rise of the ST131 *E. coli* clone is the subject of this review.

EPIDEMIOLOGY

Human infection and colonisation

Published research detailing the geographical distribution and antimicrobial resistance of human infection and colonisation by *E. coli* ST131 is summarized in Table 1.

EUROPE

ST131 *E. coli* is widely disseminated amongst 'antibiotic-resistant' community and hospital-onset *E. coli* in the UK. Originally identified as the 'CTX-M ESBL-producing UK epidemic strains A-E',[12] between 2003 and 2004, these strains have subsequently been confirmed as ST131.[38, 171] In one UK region ST131 comprised 64% of community-acquired and 84% of hospital-acquired cefpodoxime-resistant *E. coli* infections.[172] A UK national study of fluoroquinolone-resistant, non-ESBL-producing *E. coli* bacteraemia isolates illustrates the rapid emergence of this strain with isolates first identified only in 2004.[173] High rates of asymptomatic carriage of fluoroquinolone-resistant ST131 strains have been demonstrated in Northern Ireland nursing home patients.[174] In the Republic of Ireland, ST131 was also widely disseminated amongst CTX-M ESBL-producing *E. coli*.[175] No data exist on ST131 among relatively antibiotic 'susceptible' strains.

The epidemiology of the clone throughout mainland Europe is less well characterised. Current data suggest a heterogeneous distribution of infection and carriage, with prominence of the clone amongst antibiotic-resistant isolates. A collection of fluoroquinolone-resistant *E. coli* from eight European countries showed ST131 comprised 24% of this entire group. However, the number of isolates varied markedly between countries with Spain and Italy most prominent.[176] Spanish ESBL-producing *E. coli* data from 2004 revealed 9% of isolates were ST131.[18] A follow-up national study in 2006 demonstrated that 13% of ESBL-producing *E. coli* were ST131 and that they had a nationwide distribution.[177, 178] More recent data from a single region in Spain found that 22% of similar isolates from 2006-2008 were ST131 - 50% originated from nursing home patients.[179]. A study of a single region in Italy found that 61% of isolates selected from a collection with fluoroquinolone resistance and harbouring ESBL genes, were ST131.[180] French data demonstrate the emergence of this clone primarily amongst resistant isolates. ST131 was first identified in France in 2001 and rose to comprise 46% of ESBL-producing *E. coli* from 2006-2007 in one series.[181, 182] Nationwide data from community-onset ESBL-producing *E. coli* infections identified that 25% were ST131, although only one of 40 patients was felt to have 'true community-acquired' infection.[183] Data on non-ESBL-producing *E. coli* from UTIs from 2002-2003 revealed that only 3% were ST131, with the authors calculating an overall rate of 1.5% of UTIs caused by the clone.[181] Similarly, carriage of ST131 without CTX-M ESBLs has been identified in a 7% of healthy volunteer stool in France.[184] In Norway 20% of all national CTX-M-producing *E. coli* in 2003 were ST131.[185] Belgian data from 2006-2007 demonstrate a high prevalence of the clone in community-acquired ESBL-producing isolates. All CTX-M-15-carrying *E. coli* assayed, comprising 62% of all isolates, were ST131.[186]

The epidemiology of other European nations can only be inferred from case reports and smaller studies. Primarily hospital-based outbreaks have been described in Croatia,[187] Portugal[11] and Germany.[188] The clone has also been identified in Austria, Germany, Hungary, Russia, Switzerland and Turkey.[169, 176, 189]

The Americas

The epidemiology of ST131 is well characterised in Canada, with low rates in susceptible *E. coli* and high rates in resistant isolates. Two studies comprising UTI isolates, with little antimicrobial resistance, from the years 2005-2007, have demonstrated rates of ST131 in isolate collections of <3% and 1%.[168, 190] In contrast, in ambulatory patient isolates selected for fluoroquinolone or trimethoprim-sulfamethoxazole resistance from 2002-2004, ST131 comprised 23% of all isolates and 44% of fluoroquinolone-resistant isolates.[191] Blood culture isolates of ESBL-producing *E. coli* from a single region mirror the UK experience, with emergence of the strain in 2003 and a rapid rise to comprise 41% of isolates from 2004-2007. An overall rise in the incidence of ESBL-producing *E. coli* bacteraemia was also attributed to the emergence of the clone.[3]

Recent data from North America suggests ST131 as "the major cause of significantly antimicrobial-resistant *E. coli* infections in the United States".[192] A geographically widespread selection of isolates primarily from blood stream infections suggested that ST131 comprised 67-69% of isolates resistant to fluoroquinolone or extended-spectrum cephalosporins. In this study, no susceptible samples were ST131.[192] Recent studies from Chicago and Pittsburgh also identified high rates amongst resistant isolates. ST131 comprised 53% of CTX-M-ESBL-producing *E. coli* in Chicago and 30% of ESBL-producing *E. coli* in Pittsburgh, with a range of accompanying ESBL genes.[193, 194] ST131 *E. coli* has also been identified in renal transplant recipients and haematology patients in Texas, both groups with high background antimicrobial use.[195, 196]

A single report has identified ST131 in South America. The clone comprised 8% of 28 ESBL-producing *E. coli* hospital associated isolates from Rio De Janeiro, Brazil.[197]

Asia and the Middle East

ST131 has been frequently identified among antimicrobial-resistant isolates in Japan and Korea. A national survey in Japan identified the clone in 21% of ESBL-producing *E. coli* from 2002-2003.

Interestingly a greater genetic diversity within the clone and a greater variety of accompanying CTX-M ESBL genes was found in this region than elsewhere.[198] The clone comprised 33-63% of fluoroquinolone-resistant isolates from various Japanese regions.[199] Amongst community-onset infection ciprofloxacin-resistant isolates in Korea, the clone comprised 25% of isolates, only 19% of which harboured an ESBL gene[200]

In a small Cambodian sample, ST131 clones comprised 27% of community-onset UTIs due to ESBL-producing *E. coli* during 2004-2005.[181] Infrequent isolates have been detected among larger collections of clinical isolates in China[199] and the Philippines.[201] Faecal carriage was identified in a small number of hospital patients with ESBL-producing *E. coli* in stool in Lebanon.[169] The epidemiology in other Asian countries has been inferred from studies of returned travellers and from the high proportion of ESBL-producing *E. coli* ST131 isolates from India, Pakistan, Iran and Lebanon.[151] Supporting these data, the SMART study showed remarkably high background rates of 79% ESBL production amongst *E. coli* isolated from intra-abdominal infections in India.[202]

AUSTRALIA

Two studies from a single region of Australia recently confirmed the presence of the ST131 clone in this country. In one study of *E. coli* selected for fluoroquinolone or cephalosporin resistance, 31% of isolates were ST131. Less than half were CTX-M-producing.[203] In a second study 35% of fluoroquinolone-resistant isolates from a mix of hospital and community clinics were ST 131.[204]

AFRICA

Little data exist on the presence of the clone in Africa. Two small samples have suggested high rates amongst ESBL-producing *E. coli*. In Cape Town, South Africa, 43% of 23 such isolates were ST131 and expressed either CTX-M-14 or CTX-M-15 enzymes.[205] In the Central African Republic, 50% of CTX-M-15-producing *E. coli* were ST131.[181] A high proportion of ST131 have also been identified in a small number of travel-related ESBL-producing *E. coli* infections from Africa.[151]

Non-human carriage and infection

ST131 is represented amongst resistant isolates in companion and non-companion animals, although the extent is unclear thus far. A collection from eight European countries confirmed the presence of ST131, comprising 6% of ESBL-producing *E. coli* isolates recovered from companion animals.[206] Australian data show a surprisingly low incidence amongst fluoroquinolone-resistant isolates from companion animals (7.2% were ST131) compared with humans (35% were ST131).[204] Johnson *et al.* demonstrated intra-household sharing of the clone between domesticated animals; however transmission from companion animals to humans has not been confirmed.[207]

In non-companion animals, ST131 has been identified among ESBL-producing isolates in seagulls[49] and rats,[50] both of which have close contact with human populations. Two Spanish studies have suggested a low prevalence of the clone amongst poultry and pig farms in that nation.[208, 209] Mora found that the clone comprised 1.5% of *E. coli* strains recovered from Spanish poultry between 2007 and 2009. Surprisingly, in this study, the prevalence amongst *E. coli* recovered from retail chicken meat was considerably higher, comprising 7% of strains. In addition, a cluster of poultry and human strains was identified by PFGE, all of which carried the CTX-M-9 gene and a similar virulence profile, suggesting recent crossover between human and avian hosts.[209] The high similarity of an isolate from raw chicken and two human infections in the same geographical region in Canada was suggestive of transmission from foodstuff to humans.[190] Although these links are tantalising, there remains to be found a solid molecular epidemiologic connection between human infection and prior consumption of food containing ST131 *E. coli*.

Molecular epidemiological observations

Thus far, there are 48 entries of ST131 voluntarily submitted to the largest publicly accessible *E. coli* MLST database, with isolation dates ranging from 1992 to 2009. Notably, only a handful of other STs have a greater number of entries. This may equally reflect the current interest in ST131 and/or the ubiquity of this ST amongst *E. coli*. The majority of the isolates originate from human infection, primarily UTIs. In addition, ST131 *E. coli* from domesticated and farm animals, bird and food produce are also recorded in this database.[210]

Utilising the discriminating power of PFGE to analyse MLST-defined ST131 isolates has given considerable insight into the origin of the clone. Collections from focal outbreaks, and those selected for suspected clonality, have confirmed genetic similarity in excess of 85% on PGFE.[169, 179] In contrast, collections with a less highly-selected sample from human or animal origin have shown ST131 isolates with considerable diversity (<65% similarity by PFGE), at times unrelated by

traditional definitions. Even in such broad collections, small groups of identical or very closely related isolates are identified, often at distant locations.[171, 206] This pattern likely reflects the dual phenomenon of recent divergence of the clone from a common ancestor together with ongoing transmission of the clone.[169] Clinical reports support this hypothesis. There is convincing description of direct transmission between humans[211, 212], between animals and in contrast, surprising diversity amongst isolates from closely associated patient groups.[195] The ancestry and significance of occasional widely divergent or unrelated ST131 isolates remains unclear.[206]

Elucidating the worldwide distribution, transmission and reservoirs of ST131 is of importance in understanding the potential mechanisms of its dissemination and control. To date, this epidemiology has not been clearly defined. Since the initial descriptions in 2008, research has focused on identifying this strain in particular groups or collections selected for antimicrobial resistance phenotype or epidemiological clustering. There have been fewer opportunities to study this strain in unselected collections of pathogenic and non-pathogenic isolates.

Reservoirs of ST131

Potential reservoirs of ST131 including food or water sources, and travel from nations with a high prevalence of the clone, have been proposed in order to explaining the rapid emergence of the clone on multiple continents.[80] To date, reservoirs have been detected only at a local level, with high carriage and infection rates in nursing home residents in several nations.[174, 179] Investigations have only found sporadic isolates of ST131 amongst commercial animals and food-sources, although studies are limited.[190, 209] The potential spread of ST131 after introduction from international travellers has only been demonstrated indirectly. Pitout found the highest proportion of ST131 clones amongst travellers with ESBL-producing infections in those returning from the Indian Subcontinent and the Middle East.[151] Freeman and colleagues demonstrated a strong relationship between travel to India and community-onset CTX-M-15-producing *E. coli* infection in New Zealand.[54] Countries implicated in these reports, such as India and Pakistan, have known high rates of ESBL-producing *E. coli* infection but no data on the prevalence of the ST131 clone as yet.[202]

ANTIBIOTIC RESISTANCE

The ST131 ‘pandemic’ was initially described amongst *E. coli* harbouring the CTX-M-15 ESBL gene on a relatively homogenous plasmid.[169, 170] Subsequent investigation identified a high incidence of the clone amongst fluoroquinolone resistant non-ESBL-producing isolates and a low incidence amongst collections of susceptible *E. coli* isolates.[173, 176, 191, 195, 199] With further work, many authors have now confirmed surprising diversity amongst key transferable resistance elements including ESBL genes, fluoroquinolone resistance genes and the plasmid scaffold

harbouring them.[188, 200, 213] This diversity amongst a 'clonal' *E. coli* offers insight into the evolution of the clone and resistance. Lee *et al*. suggested the acquisition of transferable resistance elements as independent events from ST131 dissemination.[200] However, the timing and sequence of resistance acquisition remains unclear. Potential explanations offered include the spread of ciprofloxacin-resistant isolates, which then acquire a CTX-M gene, or possibly the simultaneous spread of clonal organisms and genes.[177, 199, 200] Johnson *et al.*, analysing North American isolates, demonstrated both vertical and horizontal transfer of the $bla_{\text{CTX-M-15}}$ genes. The gene was found in isolates closely related by PFGE – however, even within these clusters there was $bla_{\text{CTX-M-15}}$ discordance, suggesting horizontal gene transfer or potentially gene loss.[192] Given the clone's propensity for acquisition of resistance, a fine-tuning or evolutionary convergence between the clone, plasmids and acquisition of ESBL genes is likely.[170]

ESBL and AmpC enzymes

Resistance to β-lactam antibiotics in ST131 can be mediated by β-lactam-hydrolysing enzymes from two Ambler classes (A and C) and four distinct families. Among the ESBLs, CTX-M is the most prevalent in ST131, while SHV and TEM have been infrequently detected.[39, 25, 47]Of the AmpC β-lactamases, CMY has been most frequently reported.[180, 185, 194, 203, 214] Carriage of the genes encoding these β-lactamases is usually on a large plasmid (64 – 160 kb) frequently carrying genes encoding additional non-extended-spectrum β-lactamases, $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$, and the aminoglycoside-modifying enzyme AAC(6')-1b-cr.[3, 169, 170, 215]

CTX-M-15, the enzyme most closely associated with ST131, was first identified in India in 1999.[216-218] It is now the most widely distributed CTX-M worldwide.[219] The enzyme is responsible for resistance to the penicillins, cephalosporins (excluding the cephamycins) and monobactams. CTX-M takes its name from the enzyme's propensity to confer a higher level of resistance to cefotaxime than to ceftazidime (the M refers to its discovery in Munich).[220] Other CTX-M-type β-lactamases reported in association with the clone include CTX-M-2, CTX-M-3, CTX-M-9, CTX-M-14, CTX-M-27, CTX-M-32, CTX-M-61[181, 198, 209] A chromosomal rather than plasmid location of CTX-M-15 amongst ST131 isolates had also been reported, and could potentially be a contributing factor in the clonal spread of CTX-M-15-producing ST131 *E. coli*[170, 180]. SHV and TEM variants described in ST131 include SHV-12, SHV-5, SHV-7; TEM-24 and TEM-116[180, 181, 194, 206]. Isolates expressing these ESBLs may be susceptible to cefoxitin, β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) and carbapenems. However, the co-production of ESBLs with inhibitor-resistant β-lactamases (most prominently OXA-1) renders these strains resistant to commonly used β-lactamase inhibitors like clavulanic acid. AmpC β-

lactamases (such as CMY) are also resistant to β-lactamase inhibitors, as well as to cephamycins such as cefoxitin. Spanish data demonstrated 6% of AmpC-producing *E. coli* as ST131. The remainder had mutations leading to increased expression of chromosomally located AmpC genes.[214] The range and prevalence of ESBL and AmpC genes associated with ST131 is summarised in Table 2.

Resistance to other antibiotics

Fluoroquinolone resistance is found to be common amongst ST131 in most studies[180, 191, 195]. Johnson found that fluoroquinolone resistance and also trimethoprim-sulphamethoxazole resistance were significant markers of ST131 *E. coli* in Canada.[191, 192] This finding is not consistent through all regions, however.[203] The mechanism of fluoroquinolone resistance in ST131 isolates varies depending on the level of resistance. Amongst *E. coli*, low-level fluoroquinolone resistance is usually due to a single mutation in genes encoding fluoroquinolone targets.[221] The presence of plasmid-mediated quinolone resistance (PMQR) genes including *qnrA, qnrS* and *qnrB* may also contribute to low-level resistance, although they are infrequently described in the ST131 clone.[3, 193, 200, 222] Less common variations including *qnrB1* and *qnrB2* have also been reported associated with ST131.[180, 223] The 'dual substrate' aminoglycoside modifying enzyme AAC(6')-1b-cr also contributes to quinolone resistance via acetylation of selected fluoroquinolones.[224, 225] The effect of these plasmid-mediated genes on fluoroquinolone MICs is greater in combination than in isolation.[225]

When present, high level fluoroquinolone resistance in ST131 is generally due to chromosomal mutations of genes coding the fluoroquinolone targets *gyrA, gyrB, parC* and *parE*, as described in other *E. coli.*[221, 226] Studies of a ciprofloxacin-resistant clone (MIC_{90} ≥32 mg/L) showed multiple mutations in *gyrA* at codons Ser83 and Asp87, generating Asp-87→ Asn, Asp-87→ Gly or Asn-87 → Try amino acid changes and further single or double mutations of *parC* at Ser-80 and/or Glu-84 codons (Ser-80 → Ile and Glu-84 → Val or Glu-84 →Gly).[180]

The aminoglycoside-modifying enzyme AAC(6')-1b-cr is frequently associated with ST131.[3, 169, 170, 215]. (Table 2) Other aminoglycoside resistance enzymes have been detected less frequently and sometimes in combination.[227] Resistance to aminoglycosides remains variable despite the presence of this gene. In one study where 69% of 96 ST131 *E. coli* isolates possessed this enzyme, 35%, 49% and 35% of isolates were resistant to gentamicin, tobramycin and amikacin, respectively.[222]

Plasmids

The initial descriptions of ST131 demonstrated the IncFII group of plasmids harbouring CTX-M-15.[170] IncFII plasmids may also encode other types of β-lactamases found in ST131 *E. coli* including SHV-12 and CMY-2.[180, 185, 206] Greater clonal complexity among plasmids encoding CTX-M-15 is now apparent, with the multireplicons FIA, FIB and FII having been described in CTX-M-15-carrying plasmids of ST131 *E. coli*.[170, 185, 193, 228] In a Norwegian study of 23 ST131 strains, the CTX-M-15 gene was related to IncFII, FIB and FIA (87%, 44% and 42%, respectively).[185]

The full sequences of two CTX-M-15-carrying plasmids of representative ST131 *E. coli* have been characterised and shown to demonstrate extensive resistance gene profiles. The plasmid of one isolate, pEK499 (strain A: 117,536bp), a fusion of type FII and FIA replicons, harboured resistance genes for 10 antibiotics from 8 classes; $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{TEM-1}}$, *aac*6'-Ib-cr, *mph (A)*, *catB4*, *tet*(A) and the integron borne *dfr*A7, *aad*A5 and *sulI* genes. These were responsible for cephalosporin, β-lactamase inhibitor, aminoglycoside, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole resistance.[227]

DETECTION OF O25B-ST131

The three major characteristics of O25b-ST131 *E. coli* are its serogroup O25b, phylogenetic group B2 and sequence type 131. Each of these characteristics has been used to aid detection. Of note, a variety of molecular techniques have been used to determine clonality in previously described clones. The ST131 'pandemic' is amongst the first examples where MLST has been the defining technique in defining a widespread bacterial strain. The power of this technique is demonstrated in several studies where re-analysis by MLST of previously defined PFGE groups has confirmed a much broader clonality than originally suspected.[171, 191] This increased resolution does complicate comparison of the scope of ST131 to previous outbreaks, however.

MLST

MLST first delineated the pandemic clone and remains the 'gold standard' for identification. This requires the sequencing of pre-specified regions of highly conserved housekeeping genes, allowing comparison of nucleotide sequence with publically accessible databases. Hitherto, two separate schemas for sequencing and classification are available. That of Achtman *et al.*[9] defined and continues to maintain the database most frequently utilized in ST131 studies (http://mlst.ucc.ie/mlst/dbs/Ecoli).[168, 169, 172, 194, 203, 229] This scheme is based on the allele

of seven housekeeping genes, *adk* (adenylate kinase), *fum*C (fumarate hydratase), *gry*B (DNA gyrase), *icd* (isocitrate dehydrogenase), *mdh* (malate dehydrogenase), *pur*A (adenylosuccinate synthetase) and *rec*A (ATP/GTP binding motif). An alternative *E. coli* MLST scheme also using 7 housekeeping genes operated by Michigan State University, USA (http://www.shigatox.net/mlst) has been used.[3, 151] All but one of the housekeeping genes used in this scheme differ from the method proposed by Achtman *et al.*.[9]

PCR based rapid detection methods

Rapid detection methods have been developed to overcome the labour intensity of MLST. Rapid detection of ST131 using a single-nucleotide polymorphism (SNP) method based on only two housekeeping genes from the Atchman MLST schema (*mdh* and *gryB*) has been developed. The O25b variants showed the SNP on C288T and C525T for *mdh*; and C621T, C729T and T735C for *gyrB*.[191] This method has shown 100% sensitivity. When verified on a broader sample, it is likely that this method can be used as an alternative option to full MLST.

PCR-based methods to detect the phylogenetic[230] and O25 type,[229] followed by the confirmation of selected samples using MLST, have also been used.[169, 185, 189, 195, 229] This technique for detecting the O serotype O25b, is based on a method originally used to type important *E. coli* causing septicaemia.[231] This O25b typing uses the specific primers rfb1bis.f (5'-ATACCGACGACGCCGATCTG-3') and rfbO25b.r (5'-TGCTATTCATTATGCGCAGC-3').[229] A more accurate duplex PCR-based method to detect this clone was developed by the same group. This duplex PCR-based detection method for O25b-ST131 uses allele-specific PCR for the *pabB* gene unique to phylogenetic group B2 subgroup I isolates of O type 25b.[181] This duplex PCR has been successfully used as a rapid screening method for O25b-ST131 *E. coli* in many countries.[181, 193, 203, 232] A PCR method on a real-time platform has recently been described. This assay utilises amplicon melt curve analysis of two regions of the *pabB* gene. A third amplicon based on the group 1 *CTX-M* gene can be used to simultaneously detect the presence of $bla_{\text{CTX-M-15}}$.[233]

A third technique using triplex PCR to specifically detect CTX-M-15-producing O25b-ST131 *E. coli* is also described, based on the detection of the operon afa FM955459, *rfb*O25b and the 3' end of $bla_{\text{CTX-M-15}}$.[179]

REPETITIVE SEQUENCE PCR

Semi-automated repetitive sequence based PCR typing technique (Diversilab®, bioMerieux) has been found to reliably identify the pandemic clone.[203, 222, 234, 235] Although ≥ 95 % similarity to a known ST131 strain was used to define presumed ST131 by Diversilab in a Canadian study,[222, 235] other authors have shown that ST131 strains may have similarities as low as 92%.[203, 234]

PFGE

PFGE has been used to determine relationships amongst the ST131 complex rather than identify the clone in broader collections. The similarity of ST131 on PGFE depends on the origin of the collection. The majority of ST131 strains have similarities ≥ 80% by PFGE, corresponding to 4-6 bands difference.[236] However a minority of isolates show quite a diverse PFGE pattern. For example, the similarities of ST131 *E. coli* from the UK, Chicago and Japan were only 73%,[171] 67%[193] and 70%,[198] respectively.

VIRULENCE

E. coli ST131 is primarily an extra-intestinal pathogenic *E. coli* (ExPEC) harbouring virulence genes required for successful pathogenic invasion of a human or animal host. These virulence genes allow the clone to attach, to avoid and/or subvert host defence mechanisms within extra-intestinal sites, to scavenge limiting nutrients such as iron from the host; and to incite a noxious host inflammatory response, cumulatively leading to extra-intestinal diseases. The putative virulence genes possessed by extra-intestinal pathogenic *E. coli* can be classified into at least five categories based on their function: adhesins, toxins, protectins (capsule synthesis), siderophores and other additional virulence genes. There are ten commonly described virulence genes in ST131 *E. coli*. They include *iha* and *fim*H (encoding the adhesion-siderophore receptor and type I fimbriae, respectively), *sat* (secreted auto transporter, a type of toxin), *kps*M (encoding protectin II involved in group II capsular polysaccharide synthesis), *fyu*A and *iut*A (encoding siderophores involved in synthesis and uptake of ferric yersiniabactin and aerobactin, respectively), *usp* (uropathogenic-specific protein), *tra*T (surface exclusion, serum resistance associated), *omp*T (outer membrane protease) and *mal*X (pathogenicity island marker).[169, 191] The adhesins, iha and fimH were identified in 91-100% of O25b-ST131.[169] In addition to iha, Canadian O25b-ST131 *E. coli* isolates possessed the P fimbria subunit F10 allele (98%).[191] Unlike the other typical ExPEC *E. coli* including CGA and O15:K52:H1 *E. coli*; O25b-ST131 *E. coli* did not possess typical fimbriae and pilus tip adhesion molecules for pyelonephritis such as papA, P fimbriae structural subunit F16 allele and papG II.[191] In Korean isolates however, the *papG III* allele was identified in all ST131

studied.[200] The *sat* gene was present in 95–100% of O25b-ST131 *E. coli*.[169, 191] This is also a common toxin possessed by the other two types of *E. coli* (CGA and O15:K52:H1 *E. coli*).[191] The *fyu*A and *iut*A genes encoding the two siderophore virulence factors were present in 95–100% of O25b-ST131 *E. coli*.[169, 191] The *kpsM* II gene was detected in 94% of O25b-ST131 CTX-M-15-producing *E. coli*.[169] In contrast, this gene appeared less frequently (54%) amongst O25b-ST131 *E. coli* in Canada that were mostly non-ESBL producers but fluoroquinolone-resistant[191].

The other common *E. coli* virulence genes *usp, traT, ompT* and *malX* also appeared in nearly all ST131 *E. coli*.[169, 191] A clinical report of septic shock and emphysematous pyelonephritis in a previously healthy individual with CTX-M-15-producing ST131, described the presence of these 10 virulence genes plus *afa* and *dra* (central region of Dr antigen-specific fimbria, associated with binding and invasion in the mammalian urinary tract[7]).[211] These latter two virulence genes occurred in approximately 20% of ST131 isolates tested.[169]

The *ibeA* gene, encoding an invasion determinant associated with neonatal meningitis, has been detected in 34% of non-ESBL-producing ST131 *E. coli* blood culture isolates from north-west Spain.[209] This gene has only been infrequently reported in other collections.[191, 200]

The ST131 clone has also been identified amongst adherent-invasive *E. coli* (AIEC) from intestinal and extra-intestinal disease. This pathovar, distinguished from other ExPEC strains by a unique phenotype of adhesion and invasion properties, is associated with inflammatory bowel disease.[237] The intestinal AIEC phenotype ST131 carried multiple virulence genes infrequently described in the clone, including *papC*, *hlyA and cnf1*.[209, 238]

Clermont has demonstrated *in vitro* and *in vivo* virulence of the ST 131 clone. Bio-film formation identified *in vitro* is a potential contributor to the long-term persistence of the clone in various environments and its resistance to host immune defences. High virulence in a ‘mouse lethality’ model of extra-intestinal virulence, was speculated to be due to unspecified virulence genes harboured by the clone.[229]

HUMAN INFECTION

The spectrum of clinical infection caused by the ST131 clone appears broadly similar to that of other *E. coli*. UTI, representing the most common site of human infection with *E. coli*, is predominant. Description ranges from uncomplicated cystitis to severe infection complicated by bacteraemia, renal abscess and emphysematous pyelonephritis.[190, 211] Pitout *et al.* identified a propensity for urinary sepsis above other sites of infection when comparing ST131 and non-ST131

E. coli bacteraemia.[3] Johnson *et al.*, studying urinary tract origin isolates, found no clear correlation between ST131 and any particular clinical syndrome of renal tract infection.[195]

Other sites of infection have included the respiratory tract, ascitic fluid, intra-abdominal abscess, bones/joints and bacteraemia without a clinically apparent focus.[198, 212, 239]

ST131 has also been reported as a prominent cause of *E. coli* neonatal sepsis.[212] An exception to the usual spectrum of *E. coli* infection has been the description of *E. coli* ST131 pyomyositis amongst patients with haematological malignancy.[196]

Two reports illustrate direct transmission or the sharing of an identical ST131 clone between humans. Transmission of ST131 *E. coli* from an elderly father with pyelonephritis to his adult daughter after brief contact, caused her to suffer a similar illness.[211] Similarly, an identical isolate was recovered from an osteoarticluar infection in a young child and a faecal sample from her mother.[212]

Treatment

As mentioned above, the ST131 clone can harbour a diverse range of antimicrobial resistance mechanisms. Few descriptions of infections with the clone include details of antimicrobial therapy. Isolates harbouring *CTX-M* genes have been successfully treated with carbapenems alone or in combination with amikacin.[196, 211] For the clinician, even with identification and susceptibilities of a pathogenic *E. coli,* the sequence type of the isolate is unlikely to be known. Hence, comment on therapy is based on the commonly encountered antibiotic resistance phenotypes of ST131, which would be expected to respond in a similar manner to other sequence types with the same antimicrobial phenotype.

Non-ESBL-producing, fluoroquinolone-resistant isolates

Fluoroquinolone resistance is a hallmark of ST131 in many series. Although not harbouring an ESBL gene, such clones frequently carry resistance to other antibiotics. Among UTI isolates, the incidence of co-resistance to trimethoprim-sulfamethoxazole was 42% in Canada[191], 47% in Korea[200] and 70% in a European collection (including other STs)[176]. Carriage of non-extended-spectrum β-lactamase enzymes confers resistance to narrow-spectrum β-lactams, with ampicillin resistance rates ranging from 90-94%.[176, 191, 200] Fortunately, almost all isolates not producing ESBLs or AmpC remain susceptible to the third-generation cephalosporins such as ceftriaxone and cefotaxime.[195, 200] In severe infection with a strain not producing ESBLs or

AmpC, these would be potentially reliable treatment options. Oral therapy with an agent such as amoxicillin-clavulanate or trimethoprim-sulfamethoxazole if susceptibility is confirmed, could also be used in less severe infection such as uncomplicated UTI.

ESBL-PRODUCING ISOLATES

Parenteral therapy

Using older breakpoints, ESBL-producing *E. coli* isolates may test within the susceptible MIC range to some third-generation cephalosporins. In this circumstance many regions' laboratory standards suggest reporting resistance to these agents due to uncertainty about their efficacy in this setting.[240] Concern arises from studies suggesting poorer outcomes with third and fourth-generation cephalosporin therapy against ESBL-producers.[241, 242] Some authors suggest that β-lactam/β-lactamase inhibitor combinations may be effective where *in vitro* susceptibility of the isolate is demonstrated.[243, 244] The parenteral combination piperacillin-tazobactam has been used for UTIs and other infections including bacteraemia, skin structure infection and pneumonia, although published experience is limited.[243, 244]

Amongst ST131 clones, including those not producing ESBLs, concurrent aminoglycoside resistance is frequent. Reported rates of gentamicin resistance range from 44% amongst non-ESBL-producing isolates in Korea[200] to 86% resistance in CTX-M ESBL-producing isolates.[3] Amikacin resistance is less well characterised but also present in high rates amongst ESBL-producing isolates.[169] Even in the setting of *in vitro* susceptibility, uncertainty remains about therapeutic efficacy in severe infection such as blood stream infection.[245]

Carbapenems are the treatment of choice in serious ESBL-producing infection.[246] Several studies demonstrate successful therapy of UTI and non-urinary tract serious infection with meropenem or imipenem/cilastatin.[247] [248] Ertapenem, a newer narrower spectrum agent, has a limited body of experience, which also suggests successful therapy with ESBL-producing *E. coli* infection.[249, 250] There is a report of the emergence of carbapenem resistance in *E. coli* whilst a patient was ertapenem therapy.[251]

Tigecycline is a glycylcyline derived from minocycline with good *in vitro* activity against ESBL-producing *E. coli*.[252] There is some uncertainty about its potential drug concentrations achieved in the urinary tract.[253] However, a case report has documented successful outcomes in UTI caused by ESBL-producing *E. coli* and other highly resistant Enterobacteriaciae.[254] Temocillin, a derivative of ticarcillin with stability to β-lactamase hydrolysis and *in vitro* activity against the

majority of ESBL-producing Enterobacteriaceae, is a potential therapeutic option in this setting. There is limited published experience in treatment of a variety of ESBL-producing infections.[255]

Oral therapy

The oral combination amoxicillin-clavulanate has been used effectively in uncomplicated ESBL-producing *E. coli* cystitis when *in vitro* susceptibility is confirmed.[256] Of note, ESBL strains co-producing the non-extended-spectrum beta-lactamase OXA-1 may be resistant to b-lactamase inhibitor combinations.[257]

Fosfomycin is an oral antimicrobial that inhibits cell wall biosynthesis. It has been used for the treatment of ESBL-producing *E. coli* cystitis with a high success rate.[256] Of concern, a recent report demonstrates a rapid rise in resistance rates amongst ESBL-producing ST131 clones to 22% in Spain, closely tied to increasing use of fosfomycin.[258]

Nitrofurantoin is a synthetic nitrofuran antimicrobial with a long history of use in uncomplicated UTI.[259] No papers directly describe susceptibility of ST131 isolates. Amongst a European collection of fluoroquinolone resistant non-ESBL-producing isolates including ST131, 86% were susceptible to this agent.[176] Amongst Spanish ESBL-producing *E. coli,* 87% were susceptible.[178] It must be noted that nitrofurantoin is only useful in cystitis, not in renal infection per se.

CONCLUSION

Emerging from ‘molecular obscurity’ in the first decade of this century, ST131 *E. coli* is now a worldwide pathogen causing potentially severe antimicrobial-resistant infection. The dissemination of this clone has increased resistance to many low-cost and easily available antimicrobials commonly used to treat *E. coli* infection. Due to the rapid evolution of this worldwide pandemic, relatively little is known about this foe.

Molecular epidemiological study is increasingly describing the clone’s widespread but heterogeneous distribution amongst humans and animals. The vast majority of these data emanate from the developed world. Little is known about the distribution of ST131 in many parts of the developing world, areas suspected of having high rates of infection and which have even been postulated as reservoirs of the pathogen.[80] These areas, in addition, have a population particularly

vulnerable to morbidity and mortality from resistant infection due to the limited health care resources available.

Two key elements of knowledge required for potential control as a public health measure require elucidation. The first is a deeper understanding of the genetics of the ST131 clone, including greater insight into why ST131 has become so finely tuned that it can acquire resistance, virulence and rapidly disseminate on a vast scale. Research in this area should also increase our understanding of the risk of horizontal transmission of mobile resistance elements amongst ST131, between varying *E. coli* clones, and potentially to other Enterobacteriaceae. The second element is knowledge of the dynamics of transmission and dissemination of ST131 on a population basis. We have little firm information on many of the classical descriptors of communicable disease control: reservoirs, mode of transmission, incubation period, period of communicability, susceptibility and methods of control.[260]

Given the rapid spread of the ST131 clone and its demonstrated ability to cause severe infection in otherwise healthy individuals, consideration must be given to the planning of public health measures to attempt to control infection. A parallel could be drawn to community-associated methicillin-resistant *Staphylococcus aureus*. In order to successfully plan and execute interventions we will need further information on key aspects of this pathogen and the dynamics of transmission.

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All other authors: none to declare.

TABLES

Table 1

Geographical distribution and antimicrobial resistance of *E. coli* ST131 in humans

Country/Region	Specific Location	Date Range of Samples	Isolate Source	Selection criteria Used by Study	Number of isolates	% of isolates that were ST131 (n)	% ST131 Community Onset	%ST131 fluoroquinolone resistant	%ST131 SXT resistant	% ST131 harbouring ESBL
Multinational[170]	Europe, Canada & Mid East	2000-06	Laboratory collection	ESBL CTX-M-15	43	42(18)	0	NS	NS	100
Multinational[169]	Europe, Asia & Canada	NS	Clinical isolates & Laboratory collection	ESBL CTX-M-15	41	88(36)	39	97	53	100
Multinational[151]	Worldwide excluding India/Pakistan & Bangladesh	2004-06	Traveller returned from region. Majority UTI	ESBL	84	19(16)	NS	NS	NS	100
Europe[176]		2003-06	Community acquired UTI	fluoroquinolone resistant	148	24(35)	100	100	NS	NS
Belgium[186]		2006-07	Clinical Isolates	ESBL CTX-M-15	43	72(31)	90	NS	NS	100
Croatia[187]		2002-05	Clinical isolates	ESBL CTX-M	12	42(5)	NS	100	NS	100
France[181]		1994-2003	Laboratory collection	ESBL	128	6(8)	NS	NS	NS	100
France[181]	Tenon	2002-03	UTI	Non-ESBL +B2 phylotype	129	3(4)	NS	NS	NS	0
France[182]		2005	Bacteraemia	3GC resistant	41	15(6)	NS	NS	NS	100
France[183]		2006	Community onset UTI	ESBL	48	21(10)	10	100	60	100
France[181]		2006-07	Laboratory collection	ESBL	41	46(19)	NS	NS	NS	100
France[184]	Paris	2006	Stool from healthy volunteers	None	100	7(7)	100	57	NS	0
Ireland[175]		2003-07	Majority UTI	ESBL	371	<10	NS	NS	NS	100
Italy[180]	Rome	2006	Bacteraemia & UTI	fluoroquinolone resistant+ESBL	18	61(11)	NS	100	NS	100

Northern Ireland[174, 261]	Belfast	2004-06	Stool samples from residents of LTCF	ESBL+fluoroquinolone resistant	119	≥54(≥64)	0	100	NS	100
Norway[185]		2003	Clinical isolates	ESBL	45	20(9)	NS	NS	NS	100
Spain[177]		2004	Clinical isolates	ESBL	91	9(8)	NS	NS	NS	100
Spain[214]	Madrid	2004-07	Majority UTI	Amp-C	121	6(7)	NS	NS	NS	0
Spain[178]		2006	Clinical Isolates	ESBL CTX-M-15	37	86(32)	NS	NS	NS	100
Spain[179]	Lugo	2006-07	Majority UTI	ESBL	105	22(23)	NS	>96	>96	100
Spain[179]	Lugo	2007-08	Majority UTI	ESBL	249	22(54)	<50	NS	NS	100
Spain[258]	Madrid	2008	UTI	ESBL+Fosfomycin resistant	26	92(24)	NS	NS	NS	100
Turkey[189]	Izmir	2004-05	Community acquired UTI	ESBL	17	6(1)	100	100	100	100
Turkey[181]		2006	Laboratory collection	ESBL	10	20(2)	NS	NS	NS	100
United Kingdom[173]		2001-05	Bacteraemia	fluoroquinolone resistant+Non-ESBL+*aac*(6')-Ib-cr	10	50(5)	NS	100	NS	0
United Kingdom[38, 171]		2003-04	Clinical isolates	ESBL	287	≥65(≥188)	NS	NS	NS	100
United Kingdom[172]	Northwest England	2004-05	UTI & Bacteraemia	Cefpodoxime resistant	88	59(52)	NS	NS	NS	98
United Kingdom[181]		2004-07	Laboratory collection	ESBL	103	81(84)	NS	NS	NS	100
Brazil[181]		2001-05	Laboratory collection	ESBL	5	0	NS	NS	NS	100
Canada[3]	Calgary	2000-07	Bacteraemia	ESBL	67	31(21)	62	100	67	100
Canada[191]		2002-04	UTI	fluoroquinolone resistant or SXT resistant	199	23(46)	100	96	46	<2
Canada[181]		2004-06	Laboratory collection	ESBL	41	41(17)	NS	NS	NS	100
Canada[190]	Montreal	2005-07	UTI in women	Varied resistance	353	<1(2)	NS	NS	NS	NS

				sought						
Canada[168]	Montreal	2006	Women with UTI	None	256	<3	100	100	NS	NS
Canada[222]		2007	Clinical Isolates	ESBL	209	46(96)	57	NS	NS	100
USA[195]	Texas	2003-05	Bacteriuria in renal transplant recipients	None	40	35(14)	NS	86	NS	0
USA[192]		2007	Majority Bacteraemia	Varied resistance sought	127	†17(54)	NS	NS	NS	56
USA[194]	Pittsburg	2007-08	Clinical isolates	ESBL	70	30(21)	NS	NS	NS	100
USA[193]	Chicago	2008	Majority UTI	ESBL	30	53(16)	NS	100	38	100
Subcontinent[151]	India, Pakistan & Bangladesh	2004-06	Traveller returned from region. Majority UTI	ESBL	31	61(19)	NS	NS	NS	100
Cambodia[181]	Phnom Penh	2004-05	UTI	ESBL	30	27(8)	NS	NS	NS	100
China[199]		1998-2000	Laboratory collection	fluoroquinolone resistant	12	≥17(≥2)	NS	100	NS	NS
Japan[198]		2002-03	Laboratory collection	ESBL	142	19(27)	NS	NS	NS	100
Japan[199]		2003-07	Clinical isolates	fluoroquinolone resistant	128	≥30(≥38)	NS	100	NS	NS
Korea[200]		2006-07	Community Onset UTI	fluoroquinolone resistant	129	25(32)	100	100	50	19
Korea[199]		2005	Laboratory collection	fluoroquinolone resistant	21	≥33 (≥7)	NS	100	NS	NS
Philippines[201]	Manilla	2007	Clinical isolates	ESBL	15	7(1)	NS	NS	NS	100
Thailand[181]		1999	Laboratory collection	ESBL	5	0	NS	NS	NS	100
Australia[204]	Queensland	2007-08	Majority UTI	fluoroquinolone resistant	582	35(205)	NS	100	NS	NS
Australia[203]	Queensland	2008-09	Clinical isolates	Cephalosporin resistant or fluoroquinolone resistant	49	31(15)	NS	47	NS	53
Central African Republic[181]	Bangui	2004-06	Laboratory collection	ESBL	10	50(5)	NS	NS	NS	100

NS=Not specified by the authors, UTI=Urinary tract infection (or bacteriuria if not specified), ESBL=Extended Spectrum beta-Lactamase, SXT = Trimethoprim + Sulfamethoxazole, 3GC=3rd Generation Cephalosporin

≥, < & > are used to estimate when the text does not give an exact number for the relevant isolate

[†]Estimated at 17% of entire collection of *E. coli* isolates.

Table 2

ESBL, AMPC AND AAC ANTIMICROBIAL RESISTANCE GENES CARRIED BY ST131

Location	Number of ST131 with Extended Spectrum Phenotype	CTX-M-3 %(n)	CTX-M-14 %(n)	CTX-M-15 %(n)	Other CTX-M Genes %(n)	Other Extended Spectrum Genes %(n)	*aac*(6')-Ib-cr % of ST131
[†]Multiple Continents[151, 169, 170]	70			99(69)	CTX-M-1=1(1)		[‡]100
	8						
Australia[203]				50(4)	Untyped CTX-M=(25)2	CMY-2=25(2)	
[§]Belgium[186]	31			100(31)			
Cambodia[181]	8		75(6)	13(1)	CTX-M-27=13(1)		
Canada[3, 181, 222]	134		11(15)	87(117)	CTX-M-2=<1(1), CTX-M-61=<1(1)		[‡]75
Central African Republic[181]	5			100(5)			
Croatia[187]	5			100(5)			
France[181, 182]	33		21(7)	85(28)		TEM-24=3(1)	
India/Pakistan/Bangladesh[151]	19			100(19)			
Italy[180]	11		0	91(10)		SHV-12=9(1)	100
Japan[198]	27		74(20)		CTX-M-2=11(3), CTX-M-35=15(4)		
Korea[200]	6		17(1)	67(4)	CTX-M-22=17(1)		
Norway[185]	9			89(8)	CTX-M-1=11(1)		
Spain[177, 179, 209, 214, 258]	[¶]82		10(8)	66(54)	CTX-M-9=9(7), CTX-M-10=2(1), CTX-M-32=4(3)	SHV-12=1(1), CMY-2=2(3),c-AmpC=5(4)	[‡]100
Turkey[181, 189]	3	33(1)		66(3)			
UK[38, 181]	272	19(52)		[§]81(220)			
USA[193, 194]	37		14(5)	78(29)		SHV-5 or7=8(3)	[‡]63

c-AmpC=Chromosomal AmpC gene

† Isolates selected for CTX-M-15 genotype by researcher

‡Data only available on a selection of isolates from this country

§Some isolates CTX-M-28

¶One isolate contained CTX-M-14 and CTX-M-15

UPDATE ON *ESCHERICHIA COLI* ST131

EPIDEMIOLOGY

Since 2010 a number of publications have further delineated and tracked changes in the epidemiology of ST131. Many of these studies suggest increasing rates of ST131 amongst fluoroquinolone resistant and/or ESC-R-EC, including a number of regions now reporting that more than half of all ESBL-EC are ST131.

In North America, three studies have characterised the current epidemiology of ST131 amongst systematically collected unselected *E. coli* originating from bacteraemia or UTI (range 249 to 599 isolates per study). The ST131 clone comprised 23-28% of all *E. coli* isolates, the majority of which were phenotypically fluoroquinolone resistant and 3GC susceptible.[262-264] Amongst ESBL-EC the rate is likely higher, with 54% of isolates identified as ST131 in a large multi-centre study of community onset *E. coli* in five US hospitals.[265] Similarly, Canadian surveillance data demonstrated a continuing trend of increasing fluoroquinolone resistance and ESBL production amongst *E. coli,* attributed to expansion of the ST131 clone.[266] By 2011, 72% of the 227 ESBL-EC collected were ST131, in Canada.[267]

Fewer studies from Asia have reported ST131 data. A relatively low rate of 20% ST131 was identified amongst 103 community onset $bla_{\text{CTX-M}}$ producing ESBL *E. coli* in Seoul, Korea, during 2006-2011.[268] A second study identified a rate of 29% ST131 amongst 38 episodes of acute pyelonephritis caused by ESBL-EC.[269] Taiwanese data indicates that ST131 comprised 29.5% of isolates amongst 122 episodes of ESBL-EC bacteraemia over 2005-2010, with a noted numerical increase in ST131 infections over the study period.[270]

In Europe, a number of centres have also continued to publish their experiences. In Copenhagen, Denmark, amongst 115 consecutive ESBL-EC isolates, the prevalence of ST131 was 38% and accounted for the largest single ‘group’ of isolates in the study. In total, 91% of these were community acquired, which was significantly higher than for other ESBL-EC. Over 60% of ST131 belonged to a single pulsotype (812).[271]

Swedish national ESBL-EC surveillance data showed 34-38% of 1002 ESBL-EC isolates were ST131 over the years 2007-2011, with no clear temporal trend.[272] In Southern Sweden, ST131 comprised 26% of community ESBL-EC and 20% of hospital ESBL-EC during 2008-2009, although with overall low incidence of ESBL-EC in the population (2.5% in hospital, 1.6% in the community). The ST131 incidence was significantly higher than in the earlier period of 2003-2005.[273]

A collaborative study amongst four rehabilitation centres in Europe and Israel defined a high rate of ST131 amongst this hospitalised population with ST131 comprising 41% of 376 3GCR-EC isolates. This included a rate of 41% and 52% in the two Israeli sites, the first time ST131 has been identified in this country.[274] A small study of older isolates from neighbouring Egypt (2007-2008) found that 20% of inpatient and 17% of outpatient *E. coli* were phenotypic ESBLs with 19% (n=5) of these identified as ST131.[275]

CARBAPENEM RESISTANT ST131

Since 2010, descriptions of the ST131 clone harbouring a carbapenemase enzyme have been documented in the literature. Many authors have highlighted the grave threat posed by a combination of a pathogenic community disseminated clone and carbapenem resistance. Reports include ST131 harbouring prevalent and emerging carbapenemases including bla_{NDM}[276] , bla_{KPC}[277], bla_{OXA-48}[278], bla_{IMP}[279] and bla_{VIM}[280] . The majority of reports have been of singleton isolates recovered from individual patients or amongst much larger collections of CRE isolates. Of concern was a recent report from Italy describing a cluster of patients with bla_{VIM-1} harbouring ST131, closely related by PFGE, isolated over a one year period.[280] A similar report from Taiwan describes PFGE related ST131 isolates harbouring bla_{IMP-8} isolates from four non-hospitalised patients without any epidemiological link.[279]

RISKS AND RESERVOIRS

Since 2010, several publications have focused on identifying risks and reservoirs of ST131 *E. coli.* Residence in a long term care facility (LTCF), also referred to as a 'high level care' nursing home in Australia, has been strongly implicated as a risk for ST131 in a number of recent well-designed studies. In Minnesota, USA, a high risk for infection with an ST131 clone (OR=10) was identified amongst residents of LTCFs on multivariate analysis of 299 *E. coli* originating from a urinary tract source.[263] In a second study of the same region, ST131 comprised 76% of all LTCF *E. coli*.[281] In Paris, France, multivariate analysis identified a risk (OR=4.4) of LTCF residence comparing ST131 and non-ST131 strains amongst 152 patients with bla_{CTX-M} harbouring *E. coli* infection.[282] Supporting data also comes from several surveys showing high rates of ST131 amongst elderly patients in Ireland[283, 284] and nursing home patients in Germany.[285]

Whilst ST131 has been classically 'community associated', recent studies have associated healthcare exposure with ST131, primarily in North America.[263, 264, 284] One study demonstrated that 66% of ST131 *E. coli* infections were healthcare associated and 34% community associated. This was approximately inverse to other predominant clones such as ST95 and ST73.[281] Interestingly, multivariate analysis has demonstrated that the majority of this

relationship is through associations such as antimicrobial resistance phenotype and antimicrobial exposure rather than exposure to the healthcare environment.[263]

In Israel, widespread transmission of a variety of ST131 sub-clones (defined by PFGE) was demonstrated amongst residents of rehabilitation wards.[95] In contrast, in Taiwan few unique characteristics of patients with ESBL-EC bacteraemia caused by ST131 could be identified when compared to non ST131 ESBL-EC. There was no a significant difference in the odds of ST131 for community onset infections caused by ST131 (OR=1.3, p=0.47), and a significant odds ratios for ST131 amongst non-catheter associated UTI (OR=2.7).[270]

A recent Spanish case-control study demonstrated differing risks between ESBL producing ST131 and non-ESBL producing ST131. For each phenotype, the authors compared patients harbouring ST131 clones to those with *E. coli* of the same phenotype that was not ST131. Amongst the non-ESBL isolates, risks were similar to those defined in North America. They included female gender, diabetes mellitus, bedridden status and antimicrobial use (fluoroquinolone or amoxicillin + clavulanate). In contrast, amongst ESBL harbouring ST131, previous antimicrobial use and healthcare acquisition were protective for ST131. Male gender was the only delineated risk for ST131 ESBL-EC.[286]

H30 AND OTHER ST131 SUB-CLONES

Recent insight into subclonality within the ST131 clone has been a significant advance in our understanding of the ST131 pandemic. This has been investigated by characterisation of ST131 collections with pulse field gel electrophoresis (PFGE) and/or characterisation of polymorphisms in the *fim*H gene, which encodes Type 1 fimbrial adhesin.

A large study of 579 temporally (1967-2009) and geographically dispersed ST131 isolates was analysed by PFGE. This study demonstrated considerable temporal clustering of pulsotypes, with emergence of a select group of ‘high prevalence’ pulsotypes in the later part of the survey period. Three key pulsotypes emerged over 1990-2005. Two in particular (968 and 800) continued to dominate collections after this time, and feature heavily in other contemporary studies.[263, 264, 287] This finding suggested a refinement or evolution of ST131, as the clone has spread. Of note in this study, was a demarcation of pulsotypes between human isolates and those from food, food animals and companion animals. This indicates that animals are not the predominant source of ST131 in humans.[287]

Characterisation of polymorphisms in the *fim*H gene has been another focus of recent efforts to understand ST131. This gene is of interest because of two key traits. Firstly, it is more diverse (i.e. less strictly conserved) than traditional MLST housekeeping genes, and thus has been successfully

utilised in a two-locus typing schema for all *E. coli.*[40] Secondly, unlike MLST's housekeeping genes, *fimH* polymorphisms are of functional significance in the uropathogenesis of *E. coli*. Researchers have suggested that polymorphisms in the gene are a pathoadaptive trait and may be positively selected due to the integral function of *fim*H in adhesion to urothelium.[288]

Applying *fim*H typing to ST131 has given surprising insight into the clone and served to define a core fluoroquinolone resistant sub-clone of ST131. This sub-clone harbours the *fim*H polymorphism, H30, as classified by the schema of Weissman. This is defined by a single amino acid change from the consensus *fim*H sequence at the 166th position (R→H).[40] In-vitro studies indicate that this change significantly enhances the ability of *E. coli* to bind to urothelium, possibly suggesting a competitive advantage of the H30 clone.[288] Studies into the origin of this clone, using polymorphisms in fluoroquinolone resistance determining *gyr*A and *par*C genes, show a high level of conservation amongst H30. The authors suggest a possible single-strain of ST131 *E. coli* led to the emergence and dissemination of this now widespread sub-clone.[289]

Since the description of H30 sub-clones, several epidemiological studies defined its emergence amongst ST131. A multi-national retrospective study of ST131 isolates defined the 'abrupt emergence' of the sub-clone after 2000. Although not identified in isolates before 2000, it now makes up the majority of fluoroquinolone resistant ST131 (58%) and almost all ESBL positive isolates (85%).[289] Contemporary studies in two American health systems demonstrate a very high prevalence of the H30 sub-clone. This sub-clone constituted 23% and 24% of all *E. coli* in a veterans' hospitals and in Olmsted county Minnesota respectively.[263, 264] The latter study also demonstrated that the H30 sub-clone was spread throughout the dominant 968, 800 pulsotypes and many minority pulsotypes of ST131.

Given the apparent selective advantage of the H30 clone and the 968 and 800 pulsotypes, research efforts are now focused on deeper analysis of the unique genetic traits of these sublcones.

ST131 IN AUSTRALIA AND NEW ZEALAND

A number of publications have broadened our understanding of ST131 in Australia and New Zealand. Three related studies defined the epidemiology of ST131 in a single local region of Australia (Central West New South Wales). Rates of ST131 amongst *E. coli* appear divergent amongst population groups. Children (<6 years) and men had a prevalence of 8% (18/212) and 13% (49/389) ST131 respectively, amongst isolates of *E. coli* from UTI.[83, 290] In women of child bearing age, the incidence was considerably higher at 21% ST131.[46] All three studies showed a gradient, with higher rates of ST131 amongst pyelonephritis than cystitis isolates, demonstrating the

clinical virulence of this clone. Beyond demographics, study design limited the ability of these studies to elucidate risk factors for ST131.

Specific patient groups and clinical syndromes have been investigated in two recent studies. In a small laboratory based study, researchers compared fluoroquinolone resistant ST131 in humans and companion animals in Australia. Amongst 29 isolates they identified a commonality in PFGE profile, virulence genes and resistance genes, concluding that this represented exchange of ST131 clones between humans and animals in Australia. The direction of this exchange (human to animal, or vice versa) could not be determined.[291]

A recent clinical study from New Zealand was a retrospective analysis of 258 episodes of bacteraemia from trans-rectal-ultrasound guided prostate biopsy (TRUS biopsy). The authors found that 41% of all *E. coli* causing this syndrome are ST131, compared with 13% of bacteraemia causing *E. coli* from other sources. Almost all TRUS biopsy patients had been exposed to a fluoroquinolone as prophylaxis before biopsy. In total 83% of the ST131 were fluoroquinolone resistant compared with 25% of non-ST131 isolates.[292] In a follow-up study, the authors demonstrated that there were no significant differences in demographics or co-morbidity score between patients with ST131 and non-ST131 *E. coli* after TRUS biopsy.[82]

CHAPTER 3. EPIDEMIOLOGY OF COMMUNITY ONSET *ESCHERICHIA COLI* INFECTION RESISTANT TO EXPANDED-SPECTRUM CEPHALOSPORINS.

INTRODUCTION

E. coli resistant to 3GC antimicrobials include those harbouring an ESBL enzyme or other mechanisms of resistance such as plasmid borne AmpC like genes. These isolates are usually resistant to 3rd, 4th and 5th generation cephalosporin agents, and thus generally referred to by term 'Extended' or 'Expanded' spectrum cephalosporin resistant *E. coli* (ESC-R-EC). Defining risk factors for community onset ESC-R-EC (CO-ESC-R-EC) infection is important. At a clinical level, knowledge of risks can guide clinicians' choice of empiric antimicrobials to avoid potentially ineffective therapy in sepsis. It can also facilitate the use of narrow-spectrum agents where risks for ESC-R-EC are not present. At a population level, knowledge of epidemiology and patient-level risks, can direct efforts to study and control spread of ESC-R-EC and help delineate reservoirs and temporal trends for ESC-R-EC within communities.

Risk factors for CO-ESC-R-EC have been defined in many regions of the world.[270, 293-296] Viewed as a whole, these studies highlight the importance of applying contemporaneous local data when considering risks. Many risks are temporally, geographically or population specific. In one illustrative example, a validated risk-prediction tool for ESBL producing Enterobacteriaceae developed in Italy was trialled in North America. Although it performed reasonably, key demographic factors (Age ≥70 years and Charlson comorbidity index ≥4) were not applicable in the later cohort. Immunosuppression, which was not identified in the Italian model, was a significant risk in North America.[293, 296] In addition, unique reservoirs of 3GCR-EC may occur in certain populations. In the Netherlands, retail poultry has been implicated as a significant source of 3GCR-EC in the community. This appears to be due to specific factors including patterns of antimicrobial use in the farming of chickens in the Netherlands.[297] A clear link to animals has been difficult to prove in other regions.

From a local perspective, specific characteristics of Australia and New Zealand may further decrease the applicability of risks identified in other regions. Firstly, the background rate of community 3GCR-EC is far lower than in the setting of many risk-factor studies. For instance, according to the European Antimicrobial Resistance Surveillance Network's (EARS-NET) most

current data (2011), the background rate of 3GCR amongst invasive *E. coli* in Italy, where the risk prediction rule was derived, is approximately 20%.[298] Antimicrobial use patterns also vary greatly. As discussed in the publications in Chapters 3 and 4, within Australia and New Zealand, fluoroquinolone use amongst humans and animals is significantly lower than in most other areas of the world.[299] The use of 3GC antimicrobials in food production is also banned. This has led to a presumption of low rates of 3GCR Enterobacteriaceae contamination of meat products, although this has never been studied in a systematic manner.[300]

Finally, the Australian and New Zealand population includes high rates of immigrants from India and China, two countries which pose a high risk for GIT carriage of ESBL-EC after return from travel.[47] Australian census data from 2010 shows that following the United Kingdom and New Zealand, these countries make up the 3rd and 4th largest immigrant groups in Australia. Together they comprise 12% of all overseas born Australians.[301]

Given the notable differences between population demographics, antimicrobial use and other potential risk factors for ESC-R-EC in our region compared with other regions globally, we sought to determine characteristics of CO-ESC-R-EC in our population. This included locally applicable risk factors and the molecular epidemiology of infecting isolates. This was completed through a multi-centre collaboration to undertake a case-control study of CO-ESC-R-EC infection.

PUBLICATION: COMMUNITY ONSET *ESCHERICHIA COLI* INFECTION RESISTANT TO EXPANDED-SPECTRUM CEPHALOSPORINS IN LOW-PREVALENCE COUNTRIES (ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, VOLUME 58, APRIL 2014)

See Appendix A for published version

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ABSTRACT

Background

By global standards the prevalence of community onset expanded-spectrum cephalosporin resistant *Escherichia coli* (ESC-R-EC) remains low in Australia and New Zealand. Of concern, our countries are in a unique position with high extramural resistance pressure from close population and trade links to Asia-Pacific neighbours with high ESC-R-EC rates. We aim to characterize the risks and dynamics of community onset ESC-R-EC in our low-prevalence region.

Methods

A case-control methodology was used. Patients with ESC-R-EC or susceptible *E. coli* isolated from blood or urine were recruited at six geographically dispersed tertiary hospitals in Australia and New Zealand. Epidemiological data was prospectively collected and bacteria were retained for analysis.

Results

In total, 182 patients (91 cases and 91 controls) were recruited. Multivariate logistic regression identified risk factors for ESC-R amongst *E. coli* including birth on the Indian subcontinent (OR=11.13, 2.17-56.98, p=0.003), urinary tract infection in the past year (per infection OR=1.430, 1.13-1.82, p=0.003), travel to South East Asia, China, Indian subcontinent, Africa and the Middle East (OR=3.089, 1.29-7.38, p=0.011), prior exposure to trimethoprim+/-sulfamethoxazole &/or an expanded-spectrum cephalosporin (OR=3.665, 1.30-10.35, p=0.014) and healthcare exposure in the previous six months (OR=3.16, 1.54-6.46, p=0.02).

Amongst our ESC-R-EC the $bla_{\text{CTX-M}}$ ESBLs was dominant (83% of ESC-R-EC), and the worldwide pandemic clone ST-131 was frequent (45% of ESC-R-EC).

Conclusion

In our low prevalence setting, ESC-R amongst community onset *E. coli* may be associated with both 'export' from healthcare facilities into the community and direct 'import' into the community from high-prevalence regions.

INTRODUCTION

Despite a dramatic global rise in the prevalence of expanded-spectrum beta-lactamase (ESBL) producing *E. coli*, expanded-spectrum cephalosporin resistant *Escherichia coli* (ESC-R-EC) infections in the Australia, New Zealand, North America and selected European countries remain at relatively low levels. Recent Australian national data shows that 3.2% of community isolates carry such resistance. Approximately 80% of these harbor a globally dominant $bla_{CTX\text{-}M}$ ESBL gene and 12% a plasmid borne AmpC type mechanism[44]. European surveillance data shows a significant proportion of countries have rates below 10% ESC resistance amongst invasive *E. coli* isolates[26]. In the United States, a recent large sample of *E. coli* indicated 3.9% were ESBL-producing[265]. Although these low rates offer reassurance in the near-term, a year-on-year rise in incidence of community onset ESC-R-EC in low-prevalence countries is of concern[26, 302].

Australia and New Zealand are in a globally unique position. We have low rates of use of antimicrobials traditionally identified as a risk for ESC-R-EC. This includes very low fluoroquinolone use amongst humans and a ban on the use of ESC and fluoroquinolones in food production[299, 300]. In contrast, we have considerable extramural pressure on antimicrobial resistance rates. Our countries are located within the Asia-Pacific region, in which we share a mobile population[303] and frequent commerce (although no land-borders). A high proportion of our regional neighbours have rates of ESC-R amongst *E. coli* in excess of 25%[52, 202].

The aim of our study was to define the risk factors for, and dynamics of, ESC-R amongst community onset *E. coli* infections in the low-prevalence settings of Australia and New Zealand by using a case-control methodology. Furthermore, we characterize the resistance genes and membership of the worldwide pandemic clone ST131 in implicated isolates.

PATIENTS AND METHODS

The COOEE Study (**CO**mmunity **O**nset **E**SBL and AmpC ***E****. coli* Study*)* was a multisite case-control study, with prospective recruitment of patients and data collection. Six geographically dispersed tertiary centres in Australia (n=5) and New Zealand (n=1) participated. The human research ethics committees at The University of Queensland and participating sites approved this study.

Definitions

E. coli was **community onset** where a patient was resident in the community (including nursing homes), or hospitalized less than 48 hours at the time of onset; **Expanded-spectrum cephalosporin resistance** included all 'non-susceptible' isolates and was identified phenotypically. For ceftriaxone, a minimum inhibitory concentration (MIC) >1 mg/L was used. For ceftazidime, laboratories used an MIC >1 mg/L or MIC >4 mg/L, depending on their use of EUCAST or Clinical

and Laboratory Standards Institute (CLSI) criteria respectively[304, 305]; **Site of infection** was determined by the researcher from available information. For urinary tract infections (UTI), guidance was given as follows: 'Asymptomatic'= a positive urine culture, with no attributable symptoms; 'Lower tract infection'=lower urinary tract symptoms only, such as urgency, frequency and dysuria; 'Upper urinary tract infection'=temperature ≥38^{o}C, flank pain or costo-vertebral angle tenderness and/or any bacteremia from a urinary source. **Immune Suppression** referred to use at the time of the sample collection of corticosteroids (>15mg/day prednisolone or equivalent), calcineurin inhibitors, other non-biologics (e.g. mycophenylate, methotrexate), cytotoxic agents, biological agents or radiation therapy; **Charlson co-morbidity index**[306] was calculated on data available from the survey, with the exception of neurological impairment (dementia and hemiplegia) which was inadvertently omitted from the survey questioning. A **McCabe** score was assigned based on the investigator's estimate of participant survival (<1month, 1month-2years or >2years)[307]. **International travel** (excluding travel between Australia and New Zealand) was classified into geographical regions as follows *South Pacific Islands*, *South East Asia*, *Indian subcontinent*, *China*, *Japan*, *North America*, *Europe, Africa/Middle East*. **High-risk travel** (regions of Indian subcontinent, South East Asia, Africa, Middle East and China) was defined *a priori* based on Australian data[47]. **Healthcare exposure** was assessed by the Friedman criteria[308] with two modifications; a) day procedures were recorded b) the criteria were assessed in three 'discrete' time periods (<1 month ago, 1-6 months ago and 7-12 months ago). In addition, exact dates and details of any hospital admissions or surgical procedures were recorded and the interval (in days) from the termination of healthcare contact to the date of first medical review with the enrolling *E. coli* infection, was calculated.

Further definitions are found in Supplementary Material.

Clinical methods

A case-control methodology was used. **Case** patients with community onset ESC-R-EC in a culture of blood or urine were identified in the microbiology laboratory of participating hospitals. **Control patients** had community onset ESC susceptible *E. coli* isolated from the same specimen (urine or blood) as the case might be. Controls were not matched by any of clinical presentation, co-morbidity or demographic factors. They were selected as the next appropriate patient, after an enrolled case patient, within the same laboratory's specimen registration system. If the next appropriate control patient could not be recruited, the process was repeated at the same time-of-day and day-of-week, in a later week of the study. A single control was recruited for each case.

Inclusions & exclusions

A laboratory specific protocol was developed by each site to identify all potentially appropriate patients aged ≥16 years with an isolate of ESC-R-EC managed at the participating site. Patients cared for by external healthcare providers such as family doctors and external clinics (utilizing the participating laboratories as an external provider) were not considered for recruitment, due to the complex human-research ethics requirements in our jurisdiction. Initial screening to determine likely community onset and the presence of exclusion criteria was by review of available electronic laboratory data and/or contact with the clinician caring for the patient. Two exclusion criteria were applied: 1) Inability of the patient to give informed consent to participate 2) Extra-anatomical urinary drainage such as indwelling urinary catheter (in the community), intermittent catheterisation, ileal conduit or similar. These two groups, whom local clinicians already identified as high-risk for resistant infection, appeared to have relatively distinct demographic and health profiles. Hence, they were excluded in order to focus study resources on a more generalized population group.

Data collection

Hospitalized patients, or those attending ambulatory clinics, were approached for recruitment and data collection in person, whereas the remainder were contacted by telephone. By telephone, at least three contact attempts on different days were made. After informed consent, including explanation of the aims of this study, a structured interview was conducted using a standardized data collection form completed by a non-blinded investigator. Data was primarily self-reported by participants. Where the participant was uncertain of details (e.g. dates of hospitalization or antimicrobial use) or the investigator was unclear from the answer provided, they were able to review patient's medical records held at their institution.

For intermittent exposures (e.g. travel, healthcare exposure, antimicrobials etc.) participants were asked to recall 12 months before presentation. Exact dates of exposure were recorded. If the exact date was not recalled it was estimated ('start' of month = 1st, 'middle' or none specified = 15th, 'end' of month = last day).

Data was forwarded to a central co-ordinator where it was checked and entered into a secure database. Any omissions or discrepancies were clarified with the individual sites.

Laboratory methods

All phenotypic susceptibility data presented in this study has been assessed by EUCAST criteria[304]. All non-susceptible isolates were considered 'resistant' for the purpose of this

analysis. *E. coli* isolates from each patient were forwarded to the research laboratory with phenotypic identification and antimicrobial susceptibility undertaken by disk diffusion susceptibility testing (DST), automated system (VITEK2) or agar dilution, based on the criteria in use by the laboratory at the time. Where a susceptibility to an ancillary antimicrobial (e.g nitrofurantoin) was not available, this was tested by DST in the research laboratory. Where an isolate was originally tested by CLSI, DST using EUCAST criteria was undertaken (in the research laboratory), for agents where the non-susceptibility breakpoint differed between these two criteria (ceftazidime, cefepime, amikacin, gentamicin, ciprofloxacin and nitrofurantion). Where stated, MICs were performed by Etest (bioMerieux, France). For each isolate, a summative antimicrobial resistance score was calculated from 11 antimicrobials tested (ampicillin, amoxicillin+clavulanate, ceftriaxone, ceftazidime, cefepime, meropenem, trimethoprim+sulfamethoxazole (SXT), ciprofloxacin, nitrofurantoin, gentamicin and amikacin).

After overnight culture, bacterial DNA was extracted using an UltraClean microbial DNA isolation kit (MO BIO Laboratories, USA). ESC resistance genes were investigated by PCR using previously published primers and conditions[194, 309, 310]. A step-wise approach based on local epidemiology of resistance mechanisms was employed. All isolates were investigated for $bla_{\text{CTX-M-1}}$ group and $bla_{\text{CTX-M-9}}$ group genes. Isolates negative for these were investigated for $bla_{\text{CTX-M}}$ (consensus sequence), bla_{CMY}, bla_{DHA}, bla_{TEM}, bla_{SHV} and bla_{VEB}. All isolates were screened for carbapenemase genes using an in-house multiplex PCR (bla_{NDM}, bla_{KPC} and bla_{IMP})[311] and a singleplex PCR for $bla_{\text{OXA-48}}$ like enzymes[312]. All PCR amplicons were sequenced in forward and reverse direction using an ABI3730XL (Life Technologies, USA) capillary sequencer and compared to published sequences on Genebank (www.ncbi.nlm.nih.gov/genbank).

Presumptive identification of ST131 *E. coli* was determined by use of semi-automated rep-PCR (DiversiLab, bioMerieux, France). Isolates clustering within 95% similarity to multi-locus sequence type (MLST) confirmed ST131 reference clones, using a Pearson correlation co-efficient, were considered members of this clone[235]. A random selection of isolates (n=4) were confirmed as ST131 by formal MLST[9] .

Statistical methods

Sample size was calculated with overseas travel as a risk for resistant infection. With an estimated annual rate of overseas travel of 250/1000 population[303], a sample size of 95 cases with matched controls was required to detect this risk with an odds ratio ≥2.5 (power of 0.8 and two sided alpha of 0.05).

Continuous data on healthcare exposure was right-censored at 365 days. Univariate comparison was undertaken by X^2 squared test, Fischer's exact, Wilcoxon ranksum and logistic regression as indicated. Interactions were examined. A multivariate logistic regression model was constructed with variables significant on univariate analysis at a p=0.2 level. Backwards selected variables were retained in the final logistic regression model if their significance remained below p=0.2. Models were assessed by calculation of a ROC and Hosmer-Lemshow goodness of fit. All statistical tests were two tailed, and $p<0.05$ was considered significant. STATA version 12.1 (Statacorp, USA) was used.

RESULTS

In total 182 patients (91 cases, 91 controls) were recruited between March 2011 and October 2012 (Figure 1). Patients were recruited over 12 continuous months at five sites, and over nine months at one site. Sites contributed between 8 and 58 patients.

Bacteremia was detected in 33 patients (18%) and isolated positive urine cultures in the remaining 149 (82%). An uneven number of bacterememias occurred as one control patient recruited with a positive urine culture, subsequently manifested a positive blood culture. Patient's residence before presentation, clinical syndrome of presentation, and characteristics of hospital presentation did not differ significantly between case and control patients (Figure 1).

A further 43 patients with presumed community onset ESC-R-EC infection and no overt exclusion criteria were not recruited (declined to participate n=19, not contactable n=18, other n=6). On comparison with recruited study participants, the median age (56 years, p=0.81) and gender (11/43, 26% male, p=0.39) did not differ significantly from the recruited patients and they were not analyzed further.

Close temporal matching of cases and controls was not frequent. Samples from 9 controls originated from the same calendar day as the matched case. For the entire cohort, there was a median interval of 22 days between case and control sample collection.

Phenotype, resistance genes and ST131

All case patients' *E. coli* isolates demonstrated phenotypic ESC resistance (ceftriaxone + ceftazidime = 60 (68%), ceftriaxone only = 28 (32%), ceftazidime only = 3 (3%)). For the three *E. coli* with isolated ceftazidime resistance the MICs of ceftazidime in the study laboratory were; >256 mg/L, 2 mg/L and 0.25 mg/L. All control patient isolates were susceptible to ceftriaxone and ceftazidime. For all antimicrobials studied, with the exception of meropenem (100% susceptible)

and amikacin (resistance: case=4/91, 4%, control=0/91, 0%, p=0.121), resistance was significantly more likely in the ESC-R than ESC susceptible (ESC-S) isolates. For ESC-R-EC there was significant resistance to oral therapeutic options investigated including amoxicillin+clavulanate (ESC-R=59/91, 65% vs. ESC-S=15/91, 16% p=<0.001), ciprofloxacin (57/91, 63% vs. 6/91, 7% p<0.001) and SXT (64/91, 70% vs. 20/91, 22% p<0.01).

E. coli isolates were available for further analysis from 89 cases (98%) and 90 (99%) controls. Carbapenemases were not detected in any isolates. Expanded-spectrum cephalosporinase genes were detected in 87 of 89 (98%) ESC-R-EC as follows: ESBLs $bla_{\text{CTX-M-1}}$ group (36/89, 40%), $bla_{\text{CTX-M-9}}$ group (35/89, 39%), $bla_{\text{CTX-M-1}}$ & $bla_{\text{CTX-M-9}}$ group (3/89, 3%), $bla_{\text{SHV-5}}$ n=1 (1%); non-ESBLs $bla_{\text{CMY-2}}$ n=11 (12%), $bla_{\text{DHA-1}}$ n=1 (1%). The two remaining isolates included two of the three *E. coli* with isolated ceftazidime resistance (MICs 2 mg/L and 0.25 mg/L) and contained only $bla_{\text{TEM-1}}$, a non-expanded-spectrum beta-lactamase. ESC non-susceptibility most likely originated from hyper-production of this enzyme, with loss of this trait during passage and storage in the case of the lower MIC isolate.

The worldwide pandemic ST131 clone was presumptively identified in 46 patients (24%) who were significantly more likely to be case patients than controls (40/89, 45% vs. 6/90, 7%, p<0.001). Amongst ESC-R-EC, ST131 was not associated with any non-CTX-M enzymes. They constituted 54% of the entire CTX-M group isolates. In total 24 (60%) harboured a CTX-M-1 group enzyme and 19 (48%) a CTX-M-9 group enzyme (p=0.173 for comparison). This included three isolates (8%) harbouring both enzymes. There was no significant difference in the proportion of ST131 by sample type (blood vs. urine p=0.514) or hospital site (p=0.574). With the exception of the smallest site (where 0 of 8 were ST131), the clone constituted 19-32% of isolates from each site.

Demographics, comorbidities and antimicrobial use

Age was compared by visual inspection of histograms. Cases and controls had a similar bimodal distribution with peaks at approximately 25 and 65 years. Median and 25-75th centiles for cases and controls respectively were 61 (21-82) and 59 (19-87) years (p=0.769). Univariate comparison of demographic factors and medical co-morbidities between cases and controls is in Table 1. Male sex was the only variable with a significant difference (Odds Ratio (OR)= 2.3, 95% CI = 1.5-4.6, p=0.018).

Risk from previous urinary tract infection, renal allograft transplant and anatomical abnormality of the renal tract was investigated (Table 1). The number of urinary tract infections in the previous year was significantly associated with ESC-R-EC with an odds ratio of 1.32 (1.08 -1.63, p=0.008) per infection.

Results of univariate analysis of antimicrobial use in the previous year is in Table 1. Where the patient could not recall the antimicrobial taken, it was recorded as 'unknown'. Exposure to trimethoprim +/- sulfamethoxazole (SXT) (OR=3.02, 1.13-8.12, p=0.028) was a significant risk for ESC-R-EC. In addition, 7 of 7 patients who had been exposed to an expanded-spectrum cephalosporin (ceftriaxone, ceftazidime or cefepime) had an ESC-R-EC isolated.

Healthcare exposure

Healthcare exposure was analyzed using two distinct sets of data. Firstly, healthcare exposure, classified using Friedman criteria for healthcare associated infection, was analyzed in three time windows, with and without the inclusion of day procedures. Exclusion of day procedures performed marginally better at predicting ESC-R; exposure 0-1 month ago (OR=3.56, 1.14-11.14, p=0.029) and 2-6 months ago (OR=2.99, 1.50-5.98, 0.002) were associated with ESC-R-EC; whereas exposure 7-12 months ago (p=0.705) was not (full details are in the Supplementary Material).

Secondly, a continuous model of the temporal risk of ESC-R-EC after healthcare exposure was generated using the exact time interval since last hospital admission. Day procedures were excluded based on the results of the first analysis. This smoothed curve of odds ratios shows the lower bound of the 95% confidence approaching an odds ratio of 1.0 at approximately 4-5 months (Figure 2).

Travel, community and occupational exposure

Travel in the previous year was analyzed by region. Travel to the Indian subcontinent approached but did not achieve significance (p=0.09). Birth on the Indian subcontinent was a significant risk (OR=6.119, 1.32-28.44, p=0.021) (Table 1).

Occupational exposure to animals, medical patients and potential household risks were assessed, as was consumption of a variety of meats. No factors were significant (Table 1). Probable household transmission of ESC-R-EC was suggested in one case where the partner of an enrolled patient had an infection with a highly similar isolate (99% identical by rep-PCR using Diversilab) three months prior.

Multivariate analysis

For the multivariate model, healthcare exposure in the previous six months, excluding day-procedures, was selected as a pragmatic option (univariate OR=2.95, 1.59-5.46, p=0.001). This dichotomous measure was non-significantly different to the four-categories measurement used earlier (likelihood ratio test p=0.821). Travel to high-risk regions was selected from the travel group (OR 1.97, 0.94-4.11, p=0.071). Use of an expanded spectrum cephalosporin was combined with use of trimethoprim or SXT in order to enter the former into the model, given its accepted prominence as a risk factor for ESC-R-EC.

Significant variables on multivariate analysis were healthcare exposure, excluding day-procedures in the previous six months (p=0.002), birth on the Indian subcontinent (p=0.004), travel to high-risk regions (p=0.011), SXT/ESC use (p=0.014) and number UTIs in the previous year (p=0.003) (Table 2). Assessment of the final model demonstrated an area under the ROC curve of 0.77, and a non-significant Hosmer-Lemshow goodness of fit (p=0.289).

Interactions and alternative models

A significant correlation occurred between travel to high-risk regions and region of birth. Those born in high-risk regions were more likely to undertake high-risk travel than those born elsewhere (17/28, 61% vs. 21/154, 14%, p<0.001). This was particularly noted for birth and travel to the Indian subcontinent (7/13, 54% vs. 31/169, 18%, p=0.002). This correlation, and the use of differing parameters for healthcare contact and antimicrobial exposure are explored in alternative multivariate models (Supplementary Material). Specific population subgroups were also trialled in the model (symptomatic patients only, ESC-R $bla_{\text{CTX-M}}$ only and ESC-R ST131 only). None performed better than the final model, although the significance of healthcare exposure, male sex and region of birth/travel varied, depending on the model parameters selected.

Healthcare associated and non-healthcare associated ESC-R-EC

A difference in risk between healthcare associated (HA) ESC-R-EC and non-healthcare associated (non-HA) ESC-R-EC was investigated by analysis of risks within the healthcare associated (n=73) and non-healthcare associated (n=109) cohorts separately (full details are in Supplementary Material). Several of the identified risks for ESC-R-EC appeared to be most concentrated in one cohort. Travel to high risk regions (p=0.001), birth on the Indian sub-continent (p=0.006) and male sex (p=0.018) were only significant amongst the non-HA group. Conversely, a risk from SXT/ESC use was significant only in the HA group (p=0.026). The number of UTI's in the previous year was non-significantly different amongst either group separately.

Correlates of ESC resistance enzymes class

Correlates of ESC resistance enzyme class were investigated by comparison of patients harbouring *E. coli* with CTX-M group enzyme and other enzymes ('non-CTX-M'=CMY, DHA, SHV, TEM). Full details are contained in Supplementary Material.

There was no significant difference in the site of infection between CTX-M and non-CTX-M harbouring participants (p=0.473), and although bacteremia was more frequent in the CTX-M group, this did not reach statistical significance (13/74, 18% vs. 0/15, 0% p=0.114). A significantly higher median resistance score was present in CTX-M compared with non-CTX-M isolates (median=6, IQR=5-7 vs. 4, 4-5, p=0.001). Notable differences included higher rates of resistance

amongst the CTX-M group to the non-beta-lactam oral agents ciprofloxacin (56/74, 76% vs. 1/15, 7%, p<0.001) and SXT (60/74, 81% vs. 4/15, 27% p <0.001).

In regard to potential risk factors, the CTX-M group was significantly more likely to have healthcare exposure in the previous six months than the non-CTX-M group (45/74, 61%, vs. 3/15, 20%, p=0.005), although not in the previous 12 months (p=0.72). Other factors used in the multivariate model, trended towards significance amongst the CTX-M group, included more high-risk travel (p=0.052) and fewer reported UTIs in the previous 12 months (p=0.054). On comparison of factors not included in the multivariate model, 'any overseas travel' was more likely in the CTX-M group (27/74, 36% vs. 1/15 7%, p=0.033).

DISCUSSION

This multicentre prospective case-control study of community onset ESC-R-EC has several key findings that have implications for risk based empiric antibiotic prescribing, infection control practices and control of ESC-R-EC within communities.

Firstly, we established that 6 months is a practical, evidence-based definition for the duration of increased risk of a community onset *E. coli* isolate harbouring ESC-R after healthcare exposure. The time-dependent relationship of healthcare exposure and resistance seem intuitive in nature, however previously there has been little supporting data; hence, authors have used a variety of definitions from 1-6 months[294, 313, 314].

Overall, the significant contribution of healthcare exposure (OR=3.15) as an ongoing 'exporter' of resistant infection in a low-prevalence setting highlights the importance of controlling ESC resistance in the healthcare system. Supporting this hypothesis, United Kingdom data has recently demonstrated a broad-based decrease in the rate of ESC resistance amongst invasive Enterobacteriaceae following a reduction in ESC and fluorquinolones use within the hospital system[315].

The recognition of 'importation' of ESC-R-EC after travel to countries with a high community incidence of ESBLs is starting to be defined[316], although fewer studies have identified infection rather than carriage[27, 294, 317]. Whilst the pathophysiology seems clear, the temporality of this remains to be confirmed. In our study, analysis of temporality, as presented for healthcare exposure, was precluded by the imprecision of the composite 'high-risk' group and the small numbers involved. However, in absolute terms, 21 of 24 (87.5%) case participants with travel to high-risk regions departed these regions within the six months before presentation of infection. This fits with

our previous research demonstrating mostly short lived carriage of ESBL *E. coli* following travel overseas and other studies demonstrating a decrease in the risk of resistant infection beyond six weeks after return from travel[317, 318].

Investigation of risks for community acquisition in the low prevalence countries of Australia and New Zealand showed that one quarter (n=23) of ESC-R-EC patients reported neither healthcare exposure nor high-risk travel, suggesting there are as yet undefined risk factors for transmission within the community[294, 319].

Whilst there was some correlation between birth and travel regions, the identification of birth on the Indian subcontinent (OR=11.12) as a risk for ESC-R-EC in our cohort appears genuine. The etiology of this risk could stem from prolonged carriage of ESC-R-EC after travel more than one year previous, leading to delayed community onset ESC-R-EC infection. Alternatively, our observation of a mostly short interval between travel and infection, supports the possibility of domestic (within Australia and New Zealand) transmission of this resistance. Transmission of ESC-R-EC may occur from others within the household or community who have had recent travel to the Indian subcontinent. Although the true magnitude of risk and the broader applicability require further study, this observation is consistent with a previously published study from one of our participating sites and other descriptions of household transmission[55, 94]. Recently, 'birth outside of Europe' was identified as a risk for CTX-M producing *E. coli* in another study, although comparison with our data is complicated, as the European study did not fully account for recent travel[319].

Our molecular epidemiology data serves to confirm a number of key observations made in other regions. The first is a distinct difference in the epidemiology of CTX-M ESBLs and other expanded-spectrum cephalosporinase enzymes, which may be mediated by differing modes of acquisition, phenotype and characteristics of the *E. coli* strains harbouring them[194, 320]. Second, the high proportion of ST131 amongst ESBLs is no surprise given its global prevalence[165]. More surprising is its predominance without significant fluoroquinolone use (<6% of all participants in this study), one of the likely drivers in other regions[321]. Exposure to this class of antimicrobials within Australia and New Zealand is very low[299].

Male sex has been defined as a risk for community onset ESC-R-EC by other researchers[3, 25, 182, 313, 322] and became significant in some of our alternative models. The patient population of studies with this finding gives a clue to the etiology of this risk. On the whole, they are of older age with frequent healthcare exposure. This contrasts with studies containing a more traditional UTI population of young females that do not identify male sex as a risk[27, 317]. In addition to males

experiencing an age dependent rise in overall rates of *E. coli* infection[23], a limitation of case-control studies may also contribute to this finding. Aging patients certainly experience changes in nuances and dynamics of healthcare exposure and other potential risk factors for ESC-R-EC not identified with dichotomous measures such as hospitalization and antimicrobial use that are most often collected.

The strengths of our study include its prospective collaborative nature, a geographically broad sample and the case-control methodology used. The low background rates of ESC-R-EC in Australia and New Zealand have likely led to more discrete exposures and easier delineation of temporal risks than in communities where participants are frequently exposed to this form of resistance.

Limitations of our study include the moderate sample size, rate of non-recruitment; and risk of bias due to an absence of investigator or patient blinding and reliance on patient recall for many exposures. Other than the demographic details presented we have not been able to assess any factors that may have influenced recruitment vs. non-recruitment and subsequently biased the results. Recruiting a higher ratio of controls (1:2 or 1:3) would have increased our study power, and may have delineated further unidentified risks. The use of a third group of uninfected patients (a case-case-control design), would have allowed for delineation of risk factors associated with de novo acquisition of ESC-R E. coli, as opposed to delineation of risk factors for ESC-R within those that have *E. coli*[323]. However, pragmatic limitations precluded these options.

Some unique features of Australia and New Zealand may limit extrapolation of our findings to other regions. The exclusion of day-procedures in this study's definition of healthcare exposure, correlated with our local epidemiology, and would need to be reconsidered elsewhere. Furthermore, if $bla_{\text{CTX-M}}$ was not the predominant ESC resistance mechanism in a local population, risk may differ.

The use of only hospital patients for recruitment allowed consistent access to participants and samples, although it may limit the applicability of some risks to the wider community. The exclusion of patients unable to consent meant that we could not define risks for patients in long-term care facilities, a known reservoir of ESBL *E. coli* in Australia and overseas[48, 261, 263].

In conclusion, we have defined a critical risk-period ESC-R amongst community onset *E. coli* after healthcare exposure, and demonstrated that ESC-R *E. coli* infection in a low-prevalence setting may be driven by 'export' from healthcare exposure in the previous six months and 'importation' after travel to regions with a high incidence of community ESBLs.

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PRI, NR, MCP, JTF, EA, SMH, HES, MJ, EG, MDA, KS - None

FIGURES AND TABLES

Table 1

Univariate analysis of demographics, comorbidities, antimicrobial use, region of travel and birth, occupational and household exposure.

Variable	Frequency in ESC-R Cases (%) n=91	Frequency in ESC-S Controls (%) n=91	Odds Ratio	(95% CI)	p value
Demographics & co-morbidities					
Male sex	**30 (33)**	**16(18)**	**2.31**	**(1.51-4.62)**	**0.018***
Age <30 or >59 years	66 (73)	60 (66)	1.36	(0.72-2.57)	0.336
Immune suppression	19(20)	10(11)	1.99	(0.87-4.60)	0.105*
Charlson score ≥1	44(48)	34(37)	1.57	(0.87-2.83)	0.135*
Active malignancy	11(13)	9(8)	1.43	(0.55-3.73)	0.469
Renal failure	11(13)	9(10)	1.25	(0.49-3.19)	0.636
McCabe score ≥ 2+	78 (86)	76(84)	1.18	(0.53-2.65)	0.681
Indigenous	7(8)	6(7)	1.18	(0.38-3.66)	0.774
Heart disease	7(8)	7(8)	1		
Long term care facility resident	1 (1)	1(1)	1		
Smoker	12(13)	14 (15)	0.83	(0.36-1.92)	0.672
Liver disease	3(3)	4(4)	0.74	(0.16-3.41)	0.701
Lung disease	5(5)	7(8)	0.70	(0.21-2.85)	0.552
Pregnant or post-partum	3(3)	7(8)	0.41	(0.10-1.63)	0.206
Renal Tract Background					
Renal transplant	8(9)	4(4)	2.1	(0.61-7.22)	0.241
Anatomical or structural abnormality	23(25)	15(16)	1.71	(0.83-3.55)	0.147*
UTIs in past 12 months (per UTI)**	**Median = 1**	**Median = 0**	**1.32**	**(1.08-1.63)**	**0.008***
	(IQR = 0-3)	**(IQR = 0-1)**			
UTIs in lifetime (per UTI)**	Median = 2	Median = 2	1.03	(0.90-1.18)	0.657
	(25-75% = 0-5)	(25-75% = 0-5)			
Healthcare Exposure					
Any healthcare exposure in the past 6 months	56(61)	37(41)	2.33	(1.29-4.23)	0.005
Antimicrobial use					
Any antimicrobials in past 12 months	69(76)	62(68)	1.47	(0.76-2.81)	0.249
Trimethoprim+/- sulfamethoxazole	**16(17.58)**	**6(6.59)**	**3.022**	**(1.13-8.12)**	**0.028***
Expanded-spectrum cephalosporins	7 (8)	0	NA		0.014*
Fluoroquinolones	7(8)	3 (3)	2.44	(0.61-9.77)	0.206
ß-lactam + ß-lactamase inhibitor	16(17.58)	11(12.09)	1.552	(0.68-3.56)	0.300
Carbapenems	3(3.3)	2(2.2)	1.517	(0.25-9.30)	0.652
Aminoglycosides	5(5)	4(4)	1.26	(0.33-4.87)	0.733
Macrolide	6(6.59)	5(5.49	1.214	(0.36-4.13)	0.756
'Unknown' antimicrobials	35(38)	33(36)	1.1	(0.60-2.00)	0.759

Narrow spectrum cephalosporins	16(17.58)	15(16.48)	1.081	(0.50-2.34)	0.844
Narrow spectrum penicillins	10(10.99)	14(15.38)	0.679	(0.28-1.62)	0.383
Travel by region					
Any overseas travel	28 (30.8)	22 (24.18)	1.39	(0.72-2.68)	0.32
High risk regions [9]	24 (26)	14(15)	1.97	(0.94-4.11)	0.071*
Indian Subcontinent[1]	6(6.59)	1(1.1)	6.928	(0.75-53.87)	0.09
North America [2]	5(5.49)	2(2.20)	2.199	(0.49-13.69)	0.264
Africa+ Mid East [3]	3(3.3)	2 (2.2)	1.517	(0.25-9.30)	0.652
South East Asia [4]	15(16)	13(14)	1.18	(0.53-2.65)	0.681
South Pacific [5]	3(3.30)	3(3.30)	1		
Europe [6]	3(3.30)	5(5.49)	0.586	(0.14-2.53)	0.474
China [7]	4(4.4)	0			0.121
Japan[8]	1(1.1)	0			0.500
Birth by region[a]					
High risk regions	18(20)	10 (11)	2.0	(0.87-4.60)	0.105
Indian Subcontinent	**11(13)**	**2(2)**	**6.12**	**(1.32-28.45)**	**0.021***
Australia + New Zealand	58 (64)	59(65)	0.95	(0.52-1.75)	0.877
Europe	15(16)	18(20)	0.80	(0.38-1.71)	0.564
South East Asia	3(3)	4(4)	0.74	(0.16-3.41)	0.701
Africa+ Mid East	2(2)	4(4)	0.49	(0.09-2.74)	0.415
China	2(2)	0			0.497
South Pacific	0	3(3)			0.246
Latin America	0	1(1)			1.0
Occupation and household exposure					
Partner with recent ESC-R-EC infection	2 (2)	Not assessed			
Occupational healthcare exposure	10 (11)	7 (8)	1.48	(0.54-4.08)	0.447
Pet cat/dog at home	32(35)	33(36)	0.95	(0.52-1.75)	0.877
Occupational animal exposure	4 (4)	5 (5)	0.79	(0.21-3.05)	0.733
Pre-schoolers at home (<5yo)	7(8)	9 (10)	0.76	(0.27-2.13)	0.601
Food consumption					
Any meat in past 12 months	89 (98)	87 (98)	2.05	(0.37-11.46)	0.415
Poultry	88 (97)	83 (92)	2.47	(0.62-9.89)	0.206
Processed/ preserved meats	51 (56)	52 (58)	0.93	(0.52-1.68)	0.814
Pork	60 (66)	63(70)	0.83	(0.44-1.55)	0.558
Red meat	76 (84)	78 (88)	0.72	(0.31-1.66)	0.433

*Entered into multivariate model

**Infections were recorded numerically 0-5+, with all 5+ results considered 5 for analysis. Summaries presented as median.

A. Destinations of travel by region were 1. India, Pakistan, Nepal, Bangladesh 2. USA, Canada 3. Zimbabwe, Kenya, Sudan, Liberia, Turkey, Afghanistan 4. Malaysia, Singapore, Thailand, Laos, Cambodia, Vietnam, Burma, Indonesia and The Philippines 5. New Caledonia, PNG, Fiji, Samoa, Cook Islands and boat cruises through the South Pacific 6. UK, Italy, Holland, Portugal, Poland. 7. China, Hong Kong, Macau 8. Japan 9. High risk regions include the Indian subcontinent, Africa, the Middle East, South East Asia and China regions.

Table 2
Multivariate logistic regression

Variable	Odds Ratio	(95% CI)	p value
Healthcare exposure in the previous 6 months	3.16	(1.54-6.46)	0.002
UTIs in previous year (per UTI)	1.43	(1.16-1.82)	0.003
Birth on the Indian subcontinent	11.13	(2.17-56.96)	0.004
Travel to high-risk regions	3.09	(1.29-7.38)	0.011
Trimethoprim +/- Sulfamethoxazole &/or ESC use	3.67	(1.30-10.35)	0.014
Male sex	2.17	(0.97-4.84)	0.060

Figure 1
Participant identification, recruitment and characteristics of presentation and clinical syndrome.

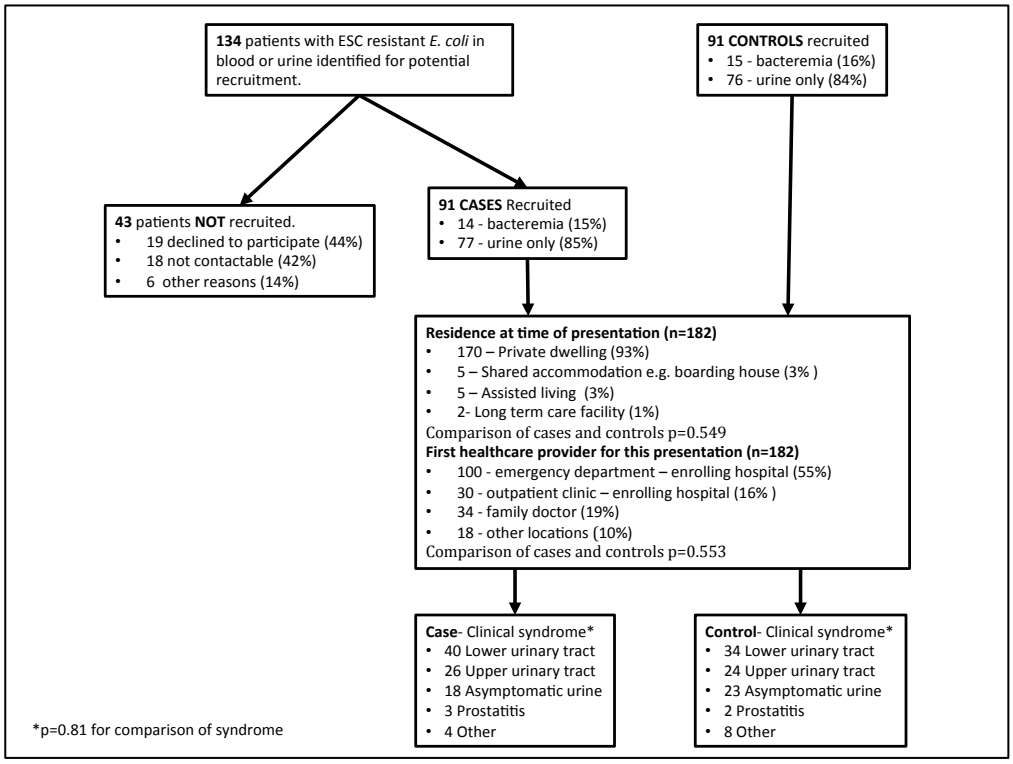
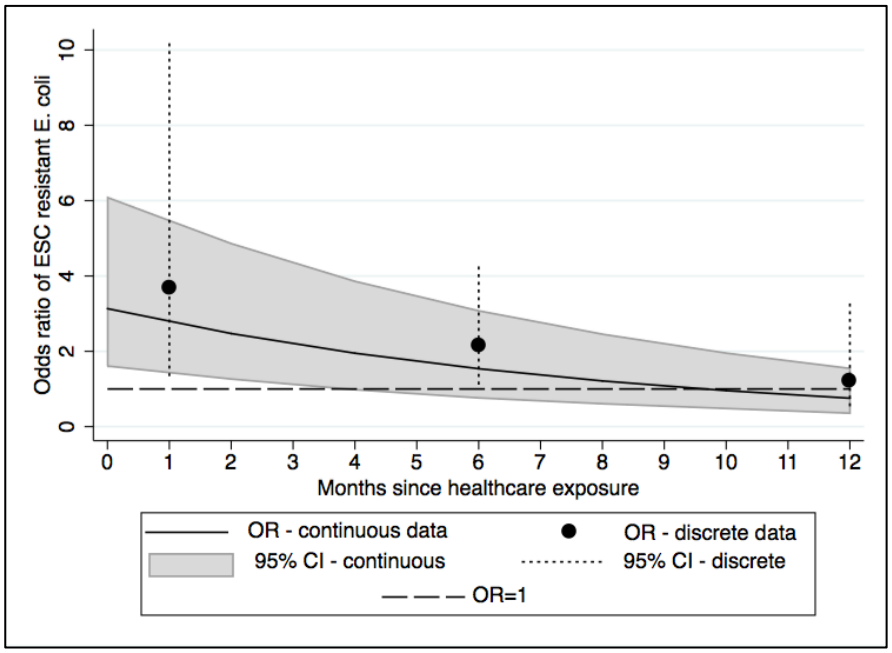


Figure 2.
The risk of ESC-R-EC over a 12-month period after the most-recent episode of healthcare exposure, excluding day procedures, estimated with two data sets. 1) Smoothed curve calculated using continuous data on months since hospital admission (black line, 95% CI in grey) 2) Discrete intervals using Friedman criteria (black dots, 95% CI as vertical dashes). The dashed line represents no increased risk (Odds Ratio=1.0)



CHAPTER 4. MOLECULAR EPIDEMIOLOGY OF COMMUNITY ONSET *ESCHERICHIA COLI* INFECTION

INTRODUCTION

The combination of bacterial isolates and extensive prospectively collected epidemiological data afforded by the design of the case-control study presented in Chapter 3, provides a unique opportunity to explore the molecular epidemiology of *E. coli* infections in our region. Whilst local researchers have investigated a variety of aspects of the molecular epidemiology ESC-R-EC, there is less data on the molecular epidemiology of other phenotypes of *E. coli*.[50, 55, 203] Furthermore, as defined in Chapter 3, the distribution of ST131 in our region differs from that in other areas, suggesting the clonal structure of *E. coli* infections in general may also differ from those discussed in recently published North American and European works.[191, 262, 264, 283]

Insight into the overall clonal structure of community onset *E. coli* infections is also important for contextualising risk factors identified in Chapter 3. Relating the difference in clonal structure of 3GCS-EC and 3GCR-EC to epidemiological features gives insight into potential drivers of the spread of resistance. Furthermore, a key tenet of any plan to target patient groups with high rates of 3GCR-EC, or specific risk factors for 3GCR-EC, would be determining if the pathogenic isolates represent clonal spread or diverse strains.

Many techniques for characterisation of the molecular epidemiology of clinical bacterial isolates have been described.[324] We utilised a hybrid of three techniques in order to achieve a resolution optimal for our study aims, whilst minimising cost and workload. All isolates underwent semi-automated rep-PCR, which is a validated technique for delineating bacterial relatedness at a clinical level.[325] This technique relies on amplification of short conserved repetitive extragenic palindromic 'rep' regions of DNA in Enterobacteriaceae.[326]

One limitation, originating from the proprietary nature of the DiversiLab re-PCR platform used, is a lack of generalisability or comparability to other collections and publications. To overcome this limitation, we used MLST to relate clusters identified on rep-PCR to broader global data. The 95% cut-off selected for this analysis was based upon a previous publication comparing these two techniques.[235, 327] Within the ST131 clonal group, which is not otherwise subtyped by rep-PCR, we used characterisation of the *fim*H gene to subtype isolates. The basis for this typing method was discussed in Chapter 2.

The case-control design of our study provided delineation of molecular epidemiology within ESC-R-EC and ESC susceptible *E. coli* groups. However, overall population rates or burdens of each clone could not be directly calculated due to the epidemiologically 'unbalanced' proportion of resistant isolates in the sample. To overcome this, we used external data to adjust for the relative prevalence of ESCR and ESC susceptible *E. coli* within our overall study population (community-onset *E. coli* infection). This back-calculation is based upon rates of 3GCR-EC identified in the AGAR 2010 community onset *E. coli* survey. Importantly, the AGAR survey population geographically and temporally mirrors our study population. Supporting this similarity, the distribution of ESBL genes identified, and resistance rates to a variety of antimicrobials, closely match those found in our study.[44] In undertaking this analysis, we are cognisant of the limits of data we can infer given the overall sample size. In particular, we were only able to analyse the data for the whole of Australia, rather than by state or region. In addition any selection bias (as discussed in chapter 2) may have had an unforseen impact on our results.

Hence, we aimed to take the epidemiological data from the case-control study (Chapter 3), in concert with analysis of bacterial isolates, to gain further insight into the molecular epidemiology of *E. coli* infection in our region and globally.

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ABSTRACT

Background

The clonal composition of *Escherichia coli* causing extra-intestinal infections includes ST131 and other common uropathogenic clones. Drivers for the spread of these clones and risks for their acquisition have been difficult to define.

Methods

We combined molecular epidemiology with clinical data from 182 patients enrolled in a case-control study of community onset expanded-spectrum cephalosporin resistant *E. coli* (ESC-R-EC) in Australia and New Zealand. Genetic analysis included antimicrobial resistance mechanisms, clonality by DiversiLab (rep-PCR) and multilocus sequence typing (MLST), and subtyping of ST131 by identification of polymorphisms in the *fim*H gene.

Results

The clonal composition of expanded-spectrum cephalosporin susceptible and ESC-R-EC isolates differed, with six MLST clusters in susceptible isolates (median=7 isolates/cluster), and three clusters amongst ESC-R-EC, including 40 (40/89, 45%) ST131 isolates.

Population estimates indicate ST131 comprises 8% (7.6-8.3%) of all *E. coli* within our population. The fluoroquinolone susceptible H41 sub-clone comprised 4.5% and the H30 sub-clone 3.5% of *E. coli*. The H30 sub-clone comprised 39% of all ESC-R-EC and 41% of all fluoroquinolone resistant *E. coli*, within our population. Patients with ST131 were also more likely than non-ST131 to present with an upper than lower urinary tract infection (RR 1.8, 95%CI 1.01-3.1).

Conclusion

ST131 and the H30 sub-clone were predominant amongst ESC-R-EC but infrequent among susceptible isolates where the H41 sub-clone was more prevalent. Within our population, the proportional contribution of ST131 to fluoroquinolone resistance is comparable to that of other regions. In contrast, the overall burden of ST131 is low by global standards.

INTRODUCTION

Using contemporary molecular typing techniques, a broad picture of the genetic diversity of *Escherichia coli* causing urinary tract and other invasive infections is beginning to emerge. Recent studies have demonstrated that collections of *E. coli* from urine and blood are largely clonal in composition[191, 262, 264, 283]. These clonal components invariably include the global pandemic clone, Sequence Type 131 (ST131) *E. coli*, and other frequently described uropathogenic *E. coli* (e.g. ST95, ST69, ST127). ST131 *E. coli* has been implicated as a major contributor to fluoroquinolone resistant and expanded-spectrum cephalosporin resistant *E. coli* (ESC-R-EC) infections globally[165].

Clinical and epidemiological risk factors for colonisation or infection with these clones, in particular ST131, have been difficult to define. Recently identified risk factors for ST131 include long term care facility (LTCF) residence or bedridden status[263, 282, 286], exposure to antimicrobials[263], ethnicity[328], female sex[286] and infection characteristics[263, 270].

In our region, a range of ST131 clones have been identified amongst animals and humans, from a variety of patient groups[46, 83, 203, 204, 290, 292]. Few facets of epidemiology have been investigated, with one study reporting no difference between the co-morbidities of patients infected with ST131 and non-ST131 *E. coli* after prostate biopsy[82], and another demonstrating some possible sharing of ST131 clones between human and companion animals[291]. There have been no population estimates of prevalence.

We previously described risk factors for community onset expanded-spectrum cephalosporin resistant *E. coli* (ESC-R-EC) in Australia and New Zealand. These risk factors included healthcare contact, travel to high-risk regions (Indian subcontinent, South East Asia (SE Asia), China, Africa and the Middle East), trimethoprim +/- sulphamethoxazole use (SXT) and/or expanded-spectrum cephalosporin use (ceftriaxone, ceftazidime or cefepime), UTIs in the previous year, and birth on the Indian subcontinent. We also demonstrated that ST131 *E. coli* was spread broadly in our region, although with a relatively uncommon distribution. It resided almost exclusively amongst ESC-R-EC where the prevalence was 45%, compared to 7% amongst ESC susceptible *E. coli* (ESC-S-EC) isolates. In addition there was a non-significant difference in the proportion containing $bla_{\text{CTX-M-9}}$ group and $bla_{\text{CTX-M-1}}$ group enzymes[329].

In this follow-up study we aim to define the clonal composition and molecular characteristics of community onset expanded-spectrum cephalosporin susceptible and resistant *E. coli* infections. Furthermore, we aim to understand the sub-clonality of ST131 and elucidate factors that may influence the distribution of the ST131 world-wide pandemic in our region. To do this we have

combined epidemiological data, collected as part of our case-control study, with genetic characterisation of *E. coli* isolates from the study patients.

MATERIALS AND METHODS

Clinical data and bacterial isolates

All bacterial isolates and clinical data are from The COOEE Study (**CO**mmunity **O**nset **E**SBL and AmpC ***E****. coli* Study*)*, a multisite case-control study, with prospective recruitment of patients and data collection. It is described in detail elsewhere[329]. In brief, six geographically dispersed tertiary centres in Australia (n=5) and New Zealand (n=1) recruited patients over a 9-12 month period during 2011 and 2012. In total, 182 patients (91 ESC-R-EC cases, 91 ESC-S-EC controls) were recruited. Bacterial isolates were recovered from 98% (179/182) of patients.

Definitions

Definitions have been described extensively elsewhere[329]. In brief, **Case** patients had community onset ESC-R-EC in a culture of blood or urine. **Control** patients had ESC-S-EC isolated, and were selected from the same laboratory as the 'case' patients. Patients who were unable to give informed consent to participate or who had extra-anatomical urinary drainage (e.g. indwelling urinary catheter (in the community), intermittent catheterisation, (ileal conduit or similar) were excluded from recruitment

E. coli was **community onset** where a patient was resident in the community (including long-term care facility) or hospitalized less than 48 hours at the time of onset; **Expanded-spectrum cephalosporin resistance** was identified phenotypically. For ceftriaxone, a susceptibility breakpoint of >1 mg/L was used. For ceftazidime, laboratories used a breakpoint of MIC >1 mg/L or MIC >4 mg/l depending on their use of EUCAST or Clinical and Laboratory Standards Institute (CLSI) criteria respectively[304, 305]. **Site of infection** was determined by the researcher from available information. Urinary tract presentations were classified as 'Asymptomatic', 'Lower tract infection' or 'Upper urinary tract infection' as defined previously[329]. All other infections (non-urinary source and prostatitis) were classified into a combined 'Other' group.

The human research ethics committees at The University of Queensland and participating sites approved this study.

Antimicrobial susceptibility phenotype

As described previously, all phenotypic data presented has been assessed by EUCAST criteria[304, 329]. All non-susceptible isolates were considered resistant for the purpose of this analysis. An aggregated resistance score (0-11) was calculated with all antimicrobial phenotypes included in the study (ampicillin, amoxicillin+clavulanate, ceftriaxone, ceftazidime, cefepime, meropenem trimethoprim+sulfamethoxazole, ciprofloxacin, nitrofurantoin, gentamicin and amikacin).

Molecular methods

Bacterial isolates were recovered from storage at -80^{o}C in the research laboratory. After overnight culture, bacterial DNA was extracted using an UltraClean microbial DNA isolation kit (MO BIO Laboratories, USA). As outlined previously, ESC resistance genes were investigated by PCR using published primers and conditions.

Phylogenetic group was determined by multiplex PCR[230]. Semi-automated rep-PCR using a DiversiLab (bioMerieux, France) was undertaken as per the manufacturer's instructions. Dendrograms were constructed from rep-PCR patterns using a Pearson correlation coefficient (DiversiLab 3.4 software). All clusters with ≥ 4 isolates demonstrating ≥ 95% similarity[327] were then identified by MLST[9] as follows: The two most distant isolates within each cluster underwent MLST typing. Where multiple isolates were equally distant, isolates were selected to maximize the diversity in geographical origin and phenotype. If isolates from a rep-PCR cluster were discordant by MLST, additional isolates underwent MLST to attempt to define the cluster. The worldwide pandemic clone, ST131 *E.* coli, was presumptively identified using the same approach and 95% cut-off, although a number of MLST confirmed ST131 reference clones were also included in the dendrogram[235]. Typing of the *fim*H allele encoding the type 1 fimbrial adhesin was undertaken in ST131 isolates. The gene was sequenced in forward and reverse direction based on published primers and conditions[330] using an ABI3730XL (Life Technologies, USA) capillary sequencer. Amino acid substitutions up to the 265th position of the putative mature peptide sequence were classified as per the schema of Weissman[40].

Calculation of whole-population estimates

Estimates of population prevalence of MLST defined clones were back-calculated by adjusting to an overall population prevalence of 3.4% ESC-R-EC, as measured in the 2010 Australian Group on Antimicrobial Resistance (AGAR) outpatient survey[44]. The survey sampled a population very similar to that of our study: outpatient urine samples from 30 hospital-based laboratories, including from 4 of 6 sites in our study. Estimates are provided with a range assuming a possible 50% difference in population ESC-R-EC rates from the AGAR survey (i.e. 3.4%, range 2.6 - 4.25%).

Statistical methods

Variables were compared using X^2 squared test, Fischer's Exact, Wilcoxon Rank Sum and calculation of relative risks (RR) where appropriate. Continuous data is presented as a median and 25-75th centile.

Statistical tests were two tailed, and $p<0.05$ was considered significant. STATA version 12.1 (Statacorp, USA) was used.

RESULTS

In total, 179 bacterial isolate were included in this study (89 ESC-R, 90 ESC-S). Bacteremia was detected in 29(16%) patients with the remainder having isolated urine cultures. All isolates were community onset, including 2/179 (1%) originating from residents of long-term care facilities.

Expanded-spectrum cephalosporin resistance genes were present amongst 87/89 (98%) of the available ESC-R isolates (36/89, 40% *bla*$_{\text{CTX-M-1}}$ group; 35/89, 39% *bla*$_{\text{CTX-M-9}}$ group; 3/89, 3% *bla*$_{\text{CTX-M-1}}$ & *bla*$_{\text{CTX-M-9}}$ groups; 11/89, 12% *bla*$_{\text{CMY-2}}$; 1/89 1% *bla*$_{\text{DHA-1}}$; 1/89 1% *bla*$_{\text{SHV-5}}$)[329].

Phylogenetic group

Phylogenetic grouping showed a high proportion of pathogenic B2 and D group *E. coli* (Table 1). The clinical syndrome varied significantly for each phylogroup, however, when comparison was limited to lower and upper urinary tract infections (the two categories which constituted the vast majority of presentations) only B2 isolates demonstrated a significant risk for upper rather than lower urinary tract infection (RR=2.3, 95% CI=1.2-4.0). The other phylogroups, A/B1 and D demonstrated a non-significant risk, favouring lower tract infection (p=0.083 and p=0.093). There was no significant difference in phylogenetic group, comparing urine and bacteraemia isolates (p=0.820).

Multilocus sequence typing

A dendrogram was constructed for ESC-S-EC (Figure 1), ESC-R-EC (Figure 2) and all isolates combined (not shown). There was no obvious geographical or temporal clustering. Twenty-nine isolates underwent full MLST. In total, nine clusters comprising 60% (108/179) of all isolates were related to six MLSTs (ST131, ST95, ST73, ST69, ST127, ST80) and two clonal complexes sharing 6 of 7 alleles (ST14 complex, ST648 complex). Whilst the proportion of clonal isolates between

ESC-R-EC and ESC-S-EC groups was similar (56/89, 63% vs. 52/90, 58% p=0.482), there was a marked difference in the composition of Sequence Types (STs). Amongst ESC-S-EC, six MLST defined clusters each contained 5-13 isolates (median=7 isolates). Within the ESC-R-EC, only three clusters were identified with a single cluster, ST131, containing 40 isolates.

ST131 and *fim*H typing

As reported previously, 46/189 (24%) isolates clustered within the ST131 worldwide pandemic clone, including 40/89 (45%) ESC-R-EC and 6/90 (7%) ESC-S-EC (p<0.001). All ESC-R-EC ST131 harboured CTX-M enzymes, and constituted 54% of the entire CTX-M group; 24 (60%) harboured a CTX-M-1 group enzyme and 19 (48%) a CTX-M-9 group enzyme (p=0.173 for comparison). This included three isolates (8%) harbouring both enzymes. Amongst the 34 non-ST131 CTX-M harbouring isolates, 19 (56%) were CTX-M-9 group and 15 (44%) CTX-M-1 group (p=0.472).

The *fim*H gene was typed as *fim*H 30 (H30) in 37 (80%) ST131 isolates, with the remaining 9(20%) typing as *fim*H 41 (H41) indicating a consensus FimH peptide sequence. By rep-PCR, H41 ST131 isolates formed distinct clusters within ST131, which branched at approximately 97% similarity from the majority of H30 isolates (Supplementary Material).

There was a significant difference in the rate of ESC resistance between H30 and H41 isolates (H30 35/37, 95% ESC-R vs. H41 4/9, 44% p=0.009) and concordance between H30 and fluoroquinolone resistance was 100% (H30=37/37, 100% ciprofloxacin resistant, H41=0/9, 0% ciprofloxacin resistant).

With the exception of the smallest site, which had no patients with ST131 and small numbers overall (n=8), the clone and the H30 sub-clone were present in all sites (Figure 3). There was no significant difference in the proportion of ST131 (p=0.574) or H30 (p=0.774) across study sites.

Clonal prevalence estimates

When adjusted for the population rate of ESC-R-EC, the (estimated) most prevalent MLST clones in our study population were ST95 (14%, 13.9-14.1%), ST73 (13%, 12.8-13%), ST14 complex (9%, 8.6-8.7%) and ST131 (8%, 7.6-8.3%) (Figure 4 and Supplementary Material).

Within ST131 the H41 sub-clone comprised 4.5% (4.5-4.5%) and the H30 sub-clone 3.5% (3.2-3.8%) of all *E. coli* in the population. The ciprofloxacin resistant H30 sub-clone constituted 41% of all ciprofloxacin resistant community onset *E. coli* isolates. In total ESC-S ST131 constituted 6.4% (6.4-6.5%) of community onset *E. coli* isolates. (Supplementary Material)

Characteristics of ST131

Comparison of site of infection for various clonal groups is in Table 2. This demonstrated ST131 clones were significantly more likely to cause upper than lower urinary tract infection (ST131, 19/35 54% upper vs. non-ST131 30/87 34% upper, Relative Risk=1.8, p=0.04) (Table 1). When compared only to other phylogroup B2 isolates (n=57), ST131 did not constitute a significantly greater proportion of upper UTIs (Upper UTI - ST131 19/35 54% vs. non-ST131 24/57 42%, p=0.256).

Analysis within the 'upper tract' and 'lower tract' groups showed no significant difference in the characteristics of clinical presentation between ST131 and non-ST131 harbouring patients. No patient in either group had evidence of renal abscess, or secondary sites of infection. The duration of symptoms before presentation, comparing ST131 to non-ST131 infections, was equivalent for lower tract infections (3 days, 1-7.5d vs. 2, 1-7d p=0.486) and upper tract infections (2d, 1-5d vs. 2d, 1-4d p=0.536).

There was no significant difference in the proportion of upper-tract infection between H30 and H41 sub-clones (Upper UTI - H30=15/28 54%, H41=4/7 57%, p=1.0).

Antimicrobial resistance

As expected, given ST131's prevalence amongst resistant isolates, the median resistance scores amongst ST131 isolates was significantly higher than for non-ST131 isolates across the whole cohort (median=6, 25-75% = 4-7 vs. 2, 0-5 p<0.001). When stratified into a variety of relevant groups, the resistance scores did not differ significantly between ST131 and non-ST131 isolates within any group (ESC-S-EC p=0.077, ESC-R-EC p=0.116 and CTX-M harbouring ESC-R-EC p=0.899). Although total resistance scores were similar, significant differences in rates of resistance to individual antimicrobials included greater resistance to ciprofloxacin (p<0.001) and SXT (p=0.013) amongst the ESC-R-EC ST131 group. When only the CTX-M harbouring isolates were analysed, the difference in ciprofloxacin remained significant (p=0.010) (Supplementary Material).

DISCUSSION

Our study provides the first comprehensive molecular epidemiology profile of susceptible and resistant *E. coli* in our region. Previous studies in our region have investigated selected groups such

as fluoroquinolone resistance or particular clonal groups, limiting their ability to ascertain a broad profile[46, 291].

At first glance, the global pandemic clone ST131 appears to be dominant in our population. However, this must be seen in the perspective of local rates of ESC-R-EC (Figure 4). Our estimates indicate ST131 makes up approximately 8% (7.6-8.3%) of community onset *E. coli* isolates. Although less than half of the ST131 would be ciprofloxacin resistant H30 sub-clones, ST131 still constitutes a disproportionate 41% of all ciprofloxacin resistant community onset *E. coli* isolates, as demonstrated in Figure 3.

Whilst the proportion of ST131 amongst ciprofloxacin resistant *E. coli* is comparable to, the estimated absolute rate of ST131 is considerably lower than, that found in other contemporaneous studies[165]. Three North America studies reported rates ranging from 23 to 28% ST131 amongst *E. coli*[262-264]. A recent Australian cohort (from a single region amongst a selected patient population) demonstrated a rate of 21% ST131 amongst females of reproductive age with UTI[46]. Comparing across all these studies the most notable variable was the background rate of fluoroquinolone resistance amongst *E. coli*. The rate in the North American studies ranged from 27-29% resistance, and the Australian study's 13% rate was high compared to our sample (7% amongst ESC-S-EC) and other Australian data[43, 44, 329].

The potential relationship between the background rate fluoroquinolone resistant *E. coli* and ST131 prevalence is revealing. By global standards, Australia and New Zealand have very low rates of fluoroquinolone resistance amongst *E. coli*. These have been achieved through regulatory control of fluoroquinolone use in humans and animals[299]. By way of example, 72% of our study population had used antimicrobials in the previous year, though only 5% were exposed to a fluoroquinolone. We hypothesize that on a population basis, the low background rate of fluoroquinolone use has kept the ST131 clone at bay amongst the majority of Australian and New Zealand *E. coli.*

The *fim*H subtyping of ST131 supports this hypothesis. The dominance of the H30 sub-clone amongst ESC-R ST131 isolates reflects an emerging global picture[289]. Conversely, the overall dominance/expansion of the fluoroquinolone susceptible H41 sub-clone has not been described previously. This sub-clone is somewhat genetically distinct from other 'clades' of ST131[331] and strongly associated with the otherwise infrequent O16:H5 serotype[332, 333]. In our setting, the H41 clone may have a selective advantage given recent research demonstrating high virulence coupled with fluoroquinolone susceptibility[332, 333]. This requires further exploration.

Whilst the identification of antimicrobial use as one of the drivers/protectors for clones amongst the whole population can be supported, it does not fully explain the 40% ST131 rate seen amongst ESC-R-EC within our population, a rate similar to that of many other regions[222, 271, 334]. The data on ESC-R-EC ST131, 88% of which are the H30 sub-clone provides some answers. Foremost, the findings of this study must be considered in the context of our previous analysis of risk for ESC-R-EC within our population. We defined multiple risk factors for ESC-R-EC, and the majority of these remained a risk for ESC-R ST131 in a sensitivity analysis[329]. Hence, within the population as a whole, factors including healthcare contact, high-risk travel, birth on the continent and previous UTIs are all risk for ST131 in our region. This risk is mediated through ST131's very tight association with CTX-M ESBLs.

A confluence of virulence and resistance is important in this association. ST131 was associated with almost twice the risk of upper tract infection compared with lower tract (RR=1.8), supporting an emerging body of literature demonstrating increased clinical virulence of ST131[46, 83, 264], despite difficulty demonstrating this in non-clinical settings[84, 85].

A key strength of our study is the ability to compare prospectively collected epidemiological data with the molecular epidemiological characteristics of our isolates amongst a geographically dispersed sample. The presence of equal numbers of ESC susceptible and resistant isolates also allowed clear differentiation of the clonal structure of each group which would not have otherwise been possible, given the low prevalence of ESC-R-EC in our region. The uniquely low rate of fluoroquinolone use in our region has provided us insight into the dynamics of the ST131 clone with little selective pressure from this antimicrobial class.

Limitations of our study include the lack of laboratory virulence data to allow greater exploration of our hypotheses and findings. Our power to detect significance of association was also limited by a moderate sample size and the sub-group nature of this analysis. Our reliance on back-calculation to determine population rates may have led to a lower accuracy of these figures than other methods provide. Finally, a number of our findings are hypothesis generating and require further studies for confirmation.

In conclusion, we delineate a markedly different clonal composition between ESC-S and ESC-R-EC groups in Australia and New Zealand. Overall, ST131 is less frequent than in other regions of the world. The fluoroquinolone susceptible H41 sub-clone of ST131 is most prevalent, although the H30 sub-clone dominates ESC-R-EC. ST131 was significantly associated with upper urinary tract infection presentations, suggesting enhanced virulence. We hypothesize that the factors contributing

to the low background rate of fluoroquinolone resistant *E. coli* in our region may have also afforded protection from wider spread of the pathogenic ST131 clone beyond ESC-R-EC.

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FIGURES AND TABLES

Table 1

Comparison of clinical presentations within phylogenetic groups and MLST defined clones.

	Clinical Syndrome							
	Asymptomatic	**Lower UTI**	**Upper UTI**	**Other***	**Total**			
	Count (%)	**Count (%)**	**Count (%)**	**Count (%)**	**Count**	**p value[1]**	**RR[2] of upper UTI (95%CI)**	**p value[2] (upper vs. lower)**
Phylogenetic group								
A/B1**	9(45)	8(40)	1(5)	2(10)	20	0.026	0.3	0.083
B2	27(21)	49(38)	43(34)	9(7)	128	0.017	2.3 (1.1-4.9)	0.010
D	4(13)	16(52)	5(16)	6(19)	31	0.043	0.5	0.093
Total	41(23)	74(41)	50(23)	17(9)	179			
MLST groups								
ST131	10(22)	16(35)	19(41)	1(2)	46	0.038	1.8 (1.01-3.1)	0.044
ST131 H30	8(22)	13(35)	15(41)	1(3)	37	0.149	1.5	0.099
ST131 H41	2(22)	3(33)	4(44)	0	9	0.682	2.0	0.345
ST95	0	5(33)	9(60)	1(7)	15	0.014	2.7 (0.96-7.5)	0.050
ST73	2(15)	5(38)	6(46)	0	13	0.437	1.8	0.310
ST14 cplx.	4(40)	4(40)	1(10)	1(10)	10	0.407	0.4	0.647
ST69	0	3(43)	3(43)	1(14)	7	0.412	1.5	0.683
All groups[3]	21(19)	39(36)	39(36)	9(8)	108	0.011	2.2 (1.2-4.0)	0.003

UTI = Urinary Tract Infection 'cplx' = clonal complex

[1]The p value has been calculated across the four clinical presentation categories.

[2]The relative risk (RR) and p value compare only upper and lower urinary tract infection (excluding other illnesses) across the three phylogroups. A 95% confidence intervals is given for significant values.

[3]All isolates that clustered within an MLST defined group

* Includes: Intra-abdominal source n=4, prostatitis n=5, bacteremia without focus n=4, others n=5

**A single B1 isolate was combined with the A group for purpose of analysis.

Figures 1 & 2

Dendrogram constructed using a Pearson correlation coefficient, based on DiversiLab rep-PCR pattern of ESC susceptible (Figure 1) and ESC resistant (Figure 2) *E. coli*. Details of figure from left to right: Dendrogram, isolate number, virtual gel. Numbered columns from left to right: I = Hospital location, II=Phylogenetic group III=Fluoroquinolone susceptibility IV= (only in Figure 2) Expanded-spectrum cephalosporinase type, V=MLST typing*

*Isolates which have been presumptively related to an MLST only by rep-PCR pattern are indicated by 'like' after the ST e.g. 'ST131 like'

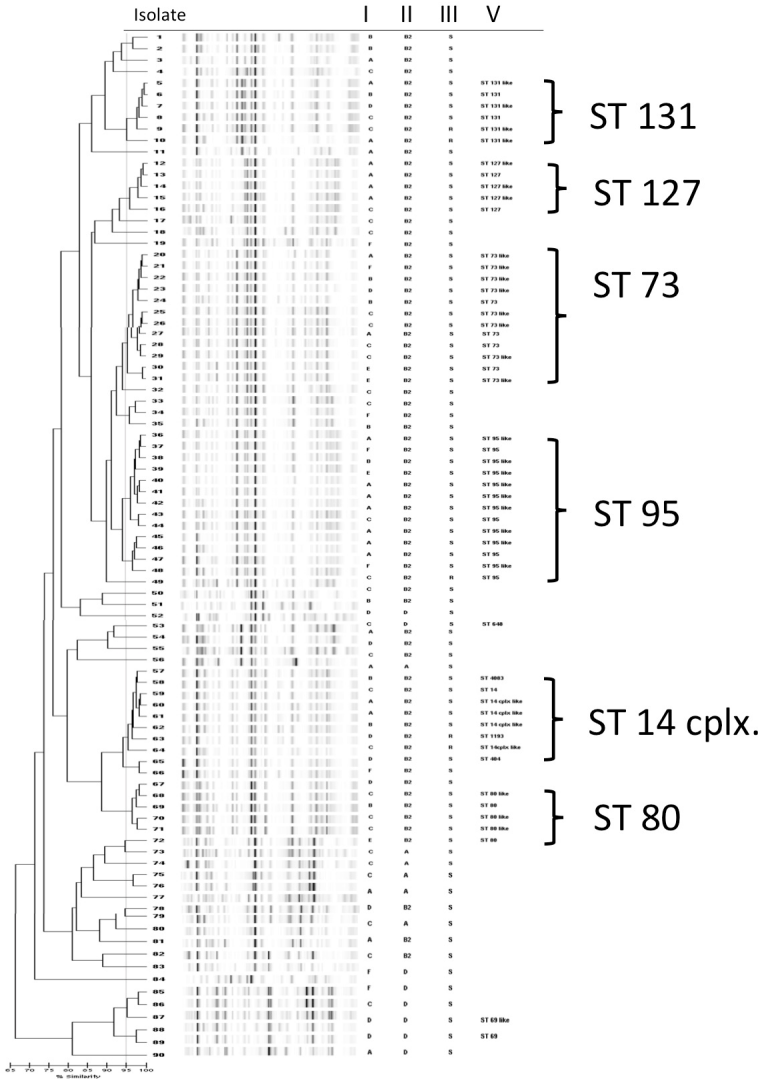


Figure 1
ESC susceptible *E. coli* (n=90)

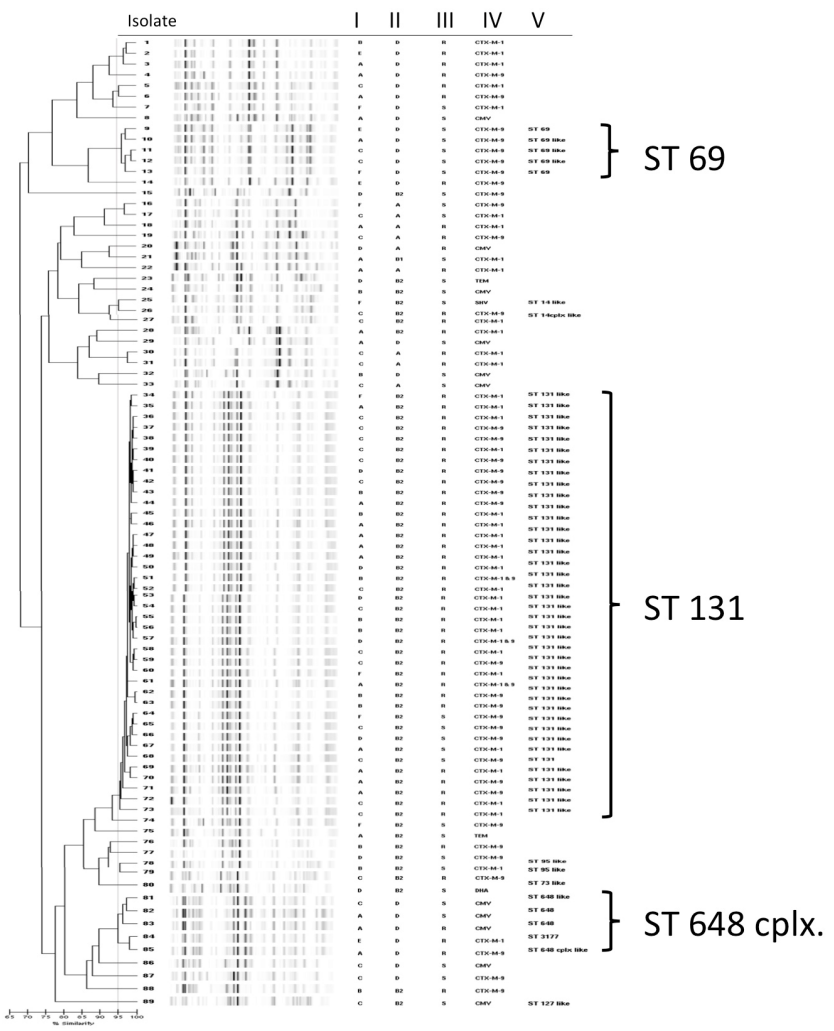


Figure 2
ESC resistant *E. coli* (n=89)

Figure 3

Distribution of ST131 and the *fim*H 30 sub-clone across the study sites for 3GC susceptible and 3GC resistant *E. coli*. '*fim*H Cons' indicated clones with consensus sequence.

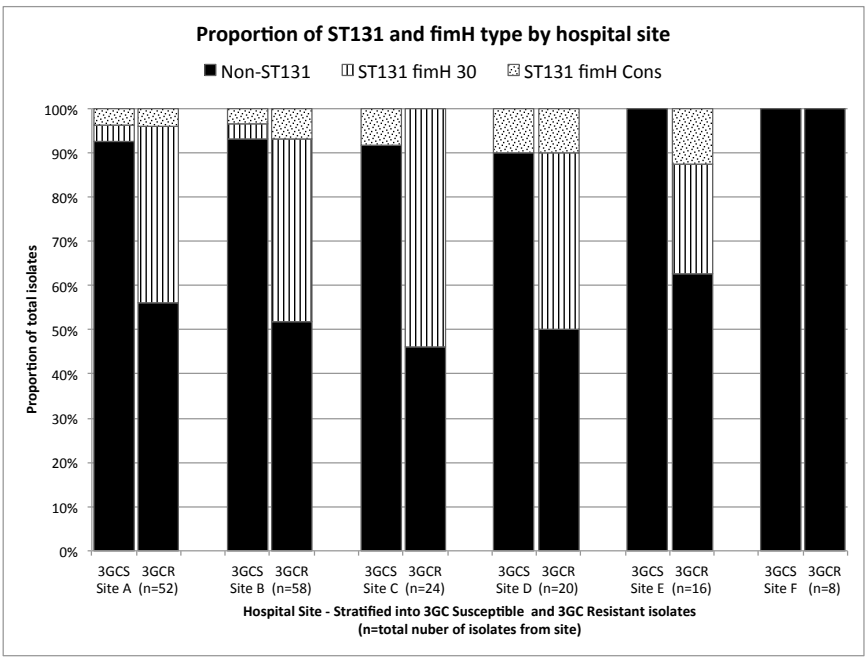
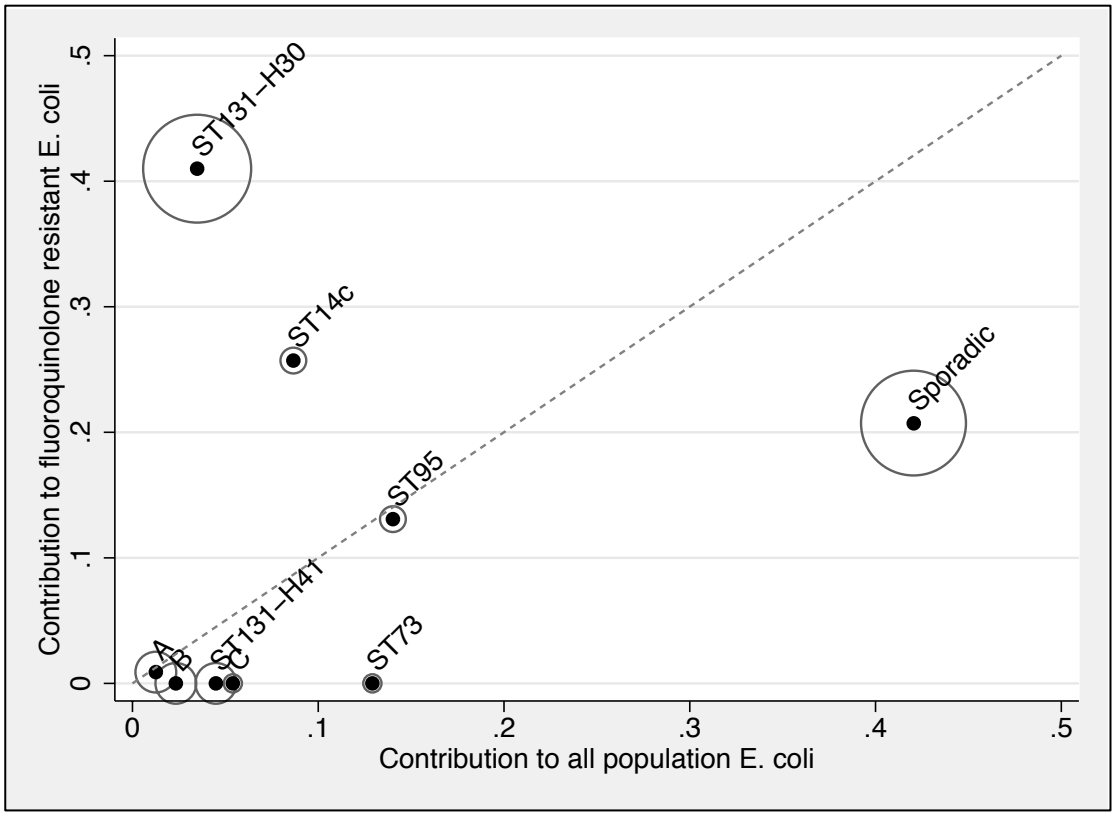


Figure 4

Relative contribution of clones to the burden of fluoroquinolone and expanded-spectrum cephalosporin resistance in our population.

The x-axis represents the proportional contribution of each clone to the total number of *E. coli* isolated within our population. The y-axis represents the proportional contribution to fluoroquinolone resistant *E. coli* in our population. The size of the outlining circle on each data-point represents the relative contribution to expanded-spectrum cephalosporin resistance within our population *E. coli*. (Dashed reference line represents a balanced contribution to fluoroquinolone resistance and overall *E. coli* burden).



Sporadic= isolates not clustered to an identifiable clone, ST14c = ST14 clonal complex. For clarity some ST labels have been shortened as follows: A=ST648, B=ST69 C=ST127 & ST80 (which closely share the same data-point)

CHAPTER 5. RETURNED TRAVELLERS AND CARRIAGE OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI.*

INTRODUCTION

Recent clinical studies have demonstrated a high rate of gastrointestinal carriage of antimicrobial resistant *E. coli* in travellers returning from countries of high-incidence to countries of lower incidence. Rates of carriage are frequently in the order of 25% of returned travellers.[47, 164, 335, 336] High-risk regions invariably include the Indian subcontinent, the Middle East and other parts of Asia. While most studies have focused on 3GCR-EC, Kennedy *et al* included fluorquinolone and aminoglycoside resistance in their sample.[47]

Whilst carriage of resistance can frequently be demonstrated, only a smaller number of studies, including the study presented in Chapter 3, have linked travel to high-risk countries with resistant *E. coli* infection.[27, 294, 317, 337] These studies have used a variety of definitions for the duration of risk after travel ranging from one month to one year.[294, 337] The use of varied definitions highlights a gap in our knowledge of gastrointestinal carriage of antimicrobial resistant *E. coli.* There is little data on the actual duration of carriage after travel, or on any specific risk factors for resistant infection related to travel.

The majority of work on GIT carriage of antimicrobial resistant *E. coli* originates from healthcare associated carriage. Whilst these studies offer some background for understanding carriage after travel, there are several reasons to question the specific applicability of durations of carriage found in healthcare related studies. This includes differences in mechanisms of acquisition of the resistance, patient characteristics, burden of comorbidities and exposure to antimicrobials. On the whole, healthcare based studies have demonstrated consistent findings across a number of patient groups. In a large retrospective study of patients who were screened on re-admission to hospital, the median time to 'loss' of carriage was 6.6 months. In addition, patients who had a clinical infection rather than a colonising isolate were significantly less likely to clear the strain.[338] In a smaller prospective study with active screening, the median duration of carriage was approximately 3 months.[339] Amongst LTCF residents, the median duration of colonisation with a variety of antimicrobial resistant *E. coli* was approximately 6 months.[340] In contrast, a recent New Zealand study demonstrated a longer duration of colonisation, with 75% of patients remaining colonised at 12 months and 50% at 3 years after first detection. Analysis within this cohort could not identify any risk-factors for this extended colonisation.[341]

Amongst returned travellers, a small number of studies on duration of carriage have been published. Tangden found approximately 25% of travellers had persisting colonisation with ESBL-EC six months after travel.[164] In another study designed specifically to map long-term carriage after travel, 24% of patients continued to carry ESBL *E. coli* at a 3-8 month assessment, including almost half in whom ESBL strains identified on follow-up had not been present on initial assessment. In this group, a small subset (10%), of patients still had carriage at 3 years after travel.[342] Case reports have also described prolonged carriage of carbapenenemase harbouring *E. coli* after travel.[343]

Given the relative paucity of data available, we sought to understand the dynamics of carriage of antimicrobial resistant *E. coli* through in-depth analysis of a previously collected sample.[47] This sample was collected as part of a study conducted in Canberra, Australia. The study enrolled 106 previously healthy volunteers who were 16 years or older and travelling overseas for at least one week. Patients provided epidemiological details via a survey and then submitted rectal or peri-anal swabs before travel and at intervals after travel. Antimicrobial resistant isolates from these swabs were retained by the investigators and have been analysed further in work presented in this chapter.

Our aim was to explore temporality and clonality of isolates present during prolonged carriage after travel. This included the level of diversity of clones present and any evidence of ESBL gene sharing via horizontal transfer of mobile plasmids. Such analysis offers another perspective on the findings in Chapter 3 by linking the defined clinical risks to a demonstrable molecular epidemiology of carriage.

PUBLICATION: PROLONGED CARRIAGE OF RESISTANT *E. COLI* BY RETURNED TRAVELLERS: CLONALITY, RISK FACTORS AND BACTERIAL CHARACTERISTICS (EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES, VOLUME 31, SEPTEMBER 2012)

See Appendix A for published version

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ABSTRACT

Purpose

To delineate the potential risks and dynamics of prolonged carriage of resistant *E. coli* in returned travellers.

Methods

A sample of 274 previously collected *E. coli,* resistant to ceftriaxone (CRO), ciprofloxacin, gentamicin and/or nalidixic acid, and recovered from 102 travellers was studied. Travellers were assessed pre-travel then longitudinally (maximum 6 months) with peri-rectal/rectal swabs. Clonality was determined by REP-PCR and the presence of O25b-ST131 was assessed. Comparison was made longitudinally for individuals and between identified co-travellers.

Results

The risk of prolonged carriage was lower for CRO than ciprofloxacin or gentamicin resistance. Repeated isolation of the same phenotype at different time points occurred in 19% of initial CRO resistant carriers compared with 50% ciprofloxacin or gentamicin resistant carriers. The duration of carriage was also longer for the latter resistance phenotypes (75^{th} quartile 8 vs. 62 & 63 days respectively). In multivariable analysis, risks for prolonged carriage included antimicrobial use whilst travelling (3.3, 1.3-8.4) and phylogenetic group B2 (9.3, 3.4-25.6) and D (3.8, 1.6-8.8). Clonality amongst longitudinal isolates from the same participant was demonstrated in 92% of participants assessable and most marked amongst CRO resistant isolates. ST-131 was surprisingly infrequent (3% of participants).

Conclusion

Prolonged carriage of ciprofloxacin and gentamicin resistant isolates is more frequent and prolonged than CRO resistance after travel. Risks for prolonged carriage indicate a contribution of host and bacterial factors to this carriage. These require further elucidation. The strong clonality identified suggests carriage of the 'phenotype' was mediated by persistence of bacteria/plasmid combinations rather than persistence of the plasmid after horizontal transfer to other bacteria.

INTRODUCTION

A number of recent publications have identified carriage of multi-resistant Enterobacteriaceae, primarily *E. coli*, in the gastrointestinal flora of returned travellers.[47, 164, 335, 336, 344] Individuals harbouring antibiotic resistant organisms have frequently travelled from countries of low resistance incidence to countries of high incidence e.g., Northern Europe to India. Key geographical regions for acquisition of multi-resistant Enterobacteriaceae include South-East Asia, the Indian Sub-continent and Africa.

One limitation of current studies is that they provide only a 'snapshot' of a narrow resistome immediately after travel, with only two studies thus far providing any longitudinal data.[47, 164] Such data helps us to better understand the link between carriage of resistant Enterobacteriaceae in travellers and subsequent infection in the carrier (or others within the community). Factors impacting on this link may include the risk of acquisition whilst travelling, clonal dynamics and duration of carriage, risk factors for prolonged carriage and the potential for spread of resistance by carriers within the home community or healthcare setting.

Even from the perspective of the more frequently described health-care associated carriage of resistant Enterobacteriaceae, longitudinal carriage studies are limited and varied. Median durations of carriage of *E. coli* range from 80-178 days with single and multiple clones identified in differing settings.[48, 340, 345-348] Furthermore, the dynamics of healthcare associated carriage may vary considerably from travellers, given the differing mechanism of acquisition and population involved. Complicating our understanding of prolonged carriage of antibiotic resistant Enterobacteriaceae, is the potential that any given resistance phenotype may be mediated by persistence of a stable bacteria/plasmid combination or persistence of the plasmid after horizontal transfer to other bacteria.[349]

In this study we aimed to define longitudinal and clonal aspects of prolonged carriage of antimicrobial resistance in a cohort of returned travellers. Natural history and clonality of individual carriers were investigated using a sample of patients selected as unlikely to have acquired new resistance after return from travel, by virtue of residence in an area of low background incidence of resistance and absence of antimicrobial exposure or re-travel.[23, 47] Shared clonality between travel partners and the incidence of the ST-131 O25B worldwide pandemic clone were explored in the entire cohort, comprising all samples collected during the study.

MATERIALS & METHODS

Bacterial Isolates & Data collection

Bacterial isolates used for this study consisted of 274 *Escherichia coli* from 102 participants collected during a previous prospective study of returned travellers residing in Canberra,

Australia.[47] The original study investigated the rate and duration of colonisation with resistant *E. coli* following international travel. Isolates presented were resistant to ciprofloxacin (cip-R), gentamicin (gent-R) and/or ceftriaxone (CRO-R). In addition, nalidixic acid resistant *E. coli* also recovered from this cohort were included in this analysis. A full description of the methods of bacterial isolation and clinical data collection is contained in the original publication.[4] In brief, 102 prospectively enrolled travellers who completed the study were asked to collect rectal or perianal swabs within 14 days before an overseas trip and within 14 days after return to Australia. If isolates resistant to ciprofloxacin, gentamicin or ceftriaxone were detected on the first return swab, participants were asked to collect regular monthly swabs until resistant bacteria were not identified on two sequential swabs or for a maximum of six months. Swabs were subcultured on three media, HBA-gentamicin (Oxoid, Australia), MacConkey agar (Oxoid, Australia) containing a nalidixic acid disc (Oxoid, UK) and chromID(ESBL) (bioMérieux, France), after an initial overnight broth enrichment. Resistant colonies were selected from each plate and underwent identification and susceptibility testing using Vitek2 (bioMérieux, USA). If all colonies on a given plate appeared morphologically identical, then only a single colony was sampled. If morphological differences between colonies were apparent, each variant was sampled. Mechanisms of ceftriaxone resistance were confirmed by PCR and sequencing and are presented in the original study. They comprised ESBL and AmpC enzymes.[4]

All participant and travel data used in this study was collected via a questionnaire completed directly by participants during the original study. Variables included travel destinations, antimicrobial use, intercurrent illness and food/water consumption whilst travelling. For this analysis, "high risk" regions were defined as regions from which >50% of travellers during the original study returned with a resistant isolate. As travellers frequently visited more than one destination, the duration of stay in each region was calculated and included.

Molecular Methods

The phylogenetic group was determined on all isolates using triplex PCR.[230] Determination of ST-131 O25B world-wide pandemic clone was undertaken by detection of *pabB* and *trpA* alleles via multiplex PCR with a positive control MLST confirmed as ST-131.[181] Repetitive extragenic palindromic PCR (REP-PCR) was undertaken using published methods.[322] The template was purified DNA (Mo-Bio, USA), using a BioRad C1000 Thermal Cycler. Primers REP-1 (5'-IIIGCGCCGICATCAGGC-3') and REP-2 (5'-ACGTCTTATCAGGCCTAC-3') were used. The products were separated on a 1% agarose gel (45v, 3 hours) and stained with ethidium bromide. Manual visual comparison was used to identify clonal isolates and any difference greater than two

non-shared bands was considered non-clonal. Bacteria for comparison were always separated on the same agarose gel.

All isolates of the same phylogenetic group harboured by the same participant or shared by travel partners were considered potentially clonal. For CRO-R isolates, all potentially clonal isolates were subject to REP-PCR. For cip-R and gent-R resistant isolates, if three or more potentially clonal isolates of identical phylogenetic group and phenotypic antimicrobial susceptibility (amoxicillin, amoxicillin/clavulanate, cephazolin, ceftriaxone, ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole) occurred within 8 weeks, interval isolates were skipped (e.g., sample 2 of samples 1, 2 & 3 skipped). If differing presumptive clonality was identified amongst the isolates analysed, then the interval isolate was subject to REP-PCR.

Definitions

Duration of carriage was calculated from the date of return to Australia until the date of collection of last positive swab. *Clearance of carriage* was assumed if there was collection of at least one swab not containing the given antimicrobial resistance phenotype, without the occurrence of any subsequent positive swabs. *Clonal carriage* was defined as identification of clonal isolates (as defined by REP-PCR) at two time points (most > 4 weeks apart). As above, any isolates with greater than two non-shared bands were considered unrelated.

Censoring and exclusions for longitudinal analysis

Participant results were censored (exclusion of all subsequent swab results) after events that may have potentiated new acquisition of resistance including further overseas travel or receipt of antimicrobial therapy for suspected or proven infection (n=6 urinary tract infection, n=2 other infection site). Participants who were still on doxycycline for malaria prophylaxis at the time of the return swab were not excluded. Participants harbouring clonally related isolates pre- and post-travel were excluded. To assess this, all pre-travel isolates were compared to post-travel isolates using REP-PCR in a similar manner to the longitudinal analysis (n=7 participants). All participants remaining who had assessable swabs collected at two or more time points after travel were included.

Statistical Methods

Kaplan-Meier plots were used to illustrate duration of resistance. Subjects were censored if they remained resistant at their final data collection time-point. Parametric survival models were used to estimate the magnitude of differences in the distribution of resistance duration due to the three antibiotics. More specifically, log normal accelerated failure time models were used as the log normal provided the best fit to the observed data from a number of common alternatives (including

Weibull and log logistic models) and estimates from accelerated failure time models could be reported as proportional increases in resistance durations. Robust variances were used to take into account within-patient correlations of resistance to the three antibiotics of interest.

Potential risk factors were assessed for possible association with duration of resistance to any of the three antibiotics, by including these variables as covariates in univariable survival models. All variables that showed some evidence of association in univariable analysis ($p<0.1$) were included in multivariable analysis. Backwards elimination was used to remove non-significant ($p>0.1$) variables until the best predictive model was obtained. SAS version 9.1 for Windows and Stata/IC 10.1 for Windows were used for analysis.

RESULTS

DURATION, CLONAL DYNAMICS AND RISKS OF PROLONGED CARRIAGE.

Of the 102 original participants in the study, 50 returned carrying antimicrobial resistant *E. coli* of interest (CRO-R, cip-R and/or gent-R), with 44 included in the final analysis after censoring and exclusions.(Fig. 1) Three participants reporting ongoing use of doxycycline for malaria prophylaxis at the initial return swab were not excluded.

Duration and Risks for Prolonged Carriage of Resistance

Upon initial assessment, after return from travel, the carriage of the three specified phenotypes was CRO-R 26% (n=27), cip-R 27% (n=28), gent-R 35% (n=36). When compared with CRO resistance, cip-R and gent-R were associated with 2.1 (95% CI, 1.1 to 4.1, p = 0.027) and 3.5 (95% CI, 1.6 to 7.5, p = 0.001) times the duration of carriage of resistance respectively. There was no significant difference between the duration of cip-R and gent-R. The median durations and inter-quartile ranges for recovery of resistance from travellers was 3 (IQR, 1 to 8) days for CRO-R, 5 (IQR, 1 to 62) days for cip-R and 8 (IQR, 3 to 63) days for gent-R. This is represented longitudinally on a Kaplan-Meier curve (Figure 2), demonstrating the major difference is in the upper quartile of participants.

Potential risks for prolonged carriage of any resistance were analysed by three groups of factors: participant/travel characteristics, duration/location of travel and bacterial factors. Results of univariable and multivariable survival models are presented in Tables 1 and 2.

Clonal dynamics of resistance

Clonality was almost always present in prolonged carriage of a given antimicrobial phenotype. Of 25 participants with isolation of bacteria of the same phenotype at two time points, the carriage of exclusively clonal isolates was demonstrated in 14 (56%) participants. Carriage of a mixture of clonal and non-clonal isolates occurred in 9 (36%). Three participants (12%) carried more than one prolonged clone simultaneously. Only 2 (8%) had no clonal relationship between bacteria isolated. In both cases bacteria were only recovered on the return swab and a single subsequent swab. These patterns are illustrated in Figure 3.

The dynamics of clonality differed between CRO-R and cip-R or gent-R resistant isolates. With the exception of a single bacterial isolate, repeated isolation of CRO-R Enterobacteriaceae was invariably due to the presence of clonal bacteria. The carriage of ciprofloxacin and gentamicin resistance demonstrated more diversity, although by month 5, all isolates recovered were clonal with earlier isolates.(Figure 4)

Travel Partners

From the cohort of 102 travellers, 70 (68%) travelled with other study members.(Figure 1) This included 29 'pairs' (travel & sexual partners) and six 'mixed groups' containing participants of other relationships (friends travelling together, families, and one unknown relationship). For analysis, pairs within mixed groups were considered, within the 'pairs' cohort.

Analysis of the 29 'pairs' revealed 14 (48%) with neither partner returning with resistance, 8 (28%) with a single partner doing so and 9 (31%) with both partners harbouring resistance. Thus, if one person was colonised, there was a 53% (9/17) chance of the partner also harbouring one of the three resistant phenotypes sought. However, using clonal analysis, just 2 of 9 couples (22%) shared clones.

Shared clonality was also identified amongst one of six 'mixed' groups of travellers.

ST-131 World Wide Pandemic Clone.

The presence of the clone was assessed amongst all 274 *E. coli* isolates recovered from 102 participants. This included all CRO-R, gent-R, cip-R and/or nalidixic acid resistant isolates recovered from participants throughout the study duration. (Figure 1) Pre-travel prevalence of the clone was 2% (2/102). An additional two individuals acquired ST-131 *E. coli* while travelling. With the exclusion of the pre-travel carriers, only one prolonged clonal carrier was colonised with a ST-131 strain. All ST-131 *E. coli* isolated were phylogenetic group B2.

Carriage of multi-resistant Enterobacteriaceae upon return from travel is a real and concerning phenomenon. The most serious outcome of such carriage is infection with resistant organisms after return home. Although we did not specifically assess infections in returned travellers, two recent studies have demonstrated this risk in diverse populations. Overseas travel afforded a relative risk of 2.7 for any infection after TRUS biopsy[337], and a relative risk of 5.7 for ESBL *E. coli* infection in a regional Canadian study.[27] Our analysis helps to delineate the complex link between acquisition and carriage during travel and infection after return.

The rapid decline in carriage of resistant isolates after travel is to some extent encouraging, however persistence is significant. Analysis of the longitudinal nature of carriage highlights the marked persistence of cip-R & gent-R isolates beyond CRO resistance, with 10% of participants in the longitudinal arm of this study harbouring cip-R &/or Gentamicin resistance at six months after return. This is noteworthy, given that fluoroquinolones and aminoglycosides are heavily relied upon for the treatment of *E. coli* infection, including urinary sepsis, in many national guidelines.[13, 350] The prolonged duration of carriage of fluoroquinolones resistance identified, concurs with descriptions of healthcare associated carriage.[340, 345, 348] Explanations for the shorter duration of CRO resistance potentially include the higher fitness cost of maintaining this resistance plasmid for bacterium, in the absence of ongoing selection pressure and genetic differences between the host bacteria that harbour each resistance element.

Risk factors for prolonged carriage are intriguing. Antimicrobial use whilst travelling was strongly associated, leading to a 3.4 times increase in duration of carriage. Kennedy, in travellers[47], and many authors in other settings, have identified antimicrobial use as a risk for acquisition of resistance, an intuitive conclusion. However, this study examines a group who all harboured resistance and did not have further antimicrobial exposure to potentiate this risk. Another mechanism apart from simple selection of antimicrobial resistance may apply. We hypothesize potential modification of intestinal microbiota after antimicrobial use, leading to the loss of other potentially competitive non-resistant *E. coli* and other integral commensal bacteria.[351]

The analysis of clonality answers interesting questions about acquisition and carriage of such resistance. Whilst the 'mobility' of resistance elements, particularly CRO resistance plasmids, was initially hypothesized in this study and is frequently discussed and demonstrated in 'high stress' situations such as healthcare settings and antimicrobial use[349], it appears not to be significant in

travellers. In fact clonality was almost absolute for plasmid mediated CRO resistance. The strong clonality of isolates amongst all phenotypes gives us insight into the environment of acquisition. The identification of clonality amongst travel partners and mixed groups (where direct transmission from person to person was unlikely) suggests exposure and ingestion of a common source of resistant isolates, potentially food or water. This is also supported by the relatively low rate (22%) of shared clonality between partners. The pattern of contraction of a variety of resistant isolates on return to persistence of a single (or very few) clones, implies the presence of only a limited number of clones in circulation able to colonise and persist amongst individuals even in such 'environments' with high resistance burden. The shared clonality amongst partners/mixed groups and the correlation with period of exposure in 'high-risk' regionsm supports this hypothesis. This can be compared to the observation of clonality amongst *E. coli* causing urinary tract infection, where a small handful of adapted clonal groups are thought to cause a significant proportion (10-20%) of all such infections[168]. Although not investigated in this study, other than ST131, further exploration of clonality across travellers and regions would be worthwhile.

The identification of 'persistent' clones also highlights the issue of potential infection and both community and healthcare related transmission of resistant isolates. It is unknown whether travellers may be the point of introduction of antibiotic resistant bacteria into a community, as compared to imported food, animals or de novo development of resistance via antibiotic use. Importation of antibiotic resistant bacteria into hospitals has been well documented via individuals who have been treated in hospitals in high-risk areas.[96] With respect to travellers, we speculate that a long duration after return from travel, e.g., 3-6 months, may be the most problematic in a healthcare system, given these clones have been selected as the best adapted for colonisation and persistence.

The relationship between persistence of colonisation and subsequent infection needs further exploration. The phylogenetic groups, as identified in this study, represent a broad family of bacterial characteristics related to virulence. The markedly increased duration of the more virulent B2 and D groups, compared to commensal *E. coli* (predominantly groups A & B2)[352], indicates bacterial genetic factors other than the presence of resistance genes, which may aid in persistence. Potential mechanisms requiring exploration include biofilm formation, competitive bacterial toxin production, e.g., colicins, and virulence factors including siderophore and fimbriae production mediating competitive advantages in iron capture and adhesion. Furthermore, the relation of factors

that determine persistence to those that determine the classical virulence of invasion and infection, also requires elucidation.

Limitations of this study include the exploratory nature, using a previously collected sample. The collection method of recovering a single isolate from the plate has led to some limitation in determining clonality. To investigate this, a model was constructed using the assumption that clones recovered on a given swab were present but not identified on all previous swabs due to this methodological issue. This model indicated that approximately one third of clones were not recovered on any given swab, suggesting that with the repeat sampling undertaken, there was a low chance of failing to identify truly persisting clones (data not shown).

In the multivariable analysis, only a limited number of factors were assessed. There may be other significant participant and isolate features that were not included in this analysis. Furthermore, the assumption that resistance was only acquired whilst travelling and did not occur after return is relied heavily upon in the data. We believe this was reasonable given the very low background rates of resistance in Canberra[23, 47]; however is likely not absolute. Acquisition of resistant clones after return (or the emergence of 'low-level' pre-travel resistant clones due to antimicrobial use) may have led to over-estimation of duration of carriage and diversity of clonality. A control group of matched non-travellers from Canberra would have been optimal in assessing this situation.

Conclusion

Prolonged gastrointestinal carriage of resistant bacteria after return from travel is a complex phenomenon. The duration of carriage of CRO resistance was significantly shorter than Ciprofloxacin or Gentamicin resistance. Risk factors for prolonged carriage of resistance include antimicrobial use whilst travelling and the duration of travel in 'high risk' regions. Clonality was present amongst all phenotypes, however almost absolute amongst CRO resistance. The contraction to a small number of clones and shared clonality amongst travel partners suggests a limited number of clones adapted to prolonged carriage circulating in regions of acquisition. ST-131, the world wide pandemic clone, was surprisingly infrequent amongst the phenotypes assessed. The identification of clonality amongst travel partners and mixed groups (where direct transmission from person to person was unlikely) suggests exposure and ingestion of a common source of resistant isolates, potentially food or water.

Acknowledgements

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Conflict of Interests

BR, KK, HS, MJ & DP declare that they have no conflicts of interest.

FIGURES AND TABLES

Table 1

Univariable analysis of risk factors for prolonged carriage of resistance.

Variable	Estimate	Lower 95% CI	Upper 95% CI	P-value
Age at departure	1.00	0.96	1.05	0.86
Female gender	1.17	0.34	4.01	0.80
Whilst travelling				
Antibiotic use	9.12	3.16	26.05	<0.0001
Diarrhoea	2.34	0.70	7.77	0.17
Consumed 'tap' water	0.53	0.16	1.79	0.30
Duration and destination				
Total travel (per week)	1.30	1.06	1.60	0.012
High risk regions (per week)	1.32	1.08	1.63	0.008
Other regions (per week)	1.03	0.72	1.46	0.87
India/Sri Lanka/Nepal[a]	1.03	1.00	1.06	0.022
SE Asia/Pacific[a]	0.98	0.91	1.05	0.55
Middle East/Africa[a]	1.03	0.97	1.10	0.33
China/Hong Kong/Taiwan/Korea[a]	1.02	0.93	1.11	0.70
South America/Mexico[a]	1.00	0.93	1.08	0.93
Bacterial factors				
Multiple resistance[b]	1.82	0.54	6.23	0.33
Phylogenetic group A	0.82	0.23	2.86	0.76
Phylogenetic group B1	1.62	0.47	5.58	0.44
Phylogenetic group B2	7.03	1.65	29.96	0.008
Phylogenetic group D	5.16	1.68	15.80	0.004

[a] Per day in this region

SE Asia (Malaysia, Thailand, Singapore, Vietnam, Philippines, Laos, Indonesia, Cambodia, Papua New Guinea, Solomon Islands), Middle East/Africa (Jordan, Israel, UAE, Egypt, Zambia, Tanzania, Kenya)

[b]Resistance to at least two of Ciprofloxacin, Gentamicin, and Ceftriaxone

Table 2
Multivariable analysis of risk factors for prolonged carriage

Variable	Estimate	95% CI	P-value
Travel in high risk regions (per week)	1.27	1.09 to 1.49	0.002
Antibiotic use	3.34	1.33 to 8.36	0.01
Phylogenetic group B2	9.32	3.39 to 25.6	<0.0001
Phylogenetic group D	3.81	1.64 to 8.82	0.002

Figure 1
Selection and exclusion of 102 participants for the three arms of this study. (The same participants and bacterial isolates were used in each arm)

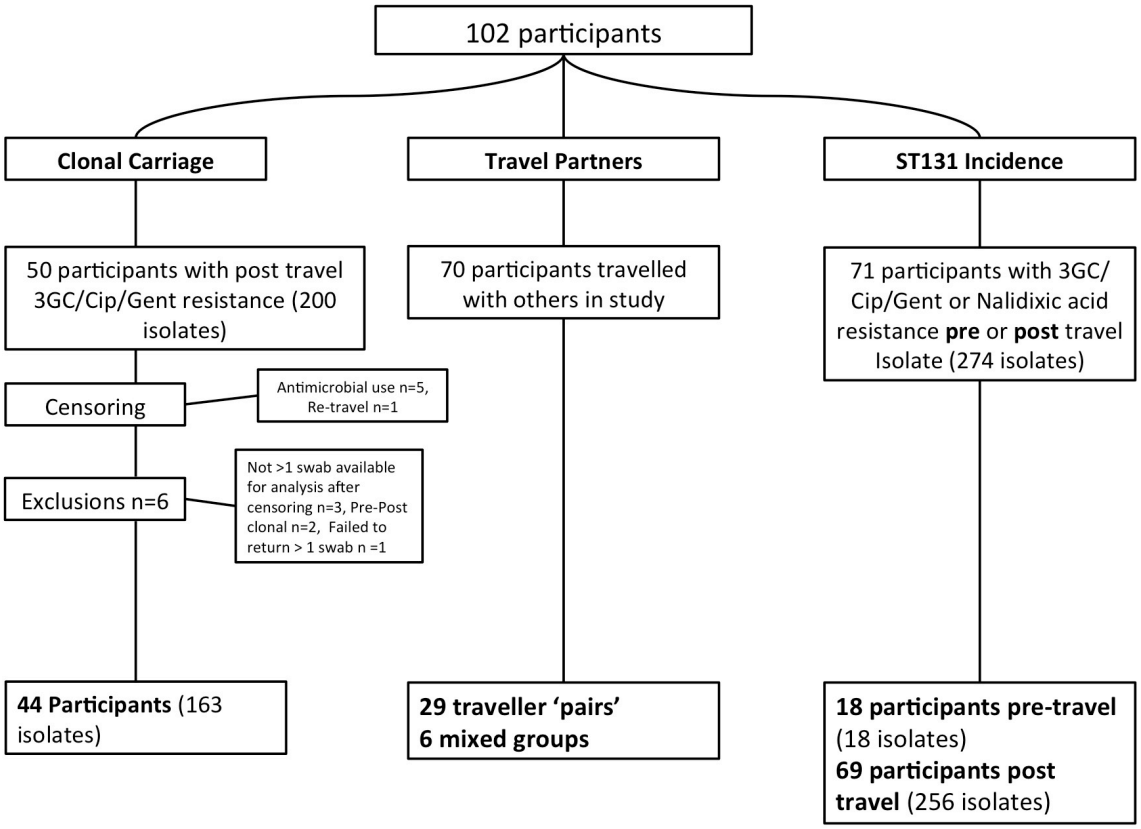


Figure 2
Kaplan-Meier survival curve comparing duration of carriage of the three resistance phenotypes across 44 selected participants. (p=0.007)

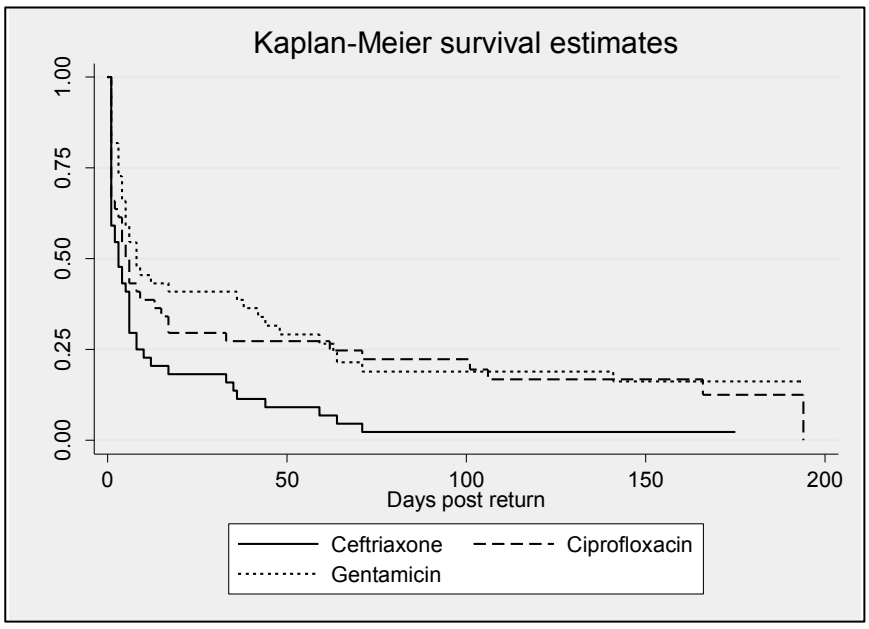


Figure 3

Travellers representative of different patterns of carriage of resistant isolates. A time-line is displayed across the top (days). Arrows represent the submission of swabs by travellers. Shaded squares indicate bacterial clones. The diamond in each square shows the time points at which the bacteria were isolated in a sample.

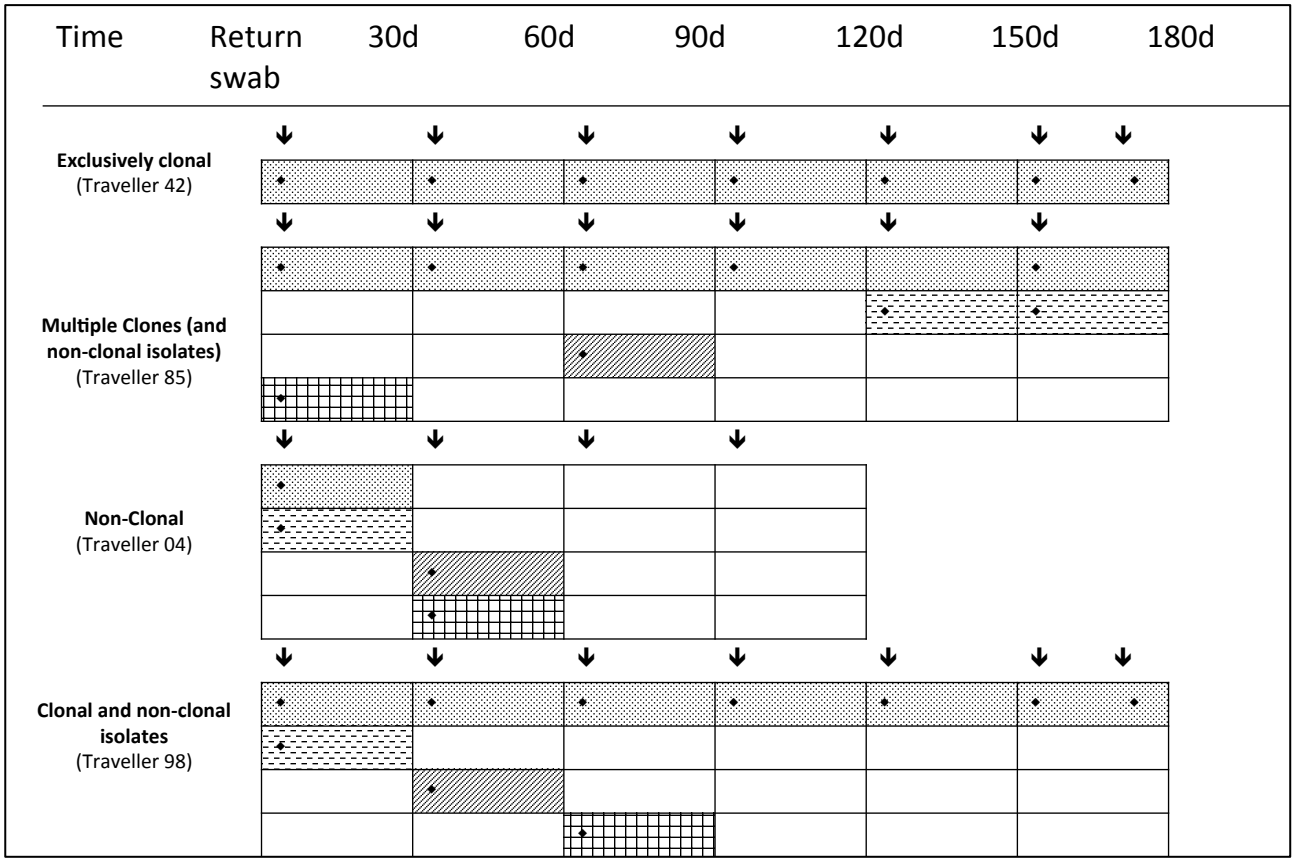
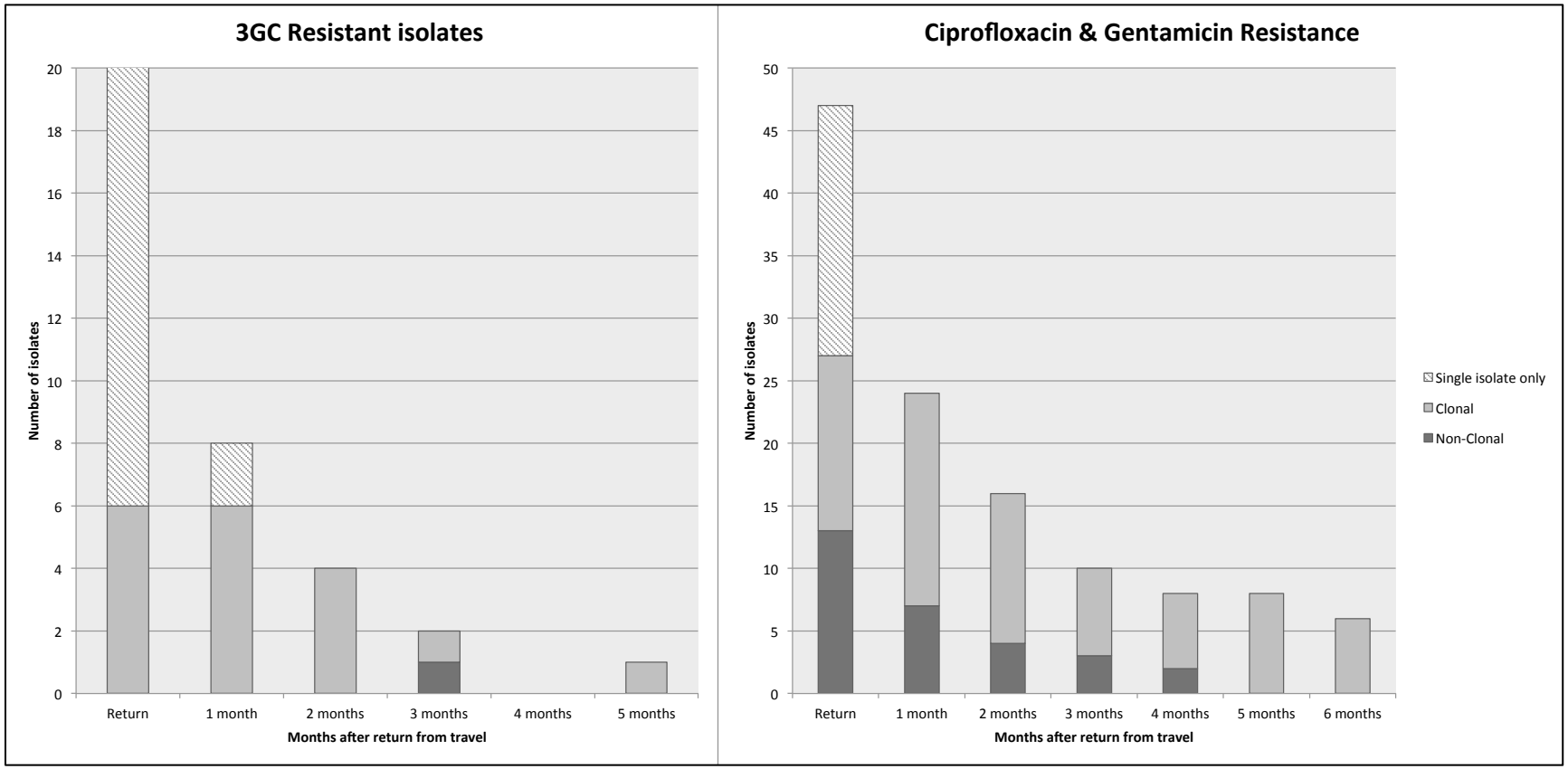


Figure 4

Graphical representation of the clonality of isolates amongst each individual

Left - Comparison of CRO resistant isolates to other CRO resistant isolates amongst the same individual. **Right**- Comparison of Ciprofloxacin and/or Gentamicin resistant isolates to other isolates of the same phenotype amongst the same individual. (Isolates harbouring CRO resistance have been excluded. Phenotypes only recovered at a single time point could not be assessed for clonality and are indicated in white/hash.)



CHAPTER 6. INFECTION CONTROL MANAGEMENT OF MULTI-RESISTANT *ESCHERICHIA COLI*

INTRODUCTION

As outlined in Chapter 1, our understanding of the infection control requirements of ESBL-E is evolving. Given the recent suggestion of relatively low transmission of ESBL-E in acute care settings, we present a national survey of infection control practice for ESBL harbouring Enterobacteriaceae, CRE and patients with overseas healthcare contact.

This survey was conducted as an online open-invitation survey using a commercially available web-based survey tool. One consideration in the timing of this survey is that it was conducted before the drafting of national guidelines on the management of CRE in healthcare settings,[63] thus it may serve as a baseline for further surveys on this topic in the future. Patients with overseas healthcare contact were included, given the emerging threat this group pose and their infrequent inclusion in general infection control recommendations.

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ABSTRACT

Introduction

Despite the global expansion of extended spectrum beta-lactamase harbouring Enterobacteriaceae (ESBL-E) and carbapenem resistant Enterobacteriaceae (CRE), only limited research on the infection control management of patients with these organisms is available.

Methods

We present a national survey of infection control practice amongst adult acute-care hospitals in Australia, for ESBL-E, CRE and the emerging threat of patients with overseas healthcare contact.

Results

In total, 97 health services responded, representing 9% of all eligible hospitals. The proportion of hospitals that reported the use of contact precautions (CP) was 96% (93/97) for ESBL-E, 81% (79/97) for CRE and 72% (48/67) for patients transferred from an international hospital. For ESBL-E, hospitals frequently employed risk-stratification to limit the use of CP (40/97, 41%).

On multivariate analysis, predictors of a strategy to limit use of CP for ESBL-E were government funding (OR=4.8, p=0.003) and a metropolitan location (OR=3.2, 0.014); predictors of any use of CP for CRE, were location in a state with a specific legislation on CRE (p=0.030) and the presence of a written policy on CRE (p=0.011).

Conclusion

Infection control management of MRGNBs varies considerably across Australian hospitals surveyed. A lower rate of reported CP use for CRE than ESBL-E was unexpected and indicates the vulnerability of some Australian hospitals. Multivariate analysis reveals various drivers influencing infection control practice in Australia.

INTRODUCTION

A marked expansion of community onset ESBL harbouring Enterobacteriaceae (ESBL-E), and the burgeoning of carbapenem resistant Enterobacteriaceae (CRE) has occurred in the past decade.[80, 353] Furthermore, patients with overseas healthcare contact are increasingly identified as a vector for the global movement of new antimicrobial resistance mechanisms including those mediating CRE.[96]

The majority of current infection-control guidelines include recommendations for the control of multi-resistant gram-negative bacilli (MRGNBs), including CRE and ESBL-E. Fewer guidelines include recommendations for patients with overseas healthcare contact.[354] Given the small number of published studies on which to base recommendations[355] and the rapidly changing epidemiology of MRGNBs, guidelines in this area risk being out-paced by on-the ground events.

Australia is a low-prevalence country for ESBL-E, with a 2010 national survey of community onset isolates indicating 3.4% of *E. coli* and 3.6% of *K. pneumoniae* were ESBL producing.[44] CRE in Australia originate from two key sources: low-level endemicity of metallo-beta-lactamase (MBL) producing Enterobacteriaceae within critical-care areas and some speciality units on the country's eastern coast;[61] and residents returning after overseas healthcare contact.[66, 71] We have previously described variation in the infection control practice used for patients hospitalised with expanded-spectrum cephalosporin resistant *E. coli* as part of an Australasia-wide study.[329, 356] Such a disparity has been noted in other reports.[357, 358]

In follow-up, we present a national survey of practice in infection control management of patients harbouring ESBL-E, CRE and patients with overseas healthcare contact, amongst acute-care adult hospitals in Australia. Our aim was to define the scope of variation in infection control practice for these groups in Australia, and to identify factors that determine which policy and practice is applied in differing health services.

METHODS

The study population was adult acute care hospitals within Australia. At the time of the survey, Australia (population 23 million) was serviced by approximately 700 publically funded hospitals and 300 private hospitals across its six states and two administrative territories.[359, 360]

A draft survey was constructed including questions based on those used in a previously published work.[357] The survey was piloted on five experienced infection control practitioners and modified based on their feedback. The survey questioned health service's practice of infection control, rather

than the details of written policy. Where a service used varied practice within their network, they were asked to answer for the area which best fitted the description of an 'adult acute care' facility. The full survey is in Supplementary Material.

The survey was conducted as an open invitation on-line survey, using a web-based interface to collect responses. An email invitation was disseminated via two frequently used national email discussion groups: one hosted by the Australasian College for Infection Prevention and Control, and the other hosted by the Australasian Society for Infectious Diseases.[361] The former emails went to approximately 500 email addresses (personal communications, Mr Michael Wishart, HSN Hospital, QLD, Australia) and the latter to approximately 900 addresses (personal communication, Dr Ashley Watson, Canberra Hospital, ACT, Australia). Follow-up emails were disseminated via these channels. The survey was open for a two-month period (November 2012 to January 2013). A small token of appreciation (a gift hamper) was offered to one randomly selected responding site.

Human research ethics approval for the conduct of this study was received from The University of Queensland.

We requested that the nominal 'head' of infection control complete or delegate completion of the survey at each site, to minimise multiple responses. If multiple responses from a single site were received, these were collapsed to a single survey as follows: Answers were classified as **concordant**, **relative agreement** (e.g. difference in details only) or **discordant**. Answers in the latter two groups were combined using the following rules: a) affirmative responses (indicating the presence of a given policy) were presumed to be correct; b) the most restrictive application of a policy or most conservative numerical was presumed to be correct. Infection control services were not re-contacted, as permission for this had not been sought in the ethics approval.

Where a single respondent answered for a health service/network of multiple adult acute-care hospitals, this was maintained as a single answer, with demographics from the single largest hospital used for analysis.

External data sources

Data is primarily as reported by the respondent. Key demographics (hospital size, funding and referral services) were confirmed with public data sources (Supplementary Material). Denominator data for Australian hospitals was extracted from the Australian Institute of Health and Welfare (AIHW) annual report 2011-2012.[360]

Definitions

Contact precautions was defined as the use of any combination of gloves, gown and or a single or cohort room.[86] Infection control practice was considered **Inclusive** when all patients with a given resistance phenotype were managed in contact precautions or **Permissive** if non-use of contact precautions was allowed in some circumstance (risk stratification by bacterial species or patient characteristic) or was not used at all. **Hospital type** was stratified by funding source; *Public hospitals* are fully funded by the Australian state and/or federal governments. They provide the vast majority of supra-regional referral services in Australia; *Private hospitals* draw funding from patient billing revenue and primarily service patients covered by voluntary private-health insurance or other third parties; **Supra-regional referral services** were highly-specialised referral services as follows: **Transplant service** (solid organ or allogeneic bone-marrow transplant services) and **other supra-regional services** (major burns, spinal injury and cystic fibrosis services). An **Infectious Disease** (ID) service was an ID physician providing consultation or in-patient services at the hospital. A **written policy** specifically pertained to the resistance phenotype (or patient group) queried, rather than a generic 'MRO' type policy. **Hospital size** was classified by the AIHW 'Peer Group' system.[362] As private hospitals are not classified by this system, two researchers (BR, SH) independently assigned a peer group after review of any available hospital demographic data (from the survey and publicly available information on the hospital's website). Disagreement was resolved by discussion. **Principal referral** hospitals (A1 by AIHW classification) are major city hospitals with more than 20,000 and regional hospitals with more than 16,000 (casemix-adjusted) separations per year. **Large hospitals** (A1, A2, B1, B2) included principal referral, specialist women's hospitals, large metropolitan (>10,000 casemix-adjusted separations), and large regional hospitals (>8000 or >5000 casemix-adjusted separations, depending on location).

Relevant legislation and recommendations

At the time of the survey there were no national infection control management recommendations or legislation specifically pertaining to CRE or patients with overseas healthcare contact. Some recommendations for ESBL-E are provided in the national infection control guidelines[86]. Two Australian states work within state-level legislation (operational directives). One encompasses all 'multi-resistant organisms' (**MRO Directive**),[363] and the other specifically CRE (**CRE Directive**).[364] See Supplementary Material for a comparison of state and national documents.

Statistical Methods

Univariate analysis was undertaken using X^2, Fisher's exact test and calculation of odds ratios (OR). Multivariate logistic regression included all variables significant on univariate analysis at a p=0.2 level. Using backwards selection variables was retained in the final logistic regression model

if their significance remained below p=0.2. Models were assessed by calculation of a ROC and Hosmer-Lemshow goodness of fit test. Robust estimates of variance were used to account for a potential lack of independence between hospitals, given some operate in shared jurisdictions where standardisation of policy may have occurred. In addition, when the geographical variable of state-based legislation was entered into multivariate analysis, it was maintained as a tripartite set (MRO policy, CRE Policy, No Policy). All statistical tests were two tailed, and $p<0.05$ was considered significant. STATA version 12.1 (Statacorp, USA) was used.

RESULTS

Valid responses were received from 97 unique hospitals or health services. Eight further responses were excluded as they originated from institutions that did not meet the study population (hospital type: exclusively paediatric n=3, elective day-procedure sites or sub-acute care only n=3, psychiatry only n=2)

Hospital demographics and national coverage

Responses were received from 68 public hospitals and 29 private hospitals. This included 75% (31/41) of all sites accredited to train fellows in adult infectious diseases within Australia.[365]

Approximately 9% of all Australian hospitals within the survey population responded (68/736, 9% public hospitals, 25/285, 9% private hospitals - excluding private hospitals in three states that do not provide denominator data).

In total, 58(60%) hospitals were situated in metropolitan locations, 32(33%) in regional locations and 7(7%) in remote locations. Stratified by state location, the geographical distribution of respondents approximately mirrored that of the Australian population (Supplementary Material).

Respondents

In total there were 108 respondents, including 9 sites with duplicate responses. The majority of survey respondents identified their role as a nursing trained infection control practitioner (81/108, 75% - including nurse-managers and clinical nurse specialists), or an MD trained clinician (22/108, 20% - including infectious disease, infection control or clinical microbiology specialists). The remainder of respondents were in managerial or academic positions (5/108, 6%).

There was at least one responding MD for 19/97(20%) health services. Significantly more principal referral centres compared with other centres, had an MD respond (18/43, 42% vs. 1/54, 2% p=0.002).

Control of Specific Organisms and Patients

ESBL ENTEROBACTERIACEAE

Contact precautions (CP) were used by 93/97 (96%) hospitals in the management of patients ESBL-E. (Table 1) The most common implementation of these precautions was 'inclusive' (48/97, 49%), with CP used for every patient with any ESBL-E. A variety of 'permissive' practices, including the use of risk stratification by organism genera and/or patient characteristics, were used in other sites (Figure 1). Data was missing from 5/97 (5%) sites.

A descriptive analysis of factors predicating the presence of a permissive practice for the application of CP in patients with ESBL-E, as compared with an inclusive practice, is in Table 2. On multivariate analysis, the predictors of use of a permissive practice were hospital funding type (p=0.003) and a metropolitan location (p=0.014) (Table 3).

CARBAPENEM RESISTANT ENTEROBACTERIACEAE

Use of CP for patients harbouring CRE was reported by 79/97 (81%) hospitals (Table 1). An inclusive practice was most common, occurring in 56/97 (58%) hospitals (Figure 1). Descriptive analysis of the predictors of use of any CP on patients harbouring CRE is in Table 2. On multivariate analysis, the significant predictors of any use of CP for patients harbouring CRE were the presence of a written policy on CRE infection control (p=0.011) and location in the state with a CRE directive (p=0.030) (Table 3).

INTERNATIONAL TRANSFER PATIENTS

In total, 67/97 (69%) hospitals provided information on infection control management of patients received after an international transfer between hospitals (IT-patients). 25/97 (26%) indicated they did not know the policy or had never faced this situation and 5/97 (5%) did not respond to the question (Table 1).

CP was used in 72% (48/67) of hospitals that provided details (Figure 1). Descriptive analysis is in Table 2. On multivariate analysis, positive predictors of the use of any CP for IT-patients was the presence of a written policy on IT-patients (p=0.025) and location in the state with a CRE directive (p=0.003) (Table 3).

Some form of enhanced infection control management (CP &/or screening) was applied to patients reporting overseas healthcare contact (although not directly transferred from a hospital) by 45/97 (46%) hospitals, with 46/97 (47%) reporting no use of precautions and 6/97 (6%) not responding.

'AMP-C' TYPE ORGANISMS

For patients harbouring AmpC producing organisms, 48/97 (49%) hospitals reported no use of CP, 41/97 (42%) an identical management strategy to ESBL harbouring organisms, 7/97 (7%) a stand-alone strategy and 1/97 (1%) did not respond.

Policy basis and origin

The most commonly utilised publications in the preparations of infection control policy for MRGNBs was the Australian National Health and Medical Research Council (NHMRC) guideline[86] (87/97, 90%) and departmental guidelines or directives issued by State or Territory governments (80/97, 82%). Hospitals based in the states with compulsory directives were significantly more likely to identify this source than other states (36/38, 95% vs. 44/59, 76% p=0.015). International guidelines were used less frequently, including those originating from the American CDC[87] (43/97, 44%) and other countries' guidelines (21/97, 22%).

Variability of survey response

Analysis of responding health services showed a disproportionate number of responses from 'large' public hospitals. This group comprised 68% (48/68) of survey respondents within the public hospital group, although numerically accounted for only 18% (131/736) of public hospitals within Australia. Within responses on IT-patient management missing data was most often from smaller hospitals. In total, 86% (37/43) of principal referral hospitals provided details on their management compared with 56% (30/54) of other sites (p=0.001 for comparison).

Analysis by respondent type showed significant differences in reported CRE management. All sites with an MD respondent (19/19) reported use of CP for CRE, whereas only 60/78 (77%) sites with a non-MD respondent reported this use (p=0.019 for comparison). When further analysis was undertaken within the subgroup of principal referral hospitals, in order to account for the disproportionate number of MD respondents within this group, this difference was still apparent, although not statistically significant due to the small numbers involved (18/18, 100% MD respondents used CP for CRE vs. 20/25, 80% non-MD respondent sites p=0.064).

Analysis of intra-site correlation for the nine sites that submitted multiple responses to the survey is contained in Supplementary Material. Across (seven) major themes in the survey, no single site showed concordance for all answers. Across all sites, 51% (32/63) of details reported were concordant, 27% (17/63) were in relative agreement and 22%(14/63) were discordant.

The highest discordance was on the presence of written policies (7/9, 88% discordant) and the use of CP for IT-patients (3/9, 33% discordant). Relative agreement occurred most often when describing CP use for ESBL-E and CRE (each 6/9 67% relative agreement). In the relative agreement group, differences between respondents were in the description of details of risk stratification practice and the use of cohort rooms.

DISCUSSION

Our national survey demonstrates widely varied infection control practice for MRGNBs across at least 97 hospitals in all regions of Australia. Such variation has been demonstrated in other parts of the world.[357, 358]

Foremost, our unexpected finding is that CP use is reported more frequently for ESBL-E than for CRE. Several explanations are apparent. First, our data indicates many infection control policies and/or practitioners rely on the use of a laboratory 'ESBL phenotype' to trigger CP use, as evidenced by the lower rates of any CP use for 'AmpC' organisms compared with ESBL-E (50% vs. 96%). A number of CRE will not possess this phenotype (nor even an MBL phenotype) and will not be detected unless decreased carbapenem susceptibility is the trigger for infection control intervention.

Second, misclassification may have occurred due to unfamiliarity with the term 'CRE'. This was unforseen in the survey design and specific examples of 'carbapenem' and 'Enterobacteriaceae' were not given. This finding is supported by the significantly higher proportion of MD trained respondents' (who are all trained and certified sub-specialists), compared to others', reporting of use of CP for CRE. Other respondents (94% from a nursing background) would have a more varied clinical and academic background than the MDs, and may not have managed patients with CRE or have been familiar with aspects of CRE, given its relative infrequency in Australia.[43]

To some extent, both possibilities indicate a number of vulnerable Australian hospitals, where patients with CRE may not have an appropriate assessment of their infection control needs. Within misclassification, it is difficult to determine for what proportion the issue is simply a lack of knowledge of current terminology, as opposed to a greater knowledge gap around the potentially varied clinical and laboratory characteristics of CRE and the serious implications of the phenotype.

Our findings on the predictors of ESBL-E and CRE practice offer insight into some of the pragmatic aspects of determining MRGNB policy.

For ESBL-E infection control, practitioners have potentially sought a balance between the low risk of nosocomial transmission of ESBL-E[94] and the various burdens of CP. Hospitals that were likely to have a high load of ESBL-E harbouring patients, due to their generally higher acuity (metropolitan and public hospitals), were more likely to use a permissive policy to limit use of CP for this phenotype. Interestingly, the only four sites that did not use any CP for ESBL-E were principal referral sites with a supra-regional referral patient load.

Predictors of CP use in CRE illustrate the benefits and risks of layering state-based legislation on healthcare services. When used in a targetted manner, this can be effective in filling a void in national policy. Operating under a CRE directive was a significant predictor of the use of CP for CRE (and for IT-patients who are also covered by the policy).

In contrast, the general MRO directive appears to be ineffective. Despite 90% of sites in the state indicating they used directives to formulate policy, it did not have a significant impact on infection control practice for the emerging threats of CRE and IT-patients. Even for ESBL organisms (which are specifically mentioned in the document), sites have either disregarded, or gradually moved away from the stipulated management.

IT-patients are an emerging risk group with which other regions are also grappling.[366] For the use of CP, the significant predictive value of the presence of a written policy for IT-patients, should be interpreted in light of the 31% missing-data and 33% discordance rate on answers to this question. There is some misclassification. Taken as a whole, these pieces of data indicate a limited knowledge of this risk-group and of details of policy even where this does exist.

A key strength of our study is the national sample and the broad mix of hospital types, including thought leading sites where future infectious disease and infection control physicians train. Other studies in this area have looked either within smaller more homogenous groups or across multiple countries.[357, 358]

A limitation of our study is selection bias. ‘Large’ hospitals were over-represented amongst respondents. Similarly, the methodology biased our sample towards a subset of infection control departments; those that are active on infection control, email bulletins and were motivated to participate.

Our survey design captured practice rather than exact policy content. Thus, the answers reflect one or few individuals’ practice, and may not be representative of the majority at the site. This is demonstrated by the analysis of multiple responders. With the exception of the questions on written policy (for which the question text required a subjective decision), discordant answers occurred in

aspects of policy that may be less commonly used (CRE, IT-Patients and AmpC organisms). However, differing interpretations of policy were very frequent. Exploration of the relationship between rates of discordant answers and actual variation in practice should be an area of future research.

Misclassification complicates interpretation of our results. For CRE and IT-patients, it is difficult to untangle whether findings represent a gap in knowledge of terminology/policy content or a true absence of policy. We believe misclassification is a lesser problem for ESBL-E data as the terminology is common in Australian infection control literature.[86]

An important factor not easily accounted for in our analysis is the exchange of information and centralisation of policy amongst local regions or private sector groups, independent of binding-directives or published recommendations.

Finally, the applicability of our findings may vary by country. The impact of state-based policy will differ depending on the governance structure of the country involved. The frequency, nature and risk of IT-patients will vary by country and travel patterns.

In conclusion, infection control management of MRGNBs varies widely amongst adult acute care hospitals in Australia. We demonstrate a vulnerability of some Australian hospital's infection control practice in the management of CRE harbouring and IT-patients. This is due to a limited knowledge of aspects of these risk-groups amongst some practitioners, an absence of policy and a lack of knowledge of policy. We await the likely positive impact of recently released national recommendations on infection control management of CRE in improving and harmonising practice within Australia.[63]

Acknowledgements
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Conflicts of Interest
BAR is supported by an Australian Post Graduate Award Scholarship

DLP has received honoraria from AstraZeneca, Merck and Pfizer

SMH is supported by an Australian Post Graduate Award Scholarship

TMB - None

FIGURES AND TABLES

Table 1

Infection control management of ESBL-E, CRE and internationally transferred patients (n=97).

	ESBL-E			CRE			International Transfer of patients		
Precautions used	**Use**	**Do not use**	**Unknown**	**Use**	**Do not use**	**Unknown**	**Use**	**Do not use**	**Unknown**
Any CP use	93(96)	4(4)	0	79(81)	18(19)	0	48 (49)	19(20)	30 (31)
Glove and Gown	88(91)	8(9)	0	73(75)	20(21)	4(4)	36 (37)	31(32)	30 (31)
Single Room*	91(94)	5(5)	1(1)	75(77)	18(19)	4(4)	48 (49)	19(20)	30 (31)
Cohort Room	28(29)	69(71)	0	21(22)	76 (78)	0	NA	NA	NA

*For International transfer single room & cohort were not differentiated in the survey.

ESBL-E = Extended spectrum beta-lactamase harbouring Enterobacteriaceae, CRE=Carbapenem resistant Enterobacteriace

Table 2.
Descriptive analysis of predictors of infection control management.

The second column contains data on the proportion of hospitals with the specified characteristic amongst the entire cohort. The three groups of data following this are descriptive analysis of the use of CP as follows: i) A permissive policy for ESBL-E ii) Any use of CP for CRE iii) Any use of CP for IT-patients.

		ESBL-E				CRE				International transfers of patients			
Predictive Characteristics	**Count amongst all hospitals (%) N=97**	**Count with permissive use of CP for ESBL-E (% of total with permissive use) n=44**	**Count with inclusive use of CP for ESBL-E (% of total with inclusive use) n=48**	**Odds Ratio (95%CI)**	**p value**	**Count with any use of CP for CRE (% of total using CP for CRE) N=79**	**Count who do not use CP for CRE (% of total not using CP) N=18**	**Odds Ratio (95%CI)**	**p value**	**Count with any use of CP for International transfers (% of total with using CP) N=48**	**Count who do not use CP for International transfers (% of total not using CP) N=19**	**Odds Ratio (95%CI)**	**p value**
Public (vs. private)	**68 (70)**	37(84)	31(65)	**4.1(1.5-11.1)**	**0.005**	54(68)	25(32)	**0.6(0.2-2.1)**	**0.436**	33(69)	15(29)	**0.6(0.2-2.1)**	**0.411**
Metropolitan location (vs. regional and rural)	**58 (60)**	32 (73)	26 (54)	**2.7 (1.1-6.4)**	**0.028**	51 (72)	7 (39)	**2.9 (1.0-8.3)**	**0.052**	33 (69)	13 (68)	**1.0 (0.3-3.2)**	**0.979**
ID service	**57 (59)**	29(66)	28(58)	**1.5 (0.6-3.5)**	**0.347**	49(62)	8(44)	**2.0(0.7-5.8)**	**0.179**	30(63)	16(84)	**0.3(0.1-1.2)**	**0.097**
Written policy on phenotype/patient group	**ESBL-E =38 (39) CRE = 22 (23) IT-Patients – 50 (52)**	17(39)	21(44)	**0.8(0.4-1.9)**	**0.621**	22(23)	0(0)		**0.011**	36 (75)	8(42)	**3.2(1.4-7.6)**	**0.006**
Written policy on MBL	**21 (22)**	NA	NA	**NA**	**NA**	18 (23)	3(17)	**1.5 (0.4-5.7)**	**0.571**	NA	NA	**NA**	**NA**
MRO directive	**21 (22)**	9(20)	12(25)	**0.8(0.3-2.1)**	**0.606**	19(24)	2(11)	**3.6(0.7-17.1)**	**0.116**	9(19)	7(37)	**0.7(0.2-2.3)**	**0.522**
CRE directive	**17 (18)**	9(20)	8(17)	**1.3(0.4-3.7)**	**0.642**	17(100)	0(0)		**0.030**	16(33)	0(0)		**0.003**
Regular international transfers*	**28 (33)**	16(41)	12(25)	**1.7(0.7-4.4)**	**0.244**	27(39)	1(6)	**10.3(1.3-83.1)**	**0.029**	21(46)	7(41)	**1.2(0.4-3.7)**	**0.753**
Large hospital	**59 (61)**	32(73)	27(56)	**2.25(0.9-5.4)**	**0.069**	49(62)	10(56)	**1.3(0.5-3.7)**	**0.614**	32(68)	15(79)	**0.5(0.2-1.9)**	**0.330**
Principal referral vs.	**43 (44)**	25(57))	18(34)	**2.6(1.1-5.9)**	**0.026**	38(48)	5(28)	**2.4(0.8-7.4)**	**0.126**	25(52)	12(63)	**0.6(0.2-1.9)**	**0.416**

other													
Transplant service **	**20 (21)**	14/25(56)	6/18(33)	**2.3(0.6-8.4)**	**0.196**	19/38(50)	1/5(20)	**4.0(0.4-40.2)**	**0.239**	13(52)	5(42)	**1.5(0.4-6.2)**	**0.562**
Other supra-regional referral services**	**21 (22)**	14/25(56)	7/18(39)	**2.3(0.6-8.4)**	**0.196**	20/38(53)	1/5(20)	**4.4(0.4-44.7)**	**0.205**	15(60)	4(33)	**3.0(0.7-12.9)**	**0.141**

CP= Contact Precautions, ESBL-E = Extended spectrum beta-lactamase harbouring Enterobacteriaceae, CRE= Carbapenem resistant Enterobacteriaceae MBL= Metallo beta-lactamase harbouring bacteria

***** Hospitals considered to have 'Regular international transfer' of patients hospitals reported a frequency of 'less than monthly' or greater. ****** Variables analysed only within principal referral hospitals.

Table 3

Multivariate analysis of the predictors of a permissive practice for ESBL-E, and any use of precautions for CRE and international transfer patients

Predictive Characteristics	Permissive policy for CP use for ESBL-E n=44/92 (48%) with permissive policy n=5 with missing data		Any CP use for CRE n=79/97 (81%) use CP		Any CP use for International transfer patients n=38/67 (72%) n=30 with missing data	
	Multivariate OR (95%CI)	**p value**	**Multivariate OR (95%CI)**	**p value**	**Multivariate OR (95%CI)**	**p value**
Public (vs. private)	**4.8 (1.7-13.4)**	**0.003**				
Metropolitan location (vs. regional and rural)	**3.2 (1.3-8.1)**	**0.014**				
ID service					**0.2 (0.3-0.9)**	**0.041**
Specific written policy*				**0.011**	**3.4 (1.2-9.7)**	**0.025**
MRO directive			2.8 (0.4-14.8)	0.218		
CRE directive				**0.030**		**0.003**
Regular international transfers			4.6 (0.5-40.3)	0.166		
Non-Transplant Supra-Regional Services**					3.4(0.7-15.5)	0.118

ID service = Infectious Disease service, ESBL-E = Extended spectrum beta-lactamase harbouring Enterobacteriaceae, CRE=Carbapenem resistant Enterobacteriaceae, MRO = Multi-resistant organism

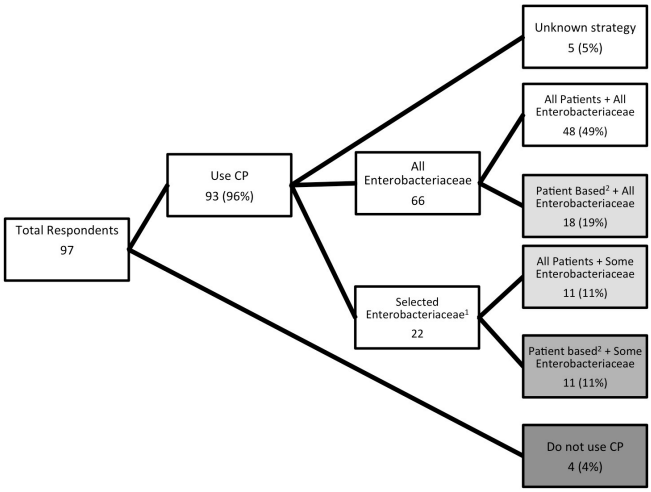
*Written policy on infection control of phenotype or patient group.

**Cystic Fibrosis, Burns, Spinal Injury

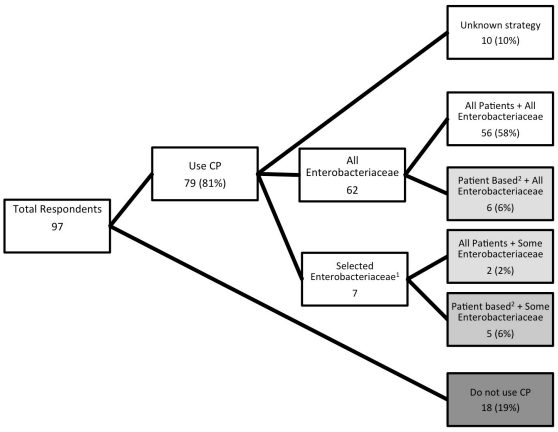
Figure 1

Breakdown of strategies for the use of contact precautions for ESBL-E, CRE and IT-Patients. Boxes shaded in white indicate an inclusive or unknown strategy. Boxes shaded in grey indicate a permissive strategy.

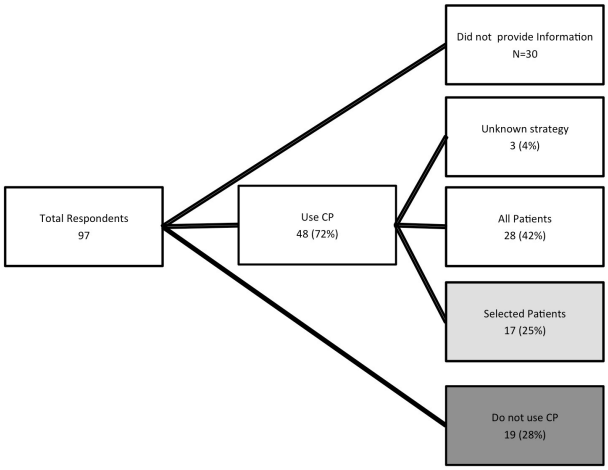
Panel A – ESBL Enterobacteriaceae



Panel B – Carbapenem Resistant Enterobacteriaceae



Panel C – International Transfer of Patients



[1] Organisms considered for CP amongst ESBL-E=*Escherichia coli* + *Klebsiella pneumoniae* (11/22, 50%), *K. pneumoniae* (5/22, 23%), *E. coli* (2/22, 10%) and neither/unsure (5, 23%). Amongst CRE = *E. coli* + *K. pneumoniae* (n=2/7, 29%), *K. pneumoniae* only (n=3/7,43%), neither/unsure (n=2/7, 29%).

[2]Patient-assessment for CP for ESBL-E included; Ward location (15/29, 51%); and clinical features of the patient (e.g. sample of origin for isolate, presence of diarrhoea) (23/29, 79%); Three sites 3/29 (10%) indicated neither of these factors was used in their assessment. For CRE patient-based assessment included the ward location (3/11, 27%) and clinical features (8/11, 73%). Three sites (3/11, 27%) indicated neither of these was used for their assessment.

CHAPTER 7. CONCLUSION

The previous decades have seen a marked expansion of antimicrobial resistant *E. coli,* increasing the burden of antimicrobial resistant infections amongst humans worldwide. Two plasmid-borne beta-lactamase antimicrobial resistance mechanisms, ESBLs and carbapenemase, have been major contributors to this change. The research program presented in this thesis aimed to gain further insight into significant aspects of antimicrobial resistant *E. coli* worldwide.

A review of the published literature defined key phenomenon pertinent to the spread of antimicrobial resistance. The ST131 *E. coli* pandemic clone represents a new convergence of virulence and resistance. As delineated in the review of ST131, this facilitated a rapid geographical spread of the ST131 clone, harbouring a surprising diversity of resistance mechanisms. The ultimate consequence of this spread is an ongoing incursion of ESBL harbouring and/or fluoroquinolone resistant *E. coli* into many clinical infections.[165]

Spread of antimicrobial resistance due to human travel is not new. However, in the previous decade several factors have increased the risk to humans posed by this mechanism of dissemination. Firstly, human travel patterns have changed with a greater number of people undertaking long distance and/or intercontinental travel. This includes more travellers with potential overseas healthcare contact either due to pre-existing medical conditions or from seeking medical treatment abroad. Secondly, we have seen the emergence of several new carbapenemase mechanisms such as bla_{NDM} and $bla_{\text{oxa-48,}}$ initially in geographically defined distributions.[96] The review presented on this topic has acted as a fulcrum for discussion and research in this sphere, with many authors citing this work as background for further scientific study.

With Australia and New Zealand a central focus of the clinical and laboratory research, new insights into ESC-R-EC, both regionally and worldwide have been gained. The unique geography, demography and antimicrobial use patterns in our region have led to a distinct epidemiology of 3GCR-EC. From the case-control study we demonstrate a persisting mix of 'old' and 'new' epidemiology. Whilst traditional risks, such as healthcare contact and antimicrobial use, remain highly significant, we see risks defining this new epidemiology including overseas travel, and transmission in ethnic groups that originate from regions of high community transmission. Within this analysis, the modelling of healthcare associated 3GCR-EC shows, for the first time, the persistence of risk after healthcare contact, and the implications this has within and outside healthcare institutions.[329]

Work on the dynamics and carriage of resistant *E. coli* in returned travellers gives further insight into the carriage and spread within the community after return from high-incidence countries. Delineation of prolonged clonal carriage after travel indicates a potential source of transmission within communities that include frequent travellers to high incidence areas. The prolonged carriage and potential introduction of fluoroquinolone and aminoglycoside resistance have significant implications in communities where this resistance is otherwise infrequent. At the same time, we offer some optimism demonstrating a relatively rapid clearance of GIT carriage of ESBL-EC from selected carriers.[318]

Within returned travellers and the case-control study group, laboratory research included a significant focus on ST131 *E. coli,* given its global importance. This offered several novel insights into our region. The first was the surprisingly low overall prevalence of the clone and the implication of a relationship this may have with low overall rates of fluoroquinolone use.

The second was the globally comparable rates of ST131 and the H30 sub-clone within ESC-R-EC and the relative homogeneity amongst ST131 and other ESBL harbouring *E. coli* in our region.[367] This confirms the rise of ST131 as the preeminent ESBL *E. coli* in almost all corners of the globe.

Finally, the analyses of the infection control management of patients harbouring ESBL-E, CRE and those with overseas healthcare contact, complemented the clinical and laboratory research within this thesis. It identified key challenges for the infection control community in Australia and abroad. Locally, there is an apparent lack of understanding of new mechanisms of resistance, such as CRE, and a potential underestimation of the risk of introduction and spread into health services.[368] Secondly, whilst we appropriately defined many permutations of infection control policy for these pathogens, many points of difference between policies lack rigorous scientific research to support the approach taken.

In summary, key findings of the research program within this thesis include

- Demonstration that healthcare contact remainings the pre-eminent risk for ESC-R-EC in Australia and the emergence of new risk factors related to travel and country of birth.
- Identification of a unique molecular epidemiology of antimicrobial susceptible and resistant *E. coli* in the Australian region.
- Demonstration of a relatively short average duration of carriage of ESC-R-EC in returned travellers harbouring these isolates in their gastrointestinal flora.

- Demonstrating wide spread variation in infection control management of patients with ESC-R-EC and CRE within Australia.

FUTURE RESEARCH

Epidemiology

This thesis has focused on aspects of antimicrobial resistant *E. coli* in a cross-sectional manner. Equally important in the thesis is the delineation of the temporal and geographical trends of resistance. Current information gleaned by comparing widely spaced snapshot samples has obvious limitations. Expansion of epidemiological investigation beyond humans is also important. This includes in-depth investigation of *E. coli* resistance in animals, including ESC and other antimicrobial classes, food and companion animals. The dynamics of bacterial sharing between humans and animals via food or other contact is at present poorly understood.

From a regional perspective, whilst animal antimicrobial use patterns may differ from many other areas, there has been no recent comprehensive investigation of this topic to confirm ongoing low rates of resistance, as have been presumed in Australia and New Zealand. Finally, the case-control study indicates undefined risks for transmission within our community that require further elucidation.

Modification of risk factors and active decolonisation

A number of the new risk factors for ESC-R-EC infection such as overseas travel and membership of certain ethnic groups are complex in nature. Within these exposures some components (e.g. water or food consumption, hygiene practices) likely represent the major contributors to the risk of ESC-R-EC colonisation and infection. These components could be identified and trialled as modifiable risk factors. Furthermore, the potential role of decolonisation, either through pharmacological or non-pharmacological measures, could be investigated for high-risk patients who have become colonised.

Infection control

Although we now have a basic understanding of the transmissibility of ESBL-E in some clinical environments, much empiric research is still required. A greater diversity of clinical settings must be studied to more broadly understand ESBL-E transmission in healthcare settings and the community. Likewise, differences in the transmissibility of carbapenem resistant *E. coli*, compared with ESBL-E must also be investigated. Rigorous studies to support strategies such as risk stratification of patients are also required.

Genetics and genomics

In the years since publication of this review, there has been a considerable increase in scientific effort to understand the genetic basis and clinical implications of the ST131 clone. A number of

these studies are now coming to fruition, providing insight into the clone's pathogenicity and propensity to acquire resistance. However, further research is still required to understand the genetic mechanisms supporting the rapid global spread of this clone, and its apparent selective advantage over other *E. coli.*

One area of bacterial genetics poorly investigated is that of colonising bacteria. As our traveller analysis demonstrated, there was considerable variability in the persistence of given clones. Investigation of the genetic mechanisms that predispose bacteria to colonisation and genetic and metabolic changes that occur in bacteria with prolonged colonisation, will give considerable insight into this phenomenon and may identify future therapeutic targets.

Thus far, there is also little data available to understand the human genetic contribution to antimicrobial resistant *E. coli* infection. Potentially, some of the difference in incidence of resistance across communities may originate from the genetic background of the colonised or infected people.

Therapy

Whist therapy of ESC-R-EC was not covered in this thesis, further study in this area remains crucial. Few controlled studies have been undertaken to define optimal antimicrobial therapy for severe ESC-R-EC infection. Likewise there are no rigorously investigated oral therapy options defined for non-severe ESC-R-EC infection. Given our emerging insight into the genetics of clones such as ST131, non-antimicrobial therapies also warrant further investigation. Mechanisms governing bacterial pathogenicity such as cell adhesion and migration may be important future therapeutic targets.

Economic implications

It is important to establish the true economic impact of antimicrobial resistant *E. coli* in the community and hospital. When available, data of this type acts as a strong imperative for funding further research in the area. In addition it allows for detailed cost-effectiveness assessment of any proposed therapies or other control measures.

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APPENDIX A - SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIAL FOR CHAPTER 3

***Escherichia coli* infection resistant to expanded-spectrum cephalosporins in low-prevalence countries**

Antimicrobial Agents and Chemotherapy, Volume 58, 2014

Benjamin A Rogers, Paul R Ingram, Naomi Runnegar, Matthew C Pitman, Joshua T Freeman, Eugene Athan, Sally N Havers, Hanna E Sidjabat, Mark Jones, Earleen Gunning, Mary De Almeida, Kaylene Styles, David L Paterson, on behalf of the Australasian Society for Infectious Diseases Clinical Research Network

A. Additional Definitions

Chronic renal failure was a baseline creatinine clearance <50ml/min/m^2; **Chronic heart failure**[369], **chronic lung disease**[370] were based on published functional definitions; **Chronic liver disease** included a history of cirrhosis or hepatic decompensation; **Active malignancy** was any malignancy, except isolated skin malignancy;; **Indigenous** patients were members of the Australian Aboriginal, Maori or Torres Strait islander community. **Meat consumption** (red meat, poultry, pork and processed meat) was any consumption in the month preceding infection.

B. Healthcare exposure analysis using the Friedman classification

Details of healthcare exposure using the Friedman classification with and without the inclusion of day-procedures as healthcare exposure.

Time since exposure[1]	**Frequency in ESC-R Cases (%) n=91**	**Frequency in ESC-S Controls (%) n=91**	**Odds Ratio**	**(95% CI)**	**p value**
Including day surgical and medical procedures[2]					
No healthcare exposure	26 (29)	42 (46)	Reference		
Healthcare <1 month ago	16 (18)	7 (8)	3.692	(1.34-10.18)	0.012
2-6 months ago	40(44)	30 (33)	2.154	(1.09-4.25)	0.027
7-12 months ago	9 (10)	12 (13)	1.212	(0.45-3.27)	0.705
Excluding day surgical and medical procedures[3]					
No healthcare exposure	55 (60)	34 (37)	Reference		
Healthcare <1 month ago	11 (12)	5 (5)	3.559	(1.14-11.14)	0.029
2-6 months ago	37(41)	20 (22)	2.993	(1.50-5.98)	0.002
7-12 months ago	9 (10)	11 (12)	1.323	(0.50-3.52)	0.575

[1]Durations are calculated from the date-of-discharge to date of first presentation of the current infection.

[2]With day-procedures: AUC = 0.62, Sensitivity = 62% Specificity = 59%.

[3]Without day-procedures: AUC = 0.64, Sensitivity=53% Specificity = 73%.

C. Comparison of the final multivariate model and models using alternative definitions.

Parameters changed in the alternative models are underlined. Where the significance (of the odds ratios) has moved across the pre-defined (p=0.05) threshold when compared with the final model, this is shaded in grey.

Final Model	**Final Model** **Odds Ratio (95% CI) p value**	**Alternative 1 Healthcare Including day procedures**	**Alternative 1** **Odds Ratio (95% CI) p value**	**Alternative 2 Any healthcare exposure in the previous year**	**Alternative 2** **Odds Ratio (95% CI) p value**	**Alternative 3** **Birth in high-risk regions**	**Alternative 3** **Odds Ratio (95% CI) p value**	**Alternative 4** **Travel to Indian subcontinent**	**Alternative 4** **Odds Ratio (95% CI) p value**	**Alternative 5 Exposure to TMP/SMX without combining ESC**	**Alternative 5** **Odds Ratio (95% CI) p value**
Healthcare exposure in the previous 6 months (excluding day prodecdures	3.16 (1.54-6.46) 0.002	Healthcare exposure in the previous 6 months	2.22 (1.11-4.43) 0.023	Any healthcare exposure in the previous year	1.90 (0.90-3.99) 0.092	As per final model	2.68 (1.33-5.41) 0.006	As per final model	2.58 (1.30-5.10) 0.006	As per final model	3.24 (1.59-6.60) 0.001
UTIs in previous year (per UTI)	1.43 (1.16-1.82) 0.003	As per final model	1.44 (1.14-1.83) 0.002	As per final model	1.44 (1.14-1.83) 0.002	As per final model	1.38 (1.09-1.75) 0.008	As per final model	1.39 (1.10-1.75) 0.005	As per final model	1.43 (1.13-1.81) 0.003
Birth on the Indian subcontinent	11.13 (2.17-56.96) 0.004	As per final model	10.63 (2.08-54.26) 0.004	As per final model	9.67 (1.91-48.95) 0.006	Birth in high-risk regions	2.05 (0.6-5.52) 0.0157	As per final model	8.48 (1.43-50.41) 0.019	As per final model	10.60 (2.08-54.09) 0.005
Travel to high-risk regions	3.09 (1.29-7.38) 0.011	As per final model	2.60 (1.10-6.13) 0.029	As per final model	2.65 (1.12-6.28) 0.027	As per final model	2.76 (1.13-6.74) 0.026	Travel to the Indian subcontinent	2.99 (0.23-39.38) 0.23	As per final model	2.88 (1.21-6.85) 0.016)
Trimethoprim +/- Sulfamethoxazole &/or ESC	3.67 (1.30-10.35) 0.014	As per final model	3.75 (1.33-10.54) 0.012	As per final model	3.57 (1.27-10.08) 0.016	As per final model	3.48 (1.24-9.79) 0.018	As per final model	3.10 (1.12-8.60) 0.003	Trimethoprim+/- Sulfamethoxazole	2.66 (0.91-7.75) 0.073
Male sex	2.17 (0.97-4.84) 0.060	As per final model	2.20 (0.99-4.87) 0.052	As per final model	2.34 (1.07-5.13) 0.034	As per final model	2.30 (1.05-5.06) 0.037	As per final model	2.52 (1.14-5.54) 0.022	As per final model	2.31 (1.04-5.12) 0.039
Characteristics: ROC AUC GOF=Goodness of fit	0.772 GOF=0.289		0.766 GOF = 0.36		0.762 GOF = 0.15		0.752 GOF=0.12		0.7614 GOF=0.35		0.763 GOF=0.28

D. Comparison of multivariate model using alternative patient groups

The group used in the alternative model is contained in the first row. Where the sign odds ratios) has moved across the pre-defined (p=0.05) threshold when compared model, this is shaded in grey.

Final Model	**Final Model** **Odds Ratio (95% CI) p value**	**Alternative 1 Exclusion of asymptomatic patients Case n=73 Control n=68** **Odds Ratio (95% CI) p value**	**Alternative3 Inclusion of only CTX-M *E. coli* cases Case n=74 Control n=91** **Odds Ratio (95% CI) p value**	**Alternative3 Inclusion of only ST131 *E. coli* cases Case n=40 Control n=91** **Odds Ratio (95% CI) p value**
Healthcare exposure in the previous 6 months (excluding day prodecdures	3.16 (1.54-6.46) 0.002	3.66 (1.61-8.32) 0.002	6.62 (2.81-15.61) <0.001	7.67 (2.66-4.15) <0.001
UTIs in previous year (per UTI)	1.43 (1.16-1.82) 0.003	1.47 (1.10-1.95) 0.008	1.30 (0.99-1.72) 0.062	1.39 (1.01-1.92) 0.044
Birth on the Indian subcontinent	11.13 (2.17-56.96) 0.004	9.19 (2.02-57.42) 0.004	18.1 (3.21-102.04) 0.001	34.11 (5.25-221.49) <0.001
Travel to high-risk regions	3.09 (1.29-7.38) 0.011	2.60 (1.01-6.65) 0.047	5.72 (2.10-15.54) 0.001	4.10 (1.10-15.29) 0.036
Trimethoprim +/- Sulfamethoxazole &/or ESC	3.67 (1.30-10.35) 0.014	3.02 (0.94-9.73) 0.064	3.91 (1.29-12.21) 0.016	3.52 (0.91-13.70) 0.069
Male sex	2.17 (0.97-4.84) 0.060	2.56 (1.06-6.16) 0.036	2.09 (0.88-4.98) 0.095	2.17 (0.77-6.15) 0.143
Characteristics: ROC AUC GOF=Goodness of fit	0.772 GOF=0.289	0.780 GOF = 0.247	0.818 GOF = 0.138	0.830 GOF = 0.018

E. Differences in risks between healthcare associated (HA) and non-healthcare associated (non-HA) ESC-R-EC groups after stratification by healthcare contact in the previous six months excluding day-procedures.

	Healthcare associated cohort (n=73)			Non-healthcare associated cohort (n=109)		
Potential Risk	**Count of cases with characteristic n=48 (%)**	**Count of controls with characteristic n=25 (%)**	**p value for comparison case vs. controls**	**Count of cases with characteristic n=43 (%)**	**Count of controls with characteristic n=66 (%)**	**p value for comparison case vs. controls**
Birth on the Indian subcontinent	2 (4)	0 (0)	0.543	9 (21)	2 (3)	0.006
Travel to high-risk regions	4 (8)	3 (12)	0.685	20 (47)	11 (17)	0.001
Trimethoprim +/- Sulfamethoxazole &/or ESC use	15 (31)	2 (8)	0.039	5 (12)	4(6)	0.313
Male sex	16 (33)	7 (28)	0.642	14 (33)	9(14)	0.018
UTIs in previous year (Count, 25-75%)	1, 0-3	1, 0-1	0.347	0, 0-3	0, 0-1	0.146

F. Analysis of correlates of ESC resistance enzyme class

Comparing characteristics of patients harbouring CTX-M group enzymes to those harbouring 'Non-CTX-M' group enzymes.

Variable	Frequency in CTX-M group (%) n=74	Frequency in non-CTX-M group (%) n=15	p value CTX-M vs. Non-CTX-M
Variables included in the multivariate model			
Male Sex	26 (35)	4(27)	0.527
Charlson score ≥1	38 (51)	6(40)	0.423
Immune suppression	17 (23)	1(7)	0.288
Anatomical or structural abnormality	18(24)	4(26)	1.0
UTIs in past 12 months (Median & (25-75th centile))	0 (0-2)	2(0-4)	0.054
SXT use	14 (19)	2(13)	1.0
ESC use	5 (7)	2(13)	0.336
High risk travel	23(31)	1(7)	0.061
Birth on Indian Subcontinent	11 (15)	0(0)	0.199
Healthcare Exposure (6 months)	45(61)	3(20)	0.005
Other variables			
Age (Median & (25-75th centile))	60 (41-71)	70(58-78)	0.061
Any overseas travel	27 (36)	1(7)	0.031
Any antimicrobial use	54 (73)	14(93)	0.108
Renal transplant	8 (11)	0 (0)	0.342
Fluoroquinolone use	6(8)	1(7)	1.0
ß-lactam + ß-lactamase inhibitor use	9 (12)	5(33)	0.055
Carbapenem use	3(4)	0(0)	1.0
Aminoglycosides use	5(7)	0(0)	0.584
Macrolide use	4(5)	1(7)	1.0
Narrow spectrum cephalosporin use	13(18)	2(13)	1.0
Narrow spectrum penicillins use	8(9)	0(0)	0.342

SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Sequence Type 131 *fim*H30 and *fim*H41 Subclones Amongst *Escherichia coli* Isolates in Australia and New Zealand

Under Review, Antimicrobial Agents and Chemotherapy, 2014

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Supplementary 1.
Comparison of phenotypic resistance of ST131 and non-ST131 isolates amongst different cohorts.

	Whole 3GC resistant cohort Count of resistant isolates (%)			**CTX-M harbouring** Count of resistant isolates (%)		**Whole 3GC susceptible cohort** Count of resistant isolates (%)		
	ST131 n=40	**Non-ST131 n=49**	**p value**	**Non-ST131 N=34**	**p value***	**ST131 N=6**	**Non-ST131 N=84**	**p value**
Individual agents								
Ampicillin	40(100)	49(100)	**1.0**	34(100)	**1.0**	4(67)	46(55)	**0.689**
Amox + clav	23(57)	34(69)	**0.245**	17(56)	**0.889**	4(66)	2(33)	**0.261**
Ceftriaxone	40(100)	46 (94)	**0.111**	34(100)	**1.0**	NA	NA	**NA**
Ceftazidime	26(65)	35(71)	**0.516**	20(59)	**0.585**	NA	NA	**NA**
Cefepime	23(58)	26(53)	**0.675**	23(68)	**0.370**	0	0	**1.0**
Amikacin	2(5)	2(4)	**1.0**	2(6)	**1.0**	0	0	**1.0**
Gentamicin	14(35)	25(51)	**0.130**	24(71)	**0.005**	2(33)	1 (1)	**0.011**
Ciprofloxacin	35(87)	22(45)	**<0.001**	21(62)	**0.010**	2(33)	4(5)	**0.050**
SXT	34(85)	30(61)	**0.013**	26(76)	**0.351**	2(33)	18(21)	**0.611**
Nitrofurantoin	3(8)	5(10)	**0.726**	4(12)	**0.696**	0	3(4)	**1.0**
Meropenem	0	0	**NA**	0	**NA**	0	0	**NA**

Combinations								
Total resistance score								
Median (25-75%)	6(4-7)	5 4-6)	**0.116**	5(5-8)	**0.899**	2(1-3)	1(0-2)	**0.077**
Ciprofloxacin + SXT resistance	30(75)	19(39)	**<0.001**	19(73)	**0.083**	0	3(4)	**1.0**

Amoxicillin + clav = Amoxicillin + clavulanic acid

SXT= Trimethoprim + sulfamethoxazole

***** Comparator group is the ESC-R ST131 isolates (first column of this table).

Supplementary 2

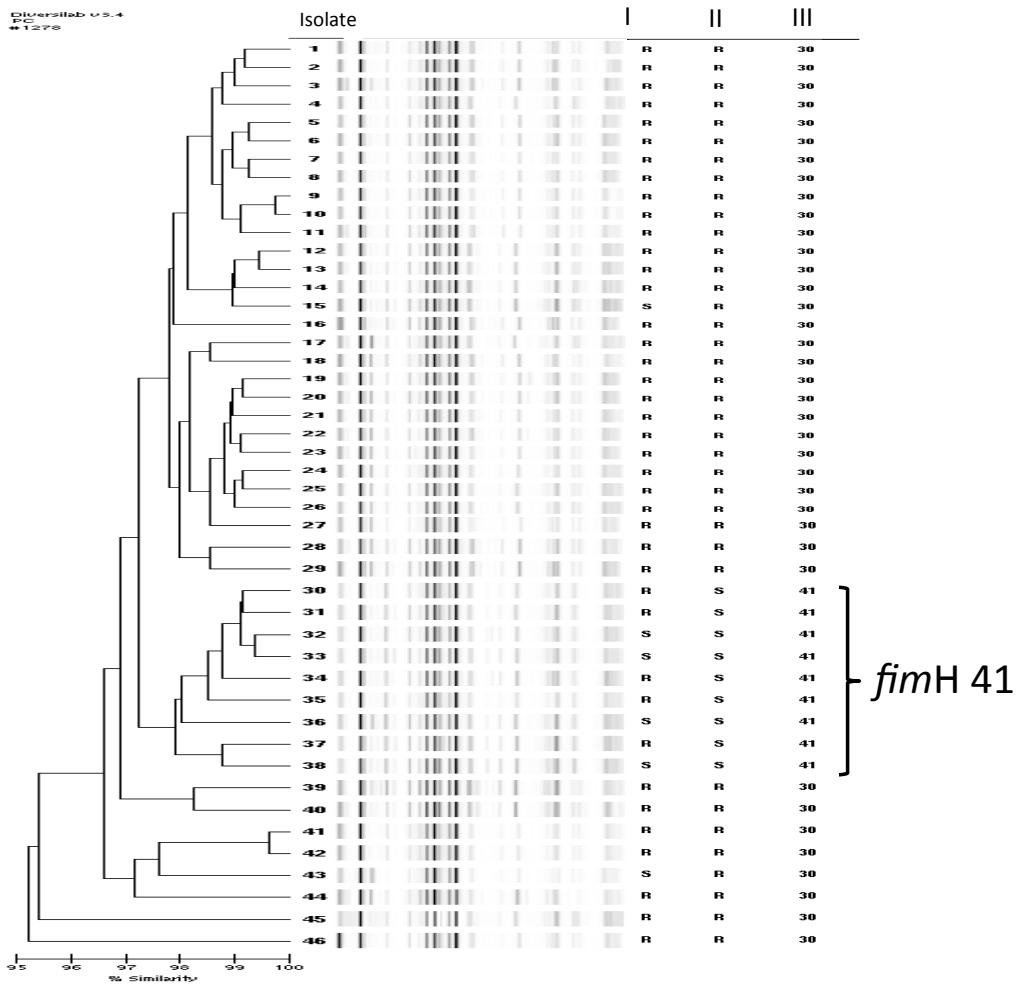
Estimated population burden of MLST defined clones including total *E. coli*, fluoroquinolone resistant *E. coli* and expanded-spectrum cephalosporin resistant *E. coli*.

Clone	Count amongst ESC-S-EC (n=90)	Count amongst ESC-R-EC (n=89)	Count of FQ resistant amongst ESC-S-EC	Count of FQ resistant amongst ESC-R-EC	Adjusted population prevalence [1]	Lower estimate [1]	Upper estimate [1]	Proportional contribution to fluoroquinolone resistance	Proportional contribution to 3GC resistance
All ESC-R isolates	0	89	0	56	0.034	0.026	0.043	0.249	1
All ST131	6	40	2	35	0.08	0.076	0.083	0.406	0.449
ST131 - H30	2	35	2	35	0.0348	0.032	0.038	0.406	0.393
ST131- H41	4	5	0	0	0.045	0.045	0.045	0	0.056
ESC-S ST131	6	0	4	0	0.064	0.064	0.065	0.025	0
ST95	13	2	1	1	0.14	0.141	0.139	0.130	0.022
ST73	12	1	0	0	0.129	0.130	0.128	0	0.011
ST14c	8	2	2	1	0.087	0.087	0.086	0.255	0.022
ST80	5	0	0	0	0.054	0.054	0.053	0.0	0
ST127	5	1	0	0	0.054	0.054	0.054	0.0	0.011
ST69	2	5	0	0	0.023	0.023	0.024	0.0	0.056
ST648	1	5	0	2	0.013	0.012	0.013	0.008	0.056
Sporadic	38	33	1	18	0.42	0.421	0.420	0.207	0.371

[1]Prevalence estimates are based on a 3.4% ESC-R *E. coli* rate amongst the population. Lower estimates are based on 2.6% ESC-R and upper on a 4.3% ESC-R *E. coli* rate. ESC-S-EC = expanded-spectrum cephalosporin susceptible *E. coli.* ESC-R-EC = expanded-spectrum cephalosporin resistant *E. coli*. FQ = fluoroquinolone, ST14C= ST14 complex, Sporadic = isolates not typed to an MLST cluster.

Supplementary 3

Dendrogram constructed using a Pearson correlation coefficient, based on DiversiLab rep-PCR pattern of ST131 *E. coli*. Details of figure from left to right: Dendrogram, isolate number, virtual gel. Numbered columns from left to right: I = expanded-spectrum cephalosporin susceptibility, II=fluoroquinolone susceptibility, III=*fim*H type.



SUPPLEMENTARY MATERIAL FOR CHAPTER 6

Predictors of use of infection control precautions for multi-resistant gram-negative bacilli in Australian hospitals: Analysis of a national survey

American Journal of Infection Control, In-Press, May 2014

Rogers BA, MBBS, FRACP[1], Havers SM, BNurs MPH[1], Brown TM, BNurs MPH[1], Paterson DL, MBBS, FRACP, FRCPA, PhD[1]

Supplementary Material

1. Synopsis of infection control guidelines and directives
2. External data used to confirm hospital demographics
3. Numerical comparison of survey respondents to national demographics
4. Analysis of multiple responders

1. Comparison of National Infection Control Guidelines and relevant state-based directives.

Issuing Body	**Australian National Health and Medical Research Council**	**Ministry of Health, New South Wales**	**Government of Western Australia, Department of Health**
Title	Guidelines for the Prevention and Control of Infection in Healthcare	Infection Control Policy: Prevention & Management of Multi-Resistant Organisms (MRO)	Infection Prevention and Control of Carbapenem-resistant Enterobacteriaeceae (CRE) in Western Australian Healthcare Facilities
Issued	2010	2007	2012 (Released October, circulated in draft since June)
Applicability	Non-binding recommendations	Binding for all public hospitals. Recommended for private hospitals	Binding for all facilities in the state.
MRGNBs specified in text.	Enterobacteriaceae, Pseudomonas and Acinetobacter mentioned. ESBL producing organisms mentioned	Multi-resistant Acinetobacter, Pseudomonas and coliforms. ESBL producing organisms mentioned	CRE Examples of clinically important Enterobacteriaceae given as: Escherichia, Klebsiella, Enterobacter, Citrobacter, Proteus and Morganella
Definition of Resistance	Gram-negative bacteria with multiple classes of drug resistance or resistant mechanisms to critically important antibiotics	A bacterium that is resistant to two or more commonly used antibiotics from different classes (to which it would not be expected to be susceptible)	Enterobacteriaceae that are non-susceptible to carbapenem antibiotics
Other pathogens included	Many pathogens.	MRSA, VRE	None.
General recommendation (in addition to standard precautions)	Contact precautions for all patients with MRO	Contact precautions for direct contact with patients infected or colonised with MRO (+ other precautions as required by potential routes of transmission e.g. droplet precautions)	Contact precautions for all CRE positive patients
Qualification of recommendation	Consider local circumstances and patient treatment plans in application of these recommendations in local guidelines	None provided	Risk based application may be used for non-carbapenemase producing CRE
Room placement	Single room (or cohort with patients with same 'strain')	Single room with en suite if this is available. Cohort only after consultation with experts.	Single, non-carpeted room with en suite. Cohort only after consultation with experts
Gloves and Gown	Before entering the patient care area	If direct contact with patient or environment.	If direct contact with patient or environment.

Epidemiological Surveillance	Recommended for all facilities in some form. Also recommends increased surveillance for MROs with high incidence or prevalence.	Active and passive epidemiological surveillance recommended for all facilities	Laboratory based reporting and referral to reference lab of all CRE isolates. Microbiological screening of patient contacts.
Reporting	Not applicable	Laboratories must have protocol to detect and notify MROs to relevant clinical team. No wider notification.	Notification of all carbapenemase producing Enterobacteriaceae to state based communicable diseases body.
Patient risk assessment and microbiological screening	Optional, based on local epidemiology. Targeted (patient and/or ward) microbiological screening for MROs with high incidence or prevalence	Risk-assessment of whole service/ward location (e.g. ICU, Maternity ward) rather than patients. Microbiological screening of specific patient groups optional based on healthcare facility policy.	All patients entering facilities must be clinically risk-assessed. Microbiological screening of patient in specific circumstances.
Inter-facility transfer & Overseas healthcare contact	Only covers management of patients with known MRO colonisation.	No mention of international origin of patients. Routine application of CP not recommended. Patients at 'high risk of MRO' should be screened and can be managed in CP whilst awaiting results.	Single room, CP and microbiological screening for any person with hospitalisation of residence in a LTCF overseas in the past 12 months.
Other Details in policy	Very extensive on many aspects of infection control	Hand hygiene, antimicrobial stewardship, environmental cleaning. Extensive detail on aspects of MRSA	Antimicrobial stewardship, outbreak management. management of CRE in LTCFs, Environmental cleaning.
Other related directives.	Not applicable	NSW Ministry of Health, Infection Control Policy, (2007)	WA Department of Health, MRSA (2005) and VRE (2011, 2012) Directives
URL.	http://www.nhmrc.gov.au/guidelines/publications/cd33	http://www0.health.nsw.gov.au/policies/pd/2007/PD2007_084.html	http://www.health.wa.gov.au/circularsnew/circular.cfm?Circ_ID=12908

2. External data used to confirm hospital demographics

Detail	Organisation	URL
Basic Hospital Demographics	National Health Performance Agency	http://www.myhospitals.gov.au
Hospital Size Classification	Australian Institute of Health and Welfare	http://www.aihw.gov.au/hospitals-data/
Allogeneic bone-marrow transplant service	Australian Bone Marrow Donor Registry	http://www.abmdr.org.au/dynamic_menus.php?id=2&menuid=9&mainid=2
Burns Service	Australian & New Zealand Burns Association	http://anzba.org.au/resources/burn-units/
Spinal Service	Royal Australian College of Physicians Spinal Cord Injury Special Interest Group	http://www.racp.edu.au/index.cfm?objectid=66166EBC-BDF4-C80F-208C08FF6FE1485C
Solid Organ Transplants (excluding kidney)	The Transplantation Society of Australia and New Zealand	http://www.tsanz.com.au/downloads/16thMayTSANZConsensusStatementVs1.2_000.pdf

3. Distribution of survey respondents compared to various national demographics

State	Population (million) (% of total Australian population*)	Total number of hospitals responding (% of all respondents)	Private hospitals responses Count (% of total responses within private hospitals)	Public hospital responses Count (% total responses within public hospitals)	ID training responses on total count/ all accredited sites (% of accredited sites in state)	Proportion of responding sites in principal referral category	Proportion of responding sites in metropolitan location
New South Wales	7.4 (32)	21 (22)	5 (17)	16 (24)	10/12 (83)	13(62)	17 (81)
Victoria	5.7 (25)	24 (25)	4 (17)	20 (29)	7/10 (70)	9 (38)	9 (38)
Queensland	4.6 (20)	21 (22)	11 (38)	10 (15)	4/6 (66)	9 (43)	12 (57)
Western Australia	2.5 (11)	17 (18)	5 (17)	12 (18)	3/3 (100)	5 (29)	11 (65)
South Australia	1.7 (7)	5 (5)	0	5 (7)	3/4 (75)	3 (60)	3 (60)
Tasmania	0.5 (2)	4 (4)	3 (10)	1 (1)	1/1 (100)	1(25)	3 (75)
ACT	0.4 (2)	2 (2)	1 (3)	1 (1)	1/1 (100)	1(50)	2 (100)
NT	0.2 (1)	3 (3)	0	3 (4)	2/2 (100)	2(66)	1 (33)
Total	23 million	97	29	68	31/41 (76)	43	58

* Data from: Australian Demographic Statistics, Dec 2012. Canberra: Australian Bureau of Statistics; 2013.

4. Analysis of concordance amongst answers from multiple responders from the same hospital site.

Answer/Policy aspect	**Concordant**	**Relative Agreement**	**Discordant**	**Details of Relative Agreement**
Hospital Services & Policies				
Presence of supra-regional referral services*	8 (89)	NA	1 (11)	
Presence of Infectious Disease service	8 (89)	NA	1 (11)	
Presence of written policies (ESBL, CRE, AmpC, IT-Patient)	2 (22)	NA	7 (78)	
CP Use				
ESBL CP use	3 (33)	6(67)	0	Five sites – differing risk stratification criteria Two sites- differing cohorting
CRE CP use	2 (22)	6 (67)	1(11)	Two sites - differing risk stratification criteria Three site - differing cohorting
IT-patient CP use	3 (33)	3 (33)	3 (33)	Differing risk-stratification criteria
AmpC CP use	6(67)	2(22)	1(11)	Two sites differing reporting of policy details
Analysis of concordance by hospital				
Hospital (number of respondents)	**Concordant**	**Relative Agreement**	**Discordant**	
Hospital 1 (2)	2	2	3	
Hospital 2 (2)	3	3	1	
Hospital 3 (2)	5	2	0	
Hospital 4 (3)	2	2	3	
Hospital 5 (3)	2	3	2	
Hospital 6 (2)	6	1	0	
Hospital 7 (2)	5	1	1	
Hospital 8 (2)	5	1	1	
Hospital 9 (2)	2	2	3	
Total (%)	**32 (51)**	**17 (27)**	**14 (22)**	

CP= Contact Precautions, ESBL-E = Extended spectrum beta-lactamase harbouring Enterobacteriaceae, CRE= Carbapenem resistant Enterobacteriaceae, IT-patient = Patient who has undergone international hospital-to-hospital transfer.

*Only supra-regional referral services that could not be externally validated are included

APPENDIX B – PUBLICATIONS INCLUDED IN THESIS

1. **Rogers BA**, Aminzadeh Z, Hayashi Y, Paterson DL. Country-to-country transfer of patients and the risk of multi-resistant bacterial infection. Clin Infect Dis. 2011 Jul 1;53(1):49-56.

2. **Rogers BA**, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011 Jan;66(1):1-14.

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4. **Rogers BA**, Ingram PR, Runnegar N, Pitman MC, Freeman JT, Athan E, Havers S, Sidjabat H, Gunning E, De Almeida M, Styles K, Paterson DL. Sequence Type 131 fimH30 and fimH41 Sub-clones Amongst Escherichia coli Isolates in Australia and New Zealand, In J Antimicrob Agents, 2015, 45; 351-8, doi: 10.1016/j.ijantimicag.2014.11.015

5. **Rogers BA**, Kennedy KJ, Sidjabat HE, Jones M, Collignon P, Paterson DL. Prolonged carriage of resistant *E. coli* by returned travellers: clonality, risk factors and bacterial characteristics. Eur J Clin Microbiol Infect Dis. 2012 Sep;31(9):2413-20

6. **Rogers BA**, Havers SM, Brown TM, Paterson DL, Predictors of use of infection control precautions for multi-resistant gram-negative bacilli in Australian hospitals: Analysis of a national survey, Am Jour Infect Cont, 2014, 42, 963-9. doi: 10.1016/j.ajic.2014.05.035

REVIEW ARTICLE

Country-to-Country Transfer of Patients and the Risk of Multi-Resistant Bacterial Infection

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Management of patients with a history of healthcare contact in multiple countries is now a reality for many clinicians. Leisure tourism, the burgeoning industry of medical tourism, military conflict, natural disasters, and changing patterns of human migration may all contribute to this emerging epidemiological trend. Such individuals may be both vectors and victims of healthcare-associated infection with multiresistant bacteria. Current literature describes intercountry transfer of multiresistant *Acinetobacter* spp and *Klebsiella pneumoniae* (including *Klebsiella pneumoniae* carbapenemase– and New Delhi metallo-β-lactamase–producing strains), methicillin-resistant *Staphylococcus aureus,* vancomycin-resistant enterococci, and hypervirulent *Clostridium difficile*. Introduction of such organisms to new locations has led to their dissemination within hospitals. Healthcare institutions should have sound infection prevention strategies to mitigate the risk of dissemination of multiresistant organisms from patients who have been admitted to hospitals in other countries. Clinicians may also need to individualize empiric prescribing patterns to reflect the risk of multiresistant organisms in these patients.

Although the world may not be truly getting smaller, the increasing use of air transport could give this impression. The exponential growth of international air travel means almost 1 billion passengers are projected to take an international flight during 2011 [1]. An intercontinental journey now takes a matter of hours, rather than the weeks or months of old. Any medical practitioner could be faced with a person who may have been in the hospital in any part of the world in preceding days. Confounding this, practitioners are increasingly managing patients who have traveled vast distances primarily for the purpose of seeking medical or surgical treatment for an illness. This emerging and diverse category of patient has previously been described in various relevant contexts [2–5]. Collectively we term members of this group the "intercountry" patient. It includes the military and civilian aeromedical evacuee, the "medical tourist" who travels specifically to seek medical treatment internationally, and a larger, less well-defined group of informal medical tourists: those whose medical care is divided between countries for a variety of social, familial, or financial reasons.

Diseases such as malaria and arbovirus infection are classically described in returned travelers. The acquisition of blood-borne viruses such as the human immunodeficiency virus, hepatitis B, and hepatitis C has been associated with medical care in some developing healthcare systems [6]. The intercountry patient is also at high risk of the more prosaic infection, however. Healthcare-associated multiresistant bacterial infection is greatly heterogeneous, and not necessarily divided along lines of economic development and industrialization. Even ubiquitous nosocomial pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae* will harbor vastly differing antimicrobial resistance patterns depending on the location of acquisition [7, 8]. Unsuspected resistance has implications on many levels. At an individual level, inadequate empirical

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Table 1. Factors Predisposing to Increased Risk of Infection and Carriage of Multiresistant Bacterial Organisms in Aeromedical Evacuees and Medical Tourists

Facilities
● Hospital accreditation varies vastly between nations, providing variable levels of oversight for institutional infection control and antimicrobial use.
● Medical tourists may undergo procedures in unlicensed settings occasionally using unproven and experimental techniques.
● Evacuees may transit through multiple health facilities in a short space of time during the process of evacuation. For example, contemporary US military evacuees averaged 4 facilities in 7 days [16].
● Confined spaces and limited facilities of transport vehicles used for evacuation may make some regular infection control practices impossible.
● Barriers including language and differing clinical practice may limit the scope of information exchanged with a patient.
Patients
● Common scenarios for evacuation such as road trauma and combat injuries have high background rates of secondary infection [17].
● High acuity of illness in transferred patients means they may be transferred directly from intensive care units, which traditionally have high rates of multiresistant organisms.
● Medical tourists undergoing solid-organ transplant or cancer therapy acquire the additional risk factor of immunosuppression while abroad.
● Medical tourism packages are frequently combined with a vacation, putting patients at risk for exposure to a broader range of community pathogens.

antimicrobial therapy of severe bacterial infection is now clearly linked to decreased survival [9]. At an institutional level such patients may be the index case for nosocomial outbreaks of new pathogens. At a community level, inadvertent human carriage has been associated with the global spread of antimicrobial resistance in community pathogens [5].

This review categorizes patients at risk of transferring multiresistant bacterial pathogens from healthcare facilities in one country to another. We outline known examples of country-to-country transfer of specific multiresistant bacterial pathogens of emerging and contemporary importance. Data were identified from a systematic search of the Medline and Ovid databases, the reference lists of published works, and web-based resources identified in these works.

PATIENTS AT RISK

Aeromedical Evacuation of Civilians

Aeromedical evacuation, defined as international patient transfer to a medical facility by long-distance air flight, is increasingly common. It is noteworthy that people with an increasing burden of comorbid disease are now traveling internationally and may have been hospitalized while abroad [10]. Collated statistics on civilian aeromedical evacuation are not readily available, although anecdotal reports indicate rising numbers [11]. A French insurer reported over 400 evacuations and repatriations in a single year [12]. The indications for transfer from a foreign hospital include a broad spectrum of medical and surgical conditions, occurring in both the military and civilian domains. Recently, mass mobilization of civilian evacuation services has been utilized in the setting of natural disasters such as the southeast Asian tsunami in 2004 and man-made disasters such as the Bali bombing terrorist attacks in 2002 [13, 14]. In both disasters, there were reports of transfer of multiresistant Gram-negative bacilli to institutions with low background rates of these organisms [4, 15].

There are factors in the dynamics of aeromedical evacuation that may increase the risk that such patients harbor multiresistant bacterial pathogens (Table 1). Two European studies have investigated rates of carriage of multiresistant organisms (MROs) in patients repatriated via air transfer. Although one study showed rates of methicillin-resistant *S. aureus* (MRSA) and multiresistant Gram-negative bacilli colonization in patients undergoing aeromedical evacuation to be similar to rates at their receiving institutions [18], a second study showed far higher rates [19]. The risk of MRSA was found to be highest in those with a prolonged intensive care unit (ICU) stay prior to transfer [18] while risk of multiresistant Gram-negative bacilli was found to be highest in patients transferred from Asia and Eastern Europe [19].

Aeromedical Evacuation of Military Personnel

Military patient movements are frequent, with the United States Air Force Aeromedical Evacuation system reporting over 40,000 patient movements globally during an 18-month period [20]. Recent military operations reveal a contemporary view of evacuees from the theater of war. High rates of infection caused by multiresistant Gram-negative bacilli have been reported in injured military personnel evacuated from Iraq and Afghanistan [21, 22]. Some studies estimate bacterial infection complicating 15% to 25% of admissions [17, 23]. Etiological investigation of infections has implicated both environmental contamination of field hospitals and frequent nosocomial transmission within the military health system [16, 24]. Risk factors for infection during evacuation included abdominal injuries, soft tissue injuries, and a high overall injury severity score. Additionally, the occurrence

of such infection increased the likelihood of the evacuee requiring ICU management [17]. At receiving institutions in North America, increasing MRO infection has led to a marked escalation in the use of broader spectrum and higher cost antimicrobials in the military healthcare system [21].

Medical Tourists

Medical tourism has been defined as ''organized travel outside one's natural health care jurisdiction for the enhancement or restoration of the individual's health through medical intervention'' [25]. With increasing globalization, such travel is now increasingly common [26]. For many years, patients have traveled internationally to access new and advanced treatment unobtainable in their home country. Patients are now increasingly traveling from developed countries to centers in South and Central America, South Africa, and Asia where treatment may be obtained at a lower cost, without the delay incurred by publicly funded health systems in their home location, or with greater privacy for cosmetic and other procedures [27, 28]. Destinations for medical tourists now encompass most corners of the globe. Patients travel internationally for procedures ranging from cosmetic surgery to fertility treatment, major joint replacement, and even life-saving cardiac surgery and organ transplant. Some health insurers in North America utilize foreign medical care in order to defray cost [26]. The American Medical Association and the American College of Surgeons have recently issued position statements pertaining to medical tourism [29, 30]. The exact numbers of medical tourists has not been documented. Recent estimates suggest that by 2012, 1.6 million patients per year will travel from North America to receive healthcare in another country [26–28]. Thailand, Hungary, India, and Singapore are all expected to receive 1 million or more medical tourists by 2012 [31].

Certain aspects of medical tourism may increase the risk of acquisition and complicate the management of MRO infection (Table 1). There have been no prospective studies of the infections associated with medical tourism, likely due to the difficulty in prospectively capturing this group. Available data come from retrospective case series and surveys of patients and physicians [32]. The largest published experiences arise from solid-organ transplantation owing to the obligate need for medical contact in the recipient's home country [33–36]. Illustrating the difficulty of such studies, a single-center experience from North America found fewer bacterial infections in those who received transplants abroad compared with local recipients. The authors felt this was significantly confounded by the inability to measure the incidence of early transplant infection in the patients who received transplants abroad [33]. Few series have specified the infecting bacterial pathogens. Canadian experience reported 8 of 20 patients with bacterial infection after renal transplant abroad, although the location of the transplant procedure was not specified. Four of these patients suffered infection with bacteria likely producing an extended-spectrum ß-lactamase (ESBL) [36].

Care Shared Across Countries

Similar in nature is a broader group of informal medical tourists: patients for whom care of an acute or chronic condition is spread across multiple nations. Factors influencing the country of care may include the proximity to friends and family, financial factors, and access to advanced facilities. The term ''diaspora'' has been used in reference to large permanent expatriate populations from many nations. Recently this term has been applied to thriving expatriate Indian and Pakistani communities, which likely number more than 24 million and 7 million individuals, respectively [37, 38]. A nation's diaspora may maintain strong familial and cultural links to their nation of origin, including frequent return travel and potentially medical treatment for acute and chronic conditions spread across multiple nations [39–41]. This group is likely more numerous than medical tourists or aeromedical evacuees.

INFECTIONS OF CURRENT CONCERN

Gram-Negative Bacilli

Acinetobacter Species

In a large outbreak of carbapenem-resistant *A. baumannii* in a Belgian hospital, the 2 index patients were evacuated from a Greek ICU after road trauma. Despite increased infection prevention precautions, 17 subsequent cases of a clonal isolate were identified over the next 6 months [42]. A smaller outbreak was also described in northern Italy, also secondary to evacuees from Greece [43].

A widespread carbapenem-resistant *Acinetobacter* outbreak in medical facilities involved in the treatment of aeromedical evacuees from military operations in Iraq and Afghanistan has been reported [22, 44]. More than 100 cases of bacteremia over an 18-month period occurred within military hospitals in the United States and Germany [44]. Although *Acinetobacter* is associated with traumatic injury in many settings, molecular and clinical studies have shown that the majority of infection in this outbreak was due to nosocomial acquisition [16, 24]. The United Kingdom has also reported the introduction of new strains of *Acinetobacter* from evacuees from Iraq [22]. In the setting of traumatic burns and blast injuries after the 2002 terrorist attacks in Bali, frequent *Acinetobacter* infection was noted in patients evacuated to Australia. Subsequent nosocomial spread in receiving hospitals was again reported [15].

Bacteria Harboring KPC and NDM Carbapenem Resistance Genes

Epidemiological investigation suggests that introduction of the *Klebsiella pneumoniae* carbapememase (KPC) gene into several

regions has been due to carriage by the intercountry patient. Israel was the first nation outside the United States to report a large outbreak of KPC-harboring *K. pneumoniae*. Widespread healthcare-associated transmission occurred of a strain identified as of North American origin [45]. Greece has identified widespread clonal KPC-producing *K. pneumoniae* indistinguishable from contemporary Israeli clones [46]. In neither case was a single point of introduction identified. The likely index case in a single-center outbreak in Germany was a patient who had been previously hospitalized in Greece [47]. Many additional countries including the United Kingdom and France have reported episodes of colonization or infection of patients transferred from endemic countries [48, 49] (Figure 1).

The New Delhi metallo-ß-lactamase gene (NDM-1) also confers almost complete ß-lactam resistance. NDM-1 has been identified in a broad range of Gram-negative bacteria including *K. pneumoniae*, *Escherichia coli,* and *Citrobacter freundii*. Almost all isolates are also resistant to aminoglycosides, fluoroquinolones, and other classes of antimicrobials. Of concern, some isolates exhibited resistance to the agents of last resort, tigecycline and colistin [50]. The NDM-1 gene was first described in Sweden [51] and the United Kingdom [52], and was strongly associated with healthcare received on the Indian subcontinent. In the United Kingdom, 9 of 19 patients had recently been hospitalized in India or Pakistan for treatment ranging from solid-organ transplantation to cosmetic surgery. Subsequently, imported cases associated with healthcare contact in India and Bangladesh have been reported in other regions including the United States, Australia, Canada, Japan, and several European nations [39, 53, 54]. Cases have also been identified among patients repatriated to locations in Western Europe from hospitals in Balkan nations, and a cluster of cases was identified in Kenya [41, 55]. These epidemiological observations require further elucidation (Figure 1).

Bacteria Harboring ESBL Enzymes

Carriage of bacteria harboring ESBL enzymes by the intercountry patient is well established and still remains a significant risk [56]. Early reports include intercontinental transfer of common nosocomial ESBL-producing bacteria such as *K. pneumoniae* [57]. Current literature reflects the emergence of *E. coli* harboring CTX-M ESBLs, with healthcare-associated acquisition responsible for approximately 15% of travel-related infections due to ESBL producers in some studies [58, 59].

Gram-Positive Organisms

Methicillin-Resistant S. aureus

Almost 50 years after its emergence, the spread of MRSA by the intercountry patient still poses a threat to institutions that have maintained low MRSA prevalence. The prevalence among hospital-acquired *S. aureus* isolates in the Netherlands and Scandinavia remains <1%, contrasting with levels in other European nations and North America (6%–63%) [7]. Two outbreaks in the Netherlands were directly linked to the transfer of patients from institutions in France and Turkey where MRSA is endemic [60]. A study in Sweden demonstrated that one-quarter of 1733 MRSA cases reported between 2000 and 2003 were likely acquired abroad; over half of these were healthcare associated [61]. The potential intercountry spread of MRSA via healthcare workers, rather than patients, is illustrated by the report of a Swiss physician found to have a new nasopharyngeal colonization with a North American clone of MRSA after returning from a clinical fellowship in North America [62].

Vancomycin-Resistant Enterococci (VRE)

Reports of intercountry spread of VRE come primarily from molecular epidemiologic assessments. VRE clonal complex-17 (CC-17), a group consisting of a number of closely related VRE sequence types, has been responsible for VRE dissemination in countries including the UK, Australia, and North America [63]. Investigators linked a sharp rise in the rate of VRE in southwest Germany to the likely importation of CC-17 VRE to their hospital system [64]. An outbreak due to CC-17 has also been reported in Turkey [65]. Neither report identified a single point of introduction.

Clinical reports of VRE transfer between nations have been prevalent in Europe [66]. Low-incidence Nordic countries (where VRE accounts for <1% of enterococcal isolates) have had sporadic importation and outbreaks from other nations since the early 1990s [66]. Molecular studies were strongly suggestive of intercountry spread of a distinctive VRE clone from North America to Norway and also to Ireland [67]. In a prospective study from the Netherlands, VRE was identified in approximately 3% of patients repatriated from a number of countries, with Asian origin being a significant risk [19].

Hypervirulent Clostridium Difficile

Since the initial description of hypervirulent ribotype O27, there have been repeated descriptions of transfer of the strain via the intercountry patient [68, 69]. A retrospective study in 2007 identified the transfer of a patient infected with the hypervirulent strain from the United Kingdom to Ireland very soon after the initial descriptions in 2005; fortunately, no outbreak occurred [68]. Introduction of the strain into France in 2006, which now has sustained transmission, was speculated to be due to transfer with patients from neighboring Belgium [70]. Australia has reported a single case of importation in a patient recently hospitalized in North America [69].

APPROACH TO THE PATIENT

All hospitals should have a predefined approach to management of patients transferred from other institutions, including

Table 2. Recommendations for the Management of Patients Who Have Been Hospitalized Internationally

1. Maintain vigilance. Ask specifically about healthcare contact whenever a patient reports international travel within the previous 12 months.
2. Preemptive isolation and screening should be used in patients with a history of international hospitalization and who have a high risk of carriage of multiresistant organisms. Isolate patients who have had direct hospital-to-hospital transfer or recent international hospitalization involving prolonged hospital stay, intensive care or critical care admission, major trauma, burns, or receipt of chemotherapy or immunosuppression (eg, solid-organ or stem-cell transplant).
3. Screening needs to be customized to the receiving institution: Focus screening on organisms that are not already endemic at your site. Basic screening may include axillary, inguinal, and nose/throat swabs PLUS rectal swab or stool sample PLUS clinical specimens including catheter urine, surgical drain, or wound discharges—screen for MRSA-, VRE-, and ESBL-producing or carbapenem-resistant GNB.Only screen for *Clostridium difficile* if diarrhea is present.
4. Receive transferred patients in an area of the hospital equipped to manage isolation for multiresistant organisms. Patients may initially require management in an area of higher acuity than required for their medical care (eg, patients for rehabilitation may need to go to an acute ward until screened).
5. All receiving institutions should have a readily accessible infection prevention policy defining at-risk patients, screening procedures, and preemptive isolation criteria. If your institution frequently receives patients from a particular location, a customized protocol should be developed and maintained for this location: Including an outline of the current pathogens of concern and empirical therapy is recommended in the case of infection.

NOTE. ESBL, extended-spectrum β-lactamase; GNB, Gram-negative bacilli; MRSA, methicillin-resistant *Staphyloccus aureus*; VRE, vancomycin-resistant enterococci.

those in other countries (Table 2). Preemptive contact isolation may be considered when there is a risk of introduction of an MRO not currently found in the institution receiving the patient. In institutions with few or no endemic MROs, there will likely be a greater willingness to institute preemptive contact isolation. In some institutions, there may already be a high prevalence of MROs and it may seem to matter little that a patient has come from another institution which also has endemic MROs. However, introduction of new mechanisms of antibiotic resistance or new ''hospital adapted'' bacterial strains may pose risks of amplifying antibiotic resistance. An example may be the receipt in a hospital in the United States with endemic KPC producers of a patient from a hospital in India or Pakistan where NDM producers are endemic.

In the setting of management of an individual patient with suspected bacterial infection we suggest a considered approach to the use of empirical therapy (Table 3).

Healthcare staff must adopt a pragmatic and nonjudgmental approach to the management of the intercountry patient who has acquired an MRO infection. This may be challenging in the setting of a patient who has sought a healthcare intervention believed inappropriate or unethical by the home treating clinician, such as commercial organ transplantation, or experimental or cosmetic procedures. This attitude is crucial in order to avoid the patient feeling stigmatized and to facilitate open

Table 3. Approach to Suspected Bacterial Sepsis in Patients Previously Hospitalized in Another Country

1. Ensure appropriate microbiology samples for the clinical presentation (eg, blood cultures, urine culture, respiratory tract cultures) if required. Notify the microbiology laboratory of the patients' origin. They may broaden their testing beyond their normal scope (eg, detection of NDM-1, *Clostridium difficile* ribotype).
2. If screening has identified MROs: These bacteria must be targeted in empirical therapy. If susceptibilities are available use these to guide antimicrobial selection. If susceptibilities are not available, empirical therapy may include agents such as linezolid or daptomycin (for VRE and MRSA) and polymyxin B, colistin, or amikacin for multidrug-resistant gram-negative bacilli. If available, consultation with an infectious disease physician or clinical microbiologist may be helpful in selecting the optimal agent for identified pathogens.
3. If no screening results are available: Therapy must target the prevalent pathogens at the transferring institution. When possible, ascertain these by direct discussion with this institution as recent outbreaks may not be publicized. See suggestions above for empirical therapy.
4. If screening does not detect an MRO: Treat as per local guidelines. However, screening is not 100% sensitive. If the patient fails to improve on empirical therapy then reassess for occult sites of infection and reculture as extensively as possible. Consider empirical therapy for organisms prevalent at the transferring institution as outlined above.

NOTE. MRO, multiresistant organism; MRSA, methicillin-resistant *Staphyloccocus aureus*; NDM-1, New Delhi metallo-β-lactamase; VRE, vancomycin-resistant enterococci.

communication of information between the patient, family, and clinician. International institutions may operate with constraints and resource limitations that are not present in the patient's country of residence. Furthermore, the patient may have felt they had no option but to utilize the facilities available in a foreign country due to the urgency of care required or the financial cost of care at home.

Local, national, and international regulations may pertain to the notification, transit, and control of patients harboring MROs [71]. This is a complex and evolving area that varies between jurisdictions.

CONCLUSION

The management of patients transferred from other institutions is a daily reality for almost all healthcare practitioners. The patient with international healthcare contact may present to healthcare institutions in a variety of forms, ranging from the overt (eg, aeromedical evacuee) to the unsuspected (eg, elective surgical day case). In some settings, ready identification of a patient's origin in foreign hospitals is difficult and requires specific questioning. Similarly, a multitude of communication barriers may lead to difficulty obtaining information pertaining to a patient's medical care in another country.

Contemporary molecular epidemiological techniques have allowed us considerable insight into the origins and movement of healthcare-associated MROs. The range of potential MROs acquired by the intercountry patient is broad. We have outlined a small number with current significance. A key concept is the dynamic nature of such outbreaks. These may emerge and disseminate before reaching the general medical literature. At times, outbreaks may go undetected in their country of origin until exported with the intercountry patient [46, 50].

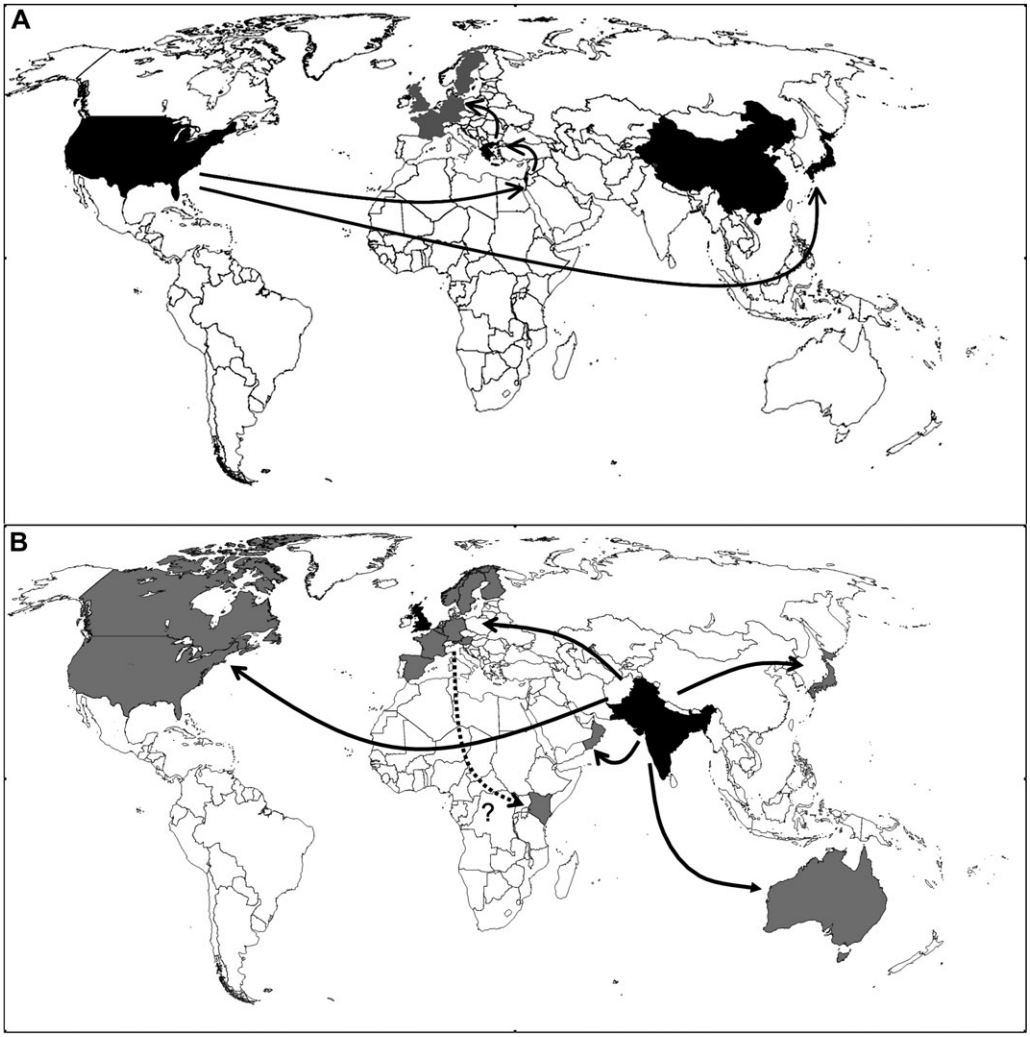

Figure 1. Schematic representation of epicenters (*black*) and reported/potential importations (*gray*) of *Klebsiella pneumoniae* carbapenemase (*A*) and New Delhi metallo-β-lactamase-1 (*B*) ß-lactamase-producing organisms [39, 41, 45–49, 53–55].

Furthermore, emerging data now suggest that the risk of acquisition of some MROs may extend to those without healthcare contact during travel to countries of high endemicity [72, 73]. Given the large pool of international travelers, this area requires further exploration to better define risk factors and the potential magnitude of this problem.

For an individual patient, the significance of an MRO infection will largely depend on his or her current medical condition and may range from an incidental finding to a life-threatening infection. For an institution, the significance of importation of MROs depends on the preexisting milieu of MROs and the likelihood of spread, determined by infection prevention practices.

In conclusion, there are many factors that may complicate the identification and management of infections with MROs in the intercountry patient. Clinical vigilance in the form of sensitive and thorough questioning coupled with a high standard of baseline infection prevention practice in all patients must be the first line of defense.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgments section.

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Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain

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Escherichia coli sequence type 131 (ST131) is a worldwide pandemic clone, causing predominantly community-onset antimicrobial-resistant infection. Its pandemic spread was identified in 2008 by utilizing multilocus sequence typing (MLST) of CTX-M-15 extended-spectrum β-lactamase-producing *E. coli* from three continents. Subsequent research has confirmed the worldwide prevalence of ST131 harbouring a broad range of virulence and resistance genes on a transferable plasmid. A high prevalence of the clone (∼30%–60%) has been identified amongst fluoroquinolone-resistant *E. coli*. In addition, it potentially harbours a variety of β-lactamase genes; most often, these include CTX-M family β-lactamases, and, less frequently, TEM, SHV and CMY β-lactamases. Our knowledge of ST131's geographical distribution is incomplete. A broad distribution has been demonstrated amongst antimicrobial-resistant *E. coli* from human infection in Europe (particularly the UK), North America, Canada, Japan and Korea. High rates are suggested from limited data in Asia, the Middle East and Africa. The clone has also been detected in companion animals, non-companion animals and foods. The clinical spectrum of disease described is similar to that for other *E. coli*, with urinary tract infection predominant. This can range from cystitis to life-threatening sepsis. Infection occurs in humans of all ages. Therapy must be tailored to the antimicrobial resistance phenotype of the infecting isolate and the site of infection. Phenotypic detection of the ST131 clone is not possible and DNA-based techniques, including MLST and PCR, are described.

Keywords: β-lactamases, molecular epidemiology, bacterial infections

Introduction

Escherichia coli is a finely tuned, ubiquitous human pathogen. It is a common cause of urinary tract infection (UTI) and bacteraemia in humans of all ages. In addition, it is a frequent cause of varied organ infections, ranging from the biliary system to the CNS. The spectrum of pathology can range from a spontaneously resolving cystitis to life-threatening sepsis syndrome.[1] Not confined to the community, *E. coli* infection is also a common hospital-acquired pathogen.[2]

Over the past five decades, we have witnessed increasing antimicrobial resistance in *E. coli* in the community setting. Initially, resistance was described to particular agents, such as ampicillin, trimethoprim, sulphur-based antimicrobials or tetracyclines.[3] More recently, the horizon of resistance has broadened, with the emergence of broad resistance to large families of agents. In particular, plasmid-mediated extended-spectrum β-lactamases (ESBLs) have become prominent in community-onset *E. coli* infection.[4,5] In addition to the resulting resistance to most β-lactam antibiotics, ESBL producers are frequently also resistant to aminoglycosides and fluoroquinolones.

There are a variety of reasons for the increased prevalence of antibiotic-resistant *E. coli*. *E. coli* is an organism known for its mobile genome and propensity to exchange genetic material.[6] However, the dissemination of 'clonal' organisms harbouring resistance is also well documented. Clonal outbreaks of *E. coli* clinical infection previously described include 'Clonal Group A' (CGA) in North America[7] and O15:K52:H1 in multiple nations.[7,8] It is estimated that 10%–20% of all *E. coli* UTIs may be caused by a small set of clonal groups.[9] In 2008, two research groups analysing the population biology of ESBL-producing *E. coli* almost simultaneously described 'serogroup O25b, sequence type 131 (ST131)' occurring in multiple countries on three continents. This previously unremarkable molecular clone harboured a CTX-M ESBL gene and a larger armamentarium of virulence genes.[10,11] Since this discovery in 2008, research has retrospectively documented a 'pandemic' emergence amongst ESBL-producing and other antimicrobial-resistant clinical isolates in the middle of this decade. Previous to this, only sporadic isolates of this clone can be identified in multilocus sequence typing (MLST) databases and published series. The rapid and apparently boundless rise of the ST131 *E. coli* clone is the subject of this review.

Epidemiology

Human infection and colonization

Published research detailing the geographical distribution and antimicrobial resistance of human infection and colonization by *E. coli* ST131 are summarized in Table 1.

Table 1. Geographical distribution and antimicrobial resistance of *E. coli* ST131 in humans

Country/Region	Specific location	Date range of samples	Isolate source	Selection criteria used by study	Number of isolates	Percentage of isolates that were ST131 (*n*)	Percentage of ST131 that were community onset	Percentage of ST131 that were fluoroquinolone resistant	Percentage of ST131 that were SXT resistant	Percentage of ST131 that harboured ESBL
Multinational[11]	Europe, Canada and Middle East	2000–06	laboratory collection	ESBL CTX-M-15	43	42 (18)	0	NS	NS	100
Multinational[10]	Europe, Asia and Canada	NS	clinical isolates and laboratory collection	ESBL CTX-M-15	41	88 (36)	39	97	53	100
Multinational[45]	worldwide, excluding India/Pakistan and Bangladesh	2004–06	traveller returned from region; majority UTI	ESBL	84	19 (16)	NS	NS	NS	100
Europe[18]		2003–06	community-acquired UTI	fluoroquinolone resistant	148	24 (35)	100	100	NS	NS
Belgium[28]		2006–07	clinical isolates	ESBL CTX-M-15	43	72 (31)	90	NS	NS	100
Croatia[29]		2002–05	clinical isolates	ESBL CTX-M	12	42 (5)	NS	100	NS	100
France[23]		1994–2003	laboratory collection	ESBL	128	6 (8)	NS	NS	NS	100
France[23]	Tenon	2002–03	UTI	non-ESBL + B2 phylotype	129	3 (4)	NS	NS	NS	0
France[24]		2005	bacteraemia	3GC resistant	41	15 (6)	NS	NS	NS	100
France[25]		2006	community-onset UTI	ESBL	48	21 (10)	10	100	60	100
France[23]		2006–07	laboratory collection	ESBL	41	46 (19)	NS	NS	NS	100
France[26]	Paris	2006	stools from healthy volunteers	none	100	7 (7)	100	57	NS	0
Ireland[17]		2003–07	majority UTI	ESBL	371	<10	NS	NS	NS	100
Italy[22]	Rome	2006	bacteraemia and UTI	fluoroquinolone resistant + ESBL	18	61 (11)	NS	100	NS	100
Northern Ireland[16,109]	Belfast	2004–06	stool samples from residents of LTCFs	ESBL + fluoroquinolone resistant	119	≥54 (≥64)	0	100	NS	100
Norway[27]		2003	clinical isolates	ESBL	45	20 (9)	NS	NS	NS	100
Spain[19]		2004	clinical isolates	ESBL	91	9 (8)	NS	NS	NS	100
Spain[61]	Madrid	2004–07	majority UTI	Amp-C	121	6 (7)	NS	NS	NS	0
Spain[20]		2006	clinical isolates	ESBL CTX-M-15	37	86 (32)	NS	NS	NS	100
Spain[21]	Lugo	2006–07	majority UTI	ESBL	105	22 (23)	NS	>96	>96	100
Spain[21]	Lugo	2007–08	majority UTI	ESBL	249	22 (54)	<50	NS	NS	100
Spain[106]	Madrid	2008	UTI	ESBL + fosfomycin resistant	26	92 (24)	NS	NS	NS	100
Turkey[31]	Izmir	2004–05	community-acquired UTI	ESBL	17	6 (1)	100	100	100	100
Turkey[23]		2006	laboratory collection	ESBL	10	20 (2)	NS	NS	NS	100
UK[15]		2001–05	bacteraemia	fluoroquinolone resistant + non-ESBL + aac(6′)-Ib-cr	10	50 (5)	NS	100	NS	0
UK[12,13]		2003–04	clinical isolates	ESBL	287	≥65 (≥188)	NS	NS	NS	100
UK[14]	north-west England	2004–05	UTI and bacteraemia	cefpodoxime resistant	88	59 (52)	NS	NS	NS	98

UK[23]		2004–07	laboratory collection	ESBL	103	81 (84)	NS	NS	NS	100
Brazil[23]		2001–05	laboratory collection	ESBL	5	0	NS	NS	NS	100
Canada[34]	Calgary	2000–07	bacteraemia	ESBL	67	31 (21)	62	100	67	100
Canada[33]		2002–04	UTI	fluoroquinolone resistant or SXT resistant	199	23 (46)	100	96	46	<2
Canada[23]		2004–06	laboratory collection	ESBL	41	41 (17)	NS	NS	NS	100
Canada[32]	Montreal	2005–07	women with UTI	varied resistance sought	353	<1 (2)	NS	NS	NS	NS
Canada[9]	Montreal	2006	women with UTI	none	256	<3	100	100	NS	NS
Canada[69]		2007	clinical isolates	ESBL	209	46 (96)	57	NS	NS	100
USA[38]	Texas	2003–05	bacteriuria in renal transplant recipients	none	40	35 (14)	NS	86	NS	0
USA[35]		2007	majority bacteraemia	varied resistance sought	127	17 (54)[a]	NS	NS	NS	56
USA[37]	Pittsburgh	2007–08	clinical isolates	ESBL	70	30 (21)	NS	NS	NS	100
USA[36]	Chicago	2008	majority UTI	ESBL	30	53 (16)	NS	100	38	100
Indian subcontinent[45]	India, Pakistan and Bangladesh	2004–06	traveller returned from region; majority UTI	ESBL	31	61 (19)	NS	NS	NS	100
Cambodia[23]	Phnom Penh	2004–05	UTI	ESBL	30	27 (8)	NS	NS	NS	100
China[42]		1998–2000	laboratory collection	fluoroquinolone resistant	12	≥17 (≥2)	NS	100	NS	NS
Japan[41]		2002–03	laboratory collection	ESBL	142	19 (27)	NS	NS	NS	100
Japan[42]		2003–07	clinical isolates	fluoroquinolone resistant	128	≥30 (≥38)	NS	100	NS	NS
Korea[43]		2006–07	community-onset UTI	fluoroquinolone resistant	129	25 (32)	100	100	50	19
Korea[42]		2005	laboratory collection	fluoroquinolone resistant	21	≥33 (≥7)	NS	100	NS	NS
Philippines[44]	Manila	2007	clinical isolates	ESBL	15	7 (1)	NS	NS	NS	100
Thailand[23]		1999	laboratory collection	ESBL	5	0	NS	NS	NS	100
Australia[48]	Queensland	2007–08	majority UTI	fluoroquinolone resistant	582	35 (205)	NS	100	NS	NS
Australia[47]	Queensland	2008–09	clinical isolates	cephalosporin resistant or fluoroquinolone resistant	49	31 (15)	NS	47	NS	53
Central African Republic[23]	Bangui	2004–06	laboratory collection	ESBL	10	50 (5)	NS	NS	NS	100

NS, not specified by the authors; UTI, urinary tract infection (or bacteriuria if not specified); ESBL, extended-spectrum β-lactamase; LTCFs, long-term care facilities; SXT, trimethoprim/sulfamethoxazole; 3GC, third-generation cephalosporin.
≥, < and > are used to estimate when the text does not give an exact number for the relevant isolate.
[a]Estimated at 17% of entire collection of *E. coli* isolates.

Europe

ST131 *E. coli* is widely disseminated amongst 'antibiotic-resistant' community and hospital-onset *E. coli* in the UK. Originally identified as the 'CTX-M ESBL-producing UK epidemic strains A–E',[12] between 2003 and 2004, these strains have subsequently been confirmed as ST131.[12,13] In one UK region, ST131 comprised 64% of community-acquired and 84% of hospital-acquired cefpodoxime-resistant *E. coli* infections.[14] A UK national study of fluoroquinolone-resistant, non-ESBL-producing *E. coli* bacteraemia isolates illustrates the rapid emergence of this strain, with isolates first identified only in 2004.[15] High rates of asymptomatic carriage of fluoroquinolone-resistant ST131 strains have been demonstrated in Northern Ireland nursing home patients.[16] In the Republic of Ireland, ST131 was also widely disseminated amongst CTX-M ESBL-producing *E. coli*.[17] No data exist on ST131 among relatively 'antibiotic-susceptible' strains.

The epidemiology of the clone throughout mainland Europe is less well characterized. Current data suggest a heterogeneous distribution of infection and carriage, with prominence of the clone amongst antibiotic-resistant isolates. A collection of fluoroquinolone-resistant *E. coli* from eight European countries showed ST131 comprised 24% of this entire group. However, the number of isolates varied markedly between countries, with Spain and Italy most prominent.[18] Spanish ESBL-producing *E. coli* data from 2004 revealed that 9% of isolates were ST131.[18] A follow-up national study in Spain in 2006 demonstrated that 13% of ESBL-producing *E. coli* were ST131 and that they had a nationwide distribution.[19,20] More recent data from a single region in Spain found that 22% of similar isolates from 2006–08 were ST131—50% originated from nursing home patients.[21] A study of a single region in Italy found that 61% of isolates selected from a collection with fluoroquinolone resistance and harbouring ESBL genes were ST131.[22] French data demonstrate the emergence of this clone primarily amongst resistant isolates. ST131 was first identified in France in 2001 and it rose to comprise 46% of ESBL-producing *E. coli* from 2006 to 2007 in one series.[23,24] Nationwide data from community-onset ESBL-producing *E. coli* infections identified that 25% were ST131, although only 1 of 40 patients was felt to have 'true community-acquired' infection.[25] Data on non-ESBL-producing *E. coli* from UTIs from 2002–03 revealed that only 3% were ST131, with the authors calculating an overall rate of 1.5% of UTIs caused by this clone.[23] Similarly, carriage of ST131 without CTX-M ESBLs has been identified in 7% of healthy volunteer stools in France.[26] In Norway, 20% of all national CTX-M-producing *E. coli* in 2003 were ST131.[27] Belgian data from 2006 to 2007 demonstrate a high prevalence of ST131 in community-acquired ESBL-producing isolates. All of the CTX-M-15-carrying *E. coli* that were assayed, comprising 62% of all isolates, were ST131.[28]

The epidemiology of other European nations can only be inferred from case reports and smaller studies. Primarily hospital-based outbreaks have been described in Croatia,[29] Portugal[11] and Germany.[30] The clone has also been identified in Austria, Germany, Hungary, Russia, Switzerland and Turkey.[10,18,31]

The Americas

The epidemiology of ST131 is well characterized in Canada, with low rates in susceptible *E. coli* and high rates in resistant isolates. Two studies comprising UTI isolates, with little antimicrobial resistance, from the years 2005–07, have demonstrated rates of ST131 in isolate collections of <3% and 1%.[9,32] In contrast, in ambulatory patient isolates selected for fluoroquinolone or trimethoprim/sulfamethoxazole resistance from 2002 to 2004, ST131 comprised 23% of all isolates and 44% of fluoroquinolone-resistant isolates.[33] Blood culture isolates of ESBL-producing *E. coli* from a single region in Canada mirror the UK experience, with emergence of the strain in 2003 and a rapid rise to comprise 41% of isolates from 2004 to 2007. An overall rise in the incidence of ESBL-producing *E. coli* bacteraemia was also attributed to the emergence of the clone.[34]

Recent data from North America suggest ST131 as 'the major cause of significantly antimicrobial-resistant *E. coli* infections in the United States'.[35] A geographically widespread selection of isolates primarily from bloodstream infections suggested that ST131 comprised 67%–69% of isolates resistant to fluoroquinolone or extended-spectrum cephalosporins. In this study, no susceptible samples were ST131.[35] Recent studies from Chicago and Pittsburgh also identified high rates amongst resistant isolates. ST131 comprised 53% of CTX-M ESBL-producing *E. coli* in Chicago and 30% of ESBL-producing *E. coli* in Pittsburgh, with a range of accompanying ESBL genes.[36,37] ST131 *E. coli* has also been identified in renal transplant recipients and haematology patients in Texas, both of which are groups with high background antimicrobial use.[38,39]

A single report has identified ST131 in South America. The clone comprised 8% of 28 ESBL-producing *E. coli* hospital-associated isolates from Rio de Janeiro, Brazil.[40]

Asia and the Middle East

ST131 has been frequently identified among antimicrobial-resistant isolates in Japan and Korea. A national survey in Japan identified the clone in 21% of ESBL-producing *E. coli* from 2002 to 2003. Interestingly, a greater genetic diversity within the clone and a greater variety of accompanying CTX-M ESBL genes was found in this region than elsewhere.[41] The clone comprised 33%–63% of fluoroquinolone-resistant isolates from various Japanese regions.[42] Amongst ciprofloxacin-resistant isolates causing community-onset infections in Korea, ST131 comprised 25% of isolates, only 19% of which harboured an ESBL gene.[43]

In a small Cambodian sample, ST131 clones comprised 27% of community-onset UTIs due to ESBL-producing *E. coli* during 2004–05.[23] Infrequent isolates have been detected among larger collections of clinical isolates in China[42] and the Philippines.[44] Faecal carriage was identified in a small number of hospital patients with ESBL-producing *E. coli* in stools in Lebanon.[10] The epidemiology in other Asian countries has been inferred from studies of returned travellers, and from the high proportion of ESBL-producing *E. coli* ST131 isolates from India, Pakistan, Iran and Lebanon.[45] Supporting these data, the SMART study showed remarkably high background rates of 79% ESBL production amongst *E. coli* isolated from intra-abdominal infections in India.[46]

Australia

Two studies from a single region of Australia recently confirmed the presence of the ST131 clone in this country. In one study of

E. coli selected for fluoroquinolone or cephalosporin resistance, 31% of isolates were ST131; <50% were CTX-M producing.[47] In a second study, 35% of fluoroquinolone-resistant isolates from a mix of hospital and community clinics were ST131.[48]

Africa

Little data exist on the presence of ST131 in Africa. Two small samples have suggested high rates amongst ESBL-producing *E. coli*. In Cape Town, South Africa, 43% of 23 such isolates were ST131 and expressed either CTX-M-14 or CTX-M-15 enzymes.[49] In the Central African Republic, 50% of CTX-M-15-producing *E. coli* were ST131.[23] A high proportion of ST131 have also been identified in a small number of travel-related ESBL-producing *E. coli* infections from Africa.[45]

Non-human carriage and infection

ST131 is represented amongst resistant isolates in companion and non-companion animals, although the extent is unclear thus far. A collection from eight European countries confirmed the presence of ST131, comprising 6% of ESBL-producing *E. coli* isolates recovered from companion animals.[50] Australian data show a surprisingly low incidence amongst fluoroquinolone-resistant isolates from companion animals (7.2% were ST131) compared with humans (35% were ST131).[48] Johnson *et al.*[51] demonstrated intrahousehold sharing of the clone between domesticated animals; however, transmission from companion animals to humans has not been confirmed.

In non-companion animals, ST131 has been identified among ESBL-producing isolates in seagulls[49] and rats,[50] both of which have close contact with human populations. Two Spanish studies have suggested a low prevalence of the clone amongst poultry and pig farms in that nation.[52,53] Mora *et al*. found that the clone comprised 1.5% of *E. coli* strains recovered from Spanish poultry between 2007 and 2009.[53] Surprisingly, in this study, the prevalence amongst *E. coli* recovered from retail chicken meat was considerably higher, comprising 7% of strains. In addition, PFGE identified a cluster of poultry and human strains, all of which carried the CTX-M-9 gene and a similar virulence profile, suggesting recent crossover between human and avian hosts.[53] The high similarity of an isolate from raw chicken and two human infections in the same geographical region in Canada was suggestive of transmission from foodstuff to humans.[32] Although these links are tantalizing, there remains to be a solid molecular epidemiological connection between human infection and prior consumption of food containing ST131 *E. coli*.

Molecular epidemiological observations

Thus far, there are 48 entries of ST131 voluntarily submitted to the largest publicly accessible *E. coli* MLST database, with isolation dates ranging from 1992 to 2009. Notably, only a handful of other STs have a greater number of entries. This may equally reflect the current interest in ST131 and/or the ubiquity of this ST amongst *E. coli*. The majority of the isolates originate from human infection, primarily UTIs. In addition, ST131 *E. coli* from domesticated and farm animals, birds and food produce are also recorded in this database.[54]

Utilizing the discriminating power of PFGE to analyse MLST-defined ST131 isolates has given considerable insight into the origin of the clone. Collections from focal outbreaks and those selected for suspected clonality have confirmed genetic similarity in excess of 85% on PGFE.[10,21] In contrast, collections with less selected samples from human or animal origin have shown ST131 isolates with considerable diversity (<65% similarity by PFGE), at times unrelated by traditional definitions. Even in such broad collections, small groups of identical or very closely related isolates are identified, often at distant locations.[13,50] This pattern likely reflects the dual phenomenon of recent divergence of the clone from a common ancestor together with ongoing transmission of the clone.[10] Clinical reports support this hypothesis. There is convincing description of direct transmission between humans[55,56] and between animals,[48] and, in contrast, of surprising diversity amongst isolates from closely associated patient groups.[38] The ancestry and significance of occasional widely divergent or unrelated ST131 isolates remains unclear.[50]

Elucidating the worldwide distribution, transmission and reservoirs of ST131 is of importance in understanding the potential mechanisms of its dissemination and control. To date, this epidemiology has not been clearly defined. Since the initial descriptions in 2008, research has focused on identifying this strain in particular groups or collections selected for antimicrobial resistance phenotype or epidemiological clustering. There have been fewer opportunities to study this strain in unselected collections of pathogenic and non-pathogenic isolates.

Reservoirs of ST131

Potential reservoirs of ST131, including food or water sources, and travel from nations with a high prevalence of the clone have been proposed as explanations for the rapid emergence of the clone on multiple continents.[57] To date, reservoirs have been detected only at a local level, with high carriage and infection rates in nursing-home residents in several nations.[16,21] Investigations have only found sporadic isolates of ST131 amongst commercial animals and food sources, although studies are limited.[32,53] The potential spread of ST131 after introduction from international travellers has only been demonstrated indirectly. Pitout *et al.*[45] found the highest proportion of ST131 clones amongst travellers with ESBL-producing infections in those returning from the Indian subcontinent and the Middle East. Freeman *et al.*[58] demonstrated a strong relationship between travel to India and community-onset CTX-M-15-producing *E. coli* infection in New Zealand. Countries implicated in these reports, such as India and Pakistan, have known high rates of ESBL-producing *E. coli* infection, but no data on the prevalence of the ST131 clone as yet.[46]

Antibiotic resistance

The ST131 'pandemic' was initially described amongst *E. coli* harbouring the CTX-M-15 ESBL gene on a relatively homogenous plasmid.[10,11] Subsequent investigation identified a high incidence of the clone amongst fluoroquinolone-resistant

non-ESBL-producing isolates and a low incidence amongst collections of susceptible *E. coli* isolates.[15,18,33,38,42] With further work, many authors have now confirmed surprising diversity amongst key transferable resistance elements, including ESBL genes, fluoroquinolone resistance genes and the plasmid scaffold harbouring them.[30,43,59] This diversity amongst a 'clonal' *E. coli* offers insight into the evolution of the clone and its resistance. Lee *et al.*[43] suggested the acquisition of transferable resistance elements as independent events from ST131 dissemination. However, the timing and sequence of resistance acquisition remains unclear. Potential explanations offered include the spread of ciprofloxacin-resistant isolates, which then acquire a CTX-M gene, or, possibly, the simultaneous spread of clonal organisms and genes.[19,42,43] Johnson *et al.*,[35] analysing North American isolates, demonstrated both vertical and horizontal transfer of the $bla_{CTX\text{-}M\text{-}15}$ gene. The gene was found in isolates closely related by PFGE; however, even within these clusters there was $bla_{CTX\text{-}M\text{-}15}$ discordance, suggesting horizontal gene transfer or, potentially, gene loss. Given the clone's propensity for the acquisition of resistance, a fine-tuning or evolutionary convergence between the clone, plasmid and acquisition of ESBL genes is likely.[11]

ESBL and AmpC enzymes

Resistance to β-lactam antibiotics in ST131 can be mediated by β-lactam-hydrolysing enzymes from three Ambler classes (A, C and D) and five distinct families. Among the ESBLs, CTX-M is the most prevalent in ST131, while SHV and TEM have been infrequently detected.[25,39,47] Of the AmpC β-lactamases, CMY has been most frequently reported.[22,37,47,60,61] Carriage of the genes encoding these β-lactamases is usually on a large plasmid (64–160 kb), which frequently carries genes encoding additional non-extended-spectrum β-lactamases, $bla_{TEM\text{-}1}$ and $bla_{OXA\text{-}1}$, and the aminoglycoside-modifying enzyme AAC(6′)-Ib-cr.[10,11,34,62]

CTX-M-15, the enzyme most closely associated with ST131, was first identified in India in 1999.[63–65] It is now the most widely distributed CTX-M worldwide.[66] The enzyme is responsible for resistance to the penicillins, cephalosporins (excluding the cephamycins) and monobactams. CTX-M takes its name from the enzyme's propensity to confer a higher level of resistance to cefotaxime than to ceftazidime (the M refers to its discovery in Munich).[67] Other CTX-M-type β-lactamases reported in association with the clone include CTX-M-2, CTX-M-3, CTX-M-9, CTX-M-14, CTX-M-27, CTX-M-32 and CTX-M-61.[23,41,53] A chromosomal rather than plasmid location of CTX-M-15 amongst ST131 isolates had also been reported and could potentially be a contributing factor in the clonal spread of CTX-M-15-producing ST131 *E. coli*.[11,22] The SHV and TEM variants described in ST131 include SHV-12, SHV-5, SHV-7, TEM-24 and TEM-116.[22,23,37,50] Isolates expressing these ESBLs may be susceptible to cefoxitin, β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) and carbapenems. However, the co-production of ESBLs with inhibitor-resistant β-lactamases (most prominently OXA-1) renders these strains resistant to commonly used β-lactamase inhibitors like clavulanic acid. AmpC β-lactamases (such as CMY) are also resistant to β-lactamase inhibitors, as well as to cephamycins such as cefoxitin. Spanish data identified 6% of AmpC-producing *E. coli* as ST131. The remainder had mutations leading to increased expression of chromosomally located AmpC genes.[61] The range and prevalence of ESBL and AmpC genes associated with ST131 are summarized in Table 2.

Resistance to other antibiotics

Fluoroquinolone resistance is common amongst ST131 in most studies.[22,33,38] Johnson *et al.*[33,35] found that fluoroquinolone resistance and also trimethoprim/sulfamethoxazole resistance were significant markers of ST131 *E. coli* in Canada. This finding is not consistent through all regions, however.[47] The mechanism of fluoroquinolone resistance in ST131 isolates varies, depending on the level of resistance. Amongst *E. coli*, low-level fluoroquinolone resistance is usually due to a single mutation in genes encoding fluoroquinolone targets.[68] The presence of plasmid-mediated quinolone resistance genes, including *qnrA*, *qnrS* and *qnrB*, may also contribute to low-level resistance, although they are infrequently described in the ST131 clone.[34,36,43,69] Less common variations, including *qnrB1* and *qnrB2*, have also been reported associated with ST131.[22,70] The 'dual substrate' aminoglycoside-modifying enzyme AAC(6′)-Ib-cr also contributes to quinolone resistance via acetylation of selected fluoroquinolones.[71,72] The effect of these plasmid-mediated genes on fluoroquinolone MICs is greater in combination than in isolation.[72]

When present, high-level fluoroquinolone resistance in ST131 is generally due to chromosomal mutations of genes coding the fluoroquinolone targets *gyrA*, *gyrB*, *parC* and *parE*, as described in other *E. coli*.[68,73] Studies of a ciprofloxacin-resistant clone (MIC_{90} ≥32 mg/L) showed multiple mutations in *gyrA* at codons Ser-83 and Asp-87, generating Ser-83 → Leu, Asp-87 → Asn, Asp-87 → Gly or Asn-87 → Tyr amino acid changes, and further single or double mutations of *parC* at Ser-80 and/or Glu-84 codons (Ser-80 → Ile and Glu-84 → Val or Glu-84 → Gly).[22]

The aminoglycoside-modifying enzyme AAC(6′)-Ib-cr is frequently associated with ST131 (Table 2).[10,11,34,62] Other aminoglycoside resistance enzymes have been detected less frequently and sometimes in combination.[74] Resistance to aminoglycosides remains variable, despite the presence of the *acc(6′)-Ib-cr* gene. In one study where 69% of 96 ST131 *E. coli* isolates possessed this gene, 35%, 49% and 35% of isolates were resistant to gentamicin, tobramycin and amikacin, respectively.[69]

Plasmids

The initial descriptions of ST131 demonstrated the IncFII group of plasmids harbouring CTX-M-15.[11] IncFII plasmids may also encode other types of β-lactamases found in ST131 *E. coli*, including SHV-12 and CMY-2.[22,50,60] Greater clonal complexity among plasmids encoding CTX-M-15 is now apparent, with the multireplicons FIA, FIB and FII having been described in CTX-M-15-carrying plasmids of ST131 *E. coli*.[11,27,36,75] In a Norwegian study of 23 ST131 strains, the CTX-M-15 gene was related to IncFII, FIB and FIA (87%, 44% and 42%, respectively).[27]

The full sequences of two CTX-M-15-carrying plasmids of representative ST131 *E. coli* have been characterized and demonstrated extensive resistance gene profiles. The plasmid of one isolate, pEK499 (strain A: 117 536 bp), a fusion of type FII and

Table 2. ESBL, AmpC and aminoglycoside-modifying enzyme genes carried by *E. coli* ST131

Location	Number of ST131 with extended-spectrum phenotype	CTX-M-3 % (*n*)	CTX-M-14 % (*n*)	CTX-M-15 % (*n*)	Other CTX-M genes % (*n*)	Other extended-spectrum genes % (*n*)	*aac(6′)-Ib-cr* % of ST131
Multiple continents[10,11,45a]	70			99 (69)	CTX-M-1=1 (1)		100[b]
Australia[47]	8			50 (4)	untyped CTX-M=25 (2)	CMY-2=25 (2)	
Belgium[28d]	31			100 (31)			
Cambodia[23]	8		75 (6)	13 (1)	CTX-M-27=13 (1)		
Canada[23,34,69]	134		11 (15)	87 (117)	CTX-M-2=<1 (1), CTX-M-61=<1 (1)		75[b]
Central African Republic[23]	5			100 (5)			
Croatia[29]	5			100 (5)			
France[23,24]	33		21 (7)	85 (28)		TEM-24=3 (1)	
India/Pakistan/ Bangladesh[45]	19			100 (19)			
Italy[22]	11		0	91 (10)		SHV-12=9 (1)	100
Japan[41]	27		74 (20)		CTX-M-2=11 (3), CTX-M-35=15 (4)		
Korea[43]	6		17 (1)	67 (4)	CTX-M-22=17 (1)		
Norway[27]	9			89 (8)	CTX-M-1=11 (1)		
Spain[19,21,53,61,106]	82[c]		10 (8)	66 (54)	CTX-M-9=9 (7), CTX-M-10=2 (1), CTX-M-32=4 (3)	SHV-12=1 (1), CMY-2=2 (3), c-AmpC=5 (4)	100[b]
Turkey[23,31]	3	33 (1)		66 (3)			
UK[12,23]	272	19 (52)		81 (220)[d]			
USA[36,37]	37		14 (5)	78 (29)		SHV-5 or -7=8 (3)	63[b]

c-AmpC, chromosomal AmpC gene.
[a]Isolates selected for CTX-M-15 genotype by researcher.
[b]Data only available on a selection of isolates from this country.
[c]One isolate contained CTX-M-14 and CTX-M-15.
[d]Some isolates CTX-M-28.

FIA replicons, harboured resistance genes for 10 antibiotics from eight classes: $bla_{CTX\text{-}M\text{-}15}$; $bla_{OXA\text{-}1}$; $bla_{TEM\text{-}1}$; *aac(6′)-Ib-cr*; *mph*(A); *catB4*; *tet*(A); and the integron-borne *dfrA7*, *aadA5* and *sulI* genes. These were responsible for cephalosporin, β-lactamase inhibitor, aminoglycoside, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole resistance.[74]

Detection of O25b-ST131

The three major characteristics of O25b-ST131 *E. coli* are its serogroup (O25b), its phylogenetic group (B2) and its ST (ST131). Each of these characteristics has been used to aid detection. Of note, a variety of molecular techniques have been used to determine clonality in previously described clones. The ST131 'pandemic' is amongst the first examples where MLST has been the defining technique in describing a widespread bacterial strain. The power of this technique is demonstrated in several studies where reanalysis by MLST of previously defined PFGE groups has confirmed a much broader clonality than originally suspected.[13,33] This increased resolution does complicate comparison of the scope of ST131 to previous outbreaks, however.

MLST

MLST first delineated the pandemic clone and remains the 'gold standard' for identification. This requires the sequencing of pre-specified regions of highly conserved housekeeping genes, allowing comparison of nucleotide sequences with publically accessible databases. Hitherto, two separate schemas for sequencing and classification are available. Achtman *et al.*[6] defined and continue to maintain the database most frequently utilized in ST131 studies (http://mlst.ucc.ie/mlst/dbs/Ecoli).[9,10,14,37,47,76] This scheme is based on the alleles of seven housekeeping genes: *adk* (adenylate kinase); *fumC* (fumarate hydratase); *gryB* (DNA gyrase); *icd* (isocitrate dehydrogenase); *mdh* (malate dehydrogenase); *purA* (adenylosuccinate synthetase); and *recA* (ATP/GTP binding motif). An alternate *E. coli* MLST scheme also using seven housekeeping genes, operated by Michigan State University, USA (http://www.shigatox.net/ecmlst), has also been used.[34,45] All but one of the housekeeping genes used in this scheme differ from the method proposed by Achtman *et al.*[6]

PCR-based rapid detection methods

Rapid detection methods have been developed to overcome the labour intensity of MLST. Rapid detection of ST131 using a single-nucleotide polymorphism (SNP) method based on only two housekeeping genes from the Achtman MLST schema (*mdh* and *gryB*) has been developed. The O25b variants showed the SNP on C288T and C525T for *mdh*; and on C621T, C729T and T735C for *gyrB*.[33] This method has shown 100% sensitivity. When verified on a broader sample, it is likely that this method can be used as an alternative option to full MLST.

PCR-based methods to detect the phylogenetic[77] and O25 type,[76] followed by the confirmation of selected samples using MLST, have also been used.[10,27,31,38,76] This technique for detecting the O serotype O25b is based on a method originally used to type important *E. coli* causing septicaemia.[78] This O25b typing uses the specific primers rfb1bis.f (5′-ATACCGACGACGCCGATCTG-3′) and rfbO25b.r (5′-TGCTATTCATTATGCGCAGC-3′).[76] A more accurate duplex PCR-based method to detect this clone was developed by the same group. This duplex PCR-based detection method for O25b-ST131 uses allele-specific PCR for the *pabB* gene unique to phylogenetic group B2 subgroup I isolates of O type 25b.[23] The duplex PCR has been successfully used as a rapid screening method for O25b-ST131 *E. coli* in many countries.[23,36,47,79]

A PCR method on a real-time platform has recently been described. This assay utilizes amplicon melt curve analysis of two regions of the *pabB* gene. A third amplicon based on the group 1 CTX-M gene can be used to simultaneously detect the presence of $bla_{CTX\text{-}M\text{-}15}$.[80]

A third technique using triplex PCR to specifically detect CTX-M-15-producing O25b-ST131 *E. coli* is also described, based on the detection of the operon afa FM955459, *rfb*O25b and the 3′ end of $bla_{CTX\text{-}M\text{-}15}$.[21]

Repetitive sequence PCR

A semi-automated repetitive sequence-based PCR typing technique (DiversiLab®, bioMerieux) has been found to reliably identify the pandemic clone.[47,69,81,82] Although ≥95% similarity to a known ST131 strain was used to define presumed ST131 by DiversiLab in a Canadian study,[69,82] other authors have shown that ST131 strains may have similarities as low as 92%.[47,81]

PFGE

PFGE has been used to determine relationships amongst the ST131 complex, rather than to identify the clone in broader collections. The similarity of ST131 on PFGE depends on the origin of the collection. The majority of ST131 strains have similarities of ≥80% by PFGE, corresponding to differences of four to six bands.[83] However, a minority of isolates show quite a diverse PFGE pattern. For example, the similarities of ST131 *E. coli* from the UK, Chicago and Japan were only 73%,[13] 67%[36] and 70%,[41] respectively.

Virulence

E. coli ST131 is primarily an extraintestinal pathogenic *E. coli* (ExPEC) harbouring virulence genes required for successful pathogenic invasion of a human or animal host. These virulence genes allow the clone to do the following: to attach; to avoid and/or subvert host defence mechanisms within extraintestinal sites; to scavenge limiting nutrients, such as iron, from the host; and to incite a noxious host inflammatory response, cumulatively leading to extraintestinal diseases. The putative virulence genes possessed by ExPEC can be classified into at least five categories based on their function: adhesins; toxins; protectins (capsule synthesis); siderophores; and other additional virulence genes. There are 10 commonly described virulence genes in ST131 *E. coli*. They include *iha* and *fimH* (encoding the adhesin-siderophore receptor and type I fimbriae, respectively), *sat* (secreted autotransporter, a type of toxin), *kpsM* (encoding protectin II, involved in group II capsular polysaccharide synthesis), *fyuA* and *iutA* (encoding siderophores involved in synthesis and uptake of ferric yersiniabactin and aerobactin, respectively), *usp*

(uropathogenic-specific protein), *traT* (surface exclusion, serum resistance-associated), *ompT* (outer membrane protease), and *malX* (pathogenicity island marker).[10,33] The adhesins, *iha* and *fimH*, were identified in 91%–100% of O25b-ST131.[10] In addition to *iha*, Canadian O25b-ST131 *E. coli* isolates possessed the P fimbriae subunit F10 allele (98%).[33] Unlike the other typical ExPEC *E. coli*, including CGA and O15:K52:H1 *E. coli*, O25b-ST131 *E. coli* did not possess typical fimbriae and pilus tip adhesion molecules for pyelonephritis, such as those encoded by the *papA* allele, the P fimbriae structural subunit F16 allele and the *papG II* allele.[33] In Korean isolates, however, the *papG III* allele was identified in all ST131 studied.[43] The *sat* gene was present in 95%–100% of O25b-ST131 *E. coli*.[10,33] This is also a common toxin possessed by the other two types of *E. coli* (CGA and O15:K52:H1).[33] The *fyuA* and *iutA* genes, encoding the two siderophore virulence factors, were present in 95%–100% of O25b-ST131 *E. coli*.[10,33] The *kpsM II* gene was detected in 94% of O25b-ST131 CTX-M-15-producing *E. coli*.[10] In contrast, this gene appeared less frequently (54%) amongst O25b-ST131 *E. coli* in Canada that were mostly non-ESBL producers but fluoroquinolone resistant.[33] The other common *E. coli* virulence genes *usp*, *traT*, *ompT* and *malX* also appeared in nearly all ST131 *E. coli*.[10,33]

A clinical report of septic shock and emphysematous pyelonephritis, in a previously healthy individual with CTX-M-15-producing ST131, described the presence of these 10 virulence genes plus *afa* and *dra* (central region of Dr antigen-specific fimbriae, associated with binding and invasion in the mammalian urinary tract[84]).[55] These latter two virulence genes occurred in ~20% of ST131 isolates tested.[10]

The *ibeA* gene, encoding an invasion determinant associated with neonatal meningitis, has been detected in 34% of non-ESBL-producing ST131 *E. coli* blood culture isolates from north-west Spain.[53] This gene has only been infrequently reported in other collections.[33,43]

The ST131 clone has also been identified amongst adherent-invasive *E. coli* (AIEC) from intestinal and extraintestinal disease. This pathovar, distinguished from other ExPEC strains by a unique phenotype of adhesion and invasion properties, is associated with inflammatory bowel disease.[85] The intestinal AIEC phenotype ST131 carried multiple virulence genes infrequently described in the clone, including *papC*, *hlyA* and *cnf1*.[53,86]

Clermont *et al.*[76] demonstrated *in vitro* and *in vivo* virulence of the ST131 clone. Biofilm formation identified *in vitro* is a potential contributor to the long-term persistence of the clone in various environments and its resistance to host immune defences. High virulence in a 'mouse lethality' model of extraintestinal virulence was speculated to be due to unspecified virulence genes harboured by the clone.

Human infection

The spectrum of clinical infection caused by the ST131 clone appears broadly similar to that of other *E. coli*. UTI, representing the most common site of human infection with *E. coli*, is predominant. Description ranges from uncomplicated cystitis to severe infection complicated by bacteraemia, renal abscess and emphysematous pyelonephritis.[32,55] Pitout *et al.*[34] identified a propensity for urinary sepsis above other sites of infection when comparing ST131 and non-ST131 *E. coli* bacteraemia. Johnson *et al.*,[38] studying urinary tract origin isolates, found no clear correlation between ST131 and any particular clinical syndrome of renal tract infection.

Other sites of infection have included the respiratory tract, ascitic fluid, intra-abdominal abscess, bones/joints and bacteraemia without a clinically apparent focus.[41,56,87] ST131 has also been reported as a prominent cause of *E. coli* neonatal sepsis.[56] An exception to the usual spectrum of *E. coli* infection has been the description of *E. coli* ST131 pyomyositis amongst patients with haematological malignancy.[39]

Two reports illustrate direct transmission or the sharing of an identical ST131 clone between humans. Transmission of ST131 *E. coli* from an elderly father with pyelonephritis to his adult daughter after brief contact caused her to suffer a similar illness.[55] Similarly, an identical isolate was recovered from an osteoarticular infection in a young child and a faecal sample from her mother.[56]

Treatment

As mentioned above, the ST131 clone can harbour a diverse range of antimicrobial resistance mechanisms. Few descriptions of infections with the clone include details of antimicrobial therapy. Isolates harbouring CTX-M genes have been successfully treated with carbapenems alone or in combination with amikacin.[39,55] For the clinician, even with identification and susceptibilities of a pathogenic *E. coli*, the ST of the isolate is unlikely to be known. Hence, comment on therapy is based on the commonly encountered antibiotic resistance phenotypes of ST131, which would be expected to respond in a similar manner to other STs with the same antimicrobial phenotype.

Non-ESBL-producing, fluoroquinolone-resistant isolates

Fluoroquinolone resistance is a hallmark of ST131 in many series. Although not harbouring an ESBL gene, such clones frequently carry resistance to other antibiotics. Among UTI isolates, the incidence of co-resistance to trimethoprim/sulfamethoxazole was 42% in Canada,[33] 47% in Korea[43] and 70% in a European collection (including other STs).[18] Carriage of non-extended-spectrum β-lactamase enzymes confers resistance to narrow-spectrum β-lactams, with ampicillin resistance rates ranging from 90% to 94%.[18,33,43] Fortunately, almost all isolates not producing ESBLs or AmpC remain susceptible to the third-generation cephalosporins, such as ceftriaxone and cefotaxime.[38,43] In severe infection with a strain not producing ESBLs or AmpC, these would be potentially reliable treatment options. Oral therapy with an agent such as amoxicillin/clavulanate or trimethoprim/sulfamethoxazole, if susceptibility is confirmed, could also be used in less severe infection, such as uncomplicated UTI.

ESBL-producing isolates

Parenteral therapy

Using older breakpoints, ESBL-producing *E. coli* isolates may test within the susceptible MIC range to some third-generation cephalosporins. In this circumstance, many regions' laboratory

standards suggest reporting resistance to these agents due to uncertainty about their efficacy in this setting.[88] Concern arises from studies suggesting poorer outcomes with third- and fourth-generation cephalosporin therapy against ESBL producers.[89,90] Some authors suggest that β-lactam/β-lactamase inhibitor combinations may be effective where *in vitro* susceptibility of the isolate is demonstrated.[91,92] The parenteral combination piperacillin/tazobactam has been used for UTIs and other infections, including bacteraemia, skin structure infection and pneumonia, although published experience is limited.[91,92]

Amongst ST131 clones, including those not producing ESBLs, concurrent aminoglycoside resistance is frequent. Reported rates of gentamicin resistance range from 44% amongst non-ESBL-producing isolates in Korea[43] to 86% resistance in CTX-M ESBL-producing isolates.[34] Amikacin resistance is less well characterized, but also present at high rates amongst ESBL-producing isolates.[10] Even in the setting of *in vitro* susceptibility, uncertainty remains about therapeutic efficacy in severe infections, such as bloodstream infection.[93]

Carbapenems are the treatment of choice in serious ESBL-producing infection.[94] Several studies demonstrate successful therapy of UTI and non-urinary tract serious infection with meropenem or imipenem/cilastatin.[95,96] Ertapenem, a newer narrower spectrum agent, has a limited body of experience that also suggests successful therapy in ESBL-producing *E. coli* infection.[97,98] There is a report of the emergence of carbapenem resistance in *E. coli* whilst on ertapenem therapy.[99]

Tigecycline is a glycylcycline derived from minocycline with good *in vitro* activity against ESBL-producing *E. coli*.[100] There is some uncertainty about its potential drug concentrations achieved in the urinary tract.[101] However, a case report has documented successful outcomes in UTI caused by ESBL-producing *E. coli* and other highly resistant Enterobacteriaceae.[102] Temocillin, a derivative of ticarcillin with stability to β-lactamase hydrolysis and *in vitro* activity against the majority of ESBL-producing Enterobacteriaceae, is a potential therapeutic option in this setting. There is limited published experience in the treatment of a variety of ESBL-producing infections.[103]

Oral therapy

The oral combination amoxicillin/clavulanate has been used effectively in uncomplicated ESBL-producing *E. coli* cystitis when *in vitro* susceptibility is confirmed.[104] Of note, ESBL strains co-producing the non-extended-spectrum β-lactamase OXA-1 may be resistant to β-lactamase inhibitor combinations.[105]

Fosfomycin is an oral antimicrobial that inhibits cell wall biosynthesis. It has been used for the treatment of ESBL-producing *E. coli* cystitis with a high success rate.[104] Of concern, a recent report demonstrates a rapid rise in resistance rates amongst ESBL-producing ST131 clones to 22% in Spain, which is closely tied to increasing use of fosfomycin.[106]

Nitrofurantoin is a synthetic nitrofuran antimicrobial with a long history of use in uncomplicated UTI.[107] No papers directly describe the susceptibility of ST131 isolates. Amongst a European collection of fluoroquinolone-resistant non-ESBL-producing isolates, including ST131, 86% were susceptible to this agent.[18] Amongst Spanish ESBL-producing *E. coli*, 87% were susceptible.[20] It must be noted that nitrofurantoin is only useful in cystitis and not in renal infection *per se*.

Conclusions

Emerging from 'molecular obscurity' in the first decade of this century, ST131 *E. coli* is now a worldwide pathogen causing potentially severe antimicrobial-resistant infections. Disseminating in conjunction with this clone is resistance to many low-cost and easily available antimicrobials commonly used to treat *E. coli* infection. Due to the rapid evolution of this worldwide pandemic, relatively little is known about this foe.

Molecular epidemiological study is increasingly describing the clone's widespread but heterogeneous distribution amongst humans and animals. The vast majority of these data emanate from the developed world. Little is known about the distribution of ST131 in many parts of the developing world, areas suspected to have high rates of infection and which have even been postulated as reservoirs of the pathogen.[57] These areas, in addition, have a population particularly vulnerable to morbidity and mortality from resistant infection due to the limited healthcare resources available.

Two key elements required for potential control on a broader scale as a public health measure require fuller elucidation. The first is a deeper understanding of the genetics of the ST131 clone, including greater insight into why ST131 has become so finely tuned to acquire both resistance and virulence, and to rapidly disseminate on a vast scale. Research in this area should also increase our understanding of the risk of horizontal transmission of mobile resistance elements amongst ST131, between varying *E. coli* clones and, potentially, to other Enterobacteriaceae. The second element is knowledge of the dynamics of transmission and dissemination of ST131 on a population basis. We have little firm information on many of the classical descriptors of communicable disease control: reservoirs; mode of transmission; incubation period; period of communicability; susceptibility; and methods of control.[108]

Given the rapid spread of the ST131 clone and its demonstrated ability to cause severe infection in otherwise healthy individuals, consideration must be given to the planning of public health measures to attempt to control infection. A parallel could be drawn to community-associated methicillin-resistant *Staphylococcus aureus*. In order to successfully plan and execute interventions, we will need further information on key aspects of this pathogen and the dynamics of transmission.

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Community-Onset *Escherichia coli* Infection Resistant to Expanded-Spectrum Cephalosporins in Low-Prevalence Countries

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By global standards, the prevalence of community-onset expanded-spectrum-cephalosporin-resistant (ESC-R) *Escherichia coli* remains low in Australia and New Zealand. Of concern, our countries are in a unique position, with high extramural resistance pressure from close population and trade links to Asia-Pacific neighbors with high ESC-R *E. coli* rates. We aimed to characterize the risks and dynamics of community-onset ESC-R *E. coli* infection in our low-prevalence region. A case-control methodology was used. Patients with ESC-R *E. coli* or ESC-susceptible *E. coli* isolated from blood or urine were recruited at six geographically dispersed tertiary care hospitals in Australia and New Zealand. Epidemiological data were prospectively collected, and bacteria were retained for analysis. In total, 182 patients (91 cases and 91 controls) were recruited. Multivariate logistic regression identified risk factors for ESC-R among *E. coli* strains, including birth on the Indian subcontinent (odds ratio [OR] = 11.13, 95% confidence interval [95% CI] = 2.17 to 56.98, $P = 0.003$), urinary tract infection in the past year (per-infection OR = 1.430, 95% CI = 1.13 to 1.82, $P = 0.003$), travel to southeast Asia, China, the Indian subcontinent, Africa, and the Middle East (OR = 3.089, 95% CI = 1.29 to 7.38, $P = 0.011$), prior exposure to trimethoprim with or without sulfamethoxazole and with or without an expanded-spectrum cephalosporin (OR = 3.665, 95% CI = 1.30 to 10.35, $P = 0.014$), and health care exposure in the previous 6 months (OR = 3.16, 95% CI = 1.54 to 6.46, $P = 0.02$). Among our ESC-R *E. coli* strains, the bla_{CTX-M} ESBLs were dominant (83% of ESC-R *E. coli* strains), and the worldwide pandemic ST-131 clone was frequent (45% of ESC-R *E. coli* strains). In our low-prevalence setting, ESC-R among community-onset *E. coli* strains may be associated with both "export" from health care facilities into the community and direct "import" into the community from high-prevalence regions.

Despite a dramatic global rise in the prevalence of expanded-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli*, infections by expanded-spectrum-cephalosporin-resistant (ESC-R) *E. coli* in Australia, New Zealand, North America, and selected European countries remain at relatively low levels. Recent Australian national data show that 3.2% of community isolates carry such resistance. Approximately 80% of these harbor a globally dominant bla_{CTX-M} ESBL gene and 12% a plasmid-borne AmpC-type mechanism (1). European surveillance data show that a significant proportion of countries have ESC resistance rates rates below 10% among invasive *E. coli* isolates (2). In the United States, a recent large sample of *E. coli* isolates indicated that 3.9% were ESBL-producing strains (3). Although these low rates offer reassurance in the near term, a year-on-year rise in the incidence of community-onset ESC-R *E. coli* infections in low-prevalence countries is of concern (2, 4).

Australia and New Zealand are in a globally unique position. We have low rates of use of antimicrobials traditionally identified as a risk factor for ESC-R *E. coli*. This includes very low fluoroquinolone use among humans and a ban on the use of ESC and fluoroquinolones in food production (5, 6). In contrast, we have considerable extramural pressure on antimicrobial resistance rates. Our countries are located within the Asia-Pacific region, with which we share a mobile population (7) and frequent commerce (although no land borders). A high proportion of our regional neighbors have rates of ESC-R among *E. coli* strains in excess of 25% (8, 9).

The aim of our study was to define the risk factors for, and dynamics of, ESC-R among community-onset *E. coli* infections in the low-prevalence settings of Australia and New Zealand by using a case-control methodology. Furthermore, we characterized the resistance genes and membership of the worldwide pandemic clone ST131 in implicated isolates.

MATERIALS AND METHODS

The COOEE Study (COmmunity Onset ESBL and AmpC *E. coli* Study) was a multisite case-control study, with prospective recruitment of patients and data collection. Six geographically dispersed tertiary centers in Australia ($n = 5$) and New Zealand ($n = 1$) participated. The human

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research ethics committees at The University of Queensland and participating sites approved this study.

Definitions. *E. coli* infection was defined as "community onset" where a patient was resident in the community (including nursing homes) or had been hospitalized less than 48 h at the time of onset; "expanded-spectrum cephalosporin resistance" included all "nonsusceptible" isolates and was identified phenotypically. For ceftriaxone, MIC > 1 mg/liter was used. For ceftazidime, laboratories used MIC > 1 mg/liter or MIC > 4 mg/liter, depending on their use of EUCAST or Clinical and Laboratory Standards Institute (CLSI) criteria, respectively (10, 11); "site of infection" was determined by the researcher from available information. Guidance for urinary tract infections (UTI) was given as follows: "asymptomatic" = a positive urine culture, with no attributable symptoms; "lower tract infection" = lower-urinary-tract-only symptoms such as urgency, frequency, and dysuria; and "upper urinary tract infection" = temperature ≥ 38°C, flank pain, or costovertebral angle tenderness and/or any bacteremia from a urinary source. "Immune suppression" referred to use at the time of the sample collection of corticosteroids (>15 mg/day prednisolone or equivalent), calcineurin inhibitors, other nonbiologics (e.g., mycophenylate and methotrexate), cytotoxic agents, biological agents, or radiation therapy; the Charlson comorbidity index (12) was calculated on the basis of data available from the survey, with the exception of neurological impairment (dementia and hemiplegia), which was inadvertently omitted from the survey questioning. A McCabe score was assigned based on the investigator's estimate of participant survival (<1 month, 1 month to 2 years, or >2 years) (13). "International travel" (excluding travel between Australia and New Zealand) was classified into geographical regions as follows: South Pacific islands, southeast Asia, Indian subcontinent, China, Japan, North America, Europe, and Africa/the Middle East. "High-risk travel" (regions of the Indian subcontinent, southeast Asia, Africa, the Middle East, and China) was defined *a priori* based on Australian data (14). "Health care exposure" was assessed by the Friedman criteria (15) with two modifications: (i) day procedures were recorded, and (ii) the criteria were assessed in three "discrete" time periods (<1 month earlier, 1 to 6 months earlier, and 7 to 12 months earlier). In addition, exact dates and details of any hospital admissions or surgical procedures were recorded and the interval (in days) from the termination of health care contact to the date of the first medical review with the enrolling *E. coli* infection subject was calculated. Further definitions are provided in the supplemental material.

Clinical methods. A case-control methodology was used. Case patients with community-onset ESC-R *E. coli* in a culture of blood or urine were identified in the microbiology laboratory of participating hospitals. Control patients had community-onset ESC-susceptible (ESC-S) *E. coli* isolated from the same specimen type (urine or blood) as the case. Controls were not matched by any clinical presentation, comorbidity, or demographic factors. They were selected as the next appropriate patient, after an enrolled case patient, within the same laboratory's specimen registration system. If the next appropriate control patient could not be recruited, the process was repeated, at the same time of day and day of week, in a later week of the study. A single control was recruited for each case.

Inclusions and exclusions. A laboratory-specific protocol was developed by each site to identify all potentially appropriate patients aged ≥16 years with an isolate of ESC-R *E. coli* managed at the participating site. Patients cared for by external health care providers such as family doctors and external clinics (utilizing the participating laboratories as an external provider) were not considered for recruitment, due to the complex human-research ethics requirements in our jurisdiction. Initial screening to determine likely community onset and the presence of exclusion criteria was by review of available electronic laboratory data and/or contact with the clinician caring for the patient. Two exclusion criteria were applied: (i) inability of the patient to give informed consent to participate, and (ii) extra-anatomical urinary drainage such as an indwelling urinary catheter (in the community), intermittent catheterization, ileal conduit, or similar. These two groups whose members local clinicians had already identified as a high risk factor for resistant infection appeared to have relatively distinct demographic and health profiles. Hence, they were excluded in order to focus study resources on a more generalized population group.

Data collection. Hospitalized patients or those attending ambulatory clinics were approached for recruitment and data collection in person, whereas the remainder were contacted by telephone. By telephone, at least three contact attempts on different days were made. After informed consent, including explanation of the aims of this study, a structured interview was conducted using a standardized data collection form completed by an investigator under non-blind conditions. Data were primarily self-reported by participants. Where the participant was uncertain of details (e.g., dates of hospitalization or antimicrobial use) or the details were not clear to the investigators on the basis of the answer(s) provided, the investigators were able to review the patient's medical records held at their institution.

For intermittent exposures (e.g., travel, health care exposure, use of antimicrobials, etc.), participants were asked to recall 12 months before presentation. Exact dates of exposure were recorded. If the exact date was not recalled, it was estimated ("start of month" = the 1st of the month, "middle" or no date specified = 15th, "end of month" = last day).

Data were forwarded to a central coordinator where they were checked and entered into a secure database. Any omissions or discrepancies were clarified with the individual sites.

Laboratory methods. All phenotypic susceptibility data presented in this study have been assessed by EUCAST criteria (10). All nonsusceptible isolates were considered "resistant" for the purpose of this analysis. *E. coli* isolates from each patient were forwarded to the research laboratory, with phenotypic identification and antimicrobial susceptibility undertaken by the use of disk diffusion susceptibility testing (DST), an automated system (VITEK 2), or agar dilution, based on the criteria in use by the laboratory at the time. Where data for susceptibility to an ancillary antimicrobial (e.g., nitrofurantoin) were not available, this was assessed by DST in the research laboratory. Where an isolate was originally tested by CLSI, DST using EUCAST criteria was undertaken (in the research laboratory) for agents for which the nonsusceptibility breakpoints of these two criteria differ (ceftazidime, cefepime, amikacin, gentamicin, ciprofloxacin, and nitrofurantoin). Where stated, MICs were performed by Etest (bioMérieux, France). For each isolate, a summative antimicrobial resistance score was calculated from 11 antimicrobials tested (ampicillin, amoxicillin plus clavulanate, ceftriaxone, ceftazidime, cefepime, meropenem, trimethoprim-sulfamethoxazole [SXT], ciprofloxacin, nitrofurantoin, gentamicin, and amikacin).

After overnight culture, bacterial DNA was extracted using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories). ESC resistance genes were investigated by PCR using previously published primers and conditions (16–18). A stepwise approach based on local epidemiology of resistance mechanisms was employed. All isolates were investigated for $bla_{\text{CTX-M-1}}$ group and $bla_{\text{CTX-M-9}}$ group genes. Isolates negative for these were investigated for $bla_{\text{CTX-M}}$ (consensus sequence), bla_{CMY}, bla_{DHA}, bla_{TEM}, bla_{SHV}, and bla_{VEB}. All isolates were screened for carbapenemase genes (bla_{NDM}, bla_{KPC}, and bla_{IMP}) using an in-house multiplex PCR (19) and a singleplex PCR for $bla_{\text{OXA-48}}$-like enzymes (20). All PCR amplicons were sequenced in the forward and reverse directions using an ABI3730XL (Life Technologies) capillary sequencer and compared to published sequences in GenBank (www.ncbi.nlm.nih.gov/GenBank).

Presumptive ST131 *E. coli* isolates were determined by use of semiautomated repetitive sequence-based PCR (rep-PCR) (DiversiLab, bioMérieux, France). Isolates clustering within 95% similarity to multilocus sequence type (MLST)-confirmed ST131 reference clones, using a Pearson correlation coefficient, were considered members of this clone (21). A selection of isolates ($n = 4$) were confirmed as ST131 by formal MLST analysis (22).

Statistical methods. Sample sizes with overseas travel as a risk factor for resistant infection were calculated. With an estimated annual rate of overseas travel of 250/1,000 population (7), a sample size of 95 cases with

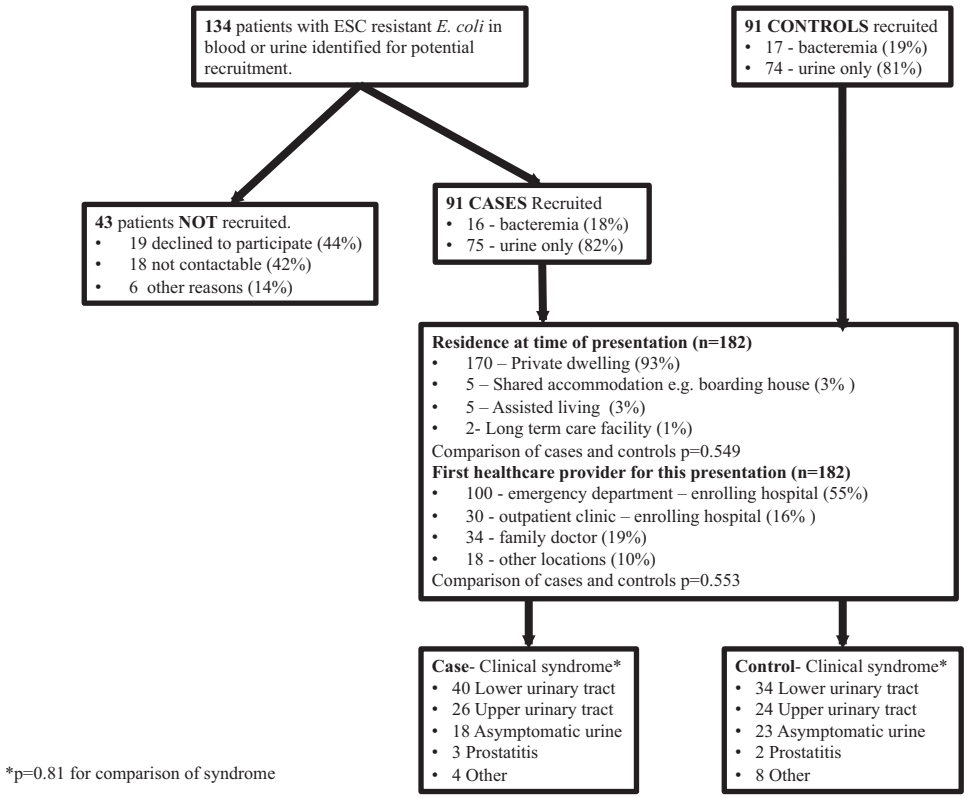

FIG 1 Participant identification and recruitment and characteristics of presentation and clinical syndrome.

matched controls was required to detect this risk with odds ratio ≥ 2.5 (power of 0.8 and two-sided alpha of 0.05).

Continuous data on health care exposure was right-censored at 365 days. Univariate comparison was undertaken by a χ^2 test, Fischer's exact test, Wilcoxon rank sum test, and logistic regression as indicated. Interactions were examined. A multivariate logistic regression model with variables significant in univariate analysis at a $P = 0.2$ level was constructed. Using backward selection, variables were retained in the final logistic regression model if their significance remained below $P = 0.2$. Models were assessed by calculation of a receiver operating characteristic (ROC) curve and Hosmer-Lemeshow goodness of fit. All statistical tests were two-tailed, and $P < 0.05$ was considered significant. STATA version 12.1 (Statacorp) was used.

RESULTS

In total, 182 patients (91 cases and 91 controls) were recruited between March 2011 and October 2012 (Fig. 1). Patients were recruited over 12 continuous months at five sites and over 9 months at one site. Sites contributed between 8 and 58 patients.

Bacteremia was detected in 33 patients (18%), and positive cultures were grown from urine samples collected from the remaining 149 (82%). Uneven numbers of bacteremias occurred as one control patient recruited with a positive urine culture subsequently manifested a positive blood culture. The residences of the patients before presentation, clinical syndromes of presentation, and characteristics of hospital presentation did not differ significantly between case and control patients (Fig. 1).

A further 43 patients with presumed community-onset ESC-R *E. coli* infection and no overt exclusion criteria were not recruited (declined to participate, $n = 19$; not contactable, $n = 18$; other, $n = 6$). On comparison with recruited study participants, the median age (56 years, $P = 0.81$) and gender (11/43, 26% male, $P = 0.39$) did not differ significantly from those of the recruited patients and they were not analyzed further.

Close temporal matching of cases and controls was not frequent. Samples from 9 controls originated from the same calendar day as those from the matched case. For the entire cohort, there was a median interval of 22 days between the dates of collection of the case and control samples.

Phenotype, resistance genes, and ST131. All case patients' *E. coli* isolates demonstrated phenotypic ESC resistance (ceftriaxone plus ceftazidime = 60 [68%], ceftriaxone only = 28 [32%], ceftazidime only = 3 [3%]). For the three *E. coli* isolates with ceftazidime resistance, the MICs of ceftazidime in the study laboratory were >256 mg/liter, 2 mg/liter, and 0.25 mg/liter, respectively. All control patient isolates were susceptible to ceftriaxone and ceftazidime. For all antimicrobials studied, with the exception of meropenem (100% susceptible) and amikacin (case = 4 resistant/91 [4%], control = 0 resistant/91 [0%], $P = 0.121$), resistance was significantly more likely in the ESC-R isolates than in the ESC-susceptible (ESC-S) isolates. For ESC-R *E. coli*, there was significant resistance to the oral therapeutic options investigated, including amoxicillin plus clavulanate (ESC-R = 59/91 [65%] versus ESC-S = 15/91 [16%], $P = <0.001$), ciprofloxacin (57/91 [63%] versus 6/91 [7%], $P < 0.001$) and SXT (64/91 [70%] versus 20/91 [22%], $P < 0.01$).

E. coli isolates from 89 cases (98%) and 90 (99%) controls were available for further analysis. Carbapenemases were not detected in any isolates. Expanded-spectrum cephalosporinase genes were detected in 87 of 89 (98%) ESC-R *E. coli* isolates as follows: for

ESBLs, the $bla_{CTX\text{-}M\text{-}1}$ group (36/89, 40%), $bla_{CTX\text{-}M\text{-}9}$ group (35/89, 39%), $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$ group (3/89, 3%), and $bla_{SHV\text{-}5}$ group ($n = 1$; 1%); and for non-ESBLs, the $bla_{CMY\text{-}2}$ group ($n = 11$; 12%) and $bla_{DHA\text{-}1}$ group ($n = 1$; 1%). The two remaining samples included two of the three *E. coli* isolates with ceftazidime resistance (MICs, 2 mg/liter and 0.25 mg/liter) and contained only $bla_{TEM\text{-}1}$, a non-expanded-spectrum beta-lactamase. ESC nonsusceptibility most likely originated from hyperproduction of this enzyme, with loss of this trait during passage and storage in the case of the isolate with the lower drug MIC.

The worldwide pandemic ST131 clone was presumptively identified in 46 patients (24%), who were significantly more likely to be case patients than controls (40/89 [45%] versus 6/90 [7%], $P < 0.001$). Among ESC-R *E. coli* isolates, ST131 was not associated with any non-CTX-M enzymes. They constituted 54% of the entire group of CTX-M isolates. In total, 24 (60%) harbored a CTX-M-1 group enzyme and 19 (48%) a CTX-M-9 group enzyme ($P = 0.173$ for the comparison). This includes three isolates (8%) harboring both enzymes. There was no significant difference in the proportions of ST131 by sample type (blood versus urine, $P = 0.514$) or hospital site ($P = 0.574$). With the exception of the smallest site (where 0 of 8 were ST131), the clone constituted 19% to 32% of the isolates from each site.

Demographics, comorbidities, and antimicrobial use. Age data were compared by visual inspection of histograms. Cases and controls had similar bimodal distributions, with peaks at approximately 25 and 65 years. Median and 25th to 75th percentiles for ages of cases and controls, respectively, were 61 years (21 to 82) and 59 years (19 to 87) ($P = 0.769$). Results of univariate comparisons of demographic factors and medical comorbidities between cases and controls are shown in Table 1. Male sex was the only variable with a significant difference (odds ratio [OR] = 2.3, 95% confidence interval [CI] = 1.5 to 4.6, $P = 0.018$).

Risk from previous urinary tract infection, renal allograft transplant, and anatomical abnormality of the renal tract was investigated (Table 1). The number of urinary tract infections in the previous year was significantly associated with ESC-R *E. coli*, with an odds ratio of 1.32 (95% CI = 1.08 to 1.63, $P = 0.008$) per infection.

Results of univariate analysis of antimicrobial use in the previous year are shown in Table 1. Where the patient could not recall the antimicrobial taken, it was recorded as "unknown." Exposure to trimethoprim or trimethoprim with sulfamethoxazole (SXT) (OR = 3.02, 95% CI = 1.13 to 8.12, $P = 0.028$) was a significant risk factor for ESC-R *E. coli*. In addition, 7 of 7 patients who had been exposed to an expanded-spectrum cephalosporin (ceftriaxone, ceftazidime, or cefepime) had ESC-R *E. coli* isolated.

Health care exposure. Health care exposure was analyzed using two distinct sets of data. First, health care exposure, classified using Friedman criteria for health care-associated (HA) infection, was analyzed in three time windows, with and without the inclusion of day procedures. Exclusion of day procedures performed marginally better at predicting ESC-R; exposure 0 to 1 month earlier (OR = 3.56, 95% CI = 1.14 to 11.14, $P = 0.029$) and 2 to 6 months earlier (OR = 2.99, 95% CI = 1.50 to 5.98, $P = 0.002$) was associated with ESC-R *E. coli* whereas exposure 7 to 12 months earlier ($P = 0.705$) was not (full details are provided in the supplemental material).

Second, a continuous model of the temporal risk of ESC-R *E. coli* infection after health care exposure was generated using the exact time interval since last hospital admission. Day procedures were excluded based on the results of the first analysis. This smoothed curve of the odds ratios shows the lower bound of the 95% CI approaching an odds ratio of 1.0 at approximately 4 to 5 months (Fig. 2).

Travel, community, and occupational exposure. Travel in the previous year was analyzed by region. Travel to the Indian subcontinent approached but did not achieve significance ($P = 0.09$). Birth on the Indian subcontinent was a significant risk factor (OR = 6.119, 95% CI = 1.32 to 28.44, $P = 0.021$) (Table 1).

Occupational exposure to animals, medical patients, and potential household risks was assessed, as was consumption of a variety of meats. No factors were significant (Table 1). Probable household transmission of ESC-R *E. coli* was suggested in one case where the partner of an enrolled patient had an infection with a highly similar isolate (99% identical by rep-PCR using Diversilab) 3 months prior.

Multivariate analysis. For the multivariate model, health care exposure in the previous 6 months, excluding day procedures, was selected as a pragmatic option (univariate OR = 2.95, 95% CI = 1.59 to 5.46, $P = 0.001$). This dichotomous measure was nonsignificantly different from the four-category measurement used earlier (likelihood ratio test $P = 0.821$). Travel to high-risk regions was selected from the travel group (OR = 1.97, 95% CI = 0.94 to 4.11, $P = 0.071$). Use of an expanded-spectrum cephalosporin was combined with use of trimethoprim and SXT, in order to enter the former into the model, given its accepted prominence as a risk factor for ESC-R *E. coli* infection.

Significant variables in multivariate analysis were health care exposure, excluding day procedures in the previous 6 months ($P = 0.002$), birth on the Indian subcontinent ($P = 0.004$), travel to high-risk regions ($P = 0.011$), SXT/ESC use ($P = 0.014$), and number of UTIs in the previous year ($P = 0.003$) (Table 2). Assessment of the final model demonstrated an area under the ROC curve of 0.77 and a nonsignificant Hosmer-Lemeshow goodness of fit ($P = 0.289$).

Interactions and alternative models. A significant correlation between travel to high-risk regions and region of birth occurred. Those born in high-risk regions were more likely to undertake high-risk travel than those born elsewhere (17/28 [61%] versus 21/154 [14%], $P < 0.001$). This was particularly noted for birth and travel to the Indian subcontinent (7/13 [54%] versus 31/169 [18%], $P = 0.002$). This correlation, and the use of differing parameters for health care contact and antimicrobial exposure, were explored in alternative multivariate models (see the supplemental material). Specific population subgroups (symptomatic patients only, ESC-R $bla_{CTX\text{-}M}$ patients only, and ESC-R ST131 patients only) were also tested in the model. None of the alternative models performed better than the final model, although the levels of significance of health care exposure, male sex, and region of birth/travel differed depending on the model parameters selected.

HA and non-HA ESC-R *E. coli*. A difference in the levels of risk of health care-associated (HA) ESC-R *E. coli* and non-HA ESC-R *E. coli* was separately investigated by analysis of risks within the HA ($n = 73$) and non-HA ($n = 109$) cohorts (full details are provided in the supplemental material). Several of the identified risks for ESC-R *E. coli* appeared to be most concentrated in one cohort. Data corresponding to travel to high-risk regions ($P = 0.001$), birth on the Indian subcontinent ($P = 0.006$), and male sex ($P = 0.018$) were statistically significant only among the mem-

TABLE 1 Univariate analysis of demographics, comorbidities, antimicrobial use, region of travel and birth, occupational and household exposure

Subject variable	Frequency in ESC-R cases (%) ($n = 91$)	Frequency in ESC-S controls (%) ($n = 91$)	Odds ratio	95% CI	*P* value
Demographics + comorbidities					
Male sex	30 (33)	16 (18)	2.31	1.51–4.62	0.018[b]
Age < 30 or > 59 yrs	66 (73)	60 (66)	1.36	0.72–2.57	0.336
Immune suppression	19 (20)	10 (11)	1.99	0.87–4.60	0.105[b]
Charlson score ≥ 1	44 (48)	34 (37)	1.57	0.87–2.83	0.135[b]
Active malignancy	11 (13)	9 (8)	1.43	0.55–3.73	0.469
Renal failure	11 (13)	9 (10)	1.25	0.49–3.19	0.636
McCabe score ≥ 2+	78 (86)	76 (84)	1.18	0.53–2.65	0.681
Indigenous	7 (8)	6 (7)	1.18	0.38–3.66	0.774
Heart disease	7 (8)	7 (8)	1		
Long-term-care-facility resident	1 (1)	1 (1)	1		
Smoker	12 (13)	14 (15)	0.83	0.36–1.92	0.672
Liver disease	3 (3)	4 (4)	0.74	0.16–3.41	0.701
Lung disease	5 (5)	7 (8)	0.70	0.21–2.85	0.552
Pregnant or postpartum	3 (3)	7 (8)	0.41	0.10–1.63	0.206
Renal tract background					
Renal transplant	8 (9)	4 (4)	2.1	0.61–7.22	0.241
Anatomical or structural abnormality	23 (25)	15 (16)	1.71	0.83–3.55	0.147[b]
UTIs in past 12 mos (per UTI)[c]	Median = 1; IQR = 0–3	Median = 0; IQR = 0–1	1.32	1.08–1.63	0.008[b]
UTIs in lifetime (per UTI)[c]	Median = 2; IQR = 0–5	Median = 2; IQR = 0–5	1.03	0.90–1.18	0.657
Antimicrobial use					
Any antimicrobials in past 12 mos	69 (76)	62 (68)	1.47	0.76–2.81	0.249
Trimethoprim ± sulfamethoxazole	16 (17.58)	6 (6.59)	3.022	1.13–8.12	0.028[b]
Expanded-spectrum cephalosporin(s)	7 (8)	0	NA		0.014[b]
Fluoroquinolone(s)	7(8)	3 (3)	2.44	0.61–9.77	0.206
β-Lactam + ß-lactamase inhibitor	16 (17.58)	11 (12.09)	1.552	0.68–3.56	0.300
Carbapenem(s)	3 (3.3)	2 (2.2)	1.517	0.25–9.30	0.652
Aminoglycoside(s)	5 (5)	4 (4)	1.26	0.33–4.87	0.733
Macrolide	6 (6.59)	5 (5.49)	1.214	0.36–4.13	0.756
"Unknown" antimicrobial(s)	35 (38)	33 (36)	1.1	0.60–2.00	0.759
Narrow-spectrum cephalosporin(s)	16 (17.58)	15 (16.48)	1.081	0.50–2.34	0.844
Narrow-spectrum penicillin(s)	10 (10.99)	14 (15.38)	0.679	0.28–1.62	0.383
Travel by region[a]					
Any overseas travel	28 (30.8)	22 (24.18)	1.39	0.72–2.68	0.32
High-risk regions	24 (26)	14 (15)	1.97	0.94–4.11	0.071[b]
Indian subcontinent	6 (6.59)	1 (1.1)	6.928	0.75–53.87	0.09
North America	5 (5.49)	2 (2.20)	2.199	0.49–13.69	0.264
Africa + the Middle East	3 (3.3)	2 (2.2)	1.517	0.25–9.30	0.652
Southeast Asia	15 (16)	13 (14)	1.18	0.53–2.65	0.681
South Pacific	3 (3.30)	3 (3.30)	1		
Europe	3 (3.30)	5 (5.49)	0.586	0.14–2.53	0.474
China	4 (4.4)	0			0.121
Japan	1 (1.1)	0			0.500
Birth by region					
High-risk region	18 (20)	10 (11)	2.0	0.87–4.60	0.105
Indian subcontinent	11 (13)	2 (2)	6.12	1.32–28.45	0.021[b]
Australia + New Zealand	58 (64)	59 (65)	0.95	0.52–1.75	0.877
Europe	15 (16)	18 (20)	0.80	0.38–1.71	0.564
Southeast Asia	3 (3)	4 (4)	0.74	0.16–3.41	0.701
Africa + Middle East	2 (2)	4 (4)	0.49	0.09–2.74	0.415
China	2 (2)	0			0.497
South Pacific	0	3 (3)			0.246
Latin America	0	1 (1)			1.0
Occupation and household exposure					
Partner with recent ESC-R *E. coli* infection	2 (2)	Not assessed			
Occupational health care exposure	10 (11)	7 (8)	1.48	0.54–4.08	0.447

(Continued on following page)

TABLE 1 (Continued)

Subject variable	Frequency in ESC-R cases (%) ($n = 91$)	Frequency in ESC-S controls (%) ($n = 91$)	Odds ratio	95% CI	*P* value
Pet cat or dog or both at home	32 (35)	33 (36)	0.95	0.52–1.75	0.877
Occupational animal exposure	4 (4)	5 (5)	0.79	0.21–3.05	0.733
Preschoolers at home (<5 yrs of age)	7 (8)	9 (10)	0.76	0.27–2.13	0.601
Food consumption					
Any meat in past 12 mos	89 (98)	87 (98)	2.05	0.37–11.46	0.415
Poultry	88 (97)	83 (92)	2.47	0.62–9.89	0.206
Processed/preserved meats	51 (56)	52 (58)	0.93	0.52–1.68	0.814
Pork	60 (66)	63 (70)	0.83	0.44–1.55	0.558
Red meat	76 (84)	78 (88)	0.72	0.31–1.66	0.433

[a] Destinations of travel by region were as follows: for the Indian subcontinent, India, Pakistan, Nepal, and Bangladesh; for North America, the United States and Canada; for Africa and the Middle East, Zimbabwe, Kenya, Sudan, Liberia, Turkey, and Afghanistan; for Southeast Asia, Malaysia, Singapore, Thailand, Laos, Cambodia, Vietnam, Burma, Indonesia, and The Philippines; for South Pacific, New Caledonia, Papua New Guinea (PNG), Fiji, Samoa, Cook Islands, and boat cruises through the South Pacific; for Europe, the United Kingdom, Italy, Holland, Portugal, and Poland; for China, China, Hong Kong, and Macau; for Japan, Japan. High-risk regions include the Indian subcontinent, Africa, the Middle East, Southeast Asia, and China regions.

[b] Entered into multivariate model.

[c] Infections were recorded numerically on a scale of 0 to 5+, with all 5+ results considered 5 for analysis. Summaries are presented as a median value and an interquartile range (IQR).

bers of the non-HA group. Conversely, data corresponding to a risk from SXT or ESC use were statistically significant only among members of the HA group ($P = 0.026$). The numbers of UTIs in the previous year were nonsignificantly different among the members of either group assessed separately.

Correlates of the classes of ESC resistance enzymes. Correlates of ESC resistance enzyme classes were investigated by a comparison of patients harboring *E. coli* with CTX-M group enzyme to those harboring other enzymes ("non-CTX-M" = CMY, DHA, SHV, TEM). Full details are provided in the supplemental material.

There was no statistically significant difference with respect to the site of infection between CTX-M-harboring and non-CTX-M-harboring participants ($P = 0.473$), and although bacteremia was more frequent in the CTX-M group, this did not reach statistical significance (15/73 [21%] versus 0/15 [0%], $P = 0.063$). A significantly higher median resistance score was present in CTX-M isolates than in non-CTX-M isolates (median = 6 [interquartile range = 5 to 7] versus median = 4 [interquartile range = 4 to 5], $P = 0.001$). Notable differences included higher rates of resistance to the non-beta-lactam oral agents ciprofloxacin (56/74 [76%] versus 1/15 [7%], $P < 0.001$) and SXT (60/74 [81%] versus 4/15 [27%], $P < 0.001$) among the members of CTX-M group.

In regard to potential risk factors, the members of the CTX-M group were significantly more likely to have had health care exposure in the previous 6 months than the members of the non-CTX-M group (45/74 [61%] versus 3/15 [20%], $P = 0.005$) although the same was not true with respect to health care exposure in the previous 12 months ($P = 0.72$). Other factors used in the multivariate model that trended toward significance among the members of the CTX-M group included more high-risk travel ($P = 0.052$) and fewer reported UTIs in the previous 12 months ($P = 0.054$). Comparisons of factors not included in the multivariate model showed that "any overseas travel" was more likely in the CTX-M group (27/74 [36%] versus 1/15 [7%], $P = 0.033$).

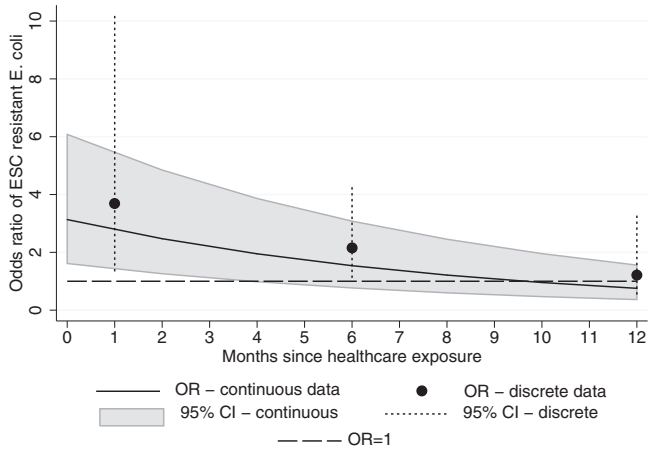

FIG 2 The risk of ESC-R *E. coli* infection over a 12-month period after the most-recent episode of health care exposure, excluding day procedures, estimated with two data sets. The smoothed curve was calculated using continuous data corresponding to the months since hospital admission (black line; 95% CI in gray). Discrete intervals determined using Friedman criteria are indicated (black dots; 95% CI as vertical dashes). The dashed line represents no increased risk (odds ratio = 1.0).

DISCUSSION

This multicenter prospective case-control study of community-onset ESC-R *E. coli* infection has several key findings that have implications for risk-based empirical antibiotic prescription and infection control practices and for control of ESC-R *E. coli* infections within communities.

TABLE 2 Multivariate logistic regression

Subject variable	Odds ratio	95% CI	*P* value
Health care exposure in the previous 6 mos	3.16	1.54–6.46	0.002
UTIs in previous yr (per UTI)	1.43	1.16–1.82	0.003
Birth on the Indian subcontinent	11.13	2.17–56.96	0.004
Travel to high-risk region(s)	3.09	1.29–7.38	0.011
Trimethoprim ± sulfamethoxazole ± ESC use	3.67	1.30–10.35	0.014
Male sex	2.17	0.97–4.84	0.060

First, we established that 6 months is a practical, evidence-based definition for the duration of increased risk of a community-onset *E. coli* isolate harboring ESC-R after health care exposure. The time-dependent relationship of health care exposure to resistance seems intuitive in nature; however, previously there has been little supporting data. Hence, authors have used a variety of definitions from 1 to 6 months (23–25).

Overall, the significant contribution of health care exposure (OR = 3.15) as an ongoing "exporter" of resistant infection in a low-prevalence setting highlights the importance of controlling ESC resistance in the health care system. Supporting this hypothesis, United Kingdom data have recently demonstrated a broad-based decrease in the rate of ESC resistance among invasive *Enterobacteriaceae* strains following a reduction in the use of ESC and fluoroquinolones within the hospital system (26).

The "importation" of ESC-R *E. coli* after travel to countries with a high community incidence of ESBLs is starting to be defined (27), although fewer studies have identified infection rather than carriage (25, 28, 29). While the pathophysiology seems clear, the temporality of this remains to be confirmed. In our study, analysis of temporality, as presented for health care exposure, was precluded by the imprecision of data from the composite "high-risk" group and the low numbers involved. However, in absolute terms, 21 of 24 (87.5%) case participants with travel to high-risk regions departed those regions within the 6 months before presentation of infection. This fits with our previous research demonstrating mostly short-lived carriage of ESBL *E. coli* following travel overseas and with other studies demonstrating a decrease in the risk of resistant infection beyond 6 weeks after return from travel (29, 30).

Investigation of risks for community acquisition in the low-prevalence countries of Australia and New Zealand showed that one-quarter ($n = 23$) of ESC-R *E. coli* patients reported neither health care exposure nor high-risk travel, suggesting there are as-yet-undefined risk factors for transmission within the community (25, 31).

While there was some correlation between birth and travel region data, the identification of birth on the Indian subcontinent (OR = 11.12) as a risk factor for ESC-R *E. coli* infection in our cohort appears genuine. The etiology of this risk could stem from prolonged carriage of ESC-R *E. coli* after travel more than 1 year previous, leading to delayed community-onset ESC-R *E. coli* infection. Alternatively, our observation of a mostly short interval between travel and infection supports the possibility of domestic (within Australia and New Zealand) transmission of this resistance. Transmission of ESC-R *E. coli* from others within the household or community who have had recent travel to the Indian subcontinent may occur. Although the true magnitude of risk and the broader applicability require further study, this observation is consistent with a previously published study from one of our participating sites and with other descriptions of household transmission (32, 33). Recently, "birth outside Europe" was identified as a risk factor for infection by CTX-M-producing *E. coli* in another study, although comparison with our data is complicated, as the European study did not fully account for recent travel (31).

Our molecular epidemiology data serve to confirm a number of key observations made in other regions. The first is a distinct difference between the epidemiology of CTX-M ESBLs and that of other expanded-spectrum cephalosporinase enzymes, which may be mediated by the differing modes of acquisition, phenotypes, and characteristics of the *E. coli* strains harboring them (16, 34). Second, the high proportion of the ST131 clone among ESBLs is no surprise given its global prevalence (35). More surprising is its predominance without significant fluoroquinolone use (<6% of all participants in this study), one of the likely drivers in other regions (36). Exposure to this class of antimicrobials within Australia and New Zealand is very low (5).

Male sex has been defined by other researchers as a risk factor for community-onset ESC-R *E. coli* infection (23, 37–40) and became significant in some of our alternative models. The patient population of studies with this finding gives a clue to the etiology of this risk. On the whole, they are of older age with frequent health care exposure. This contrasts with studies conducted with a more traditional UTI population of young females that did not identify male sex as a risk factor (28, 29). In addition to males experiencing an age-dependent rise in the overall rates of *E. coli* infection (41), a limitation associated with case-control studies may also contribute to this finding. Aging patients certainly experience changes in the nuances and dynamics of health care exposure and other potential risk factors for ESC-R *E. coli* infection not identified with the data corresponding to dichotomous measures such as hospitalization and antimicrobial use that are most often collected.

The strengths of our study include its prospective collaborative nature, a geographically broad sample range, and the case-control methodology used. The low background rates of ESC-R *E. coli* infection in Australia and New Zealand have likely led to more discrete exposures and easier delineation of temporal risks than in communities where participants are frequently exposed to this form of resistance.

Limitations of our study include the moderate sample size, rate of nonrecruitment, and risk of bias due to an absence of blind investigator or patient procedures and reliance on patient recall for many exposures. Recruiting a higher ratio of controls (1:2 or 1:3) would have increased our study power and might have delineated further unidentified risks. The use of a third group of uninfected patients (a case-case-control design) would have allowed for delineation of risk factors associated with de novo acquisition of ESC-R *E. coli*, as opposed to delineation of risk factors for ESC-R within those that have *E. coli* (42). However, pragmatic limitations precluded these options.

Some unique features of Australia and New Zealand may limit extrapolation of our findings to other regions. The exclusion of day procedures in this study's definition of health care exposure correlated with our local epidemiology and would need to be reconsidered elsewhere. Furthermore, in cases in which $bla_{\text{CTX-M}}$ was not the predominant ESC resistance mechanism in a local population, risk data might differ.

The use of only hospital patients for recruitment allowed consistent access to participants and samples, although it might limit the applicability of some risks to the wider community. The exclusion of patients unable to consent meant that we could not define risks for patients in long-term-care facilities, a known reservoir of ESBL *E. coli* in Australia and overseas (43–45).

In conclusion, we have defined a critical ESC-R risk period after health care exposure among community-onset *E. coli* infections and demonstrated that ESC-R *E. coli* infection in a low-prevalence settings may be driven by "export" from health care exposure in the previous 6 months and importation after travel to regions with a high incidence of community ESBLs.

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Sequence type 131 *fimH*30 and *fimH*41 subclones amongst *Escherichia coli* isolates in Australia and New Zealand

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ABSTRACT

The clonal composition of *Escherichia coli* causing extra-intestinal infections includes ST131 and other common uropathogenic clones. Drivers for the spread of these clones and risks for their acquisition have been difficult to define. In this study, molecular epidemiology was combined with clinical data from 182 patients enrolled in a case–control study of community-onset expanded-spectrum cephalosporin-resistant *E. coli* (ESC-R-EC) in Australia and New Zealand. Genetic analysis included antimicrobial resistance mechanisms, clonality by DiversiLab (rep-PCR) and multilocus sequence typing (MLST), and subtyping of ST131 by identification of polymorphisms in the *fimH* gene. The clonal composition of expanded-spectrum cephalosporin-susceptible *E. coli* and ESC-R-EC isolates differed, with six MLST clusters amongst susceptible isolates (median 7 isolates/cluster) and three clusters amongst resistant isolates, including 40 (45%) ST131 isolates. Population estimates indicate that ST131 comprises 8% of all *E. coli* within our population; the fluoroquinolone-susceptible H41 subclone comprised 4.5% and the H30 subclone comprised 3.5%. The H30 subclone comprised 39% of all ESC-R-EC and 41% of all fluoroquinolone-resistant *E. coli* within our population. Patients with ST131 were also more likely than those with non-ST131 isolates to present with an upper than lower urinary tract infection (RR = 1.8, 95% CI 1.01–3.1). ST131 and the H30 subclone were predominant amongst ESC-R-EC but were infrequent amongst susceptible isolates where the H41 subclone was more prevalent. Within our population, the proportional contribution of ST131 to fluoroquinolone resistance is comparable with that of other regions. In contrast, the overall burden of ST131 is low by global standards.

1. Introduction

Using contemporary molecular typing techniques, a broad picture of the genetic diversity of *Escherichia coli* causing urinary tract infections (UTIs) and other invasive infections is beginning to emerge. Recent studies have demonstrated that collections of *E. coli* from urine and blood are largely clonal in composition [1–4]. These clonal components invariably include the global pandemic clone, sequence type 131 (ST131) *E. coli*, as well as other frequently described uropathogenic *E. coli* (e.g. ST95, ST69, ST73 and ST127). ST131 *E. coli* has been implicated as a major contributor to fluoroquinolone-resistant and expanded-spectrum cephalosporin-resistant *E. coli* (ESC-R-EC) infections globally [5].

Clinical and epidemiological risk factors for colonisation or infection with these clones, in particular ST131, have been difficult to define. Recently identified risk factors for ST131 include long-term care facility (LTCF) residence or bedridden status [6–8], exposure to antimicrobials [6], ethnicity [9], female sex [8], age [6,8] and infection characteristics [6,10].

In Australia and New Zealand, a range of ST131 clones have been identified amongst animals as well as humans from a variety

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of patient groups [11–16]. Few facets of epidemiology have been investigated, with one study reporting no difference between the co-morbidities of patients infected with ST131 and non-ST131 *E. coli* following prostate biopsy [17], and another demonstrating some possible sharing of ST131 clones between human and companion animals [18]. There have been no population estimates of prevalence.

We previously described risk factors for community-onset ESC-R-EC in Australia and New Zealand. These risk factors included healthcare contact, travel to high-risk regions (Indian subcontinent, Southeast Asia, China, Africa and the Middle East), trimethoprim ± sulfamethoxazole and/or expanded-spectrum cephalosporin use (ceftriaxone, ceftazidime or cefepime), UTI in the previous year, and birth on the Indian subcontinent. We also demonstrated that ST131 *E. coli* was spread broadly in our region, although with a relatively uncommon distribution. It resided almost exclusively amongst ESC-R-EC, where the prevalence was 45% compared with 7% amongst expanded-spectrum cephalosporin-susceptible *E. coli* (ESC-S-EC) isolates. In addition, there was a non-significant difference in the proportion containing $bla_{\text{CTX-M-9}}$ group and $bla_{\text{CTX-M-1}}$ group enzymes [19].

The aim of this follow-up study was to define the clonal composition and molecular characteristics of community-onset ESC-S-EC and ESC-R-EC infections. A further aim was to understand the subclonality of ST131 and to elucidate factors that may influence the distribution of the ST131 worldwide pandemic in our region. To do this, epidemiological data, collected as part of a case–control study, were combined with genetic characterisation of *E. coli* isolates from the study patients.

2. Materials and methods

2.1. Clinical data and bacterial isolates

All bacterial isolates and clinical data are from The COOEE Study (COmmunity Onset ESBL and AmpC *E. coli* Study), a multisite case–control study with prospective recruitment of patients and data collection. The study has been described in detail elsewhere [19]. In brief, six geographically dispersed tertiary centres in Australia ($n = 5$) and New Zealand ($n = 1$) recruited patients over a 9–12-month period during 2011 and 2012. In total, 182 patients (91 ESC-R-EC cases and 91 ESC-S-EC controls) were recruited. Each site recruited a single unmatched control for each case. Bacterial isolates were recovered from 98% (179/182) of patients, with the remainder unable to be recovered from the clinical laboratory.

Expanded-spectrum cephalosporin resistance genes were present amongst 87/89 (98%) of the available ESC-R-EC isolates [36 (40%) $bla_{\text{CTX-M-1}}$ group, 35 (39%) $bla_{\text{CTX-M-9}}$ group, 3 (3%) $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ groups, 11 (12%) $bla_{\text{CMY-2}}$, 1 (1%) $bla_{\text{DHA-1}}$ and 1 (1%) $bla_{\text{SHV-5}}$] [19].

2.2. Definitions

Definitions have been described extensively elsewhere [19]. In brief, case patients had community-onset ESC-R-EC in a culture of blood or urine. Control patients had ESC-S-EC isolated and were selected from the same laboratory as the 'case' patients. Patients who were unable to give informed consent to participate or who had extra-anatomical urinary drainage [e.g. indwelling urinary catheter (in the community), intermittent catheterisation, ileal conduit or similar] were excluded from recruitment.

Escherichia coli was considered community-onset when a patient was resident in the community (including LTCFs) or was hospitalised for <48 h at the time of onset. Expanded-spectrum cephalosporin resistance was identified phenotypically. For ceftriaxone laboratories used a minimum inhibitory concentration (MIC) breakpoint of ≤1 mg/L. For ceftazidime, laboratories used an MIC breakpoint of ≤1 mg/L or ≤4 mg/L depending on their use of European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI) criteria, respectively, within the participating laboratory [20,21]. Site of infection was determined by the researcher from available information. This included the researcher's clinical assessment if they were involved with patient care, or information relayed by the treating clinician if the researcher was not involved in care. Urinary tract presentations were classified as 'asymptomatic', 'lower tract infection' or 'upper urinary tract infection' as defined previously [19]. All other infections (non-urinary source and prostatitis) were classified into a combined 'Other' group.

The Human Research Ethics Committees at The University of Queensland (Brisbane, Queensland, Australia) and participating sites approved this study.

2.3. Antimicrobial susceptibility phenotype

As described previously, all phenotypic data presented here have been assessed by EUCAST criteria [19,20]. All non-susceptible isolates were considered resistant for the purpose of this analysis. An aggregated resistance score (0–11) was calculated with all antimicrobial phenotypes included in the study [ampicillin, amoxicillin/clavulanic acid, ceftriaxone, ceftazidime, cefepime, meropenem trimethoprim/sulfamethoxazole (SXT), ciprofloxacin, nitrofurantoin, gentamicin and amikacin].

2.4. Molecular methods

Bacterial isolates were recovered from storage at −80 °C in the research laboratory. Following overnight culture, bacterial DNA was extracted using an UltraClean® Microbial DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA). As outlined previously, expanded-spectrum cephalosporin resistance genes were investigated by PCR using published primers and conditions.

Phylogenetic group was determined by multiplex PCR [22]. Semi-automated repetitive sequence-based PCR (rep-PCR) using a DiversiLab® system (bioMérieux, 69280 Marcy-l'Étoile, France) was undertaken according to the manufacturer's instructions. Dendrograms were constructed from rep-PCR patterns using a Pearson correlation coefficient (DiversiLab 3.4 software). All clusters with at least four isolates demonstrating ≥95% similarity [23] were then identified by multilocus sequence typing (MLST) [24] as follows. The two most distant isolates within each cluster underwent MLST. Where multiple isolates were equally distant, isolates were selected to maximise the diversity in geographical origin and phenotype. If isolates from a rep-PCR cluster were discordant by MLST, additional isolates underwent MLST to attempt to define the cluster. The worldwide pandemic clone, ST131 *E. coli*, was presumptively identified using the same approach and 95% cut-off, although a number of MLST-confirmed ST131 reference clones were also included in the dendrogram [25]. Typing of the *fimH* allele encoding the type 1 fimbrial adhesin was undertaken in ST131 isolates. The gene was sequenced in forward and reverse direction based on published primers and conditions [26] using an ABI 3730XL (Life Technologies, Waltham, MA) capillary sequencer. Amino acid substitutions up to the 265th position of the putative mature peptide sequence were classified as per the schema of Weissman et al. [27].

2.5. Calculation of whole-population estimates

Estimates of the population prevalence of MLST-defined clones were back-calculated by adjusting to an overall population prevalence of 3.4% ESC-R-EC, as measured in the 2010 Australian Group

on Antimicrobial Resistance (AGAR) outpatient survey [28]. The survey sampled a population very similar to that of the current study, in the 12 months before this study: outpatient urine samples from 30 hospital-based laboratories including from 4 of 6 sites in the current study. Estimates are provided with a range assuming a possible 50% difference in population ESC-R-EC rates from the AGAR survey (i.e. 3.4%, range 2.6–4.25%).

2.6. Statistical methods

Variables were compared using χ^2, Fisher's exact and Wilcoxon rank-sum tests and calculation of relative risks (RRs) where appropriate. Continuous data are presented as the median and interquartile range (IQR). Statistical tests were two tailed, and *P*-value of <0.05 was considered significant. STATA v.12.1 (StataCorp LP, College Station, TX) was used for statistical analysis.

3. Results

In total, 179 bacterial isolates were included in this study (89 ESC-R-EC and 90 ESC-S-EC). Bacteraemia was detected in 29 patients (16%), with the remainder having isolated urine cultures. All isolates were community-onset, including 2/179 (1.1%) originating from residents of LTCFs.

3.1. Phylogenetic group

Phylogenetic grouping showed a high proportion of pathogenic B2 and D group *E. coli* (Table 1). The clinical syndrome varied significantly for each phylogroup, however when comparison was limited to lower and upper UTIs (the two categories that constituted the vast majority of presentations) only B2 isolates demonstrated a significant risk for upper rather than lower UTI (RR = 2.3, 95% confidence interval 1.1–4.9). The other phylogroups (A/B1 and D) demonstrated a non-significant risk favouring lower UTI ($P = 0.083$ and $P = 0.093$, respectively). There was no significant difference in phylogenetic group comparing urine and bacteraemia isolates ($P = 0.820$).

3.2. Multilocus sequence typing

A dendrogram from the rep-PCR data was constructed for ESC-S-EC (Fig. 1), ESC-R-EC (Fig. 2) and all isolates combined (data not shown). There was no obvious geographical or temporal clustering. Twenty-nine isolates underwent full MLST. In total, nine clusters comprising 60.3% (108/179) of all isolates were related to six MLSTs (ST131, ST95, ST73, ST69, ST127 and ST80) and two clonal complexes sharing six of seven alleles (ST14 complex and ST648 complex). Whilst the proportion of clonal isolates between ESC-R-EC and ESC-S-EC groups was similar [56/89 (63%) vs. 52/90 (58%); $P = 0.482$), there was a marked difference in the composition of sequence types. Amongst ESC-S-EC, six MLST-defined clusters each contained 5–13 isolates (median 7 isolates). Within the ESC-R-EC, only three clusters were identified with a single cluster, ST131, containing 40 isolates.

3.3. ST131 and fimH typing

As reported previously, 46/189 isolates (24%) clustered within the ST131 worldwide pandemic clone, including 40/89 (45%) ESC-R-EC and 6/90 (7%) ESC-S-EC ($P < 0.001$). All ESC-R-EC ST131 harboured CTX-M enzymes and constituted 54% of the entire CTX-M group; 24 (60%) harboured a CTX-M-1 group enzyme and 19 (48%) harbouring a CTX-M-9 group enzyme ($P = 0.173$ for comparison). This included three isolates (8%) harbouring both enzymes. Amongst the 34 non-ST131 CTX-M-harbouring isolates, 19 (56%) were CTX-M-9 group and 15 (44%) were CTX-M-1 group ($P = 0.472$).

The *fimH* gene was typed as *fimH*30 (H30) in 37 (80%) ST131 isolates, with the remaining 9 (20%) typing as *fimH*41 (H41), indicating a consensus FimH peptide sequence. By rep-PCR, H41 ST131 isolates formed distinct clusters within ST131, which branched at ca. 97% similarity from the majority of H30 isolates (Supplementary material).

There was a significant difference in the rate of expanded-spectrum cephalosporin resistance between H30 and H41 isolates [H30 35/37 (95%) ESC-R-EC vs. H41 4/9 (44%); $P = 0.009$] and concordance between H30 and fluoroquinolone resistance was 100% [H30 = 37/37 (100%) ciprofloxacin-resistant; H41 = 0/9 (0%) ciprofloxacin-resistant].

With the exception of the smallest site, which had no patients with ST131 and small numbers overall ($n = 8$), the clone and the H30 subclone were present in all sites (Fig. 3). There was no significant difference in the proportion of ST131 ($P = 0.574$) or H30 ($P = 0.774$) across study sites.

Table 1
Comparison of clinical presentations within phylogenetic groups and MLST-defined clones.

	Clinical syndrome [*n* (%)]					*P*-value[b]	RR of upper vs. lower UTI (95% CI)[c]	*P*-value (upper vs. lower UTI)[c]
	Asymptomatic	Lower UTI	Upper UTI	Other[a]	Total			
Phylogenetic group								
A/B1[d]	9 (45)	8 (40)	1 (5)	2 (10)	20	0.026	0.3	0.083
B2	27 (21)	49 (38)	43 (34)	9 (7)	128	0.017	2.3 (1.1–4.9)	0.010
D	4 (13)	16 (52)	5 (16)	6 (19)	31	0.043	0.5	0.093
Total	40 (22)	73 (41)	49 (27)	17 (9)	179			
MLST groups								
ST131	10 (22)	16 (35)	19 (41)	1 (2)	46	0.038	1.8 (1.01–3.1)	0.044
ST131 H30	8 (22)	13 (35)	15 (41)	1 (3)	37	0.149	1.5	0.099
ST131 H41	2 (22)	3 (33)	4 (44)	0	9	0.682	2.0	0.345
ST95	0	5 (33)	9 (60)	1 (7)	15	0.014	2.7 (0.96–7.5)	0.050
ST73	2 (15)	5 (38)	6 (46)	0	13	0.437	1.8	0.310
ST14 cplx.	4 (40)	4 (40)	1 (10)	1 (10)	10	0.407	0.4	0.647
ST69	0	3 (43)	3 (43)	1 (14)	7	0.412	1.5	0.683
All groups[e]	21 (19)	39 (36)	39 (36)	9 (8)	108	0.011	2.2 (1.2–4.0)	0.003

MLST, multilocus sequence typing; UTI, urinary tract infection; RR, relative risk; CI, confidence interval; cplx., clonal complex.
[a] Includes intra-abdominal source ($n = 4$), prostatitis ($n = 5$), bacteraemia without focus ($n = 4$) and others ($n = 4$).
[b] The *P*-value has been calculated across the four clinical presentation categories.
[c] The RR and *P*-value compare only upper and lower UTI (excluding other illnesses) across the three phylogroups. A 95% CI is given for significant values.
[d] A single B1 isolate was combined with the A group for purpose of analysis.
[e] All isolates that clustered within an MLST-defined group(see Supplementary material for full MLST groupings).

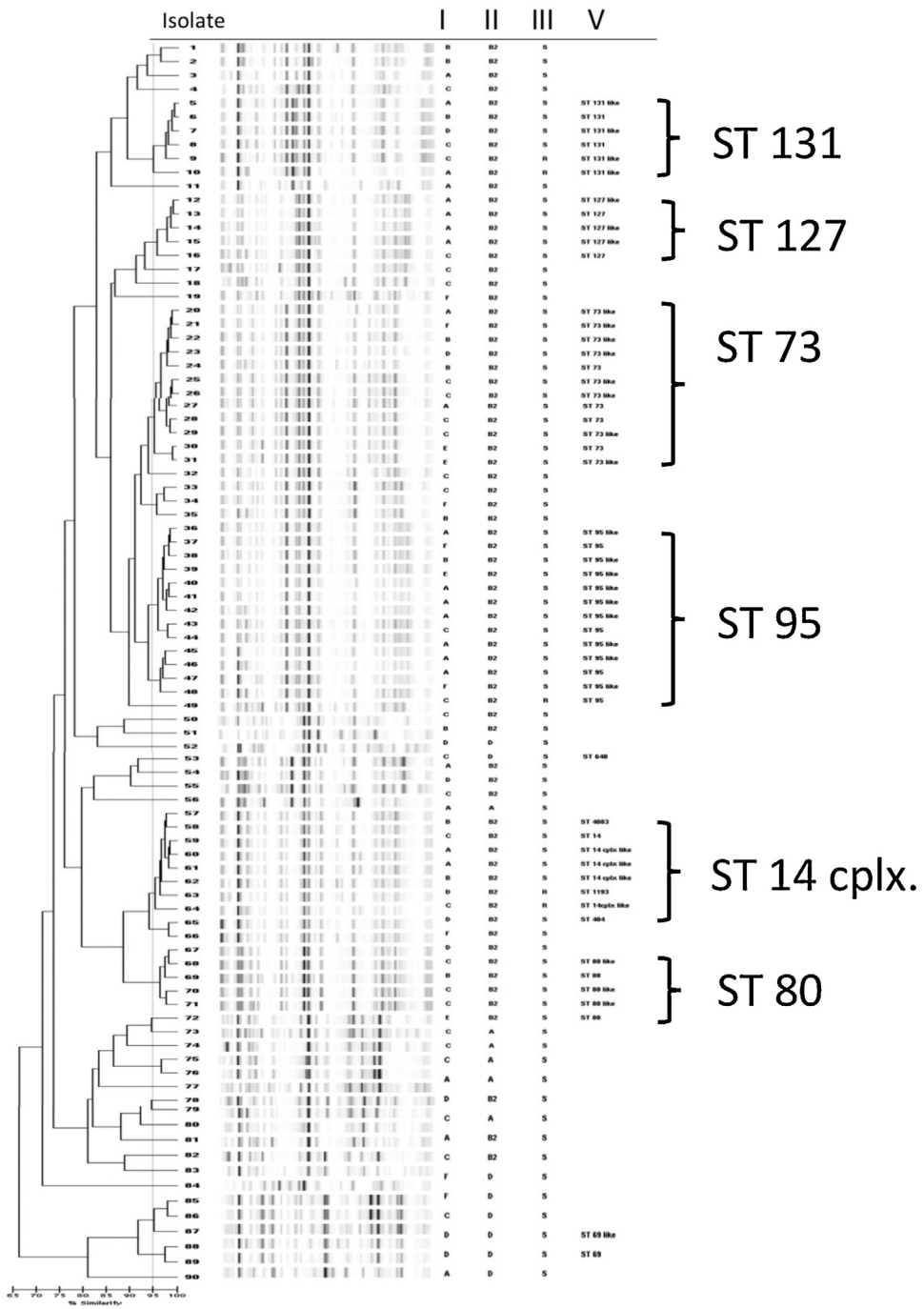


Fig. 1. Dendrogram constructed using a Pearson correlation coefficient, based on DiversiLab® repetitive sequence-based PCR (rep-PCR) pattern of expanded-spectrum cephalosporin (ESC)-susceptible *Escherichia coli*. Details of figure from left to right: dendrogram: isolate number; virtual gel. Numbered columns from left to right: I, hospital location; II, phylogenetic group; III, fluoroquinolone susceptibility; V, multilocus sequence typing (MLST) sequence type. Isolates that have been presumptively related to a MLST only by rep-PCR pattern are indicated by 'like', e.g. 'ST131 like'. cplx., clonal complex.

3.4. Clonal prevalence estimates

When adjusted for the population rate of ESC-R-EC, the estimated most prevalent MLST clones in this study population were ST95 (14%, 13.9–14.1%), ST73 (13%, 12.8–13%), ST14 complex (9%, 8.6–8.7%) and ST131 (8%, 7.6–8.3%) (Fig. 4; Supplementary material).

Within ST131, the H41 subclone comprised 4.5% (4.5–4.5%) and the H30 subclone comprised 3.5% (3.2–3.8%) of all *E. coli* in the population. The ciprofloxacin-resistant H30 subclone constituted 41% of all ciprofloxacin-resistant community-onset *E. coli* isolates. In total, ESC-S-EC ST131 constituted 6.4% (6.4–6.5%) of community-onset *E. coli* isolates (Supplementary material).

3.5. Characteristics of ST131

A comparison of the site of infection for various clonal groups is shown in Table 1. This demonstrated that ST131 clones were significantly more likely to cause upper UTI than lower UTI [ST131, 19/35 (54%) upper UTI vs. non-ST131 30/87 (34%) upper UTI; RR = 1.8, $P = 0.044$] (Table 1). When compared only with other phylogroup B2 isolates ($n = 57$), ST131 did not constitute a significantly

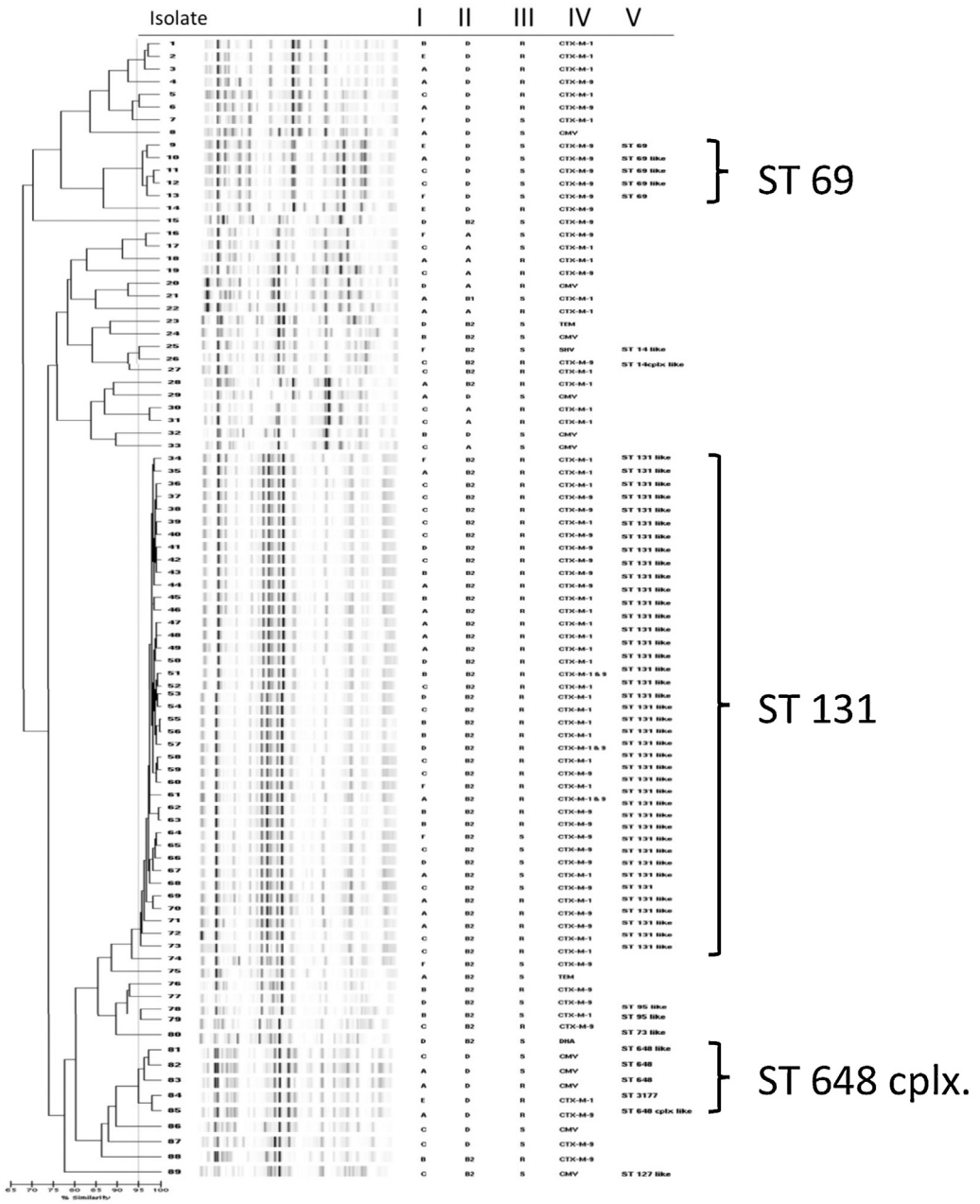


Fig. 2. Dendrogram constructed using a Pearson correlation coefficient, based on DiversiLab® repetitive sequence-based PCR (rep-PCR) pattern of expanded-spectrum cephalosporin (ESC)-resistant *Escherichia coli*. Details of figure from left to right: dendrogram: isolate number; virtual gel. Numbered columns from left to right: I, hospital location; II, phylogenetic group; III, fluoroquinolone susceptibility; IV, expanded-spectrum cephalosporinase type; V, multilocus sequence typing (MLST) sequence type. Isolates that have been presumptively related to a MLST only by rep-PCR pattern are indicated by 'like', e.g. 'ST131 like'. cplx., clonal complex.

greater proportion of upper UTIs [upper UTI, ST131 19/35 (54%) vs. non-ST131 24/57 (42%); $P = 0.256$).

Analysis within the 'upper tract' and 'lower tract' groups showed no significant difference in the characteristics of clinical presentation between ST131- and non-ST131-harbouring patients. No patient in either group had evidence of renal abscess or secondary sites of infection. The duration of symptoms before presentation comparing ST131 with non-ST131 infections was equivalent for lower UTIs [3 days (1–7.5 days) vs. 2 days (1–7 days); $P = 0.486$] and upper UTIs [2 days (1–5 days) vs. 2 days (1–4 days); $P = 0.536$].

There was no significant difference in the proportion of upper UTIs between H30 and H41 subclones (upper UTI, H30 = 15/28 (54%), H41 = 4/7 (57%); $P = 1.0$).

3.6. Antimicrobial resistance

As expected, given the prevalence of ST131 amongst resistant isolates, the median resistance score amongst ST131 isolates was significantly higher than for non-ST131 isolates across the whole cohort [median 6 (IQR 4–7) vs. 2 (IQR 0–5); $P < 0.001$]. When stratified into a variety of relevant groups, the resistance scores did not differ significantly between ST131 and non-ST131 isolates within any group (ESC-S-EC, $P = 0.077$; ESC-R-EC, $P = 0.116$; and CTX-M-harbouring ESC-R-EC, $P = 0.899$). Although total resistance scores were similar, significant differences in rates of resistance to individual antimicrobials included greater resistance to ciprofloxacin ($P < 0.001$) and SXT ($P = 0.013$) amongst the ESC-R-EC ST131 group.

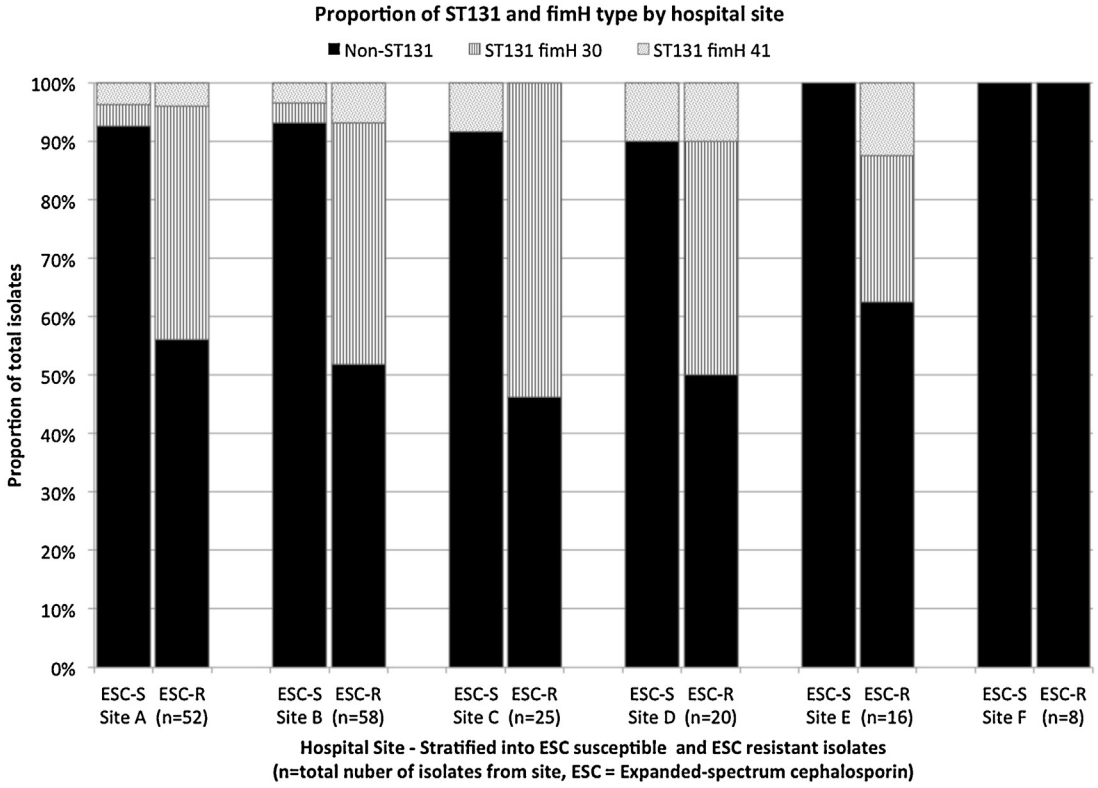
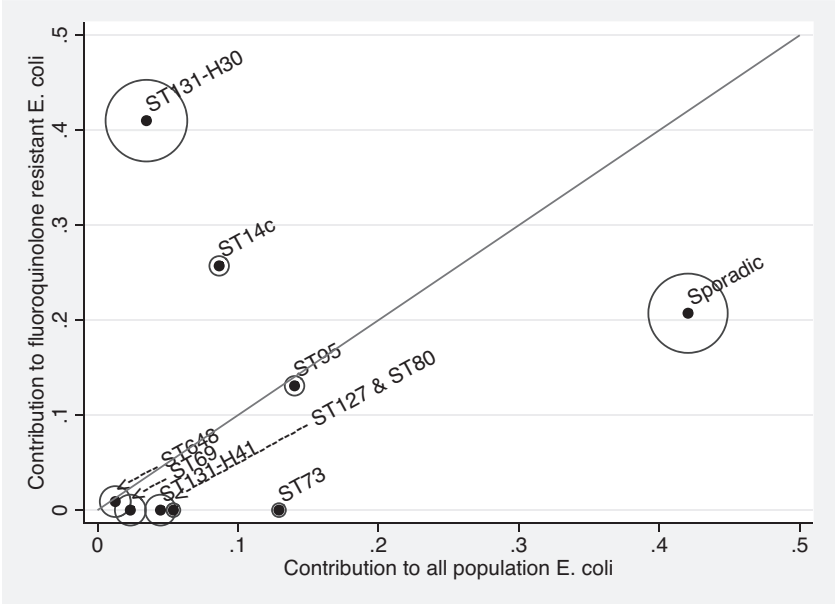


Fig. 3. Distribution of sequence type 131 (ST131) and the *fimH* subclones across the study sites for expanded-spectrum cephalosporin-susceptible (ESC-S) and expanded-spectrum cephalosporin-resistant (ESC-R) *Escherichia coli*.



Sporadic= isolates not clustered to an identifiable clone, ST14c = ST14 clonal complex. ST127 & ST80 have been represented by a single data point.

Fig. 4. Relative contribution of clones to the burden of fluoroquinolone and expanded-spectrum cephalosporin resistance in our population. The *x*-axis represents the proportional contribution of each clone to the total number of *Escherichia coli* isolated within our population. The *y*-axis represents the proportional contribution of each clone to fluoroquinolone-resistant *E. coli* in our population. The size of the outlining circle on each data point represents the relative contribution to expanded-spectrum cephalosporin resistance, as a proportion of all expanded-spectrum cephalosporin-resistant *E. coli*, within our population *E. coli*. Dashed reference line represents a balanced contribution to fluoroquinolone resistance and overall *E. coli* burden.

When only the CTX-M-harbouring isolates were analysed, the difference in ciprofloxacin remained significant ($P = 0.010$) (Supplementary material).

4. Discussion

This study provides the first comprehensive molecular epidemiological profile of susceptible and resistant *E. coli* in our region. Previous studies in our region have investigated selected groups such as fluoroquinolone resistance or particular clonal groups, limiting their ability to ascertain a broad profile [13,18].

At first glance, the global pandemic clone ST131 appears to be dominant in our population. However, this must be seen in the perspective of local rates of ESC-R-EC (Fig. 4). Our estimates indicate that ST131 makes up 8% (7.6–8.3%) of community-onset *E. coli* isolates. Although less than one-half of the ST131 would be ciprofloxacin-resistant H30 subclones, ST131 still constitutes a disproportionate 41% of all ciprofloxacin-resistant community-onset *E. coli* isolates, as shown in Fig. 3.

Whilst the proportion of ST131 amongst ciprofloxacin-resistant *E. coli* is comparable with that found in other contemporaneous studies, the estimated absolute rate of ST131 is considerably lower [5]. Three North America studies reported rates ranging from 23% to 28% ST131 amongst *E. coli* [1,4,6]. A recent Australian cohort (from a single region amongst a selected patient population) demonstrated a rate of 21% ST131 amongst females of reproductive age with UTI [13]. Comparing across all these studies, the most notable variable was the background rate of fluoroquinolone resistance amongst *E. coli*. The rate in the North American studies ranged from 27% to 29% resistance, and the rate in the Australian study of 13% was high compared with our sample (7% amongst ESC-S-EC) and other Australian data [19,28,29].

The potential relationship between the background rate of fluoroquinolone-resistant *E. coli* and ST131 prevalence is revealing. By global standards, Australia and New Zealand have very low rates of fluoroquinolone resistance amongst *E. coli*. These have been achieved through regulatory control of fluoroquinolone use in humans and animals [30]. By way of example, 72% of our study population had used antimicrobials in the previous year, although only 5% were exposed to a fluoroquinolone. We hypothesise that on a population basis, the low background rate of fluoroquinolone use has kept the ST131 clone at bay amongst the majority of Australian and New Zealand *E. coli*.

The *fimH* subtyping of ST131 supports this hypothesis. The dominance of the H30 subclone amongst ESC-R-EC ST131 isolates reflects an emerging global picture [31]. Conversely, the overall dominance/expansion of the fluoroquinolone-susceptible H41 subclone has not been described previously. This subclone is somewhat genetically distinct from other 'clades' of ST131 [32] and is strongly associated with the otherwise infrequent O16:H5 serotype [33,34]. In our setting, the H41 clone may have a selective advantage given recent research demonstrating high virulence coupled with fluoroquinolone susceptibility [33,34]. This requires further exploration.

Whilst the identification of antimicrobial use as one of the drivers/protectors for clones amongst the whole population can be supported, it does not fully explain the 45% ST131 rate seen amongst ESC-R-EC within our population, a rate similar to that of many other regions [35–37]. The data on ESC-R-EC ST131, 88% of which are the H30 subclone, provides some answers. Foremost, the findings of this study must be considered in the context of our previous analysis of risk for ESC-R-EC within our population. We defined multiple risk factors for ESC-R-EC, and the majority of these remained a risk for ESC-R-EC ST131 in a sensitivity analysis [19]. Hence, within the population as a whole, factors including healthcare contact, high-risk travel, birth on the continent and previous UTI are all risk for ST131 in our region. This risk is mediated through the very tight association of ST131 with CTX-M extended-spectrum β-lactamases (ESBLs).

A confluence of virulence and resistance is important in this association. ST131 was associated with almost twice the risk of upper UTI compared with lower UTI (RR = 1.8). Whether this virulence is greater than other B2 phylogroup isolates remains controversial, with mixed findings amongst clinical [4,13,14] and non-clinical studies of ST131 virulence [38,39].

A key strength of this study is the ability to compare prospectively collected epidemiological data with the molecular epidemiological characteristics of our isolates amongst a geographically dispersed sample. The presence of equal numbers of ESC-S-EC and ESC-R-EC isolates also allowed clear differentiation of the clonal structure of each group, which would not otherwise have been possible, given the low prevalence of ESC-R-EC in our region. The uniquely low rate of fluoroquinolone use in our region has provided us insight into the dynamics of the ST131 clone with little selective pressure from this antimicrobial class.

Limitations of this study include the lack of laboratory virulence data to allow greater exploration of our hypotheses and findings. The power to detect significance of association was also limited by a moderate sample size and the subgroup nature of this analysis. The reliance on back-calculation to determine population rates may have led to a lower accuracy of these figures than other methods. Finally, a number of the findings are hypothesis generating and require further studies for confirmation.

5. Conclusion

We delineate a markedly different clonal composition between ESC-S-EC and ESC-R-EC groups in Australia and New Zealand. Overall, ST131 is less frequent than in other regions of the world. The fluoroquinolone-susceptible H41 subclone of ST131 is most prevalent, although the H30 subclone dominates ESC-R-EC. ST131 was significantly associated with upper UTI presentation, suggesting enhanced virulence. We hypothesise that the factors contributing to the low background rate of fluoroquinolone-resistant *E. coli* in our region may also have afforded protection from wider spread of the pathogenic ST131 clone beyond ESC-R-EC.

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Competing interests: DLP has received honoraria from AstraZeneca, Merck, Shionogi, Bayer and Pfizer. All other authors declare no competing interests.

Ethical approval: The project was conducted after ethical review by The Royal Brisbane and Women's Hospital Human Research Ethics Committee [HREC10/QRBWH/388].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.11.015.

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ARTICLE

Prolonged carriage of resistant *E. coli* by returned travellers: clonality, risk factors and bacterial characteristics

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Abstract The aim of this study was to delineate the potential risks and dynamics of the prolonged carriage of resistant *E. coli* in returned travellers. A sample of 274 previously collected *E. coli* resistant to ceftriaxone (CRO), ciprofloxacin, gentamicin and/or nalidixic acid recovered from 102 travellers was studied. Travellers were assessed pre-travel then longitudinally (maximum 6 months) with peri-rectal/rectal swabs. Clonality was determined by REP-PCR and the presence of O25b-ST131 was assessed. Comparison was made longitudinally for individuals and between identified co-travellers. The risk of prolonged carriage was lower for CRO than for ciprofloxacin or gentamicin resistance. Repeated isolation of the same phenotype at different time points occurred in 19% of initial CRO-resistant carriers compared with 50% of ciprofloxacin- or gentamicin-resistant carriers. The duration of carriage was also longer for the latter resistance phenotypes (75th quartile 8 vs 62 and 63 days respectively). In multivariate analysis, risks of prolonged carriage included antimicrobial use whilst travelling (3.3, 1.3–8.4) and phylogenetic group B2 (9.3, 3.4–25.6) and D (3.8, 1.6–8.8). Clonality amongst longitudinal isolates from the same participant was demonstrated in 92% of participants who were assessable and most marked amongst CRO-resistant isolates. ST-131 was surprisingly infrequent (3% of participants). Prolonged carriage of ciprofloxacin- and gentamicin-resistant isolates is more frequent and prolonged than CRO resistance after travel. Risks of prolonged carriage indicate a contribution of host and bacterial factors to this carriage. These require further elucidation. The strong clonality identified suggests that carriage of a "phenotype" was mediated by persistence of bacteria/plasmid combinations rather than persistence of the plasmid after horizontal transfer to other bacteria.

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Introduction

A number of recent publications have identified carriage of multi-resistant Enterobacteriaceae, primarily *E. coli*, in the gastrointestinal flora of returned travellers [1–5]. Individuals harbouring antibiotic-resistant organisms have frequently travelled from countries of low resistance incidence to countries of high incidence, e.g., Northern Europe to India. Key geographical regions for acquisition of multi-resistant Enterobacteriaceae include South-East Asia, the Indian Subcontinent and Africa.

One limitation of the current studies is that they provide only a "snapshot" of a narrow resistome immediately after travel, with only two studies thus far providing any longitudinal data [2, 4]. Such data help us to better understand the link between carriage of resistant Enterobacteriaceae in travellers and subsequent infection in the carrier (or others within the community). Factors impacting on this link may include the risk of acquisition whilst travelling, clonal

dynamics and duration of carriage, risk factors for prolonged carriage and the potential for spread of resistance by carriers within the home community or healthcare setting.

Even from the perspective of the more frequently described health-care-associated carriage of resistant Enterobacteriaceae, longitudinal carriage studies are limited and varied. Median durations of carriage of *E. coli* range from 80 to 178 days with single and multiple clones identified in differing settings [6–11]. Furthermore, the dynamics of healthcare-associated carriage may vary considerably from that of travellers, given the differing mechanism of acquisition and population involved. Complicating our understanding of prolonged carriage of antibiotic-resistant Enterobacteriaceae is the potential that any given resistance phenotype may be mediated by persistence of a stable bacteria/plasmid combination or persistence of the plasmid after horizontal transfer to other bacteria [12].

In this study we aimed to define longitudinal and clonal aspects of the prolonged carriage of antimicrobial resistance in a cohort of returned travellers. The natural history and clonality of individual carriers was investigated using a sample of patients selected as being unlikely to have acquired new resistance after return from travel, by virtue of residence in an area of low background incidence of resistance and absence of antimicrobial exposure or re-travel [4, 13]. Shared clonality between travel partners and the incidence of the ST-131 O25B worldwide pandemic clone were explored in the entire cohort, comprising all samples collected during the study.

Materials and methods

Bacterial isolates and data collection

Bacterial isolates used for this study consisted of 274 *Escherichia coli* from 102 participants collected during a previous prospective study of returned travellers residing in Canberra, Australia [4]. The original study investigated the rate and duration of colonisation with resistant *E. coli* following international travel. Isolates presented were resistant to ciprofloxacin (cip-R), gentamicin (gent-R) and/or ceftriaxone (CRO-R). In addition, nalidixic acid-resistant *E. coli* also recovered from this cohort were included in this analysis. A full description of the methods of bacterial isolation and clinical data collection is contained in the original publication [4]. In brief, 102 prospectively enrolled travellers who completed the study were asked to collect rectal or perianal swabs within 14 days before an overseas trip and within 14 days after return to Australia. If isolates resistant to ciprofloxacin, gentamicin or ceftriaxone were detected on the first return swab, participants were asked to collect regular monthly swabs until resistant bacteria were not identified on two sequential swabs or for a maximum of 6 months. Swabs were subcultured on three media, HBA-gentamicin (Oxoid, Adelaide, SA, Australia), MacConkey agar (Oxoid) containing a nalidixic acid disc (Oxoid, Basingstoke, Hampshire, UK) and chromID (ESBL; bioMérieux, Craponne, France), after initial overnight broth enrichment. Resistant colonies were selected from each plate and underwent identification and susceptibility testing using Vitek2 (bioMérieux, Durham, NC, USA). If all colonies on a given plate appeared morphologically identical, then only a single colony was sampled. If morphological differences between colonies were apparent, each variant was sampled. Mechanisms of ceftriaxone resistance were confirmed by PCR and sequencing and are presented in the original study. They comprised ESBL and AmpC enzymes [4].

All participant and travel data used in this study were collected via a questionnaire completed directly by participants during the original study. Variables included travel destinations, antimicrobial use, intercurrent illness and food/water consumption whilst travelling. For this analysis "high risk" regions were defined as regions where >50% of travellers during the original study returned with a resistant isolate. As travellers frequently visited more than one destination, the duration of stay in each region was calculated and included.

Molecular methods

The phylogenetic group was determined on all isolates using triplex PCR [14]. Determination of the ST-131 O25B worldwide pandemic clone was undertaken by detection of *pabB* and *trpA* alleles via multiplex PCR with a positive control MLST, confirmed as ST-131 [15]. Repetitive extragenic palindromic PCR (REP-PCR) was undertaken using published methods [16]. The template was purified DNA (MoBio, USA), using a BioRad C1000 Thermal Cycler. Primers REP-1 (5'-IIIGCGCCGICATCAGGC-3') and REP-2 (5'-ACGTCTTATCAGGCCTAC-3') were used. The products were separated on a 1% agarose gel (45 v, 3 h) and stained with ethidium bromide. Manual visual comparison was used to identify clonal isolates and any difference greater than two non-shared bands was considered non-clonal. Bacteria for comparison were always separated on the same agarose gel.

All isolates of the same phylogenetic group harboured by the same participant or shared by travel partners were considered potentially clonal. For CRO-R isolates, all potentially clonal isolates were subject to REP-PCR. For cip-R and gent-R isolates, if three or more potentially clonal isolates of the identical phylogenetic group and phenotypic antimicrobial susceptibility (amoxicillin, amoxicillin/clavulanate,

cephazolin, ceftriaxone, ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole) occurred within 8 weeks, interval isolates were skipped (e.g. sample 2 of samples 1, 2 and 3 skipped). If differing presumptive clonality was identified amongst the isolates analysed then the interval isolate was subject to REP-PCR.

Definitions

Duration of carriage was calculated from the date of return to Australia until the date of collection of the last positive swab. *Clearance of carriage* was assumed if there was collection of at least one swab not containing the given antimicrobial resistance phenotype, without the occurrence of any subsequent positive swabs. *Clonal carriage* was defined as identification of clonal isolates (as defined by REP-PCR) at two time points (most>4 weeks apart). As above, any isolates with more than two non-shared bands were considered unrelated.

Censoring and exclusions for longitudinal analysis

Participant results were censored (exclusion of all subsequent swab results) after events that may have potentiated new acquisition of resistance including further overseas travel or receipt of antimicrobial therapy for suspected or proven infection (n=6 urinary tract infection, n=2 other infection site). Participants who were still on doxycycline for malaria prophylaxis at the time of the return swab were not excluded. Participants harbouring clonally related isolates pre- and post-travel were excluded. To assess this, all pre-travel isolates were compared with post-travel isolates using REP-PCR in a similar manner to the longitudinal analysis (n=7 participants). All participants remaining who had assessable swabs collected at two or more time points after travel were included.

Statistical methods

Kaplan–Meier plots were used to illustrate duration of resistance. Subjects were censored if they remained resistant at their final data collection time-point. Parametric survival models were used to estimate the magnitude of differences in the distribution of resistance duration due to the three antibiotics. More specifically, log normal accelerated failure time models were used, as the log normal provided the best fit to the observed data from a number of common alternatives (including Weibull and log-logistic models) and estimates from accelerated failure time models could be reported as proportional increases in resistance duration. Robust variances were used to take into account within-patient correlations of resistance to the three antibiotics of interest.

Potential risk factors were assessed for possible association with duration of resistance to any of the three antibiotics by including these variables as covariates in univariate survival models. All variables that showed some evidence of association in univariate analysis (p<0.1) were included in multivariate analysis. Backwards elimination was used to remove non-significant (p>0.1) variables until the best predictive model was obtained. SAS version 9.1 for Windows and Stata/IC 10.1 for Windows were used for analysis.

Results

Duration, clonal dynamics and risks of prolonged carriage

Of the 102 original participants in the study, 50 returned carrying antimicrobial-resistant *E. coli* of interest (CRO-R, cip-R and/or gent-R), with 44 included in the final analysis after censoring and exclusions (Fig. 1). Three participants reporting ongoing use of doxycycline for malaria prophylaxis at the initial return swab were not excluded.

Duration and risks of prolonged carriage of resistance

Upon initial assessment, after return from travel, the carriage of the three specified phenotypes was CRO-R 26% (n=27), cip-R 27% (n=28), gent-R 35% (n=36). When compared with CRO resistance, cip-R and gent-R were associated with 2.1 (95% CI, 1.1 to 4.1, p=0.027) and 3.5 (95% CI, 1.6 to 7.5, p=0.001) times the duration of carriage of resistance respectively. There was no significant difference between the duration of cip-R and gent-R. The median durations and inter-quartile range for recovery of resistance from travellers was 3 (IQR, 1 to 8) days for CRO-R, 5 (IQR, 1 to 62) days for cip-R and 8 (IQR, 3 to 63) days for gent-R. This is represented longitudinally on a Kaplan–Meier curve (Fig. 2), demonstrating that the major difference is in the upper quartile of participants.

Potential risks for prolonged carriage of any resistance were analysed by three groups of factors: participant/travel characteristics, duration/location of travel and bacterial factors. Results of the univariate and multivariate survival model are presented in Tables 1 and 2.

Clonal dynamics of resistance

Clonality was almost always present in prolonged carriage of a given antimicrobial phenotype. Of 25 participants with isolation of bacteria of the same phenotype at two time points, the carriage of exclusively clonal isolates was demonstrated in 14 participants (56%). Carriage of a mixture of clonal and non-clonal isolates occurred in 9 (36%). Three participants (12%) carried more than one prolonged clone

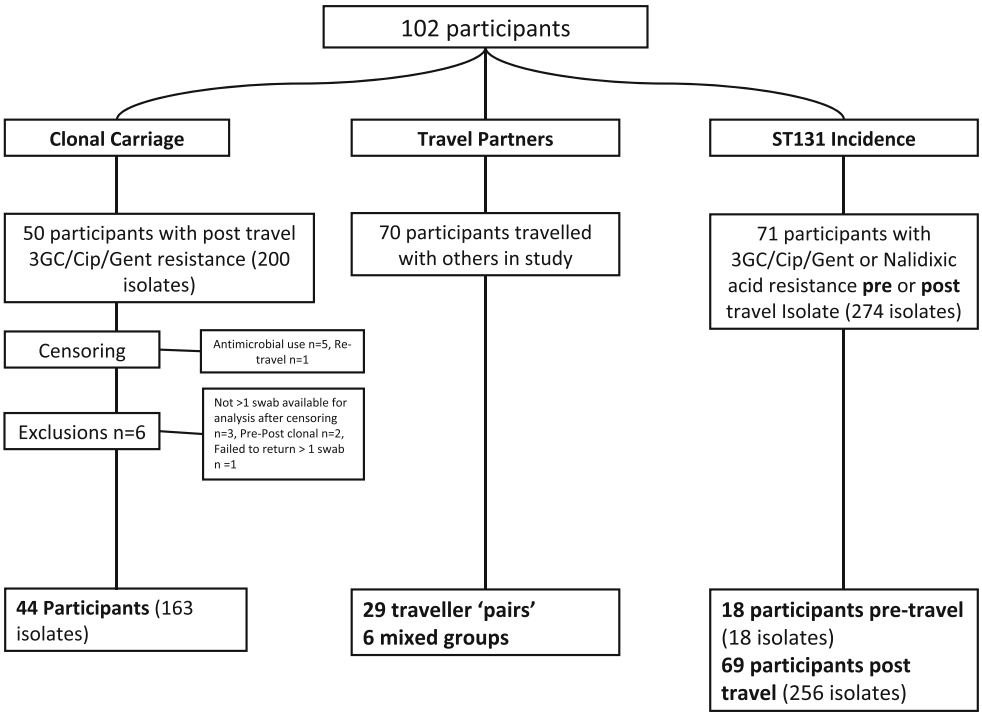

Fig. 1 Selection and exclusion of 102 participants for the three arms of this study. (The same participants and bacterial isolates were used in each arm)

simultaneously. In only 2 (8%) was no clonal relationship found between the bacteria isolated. In both cases bacteria were only recovered on the return swab and a single subsequent swab. These patterns are illustrated in Fig. 3.

The dynamics of clonality differed between CRO-R and cip-R or gent-R isolates. With the exception of a single bacterial isolate, repeated isolation of CRO-R *E. coli* was invariably due to the presence of clonal bacteria. The carriage of ciprofloxacin and gentamicin resistance demonstrated more diversity, although by month 5 all the isolates recovered were clonal with earlier isolates (Fig. 4).

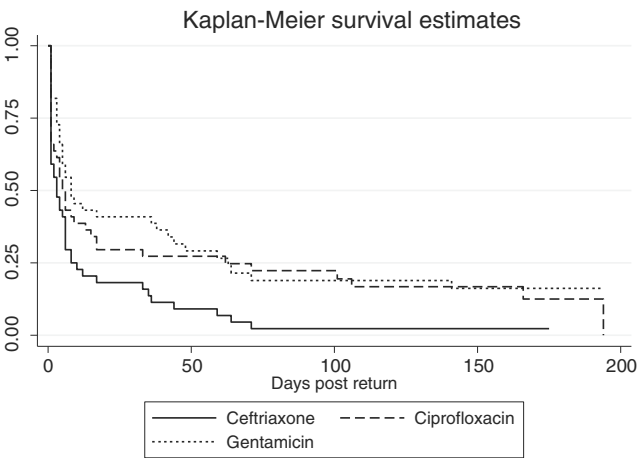

Fig. 2 Kaplan–Meier survival curve comparing the duration of carriage of the three resistance phenotypes across 44 selected participants (p=0.007)

Travel partners

From the cohort of 102 travellers, 70 (68%) travelled with other study members (Fig. 1). This included 29 "pairs" (travel and sexual partners) and six "mixed groups" containing participants of other relationships (friends travelling together, families and one unknown relationship). For analysis, pairs within mixed groups were considered, within the "pairs" cohort.

Analysis of the 29 "pairs" revealed 14 with neither returning with resistance (48%), 8 (28%) with a single partner and 9 (31%) with both partners harbouring resistance. Thus, if one person was colonised, there was a 53% chance of the partner also harbouring one of the three resistant phenotypes sought (9 out of 17). However, using clonal analysis, just 2 of the 9 couples (22%) shared clones.

Shared clonality was also identified amongst one in six "mixed" groups of travellers.

ST-131 worldwide pandemic clone

The presence of the clone was assessed amongst all 274 *E. coli* isolates recovered from 102 participants. This included all CRO-R, gent-R, cip-R and/or nalidixic acid-resistant isolates recovered from participants throughout the study duration (Fig. 1). Pre-travel prevalence of the clone was 2% (2 out of 102). An additional two individuals acquired ST-131 *E. coli* while travelling. With the exclusion of the pre-travel carriers, only one prolonged clonal carrier was colonised with an ST-131 strain. All ST-131 *E. coli* isolated were phylogenetic group B2.

Table 1 Univariate analysis of risk factors for prolonged carriage of resistance. The estimates provided represent a proportional increase in the duration of carriage (e.g. 1.00 representing no increased duration, 2.00 represents two times the duration of carriage)

Variable	Estimate	Lower 95% CI	Upper 95% CI	*P* value
Age at departure	1.00	0.96	1.05	0.86
Female gender	1.17	0.34	4.01	0.80
Whilst travelling				
Antibiotic use	9.12	3.16	26.05	<0.0001
Diarrhoea	2.34	0.70	7.77	0.17
Consumed "tap" water	0.53	0.16	1.79	0.30
Duration and destination				
Total travel (per week)	1.30	1.06	1.60	0.012
High-risk regions (per week)	1.32	1.08	1.63	0.008
Other regions (per week)	1.03	0.72	1.46	0.87
India/Sri Lanka/Nepal[a]	1.03	1.00	1.06	0.022
SE Asia/Pacific[a]	0.98	0.91	1.05	0.55
Middle East/Africa[a]	1.03	0.97	1.10	0.33
China/Hong Kong/Taiwan/Korea[a]	1.02	0.93	1.11	0.70
South America/Mexico[a]	1.00	0.93	1.08	0.93
Bacterial factors				
Multiple resistance[b]	1.82	0.54	6.23	0.33
Phylogenetic group A	0.82	0.23	2.86	0.76
Phylogenetic group B1	1.62	0.47	5.58	0.44
Phylogenetic group B2	7.03	1.65	29.96	0.008
Phylogenetic group D	5.16	1.68	15.80	0.004

SE Asia includes Malaysia, Thailand, Singapore, Vietnam, Philippines, Laos, Indonesia, Cambodia, Papua New Guinea, Solomon Islands; Middle East/Africa includes Jordan, Israel, UAE, Egypt, Zambia, Tanzania, Kenya

[a]Per day in this region

[b]Resistance to at least two out of ciprofloxacin, gentamicin and ceftriaxone

Discussion

Carriage of multi-resistant Enterobacteriaceae upon return from travel is a real and concerning phenomenon. The most serious outcome of such carriage is infection with resistant organisms after return home. Although we did not specifically assess infections in returned travellers, two recent studies have demonstrated this risk in diverse populations. Overseas travel afforded a relative risk of 2.7 for any infection after TRUS biopsy [17], and a relative risk of 5.7 for ESBL *E. coli* infection in a regional Canadian study [18]. Our analysis helps to delineate the complex link between acquisition and carriage during travel and infection after return.

The rapid decline in carriage of resistant isolates after travel is to some extent encouraging; however, persistence is significant. Analysis of the longitudinal nature of carriage highlights the marked persistence of cip-R and gent-R isolates beyond CRO resistance, with 10% of participants in the longitudinal arm of this study harbouring cip-R and/or gen-R at 6 months after return. This is noteworthy, given that fluoroquinolones and aminoglycosides are heavily relied upon for the treatment of *E. coli* infection, including urinary sepsis, in many national guidelines [19, 20]. The prolonged duration of carriage of fluoroquinolones resistance identified concurs with descriptions of healthcare-associated carriage [6, 7, 10]. Explanations for the shorter duration of CRO resistance potentially include the higher fitness cost for bacterium of maintaining this resistance plasmid in the absence of ongoing selection pressure and genetic differences between the host bacteria that harbour each resistance element.

Table 2 Multivariate analysis of risk factors for prolonged carriage

Variable	Estimate	95% CI	*P* value
Travel in high-risk regions (per week)	1.27	1.09 to 1.49	0.002
Antibiotic use	3.34	1.33 to 8.36	0.01
Phylogenetic group B2	9.32	3.39 to 25.6	<0.0001
Phylogenetic group D	3.81	1.64 to 8.82	0.002

Risk factors for prolonged carriage are intriguing. Antimicrobial use whilst travelling was strongly associated, leading to a 3.4 times increase in duration of carriage. Kennedy, in travellers [4], and many authors in other settings have identified antimicrobial use as a risk factor for the acquisition of resistance, an intuitive conclusion. However, this study examines a group who all harboured resistance and did not have further antimicrobial exposure to potentiate this risk. Another mechanism apart from simple selection of antimicrobial resistance may apply. We hypothesise potential modification of intestinal microbiota after antimicrobial use, leading to the loss of other potentially competitive non-resistant *E. coli* and other integral commensal bacteria [21].

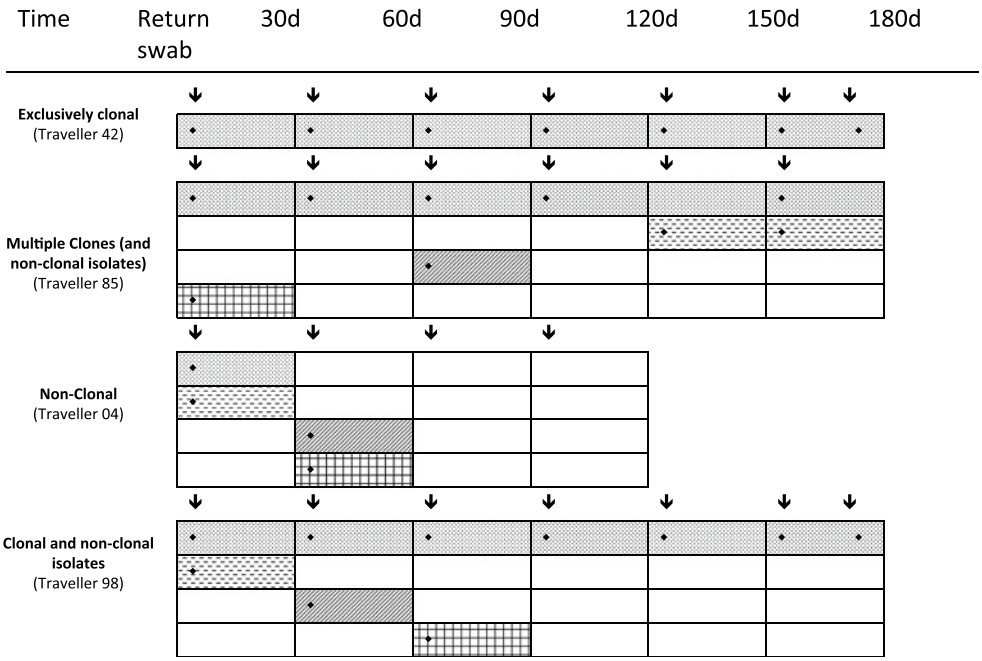

Fig. 3 Travellers representative of different patterns of carriage of resistant isolates. A time-line is displayed across the top (days). *Arrows* represent the submission of swabs by travellers. *Shaded squares* indicate bacterial clones with the *diamond in each square* showing time points at which the bacteria were isolated in a sample

The analysis of clonality answers interesting questions about the acquisition and carriage of such resistance. Whilst the "mobility" of resistance elements, particularly CRO resistance plasmids, was initially hypothesised in this study and is frequently discussed and demonstrated in "high stress" situations such as healthcare settings and antimicrobial use [12], it appears not to be significant in travellers. In fact, clonality was almost absolute for plasmid-mediated CRO resistance. The strong clonality of isolates amongst all phenotypes gives us an insight into the environment of acquisition. The identification of clonality amongst travel partners and mixed groups

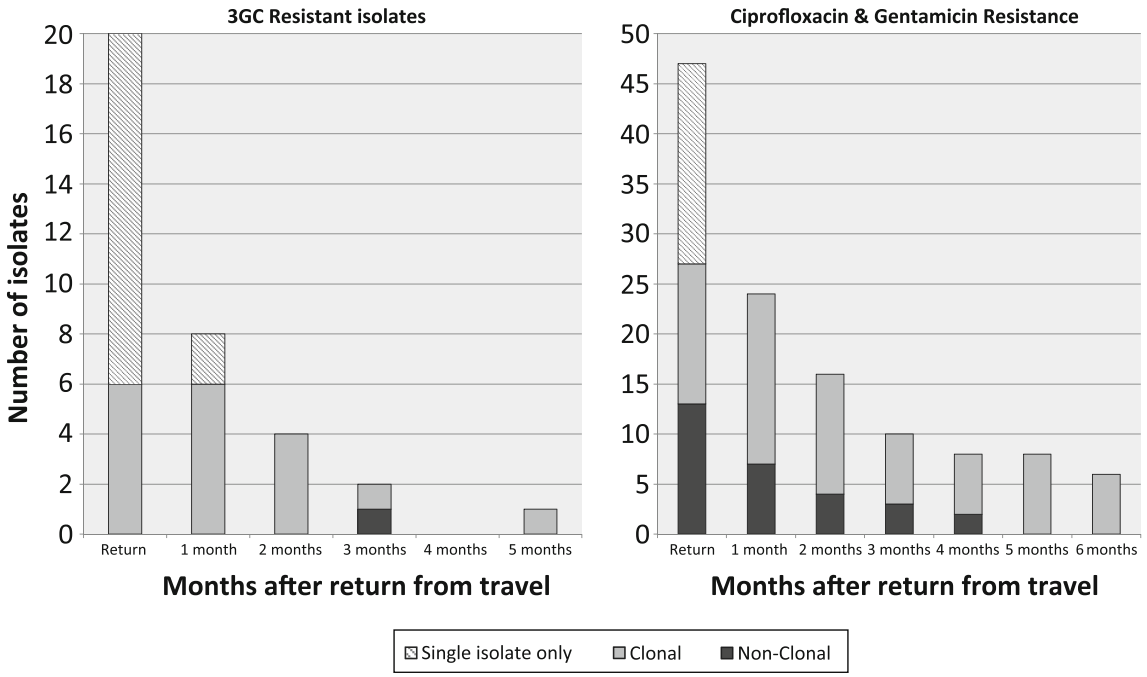

Fig. 4 Graphical representation of the clonality of isolates amongst each individual. *Left* Comparison of CRO-resistant isolates with other CRO-resistant isolates amongst the same individual. *Right* Comparison of ciprofloxacin- and/or gentamicin-resistant isolates with other isolates of the same phenotype amongst the same individual. (Isolates harbouring CRO resistance have been excluded.) (Phenotypes that were only recovered at a single time point could not be assessed for clonality and are indicated in *white/hash*)

(where direct transmission from person to person was unlikely) suggests exposure and ingestion of a common source of resistant isolates, potentially food or water. This is also supported by the relatively low rate (22%) of shared clonality between partners. The pattern of contraction of a variety of resistant isolates on return to persistence of a single (or very few) clones implies the presence of only a limited number of clones in circulation able to colonise and persist amongst individuals even in such "environments" with high resistance burden. The shared clonality amongst partners/mixed groups and the correlation with period of exposure in "high-risk" regions supports this hypothesis. This can be compared with the observation of clonality amongst *E. coli* causing urinary tract infection, where a small handful of adapted clonal groups are thought to cause a significant proportion (10–20%) of all such infections [22]. Although not investigated in this study, other than ST131, further exploration of clonality across travellers and regions would be worthwhile.

The identification of "persistent" clones also highlights the issue of potential infection and both community- and healthcare-related transmission of resistant isolates. It is unknown whether travellers might be the point of introduction of antibiotic-resistant bacteria into a community, rather than imported food, animals or de novo development of resistance via antibiotic use. Importation of antibiotic-resistant bacteria into hospitals has been well documented via individuals who have been treated in hospitals in high-risk areas [23]. With respect to travellers, we speculate that a long duration after return from travel, e.g. 3–6 months, may be the most problematic in a healthcare system, given that these clones have been selected as those best adapted for colonisation and persistence.

The relationship between persistence of colonisation and subsequent infection needs further exploration. The phylogenetic groups, as identified in this study, represent a broad family of bacterial characteristics related to virulence. The markedly increased duration of the more virulent B2 and D groups, compared with commensal *E. coli* (predominantly groups A and B2) [24], indicates bacterial genetic factors other than the presence of resistance genes that may aid in persistence. Potential mechanisms requiring exploration include biofilm formation, competitive bacterial toxin production, e.g. colicins, and virulence factors including siderophore and fimbriae production mediating competitive advantages in iron capture and adhesion. Furthermore, the relation of factors that determine persistence to those that determine the classical virulence of invasion and infection also requires elucidation.

Limitations of this study include the exploratory nature using a previously collected sample. The collection method of recovering a single isolate from the plate has led to some limitation in determining clonality. To investigate this, a model was constructed using the assumption that clones recovered on a given swab were present, but not identified on all previous swabs because of this methodological issue. This model indicated that approximately one third of clones were not recovered on any given swab, suggesting that with the repeat sampling undertaken, there was a low chance of failing to identify truly persisting clones (data not shown).

In the multivariate analysis, only a limited number of factors were assessed. There may be other significant participant and isolate features that were not included in this analysis. Furthermore, the assumption that resistance was only acquired whilst travelling and did not occur after return is relied heavily upon in the data. We believe this was reasonable given the very low background rates of resistance in Canberra [4, 13]; however, it is likely not absolute. Acquisition of resistant clones after return (or the emergence of "low-level" pre-travel resistant clones owing to antimicrobial use) may have led to over-estimation of the duration of carriage and diversity of clonality. A control group of matched non-travellers from Canberra would have been optimal in assessing this situation.

Conclusion

Prolonged gastrointestinal carriage of resistant bacteria after return from travel is a complex phenomenon. The duration of carriage of CRO resistance was significantly shorter than for ciprofloxacin or gentamicin resistance. Risk factors for prolonged carriage of resistance include antimicrobial use whilst travelling and the duration of travel in "high risk" regions. Clonality was present amongst all phenotypes, but was almost absolute amongst CRO resistance. The contraction to a small number of clones and shared clonality amongst travel partners suggests a limited number of clones adapted to prolonged carriage circulating in regions of acquisition. ST-131, the worldwide pandemic clone, was surprisingly infrequent amongst the phenotypes assessed. The identification of clonality amongst travel partners and mixed groups (where direct transmission from person to person was unlikely) suggests exposure and ingestion of a common source of resistant isolates, potentially food or water.

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Conflict of interests BR, KK, HS, MJ and DP declare that they have no conflicts of interest.

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Major article

Predictors of use of infection control precautions for multiresistant gram-negative bacilli in Australian hospitals: Analysis of a national survey

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Key Words:
Patient isolation
Bacterial infection
Cephalosporin resistance
Carbapenemase
β-lactamase

Introduction: Despite the global expansion of extended spectrum β−lactamase-harboring *Enterobacteriaceae* (ESBL-E) and carbapenem-resistant *Enterobacteriaceae* (CRE), only limited research on the infection control management of patients with these organisms is available.

Methods: We present a national survey of infection control practices amongst adult acute-care hospitals in Australia, for ESBL-E, CRE, and the emerging threat of patients with overseas health care contact.

Results: In total, 97 health services responded, representing 9% of all eligible hospitals. The proportion of hospitals that reported use of contact precautions (CP) was 96% (93 out of 97) for ESBL-E, 81% (79 out of 97) for CRE, and 72% (48 out of 67) for patients transferred from an international hospital. For ESBL-E hospitals frequently employed risk-stratification to limit the use of CP (40 out of 97; 41%). On multivariate analysis predictors of a strategy to limit use of CP for ESBL-E were government funding (odds ratio, 4.8; $P = .003$) and a metropolitan location (odds ratio, 3.2; $P = .014$); predictors of any use of CP for CRE were location in an Australian state with a specific legislation on CRE ($P = .030$) and the presence of a written policy on CRE ($P = .011$).

Conclusions: Infection control management of multiresistant gram-negative bacilli varied considerably across the Australian hospitals surveyed. A lower rate of reported CP use for CRE than for ESBL-E was unexpected and indicates a vulnerability in some Australian hospitals. Multivariate analysis revealed various drivers influencing infection control practice in Australia.

A marked expansion of community-onset extended-spectrum β-lactamase harboring *Enterobacteriaceae* (ESBL-E), and the burgeoning of carbapenem-resistant *Enterobacteriaceae* (CRE) has occurred during the past decade.[1,2] Furthermore, patients with overseas health care contact are increasingly identified as a vector for the global movement of new antimicrobial resistance mechanisms, including those mediating CRE.[3]

The majority of current infection-control guidelines include recommendations for the control of multiresistant gram-negative bacilli (MRGNBs), including CRE and ESBL-E. Fewer guidelines include recommendations for patients with overseas health care contact.[4] Given the small number of published studies on which to base recommendations[5] and the rapidly changing epidemiology of MRGNBs, guidelines in this area risk being outpaced by on-the-ground events.

Australia is a low-prevalence country for ESBL-E, with a 2010 national survey of community onset isolates indicating 3.4% of *Escherichia coli* and 3.6% of *Klebsiella pneumonia* were ESBL producing.[6] CRE in Australia originate from 2 key sources: low-level endemicity of metallo-β-lactamase-producing *Enterobacteriaceae* within critical-care areas and some specialty units on the country's eastern coast,[7] and residents returning after overseas health care contact.[8,9] We have previously described variations in the infection control practices used for patients hospitalized with expanded-spectrum cephalosporin-resistant *Escherichia coli* as part of an Australasia-wide study.[10,11] Such a disparity has been noted in other reports.[12,13]

In summary, we present a national survey of practice in infection control management of patients harboring ESBL-E, CRE, and

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patients with overseas health care contact, amongst acute-care adult hospitals in Australia. Our aim was to define the scope of variation of infection control practices for these groups in Australia and to identify factors that determine which policy and practice is applied in differing health services.

METHODS

The study population was adult acute care hospitals within Australia. At the time of the survey Australia (population 23 million) was serviced by approximately 700 publically funded hospitals and 300 private hospitals across its 6 states and 2 administrative territories.[14,15]

A draft survey was constructed, including questions based on those used in a previously published work.[12] The survey was pilot tested on 5 experienced infection control practitioners and modified based on their feedback. The survey questioned health service practice of infection control, rather than the details of written policy. Where a service used varied practices within their network, respondents were asked to answer for the area that best fit the description of an adult acute care facility. The full survey is in the Supplementary material.

The survey was conducted as an open invitation online survey, using a Web-based interface to collect responses. An e-mail invitation was disseminated via 2 frequently used national e-mail discussion groups, 1 hosted by the Australasian College for Infection Prevention and Control and the other hosted by the Australasian Society for Infectious Diseases.[16] The former sends e-mail messages to approximately 500 e-mail addresses (personal communication, Michael Wishart, HSN Hospital, Queensland, Australia, May 5, 2014) and the latter to approximately 900 e-mail addresses (personal communication, Dr Ashley Watson, Canberra Hospital, Australian Capital Territory, Australia, April 24, 2014). Follow-up e-mail messages were disseminated via these channels. The survey was open for a 2-month period (November 2012-January 2013). A small token of appreciation (a gift hamper) was offered to 1 randomly selected responding site.

Human research ethics approval for the conduct of this study was received from University of Queensland.

We requested that the nominal head of infection control complete or delegate completion of the survey at each site, to minimize multiple responses. If multiple responses from a single site were received, these were collapsed as follows: concordant, relative agreement (eg, difference in details only), or discordant. Answers in the latter 2 groups were combined using the following rules: affirmative responses (indicating the presence of a given policy) were presumed to be correct, and the most restrictive application of a policy or most conservative numerical was presumed to be correct. Infection control services were not recontacted because permission for this had not been sought in the ethics approval.

Where a single respondent answered for a health service/network of multiple adult acute-care hospitals, this was maintained as a single answer, with demographics from the single largest hospital used for analysis.

External data sources

Data are primarily as reported by the respondent. Key demographics (eg, hospital size, funding, and referral services) were confirmed with public data sources (Supplementary material 1-4). Denominator data for Australian hospitals was extracted from the Australian Institute of Health and Welfare (AIHW) annual report 2011-2012.[15]

Definitions

Contact precautions were defined as the use of any combination of gloves, gown, and or a single or cohort room.[17] Infection control practice was considered inclusive when all patients with a given resistance phenotype were managed in contact precautions or permissive if nonuse of contact precautions was allowed in some circumstance (risk stratification by bacterial species or patient characteristic) or was not used at all. Hospital type was stratified by funding source; public hospitals are fully funded by the Australian state and/or federal governments. They provide the vast majority of supraregional referral services in Australia, whereas private hospitals draw funding from patient billing revenue and primarily service patients covered by voluntary private health insurance or other third parties. Supraregional referral services were highly specialized referral services such as transplant service (ie, solid organ or allogeneic bone marrow transplant services) and other supraregional services (ie, major burns, spinal injury, and cystic fibrosis services). An infectious disease (ID) service was an ID physician providing consultation or inpatient services at the hospital. A written policy specifically pertained to the resistance phenotype (or patient group) queried, rather than a generic multiresistant organism (MRO)-type policy. Hospital size was classified by the AIHW Peer Group system.[18] Because private hospitals are not classified by this system, 2 researchers (BR and SH) independently assigned a peer group after review of any available hospital demographic data (from the survey and publicly available information on the hospital's Website). Disagreement was resolved by discussion. Principal referral hospitals (A1 by AIHW classification) are major city hospitals with >20,000 and regional hospitals with >16,000 (casemix-adjusted) separations per year. Large hospitals (A1, A2, B1, and B2) included principal referral, specialist women's hospitals, large metropolitan (>10,000 casemix-adjusted separations), and large regional hospitals (>8,000 or >5,000 casemix-adjusted separations, depending on location).

Relevant legislation and recommendations

At the time of the survey there were no national infection control management recommendations or legislation specifically pertaining to CRE or patients with overseas health care contact. Some recommendations for ESBL-E are provided in the national infection control guidelines.[17] Two Australian states work within state-level legislation (operational directives). One encompasses all MROs (MRO directive),[19] and the other specifically CRE (CRE directive).[20] See Supplementary material 1-4 for a comparison of state and national documents.

Statistical methods

Univariate analysis was undertaken using χ^2 test, Fisher exact test, and calculation of odds ratios. Multivariate logistic regression included all variables significant on univariate analysis at a $P = .2$ level. Using backward selection variables were retained in the final logistic regression model if their significance remained below $P = .2$. Models were assessed by calculation of a receiver operator curve and Hosmer-Lemshow goodness of fit test. Robust estimates of variance were used to account for a potential lack of independence between hospitals, given some operate in shared jurisdictions where standardization of policy may have occurred. In addition, when the geographic variable of state-based legislation was entered into multivariate analysis, it was maintained as a tripartite set (MRO policy, CRE policy, or no policy). All statistical tests were 2 tailed, and $P < .05$ was considered significant. STATA version 12.1 (Statacorp, College Station, Texas) was used.

Table 1
Infection control management of extended-spectrum β-lactamase harboring *Enterobacteriaceae* (ESBL-E), carbapenem-resistant *Enterobacteriaceae* (CRE), and internationally transferred patients (IT-patients) (N = 97)

	ESBL-E			CRE			IT-patients		
Precautions used	Use	Do not use	Unknown	Use	Do not use	Unknown	Use	Do not use	Unknown
Any contact precaution use	93 (96)	4 (4)	0	79 (81)	18 (19)	0	48 (49)	19 (20)	30 (31)
Glove and gown	88 (91)	8 (9)	0	73 (75)	20 (21)	4 (4)	36 (37)	31 (32)	30 (31)
Single room*	91 (94)	5 (5)	1 (1)	75 (77)	18 (19)	4 (4)	48 (49)	19 (20)	30 (31)
Cohort room	28 (29)	69 (71)	0	21 (22)	76 (78)	0	NA	NA	NA

NOTE. Data are presented as n (%).
NA, not applicable.
*For IT-patients, single room and cohort were not differentiated in the survey.

RESULTS

Valid responses were received from 97 unique hospitals or health services. Eight further responses were excluded because they originated from institutions that did not meet the study population (hospital type: exclusively pediatric n = 3, elective day-procedure sites or subacute care only n = 3, or psychiatry only n = 2).

Hospital demographics and national coverage

Responses were received from 68 public hospitals and 29 private hospitals. This included 75% (31 out of 41) of all sites accredited to train fellows in adult ID within Australia.[21]

Approximately 9% of all Australian hospitals within the survey population responded (9% public hospitals [68 out of 736] and 9% private hospitals [25 out of 285], excluding private hospitals in 3 states that do not provide denominator data).

In total, 58 hospitals (60%) were situated in metropolitan locations, 32 were located in regional locations (33%), and 7 were located in remote locations (7%). Stratified by state location, the geographic distribution of respondents approximately mirrored that of the Australian population (Supplementary material 1-4).

Respondents

In total there were 108 respondents, including 9 sites with duplicate responses. The majority of survey respondents identified their role as a nursing-trained infection control practitioner (81 out of 108; 75%, including nurse-managers and clinical nurse specialists), or a physician (22 out of 108; 20%, including ID, infection control, or clinical microbiology specialists). The remainder of respondents were in managerial or academic positions (5 out of 108; 6%).

There was at least 1 responding physician for 19 out of 97 (20%) health services. Significantly more principal referral centers compared with other centers had a physician respond (18 out of 43 [42%] vs 1 out of 54 [2%]; $P = .002$).

Control of specific organisms and patients

ESBL-E

Contact precautions were used by 93 out of 97 hospitals (96%) in the management of patients with ESBL-E. (Table 1) The most common implementation of these precautions was inclusive (48 out of 97; 49%), with contact precautions used for every patient with any ESBL-E. A variety of permissive practices, including the use of risk stratification by organism genera and/or patient characteristics were used in other sites (Fig 1). Data was missing from 5 out of 97 sites (5%).

A descriptive analysis of factors predicating the presence of a permissive practice for the application of contact precautions in patients with ESBL-E, compared with an inclusive practice, is shown in Table 2. On multivariate analysis, the predictors of use of a permissive practice were hospital funding type ($P = .003$) and a metropolitan location ($P = .014$) (Table 3).

CRE

Use of contact precautions for patients harboring CRE was reported by 79 out of 97 hospitals (81%) (Table 1). An inclusive practice was most common, occurring in 56 out of 97 hospitals (58%) (Fig 1). Descriptive analysis of the predictors of use of any contact precautions on patients harboring CRE is in Table 2. On multivariate analysis the significant predictors of any use of contact precautions for patients harboring CRE were the presence of a written policy on CRE infection control ($P = .011$) and location in the state with a CRE directive ($P = .030$) (Table 3).

International transfer patients

In total 67 out of 97 hospitals (69%) provided information on infection control management of patients received after an international transfer between hospitals, 25 out of 97 (26%) indicated they did not know the policy or had never faced this situation, and 5 out of 97 (5%) did not respond to the question (Table 1).

Contact precautions was used in 72% of hospitals that provided details (48 out of 67) (Fig 1). Descriptive analysis of infection control management is presented in Table 2. On multivariate analysis positive predictors of the use of any contact precautions for internationally transferred patients was the presence of a written policy on internationally transferred patients ($P = .025$) and location in the state with a CRE directive ($P = .003$) (Table 3).

Some form of enhanced infection control management (contact precautions and/or screening) was applied to patients reporting overseas health care contact (although not directly transferred from a hospital) by 45 out of 97 hospitals (46%), with 46 out of 97 (47%) reporting no use of precautions and 6 out of 97 (6%) not responding.

AmpC-type organisms

For patients harboring AmpC-producing organisms, 48 out of 97 hospitals (49%) reported no use of contact precautions, 41 out of 97 hospitals (42%) reported an identical management strategy to ESBL-harboring organisms, 7 out of 97 hospitals (7%) reported a standalone strategy, and 1 out of 97 hospitals (1%) did not respond.

Policy basis and origin

The most commonly utilized publications in the preparations of infection control policy for MRGNBs was the Australian National Health and Medical Research Council guideline[17] (87 out of 97; 90%) and departmental guidelines or directives issued by state or

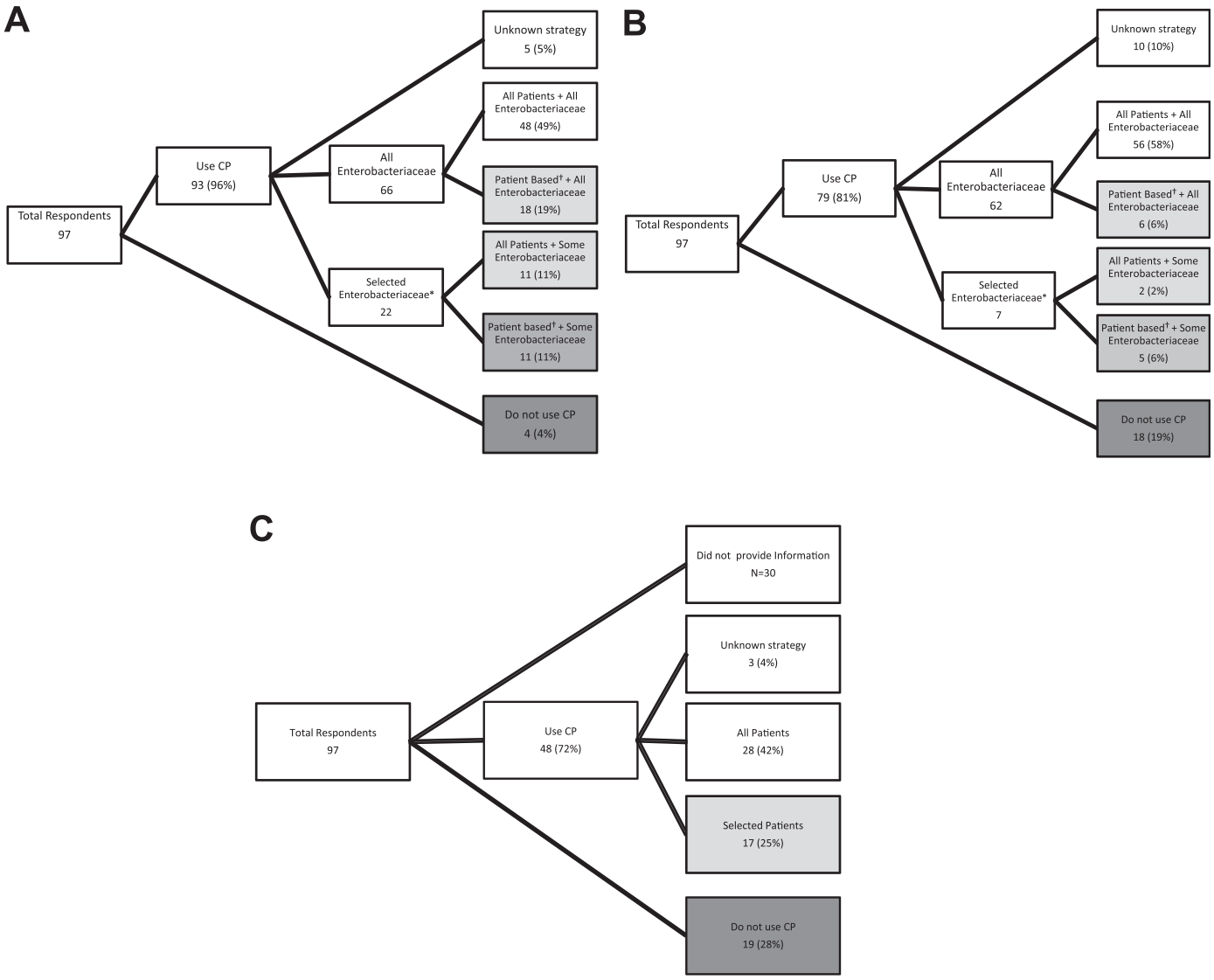

Fig 1. Breakdown of strategies for the use of contact precautions (CP) for extended-spectrum β-lactamase harboring *Enterobacteriaceae* (ESBL-E), carbapenem-resistant *Enterobacteriaceae* (CRE), and internationally transferred patients. Boxes shaded in white indicate an inclusive or unknown strategy. Boxes shaded in grey indicate a permissive strategy. (A) ESBL-E. (B) CRE. (C) Internationally transferred patients. *Organisms considered for CP amongst ESBL-E include *Escherichia coli* + *Klebsiella pneumoniae* (11 out of 22; 50%), *K pneumoniae* (5 out of 22; 23%), *Escherichia coli* (2 out of 22; 10%), and neither/unsure (5 out of 23; 23%). Organisms considered for CP amongst CRE = *Escherichia coli* + *K pneumonia* (2 out of 7; 29%), *K pneumonia* only (3 out of 7; 43%), and neither/unsure (2 out of 7; 29%). †Patient-assessment for CP for ESBL-E included ward location (15 out of 29; 51%) and clinical features of the patient (eg, sample of origin for isolate and presence of diarrhea) (23 out of 29; 79%). Three sites (out of 29; 10%) indicated neither of these factors was used in their assessment. For CRE, patient-based assessment included the ward location (3 out of 11; 27%) and clinical features (8 out of 11; 73%). Three sites (out of 11; 27%) indicated neither of these were used for their assessment.

territory governments (80 out of 97; 82%). Hospitals based in states with compulsory directives were significantly more likely to identify this source than other states (36 out of 38 [95%] vs 44 out of 59 [76%]; $P = .015$). International guidelines were used less frequently, including those originating from the Centers for Disease Control and Prevention[22] (43 out of 97; 44%) and other countries' guidelines (21 out of 97; 22%).

Variability of survey response

Analysis of responding health services showed a disproportionate number of responses from large public hospitals. This group comprised 68% (48 out of 68) of survey respondents within the public hospital group, although numerically accounts for only 18% of public hospitals (131 out of 736) within Australia. Within responses on internationally transferred patient management missing data was most often from smaller hospitals. In total 86% of principal referral hospitals (37 out of 43) provided details on their management compared with 56% of other sites (30 out of 54) ($P = .001$ for comparison).

Analysis by respondent type showed significant differences in reported CRE management. All sites with a physician respondent (19 out of 19) reported use of contact precautions for CRE, whereas only 60 out of 78 sites (77%) with a nonphysician respondent reported this use ($P = .019$ for comparison). When further analysis was undertaken within the subgroup of principal referral hospitals, to account for the disproportionate number of physician respondents within this group, this difference was still apparent, although not statistically significant due to the small numbers involved (18 out of 18 physician respondents (100%) used contact precautions for CRE versus 20 out of 25 nonphysician respondent sites [80%]; $P = .064$).

Table 2
Descriptive analysis of predictors of infection control management. The 3 groups of data are descriptive analysis of the use of contract precautions (CP) as a permissive policy for extended-spectrum β-lactamase harboring *Enterobacteriaceae* (ESBL-E), any use of CP for carbapenem-resistant *Enterobacteriaceae* (CRE), or any use of CP for internationally transferred patients (IT-patients)

		ESBL-E				CRE				IT-patients			
Predictive characteristic	Count amongst all hospitals (%) (N = 97)	Count with permissive use of CP for ESBL-E (% of total with permissive use) (n = 44)	Count with inclusive use of CP for ESBL-E (% of total with inclusive use) (n = 48)	Odds ratio (95% CI)	*P* value	Count with any use of CP for CRE (% of total using CP for CRE) (n = 79)	Count who do not use CP for CRE (% of total not using CP) (n = 18)	Odds ratio (95% CI)	*P* value	Count with any use of CP for IT-patients (% of total with using CP) (n = 48)	Count who do not use CP for IT-patients (% of total not using CP) (n = 19)	Odds ratio (95% CI)	*P* value
Public (vs private)	68 (70)	37 (84)	31 (65)	4.1 (1.5-11.1)	.005	54 (68)	25 (32)	0.6 (0.2-2.1)	.436	33 (69)	15 (29)	0.6 (0.2-2.1)	.411
Metropolitan location (vs regional and rural)	58 (60)	32 (73)	26 (54)	2.7 (1.1-6.4)	.028	51 (72)	7 (39)	2.9 (1.0-8.3)	.052	33 (69)	13 (68)	1.0 (0.3-3.2)	.979
Infectious disease service	57 (59)	29 (66)	28 (58)	1.5 (0.6-3.5)	.347	49 (62)	8 (44)	2.0 (0.7-5.8)	.179	30 (63)	16 (84)	0.3 (0.1-1.2)	.097
Written policy on phenotype/patient group	ESBL-E = 38 (39) CRE = 22 (23) IT-patients = 50 (52)	17 (39)	21 (44)	0.8 (0.4-1.9)	.621	22 (23)	0 (0)		.011	36 (75)	8 (42)	3.2 (1.4-7.6)	.006
Written policy on MBL	21 (22)	NA	NA	NA	NA	18 (23)	3 (17)	1.5 (0.4-5.7)	.571	NA	NA	NA	NA
MRO directive	21 (22)	9 (20)	12 (25)	0.8 (0.3-2.1)	.606	19 (24)	2 (11)	3.6 (0.7-17.1)	.116	9 (19)	7 (37)	0.7 (0.2-2.3)	.522
CRE directive	17 (18)	9 (20)	8 (17)	1.3 (0.4-3.7)	.642	17 (100)	0 (0)		.030	16 (33)	0 (0)		.003
Regular international transfers*	28 (33)	16 (41)	12 (25)	1.7 (0.7-4.4)	.244	27 (39)	1 (6)	10.3 (1.3-83.1)	.029	21 (46)	7 (41)	1.2 (0.4-3.7)	.753
Large hospital	59 (61)	32 (73)	27 (56)	2.25 (0.9-5.4)	.069	49 (62)	10 (56)	1.3 (0.5-3.7)	.614	32 (68)	15 (79)	0.5 (0.2-1.9)	.330
Principal referral vs other	43 (44)	25 (57)	18 (34)	2.6 (1.1-5.9)	.026	38 (48)	5 (28)	2.4 (0.8-7.4)	.126	25 (52)	12 (63)	0.6 (0.2-1.9)	.416
Transplant service†	20 (21)	14/25 (56)	6/18 (33)	2.3 (0.6-8.4)	.196	19/38 (50)	1/5 (20)	4.0 (0.4-40.2)	.239	13 (52)	5 (42)	1.5 (0.4-6.2)	.562
Other supra-regional referral services†	21 (22)	14/25 (56)	7/18 (39)	2.3 (0.6-8.4)	.196	20/38 (53)	1/5 (20)	4.4 (0.4-44.7)	.205	15 (60)	4 (33)	3.0 (0.7-12.9)	.141

MBL, metallo-β-lactamase harboring bacteria; *MRO*, multiresistant organism; *NA*, not applicable.
*Hospitals were considered to accept international transfers regularly if they reported a frequency of less than monthly or greater.
†Variables analyzed only within principal referral hospitals.

Table 3
Multivariate analysis of the predictors of a permissive practice for extended-spectrum β-lactamase harboring *Enterobacteriaceae* (ESBL-E), carbapenem-resistant *Enterobacteriaceae* (CRE), and any use of contact precautions (CP) for CRE and internationally transferred patients (IT-patients)

	Permissive policy for CP use for ESBL-E*		Any CP use for CRE†		Any CP use for IT-patients‡	
Predictive characteristic	Multivariate OR (95% CI)	*P* value	Multivariate OR (95% CI)	*P* value	Multivariate OR (95% CI)	*P* value
Public (vs private)	4.8 (1.7-13.4)	.003				
Metropolitan location (vs regional and rural)	3.2 (1.3-8.1)	.014				
Infectious disease service					0.2 (0.3-0.9)	.041
Specific written policy§				.011	3.4 (1.2-9.7)	.025
Multiresistant organism directive			2.8 (0.4-14.8)	.218		
CRE directive				.030		.003
Regular international transfers			4.6 (0.5-40.3)	.166		
Nontransplant supraregional services‖					3.4 (0.7-15.5)	.118

CI, confidence interval; *OR*, odds ratio.
*n = 44 out of 92 (48%) with permissive policy, n = 5 with missing data.
†n = 79 out of 97 (81%) use CP.
‡n = 38 out of 67 (72%), n = 30 with missing data.
§Written policy on infection control of phenotype or patient group.
‖Cystic fibrosis, burns, or spinal injury.

Analysis of intrasite correlation for the 9 sites that submitted multiple responses to the survey is presented in Supplementary material 4. Across 7 major themes in the survey, no single site showed concordance for all answers. Across all sites, 51% of details reported were concordant (32 out of 63), 27% were in relative agreement (17 out of 63), and 22% were discordant (14 out of 63).

The highest discordance was on the presence of written policies (7 out of 9; 88% discordant) and the use of contact precautions for internationally transferred patients (3 out of 9; 33% discordant). Relative agreement occurred most often when describing contact precautions use for ESBL-E and CRE (each 6 out of 9; 67% relative agreement). In the relative agreement group, differences between respondents were in the description of details of risk stratification practice and the use of cohort rooms.

DISCUSSION

Our national survey demonstrates widely varied infection control practice for MRGNBs across at least 97 hospitals in all regions of Australia. Such variation has been demonstrated in other parts of the world.[12,13]

Foremost, our unexpected finding is that contact precautions use is reported more frequently for ESBL-E than for CRE. Several explanations are apparent. First, our data indicate many infection control policies and/or practitioners rely on the use of a laboratory ESBL phenotype to trigger contact precautions use, as evidenced by the lower rates of any contact precautions use for AmpC organisms compared with ESBL-E (50% vs 96%). A number of CRE will not possess this phenotype (nor even an metallo-β-lactamase phenotype) and will not be detected unless decreased carbapenem susceptibility is the trigger for infection control intervention.

Second, misclassification may have occurred due to unfamiliarity with the term CRE. This was unforeseen in the survey design and specific examples of carbapenem and *Enterobacteriaceae* were not given. This finding is supported by the significantly higher proportion of physician-trained respondents (who are all trained and certified subspecialists), compared with others, reporting of use of contact precautions for CRE. Other respondents (94% from a nursing background) would have a more varied clinical and academic background than the physicians, and may not be familiar with aspects of CRE, given its relative infrequency in Australia.[23]

To some extent both possibilities indicate a number of vulnerable Australian hospitals, where patients with CRE may not have an appropriate assessment of their infection control needs. Within misclassification it is difficult to determine for what proportion the issue is simply a lack of knowledge of current terminology, as opposed to a greater knowledge gap around the potentially varied clinical and laboratory characteristics of CRE and the serious implications of the phenotype.

Our findings on the predictors of ESBL-E and CRE practice offer insight into some of the pragmatic aspects of determining MRGNB policy.

For ESBL-E infection control practitioners have potentially sought a balance between the low risk of nosocomial transmission of ESBL-E[24] and the various burdens of contact precautions. Hospitals that were likely to have a high load of ESBL−E-harboring patients, due to their generally higher acuity (metropolitan and public hospitals), were more likely to use a permissive policy to limit use of contact precautions for this phenotype. Interestingly, the only 4 sites that did not use any contact precautions for ESBL-E were principal referral sites with a supraregional referral patient load.

Predictors of contact precautions use in CRE illustrate the benefits and risks of layering state-based legislation on health care services. When used in a targeted manner, this can be effective in filling a void in national policy. Operating under a CRE directive was a significant predictor of the use of contact precautions for CRE (and for internationally transferred patients, which are also covered by the policy).

In contrast, the general MRO directive appears to be ineffective. Despite 90% of sites in the state indicating they used directives to formulate policy, directives did not have a significant influence on infection control practice for the emerging threats of CRE and internationally transferred patients. Even for ESBL organisms (which are specifically mentioned in the document), sites have either disregarded, or gradually moved away from, the stipulated management.

Internationally transferred patients are an emerging risk group with which other regions are also grappling.[25] The significant predictive value for the use of contact precautions of the presence of a written policy for internationally transferred patients should be interpreted in light of the 31% missing data and the 33% discordance rate on answers to this question. There is some misclassification. Taken as a whole these data indicate limited knowledge of this risk group and of details of policy even where policies do exist.

A key strength of our study is the national sample and the broad mix of hospital types, including thought-leading sites where future ID and infection control physicians train. Other studies in this area have looked either within smaller, more homogenous groups or across multiple countries.[12,13]

A limitation of our study is selection bias. Large hospitals were overrepresented amongst respondents. Similarly, the methodology biased our sample toward a subset of infection control departments that are active on infection control e-mail bulletins and were motivated to participate.

Our survey design captured practice rather than exact policy content. Thus, the answers reflect 1 or a few individuals' practice, and may not be representative of the majority at the site. This is demonstrated by the analysis of multiple responders. With the exception of the questions on written policy (for which the question text required a subjective decision), discordant answers occurred in aspects of policy that may be less commonly used (eg, CRE, internationally transferred patients, and AmpC organisms). However, differing interpretations of policy was very frequent.

Misclassification complicates the interpretation of our results. For CRE and internationally transferred patients it is difficult to untangle if findings represent a gap in knowledge of terminology/policy content or a true absence of policy. We believe misclassification is a lesser problem for ESBL-E data because the terminology is common in Australian infection control literature.[17]

An important factor not easily accounted for in our analysis is the exchange and centralization of policy amongst local regions or private sector groups, independent of binding directives or published recommendations.

Finally, applicability of our findings may vary by country. The influence of state-based policy will differ depending on the governance structure of the country involved. The frequency, nature, and risk of internationally transferred patients will vary by country and travel patterns.

CONCLUSIONS

Infection control management of MRGNBs varies widely amongst adult acute care hospitals in Australia. We have demonstrated a vulnerability of some Australian hospitals' infection control practice in the management of CRE-harboring patients and internationally transferred patients. This is due to limited knowledge of aspects of these risk groups amongst some practitioners, an absence of policy, and a lack of knowledge of policy. We await the likely positive influence of recently released national recommendations on infection control management of CRE in improving and harmonizing practice within Australia.[26]

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ajic.2014.05.035.

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APPENDIX C – ADDITIONAL RELEVANT PUBLICATIONS 2010-2014

1. Paterson DL, **Rogers BA**. How Soon is Now? The urgent need for randomized, controlled trials evaluating multi-resistant bacterial infection. Clin Infect Dis. 2010 Dec 1;51(11):1245-7.

2. Avent ML, **Rogers BA**, Cheng AC, Paterson DL. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. Intern Med J. 2011 Jun;41(6):441-9.

3. Totsika M, Moriel DG, Idris A, **Rogers BA**, Wurpel DJ, Phan MD, et al. Uropathogenic *Escherichia coli* mediated urinary tract infection. Current drug targets. 2012 Oct;13(11):1386-99.

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How Soon Is Now? The Urgent Need for Randomized, Controlled Trials Evaluating Treatment of Multidrug-Resistant Bacterial Infection

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(See the article by Kofteridis et al, on pages 1238–1244.)

Antibiotic resistance among gram-negative bacilli shows no signs of abatement. Resistance of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and the Enterobacteriaceae to multiple antibiotic classes is a growing clinical problem worldwide [1]. Two trends are particularly noteworthy. First, there has been increased recognition of successful antibiotic-resistant clones appearing in multiple geographic regions. Multilocus sequence typing analyses of contemporary collections of multidrug-resistant strains have shown that carbapenem-resistant *A. baumannii* ST92 [2], *K. pneumoniae* carbapenemase–producing *K. pneumoniae* ST258 [3], and extended-spectrum β-lactamase–producing *Escherichia coli* ST131 [4] are global problems. Second, new mechanisms of multidrug resistance are becoming evident. These include aminoglycoside 16S ribosomal RNA methylation [5] and production of the New Delhi metallo-β-lactamase [6].

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From a clinical viewpoint, the end result of these and other mechanisms of antibiotic resistance is loss of susceptibility to all penicillins (including combinations with β-lactamase inhibitors), cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. There are desperately few treatment options available for strains with this resistance profile. Possibilities for "salvage therapy" include polymyxins (eg, colistin) [7], tigecycline [1], and fosfomycin [8]. As is illustrated in the article by Kofteridis et al [9] in this issue of *Clinical Infectious Diseases*, much attention has been paid to the potential use of novel salvage regimens, including combinations of different antibiotics or use of multiple modes of antibiotic administration.

Kofteridis et al [9] evaluated the combination of aerosolized plus intravenous colistin in contrast with monotherapy with intravenous colistin. They concluded that this combination of administration routes did not provide additional therapeutic benefit in patients with ventilator-associated pneumonia (VAP) due primarily to *A. baumannii*. Before this therapeutic strategy is "written off" as ineffective, it is useful to look more deeply into the design and results of this study. Using a matched case-control study design, Kofteridis and colleagues demonstrated that 54% of patients in the dual–administration route arm had clinical cure, compared with 32.5% in the intravenous only arm ($P = .05$). There were also trends toward superiority in the end points "clinical success" (74% vs 60%; $P = .10$) and mortality in the intensive care unit (24% vs 42%; $P = .066$). In a multivariable model, trends toward superior clinical cure with the dual route of administration persisted (odds ratio, 2.375; 95% confidence interval, 0.901–6.258; $P = .08$) [9].

There are a number of potential reasons why these results should not lead to the combination of aerosolized plus intravenous colistin being discarded as a potential treatment option. First, the study methods give no indication of sample size deliberations; it may well be that the study was underpowered to truly show a significant difference between the 2 treatment options. Second, the diagnosis of VAP is always fraught with difficulties, even when quantitative cultures are used. Given that patients with airway colonization may have been labeled as having VAP, we have a real chance of bias toward the null hypothesis, because the outcomes for patients with airway colonization should not be affected by therapy, thereby necessitating an even greater sample size to show a real difference. Third, the dosages of colistin were not based on modern pharmacokinetic analyses, and it is possible that dosing regimens were not optimal.

Table 1. Potential Randomised, Controlled Trials in the Arena of Treatment of Infection with Gram-Negative Bacilli That Could Be Evaluated for Fast-Tracked Funding

β-Lactam antibiotics plus aminoglycosides versus β-lactam antibiotics alone for serious *Pseudomonas aeruginosa* infection
Pharmacodynamically optimized versus standard therapy for serious infections due to gram-negative bacilli
Combinations including colistin versus colistin alone for bacterial infections resistant to all other options
Inhaled plus intravenously administered antibiotics versus intravenous administration alone for ventilator-associated pneumonia
Short-course versus long-course therapy for bloodstream infection due to gram-negative bacilli

In addition, few details are provided about the mode of aerosolization of colistin. The science of aerosolization of drugs has advanced rapidly in the past decade [10]. A different approach to drug delivery is necessitated in the intubated versus the awake patient because of characteristics including the patient's supine position, artificial airway, and the use of humidified air [10]. With use of a traditional jet nebulizer, it is estimated that only 6%–10% of the nominal dose would be inhaled by the patient [11]. Newer technologies, such as the vibrating-mesh nebulizer, significantly improve delivery and have yielded promising results in the nebulized delivery of other antimicrobials, such as amikacin, in this setting [12].

Although the combination of aerosolized plus intravenous colistin is potentially promising for the treatment of VAP due to multidrug-resistant gram-negative bacteria, combinations of different antibiotics also need to be considered. James Rahal and Carl Urban from New York City have pioneered the study of combination therapy and have shown synergistic effects of the combination of carbapenems, rifampin and a polymyxin against carbapenem-resistant Gram negative organisms [13, 14]. A number of other combinations have been assessed by a variety of groups around the world. Numerous observational studies have been performed, in addition to this one by Kofteridis et al [9]. Unfortunately, conflicting results abound, but some studies have yielded encouraging results [15].

It is important to temper the potential advantages of combination therapy with experience from management of serious, antibiotic-susceptible *P. aeruginosa* infection. The parallels are significant. In vitro, synergy has been widely shown between certain combinations of antibiotics (eg, antipseudomonal β-lactam antibiotics plus aminoglycosides) [16]. Some observational studies have shown substantial benefits of combination therapy, including significant reductions in mortality when combination therapy is used [17]. Yet, there is a paucity of randomized, controlled trials showing superiority of combination therapy versus monotherapy. Indeed, meta-analyses of randomized, controlled trials have shown no benefit of combination therapy over monotherapy for *P. aeruginosa* or other common serious infections with gram-negative bacteria [18, 19].

We desperately need randomized, controlled trials in the field of treatment of infections with multidrug-resistant gram-negative bacilli. We acknowledge that the severity of illness and the clinical complexities of patients usually affected by multidrug-resistant gram-negative bacilli greatly hamper conduct of such studies. Furthermore, substantial resources are needed to conduct randomized, controlled trials: the pharmaceutical industry will not fund such studies until new antibiotics are developed that have significant activity against multidrug-resistant organisms. Even then, industry is likely to take the easy way out and conduct studies of conditions such as urinary tract infection or complicated intra-abdominal infections. Therapy for multidrug-resistant organisms will continue to be regarded as an off-label use, with clinicians persisting to use therapy without data from randomized, controlled trials. It is hoped that the US Food and Drug Administration would demand studies of patients at high risk of developing infection due to multidrug-resistant organisms. Whether this imposition of additional hurdles on the path to drug approval would discourage development of new drugs active against these organisms is highly contentious.

It may be several years before the pharmaceutical industry has sufficient new compounds to be studied. In the meantime, is there a way we can rigorously study existing options? The National Institutes of Health is to be congratulated for their program to fund "Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance." A randomized, controlled trial comparing the combination of colistin and imipenem versus colistin monotherapy for multidrug-resistant *A. baumannii* infection has recently been funded by this program (K. Kaye, personal communication). On the basis of the work of Kofteridis et al [9], it would seem that an randomized, controlled trial comparing the combination of aerosolized plus intravenous colistin versus monotherapy with intravenous co-

Table 2. Predicted Issues in Gram-Negative Bacteria Resistance in the Next Decade

Widespread occurrence of carbapenem resistance in hospitalized patients necessitating "routine" use of polymyxins or tigecycline
Resistance to polymyxins and tigecycline commonplace in some hospitals
Loss of improvement in intensive care unit survival rates due to impact of resistance in gram-negative bacilli
Calls for universal screening for multidrug-resistant gram-negative bacilli at hospital admission
Increased acquisition of carbapenem-resistant organisms outside of hospitals
Increased hospitalizations for community-onset urinary tract infections due to pathogens resistant to all orally administered antibiotics

listin may also be a reasonable proposition (Table 1). Studies evaluating combinations with rifampin may also be worthy of further consideration.

The sad news is that multidrug-resistant *A. baumannii* and *P. aeruginosa* have been noted for more than a decade [20]. On the basis of the global epidemiology of infection, we need to be planning randomized, controlled trials for treatment of a variety of infections with a high probability of being widespread in the next decade (Table 2). How soon should we act? Now!

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REVIEW

Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity

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Abstract

The new Australian Therapeutic Guidelines: Antibiotic, version 14 have revised the recommendations for the use and monitoring of aminoglycosides. The guidelines have clear distinctions between empirical and directed therapy as well as revised recommendations about the monitoring of aminoglycosides. This has led many clinicians to review their current practice with regard to the use of aminoglycosides. This review summarizes why aminoglycosides are still a valid treatment option and discusses the rationale for current dosing regimens in Gram-negative infections. In particular it focuses on the various methods for monitoring aminoglycosides that are currently being used. The aminoglycoside monitoring methods can be categorized into three groups: linear regression analysis (one compartment model), population methods and Bayesian estimation procedures. Although the population methods are easy to use and require minimal resources they can recommend clinically inappropriate doses as they have constant pharmacokinetic parameters and are not valid in special population groups, that is, renal impairment. The linear regression and Bayesian methods recommend more accurate dosage regimens; however, they require additional resources, such as information technology and healthcare personnel with background training in pharmacokinetics. The Bayesian methods offer additional advantages, such as calculation of doses based on a single serum concentration and optimization of the patient's previous pharmacokinetic data, in order to determine subsequent dosage regimens. We recommend the Bayesian estimation procedures be used, wherever feasible. However, they require the expertise of healthcare practitioners with a good understanding of pharmacokinetic principles, such as clinical pharmacists/clinical pharmacologists, in order to make appropriate recommendations.

Introduction

The aminoglycoside antimicrobials have a long and controversial history. First developed in the 1940s, they are derived from antimicrobial substances produced by the soil dwelling bacterial species *Streptomyces* and *Micromonospora*. The 'workhorse' of aminoglycosides, gentamicin, has been used for the treatment of serious Gram-negative bacterial infections since the early 1960s and continues in this role today.[1,2]

Aminoglycoside are bactericidal. Their primary site of action is the 30S subunit of the prokaryotic ribosome, interrupting bacterial protein synthesis. To reach this site they bind to the bacterial cell wall and undergo active transport into the cell cytosol.[3]

The three commonly used parenteral agents are gentamicin, tobramycin and amikacin. Other routes of administration include inhalation through a nebulizer (tobramycin), intraperitoneal and intraventricular administration (gentamicin). Two further agents paromomycin and neomycin are used orally for their bowel intra-luminal activity, as they are not systemically absorbed.[4] Streptomycin, the first aminoglycoside agent

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in widespread use, along with netilmicin and kanamycin are now infrequently used.

Indications for use

With the advent of broad-spectrum β-lactam antimicrobials (anti-pseudomonal penicillins, third generation cephalosporins and carbapenems), we have seen a shift away from the prolonged administration of aminoglycosides. This change has been driven by the improved safety profile and improved pharmacokinetic parameters of these newer agents. However, in the latest edition of the Therapeutic Guidelines: Antibiotic, version 14 (TG14: Antibiotic), there remains a broad group of indications for the use of aminoglycosides. The guidelines clearly delineate empirical and directed therapy. For empirical therapy the recommended treatment duration is now limited to 48 h and monitoring of plasma concentrations is not required. Aminoglycosides are used in this setting because of their rapid bactericidal activity and low rates of resistance in community and healthcare settings.[2] For prolonged directed therapy (>48 h), aminoglycosides are now reserved for a limited number of specific indications; infections when resistance to other safer antimicrobials has been shown, combination therapy for serious *Pseudomonas aeruginosa* infections and brucellosis, and in low doses as synergistic treatment of streptococcal and enterococcal endocarditis.

Common indications for parenteral aminoglycosides administration are summarized in Table 1. There are very few absolute contraindications to aminoglycosides; however, they should be used with caution with patients with pre-existing hearing, vestibular and renal impairment.[2]

Toxicity

The significant clinical toxicities of aminoglycosides are ototoxicity, nephrotoxicity and less often neuromuscular toxicity. There is no definitive evidence of differences in the degree of toxicity among the three commonly used agents (gentamicin, tobramycin and amikacin).[5,6] Patient factors that vary the risk of toxicity include pre-existing disease, severity of illness, concomitant drugs administered and genetic predisposition.[5,6] In addition, prolonged therapy of aminoglycosides has shown to be an independent risk factor for toxicity.[7]

Nephrotoxicity occurs after glomerular filtration of the agent in the proximal convoluted tubule. Ototoxicity, including vestibular and cochlear toxicity, occurs from damage to the sensory hair cells of these organs. The exact pathophysiological mechanism at both sites is incompletely understood. Neuromuscular blockade after

Table 1 Indications for parenteral aminoglycosides[2]

Surgical prophylaxis (preventive)
Duration: *single dose only*
Agent: *gentamicin used in combination or alone*
Procedures
Urological surgery
Selected abdominal surgery (excluding hernia repair)
Vascular surgery
Cardiac surgery
Empirical therapy (pathogen unknown)
Patient illness: severe
Duration: *<48 h*
Agent: *gentamicin always used in combination*
Intra-abdominal and surgical infection
Cholangitis
Acute cholecystitis
Diverticulitis
Perforated viscus
Surgical site infection
Genito-urinary infection
Pyelonephritis
Epididymo-orchitis (from a suspected urinary source)
Pelvic inflammatory disease (non-sexually acquired)
Respiratory tract infection
Community acquired pneumonia in tropical regions
Hospital acquired pneumonia
Infective endocarditis
Central nervous system
Epidural abscess
Endophthalmitis
Sepsis
Intravascular line-related
Adult with an unknown source or suspected Gram-negative bacteraemia
Children less than 4 months if age (including neonates) with an unknown source
Febrile neutropenia†
Directed therapy (pathogen known)
Duration: *prolonged, often weeks*
Agent: *see below, almost always used in combination*
Gentamicin
Brucellosis
Infective endocarditis‡ (pathogen): *Enterococcus spp.*, *Streptococcus spp*. and *Bartonella spp*.
Infective endocarditis‡ (device): infection associated with a prosthetic valve or intra-cardiac device
Enteric organism bacteraemia: *Campylobacter spp*. and *Yersinia spp*.
Pseudomonas aeruginosa infection‡
Severe listeria†‡
Treatment of biological warfare agents: pneumonic plague and tularaemia
Tobramycin
Pseudomonas aeruginosa infection in cystic fibrosis
Amikacin
Highly drug-resistant Gram-negative organisms, for example, metallo ß-lactamase producing bacteria;
Central nervous system *Nocardia spp*. infection;
(Resistant) mycobacterial infection including *M. tuberculosis*, *M. abscessus*, *M. avium* complex§

†Indication not in TG14: Antibiotic. ‡Synergistic use. §In the setting of drug-resistant tuberculosis amikacin is used at a low dose often for prolonged periods (>6 months).

administration is described, usually in conjunction with other diseases or drugs that affect the neuromuscular junction, for example, patients with myasthenia gravis.[5] Although a genetic susceptibility mutation has been reported (mitochondrial 1555A→G) in patients with kindreds with an inherited susceptibility to hearing loss,[8] the population prevalence of these mutations (1:500) is probably too infrequent to account for most cases of ototoxicity. The incidence of vestibular or cochlear toxicity varies in studies with the definition used, and most were performed before the era of high-dose-extended interval regimens. However, most reviews have reported rates of approximately 5–10% for hearing impairment and approximately 3% for vestibular toxicity.[9] The rate of ototoxicity with durations of treatment less than 48 h is not known; however, ototoxicity following single doses has been described.[10,11]

Monitoring for toxicity can be through three mechanisms; quantitative testing of end-organ effects (monitoring serum creatinine and audiometry), active bedside testing and passive reporting by the patient. There is no definitive evidence to inform optimal techniques for monitoring of toxicity.[5] As a minimum standard, before the commencement of aminoglycoside therapy patients should be informed about the possible adverse effects and asked to report if they develop subjective hearing loss, tinnitus or oscillopsia, and serum creatinine should be monitored in all patients. Where the expected duration of therapy is more than 5 days, bedside tests should be performed for cochlear and vestibular function (Table 2). The 'whisper test' has been shown to have a high sensitivity for hearing impairment within the clinically relevant frequency range,[12] but does not detect high-frequency hearing loss that often occurs earlier. Additionally, hearing loss may occur after cessation of the antibiotic course. Where available, serial audiometry (pure tone audiometry ± otoacoustic emissions) may be considered; the development of high-tone hearing impairment and impaired outer cell function is characteristic of drug-induced damage. Diagnosis is most accurate if a baseline result is available for comparison, as similar changes may be found in patients with hearing loss because of other causes, including presbycusis (age-related hearing loss). There is no definitive evidence that ceasing aminoglycosides when ototoxicity is detected minimizes further damage, but it would seem prudent to weigh up the benefits of continuing with the significant risk of ototoxicity in this situation.

Table 2 Clinical screening tests for ototoxicity

Whispered voice test
Performance
The examiner stands arm's length (0.6 m) behind the seated patient and whispers a combination of numbers and letters (e.g. 4-K-2) and then asks the patient to repeat the sequence.
The examiner should quietly exhale before whispering to ensure as quiet a voice as possible.
The examiner always stands behind the patient to prevent lip reading.
Each ear is tested individually, starting with the ear with better hearing, and during testing the non-test ear is masked by gently occluding the auditory canal with a finger and rubbing the tragus in a circular motion.
The other ear is assessed similarly with a different combination of numbers and letters.
Interpretation
If the patient responds correctly, hearing is considered normal; if the patient responds incorrectly, the test is repeated using a different number/letter combination.
The patient is considered to have passed the screening test if they repeat at least three out of a possible six numbers or letters correctly.
Action
Patients with impaired hearing should be referred for audiometry.
Dynamic visual acuity testing
Performance
With the patient seated and with the head kept still at 6 metres from the Snellen chart determine their best visual acuity with both eyes open.
Then do the same with examiner standing behind the patient and rotating the patient's head smoothly from side to side at a rate of approximately two cycles per second in a continuous, sinusoidal fashion (i.e. with no stationary periods to allow fixation).
Interpretation
A normal person will lose less than or equal to two rows on the Snellen chart.
Anything greater than this indicates an abnormality of the vestibulo-ocular reflex (i.e. in the case of gentamicin or amikacin because of bilateral vestibular injury).
Record both baseline and during rotation results to compare with prior and future tests.
Action
If there is evidence of vestibular toxicity, aminoglyoside antibiotic should be ceased where possible.
Patients should be assessed by physiotherapy and occupational therapy.
A neurological opinion should be sought if there is evidence of functional impairment.

Aminoglycoside resistance

Aminoglycoside resistance in Gram-negative bacteria can be endogenous or acquired. Examples of clinically encountered bacteria exhibiting endogenous resistance include *Stenotrophomonas maltophilia* and *Burkholderia cepacia*.

Acquired resistance can occur in almost all Gram-negative organisms, including commonly encountered bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae* and *P. aeruginosa*. Acquired resistance is frequently encoded

by genes located on a transferrable plasmid, increasing the likelihood of horizontal spread to other bacteria. It is frequently associated with other resistance genes, such as those encoding extended spectrum β-lactamases,[13] rendering bacteria resistant to multiple classes of antibiotics.

There is a wide variety of mechanisms of aminoglycoside resistance. The most common is enzymatic inactivation of the aminoglycoside through adenylation, acetylation or phosphorylation. Other less frequent mechanisms include decreased antimicrobial permeability, active efflux pumps and various changes in the ribosomal target site.[14] A newly described mechanism of resistance is methylation of 16S ribosomal RNA. This mechanism results in high level resistance to gentamicin, tobramycin and amikacin.[15]

In the drug development pipeline are next-generation aminoglycosides or 'neoglycosides', synthesized by extensive modification of current agents. They have demonstrated promising *in vitro* activity against bacteria resistant to currently available aminoglycosides.[16] However, they are unlikely to be active against strains which produce 16S ribosomal RNA methylases.

Pharmacokinetic consideration for Gram-negative infection

Traditionally, gentamicin was administered as multiple daily doses until the introduction of once-daily aminoglycoside dosing in the early 1990s. Following increased evidence from clinical studies,[17–22] it is now widely accepted that once-daily dosing is as effective and probably less nephrotoxic than conventional multiple-daily dosing regimens. The advantages of once-daily dosing include: optimization of the maximum concentration (C_{max}) : minimum inhibitory concentration (MIC) ratio,[23,24] area under the curve (AUC), post-antibiotic effect[25] and reduction in costs of medication administration (Fig. 1).[26–28]

There are two pharmacodynamic predictors of efficacy for aminoglycosides namely $AUC_{0–24}$: MIC and the C_{max} : MIC ratio and dosing should aim to optimize these parameters. Target AUC values of 80 to 100 and C_{max} 8 to 10, respectively, are reasonable based on animal and human pharmacodynamic studies.[6] In clinical practice either or both of these parameters could be used, even though they are influenced differently by host factors. C_{max} : MIC ratio is related most exclusively to volume of distribution, whereas the $AUC_{0–24}$: MIC ratio is influenced by both volume of distribution and clearance. It has been argued that getting high peaks to achieve satisfactory C_{max} : MIC ratios against *P. aeruginosa* should be the main aim of therapy, because susceptible members of this species have median MIC values of <2 mg/L.[6]

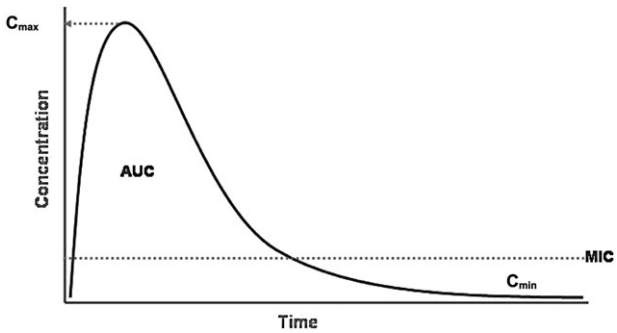

Figure 1 Pharmacokinetic parameters. $AUC_{0–24}$, area under the curve (24-h dosing interval); C_{max}, maximum concentration; C_{min}, minimum concentration; MIC, minimum inhibitory concentration (of the organism the antibiotic is targeting).

However, for organisms with a MIC of 2 mg/L, such as *P. aeruginosa*, high C_{max} of at least 20 mg/L (10 times MIC of 2 mg/L) would thus be required.[23,24] In theory, constant exposure to aminoglycosides is required where it is being used in synergy with beta-lactams for endocarditis, and this is the basis for the recommendation for multiple daily dosing in many guidelines.[2,29] However, clinical trials have demonstrated the efficacy of single daily doses for at least some pathogens.[30] Based on these studies, international guidelines recommend these regimens routinely.[31]

Therapeutic drug monitoring

Therapeutic drug monitoring is frequently used to confirm serum concentrations of aminoglycosides and to make required dosage adjustments. However, it is important to record accurate data, such as the dose, time of sample and time of start and of infusion, in order to correctly interpret serum concentrations and make the correct dosage adjustments.

When patient individualization dosing strategies are used based on the subject's weight and aimed at a therapeutic range to optimize clinical outcomes, a greater percentage of patients will achieve the targeted concentration as compared with patients who receive a fixed dose strategy.[32] Patients who attained targeted therapeutic serum concentrations of aminoglycosides early in their treatment course have shown an improved clinical outcomes.[5]

Some centres measure 'peak' and 'trough' concentrations and make empiric dosage adjustments, which was commonly performed in the era when multiple daily dosing regimens were common. These empiric adjustments result in a 'trial and error period' with different dosage regimens until optimal serum concentrations are

achieved. This approach potentially results in incorrect dosage adjustments, prolonged periods to obtain targeted concentrations and unnecessary healthcare costs.[5] To optimize the use of serum concentrations and make the interpretation of pharmacokinetic data more meaningful the second level should not be a trough concentration, but a measurable random concentration (above the limit of detection of the assay normally at or above 0.5 mg/L). If an undetectable level is reported, that is <0.5 mg/L, and one substitutes a level of 0.5 mg/L when calculating a patient's pharmacokinetic parameters, several assumptions are made which can result in inaccurate dosage regimens.

Aminoglycoside monitoring

Several nomograms and algorithms have been developed to individualize pharmacokinetic monitoring for gentamicin. Three major methods of dose individualization commonly used to target specific pharmacokinetic parameters are: (i) linear regression analysis (one compartment model), (ii) population methods and (iii) Bayesian estimation procedures.[33] Currently available algorithms and computer programs are summarized in Table 3.

Australian guidelines for monitoring

For many years, the 'Therapeutic Guidelines' has recommended several methods for monitoring gentamicin. Although their recommended method for monitoring aminoglycosides was a computerized method with a dosage adjustment to achieve a target AUC they also realized that not all hospitals would have access to these computer programs so nomograms were included. However, the new Therapeutic Guidelines have now been released and they have made several new recommendations, including the deletion of the nomograms and recommendation to only use AUC-based computerized methods.

Linear regression analysis

The first method used to fit serum concentrations to individual patient models was the Sawchuk-Zaske method using linear regression analysis. The Aminoglycoside Levels and Daily Dose Indicator (ALADDIN) is also another example of this method. These methods use an *a posteriori* drug dosing calculations where the patient's pharmacokinetic parameters are calculated from at least two measured serum concentrations and assume a one compartment model.[5] These methods require healthcare practitioners who have specialized pharmacokinetic knowledge (clinical pharmacologist/pharmacist) and patient information, such as value and timing of dose and level and duration of infusion, in order to interpret accurately the pharmacokinetic results (AUC, C_{max}, C_{min}, Cl and Vd). Based on the pharmacokinetic results the programs then determine the most appropriate dose and dosing interval adjustments for the patient. They also require additional resources, such as access to computers at the point of patient care.

Although these methods are simple they do make several assumptions: they only use serum concentration data around the dosing interval where the levels were obtained and therefore there is a loss of continuity of data; they assume that the assay error is a constant percentage which means lower serum concentrations can be overestimated.[5] The lack of population data, and hence the necessity to have two levels presents a limitation in some settings, such as paediatrics.

Population methods

A population method, alternatively called an *a priori* dosing method, determines aminoglycoside dosage based on population pharmacokinetic parameters, without using the patient's individual pharmacokinetic results.[5] Nomograms such as the Therapeutic Guidelines: Antibiotic Dose Adjustment Nomogram (TG Nomogram), the Hartford Nomogram and the Begg Nomogram, use estimates of pharmacokinetic parameters such as volume of distribution in order to estimate dosage recommendations. The nomograms have proved popular as they are easy to interpret, require no specialized pharmacokinetic knowledge for the interpretation of the results and limited use of resources (personnel and/or computers). In addition, the patient information required to interpret the nomogram is minimal and they only require information, such as value and timing of dose and level.

Although the TG Nomogram and the Hartford Nomogram have been widely used, it is important to ensure the individual patient matches the population for which the nomogram has been developed. In addition all nomograms assume stability of pharmacokinetic parameters, such as creatinine clearance, which may not occur in a sick patient. The TG Nomogram has only been validated in a small number of patients with a normal renal function and may not be suitable in patients with a creatinine clearance less than 90 mL/min,[34] whereas the Hartford Nomogram has not been validated in patients that may have altered kinetic parameters, such as, burns, pregnancy, ascites, dialysis.[24] In addition, several studies have found a lack of agreement in dose recommendations made by the population–nomogram methods.[34,36]

Table 3 Comparison of aminoglycoside programs

	TG Nomogram	Begg	Hartford Nomogram	Sawchuk-Zaske method	ALADDIN	DoseCalc	BestDose	SeBA-GEN	TCI works	MM-USCPACK version 15.2
Method	Population nomogram	Population nomogram	Population nomogram	Log linear regression analysis – one compartment model	Log linear regression analysis – one compartment model	Log linear regression analysis – one compartment model	Bayesian estimation procedures	Bayesian estimation procedures	Bayesian estimation procedures	Bayesian estimation procedures
Pharmacokinetic parameter used to calculate subsequent doses	Population data	Population data	Population data	C_{max} C_{min}	AUC	Ratio of actual : estimated C_t at any specified time	C_{max} C_{min}	AUC	C_{max} C_{min} AUC	C_{max} C_{min}
Validation	Yes[34]	No	Yes[24]	Yes[35]	Yes[36]	(Gentamicin only)[34,36]	Validation by developers (not published)	Yes[33,34]	Validation by developers (not published)	Yes[37,38]
Special population groups	No	No	No	Paediatrics Renal impairment cystic fibrosis	Paediatrics Renal impairment cystic fibrosis	Renal impairment obesity	Paediatrics Renal impairment Cystic fibrosis Burns	Renal impairment, cystic fibrosis (adults)	Paediatrics Renal impairment, cystic fibrosis (adults and paediatrics)	Renal impairment Dialysis Different ethnic groups
Antibiotics	Gentamicin Tobramycin amikacin	Gentamicin	Gentamicin	Gentamicin, tobramycin amikacin	Gentamicin, tobramycin amikacin	Gentamicin tobramycin	Gentamicin tobramycin	Gentamicin tobramycin	Gentamicin tobramycin, amikacin vancomycin	Gentamicin Tobramycin Amikacin Vancomycin
Advantages of the program	Used at the bedside	Used at the bedside	Used at the bedside	Used at the bedside	Searchable database for patient information Printable patient report for medical chart		Printable patient report for medical chart	Printable patient report for medical chart	Searchable database for patient information Graphical presentation of data Printable patient report for medical chart Shows user when there is an error in data (i.e. incorrect time recorded)	Searchable database for patient information Graphical presentation of data Generates patient report for medical chart Shows user when there is an error in data (i.e. incorrect time recorded)
Limitations of the program	Calculates dose adjustment but not dosing interval adjustment (set at 24 h)	Calculates a starting dose and dose adjustment but not dosing interval adjustment (set at 24 h)	Calculates dose interval adjustment but not dosing adjustment (set at 7 mg/kg)	Requires a log linear calculator	Stand-alone application Requires Microsoft Office Access 2003 platform to run Second level needs to be obtained within same dosing interval		Stand-alone	DOS-based	Patient report is lengthy and may contain too much information for patient's chart	Complex reports and data analysis Does not use standard units for reporting pharmacokinetic parameters

Table 3 Continued

	TG Nomogram	Begg	Hartford Nomogram	Sawchuk-Zaske method	ALADDIN	DoseCalc	BestDose	SeBA-GEN	TCI works	MM-USCPACK version 15.2
Network capability	Not applicable	Not applicable	Not applicable	Not applicable	Limited by Microsoft Office Access 2003 requirements	No (but can be run as a multi-user application launched from a Novell server)	No	No	Yes	Yes
Cost	No cost to user	No cost to user	No cost to user	No cost to user	No cost to user	Cost for private institutions/ individuals	Yes	No cost to user	No cost to user	US$595 (trial version available)
IT support	Not applicable	Not applicable	Not applicable	Not applicable	No	No	No	No	Yes	Yes
Developer	John Turnidge, The Women and Children's Hospital, Adelaide	Evan Begg Murray Barclay Stephen Duffull, Christchurch Hospital	David Nicolau, Hartford Hospital, Hartford, Connecticut	R. J. Sawchuk Darwin Zaske	John Turnidge Jan Bell, The Women and Children's Hospital, Adelaide	Alasdair Millar Frank Sanfilippo, Royal Perth Hospital	Paul Harrison, CompuThought	Stepehn Duffull and Carl Kirkpatrick University of Otago and Queensland	Stephen Duffull Carl Kirkpatrick and Lionel van den Berg, University of Otago and Queensland	Roger Jellife, School of Medicine University of Southern California
Last updated/ latest version	Has been removed from Therapeutic Guidelines v 14 because no longer recommended	1995	1995	1977	2007 Version 4	Jan 2004 Version 8.0	1998 Version 1.4			Version 15.2
Manual available	See guidelines	Published paper	Published paper	Published paper	Yes	No	Yes	Yes	Yes	Yes
How to access	Published: now withdrawn from use	Published paper	Published paper	Published paper	http:// www.asainc.net.au/aladdin	Developer	Not available, but still used in some centres	Developer	http:// www.tciworks.info	http://www.lapk.org/software.php

ALADDIN, Aminoglycoside Levels and Daily Dose Indicator; AUC, area under the curve; C_{max}, maximum concentration; C_{min}, minimum concentration; IT, information technology.

Unfortunately the patients' pharmacokinetic parameters were not described in these studies.

Bayesian estimation procedures

The Bayesian approach offers the advantage that it makes optimal use of all information contained in the population model (a priori) combined with the most current pharmacokinetic information from the patient (a posteriori) to develop the patient's most precise regimen.[5,33] Examples are the Sequential Bayesian Algorithm for Gentamicin (SeBA-GEN, The University of Queensland, Australia and the University of Otago, New Zealand) program, Target Intervention Software program (TCI works, The University of Queensland, Australia, and the University of Otago, New Zealand) and MM-USCPACK (Laboratory of Applied Pharmacokinetics, School of Medicine, University of Southern California, Los Angeles, CA, USA), which have been developed for dose adjustment of once-daily dosing of aminoglycosides.[5,33] Although these methods calculate the most precise regimen, they require healthcare practitioners (clinical pharmacologist/pharmacist) who have specialized pharmacokinetic knowledge and ready access to resources, such as computers. In addition, patient information such as the patient's sex, height, weight, age, serum creatinine, value and timing of dose and level, duration of infusion in order to accurately compute pharmacokinetic parameters (AUC, C_{max}, C_{min}, Cl and Vd). An advantage of the Bayesian estimation procedures is that they can calculate doses based on a single serum concentration and can also predict an appropriate starting dose providing some patient information has been entered into the program.

Conclusion

The aminoglycosides remain an important antibiotic class in our current therapeutic armamentarium. They continue to be recommended for short-term and prolonged therapy in selected situations. Their use can be associated with significant toxicity. A key strategy in minimizing toxicity and optimizing therapy is therapeutic drug monitoring. All of the methods for dose individualization described above have been used by hospitals and each is associated with advantages and disadvantages. The ideal method is one that predicts an accurate, clinically appropriate dose, requires minimal resources and is easy to use. The linear regression methods, that is, Sawchuk-Zaske and ALADDIN, require two serum concentrations after a dose of aminoglycoside whereas the Bayesian estimation procedures can calculate doses based on one serum concentration. However, both the linear regression and the Bayesian estimation procedures require skilled personnel, usually clinical pharmacists and or clinical pharmacologists, with an understanding of pharmacokinetics to use and interpret the information. As not all clinicians have access to these specialized pharmacy services this limits their application. Additionally, it should be stressed the software is only as good as the data entered – if the time of administration of the drug or specimen collection is inaccurate, then erroneous results may occur. The once–daily aminoglycoside nomograms (i.e. TG Nomogram and Hartford Nomogram) require only one serum concentration, are easy to interpret and require no specialized pharmacokinetic knowledge. Nevertheless, concerns have been raised about their reliability as they make the assumption that all patients have the same constant pharmacokinetic parameters, which means they are not valid in patients that do not meet these criteria, such as special population groups, for example, renal impairment. Therefore, given the large interpatient variability in aminoglycoside pharmacokinetic parameters there are many clinical situations where it is inappropriate to use nomograms. We recommend the Bayesian estimation procedures be used, wherever feasible.

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Uropathogenic *Escherichia coli* Mediated Urinary Tract Infection

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Abstract: Urinary tract infection (UTI) is among the most common infectious diseases of humans and is the most common nosocomial infection in the developed world. They cause significant morbidity and mortality, with approximately 150 million cases globally per year. It is estimated that 40-50% of women and 5% of men will develop a UTI in their lifetime, and UTI accounts for more than 1 million hospitalizations and $1.6 billion in medical expenses each year in the USA. Uropathogenic *E. coli* (UPEC) is the primary cause of UTI. This review presents an overview of the primary virulence factors of UPEC, the major host responses to infection of the urinary tract, the emergence of specific multidrug resistant clones of UPEC, antibiotic treatment options for UPEC-mediated UTI and the current state of vaccine strategies as well as other novel anti-adhesive and prophylactic approaches to prevent UTI. New and emerging themes in UPEC research are also discussed in the context of future outlooks.

Keywords: Antibiotic resistance, uropathogenic *Escherichia coli*, vaccine, virulence.

INTRODUCTION

Urinary tract infections (UTI) are among the most frequent human bacterial infections, with an estimated 40-50% of women experiencing at least one cystitis episode in their lifetime [1, 2]. UTI usually starts as a bladder infection (cystitis), but can develop to acute kidney infection (pyelonephritis), ultimately resulting in scaring and renal failure. UTI is also a major cause of sepsis, which has a mortality rate of 25% and results in more than 36,000 deaths per year in the USA [3]. Almost all patients with an indwelling urinary catheter for 30 days or longer develop catheter-associated UTI, which accounts for approximately 40% of all hospital-acquired infections [1]. Common Gram-negative and Gram-positive bacterial pathogens that cause UTI include *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, Acinetobacter baumannii, Morganella morgannii, Staphylococcus saprophyticus* and *Enterococcus* spp.

UROPATHOGENIC *ESCHERICHIA COLI* AND MAJOR VIRULENCE FACTORS

Uropathogenic *E. coli* (UPEC) is the most common etiological agent of UTI, causing more than 80% of infections. UPEC strains are often classified on the basis of O (somatic), K (capsular polysaccharide), and H (flagellar) antigens. There is a high frequency of the antigens O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 among UPEC strains, while specific K and H antigens display a less distinct pattern [4]. UPEC strains possess an arsenal of virulence factors Fig. (**1**) that contribute to their ability to cause disease, including adhesins (e.g. type 1 and P fimbriae), toxins (e.g. hemolysin) and iron-acquisition systems that utilise siderophores (e.g. enterobactin, salmochelin, aerobactin) [5-7]. Several studies have also implicated UPEC flagella as a virulence factor that contributes to UTI [8-11]. Undoubtedly, adherence to the urinary tract epithelium is a critical step in the initiation of disease; it enables UPEC to resist the hydrodynamic forces of urine flow and triggers host and bacterial cell-signalling pathways that result in inflammation and colonization, respectively.

Fimbrial Adhesins

Fimbrial adhesins of UPEC are characterised by a conserved chaperone-usher pathway that underpins their assembly [12]. Fimbriae (or pili) assembled by the chaperone-usher pathway are typically encoded by gene clusters that in addition to the chaperone- and usher-encoding genes also include genes encoding the major and minor pilin subunits. P and type 1 fimbriae are the best-characterised chaperone-usher class of fimbriae produced by UPEC. P fimbriae are strongly associated with acute pyelonephritis; they contribute to the establishment of UTI by binding to the α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside receptor epitope in the globoseries of glycolipids and activate innate immune responses in animal models and in human infection [13-16]. Type 1 fimbriae enhance colonization and host response induction in the murine UTI model, and promote biofilm formation and host cell invasion [17-19]. Type 1 fimbriae confer binding to α-D-mannosylated proteins such as uroplakins, which are abundant in the bladder [17, 20]. Both P- and type 1 fimbriae recognise their receptor targets by virtue of organelle tip-located adhesins, namely PapG and FimH, respectively [21-23].

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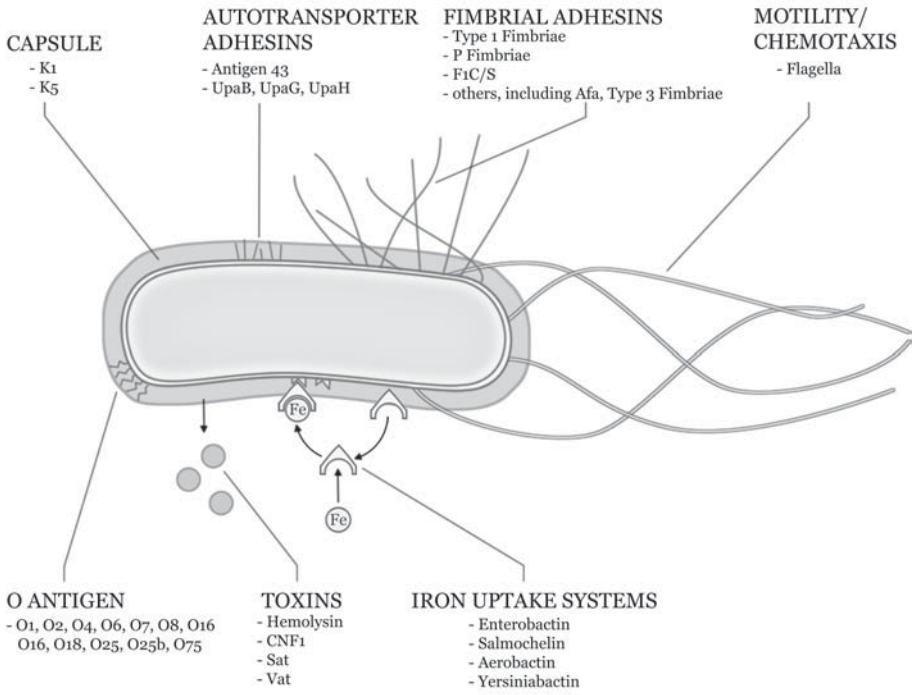

Fig. (1). Major virulence factors of UPEC. Schematic diagram of a UPEC bacterium showing examples of the major virulence factors present on the cell surface or secreted. Fimbrial and autotransporter adhesins mediate attachment and colonization of multiple host tissues. Flagella mediate motility and chemotaxis and contribute to bacterial dissemination to new sites in the urinary tract. Iron uptake systems scavenge iron from the host and facilitate its uptake into UPEC, thus promoting bacterial fitness in the iron-limiting urinary tract. Secreted toxins induce host cell lysis and can disrupt inflammatory signaling cascades in the host. Capsule and O-antigen mediate serum resistance and promote host evasion. Expression of each factor is tightly regulated by UPEC and coordinated to prevent co-expression of virulence factors with competing functions. UPEC organelles and virulence factors are not drawn to scale.

Genome sequencing of a number of UPEC strains has revealed they contain multiple fimbrial gene clusters; for example the well-defined CFT073 strain contains ten putative chaperone-usher gene clusters [24]. Some of these other chaperone-usher fimbriae have also been associated with the ability of UPEC to cause UTI. F1C fimbriae resemble type 1 fimbriae in their genetic organisation and organelle structure [25-27], however differ in their adhesin specificity. F1C fimbriae mediate binding to galactosylceramide targets present on epithelial cells in the bladder and kidneys as well as globotriaosylceramide present exclusively in the kidneys [28, 29]. Other fimbriae of the chaperone-usher family that have been characterised from UPEC include S fimbriae (which mediate adherence to sialic acid glycolipids or glycoproteins) [30], Afa/Dr fimbriae (which mediate adherence to decay-accelerating factor, collagen IV and carcinoembryonic antigen-related cell adhesion molecules) [31], F9 fimbriae [32], type 3 fimbriae [33-35] and Auf fimbriae [36].

Autotransporter Adhesins

Another class of adhesins strongly associated with UPEC virulence is represented by the autotransporter (AT) proteins. AT proteins represent the largest group of bacterial type V secreted proteins and share several common features: an N-terminal signal sequence, a passenger (α) domain that is either anchored to the cell surface or released into the external milieu, and a translocation (β) domain that resides in the outer membrane [37, 38]. In general, AT proteins differ substantially in their passenger domain sequence, which determines the unique functional characteristics of the protein and is often associated with virulence [39]. Similar to chaperone-usher fimbrial genes, UPEC contain multiple different AT-encoding genes; CFT073 strain contains eleven putative AT genes [40, 41]. Characterised UPEC AT proteins include the secreted toxin Sat (see below), the phase variable outer membrane protein antigen 43 (Ag43) [42], the trimeric AT protein UpaG [43, 44] and the surface-located UpaB, UpaC and UpaH proteins [40, 45]. AT proteins of UPEC possess a diverse range of functions including adhesion, aggregation, biofilm formation and cytotoxicity. The Ag43, UpaB and UpaH AT proteins contribute to colonization of the mouse bladder [40, 42, 45] and Ag43 expression is associated with intracellular biofilm formation [46]. The secretion of AT proteins is assisted by accessory factors such as the Bam complex (also known as the YaeT or Omp85 complex), as well as periplasmic chaperones such as SurA, Skp and DegP [47-53]. Most recently, a new translocation and assembly module (TAM) that promotes efficient secretion of AT proteins in proteobacteria has been described [54].

Toxins

UPEC also secrete a range of toxins that damage host epithelial cells. Approximately 50 % of UPEC strains produce α-hemolysin and its expression correlates strongly with symptomatic UTI [55]. The secretion of α-hemolysin causes host cell lysis and results in the release of nutrients

and other growth factors such as iron that can be utilized by the infecting bacteria. Sublytic concentrations of hemolysin may also contribute to virulence by inactivating the serine/threonine kinase Akt, which plays a central role in host cell cycle progression, metabolism, vesicular trafficking, survival, and inflammatory signaling pathways [56]. Another toxin, cytonecrotizing factor 1 (CNF1) is secreted by approximately one third of UPEC strains. CNF1 activates the small GTP-binding proteins of the Rho family by inducing glutamine deamination, resulting in the disruption of normal eukaryotic cell function [57, 58]. The serine protease autotransporter toxins Sat and Vat are also produced by some UPEC strains. Sat is a 107 kDa protein that induces a variety of cytopathic effects on bladder and kidney cells and elicits glomerular damage in the mouse UTI model [59, 60]. Vat has been best characterized from avian pathogenic *E. coli*, which is closely related to UPEC at the genomic level, and also induces cytopathic effects in epithelial cells [61]. A hybrid nonribosomal peptide-polyketide colibactin that induces cytopathic effects on epithelial cells has been described [62]. However, the genes encoding this toxin are also found in many commensal *E. coli* strains and thus a specific role in UTI has not been determined.

Iron Acquisition Systems

Iron is essential for bacterial growth and is limited in the urinary tract. Therefore, iron acquisition systems are important colonization factors for UPEC. An efficient method for the sequestration of iron is through the production of siderophores, which are low molecular weight Fe^{3+}-chelating compounds, and subsequent uptake *via* their associated membrane receptors [63, 64]. Four different siderophore systems have been identified and characterised in UPEC, namely enterobactin [65, 66], the glucosylated enterobactin derivative salmochelin [67], yersiniabactin [68-70] and aerobactin [71]. These siderophore systems are negatively regulated by ferrous iron and the ferric uptake regulator Fur, and are expressed under low iron conditions [72, 73]. UPEC strains encode and express different combinations of these siderophores [66, 74], with some strains such as the asymptomatic bacteriuria strain 83972 able to express all four siderophores [75]. In the UPEC strains CFT073 and 536, aerobactin, yersiniabactin and the heme receptors (ChuA and Hma) contribute to virulence in a mouse UTI model [76].

Outlook

Recent advances in DNA sequencing technologies have demonstrated that the *E. coli* pan-genome is open, with some studies estimating a gene pool of 13,000 genes [77]. There are currently 61 complete and 246 draft *E. coli* genomes available on the NCBI database. New *E. coli* genes continue to be identified with every new genome sequence. The majority of these new genes are carried on mobile DNA elements (plasmids, prophages, pathogenicity islands) and are of unknown function. Therefore, the full virulence gene repertoire of *E. coli* remains to be determined, and continued sequencing efforts, together with detailed molecular studies of putative virulence factors, are needed.

HOST-UPEC INTERACTIONS DURING UTI

Early Innate Responses Triggered Upon UPEC Entry and Attachment to Urothelium

The host response to UTI includes antimicrobial peptides, cytokine production, neutrophil influx, inflammation, apoptosis and exfoliation of host cells. Urothelial cells ensure sterility in the urinary tract by release of proteins, such as the Tamm-Horsfall protein that blocks bacterial adhesion, and lipocalin and lactoferrin, which dramatically limit the free iron present in the urinary tract. In addition, a first-line defense mechanism by urothelial cells against invading pathogens includes the synthesis and secretion of antimicrobial peptides, known as defensins, including alpha- and beta-defensins and cathelicidin. For example, the human cathelicidin antimicrobial peptide LL-37 protects the urinary tract from UPEC infection and its production by infected urothelial cells was recently shown to be enhanced by vitamin D supplementation [78, 79]. However, some UPEC strains have evolved strategies to subvert these first-line defenses and colonise the urothelium. Upon adhesion to mannosylated glycoproteins in the urothelium, UPEC trigger a cascade of Toll-like receptor mediated responses [80]. TLRs are a family of pathogen recognition receptors (PRRs) [81, 82], which are membrane-bound sensors that recognize pathogen associated molecular patterns (PAMPs) in the extracellular compartment and endosomes [83]. TLR4 is a key factor mediating mucosal immunity of the urinary tract against UPEC, as C3H/HeJ mice bearing a mutation in the Toll/interleukin-1 receptor (TIR) domain of TLR4 are poor at resolving experimental UTI [84]. Furthermore, *tlr4* $^{-/-}$ mice have a significantly higher bacterial burden in their bladder, compared to wild-type mice [85]. The poor ability to resolve UTI in these mice is a result of impaired cytokine and chemokine production and neutrophil recruitment. The TLR4 response initiated by UPEC is triggered by LPS, which acts in concert with P [86-88] and type 1 fimbriae [89, 90], upon initial attachment of bacteria to the urinary epithelium. Other TLRs that recognise UPEC flagellin were also shown to be involved in the host defence against UPEC. *tlr11*$^{-/-}$ and *tlr5*$^{-/-}$ mice display increased susceptibility to UPEC infection in the kidney and bladder, respectively [91, 92]. Downstream TLR adaptor molecules such as myeloid differentiation primary response protein 8 (MyD88), TIR domain-containing adaptor inducing beta interferon (TRIF), and TRIF-related adaptor molecule (TRAM) are also essential in signalling and activate the canonical NF-kB transcription factor to induce pro-inflammatory gene expression [93]. UPEC have also adapted mechanisms to evade and counter host recognition and signalling. Some UPEC strains produce the Toll/interleukin-1 receptor domain-containing protein TcpC, which has a similar structure to the TIR domain of human TLR1 and can interfere with MyD88 *via* direct binding [94]. TcpC disrupts TLR and non-TLR signalling pathways, thereby dampening innate immune responses.

UPEC Invasion of Host Cells and Intracellular Survival

In the mouse UTI model, adherence of some UPEC strains to the bladder surface results in the invasion of superficial facet cells in a type 1 pilus-dependent manner [18, 95]. Studies using the reference cystitis UPEC strain UTI89, have revealed a series of cellular events post-invasion that lead to

the rapid replication of UPEC in the cell cytoplasm and the formation of aggregates referred to as intracellular bacterial communities (IBCs) [46]. The end-point of the IBC cycle is characterised by UPEC fluxing out of superficial facet cells as long filamentous bacteria [96]. IBCs may enable UPEC evasion of the host immune response, permit re-infection and contribute to chronicity [97]. Evidence for the presence of IBCs in human UTI has also been provided; IBCs and filamentous bacteria have been observed in urine from women suffering acute cystitis [98]. The ability of UPEC to survive intracellularly is not limited to epithelial cells. Some UPEC strains were shown to survive in primary mouse and human macrophages within late endosome LAMP1 positive vesicles [99]. Another study reported that FimH-expressing UPEC are taken up into a membrane bound vesicle containing glycolipid proteins and caveolin [100]. The ability of UPEC to reside inside epithelial cells and macrophages invokes the idea that autophagy might be implicated in targeting intracellular UPEC during early or late bacterial entry. Classically a non-selective degradation system for proteins and organelles, there is mounting evidence that autophagy can indeed target intracellular pathogens [101]. The role of autophagy as an important innate immune response mechanism has been demonstrated against intracellular pathogens such as *Mycobacterium tuberculosis* [102]*, Salmonella enterica* serovar Typhimurium [103] and *Francisella tularensis* [93]. Autophagy can either result in pathogen elimination, as in the case of *M. tuberculosis* [102], or enable pathogen survival (e.g. *Coxiella burnetti* [104, 105]). The role of autophagy in UTI remains unclear. UPEC toxins such as hemolysin [106] and Sat [107] were recently shown to induce the onset of autophagy. Work from Amer *et al.,* [108] suggests that autophagy serves a protective function in macrophages infected with the UPEC strain CFT073. Chemically inhibition of CFT073-mediated autophagy in macrophages using 3-methyladenine (3-MA) increased macrophage killing of intracellular CFT073. However, CFT073 survives poorly in macrophages [99], suggesting that different UPEC strains may be affected by autophagy in different ways.

Outlook

While the mechanisms of UPEC intracellular survival are not well understood, it is clear that this opportunistic intracellular lifestyle offers UPEC a significant advantage; bacteria are protected from the immune response or from antibiotics sufficiently to be able to replicate and establish acute infection, while some bacteria may also remain quiescent for long periods contributing to persistent and chronic infections. The mouse model of experimental UTI has been an invaluable tool in understanding host-UPEC interactions during acute infection. Recent work describing how this model can be used to study chronic infection [109], is offering unique insight into how early host-pathogen interactions determine the outcome of disease and will greatly facilitate the identification of UPEC and host factors that are important in different stages of UTI.

EMERGING ANTIBIOTIC RESISTANT CLONES OF *E. COLI*

Since their discovery, antibiotics have revolutionised the treatment and prevention of bacterial infections. For the majority of community-acquired UTI, conventional antimicrobial therapy still provides effective treatment. However, the recent emergence and increasing prevalence of antibiotic resistant uropathogens has complicated the management of UTI. Antibiotic resistance genes are often carried on plasmids and mobile DNA elements that can spread rapidly and efficiently among bacterial populations. In addition, increasing human migration and travel, as well as the emergence of medical tourism (referring to the rapidly-growing phenomenon of international travel to obtain health care) are also contributing to the rapid spread of some resistant strains across the globe [110]. Many multidrug resistant *E. coli* strains are now recognized as belonging to specific clones that are commonly isolated from UTI cases originating in a specific locale, country or even globally.

E. coli Clonal Group A

First reported a decade ago, *E. coli* strains belonging to clonal group A (CGA) commonly exhibit resistance to multiple antibiotics, predominantly trimethoprim/sulfamethoxazole (TMP/SMZ) [111, 112]. While TMP/SMZ-resistant CGA strains are particularly prominent across the United States [113], they are now recognized to belong to a broadly disseminated multidrug resistant clone that is endemic worldwide [114].

E. coli ST131

Another more recent example of an emerging multidrug resistant *E. coli* clonal group is the O25b:H4-ST131 group of extraintestinal pathogens. Reports within the last five years have demonstrated the global distribution of this lineage indicating that it constitutes a major threat to public health worldwide. *E. coli* ST131 are commonly associated with production of the CTX-M-15 enzyme, currently the most widespread extended spectrum β-lactamase (ESBL) of its type in the world [115, 116]. Apart from oxyiminocephalosporins (i.e cefotaxime, ceftazidime), and monobactams, *E. coli* ST131 strains are often co-resistant to fluoroquinolones (i.e ciprofloxacin) [117-119], thus limiting further the treatment options that are currently available against this clone.

Virulence of Multidrug Resistant *E. coli* Clones

The successful dissemination of both *E. coli* CGA and ST131 clones is thought to be due to their resistance to multiple antibiotic classes as well as their virulence capacity. *E. coli* CGA strains belong to phylogenetic group D and cluster within clonal complex 69 by MLST analysis [120, 121]. The typical virulence genotype of CGA strains includes several genes commonly associated with UPEC, including genes encoding P fimbriae, group 2 capsule, and the aerobactin and yersiniabactin iron-acquisition systems [113]. In contrast, *E. coli* ST131 strains are derived from phylogenetic group B2, which also includes other recognised pathogenic *E. coli* clonal groups, and only a few virulence genes appear to be uniformly encoded in all *E. coli* ST131 strains - i.e. the *fimH* adhesin of type 1 fimbriae, the secreted autotransporter toxin (*sat*), the aerobactin receptor (*iutA*), the uropathogenic-specific protein (*usp*), and the pathogenicity island marker (*malX*) [116-118, 122]. The absence of hallmark UPEC viru-

lence factors among *E. coli* ST131, such as P or S fimbriae and toxins, is intriguing considering the widely successful emergence of the ST131 clone as a major cause of urinary tract and bloodstream infections in the community and healthcare facilities across Europe, Asia, Africa, North America and Australia [116, 118, 123-126]. In the first comprehensive analysis of a genome sequence from an *E. coli* ST131 isolate, we demonstrated the presence of unique genomic regions that encode several putative virulence factors and are absent from other characterised UPEC isolates [127]. These genomic regions are prevalent among *E. coli* ST131 (Totsika, Beatson and Schembri, unpublished data) and likely contribute to the fitness of this globally disseminated multi-resistant *E. coli* clone. In a mouse model of acute UTI, *E. coli* ST131 isolates from the United Kingdom and Australia were shown to colonise the bladder robustly and this depended on their ability to express type 1 fimbriae [127]. Type 1 fimbriae expression in *E. coli* ST131 is affected by a null mutation identified in the regulator-encoding gene *fimB* and shown to be prevalent among geographically diverse *E. coli* ST131 isolates [127]. While *fimB* inactivation resulted in altered phase-on switching of type 1 fimbriae in the majority of *E. coli* ST131, we showed that these isolates were still able to express type 1 fimbriae at a level that could mediate enhanced adherence, invasion of and intracellular survival in bladder epithelial cells *in vitro*, as well as increased colonization of the mouse bladder during acute infection *in vivo* [127]. Our study highlighted some unique genomic and virulence attributes of *E. coli* ST131, underlying the need for more studies looking into the virulence mechanisms employed by this widespread clone of multidrug resistant pathogens.

Outlook

The need to understand how different multidrug resistant *E. coli* clonal groups cause disease has become even more urgent in the face of emerging types of bacterial enzymes that confer multi- or even extensive-drug resistant phenotypes and thus restrict our already limited options for antibiotic therapy. In 2008, a new type of metallo-beta-lactamase, termed New Delhi metallo-β-lactamase-1 (NDM-1), was identified in a uropathogenic strain of *K. pneumoniae* [128]. NDM-1 can hydrolyse penicillins, cephalosporins and carbapenems and the $bla_{\text{NDM-1}}$ gene has been found in different plasmid types that co-harbor other resistance genes [129]. Since first reported, NDM-1 has spread among *Enterobacteriaceae* (and other bacteria) rapidly and widely [130]. Recently, there have been several studies reporting the acquisition of the $bla_{\text{NDM-1}}$ gene by *E. coli* ST131 strains [131, 132]. The presence of the $bla_{\text{NDM-1}}$ gene and other carbapenem-hydrolyzing β-lactamase genes, including $bla_{\text{KPC-2}}$ [133], in a widely disseminated and virulent *E. coli* clone is of great concern to public health. In addition, novel NDM-1 variants with enhanced carbapenamase activity, such as NDM-4 and NDM-5, have been very recently identified in *E. coli* [134, 135], suggesting that these enzymes are continuously and rapidly evolving. With carbapenem antibiotics considered our 'last-line' of therapy against multidrug resistant Gram-negative pathogens, the need for the development of alternative anti-infective strategies is extremely urgent.

TREATMENT OF UTI

Treatment Strategy

For clinical purposes UTI can be divided in a number of ways. They may be divided into 'lower tract' and 'upper tract' infection referring to cystitis and pyelonephritis respectively. Alternatively they may be classified as 'uncomplicated' which generally refers to non-pregnant women with cystitis in a structurally and functionally normal renal tract; or 'complicated' which refers to pyelonephritis in non-pregnant women, or any UTI in other patient groups including men, children and those with structurally or functionally abnormal renal tracts (including an indwelling catheter) [136]. This clinical division facilitates the selection of an appropriate treatment strategy. Uncomplicated infection can be treated with a short course of therapy (<5 days, depending on the susceptibility and regimen). Complicated infections may require prolonged antimicrobial therapy and/or other non-pharmacological interventions including removal of indwelling devices.

The traditional strategy for treating uncomplicated UTI is with empiric orally administered antimicrobials, commencing after a urine sample has been submitted for culture (but prior to identification and susceptibility testing of the implicated bacteria). Other strategies have also been investigated. Clinical studies of uncomplicated cystitis have observed natural resolution of symptoms in most untreated women in approximately 5 days, albeit delayed when compared with treatment with active antimicrobial therapy [137]. A comparison of multiple strategies including empiric therapy without culture, delayed therapy commenced for ongoing symptoms at 48 hours, and delayed therapy based on urine culture results demonstrated the efficacy of all strategies. Targeting therapy based on dipstick, urine culture, or delaying therapy for 48 hours, lead to a decrease on the overall use of antimicrobials [138]. Non-culture strategies rely on the high susceptibility of local bacteria to empiric antimicrobial therapy and have not been trialled in settings of high rates of multidrug resistant organisms. The treatment of complicated infection is more specifically tailored to the patient and site of infection. For the most common complication, pyelonephritis, most national guidelines recommend between 7 and 14 days intravenous and/or oral therapy depending on the agent selected [139, 140].

Antimicrobial Therapy

Over the last forty years, antimicrobial treatment of uncomplicated UTI has evolved in response to the prevalence of antibiotic resistance in *E. coli* in the community. Thus, for example, orally administered amoxicillin was superseded by non-beta-lactam antibiotics or beta-lactam antibiotics stable to the TEM-1 beta-lactamase, which became the prevalent cause of amoxicillin resistance [141]. For many years, amoxicillin/clavulanate, cephalexin, trimethoprim/ sulfamethoxazole or fluoroquinolones (for example, ciprofloxacin) were used as first line treatment of uncomplicated UTI. However, resistance of *E. coli* to each of these antibiotics is now substantial and in many parts of the world these drugs can no longer be used as empiric therapy [139]. The dissemination of specific clonal groups has contributed to this spread of

resistance *via* CGA harbouring trimethoprim/ sulfamethoxazole resistance [112] and ST131 strains harbouring fluoroquinolone resistance [142].

Oral Therapy

In most parts of the world, nitrofurantoin, fosfomycin and pivmecillinam remain active against the majority of *E. coli* strains causing uncomplicated UTI [123, 143-146]. However, in nations where there has been heavy use of these agents, resistance has emerged [147]. Whilst useful for uncomplicated lower tract infection, these agents have drawbacks. Orally administered nitrofurantoin has low efficacy for pyelonephritis due to poor tissue penetration and should not be administered to patients with impaired renal function. Fosfomycin has good penetration of renal tissue, [148] however there have been few clinical studies on its use in pyelonephritis; hence it is of uncertain efficacy in the treatment of pyelonephritis [149]. Furthermore, fosfomycin and pivmecillinam are not readily available or licenced for use in a number of countries [150]. Another potential option is the beta-lactam/beta-lactamase inhibitor combination of amoxicillin/clavulanate. It has proved successful in the treatment of lower urinary tract infection caused by ESBL producing organisms where *in vitro* activity is confirmed (MIC $\leq$8μg/ml) [151]. It has also been used for step-down therapy after intravenous treatment of pyelonephritis in similar isolates [152].

Parenteral Therapy

The extent of resistance to orally administered antibiotics, coupled with issues of drug availability and suitability, means that some patients with uncomplicated UTI or early pyelonephritis, who would previously have been treated with oral therapy, now need intravenous therapy. The common phenotype causing this clinical scenario is an isolate harbouring fluoroquinolone, trimethoprim/sulfamethoxazole resistance and an ESBL enzyme, usually of CTX-M type. These isolates also frequently co-produce enzymes that confer aminoglycoside resistance [153]. Patients with severe infection causing sepsis also require parenteral therapy, regardless of the antimicrobial resistance phenotype.

For suspected or proven resistant infection, intravenous carbapenem antimicrobials have become the treatment of choice. Whilst all the commonly available carbapenems (meropenem, imipenem, doripenem and ertapenem) are active against ESBL producing *E. coli*, intravenous ertapenem has found favour. Its prolonged half-life allows once daily administration for ambulatory intravenous therapy [154]. In addition, its narrower spectrum (without activity against *P. aeruginosa*) may decrease risk of further potentiating further resistance within the healthcare environment [155]. Other parenteral treatment options for ESBL *E. coli* include the intravenous beta-lactam/beta-lactamase inhibitor combination, Piperacillin/Tazobactam, if *in vitro* activity is present [152]. The aminoglycoside amikacin is also frequently active against CTX-M harbouring *E. coli*, even in the presence of resistance to other drugs from this class (gentamicin and tobramycin) due to the stability of amikacin against degradation by the predominant aminoglycoside resistance mechanism (*aac*(*6'*)-*1b*). As with all aminoglycosides, toxicity including ototoxicity and nephrotoxicity is problematic with prolonged amikacin administration [156].

Unfortunately, the dissemination of beta-lactamases that inactivate carbapenems ("carbapenemases") amongst *E. coli*, has further complicated management of UTI. *E. coli* may now produce bla_{KPC} or bla_{NDM} type beta-lactamases which confer resistance to all penicillins, cephalosporins and carbapenems, without any useful inhibition by currently available beta-lactamase inhibitors [157, 158]. These organisms are also typically resistant to fluoroquinolones and aminoglycosides. In this setting of extensive drug resistance there are few options for treatment. The legacy agent colistin maintains activity [159], however its use comes with a significant risk of nephrotoxicity. The relatively novel glycylcycline, tigecycline, is also frequently active *in vitro* [158, 159], although its efficacy in renal tract infection is uncertain [160].

Outlook

To address the challenge of rising drug resistance among bacteria, the Infectious Diseases Society of America (IDSA) in collaboration with the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), have recently updated their guidelines for the treatment of uncomplicated cystitis and pyelonephritis among women [139]. The proposed changes aim to provide recommendations on optimal treatment options for uncomplicated UTI taking into account the increased rates of antimicrobial resistance, among other factors. Despite the increasing trend in antibiotic resistance among uropathogenic *E. coli* seen worldwide, regional resistance rates and patterns can vary considerably [161, 162]. This variation represents an additional challenge for clinicians when choosing effective treatments for UTI in different countries and highlights the need for continuous active surveillance of antibiotic resistance patterns at the institute/regional level. The outlook for management of antimicrobial resistant UTI in the future is guarded. While some new antibiotics are relatively advanced in development, such as the novel aminoglycoside ACHN-490, they are not likely to be active against metallo-beta-lactamase producers (eg. bla_{NDM}) which also harbour emerging aminoglycoside resistance mechanisms (16S rRNA methylases) [163]. Priorities for drug discovery for UTI include orally administered antibiotics active against ESBL producers and intravenously administered antibiotics active against producers of both carbapenemases and 16S rRNA methylases. As a temporising measure, improving global access and further clinical trials of existing agents such as fosfomycin and temocillin should also be a priority.

VACCINATION APPROACHES FOR THE PREVENTION OF UTI

The need for vaccines to prevent UTI has increased as fast as antibiotic resistance among UPEC strains. The combination of successful antibiotic treatments and the versatility of UPEC strains on evading the immune system imposed economic and immunological obstacles that reduced the interest on vaccination and delayed its research and development for several years. Now that the scenario has completely changed and antibiotic treatment has struggled to catch up

with the evolving mechanisms of drug resistance, novel vaccinology approaches have been proposed to overcome old pitfalls and deliver a safe and broadly protective vaccine Fig. (**2**).

Conventional Vaccinology Approaches

Initial vaccinology approaches for ExPEC followed the same conventional rules that led to the decrease and even eradication of several diseases for more than a century. Initial attempts involved the use of capsular polysaccharides [164] and O-antigen [165] in immunotherapies, but high diversity and the limited immunogenicity and immunological memory towards polysaccharides if not protein-conjugated made the development of a polysaccharide-based vaccine extremely challenging. In an attempt to partially overcome capsular diversity, six UPEC strains representing the most important serotypes involved in UTI were combined in a heat-killed multi-microbial vaccine (SolcoUrovac), and used for the vaginal immunization of women suffering from recurrent UTIs during a phase II clinical trial. Vaccination prevented re-infection in 50% of women during the 24-week trial and significantly delayed the re-infection interval compared to the placebo-treated group. Mucosal vaccination was shown to be safe and efficient, but efficacy against heterologous strains and long-lasting mucosal immune responses was not demonstrated [166].

Conventional vaccinology approaches have led to the development of most of the vaccines that are currently licensed. None of these approaches, however, have successfully overcome the problem of high antigenic variability and the diverse virulence mechanisms of UPEC. This led to their gradual replacement with functional vaccinology approaches focused on common mechanisms in UTI pathogenesis and targeting bacterial components directly involved in virulence. Functional vaccinology approaches have attempted to block the interaction between UPEC and the host or neutralize UPEC secreted components responsible for tissue damage and/or nutrient scavenging. Hemolysin [167], type 1 [168] and P [169] fimbriae subunits, and siderophore receptors [170, 171] have been targeted by this approach. However, despite promising results obstacles including low prevalence, poor solubility and high variability have hampered progress.

Despite the diversity and low immunogenicity of the *E. coli* capsule, the potential of whole-cell formulations is high, since whole cells could display multiple antigens, may possess natural adjuvants and should allow the production of antibodies against conformational epitopes. As an alternative to whole-cell vaccines, proteomic vaccinology approaches have exploited the ability of *E. coli* to form and release outer membrane vesicles, in order to define the composition of the outer membrane and to use outer membrane vesicles as a natural vehicle to deliver a multi-component vaccine against UPEC and other extra-intestinal pathogenic *E. coli* strains through a high-yield system [172].

New Vaccinology Approaches

Iron receptors show promise as targets for vaccine formulations against UTI. Iron is essential for bacterial growth, therefore UPEC strains have evolved several highly specialized iron scavenging systems for survival in limited iron environments (e.g. urine). All iron receptors have a beta-barrel structure that spans the outer membrane and contains external loops that interact with the siderophore substrates to promote iron internalization. UPEC strains have a vast repertoire of iron scavenging systems and some strains can harbor multiple systems with functional redundancy [75]. Low solubility and functional redundancy are the two major obstacles in successfully using iron receptors in vaccine formulations. As a means to overcome this, structural vaccinology

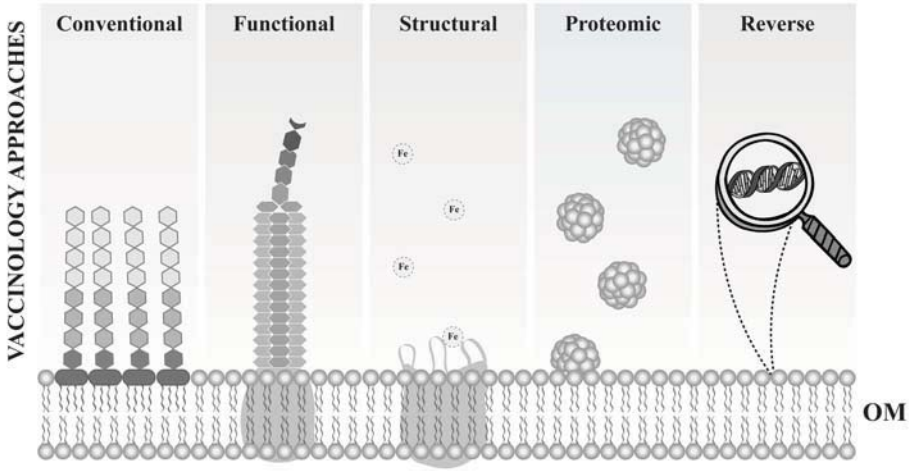

Fig. (2). Vaccinology approaches against UPEC. Several vaccinology approaches have been applied for the delivery of vaccines against UPEC strains: Conventional approaches, providing protection through the delivery of whole inactivated bacteria or major surface components, like lipopolysaccharides or capsular polysaccharides; Functional approaches, targeting mechanisms involved in the interaction to the host and pathogenesis, like fimbrial components and toxins; Structural approaches, taking advantage of structural analysis of outer membrane antigens for the identification of surface-exposed epitopes, like iron and siderophore receptors; Proteomic approaches, using OMVs for the identification of novel surface-exposed antigens or as vehicles for multi-component vaccines; Reverse vaccinology approaches, mining genome sequences for the identification of surface-exposed or secreted antigens.

approaches that use protein crystal structures and structural modeling have identified the external loops of different iron receptors, which were successfully targeted as fused recombinant antigens and conferred protection with increased coverage and prevented bacterial survival due to functional redundancy in iron receptors [173].

Although several vaccinology approaches have identified promising candidates, antigen solubility and diversity remain important obstacles in delivering a broadly protective vaccine against UTI. For these reasons, a multi-component vaccine approach seems like the most promising alternative. The genome sequence of bacteria can be viewed as the most comprehensive library for the identification of multiple vaccine candidates. It provides information on all of the proteins that can be encoded by any bacterial strain without having to rely on *in vitro* or *in vivo* culture conditions. The reverse vaccinology approach uses genomic information for the identification of immunogenic and protective vaccine candidates and has been successful in providing protective antigens against several pathogens [174, 175]. Using a novel genomic analysis approach to compare UPEC and neonatal meningitis-associated *E. coli* with and non-pathogenic *E. coli* strains, (called subtractive reverse vaccinology), nine protective antigens were identified, most of them with improved solubility, cross-protection and high prevalence among a collection of extra-intestinal pathogenic *E. coli* strains (including UPEC), indicating that their combination in a multi-component formulation could lead to the development of a novel recombinant vaccine [176].

Outlook

Now that several vaccine candidates have been identified and the feasibility of a vaccine against UPEC strains has been demonstrated, our attention has slightly shifted to the host. Research in mucosal immune responses will allow the improvement of existing or development of novel adjuvants and alternative routes of administration to facilitate delivery and boost the immune response to target antigens in the urinary tract.

ALTERNATIVE APPROACHES TO PREVENT UTI

Anti-Adhesion Strategies

Over the last decade several alternative approaches have been investigated for the treatment and prevention of UPEC-mediated UTI. Given the critical role of adhesion in colonization of the urinary tract, a number of methods aimed at preventing adhesion have been investigated; for example, anti-adhesion methods that target specific interactions between fimbrial adhesins and their cognate receptor. In this respect, the greatest progress has been made with molecules that prevent binding mediated by the tip-located FimH adhesin of type 1 fimbriae. FimH binds to a wide range of glycoproteins containing one or more *N*-linked high-mannose structures, and this binding can be inhibited by D-mannose [177] as well as a variety of natural and synthetic saccharides containing terminal mannose residues [178-184]. Other mannose derivates with higher FimH binding affinity have also been identified [185, 186]. Most recently, a series of small-molecular weight compounds (mannosides) optimized for oral administration were shown to block UPEC FimH-receptor binding to the bladder uroepithelium and subsequent colonization in the mouse UTI model [187]. An exciting aspect of this work was the observation that these mannosides enhanced the activity of trimethoprim-sulfamethoxazole against a resistant UPEC strain. The ability of mannosides to inhibit colonization by globally disseminated multidrug resistant UPEC clones, such as *E. coli* ST131, remains to be examined.

Pilicides

Pilicides are a class of synthetic molecules that prevent the synthesis of UPEC chaperone-usher fimbriae by inhibiting the function of the chaperone protein [188]. Pilicides have been designed that target the specific site of the chaperone that interacts with its cognate usher protein, the outer-membrane assembly platform that facilitates fimbrial assembly [188, 189]. An attractive feature of pilicides is the potential to target multiple different chaperone-usher fimbrial types. Thus, it may be possible to inhibit the biosynthesis of multiple different chaperone usher fimbriae from UPEC, or even other Gram-negative bacterial pathogens, simultaneously. More recently, new pilicide derivatives with improved efficacy or altered specificity have been described [190, 191].

Prophylactic Strategies Using Asymptomatic *E. coli* Strain 83972

Another approach aimed at the prevention of UTI involves the use of the prophylactic asymptomatic bacteriuria (ABU) *E. coli* strain 83972. *E. coli* 83972 is a B2 clinical isolate capable of long-term bladder colonization that has been effectively employed as a prophylactic agent for the prevention of UTI in human inoculation studies [192-199]. *E. coli* 83972 was originally isolated from a young Swedish girl with ABU who had carried it for at least three years without symptoms [192, 194]. It is well adapted for growth in the urinary tract where it establishes long-term bacteriuria [196]. *E. coli* 83972 lacks defined O and K surface antigens, is non-motile and does not produce a capsule [192, 200, 201]. In addition, it is attenuated in a number of virulence determinants, including type 1, P and F1C fimbriae [200, 202, 203]. *E. coli* 83972 has been used for prophylactic purposes in patients with chronic and recurrent UTI. In these studies the bladders of patients were deliberately colonized with *E. coli* 83972 in order to prevent the establishment of UPEC and other uropathogens. *E. coli* 83972 has been successfully used in several studies and can establish bacteriuria without jeopardizing the health of the patient. In one study, women with chronic symptomatic UTI were eradicated of their infection and then deliberately colonized with *E. coli* 83972. Stable bacteriuria was established for more than 30 days in seven of the twelve individuals [192]. In another study, a group of patients with recurrent symptomatic UTI had their bladders deliberately colonized with *E. coli* 83972. Successful long-term colonization (5 months to 3 years) was achieved in 6/12 patients with neurogenic bladder disorder [196]. Deliberate colonization with *E. coli* 83972 can reduce the frequency of UTI in patients with neurogenic bladder secondary to spinal cord injury [193, 195] and prevent catheter colonization by bacterial and fungal uropathogens [197, 199, 204]. *E. coli* 83972 is well maintained in the bladder,

does not adhere to uroepithelial cells or induce a proinflammatory cytokine response, and can outcompete other UPEC strains during growth in human urine – possibly due to its ability to produce multiple siderophores [75, 205-207]. Clinical trials using *E. coli* 83972 to pre-coat urinary catheters have been carried out in Sweden and the USA and show promising results, with these trials currently being extended [192, 193, 195, 208]. Other complementary approaches aimed at altering the adherence capacity of *E. coli* 83972 are also being investigated [209].

Outlook

The recent rapid emergence of multidrug resistant UPEC strains has necessitated the need to develop new approaches to treat UTI and other infections caused by these organisms. The prevention of UPEC adhesion can be achieved by anti-adhesive molecules that mimic human receptor targets and by compounds that disrupt adhesin assembly (i.e. pilicides). Prophylactic approaches based on the asymptomatic bacteriuria strain 83972 also show promise. A comprehensive evaluation of these approaches in defined animal infection models and human clinical trials is now required.

CONCLUSIONS

Several new and emerging themes in UPEC research should influence future directions in the field. Although traditionally regarded as an extracellular pathogen, recent work has shown that some UPEC strains can survive and replicate intracellularly within epithelial cells and macrophages. Thus, it appears that there are two types of UPEC strains that cause symptomatic UTI: (i) those represented by the prototype strain CFT073 that produce toxins and cause severe damage to the uroepithelium and (ii) those represented by the prototype strain UTI89 that can invade epithelial cells, form IBCs and survive intracellularly. The emergence of dominant UPEC clones such as ST131 suggests that these phenotypes can now be coupled with antibiotic resistance, making the overall picture of UPEC virulence even more diverse. The challenge over the next decade will be to understand the molecular mechanisms by which different UPEC strains colonise the urinary tract, dissect the corresponding host responses to infection and then exploit this knowledge in the development of new approaches to treat and prevent UTI.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Perspective

An oral carbapenem, but only now intravenous penicillin: the paradox of Japanese antimicrobials

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SUMMARY

At present there is a profound paradox in antimicrobial use and development in Japan. A tightly held domestic pharmaceutical market with significant barriers to the importation and registration of foreign agents, has spurred domestic pharmaceutical companies to develop a vast range of antimicrobials. Many Japanese developed antimicrobials are now used globally. A negative consequence of this environment, however, is the lack of availability of several 'workhorse' narrow-spectrum agents to treat patients in Japan. Absent agents include anti-staphylococcal penicillins and until recently, intravenous benzylpenicillin. In substitution for these unavailable agents, patients are frequently administered broader spectrum antimicrobials. This change offers no additional benefit to the patient and is potentially contributing to treatment failure and high rates of antimicrobial resistance amongst key bacterial pathogens in Japan. The situation in Japan illustrates the broader global challenges faced in integrating the development of new antimicrobial agents with maintaining the supply and use of older and less profitable agents.

1. The paradox of Japanese antimicrobials

A 3-year-old infant presents to a Tokyo hospital emergency clinic with acute otitis media. The experienced physician notes the severe pain and fever. She chooses to prescribe antimicrobial therapy. The doctor is aware that Japanese children do not receive protection against *Haemophilus influenzae* type B (Hib) through the national vaccine schedule, Hib being a common, albeit preventable, cause of otitis media and severe complications including mastoiditis and meningitis. She is also aware that Japan has the world's highest rate of BLNAR (beta-lactamase-negative ampicillin-resistant) *H. influenzae*, rendering most commonly used oral antimicrobials ineffective in approximately 30% of such infections in Japan.[1] Fortunately, since 2009, Japan is the only country in the world with an oral carbapenem available to combat such infections.[2] Therapy is successful.

In the neurology ward of the same hospital a patient is diagnosed with neurosyphilis. Until August 2011, the clinician would have been unable to follow almost universal treatment recommendations suggesting intravenous benzylpenicillin, as this agent was not approved for intravenous use.[3] Likewise, a patient suffering from *Clostridium difficile* diarrhea has only had access to metronidazole for this indication since February 2012.[4] Even now, a patient diagnosed with methicillin-sensitive *Staphylococcus aureus* endocarditis would not be able to receive the 'gold standard' treatment of an anti-staphylococcal penicillin, as a suitable agent is completely unavailable.

The vignettes above highlight some of the profound contrasts of Japanese antimicrobial use and development, and the impact these contrasts have on medical practice and ultimately on patients. In essence, the current commercial and regulatory environment has led to a nation of polar opposites.

On the positive side, a tightly held domestic pharmaceutical industry with major regulatory and financial hurdles to the importation and use of foreign antimicrobial agents has spurred significant local antimicrobial drug development,[5] defying the recent global trend away from new antimicrobial development.[6] Japan is the birthplace of many agents, including broad-spectrum antimicrobials used regularly by infectious disease physicians and other practitioners to treat critically ill patients the world over (Table 1). These agents include the carbapenems such as meropenem and doripenem, used to treat the emerging global epidemic of multi-resistant organisms, including those harboring extended-spectrum beta-lactamase enzymes.

Of less clear benefit, the protection afforded to the domestic industry has also facilitated the development of a large number of 'domestic' antimicrobials. Usually derivatives of existing classes, many of these agents are not used widely in other nations (Table 1).

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Table 1
Antimicrobial development and availability in Japan

Japanese developed antimicrobials with a global market	Domestic Japanese antimicrobials (without a major global market)	Agents difficult to access in Japan
Carbapenems	*Carbapenems*	*Agents with very recent approval*
Meropenem, doripenem	Biapenem, panipenem, tebipenem (oral)	Oral metronidazole: approved for *Clostridium difficile* 2012
Quinolones	*Quinolones*	Intravenous benzylpenicillin: approved 2011
Levofloxacin, norfloxacin, gatifloxacin	Pazufloxacin, enoxacin, nadifloxacin, lomefloxacin, tosufloxacin, fleroxacin, garenoxacin, sitafloxacin, prulifloxacin	TMP–SMX: approved for *Pneumocystis jiroveci* pneumonia in 2012; use for other indications is allowed "only when other agents cannot be used"
Other	*Other*	*Agents that remain unavailable*
Cefazolin Ceftaroline	Arbekacin (anti-MRSA aminoglycoside)	Anti-staphylococcal penicillins, e.g. nafcillin, oxacillin, cloxacillin, flucloxacillin
Colistin Ceftaroline[a]	Faropenem (oral 'penem' antimicrobial)	Intravenous metronidazole
Kanamycin Ceftaroline		Benzathine penicillin
		Polymyxins, e.g. colistin,[a] polymyxin B

MRSA, methicillin-resistant *Staphylococcus aureus*; TMP–SMX, trimethoprim–sulfamethoxazole.
[a] Although colistin was developed in Japan, the agent is no longer available in this nation.

Whilst the situation has not been thoroughly explored for antimicrobials, in other medical disciplines, particularly oncology, the standards by which the efficacy of 'domestic' agents has been measured have been brought into question.[5]

This environment has a clear negative aspect, however, as Japanese clinicians have not had access to a number of everyday narrow-spectrum antimicrobials (Table 1). Clinicians in most other nations use these 'workhorse' agents on a daily basis for a vast range of conditions, ranging from minor to life-threatening. Local experience in Japan shows that clinicians now substitute broad-spectrum antimicrobial therapies for the absent narrow-spectrum agents.[7] These 'substitutions' offer no additional therapeutic benefit for the patient, and come with the additional risk of potentiating antimicrobial resistance.

The evolution of this situation is illustrated by the case of the narrow-spectrum penicillins, benzylpenicillin and cloxacillin. Until the recent change to the regulatory approval, benzylpenicillin was approved in Japan only for intramuscular use at a very low dose (600 000 units every 6 h). Although discrepancies between approved and real-life indications exist in many nations, Japanese prescribers are reluctant to use therapies 'off-label' even when the indication is based on the best clinical evidence.

This reluctance stems from the existence of two conflicting sets of laws governing doctor's pharmaceutical prescribing practices. Clause 21 of the Japanese Medical Practitioners Law advises doctors of their duty to prescribe appropriate drugs as required for the clinical situation. Potentially conflicting this, Japan's Pharmaceutical Affairs Law restricts prescribing only to agents approved by these laws.[8] Consequently, off-label use of antimicrobials (or other agents), would potentially expose the doctor and hospital to financial penalties and medico-legal sanction. The health practitioner may be refused reimbursement from the national health insurance scheme for the therapy and the patient will be excluded from coverage by the universally available compensation scheme for medical adverse events.[9]

The competitive nature of the Japanese market has posed a predicament to Japanese pharmaceutical companies regarding the supply of older and cheaper antimicrobial agents. By way of example, the cost of the benzylpenicillin in Japan is low (1 million units = 213 JPY = 3 USD), rendering a costly application for expanded indication non-viable on purely financial terms. The negative financial impact could potentially be compounded if the change in licensure led to diminished use of newer domestically developed agents. The possibility of domestic (or foreign) third parties establishing themselves in the market to supply low-cost narrow-spectrum agents seems slim. In addition to the cost, it would involve navigating of the regulatory authorities, which have been described as opaque in their decision-making process, making any application a financially risky process.[5] This predicament is in contrast to newer, high-cost agents such as linezolid, tigecycline, and daptomycin, all of which have been successfully licensed in the previous decade.

The least desirable outcome of this financial and regulatory environment is illustrated by the anti-staphylococcal penicillin, cloxacillin. In 1998 the Japanese producer discontinued local manufacture of this parenteral agent. With importation an unrealistic option, for reasons outlined above, this crucial class of antimicrobial was rendered effectively unavailable in the nation. The only remaining agent is an infrequently utilized co-formulation of cloxacillin and ampicillin, licensed at a very low dose (cloxacillin total dose = 2000 mg/day).[10] Clinicians now rely primarily on cephalosporins, at times even using fourth-generation cephalosporins, carbapenems, or fluoroquinolones when treating susceptible staphylococcal infections. Many of these substitute antimicrobials have neither been formally studied nor have 'time-tested' experience in this indication.

The heavy use of broad-spectrum agents is one of the likely contributors to the high rates of antimicrobial resistance in particular organisms in Japan. Whilst such agents are invaluable in empiric therapy and the treatment of resistant infection, their indiscriminate use increases the risk of emergence of resistance via 'collateral damage'.[11] The first ever description of a mobile metallo-beta-lactamase (MBL) enzyme (bla_{IMP-1}), originated from Japan during the 1980s.[12] These MBLs confer resistance to vitally important carbapenem antimicrobials and remain endemic in Japan today.[13,14] The authors of the original description directly attributed the emergence of this enzyme to the heavy use of carbapenem agents in Japan.[12]

In addition, as noted earlier, there is also extreme resistance in *H. influenzae* to beta-lactams,[1] a phenomenon potentiated by antimicrobial overuse and poor access to the Hib vaccine. Regulation and licensing of vaccines in Japan remains in a similar bind to that outlined herein for antimicrobials. A complex political and regulatory environment has limited the availability and uptake of a number of vaccines that are routine in almost all other nations. Vaccines affording protection for bacterial pathogens including Hib and *Streptococcus pneumoniae* are classified as 'elective' rather than 'scheduled'. The consequence of this second tier classification is that there is no specific inclusion in national vaccination schedules, nor any obligation for public funding of these agents. The mechanism by which vaccines are classified into these tiers has been described as 'not fully transparent' and is discussed further in the reference provided.[15] The current lay of the land also demonstrates high rates of antimicrobial resistance in

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other common pathogens. This includes endemic methicillin-resistant *S. aureus*[16] and some of the world's highest rates of macrolide resistance in respiratory pathogens including *S. pneumoniae* and *Mycoplasma pneumoniae*.[17]

The licensing of intravenous benzylpenicillin is an encouraging step for a number of reasons. Firstly, it represents the use of an alternative pathway to expanded licensing indications in Japan. Since 2010 Japanese medical specialist societies have been able to directly apply for licensing of new agents or indications, circumventing some of the issues highlighted above. If similar approval has previously been granted in other nations (USA, UK, France, or Germany), then approval may be granted in Japan without further domestic trials. Furthermore, in addition to licensing, the regulator may request and incentivize a supplier in the local market to produce or import the drug if supply is not readily available.[8]

Secondly, this process was initiated by IDATEN, Japan's nascent community of infectious disease specialists.[18] By global standards this community is small. Despite an otherwise highly developed medical workforce, Infectious Disease has not traditionally been a recognized specialty. Few hospitals in Japan have access to a trained Infectious Diseases specialist, let alone a department with other roles including antimicrobial management and stewardship, now emerging as the standard of care in other nations.[19] The expanded licensing of benzylpenicillin (and other antimicrobials) highlights what may be a major task for this society in the future. There are now generations of clinicians in Japan who have little experience treating patients with such traditional and narrow-spectrum agents. These clinicians will require considerable encouragement, education, and support in order to begin utilizing them to their full potential.

Like many other nations and the global community as a whole, Japan faces significant challenges balancing antimicrobial development and use. The first difficulty lies in uncoupling the supply and use of off-patent and commercially unprofitable narrow-spectrum antimicrobials from purely commercial imperatives that may lead to their demise.[20] The second challenge is in creating an environment that fosters the continued development of new antimicrobial agents, although not by simply encouraging their overuse in order to provide a financial return. We hope that the recent expanded licensing of intravenous benzylpenicillin, and other agents, prompted by the emergence of a new voice from the infectious disease community in Japan, is a first step in rebalancing the nation's formulary. Enhancing Japanese clinicians' access to important narrow-spectrum antimicrobials will greatly benefit patients in Japan. Continued good work by the Japanese pharmaceutical industry in the development of new antimicrobial agents benefits the global community as a whole.

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Treatment Options for New Delhi Metallo-Beta-Lactamase-Harboring Enterobacteriaceae

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The New Delhi metallo-β-lactamase gene (bla_{NDM-1}) has emerged as a worldwide concern among isolates of Enterobacteriaceae. Its epidemiology is been strongly associated with travel and healthcare on the Indian Subcontinent. We report two cases of urinary tract infection with Enterobacteriaceae harboring a bla_{NDM-1}. Both cases presented as infection in community-dwelling individuals in Australia and were associated with travel to the Indian Subcontinent. One isolate of *Escherichia coli* harbored the previously undescribed enzyme variant bla_{NDM-3}, differing from bla_{NDM-1} by a single nonsynonymous SNP conferring a putative peptide sequence change at the 95th position (ASP→ASN). The second was an *Enterobacter cloacae* harboring bla_{NDM-1}. Further genetic characterization included identification of additional β-lactamase and aminoglycoside resistance genes. Legacy antimicrobials were used for treatment. Oral therapy with nitrofurantoin was successful in one case, while combination of colistin and rifampicin was required in the second patient. Such infection, due to extensively drug-resistant pathogens, poses significant challenges in balancing the efficacy and toxicity of potential antimicrobial therapies.

Introduction

The new delhi metallo-β-lactamase gene (bla_{NDM-1}) has recently been described as disseminated on the Indian Subcontinent and in the United Kingdom.[7,22] A number of bla_{NDM} variants have subsequently been reported.[4,5] These plasmid-borne genes code for an ambler class B, zinc-dependent β-lactamase enzyme that confers resistance to penicillin, cephalosporins, and carbapenems. Organisms harboring the bla_{NDM-1} gene invariably carry resistance to other classes of antimicrobials, including fluoroquinolones, trimethoprim/sulfamethoxazole, and aminoglycosides. Some bla_{NDM-1}-harboring isolates have been described as pan-resistant with demonstrable resistance to all currently available antimicrobials.[7] The gene has been associated with community-onset and healthcare-associated or acquired infection and colonization, by a range of common Enterobacteriaceae and other gram-negative pathogens. Due to the extensive antimicrobial resistance of bla_{NDM}-harboring isolates, treatment options are very limited.

Case Histories

We report two contrasting cases of successful treatment of bla_{NDM}-harboring Enterobacteriaceae causing urinary tract infection (UTI), illustrative of a spectrum of complexities in acquisition and management.

Case 1: Community-acquired uncomplicated cystitis

In December 2010, a 28-year-old previously well woman presented to her local doctor in Queensland, Australia, 7 days after return from travel to India. She reported symptoms of cystitis, including urinary frequency and dysuria. She had no history of UTI, renal tract abnormality, or significant medical illness. She was of Indian descent, and resided in Australia. The patient travelled in India for 2 months to visit friends and relatives. The majority of the trip was spent in metropolitan areas in the states of Gujarat and Madhya Pradesh. While in India, the patient underwent tooth extractions with a local dentist, including prescription of an unknown oral antimicrobial. She had no other healthcare contact, except a brief visit to a relative in hospital.

On presentation, she had no fever or suggestion of upper renal tract involvement. Midstream urine culture was sent to a private diagnostic laboratory. The urine yielded a pure growth of *Escherichia coli* ($>10^8$ organisms/L) on culture. Antimicrobial susceptibility testing by the private diagnostic

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laboratory indicated resistance to all agents tested (ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cephalexin, ceftriaxone, ceftazidime, meropenem ciprofloxacin, trimethoprim, gentamicin, amikacin), with the exception of nitrofurantoin. The isolate was referred to our research laboratory for further investigation. The patient was treated with a 7-day course of nitrofurantoin with rapid resolution of all clinical symptoms.

Case 2: Complicated healthcare-associated cystitis

In January 2011, a 78-year-old man presented to a physician in Tasmania, Australia, with several weeks of mild dysuria and urinary frequency. Approximately 12 weeks earlier, he had been discharged from a hospital in Calcutta, West Bengal, India, after a 10-day stay for management of hematuria. During this admission, he underwent surgical management, including urethrotomy, transurethral resection of residual prostatic tissue, and evacuation of intravesical thrombus. After discharge from hospital, an in-dwelling catheter remained *in situ* for a further 14 days.

The patient was of Indian descent. He spends 5 months each year in India and the remaining 7 months of the year in Australia, residing with family in each location. He had returned to Australia ~6 weeks before this presentation. Relevant history included a previous transurethral resection of prostate, hypertension, and cardiovascular and peripheral vascular disease.

On presentation in Australia, he was systemically well without signs of upper UTI or sepsis. A mid-stream urine culture demonstrated $1{,}000 \times 10^6$/L leukocytes and growth of both *E. cloacae* $>10^8$ CFU/L and *Pseudomonas aeruginosa* $>10^8$/L. An ultrasonography of the renal tract showed no abnormality. Both organisms were noted to be antimicrobial resistant. In particular, the *E. cloacae* was resistant to carbapenems. After confirmation of antimicrobial susceptibilities, the patient was admitted to hospital and treated with intravenous colistin methanesulfonate (and oral rifampicin), as no oral agents were available. Colistin methanesulfonate was commenced at a dose of 100 mg colistin base activity 12 hourly and oral rifampicin 300 mg 12 hourly. Therapy was complicated by deterioration in renal function on day 4, with serum creatinine rising from 77 to 146 μmol/L (reference range, 60–120 μmol/L).The colistin methane sulfonate dose was reduced to 75 mg colistin base activity 12 hourly in addition to increased hydration and withholding his regular angiotensin II receptor antagonist. He completed 14 days of combined therapy with resolution of all symptoms and demonstration of a negative urine culture 3 months after therapy.

Methods and Results

Phenotypic analysis

Phenotypic antimicrobial susceptibility was undertaken by disc diffusion as per CLSI methods[2] and Etest minimum inhibitory concentration (MIC) (bioMérieux, Marcy l'Etoile, France). Results for *E. coli* and *E. cloacae* are listed in Table 1. Phenotypic MBL activity was confirmed with the use of EDTA inhibition in both isolates.[3] Neither demonstrated synergy with clavulanic acid. The *P. aeruginosa* was susceptible to meropenem and colistin, while resistant to ceftazidime, piperacillin/tazobactam, ciprofloxacin, and gentamicin. No phenotypic MBL activity was detected.

Genotypic analysis

Antimicrobial resistance genes were investigated by PCR as described previously.[20] Genes investigated included β-lactamases and aminoglycoside resistance genes; Ambler

Table 1. Antimicrobial Susceptibility and Resistance Genes in the *NDM*-Producing Isolates

	Escherichia coli	Enterobacter cloacae
Phenotypic antimicrobial susceptibility[a]		
Imipenem	Resistant (12 mcg/ml)	Resistant (>32 mcg/ml)
Meropenem	Resistant (4 mcg/ml)	Resistant (>32 mcg/ml)
Doripenem	Resistant (12 mcg/ml)	Resistant (>32 mcg/ml)
Piperacillin + tazobactam	Resistant (>256 mcg/ml)	Resistant (>256 mcg/ml)
Cefoxitin	Resistant	Resistant
Cefotaxime	Resistant	Resistant
Ceftazidime	Resistant	Resistant
Aztreonam	Resistant	Resistant
Ciprofloxacin	Resistant	Resistant
Trimethoprim/sulfamethoxazole	Resistant	Resistant
Gentamicin	Resistant	Resistant
Amikacin	Resistant	Resistant
Tigecycline	0.19 mcg/ml	3.0 mcg/ml
Colistin	0.5 mcg/ml	<0.125 mcg/ml
Nitrofurantoin	Susceptible (16 mcg/ml)	Resistant (>512 mcg/ml)
Fosfomycin	Susceptible (3 mcg/ml)	Resistant (256 mcg/ml)
Chloramphenicol	Susceptible	Resistant
Genetic characterization		
NDM genes	$bla_{\text{NDM-3}}$	$bla_{\text{NDM-1}}$
Other extended-spectrum β-lactamase genes	$bla_{\text{CMY-6}}$, $bla_{\text{CTX-M15}}$	Nil
Aminoglycoside resistance genes	*aac-6′-Ib* and r*mtC*	*aac-6′-Ib* and *rmtC*

[a]Performed by disk as per CLSI standards or Etest, where an MIC is listed. Where no break point has been established for a given agent, only the MIC is provided.

class A ESBLs (bla_{CTX-M}, bla_{TEM} and bla_{SHV}); class B metallo-β-lactamases (bla_{NDM}, bla_{VIM}, and bla_{IMP}); class C extended-spectrum cephalosporinases (bla_{DHA} and bla_{CMY}); and the aminoglycoside resistance genes *aac-6′-Ib* and methylase genes. Results are in Table 1. The presence of bla_{NDM} was detected using forward primer (5′-GGGCCGTATGAGTG ATTGC-3′) and reverse primer (5′-GAAGCTGAGCACCG CATTAG-3′), producing a 758-bp product in *E. coli* and *E. cloacae*. In addition, a pair of flanking primers was used to amplify and sequence the entire gene, *NDM* F (5′-CTGGGTCGAGGTCAGGATAG-3′) and *NDM* R (5′-TCGCC CCATATTTTTGCTAC-3′). Amplicons were sequenced in forward and reverse direction using an Applied Biosystems 3730xl platform and compared to published sequences in GenBank (www.ncbi.nlm.nih.gov/genbank). *P. aeruginosa* did not harbor a detectable bla_{NDM} or other MBL genes, and is not discussed further.

The *E. coli* bla_{NDM} sequence contained a nonsynonymous single-nucleotide polymorphism at the 283rd position (G→A), conferring a putative peptide sequence change at the 95th position (ASP→ASN). Plasmid transformation studies reported elsewhere show similar carbapenem MICs in the parent and transformant, confirming the carbapenemase activity of this variant.[21] This variant has been designated bla_{NDM-3} (www.lahey.org/Studies/) and submitted to Genbank (accession: NDM-3 JQ734687). Further characterization is ongoing. The sequence obtained from *E. cloacae* demonstrated 100% concordance with the previously published sequences of bla_{NDM-1}.

Discussion

We present two cases of travel-associated UTI with highly resistant organisms harboring the New Delhi metallo-β-lactamase enzyme.

The first case demonstrates importation of community-acquired bla_{NDM} harboring *E. coli*. While less common than healthcare-associated infection, such acquisition has been reported in several other countries,[1,7] although not Australia. Key epidemiological factors in this case include travel and antimicrobial use.[6] Travel *per se* is associated with UTI in females.[18] Travel to countries with a high incidence of antimicrobial resistance, in particular the Indian Subcontinent, is associated with gastrointestinal carriage of antimicrobial-resistant organisms on return. Antimicrobial use while travelling was an independent risk for acquisition and prolonged carriage of such organisms.[6,17]

The second case highlights the increasing frequency and complexity of the intercountry patient. This patient could be described as an informal medical tourist seeking healthcare in multiple nations for social and familial reasons.[16] While we cannot determine the exact timing of infection, the clinical picture is likely of healthcare associated if not nosocomial infection. The organism was most likely acquired in a hospital in Calcutta or after discharge while catheterized in the community.

In infection due to highly resistant pathogens, antimicrobial choice is limited, and requires a considered analysis of risk and benefit of available agents. The case reports described illustrate an unusual clinical situation, as the patients were systemically well, at most requiring oral therapy. However, the extreme resistance of the isolates dictated that the only options for therapy were limited to infrequently used and legacy antimicrobials.

Clinical studies of cystitis have observed natural resolution in most women, although delayed when compared with susceptible antimicrobial therapy.[10] In this setting of a highly resistant pathogen, however, withholding therapy must be weighed against a potential risk of upper UTI and subsequent sepsis with difficult to treat organism. In the second case, additional factors, including a concern about infection of the prostatic surgical bed, the patient's surgically altered urinary tract, and his underlying multiple medical comorbidities, also influenced the decision to treat and the duration of therapy.

As yet, there have been few descriptions in the literature and no prospective studies of oral therapy for bla_{NDM}-harboring organisms. Virtually, all described isolates are resistant *in vitro* to commonly used non-β-lactams, such as trimethoprim/sulfamethoxazole and fluoroquinolones. Susceptibility testing to legacy oral antimicrobials such as nitrofurantoin, fosfomycin, and chloramphenicol is not included in all reports of bla_{NDM}-harboring isolates. In a single study, one-third of urinary tract *E. coli* harboring bla_{NDM-1} remained nitrofurantoin susceptible.[19] Due to poor tissue penetration, this agent only has utility in cystitis, however. Fosfomycin has been used successfully in the therapy of cystitis of other resistance phenotypes, including extended-spectrum β-lactamases, and has good reported penetration into prostate tissue.[11] Susceptibility of NDM-harboring Enterobacteriaceae to fosfomycin has been reported,[12–15] although with no clear denominator of isolates to determine a true rate of resistance. It may have been a suitable alternative in the first case presented, although unfortunately is not easily available in Australia. Susceptibility to other potentially useful legacy oral agents, including chloramphenicol[8,9] and tetracycline,[15] is also occasionally reported, again without a clear denominator.

Conclusion

We present two cases of multidrug-resistant New Delhi metallo-β-lactamase-producing Enterobacteriaceae illustrating a spectrum of acquisition and treatment complexity. Both were treated with legacy antimicrobials, one case with significant complications. While such agents have gone out of favor due to potential side effects, tolerability issues, or absence of availability (in the case of fosfomycin in Australia), at times they may be the best option available. In the setting where an isolate can be confirmed as susceptible and the clinical scenario is appropriate, such as cystitis, we suggest consideration of oral legacy antimicrobials to treat NDM-1-harboring infections.

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Disclosure Statement

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COMMENTARY

Who Is Leading This Dance? Understanding the Spread of *Escherichia coli* Sequence Type 131

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(See the article by Banerjee et al, on pages 361–369.)

Escherichia coli is part of our normal intestinal flora and a ubiquitous human pathogen. It causes a wide range of disease, including intestinal infection (from diarrhea to hemolytic uremic syndrome) and extraintestinal infection (from uncomplicated urinary tract infection to bacteremia and meningitis). Although wild-type *E. coli* is intrinsically susceptible to most antimicrobials, extraintestinal *E. coli* strains have shown the ability to develop resistance to every class of agents introduced for human use. This trend goes back to the development of sulfonamide and ampicillin resistance in the mid-twentieth century and seems unlikely to abate in the foreseeable future.[1,2]

The previous decade has heralded some changes in the dynamics of resistance in *E. coli*. Resistance to sulfamethoxazole-trimethoprim and fluoroquinolones has skyrocketed worldwide, which is a significant trend, because it compromises 2 valuable oral treatment options for infections caused by *E. coli*. Equally alarming has been a rapid increase in the incidence of *E. coli*–producing extended-spectrum β-lactamase enzymes (ESBL-EC), and the expansion of ESBL-EC from nosocomial to community acquisition that has occurred in many regions.

A breakthrough in understanding these epidemiologic shifts in resistance came in the mid-2000s, when researchers used multilocus sequence typing (MLST) to identify the dominance of a particular bacterial clone, sequence type (ST) 131, among ESBL-EC strains.[3,4] Subsequently, ST131 *E. coli* has been shown to have worldwide distribution. It spans not only clinical strains of ESBL-EC, in which ST131 frequently accounts for greater than 25% of those isolates recovered, but also a significant proportion of fluoroquinolone-resistant strains and even asymptomatic carriage of ciprofloxacin-resistant ST131 *E. coli* among healthy individuals.[5] Overall, ST131 is now regarded as the single most important clone driving multidrug resistance in the community setting.

With this in mind, the work by Banerjee et al[6] reported in this issue of *Infection Control and Hospital Epidemiology* urges us to redefine the epidemiology of *E. coli* ST131. Here, the authors conducted a population-based, molecular epidemiologic analysis of 299 consecutive clinical isolates of extraintestinal *E. coli* identified in Olmstead County, Minnesota. Because of this study design, the isolates represented a relatively unbiased sample of community-associated, healthcare-associated, and hospital-acquired infections without regard to antimicrobial susceptibility. It should be pointed out that 39% of the isolates were from individuals with healthcare-associated or hospital-acquired infections; thus, the sample represented a relatively "medicalized" population.

In this context, ST131 accounted for 27% of the isolates. The most intriguing finding was that ST131 accounted for a much greater proportion of healthcare-associated than community-associated isolates (49% vs 15%). A whopping 76% of the isolates from long-term care facility (LTCF) residents were ST131. As expected, ST131 accounted for a much bigger share of antimicrobial-resistant isolates, including over 80% of isolates that were nonsusceptible to fluoroquinolones and approximately half of isolates resistant to trimethoprim-sulfamethoxazole; however, only 11% of the ST131 isolates were nonsusceptible to ceftriaxone, which is most commonly due to production of ESBL. The independent predictors for ST131 included residence in a LTCF, urinary tract infection in the previous month, complex infection due to *E. coli*, and previous antimicrobial exposure (extended-spectrum cephalosporin, macrolides, and fluoroquinolones). These predictors could all be considered potential markers of healthcare-associated infection, although only residence in a LTCF is included in the most commonly adopted definition.[7]

In summary, the data presented by Banerjee et al[6] yield compelling odds ratios for these medical risk factors that are in favor of ST131, not against it. Although these findings seemingly contradict the largest body of work on this clone, which strongly delineates its community-associated nature,[5]

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TABLE 1. Key Information on Molecular Epidemiology Methods Used in Characterizing Sequence Type (ST) 131 *Escherichia coli*

Method	Typing targets	Resolution for ST131	Key characteristics
Multilocus sequence typing	Genomic sequence of 7 conserved housekeeping genes present in all *E. coli*. Gene functions are unrelated to bacterial virulence and pathogenesis.	First identified ST131 as a global epidemic clone.[3,4] By definition, all strains type as ST131 under the Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli).	Provides very broad molecular epidemiological characterization. Given STs may contain a large range of bacteria of quite diverse genetic makeup.
Pulsed-field gel electrophoresis	DNA restriction sites (arbitrary sequences of 4–8 base-pairs of DNA) scattered throughout the *E. coli* genome.	Types ST131 into a variety of pulsotypes (subclones). Distinguishes human and animal clones and demonstrates geographical variation in circulating ST131 clones.[16]	Highly discriminatory typing method often used for spatially and temporally related outbreaks (eg, within a given healthcare network). May fail to identify spread of less closely related strains when used alone (eg, ST131 separates into a large number of pulsotypes).
fimH typing[17]	Genomic sequence of a 469-base-pair fragment of the gene encoding a subunit of the type 1 fimbriae, which is used by *E. coli* to adhere to urothelium (the gene is occasionally absent in nonuropathogenic strains).	ST131 show a variety of *fimH* types. Some of these correlate closely with other characteristics, including fluoroquinolone resistance.[18]	Given the functional nature of this gene, potentially identifies genetic traits that play a role in bacterial pathogenesis.

a number of historical[8,9] and contemporary[10,11] studies have also demonstrated high rates of healthcare-associated and hospital-acquired ST131. However, none demonstrate these findings with the weight of the study by Banerjee et al.[6]

This contrast in epidemiology warrants further consideration. Simple explanations may be geographical and methodological. First, the epidemiology of this clone undoubtedly varies by location.[5] Second, the methodology of published studies varies. Many investigators set out to find ST131 in the context of community-associated ESBL-EC infection and carefully surveyed this particular group.[12] Others have not stratified community-onset infections by healthcare association at all. In retrospect, these studies may have been examining only the tip of the iceberg (ie, community-associated, ESBL-producing *E. coli* ST131) while largely ignoring the much more substantial remainder under the water (ie, healthcare-associated, fluoroquinolone-resistant *E. coli* ST131). Another possibility is also in play. We may be seeing a change in the epidemiology of ST131, with this unwanted resident increasingly moving from the community into acute care settings, which is a path already travelled by predecessors such as community-associated methicillin-resistant *Staphylococcus aureus*.[13]

Regardless of the "when" and "where," the question of "why" ST131 has become a resident of our healthcare system is important. Some portals of entry are clear. Banerjee et al[6] and a number of other researchers have identified LTCFs as a reservoir of ST131. Two-way traffic of patients and resistant organisms between LTCFs and acute care facilities is almost invariable, with the term "revolving door" used by some to characterize this.[11] Heavy use of fluoroquinolones should likely shoulder some blame, although the true nature of their effect is not defined. Other than correlating individuals' previous fluoroquinolone exposure to infection with fluoroquinolone-resistant ST131 clones, we have no information on the broader ecological impact of fluoroquinolone use on the spread of ST131. We do know from other settings that overall population fluoroquinolone use closely correlates with rates of resistance amongst all *E. coli*[14] and that, once established, gastrointestinal carriage of fluoroquinolone resistance is prolonged compared with the carriage of resistance to other agents, such as ESBLs.[15] It is also increasingly clear among ST131 that animals and human clones are genetically distinct.[16] This suggests that problematic fluoroquinolone use driving this particular epidemic could be in the human population rather than the result of often-cited veterinary use and food contamination.

The most elusive component of "why" relates to the molecular characteristics of the clone. Although not extensively discussed in this study, Baterjee et al[6] offer some intriguing

data. Although ST131 was originally identified as an epidemic clone using MLST, the molecular-epidemiological equivalent of a 40,000-foot view, drilling down on ST131s with the other methods gives interesting insights (Table 1). Pulsed-field gel electrophoresis (PFGE), which is traditionally used within a defined spatial and temporal context, demonstrates that approximately half of the ST131 fluoroquinolone-resistant isolates in the Banterjee et al[6] study belonged to 2 specific PFGE pulsotypes. However, the more specific method to define the core subclone of fluoroquinolone-resistant ST131 appeared to be *fimH* typing, which is a recently defined single-locus sequence typing method that uses a section of the *fimH* gene.[17] Unlike the benign housekeeping genes of MLST, *fimH* is a virulence gene that codes for a subunit of type I fimbriae crucial for bacterial adhesion to urotheleium. Here, 99% of the fluoroquinolone-resistant ST131 isolates shared a specific allele, *fimH*30, whereas this allele was absent from any of the fluoroquinolone-susceptible ST131 isolates. This allele is likely more than just an epidemiological marker. In-depth examination of *fimH30*-carrying strains by other investigators has suggested a positively selected patho-adaptive trait. These strains appear to have augmented urovirulence via an enhanced ability to bind to urothelium.[18] In essence, this fluoroquinolone-resistant ST131 *fimH30* subclone is now fine-tuned for its host and environment and appears to be out-competing other ST131 subclones and non-ST131 strains.

The study was performed in a rather rural county in North America, and the generalizability of the authors' findings remains to be seen. However, they will prompt us to redefine the way we think about this emerging and expanding epidemic, which has the potential to deprive us of most oral treatment options for infections due to this exceedingly common bacteria in the near future.

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Infectious Complications Following Transrectal Ultrasound–Guided Prostate Biopsy: New Challenges in the Era of Multidrug-Resistant *Escherichia coli*

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Transrectal ultrasound (TRUS)–guided prostate biopsy is currently considered the standard technique for obtaining tissue to make a histological diagnosis of prostatic carcinoma. Infectious complications following TRUS-guided prostate biopsy are well described, and are reportedly increasing in incidence. The role of antibiotic prophylaxis in reducing post–TRUS biopsy infections is now established, and many guidelines suggest that fluoroquinolone antimicrobials are the prophylactic agents of choice. Of note, however, recent reports suggest an emerging association between TRUS biopsy and subsequent infection with fluoroquinolone-resistant *Escherichia coli*. Against this background, we provide an overview of the epidemiology, prevention, and treatment of infectious complications following TRUS biopsy, in the wider context of increasing global antimicrobial resistance.

Keywords. prostate biopsy; *Escherichia coli*; fluoroquinolones; ESBL.

Transrectal ultrasound (TRUS)–guided prostate biopsy is the standard technique for obtaining a histological diagnosis of prostatic carcinoma [1]. The commonest indications for TRUS biopsy are a raised prostate-specific antigen (PSA) level and/or an abnormal digital rectal examination. According to recent estimates, approximately 1 million TRUS biopsies are performed annually in the United States [2].

TRUS biopsy is generally performed under local anesthetic in the outpatient setting. The patient is placed in the lateral decubitus position with both hips and knees flexed. An ultrasound probe is inserted into the rectum, to enable visualization of the prostate gland anatomy (Figure 1). A spring-loaded device is used to collect core biopsies, sampling tissue systematically from both sides of the gland. The optimum number of biopsies is controversial, although data suggest that an extended regimen (≥10 cores) detects more cancers than previously used sextant techniques [3]. If histopathology fails to reveal malignancy, repeat biopsies are often performed as a second procedure may detect carcinoma in around 20% of cases [3]. Although generally considered a safe and well-tolerated procedure, postbiopsy complications are reported in up to 50% of cases, and include pain, hematuria, hematospermia, urinary retention, and infection [4]. In the following review, we provide an overview of the published literature relating to infectious complications of TRUS biopsy, with a specific focus on antimicrobial-resistant *Escherichia coli*.

INCIDENCE AND MORBIDITY OF INFECTIOUS COMPLICATIONS AFTER TRUS BIOPSY

A variety of infectious complications may occur following TRUS biopsy, ranging from asymptomatic bacteriuria

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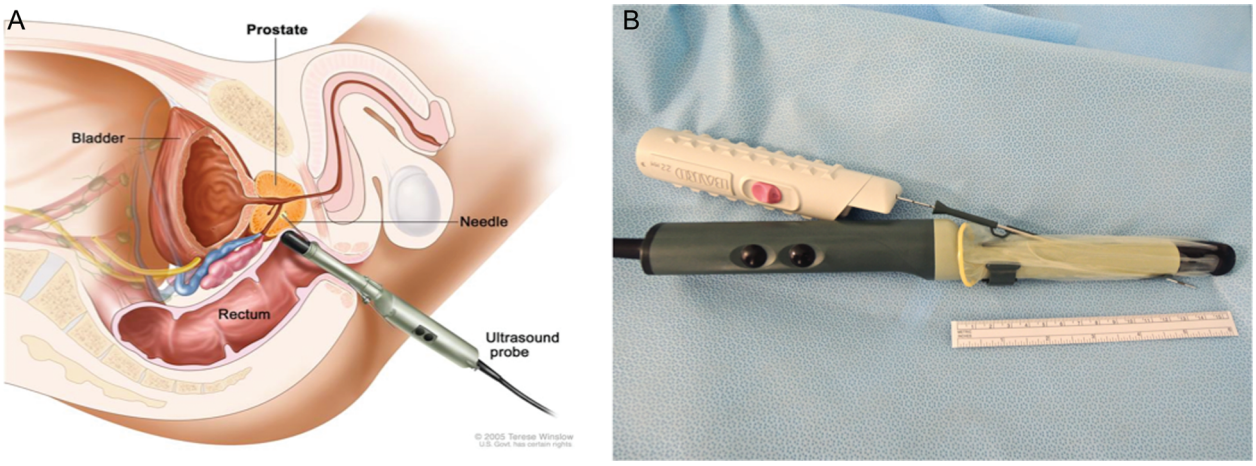

Figure 1. *A,* Anatomy of the male genitourinary tract in relation to transrectal ultrasound–guided prostate biopsy. Graphic used with permission of Terese Winslow. *B,* Typical biopsy apparatus consisting of ultrasound probe with a protective condom (gray handle) and spring-loaded sampling device (beige handle).

or urinary tract infection (UTI) through to prostatitis, bacteremia, and severe sepsis [5, 6]. The reported incidence of UTI after TRUS biopsy typically ranges between 2% and 6% with approximately 30%–50% of these patients having accompanying bacteremia [7, 8]. Bacteremia is frequently accompanied by severe sepsis, which has an overall incidence of 0.1%–2.2% following TRUS biopsy [7]. One recent study reported that among post–TRUS biopsy patients hospitalized with *E. coli* bacteremia, 25% had severe sepsis requiring intensive care unit (ICU) admission [9]. Despite relatively high rates of ICU admission in that study, men with *E. coli* bacteremia after TRUS biopsy had significantly lower 30-day mortality than other men with community-onset *E. coli* bacteremia during the same time period (0% vs 9.9%; $P = .017$). Similar findings are reported in another retrospective observational study where lower 30-day mortality was observed among men after TRUS biopsy compared to men who did not have a preceding TRUS biopsy (0.31% vs 1.09%; $P < .0001$). The authors of that study postulated that the lower mortality rate was a reflection of less comorbidity in the TRUS biopsy group [2].

For the less severe infectious complications of TRUS biopsy, it is likely that the true incidence is underestimated, as reported rates of infectious complications usually only include patients requiring hospitalization [2, 10]. The suggestion that many infectious complications are managed in the community is supported by a recent large prospective European study where, in the 2 weeks following prostate biopsy, fever was reported in 4.2% of patients whereas hospitalization occurred in only 0.8% [11]. Similarly, a retrospective observational study from Scotland reported that the majority of post–TRUS biopsy infectious complications were managed by primary care practitioners [12].

Interestingly, several recent reports suggest that the incidence of infectious complications after TRUS biopsy is increasing. One study from Ontario, Canada [10], reported increasing rates of hospitalizations within the 30-day period following TRUS biopsy, from 1.0% in 1996 to 4.1% in 2005 ($P < .0001$). Of note, more than two-thirds of postbiopsy hospitalizations were for infection-related causes. Similarly, using data derived from Medicare records [2], researchers from the United States also described an increasing frequency of infectious complications following TRUS biopsy, increasing from 0.4% in 1991 to 1.1% in 2007 ($P < .0001$). The reasons for these reported increases in postbiopsy infections are unclear, but suggested contributory factors include rising rates of antimicrobial resistance and changes in biopsy technique [2, 10].

PATHOPHYSIOLOGY AND CAUSATIVE PATHOGENS OF POST–TRUS BIOPSY INFECTIONS

Although no studies have clearly defined the pathophysiology of post–TRUS biopsy sepsis, the primary mechanism is likely to be direct inoculation of bacteria from the rectal mucosa by the biopsy needle into the prostate, blood vessels, or urinary tract. This is supported by high reported rates of bacteremia (16%–75%) and bacteriuria (36%–53%) immediately postprocedure in the absence of prophylactic antibiotics and the fact that most infections manifest clinically within 3 days of TRUS biopsy [13]. In addition, preexisting infection or inflammation may also contribute to postbiopsy infections, although the value of routine urine culture and prebiopsy treatment of asymptomatic bacteriuria remains controversial. One study

demonstrated a rate of febrile genitourinary infections of 27% (3/11) among patients with asymptomatic bacteriuria prebiopsy compared to a rate of 0.9% (12/1322) among those with negative prebiopsy urine cultures [14]. In another study, however, none of 12 patients with asymptomatic bacteriuria prebiopsy had infectious complications compared to 4 of 341 (1.2%) of those with negative urine prebiopsy cultures [15]. A similar study also reported no infectious complications among 7 patients with prebiopsy asymptomatic bacteriuria compared to infectious complications in 2 of 46 men (4.3%) with negative prebiopsy urine cultures [16].

Causative Pathogens and Antimicrobial Resistance Profiles

The commonest pathogen implicated in post–TRUS biopsy sepsis is *E. coli*, accounting for approximately 75%–90% of infectious complications in published series (Table 1).

Over the past decade, antimicrobial-resistant *E. coli* has been increasingly described in the setting of postbiopsy sepsis (Table 1). To date, the most clinically significant resistance phenotypes described in *E. coli* following TRUS biopsy are fluoroquinolone resistance and/or production of an extended spectrum β-lactamase (ESBL) (Table 1). Recent studies indicate that approximately 11%–22% of men undergoing prostate biopsy harbor fluoroquinolone-resistant organisms within the rectum prior to the administration of antimicrobials [13, 24–26]. In addition, a recent study found that the rate of fluoroquinolone-resistant *E. coli* in postbiopsy bloodstream infections was 62% [9], compared to 14% in nonbiopsy *E. coli* bloodstream isolates from males in the same geographic setting ($P < .001$). A strong association has been described between fluoroquinolone-resistant *E. coli* and sequence type (ST) 131 *E. coli*, a relatively recently described lineage of phylogenetic group B2 [27]. This globally spread "pandemic" clone is notable for its ability to harbor numerous genes associated with both antimicrobial resistance and virulence [28]. Contemporary studies have indicated that *E. coli* ST131 frequently makes up >50% of all fluoroquinolone-resistant *E. coli* isolated from UTIs [27]. Of note, a recent study also found that ST131 *E. coli* accounted for 40% of all *E. coli* bloodstream isolates after TRUS biopsy [9]. Similarly, another study reported that 70% of fluoroquinolone-resistant *E. coli* isolated from rectal swabs taken from men prior to prostate biopsy belonged to the ST131 clone [24]. Given the reported spread of this clone [27], it is likely that increasing community transmission and resultant fecal carriage has contributed to these reports of postbiopsy infections with ST131 *E. coli*.

ESBL-producing *E. coli* has also been increasingly identified in case series of postbiopsy infections [18, 21, 29]. Of importance in the setting of postbiopsy sepsis is the strong association between ESBL production and fluoroquinolone resistance in *E. coli*. The rate of coresistance to fluoroquinolones among ESBL-producing *E. coli* ranges from 50% to 100% [30]. Consequently, the preceding use of fluoroquinolone antimicrobials is a significant risk factor for subsequent ESBL-producing *E. coli* infection [31, 32]. This is relevant when considering the current recommendations to use fluoroquinolones as prophylactic agents prior to TRUS biopsy [33]. In addition to selecting for fluoroquinolone-resistant *E. coli* that may be harbored in the gastrointestinal tract prebiopsy, the use of prophylactic fluoroquinolones may also coselect for ESBL-producing *E. coli.* Aminoglycoside resistance is another important resistance phenotype, commonly encountered alongside fluoroquinolone resistance and ESBL production [27]. Awareness of aminoglycoside resistance is important when considering the appropriate choice of empiric treatment for patients presenting to hospital with post–TRUS biopsy infections, particularly given that some recent guidelines advocate aminoglycosides for the empiric treatment of urosepsis [34, 35].

POTENTIAL RISK FACTORS FOR POST–TRUS BIOPSY INFECTIONS

In view of the increasing burden on healthcare services resulting from post–TRUS biopsy infections [36], several recent studies have attempted to identify both patient and procedural factors that may predict which men are at greatest risk of infectious complications [2, 11, 20, 22]. Patient-specific risk factors identified include underlying medical comorbidities, particularly diabetes mellitus, and recent hospitalization [2, 22]. For example, one recent study [22] observed that patients who had been hospitalized in the month preceding TRUS biopsy were significantly more likely to be hospitalized with urosepsis postbiopsy than those without a history of hospitalization (odds ratio, 8.63; 95% confidence interval [CI], 1.48–50.4; $P = .02$).

Preexisting urological pathology may also increase the risk of postbiopsy infections. One study noted that patients with long-term urethral catheters were significantly more likely to develop an infectious complication postbiopsy compared to patients without catheters (19.2% vs 3.06%; $P < .0001$) [20]. However, data regarding the role of other urological abnormalities in predisposing to infections are conflicting and reports of associations with prebiopsy prostate size and preexisting prostatic malignancy have been inconsistent in their findings [11, 22, 37]. Similarly, no procedural factors have been specifically identified as risk factors for post–TRUS biopsy infections. Although one study found that an increased number of cores taken during the biopsy procedure was associated with postbiopsy infection [20], other studies have not confirmed this association [22, 37]. In addition, having a second TRUS biopsy procedure has not been associated with an increased risk of postbiopsy infection [20]. In one instance, contamination of equipment used to perform TRUS biopsy was implicated as a causal factor in an outbreak

Table 1. Published Series of Microbiologically Confirmed Post–Transrectal Ultrasound-Guided Prostate Biopsy Infections Since 2009

Study	Location	TRUS Biopsies, No.	Confirmed Infections,[a] No. (%)	Organisms Isolated on Culture, No.	Fluoroquinolone-Resistant Organisms, No. (%)	Additional Susceptibility Data, No. (%)
Young et al [17]	US	1423	5 (0.4)	*Escherichia coli*: 5/5	5/5 (100)	ESBL: 3/5 (60) Gentamicin resistant: 1/5 (20)
Hadway et al [16]	UK	256	7 (2.7)	*E. coli*: 5/7 *Citrobacter freundii*: 1/7 *Proteus* spp: 1/7	7/7 (100)	ESBL *E. coli*: 3/5 (60) ESBL *C. freundii*: 1/1 (100)
Horcajada et al [18]	Spain	411	11 (2.7)	*E. coli*: 8/11 *Klebsiella pneumoniae*: 2/11 *Morganella morganii*: 1/11	6/11 (55) (all organisms collated)	4/11 ESBL (36) (all organisms collated)
Lange et al [19]	Canada	4749	16 (0.3)	*E. coli*: 16/16	16/16 (100)	3rd-generation cephalosporin resistant: 2/16 (13) Gentamicin resistant: 3/16 (19)
Simsir et al [20]	Turkey	2033	62 (3.0)	*E. coli*: 46/62 *K. pneumoniae*: 9/62 *Psuedomonas aeruginosa*: 6/62 CoNS 1/62	*E. coli*: 3/46 (7) *K. pneumoniae*: 3/9 (33) *P. aeruginosa*: 2/6 (33)	Not reported
Zaytoun et al [8]	US	1446	9 (0.6)	*E. coli*: 7/9 *Micrococcus* spp: 1/9 *Staphylococcus aureus*: 1/9	*E. coli*: 4/7 (57)	ESBL *E. coli*: 0/7 (0) Gentamicin resistant: 1/7 (14)
Patel et al [21]	UK	316	10 (3.2)	*E. coli*: 10/10	*E. coli*: 10/10 (100)	Gentamicin resistant: 3/9 (33) (1 isolate not tested)
Loeb et al [11]	Netherlands	10 474	72 (0.7)	*E. coli*: 63/72 *P. aeruginosa*: 6/72 *Klebsiella oxytoca*: 2/72 *Enterococcus faecalis*: 1/72	14% blood isolates 6% urine isolates (all organisms collated)	Not reported
Carignan et al [22]	Canada	5798	48 (0.8)	*E. coli*: 36/48 *Enterobacter* spp: 3/48 *Citrobacter* spp: 3/48 GPC: 6/48	For all Enterobacteriaceae tested: 20/42 (48)	For all Enterobacteriaceae tested: Gentamicin resistant: 9/41 (22) 3rd-generation cephalosporin resistant: 2/28 (7)
Carmignani et al [23]	Italy	447	9 (2.0)	*E. coli*: 8/9 *Aeromonas* spp: 1/9	*E. coli*: 7/8 (88)	ESBL: 6/8 (75)

Abbreviations: CoNS, coagulase-negative staphylococci; ESBL, extended-spectrum β-lactamase; GPC, gram-positive cocci; TRUS, transrectal ultrasound.

[a] Definition of confirmed infection is positive bacterial growth from urine or blood with accompanying clinical symptoms.

of postbiopsy infections, although in this case, the causative pathogen was *Pseudomonas aeruginosa* [38].

Several studies have also attempted to assess possible risk factors specifically for post–TRUS biopsy infection with antimicrobial-resistant *E. coli*. Although only reported from smaller cohorts to date, evidence supports the link between postbiopsy sepsis with a fluoroquinolone-resistant *E. coli* and receipt of a fluoroquinolone antimicrobial in the months preceding biopsy [17, 21]. Moreover, the link between prior fluoroquinolone exposure, colonization with fluoroquinolone-resistant *E. coli*, and subsequent postbiopsy infection with fluoroquinolone-resistant *E. coli* has also recently been demonstrated [25]. In addition, travel to areas with a high prevalence of resistant pathogens has also recently been associated with post–TRUS biopsy sepsis due to ESBL-producing *E. coli* [21, 29].

PREVENTION OF INFECTIOUS COMPLICATIONS AFTER TRUS BIOPSY

Antimicrobial Prophylaxis

Strong evidence exists to support the use of antimicrobial prophylaxis prior to TRUS biopsy [4–6]. However, wide variability in practice with respect to both the choice and duration of prophylaxis has been reported [39]. A recent systematic review of relevant studies concluded that antimicrobial prophylaxis was effective in preventing infectious complications following prostate biopsy, with risk ratios (RRs) favoring prophylaxis for bacteriuria (RR, 0.25; 95% CI, .15–.42), bacteremia (RR, 0.67; 95% CI, .49–.92), fever (RR, 0.39; 95% CI, .23–.64), UTI (RR, 0.37; 95% CI, .22–.62), and hospitalization (RR, 0.13; 95% CI, .03–.55) [40]. Data were analyzed separately for fluoroquinolones, sulphonamides, and other antibiotic classes. All agents were effective vs placebo, but most data supported the use of fluoroquinolones [40]. Fluoroquinolones are particularly useful in this setting due to their broad spectrum of activity against intestinal flora and high prostatic tissue levels obtained after oral administration [41]. In addition, fluoroquinolones are recommended as first-line prophylactic agents prior to TRUS biopsy in current guidelines [33–35]. No conclusive data have been found to support either the use of long-course (3 days) over short (1 day) fluoroquinolone regimens, or multiple vs single-dose schedules [40]. The equivalence of single-dose ciprofloxacin to that of longer regimens has also been more recently reported in a large US study [42].

Role of Targeted Antimicrobial Prophylaxis

In view of increasing rates of fluoroquinolone resistance in *E. coli* in many countries and the frequent isolation of such organisms in cases of postbiopsy sepsis, a key question is whether universal use of prophylactic fluoroquinolones will remain an appropriate recommendation. Rather than a "one size fits all" model, recent data suggest that a tailored approach to prophylaxis may be more clinically useful and cost-effective. The role of prebiopsy screening for resistant pathogens, followed by culture-directed antimicrobial prophylaxis, has been assessed in several recent studies [25, 43]. In particular, it is suggested that prebiopsy screening for fluoroquinolone-resistant *E. coli* may allow identification of those men harboring such organisms in their endogenous gastrointestinal flora prebiopsy, and for whom fluoroquinolone prophylaxis may not be appropriate [44]. One recent prospective study observed infectious complications in 9 of 345 (2.6%) men who received empiric fluoroquinolone prophylaxis prebiopsy, compared to 0 of 112 men who underwent prebiopsy screening for fluoroquinolone-resistant pathogens and "directed" prophylaxis with alternative agents such as cephalosporins or trimethoprim-sulfamethoxazole [43]. Of note, these authors suggested that this targeted approach to prophylaxis resulted in a cost benefit to their institution [43].

Adjunctive Measures to Antimicrobial Prophylaxis

The role of adjunct measures in preventing postbiopsy infections remains controversial. In particular, the role of prebiopsy rectal cleansing enemas has been recently assessed [40]. The rationale for enema use is to reduce the rectal microbial burden prebiopsy and hence lessen the bacterial inoculum introduced during the biopsy procedure. To date, however, no significant differences have been found between "antibiotic" and "antibiotic + enema" groups [40]. Currently, enema use in this context is not routinely recommended in European guidelines, but use of this practice may be more common in the United States, where American Urological Association guidelines advise "physician discretion" in their use [33]. In addition, rectal disinfection with agents such as chlorhexidine or povidone-iodine has been proposed as another potential adjunct to antibiotic prophylaxis, although data supporting the efficacy of such approaches are currently limited [13]. It is possible that in the face of the increasing antimicrobial resistance, more research into this intervention may prove worthwhile.

MANAGEMENT OF INFECTIOUS COMPLICATIONS AFTER TRUS BIOPSY

At present, there are no published guidelines for the management of post–TRUS biopsy infections. However, in addition to patient-specific prophylactic regimens, consideration should be given to tailored empiric antimicrobial therapy if a patient presents with postbiopsy sepsis. Previous studies have demonstrated that inappropriate empiric therapy of *E. coli* bloodstream infections is associated with an increased risk of mortality [32]. Of note, a recent study suggested that receipt of inappropriate empiric therapy in the setting of post–TRUS biopsy sepsis is not uncommon. In this study, among 47 men presenting with postbiopsy *E. coli* bacteremia, approximately one-third received inappropriate empiric therapy, including 12 men who required admission to the ICU [9]. These authors suggested that in their setting, recent TRUS biopsy was actually a risk factor for bacteremia with multidrug-resistant *E. coli*. Therefore, broader-spectrum empiric antimicrobial coverage should be considered for post–TRUS biopsy sepsis compared to that given for other causes of community-onset urosepsis. Moreover, as discussed above, other individual risk factors that should be considered when choosing appropriate empiric therapy include prior exposure to fluoroquinolones and a history of recent travel to areas with high endemicity for resistant organisms [21, 29].

FUTURE RESEARCH DIRECTIONS

Several issues remain unresolved in the prevention and treatment of post–TRUS biopsy infections. Although initial studies indicate the possible clinical and economic benefits of a tailored approach to prophylaxis, a number of methodological, logistical, and pharmacological questions remain unclear. The optimal laboratory screening methodology for detecting clinically meaningful populations of resistant pathogens is not yet established, with published studies utilizing a variety of methods [43, 45]. In addition, the optimal duration between prebiopsy screening and biopsy is uncertain, but in order to be clinically useful, a prebiopsy screen must be taken at a sufficient time beforehand to allow culture and susceptibility results to become available. Furthermore, the most appropriate choice of alternative prophylactic agent(s) should be considered, and must take into account relevant pharmacokinetic and pharmacodynamic parameters, particularly penetration into prostatic tissue and postantibiotic effect. At present, data are lacking to guide choice of alternative agents to fluoroquinolones, although some data suggest that agents such as amikacin [46] and piperacillin/tazobactam [47] may have utility. In addition, preliminary data suggest that fosfomycin may be of benefit in preventing postbiopsy infections, particularly in patients who harbor fluoroquinolone-resistant *E. coli* [48].

Apart from antimicrobial prophylaxis strategies, another potentially useful strategy to decrease post–TRUS biopsy infections may be to increase utilization of the transperineal route, whereby tissue is sampled transcutaneously across the perineal skin, rather than the rectal mucosa. Currently, this approach is used primarily for men with >1 previous negative TRUS biopsy in the context of a persistently elevated PSA [49]. Recent data suggest that efficacy of cancer detection is equivalent to that of TRUS biopsy and, importantly, that infectious complications are no higher than with TRUS biopsy, even in the absence of prophylactic antimicrobials [49, 50]. Given that the risk of TRUS-biopsy sepsis is unlikely to be completely ameliorated, the overall risks and benefits to each individual patient prior to performing a TRUS biopsy should also be assessed. Important factors to consider include concomitant comorbid conditions, anticipated life expectancy, and suitability for subsequent treatment of any underlying malignancy. Furthermore, as illustrated by the recent debate about the overall impact of PSA screening on clinical outcomes and survival in asymptomatic men [51], optimization of the use of biomarkers in identifying those men at greatest risk of morbidity and mortality from prostate malignancy is required. Potentially newer biomarkers may allow better prediction of men who may receive the greatest potential benefit from TRUS biopsy [52].

CONCLUSIONS

Although infrequent, infectious complications following TRUS-guided prostate biopsy can result in potentially life-threatening complications. Given the large number of biopsies performed annually, the population and economic burden of postbiopsy sepsis is substantial, and is underestimated by studies assessing only hospitalized patients. Post–TRUS biopsy infections caused by resistant *E. coli* are an increasingly encountered clinical problem that pose a practical challenge to clinicians attempting to determine the most appropriate prophylactic and empiric therapy regimens. As multidrug-resistant *E. coli* increases in prevalence globally, further studies are required to investigate the utility of prebiopsy screening for resistant *E. coli* and the role of individualized decision making in the prevention and treatment of postbiopsy infections.

Note

Potential conflicts of interest. All authors: No reported conflicts.

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Rectal colonization with New Delhi metallo-β-lactamase-1-producing *Escherichia coli* prior to transrectal ultrasound (TRUS)-guided prostate biopsy

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Sir,

The global spread of New Delhi metallo-β-lactamase (NDM)-producing Enterobacteriaceae is of significant public health concern.[1] To date, NDM-producing Enterobacteriaceae have been isolated from numerous geographical regions, including Europe, North America, Australia and New Zealand.[1] In addition to hydrolysing carbapenems, NDM-producing organisms display resistance to a broad range of antimicrobial classes, primarily due to the presence of additional acquired plasmid-associated resistance genes.[2] As a result, infections caused by these organisms pose a considerable therapeutic challenge.

Transrectal ultrasound (TRUS)-guided prostate biopsy is a commonly performed urological outpatient procedure, with ~1 000 000 biopsies performed annually in the USA.[3] A variety of infectious complications have been reported following TRUS biopsy, ranging from urinary tract infection through to bacteraemia and sepsis.[3,4] Of note, the incidence of infectious complications following TRUS biopsy is reportedly increasing, with clinical and economic consequences.[3,5] The most common pathogen in the setting of post-biopsy sepsis is *Escherichia coli*, with causative strains probably originating from the patient's own endogenous flora. Over the past decade, the prevention and management of TRUS-biopsy infections due to *E. coli* has become more complicated due to increasing antimicrobial resistance in this organism. Currently, the most common resistance profiles encountered in this setting are fluoroquinolone resistance and/or extended-spectrum β-lactamase (ESBL) production.[3,6] Recent studies suggest that pre-biopsy screening for such resistant pathogens with subsequent 'tailored' prophylaxis based on antimicrobial susceptibility results may be an effective way to reduce infectious complications.[3,7] To date, however, there are no reports describing pre-TRUS biopsy isolation of carbapenemase-producing *E. coli*. Here, we describe the isolation of NDM-1-producing *E. coli* from a patient undergoing rectal screening prior to TRUS biopsy.

In early 2013, an elderly New Zealand male attended the urology outpatient clinic for a pre-biopsy rectal screen. A TRUS biopsy 15 months earlier had demonstrated the presence of low-grade prostatic carcinoma, and the patient was being monitored by regular measurement of prostate-specific antigen (PSA) levels and outpatient clinic assessments. In order to assess possible progression of his carcinoma, a repeat biopsy was recommended. In the month before attending for pre-biopsy rectal screening, the patient had returned from visiting relatives in India. He had no known healthcare contact during this trip.

Rectal screening for pre-TRUS biopsy carriage of fluoroquinolone-resistant *E. coli* was introduced in Auckland City Hospital, New Zealand in March 2012. Where possible, rectal swabs obtained from patients pre-TRUS biopsy are incubated aerobically overnight in MacConkey broth containing 1 mg/L ciprofloxacin. Isolates growing in this broth are then subcultured onto MacConkey agar containing 1 mg/L ciprofloxacin. Any *E. coli* isolates growing after 24 h are identified using the Bruker MALDI-TOF system, and antimicrobial susceptibility testing is performed using agar dilution according to CLSI guidelines.[8]

The patient's pre-biopsy rectal swab grew a fluoroquinolone-resistant *E. coli* that was also resistant to penicillins, extended-spectrum cephalosporins, carbapenems, piperacillin/tazobactam, aztreonam, aminoglycosides and trimethoprim/sulfamethoxazole. The isolate tested susceptible to fosfomycin and nitrofurantoin. Double-disc synergy testing using meropenem or ertapenem with either EDTA or dipicolinic acid as inhibitors was positive, but the modified Hodge test was negative. PCR analysis and DNA sequencing demonstrated the presence of the $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$ genes. In addition, the isolate was also found to harbour the *aac-6′-Ib* and *rmtC* genes. Multilocus sequence typing (MLST) analysis was performed as previously described (http://mlst.ucc.ie/mlst/dbs/Ecoli), and revealed sequence type (ST) 101.

Given the risk of a post-biopsy infection with extremely limited treatment options, along with the patient's advanced age and the low-grade nature of his carcinoma, a decision was made not to proceed with biopsy.

This case illustrates the increasingly complex challenges caused by antimicrobial resistance in the context of TRUS biopsy. Although for many patients colonized with fluoroquinolone-resistant *E. coli*, pre-biopsy screening and 'targeted' prophylaxis may be a useful preventative strategy,[7,9] for patients colonized with extremely resistant organisms such as NDM-producing *E. coli*, options for targeted prophylaxis and potential treatment may be so limited that the benefit of proceeding with biopsy may itself need to be called into question. Although fosfomycin has shown initial efficacy

in preventing post-TRUS biopsy infections with antimicrobial-resistant pathogens, susceptibility of NDM-producing Enterobacteriaceae to fosfomycin remains variable.[10] Given the global dissemination of bla_{NDM}-producing Enterobacteriaceae, coupled with the large number of TRUS biopsies performed in many regions, it seems inevitable that these organisms will continue to be isolated in the setting of TRUS biopsy-related sepsis. Accordingly, a pragmatic approach to undertaking TRUS biopsy seems indicated, including not only a tailoring of prophylaxis based on the patient's colonization status, but also a careful reassessment of the overall net benefit and clinical utility of the procedure itself, particularly in patients found to be colonized with highly resistant pathogens such as NDM-producing *E. coli*.

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Transparency declarations

None to declare.

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Co-selection may explain high rates of ciprofloxacin non-susceptible *Escherichia coli* from retail poultry reared without prior fluoroquinolone exposure

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Australia has never permitted fluoroquinolone use in food-producing animals. We examined local retail poultry for contamination with fluoroquinolone non-susceptible *Escherichia coli*, then explored the hypothesis that their presence may be due to co-selection of resistance determinants. Between August and November 2010, samples from 30 locally produced, uncooked retail poultry carcasses from four different processing centres underwent selective enrichment culture for ciprofloxacin non-susceptible *E. coli*. Their chromosomal- and plasmid-mediated resistance determinants were characterized, and phylogenetic analysis and transformation experiments were performed. Unexpectedly, we found nine (30 %) of our small collection of poultry samples carried fluoroquinolone non-susceptible *E. coli* of which nearly half possessed *aac(6')-Ib-cr*, a novel plasmid-mediated gene encoding an aminoglycoside acetylating enzyme that also confers fluoroquinolone resistance. All nine isolates were co-resistant to amoxicillin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole – all antibiotic classes that are registered for use in poultry reared for food production within Australia. Their unique phylogenetic relatedness suggested clonal dissemination driven by non-fluoroquinolone selective pressures. *aac(6')-Ib-cr* was successfully transformed and selected for using non-fluoroquinolone antibiotic pressure. Vertical and perhaps horizontal co-selection may be contributing to the emergence of fluoroquinolone resistance in poultry and could play a similar role in the human setting. This suggests that preservation of the usefulness of fluoroquinolones may require more than just restriction of their use in isolation from other interventions.

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INTRODUCTION

Antimicrobial use in food-producing animals combined with faecal contamination from animals during processing of carcasses may contribute to transmission of resistant pathogens and/or resistance determinants from animals to humans (Cheng *et al.*, 2012; JETACAR, 2009). This is of particular concern for fluoroquinolones, which are critical to human health yet are one of the classes of antimicrobial most commonly used in veterinary medicine (Forcella *et al.*, 2010). Australia is in the unique position of never having permitted fluoroquinolone use in food-producing animals (JETACAR, 2009). Preliminary findings from our group suggested the prevalence of fluoroquinolone non-susceptible *Escherichia coli* in Western Australian retail poultry samples was significantly higher than the 0 % previously reported in 2007 (Barlow & Gobius, 2008). In the absence of selective pressure due to fluoroquinolone use, we explored the hypothesis that this may be due to co-selection of resistance determinants.

Abbreviation: rep-PCR, repetitive element palindromic-PCR.

METHODS

Between August and November 2010, 30 locally produced, uncooked retail poultry carcasses were obtained from four Western Australian processing centres and rinsed with 500 ml peptone water. Fifty millilitres of rinse fluid was added to 50 ml double-strength *E. coli* broth and incubated at 44 °C overnight. A 500 µl sample of this broth was spread evenly onto MacConkey agar containing 1 µg ciprofloxacin ml^{-1} and incubated overnight at 37 °C. Presumptive *E. coli* were

randomly selected and then identified using standard methods. MIC values were determined by Etest (AB Biodisk; bioMérieux) and interpreted using Clinical and Laboratory Standards Institute criteria (CLSI, 2010). For all ciprofloxacin non-susceptible isolates, PCR and sequencing of *gyrA*, *gyrB*, *parC* and *parE* genes were performed as described previously (Komp Lindgren *et al.*, 2003). A multiplex PCR was used to screen for isolates with *qnrA*, *qnrB* and *qnrS* (Robicsek *et al.*, 2006b). PCR was used to detect isolates containing *qepA1*, *qepA2* and *aac(6')-Ib* (Wang *et al.*, 2003). The *aac(6')-Ib-cr* variant (a novel plasmid-mediated gene encoding an aminoglycoside acetylating enzyme that also confers fluoroquinolone resistance) was detected by sequencing the *aac(6')-Ib* gene (Park *et al.*, 2006). Phylogenetic grouping was determined with multiplex PCR (Clermont *et al.*, 2000). Genetic relatedness was investigated using repetitive element palindromic-PCR (rep-PCR) (DiversiLab; bioMérieux). Plasmid mobility was determined by transformation. Plasmids purified by alkaline lysis were electroporated into *E. coli* DH10B using a previously described method, modified by use of selective medium containing 4 mg gentamicin l^{-1} (Sidjabat *et al.*, 2009).

RESULTS AND DISCUSSION

Ciprofloxacin non-susceptible *E. coli* were isolated from nine (30 %) of the 30 poultry carcasses (originating from three of the four processing plants). The ciprofloxacin MIC for all nine isolates was >32 mg l^{-1}. Each isolate demonstrated an identical susceptibility profile, namely non-susceptibility to amoxicillin, tetracycline, trimethoprim/sulfamethoxazole and gentamicin, but susceptibility to ceftriaxone and ceftazidime. All the isolates had the same chromosomal mutations known to be associated with fluoroquinolone non-susceptibility including those within *gyrA* (S83L, D83N) and *parC* (S80I, E84G), but not *gyrB* or *parE*. Four isolates possessed the *aac(6')-Ib-cr* variant. No *qnr* or *qepA* plasmid-mediated quinolone resistance was detected. Two ciprofloxacin non-susceptible isolates (WA CH1, WA CH2) were selected for transformation experiments (Table 1). These results demonstrated that genes encoding amoxicillin, tetracycline and trimethoprim/sulfamethoxazole resistance were co-located on the same plasmid as genes encoding aminoglycoside resistance; these genes could be passed horizontally between *E. coli* strains and then be selected out in the presence of an aminoglycoside. When present, the *aac(6')-Ib-cr* variant was also shown to be co-located on the same plasmid. This variant was subject to the same horizontal transfer and selection processes in the absence of a fluoroquinolone. All nine isolates belonged to phylogenetic group D and showed a high degree of genetic relatedness (Fig. 1). All nine appeared unrelated to four ciprofloxacin-susceptible isolates from the same cohort of retail poultry samples.

Fluoroquinolone non-susceptible *E. coli* have been detected from poultry in countries in which fluoroquinolone use is currently, or has previously been permitted in the rearing of food-producing animals. This includes the USA (Johnson *et al.*, 2007), Europe (0–4 % of isolates) (Gyles, 2008), Iran (42 %) (Moniri & Dastehgoli, 2005) and Nigeria (55 %) (Fortini *et al.*, 2011), although, to our knowledge, *aac(6')-Ib-cr* has only ever been detected in retail poultry from China (Huang *et al.*, 2009). Thus, our finding of a comparatively high rate (30 %) of ciprofloxacin non-susceptible *E. coli* [nearly half of which possessed the *aac(6')-Ib-cr* gene] in poultry without prior fluoroquinolone exposure was unexpected.

Contamination of carcasses with human faecal flora from poultry processors is unlikely since all nine isolates had a unique antibiotic susceptibility profile that is dissimilar to isolates causing community-onset *E. coli* human infections in Australia (Pearson *et al.*, 2007). All nine were genetically closely related and hence unlikely to have resulted from contamination at three separate poultry processing centres.

Surreptitious veterinary use of fluoroquinolones is unlikely because Australia has strict regulatory control over antibiotic use in animals (JETACAR, 2009). A national study of antibiotic consumption demonstrated no evidence of quinolone use in stock feed between 1992 and 1997 (JETACAR, 2009).

A more plausible explanation for our findings is co-selection due to the use of non-fluoroquinolone antimicrobials in

Table 1. Antimicrobial MIC testing and molecular characterization of quinolone resistance determinants in the parent and transformant isolate

Study isolate	WA CH2		WA CH1	
	Parent	Transformant	Parent	Transformant
Phenotype (MIC, mg l^{-1})				
Gentamicin	>16	>16	>16	>16
Amoxicillin	>256	>256	>256	>256
Tetracycline	>256	>256	>256	>256
Trimethoprim/sulfamethoxazole	>32	>32	>32	>32
Ciprofloxacin	>32	<0.002	>32	0.047
Genotype				
gyrA (S83L, D83N), *parC* (S80I, E84G)	Yes	No	Yes	No
aac(6')-Ib	Yes	Yes	Yes	Yes
aac(6')-Ib-cr	No	No	Yes	Yes

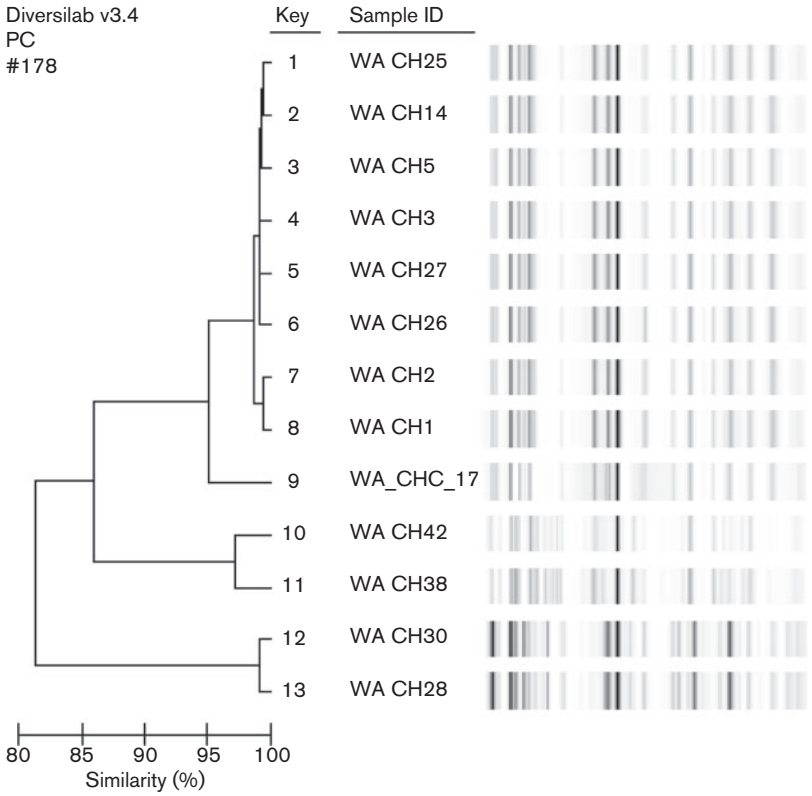

Fig. 1. Dendrogram showing results of rep-PCR typing of the nine ciprofloxacin non-susceptible *E. coli* isolates (WA CH1, -2, -3, -5, -14, -25, -26 and -27 and WA_CHC_17) compared to four ciprofloxacin-susceptible isolates (WA CH28, -30, -38 and -42) from the same cohort of retail poultry samples.

food-producing animals (Fortini *et al.*, 2011; Gyles, 2008). All nine isolates demonstrated co-resistance to antibiotic classes that are registered for therapeutic or prophylactic use in poultry reared for food production within Australia (JETACAR, 2009). Co-selection may be driven by one of two mechanisms. Firstly, a single resistance mechanism [e.g. *aac(6')-Ib-cr* or a multi-drug efflux pump] may confer resistance to multiple antibiotic classes concurrently. Secondly, two different antibiotic resistance determinants (usually co-located) may be vertically or horizontally passed between bacterial isolates. The unique genetic relatedness of all nine isolates suggests that dissemination of chromosomal quinolone resistance determinants is a clonal phenomenon (vertical co-selection), although the origins of this clone are unknown. Our transformation experiments demonstrate that dissemination of plasmid-mediated quinolone resistance determinants such as *aac(6')-Ib-cr* may also occur in the context of non-fluoroquinolone selective pressure (horizontal co-selection). Although acquisition of *aac(6')-Ib-cr* confers only a minor rise in fluoroquinolone MIC, it may promote subsequent mutations that confer high-level resistance (Robicsek *et al.*, 2006a). Plasmid heterozygosity may explain why only some isolates appeared to carry the *aac(6')-Ib-cr* variant (Novick & Richmond, 1965).

Co-selection has been proposed as an explanation for the emergence of fluoroquinolone resistance in Gram-negative bacteria in both humans (Park *et al.*, 2006) and animals (Gyles, 2008). However, in these studies, the relative contribution of co-selection versus direct selective pressure has been difficult to discern in the context of active fluoroquinolone use. As our study was performed in a country that has never permitted fluoroquinolone use in food-producing animals, our results suggest that vertical and perhaps horizontal co-selection may be contributory forces behind the emergence of fluoroquinolone resistance. This necessitates a re-evaluation of the mechanisms contributing to this problem, and our attempts to address it. If co-selection does explain our findings, then efforts to preserve the long-term future of fluoroquinolones, whether in animals or humans, may require more than just restriction of their use in isolation from other interventions.

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Global dissemination of a multidrug resistant *Escherichia coli* clone

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***Escherichia coli* sequence type 131 (ST131) is a globally disseminated, multidrug resistant (MDR) clone responsible for a high proportion of urinary tract and bloodstream infections. The rapid emergence and successful spread of *E. coli* ST131 is strongly associated with several factors, including resistance to fluoroquinolones, high virulence gene content, the possession of the type 1 fimbriae FimH30 allele, and the production of the CTX-M-15 extended spectrum β-lactamase (ESBL). Here, we used genome sequencing to examine the molecular epidemiology of a collection of *E. coli* ST131 strains isolated from six distinct geographical locations across the world spanning 2000–2011. The global phylogeny of *E. coli* ST131, determined from whole-genome sequence data, revealed a single lineage of *E. coli* ST131 distinct from other extraintestinal *E. coli* strains within the B2 phylogroup. Three closely related *E. coli* ST131 sublineages were identified, with little association to geographic origin. The majority of single-nucleotide variants associated with each of the sublineages were due to recombination in regions adjacent to mobile genetic elements (MGEs). The most prevalent sublineage of ST131 strains was characterized by fluoroquinolone resistance, and a distinct virulence factor and MGE profile. Four different variants of the CTX-M ESBL–resistance gene were identified in our ST131 strains, with acquisition of CTX-M-15 representing a defining feature of a discrete but geographically dispersed ST131 sublineage. This study confirms the global dispersal of a single *E. coli* ST131 clone and demonstrates the role of MGEs and recombination in the evolution of this important MDR pathogen.**

bacterial evolution | genomics | phylogeography | genomic epidemiology

Many multidrug-resistant (MDR) bacterial strains are now recognized as belonging to clones that originate in a specific locale, country, or even globally. *Escherichia coli* sequence type 131 (ST131) is one such recently emerged and globally disseminated MDR pandemic clone responsible for community and hospital-acquired urinary tract and bloodstream infections. *E. coli* ST131 was identified in 2008 as a major clone linked to the spread of the CTX-M-15 extended-spectrum β-lactamase (ESBL) resistance (1–3). Since then, *E. coli* ST131 has also been strongly associated with fluoroquinolone resistance, and coresistance to aminoglycosides and trimethoprim-sulfamethoxazole (4–6). Alarmingly, strains of *E. coli* ST131 resistant to carbapenems have also been reported (7, 8), further limiting treatment options for this clone.

E. coli ST131 belongs to the B2 phylogenetic subgroup I, with most isolates characterized as serotype O25b:H4 (1). Epidemiology studies using pulse-field gel electrophoresis (PFGE) have demonstrated that *E. coli* ST131 strains exhibit diversity, with some dominant PFGE pulsotypes including the UK epidemic strain A (9) and pulsotype 968 (10, 11) widely distributed across the globe. More recently, a typing scheme using the type 1 fimbriae *fimH* adhesin gene revealed that a large subclonal lineage of *E. coli* ST131 strains possess the FimH30 allele, which is also associated with specific mutations in the *gyrA* and *parC* genes that confer resistance to fluoroquinolones (12).

Several whole genome (13–16) and PCR (1, 17–20) studies have revealed that *E. coli* ST131 strains possess a variable complement of genes encoding established virulence factors commonly associated with extraintestinal pathogenic *E. coli* (ExPEC). Indeed, few virulence genes appear to be uniformly present in *E. coli* ST131 and, thus, it is likely that differences in virulence gene content contribute to the variable virulence potential that has been reported. For example, although some ST131 strains cause rapid death in a mouse sepsis infection model (21), this phenotype is not consistent among all strains (22). The *E. coli* ST131 strain EC958, which is a representative of the FimH30-fluoroquinolone resistant subgroup, has been characterized at the molecular level (15). *E. coli* EC958 contains an insertion in the type 1 fimbriae regulator gene *fimB* (15) that

Significance

***Escherichia coli* sequence type 131 (ST131) is a globally disseminated multidrug-resistant clone associated with human urinary tract and bloodstream infections. Here, we have used genome sequencing to map the temporal and spatial relationship of a large collection of *E. coli* ST131 strains isolated from six distinct geographical regions across the world. We show that *E. coli* ST131 strains are distinct from other extraintestinal pathogenic *E. coli* and arose from a single progenitor strain prior to the year 2000.**

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is also common to other strains in the FimH30 subgroup (23) and colonizes the mouse bladder in a type 1 fimbriae-dependent manner (15). In mice, *E. coli* EC958 establishes acute and chronic urinary tract infection (UTI), forms intracellular bacterial communities in the bladder (24), and causes impairment of ureter contractility (25). *E. coli* EC958 is also resistant to the bactericidal action of human serum (26).

The rapid global dissemination of *E. coli* ST131, combined with its MDR phenotype and the lack of new antimicrobial drugs in the developmental pipeline, highlights the urgent need to understand this pathogen and combat its spread. Here, we sequenced the genomes of 95 *E. coli* ST131 strains from six geographical regions (isolated from 2000 to 2011) to examine the spatial and temporal relationships of *E. coli* ST131. Our data supports the rapid and recent global dispersal of *E. coli* ST131 as a single clone.

Results and Discussion

A Global Collection of *E. coli* ST131 Strains. A collection of 99 *E. coli* strains defined as ST131, using a described PCR test specific for the O25b *rfb* gene and allele 3 of the *pabB* gene (27), were isolated between 2000 and 2011 from six countries (Australia, Canada, India, Spain, United Kingdom, New Zealand) (Dataset S1). The strains were obtained from several clinical sources and included isolates from urine ($n = 53$), blood ($n = 21$), peritoneal fluid ($n = 1$), abdominal abscess ($n = 1$), surgical wound ($n = 2$), and rectal swabs ($n = 11$). The strains were selected with an endeavor to encompass diversity with respect to geographic origin, date of isolation, and clinical source. The strains possessed a range of antibiograms, including variable resistance to aminoglycosides, second and third generation cephalosporins, fluoroquinolones, penicillins, and sulfonamides (Dataset S1). All strains were sequenced by using the Illumina HiSeq, assembled using Velvet, and *in silico* multilocus sequence typing (MLST) was performed to confirm the sequenced strains were ST131. Four strains originally defined as ST131 by *rfb* and *pabB* PCR actually belonged to ST95 (Dataset S1), thus reducing the final number of ST131 strains examined to 95.

Rapid Global Dispersal of *E. coli* ST131 as a Single Clone. Phylogenetic analysis of the 95 *E. coli* ST131 strains was carried out by using whole genome alignment and single-nucleotide polymorphism (SNP) analysis using the completely sequenced ST131 representative strain SE15 (13). A maximum likelihood (ML) tree built using all 142,750 SNPs confirmed that all ST131 strains belonged to phylogroup B2, subgroup I and showed that ST131 clustered into three well-supported clades that we refer to as A, B, and C (*SI Appendix*, Fig. S1*A*). ML trees based on the 3,186,979-bp core alignment of the assembled sequence data supported this topology (*SI Appendix*, Fig. S2*A*). Recombination is the primary contributor to interclade diversity with only 70 nucleotide substitutions found to distinguish clades B and C after removal of recombinant regions (Fig. 1*A* and Dataset S2). Neither temporal nor geographical clustering between the major clades could be observed (Fig. 1*A*); however, each clade is comprised of at least two well-supported sublineages and smaller clusters of closely-related strains that exhibit some geographical association (Fig. 1*B* and Dataset S2). This data suggested an evolutionary history more complex than a standard geographical clonal expansion, as exemplified by many occurrences of nearly identical strains isolated in different countries and continents and over different periods of time. Similar phylogeographic patterns have been observed for other successful MDR global lineages such as *Staphylococcus aureus* ST239 and the PMEN1 pneumococcal lineages (28, 29), whereas a contrasting example of clonal expansion with more defined geographical clustering has been reported for *Shigella sonnei* (30).

ST131 clade A contains the previously-sequenced SE15 strain and is the most divergent clade (~7,000 and ~8,900 SNPs from clades B and C, respectively) characterized by the *fimH41* allele and different *gyrA* and *parC* variants. ST131 clade B is very similar to clade C (distinguished by ~2,900 SNPs) and is characterized by

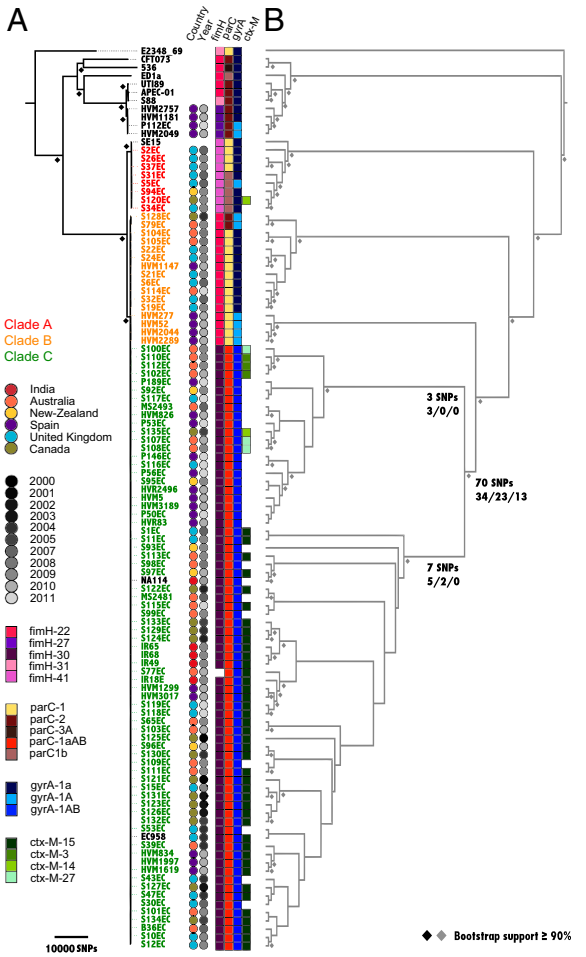

Fig. 1. Phylogenetic relationship of ST131 strains. (*A*) ML phylogram with triangles indicating bootstrap support of >90% from 1,000 replicates. The tree is rooted by using the outgroup phylogroup D strain UMN026; branch lengths correspond to the number of SNPs difference (scale bar bottom left). The phylogram was built from 119,514 substitution-only SNPs determined by read-mapping using *E. coli* SE15 as reference excluding recombinant regions, as defined by BRATNextGen analysis (34). The taxa labels for sequenced ST131 strains are colored red (clade A), orange (clade B) and green (clade C). Previously sequenced reference strains are colored black. Colored circles next to each strain correspond to country and year of isolation (see key). Squares indicate allelic profiling for *fimH*, *parC*, *gyrA*, and CTX-M (see key). A missing square indicates the gene is absent. (*B*) Several well-supported subclades are evident in the ST131 phylogeny, with the CTX-M-15 gene confined to the second subclade of clade C. The topology-only cladogram (not to scale) corresponding to the phylogram in *A* is shown in gray, with node support of >90% depicted as gray diamonds. The number of SNPs that define clade C and sublineages C1 (*Upper*) and C2 (*Lower*) are shown below relevant branches (nonsynonymous, synonymous, intergenic); refer to Dataset S2 for full list of SNPs and consequences.

an intact *fimB*, the *fimH22* allele, and *gyrA* and *parC* variants that are consistent with their fluoroquinolone sensitivity (Fig. 1*A* and Dataset S1). ST131 clade C strains make up 79% of the ST131 strains sequenced in this study and are distinguished by possession of the *fimH30* allele and the fluoroquinolone resistance alleles *gyrA*1AB and *parC*1aAB (Fig. 1*A*). All but one of the clade C strains contained an insertion within the *fimB* gene as we originally observed in the clade C strain EC958 (15). These isolates were collected in six countries from 2000 to 2011, indicating that the dominant clade C ST131 lineage originated from a single clone before the year 2000 (Fig. 1*A*). Although we cannot rule out the possibility of a bias in our strain collection, we note that the dominant group among another large collection of ST131 strains

was also found to share the same *fimH*30-*gyrA*1AB-*parC*1aAB allelic profile (12).

Analysis of the density of all SNPs along the SE15 reference chromosome revealed a nonhomogeneous distribution, with many core-genome regions associated with a density ~8.5-fold higher than the expected average (Fig. 2). Because discrete regions with a high-density of SNPs may be the result of recombination events, as opposed to mutational hotspots (31, 32), we inferred the recombination across ST131 genomes by using a Bayesian clustering approach that was previously successfully applied to *S. aureus* and *Streptococcus pneumoniae* (33, 34). We found that recombination has introduced 76.6% of the 16,424 SNPs and 2,050 small indels that differentiate the strains within the ST131 lineage (*SI Appendix*, Fig. S3 and Dataset S3). Phylogenetic analysis using only SNPs found in recombinant regions also clustered the ST131 strains into the same three-clades structure (*SI Appendix*, Figs. S1*B* and S2*B*). Overall these results reflect the significant role that recombination has played in shaping the three major ST131 lineages with subsequent point mutations driving the fine-scale diversity within each clade.

Antibiotic Resistance Is Associated with ST131 Clade C. Besides the major contribution of recombination events to the between-clade diversity of ST131, we also observed differences in the distribution of SNPs between recombinant and nonrecombinant regions (Fig. 2). SNP density across all strains combined was higher in recombinant regions with an estimated 1.19×10^{-2} SNPs per site compared with 1.39×10^{-3} SNPs per site in nonrecombinant regions. Despite the lower density of SNPs, nonrecombinant regions were characterized by a relatively higher ratio of nonsynonymous to synonymous SNPs (0.05 and 0.07 SNPs per kilobase, respectively) compared with recombinant regions (0.2 and 0.89 SNPs per kilobase, respectively). This difference was significant ($\chi^2 = 1{,}045.8$, $P < 0.00001$) and is consistent with a pairwise comparison of ST131 clade A and clade C strains (23).

Fluoroquinolone resistance is one of the major determining features of the ST131 clone and is associated with point mutations in the *gyrA* and *parC* genes (12) (Fig. 1). The three major *gyrA* alleles found in our ST131 dataset were attributed to vertically transmitted point mutations, with unique *gyrA* mutations also found in clade A strain S5EC (A669T) and clade C strain B36EC (Q453R), respectively. In contrast, the *parC*1aAB allele was introduced into clade C via recombination, replacing the *parC*1 allele and surrounding Rec_089 region that is conserved in most clade A and B strains (Dataset S3). Multiple, overlapping recombination events continue to shape the ST131 lineage as evidenced by two independent replacements of Rec_089 in subgroups of clade A (encompassing *parC*2) and clade B (*parC*3A), with a further two partial replacements of a 1.8-kb Rec_089 subfragment immediately upstream of *parC* in two clade C strains (S101EC and S113EC). Among the 34 nonsynonymous and nonrecombinant substitutions that define clade C, we could map nine to crystal structures of homologs, several of which encode amino acid changes that may impact their function (*SI Appendix*, Fig. S5 and Dataset S2). For example, there is a mutation in the gene encoding the MukB chromosome partition protein, a known interacting partner of ParC (35). In addition to established *fimH*, *parC* and *gyrA* mutations in clade C strains, our identification of further genes with clade C-specific mutations paves the way for more targeted investigations to identify key evolutionary events that underpin the success of *E. coli* ST131.

Among the SNPs that have arisen in individual ST131 clade C strains or subgroups, there are a number within potential antibiotic resistance genes that may have been selected in response to antibiotic treatment (Dataset S2). Each ST131 clade C strain (minus NA114) has between 0 and 50 (mean = 13, SD = 11) unique, nonrecombinant SNPs, 49% of which are nonsynonymous. There are numerous examples of nonsynonymous SNPs within genes that encode homologs of multidrug resistance proteins or other putative transporters that may affect antimicrobial uptake or efflux (Dataset S2). There are also several SNPs in genes encoding penicillin-binding proteins (e.g., ECSF_2363/PBP1C, ECSF_0094/PBP3), other cell wall modifying enzymes (e.g., ECSF_2495 lytic murein transglycosylase B) and examples of cell division genes (e.g., ECSF_2198), or essential genes that may be important for intrinsic resistance development. Although the majority of ST131 clade C SNPs are unique to the strain in which they are found, or exhibit patterns of descent consistent with the inferred phylogeny, we identified genes in which the same mutation appeared to have been acquired independently (Dataset S2). For example, the dihydrofolate reductase gene (ESCF_0053) acquired the trimethoprim resistance L28R mutation in two phylogenetically separated clade C strains (S116EC and S11EC), with several other nonsynonymous mutations in this gene present in different strains.

The majority of clade C strains also possess the CTX-M-15 gene (36 of 42 strains in sublineage C2), with seven other clade C strains containing different CTX-M alleles (3, 14, or 27) (Fig. 1*A* and Dataset S1). The CTX-M-15–positive strains cluster within a discrete, but temporally and geographically dispersed, sublineage within clade C (Fig. 1*B*). Although the pattern of CTX-M-15 distribution within this sublineage is suggestive of an ancestral acquisition of the CTX-M-15 gene and subsequent loss by some individual strains, this allele does not associate with any particular plasmid incompatibility group defined by sequence-based typing (*SI Appendix*, Fig. S4). Furthermore, the CTX-M-15 gene is found on assembled contiguous fragments (contigs) ranging in size from 1.4 kb to 10 kb with variable adjacent gene content (many of which have been previously identified on plasmids), suggesting that the CTX-M-15 gene has

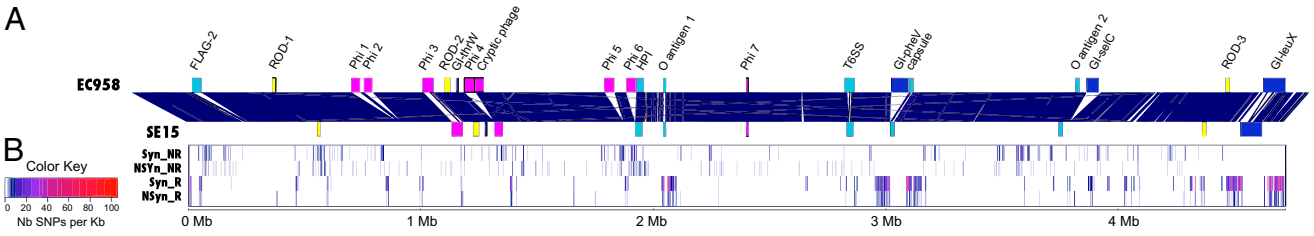

Fig. 2. Distribution of ST131-only core SNPs in recombinant versus nonrecombinant regions. (*A*) Comparison of the linear genome arrangement of the clade C strain EC958 (*Upper*) and the clade A strain SE15 (*Lower*). Solid dark-blue lines between EC958 and SE15 indicate BLAST match of ≥99% nucleotide identity between the two genomes. Genomic features of interest are highlighted for both strains as follows: prophages (pink); ST131 characteristic ROD1, ROD2, and ROD3 (yellow); previously characterized genomic islands (blue); and other regions of interest (turquoise). Labels refer to the ST131-characteristic regions defined in the genome of EC958 (15). (*B*) Heatmap showing the density of 16,424 ST131-only core SNPs along the SE15 chromosome: Syn_NR (synonymous, nonrecombinant); NSyn_NR (nonsynonymous, nonrecombinant); Syn_R (synonymous, recombinant); and NSyn_R (nonsynonymous, recombinant). ST131-only core SNPs were defined as bases called from the mapping data in all strains of the dataset with polymorphisms specific to the ST131 lineage. Recombinant region coordinates were delineated by using BratNextGen. The SNP density heatmap with (number of SNPs per 1 kbp nonoverlapping bin) is indicated by the color key. The *x* axis at the bottom of the figure represents the SE15 reference chromosome coordinates.

been independently acquired several times or that it has translocated between different plasmids or the chromosome. Both scenarios are consistent with previous reports of different types of ST131 plasmids that harbor CTX-M-15 (37).

***E. coli* ST131 Contains Many ExPEC-Associated Genes.** The complement of virulence-associated genes was determined in the 95 *E. coli* ST131 strains by examining for the presence of genes encoding chaperone-usher (CU) fimbriae (38), autotransporter (AT) proteins (39), siderophore receptors (40), toxins, colicins (41), and other genes often assessed by PCR in ExPEC (1) (*SI Appendix*, Fig. S6). The *E. coli* ST131 strains contained genes encoding type 1, Mat (ECP), Yde, ECSF_0166, EC958_4610, and Yeh fimbriae; other CU fimbriae genes including Afa and P fimbriae were variable. The complement of AT-encoding genes was highly conserved, with most strains containing genes encoding antigen 43, UpaB, UpaC, YfaL, and Sat. The ECSF_4014 AT gene was uniquely present in *E. coli* ST131 strains. Most *E. coli* ST131 strains contained a number of genes associated with iron acquisition; of note, the Yersiniabactin receptor (ECSF_1835) was found to be widely prevalent but highly diverse with 17 independent substitutions (14 nonsynonymous) confined to clade B and C strains, strongly suggesting that, like *fimH,* this gene may be under positive selection. Approximately 15% of *E. coli* ST131 strains contained genes encoding the HlyA and Cnf1 toxins. In all but clade C strain S115EC, these genes were colocated on the chromosome, which is consistent with their presence on the same genomic island in other ExPEC strains such as CFT073. In general, 131 UPEC-specific genes present in CFT073, UTI89, 536, and F11 (42) were also conserved, with only the gene encoding the putative regulator c0765 absent from all ST131 strains.

Diversity Within the ST131 Lineage Is Primarily due to Mobile Genetic Elements and Recombination of Associated Regions. *E. coli* ST131 strain EC958 contains several mobile genetic elements (MGEs) and other genomic regions not found in completely sequenced non-ST131 UPEC strains (i.e., CFT073, 536, UTI89, UMN026, IAI39), including seven prophage elements (Phi1-7), the Flag-2 lateral flagellar locus, the O-antigen loci, the *ratA*-like toxin encoding gene, the type VI secretion locus, the capsular locus, and four genomic islands (GI) in chromosomal integration hotspots (GI-*pheV*, GI-*selC*, GI-*leuX*, and GI-*thrW*) (15). The majority of these regions were highly conserved in strains from clade C but were fully or partly absent in strains from clades A and B (Fig. 3 and *SI Appendix*, Fig. S6). Exceptions included the Phi6, GI-*selC*, and capsular loci regions, which were not exclusively associated with a particular clade, suggesting a more complex evolutionary history. The Flag-2 locus was completely absent in strains from clade A and in four Spanish strains of clade B (HVM277, HVM52, HVM2044, HVM2289), replaced by the *fhiA-mbhA* scar found in *E. coli* K12 strains (43). Interestingly, these four Spanish strains form a discrete sublineage (Fig. 1*B*) and also lack prophage and genomic islands that are present in other clade B strains. In contrast to the O25b serotype of most clade B and C strains, the LPS core biosynthesis region (specifically *wbbJ-rfbE*) of clade A strains was the same as in SE15, which has been reported as serotype O150 (13). Three regions of difference (ROD) > 10 kb in length were shown to be unique in ST131 strains EC958 and SE15 (15). Although the functions of genes encoded by ROD1 are unclear, ROD1 is conserved in all ST131 strains. Similarly, ROD2 (which contains several sugar metabolism genes) was also ubiquitous but contained deletions in at least three ST131 strains (*SI Appendix*, Fig. S7). ROD3 is also conserved across all ST131 strains except for clade A. The absence of several regions within the NA114 genome that are otherwise present in closely related clade C strains such as S97EC (Fig. 3) is consistent with the assembly of this genome, which was performed by concatenating ordered contigs to produce a single pseudomolecule without gap closure and finishing (14).

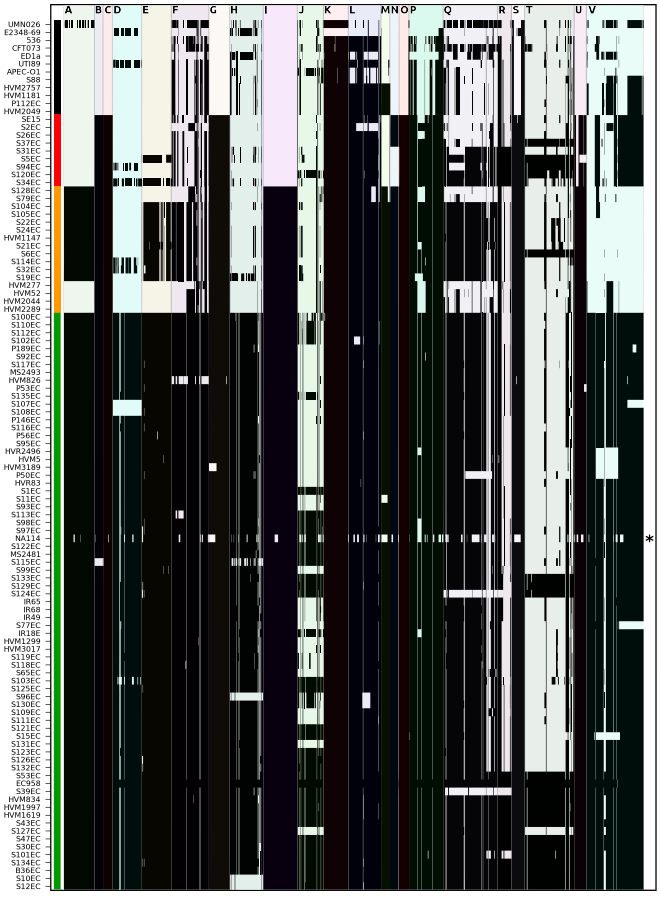

Fig. 3. Selected regions of interest in ST131 strains. ST131-characteristic regions previously defined in the genome of EC958 (15) are shown along the *x* axis with strain identifiers listed on the *y* axis according to the phylogenetic tree order displayed in Fig. 1*A*. Regions A–M are shown to scale in order of their location relative to the SE15 chromosome (Fig. 2) and correspond to: A, flag2 flagellar region (38.1 kb); B, GI-ThrW genomic island; C, ROD1; D, Phi1 prophage; E, Phi2 prophage; F, Phi3 prophage; G, ROD2; H, Phi4 prophage; I, Phi5 prophage; J, Phi6 prophage; K, High-Pathogenicity Island; L, cryptic prophage; M, O-antigen 1 region (*wbbJ-rfbE*); N, Phi7 prophage; O, RatA-like region; P, T6SS region; Q, GI-PheV genomic island; R, capsule region; S, O-antigen2 region; T, GI-SelC genomic island; U, ROD3; V, GI-LeuX. Black shading indicates a match of ≥95% nucleotide identity in a minimum window of 200 bp calculated by comparing the query sequence to the assembled contigs or the consensus from mapped reads for each strain, as implemented in seqfindr (http://github.com/mscook/seqfindr).

A total of 137 regions were defined as recombinant within the ST131 lineage (Dataset S3), with a clear propensity to be located adjacent to predicted MGEs (*SI Appendix*, Fig. S3). These recombinant regions totalled 0.94 Mb, or nearly one-fifth of the entire *E. coli* SE15 genome and include the aforementioned *fimH* and *parC* genes, which are found on recombinant regions Rec_137 (92.3 kb) and Rec_089 (18.5 kb), respectively. Although the majority of regions are less than 1,000 bp in length, ~80% of the recombinant bases are contained within 24 large recombinant regions that range in size from 10.2 kb to 166.2 kb. We could define the lineage on which the recombination event occurred in the majority of cases; however, the larger fragments, such as Rec_088, Rec_089, and Rec_137, have a more complex evolutionary history with evidence for multiple blocks of different origin, reflecting sequential, overlapping recombination events within the same region (*SI Appendix*, Fig. S3 and Dataset S3). When considering the repertoire of recombinant regions that distinguish each clade, clade A was the most distant, with a total of 0.52 and 0.6 Mb differing from clade B and C, respectively.

Fewer recombinant regions distinguish clade B from clade C, with the majority of differences contained in regions upstream of Phi3, and upstream and downstream of GI-*pheV* and GI-*leuX*, respectively (Dataset S3).

A striking feature of the recombination distribution along the chromosome is that the majority of large recombinant regions were associated with the sites of insertion of prophage and genomic island MGEs (*SI Appendix*, Fig. S3). Statistical evaluation of 10,000 replicates of the Kolmogorov–Smirnov test confirmed that the distribution of the observed distances between recombinant regions and MGEs was significantly negatively skewed compared with randomly selected regions (K–S test, mean $D = 0.370$, SD = 0.049, mean $P = 6.082 \times 10^{-6}$, SD = 8.004×10^{-5}) (*SI Appendix*, Fig. S8). This phenomenon has been observed in a comparison using the *E. coli* ST131 SE15 and NA114 genomes, for which our analysis agrees with 20 of 22 recombination regions (23), and in other comparisons of closely related *E. coli* genomes (44). In contrast, a reduced role for recombination was reported in a study comparing 12 ST131 genomes and 50 publicly available *E. coli* reference genomes (45).

Recombinant Regions Have Shaped the ST131 Lineage. Fimbrial adhesins and bacterial motility genes were significantly overrepresented in recombinant fragments (*SI Appendix*, Fig. S9). A prime example was the *fliC-fliY* flagellar locus encoded on the recombinant fragment Rec_051 (ECSF_1762 to ECSF_1776). In SE15 and other ST131 clade A strains, the *fliC* allele corresponds to the H5 serogroup. In contrast, clade B and C strains possess an H4 *fliC* allele within Rec_051, a 12.6-kb recombinant fragment that is adjacent to the Phi5 insertion in EC958. The *fim* operon containing the type 1 fimbrial *fimH* gene resides within Rec_137, which at 92.6 kb is one of the largest and most complex recombinant fragments within the ST131 lineage (Dataset S3). The subfragment of Rec_137 that encodes the region *fimC* to *uxuR* displayed characteristic recombination patterns, introducing a clade-specific *fimH allele* (*fimH*41 in clade A, *fimH*22 in clade B, and *fimH*30 in clade C). Interestingly, these three *fimH* alleles were also identified as the major signatures in a small collection of mainly American isolates, and the same recombinant region was deduced from the comparison of SE15 and NA114 (23). As observed in EC958 (15), an insertion within *fimB* was found in clade C strains, although it is not clear if this insertion was acquired by homologous recombination concomitant with the acquisition of the *fimH*30 subfragment, or subsequent to this event. The only exception in our collection was the ST131 clade C strain S77EC, which contained a large deletion encompassing part of the 3′ end of the adjacent GI-*leuX* island (Fig. 3) and the *fim* locus.

Several regions containing putative virulence genes, namely Rec_087 (ECSF_2626 to ECSF_2634) and part of Rec_088 (ECSF_2784 to ECSF_2804), which contain genes related to a Type 6 Secretion System (T6SS) and a Type 2 Secretion System (T2SS), respectively, have also undergone gene conversion. Clade B and C strains carry T6SS alleles that are distinct from clade A strains. In contrast, the T2SS locus in clade C strains appears to have been subjected to several independent recombination events, consistent with its location in a recombination hotspot downstream of the GI-*pheV* island (*SI Appendix*, Fig. S3). Between the T2SS region and GI-*pheV*, the Rec_088 recombinant fragment also encodes the group II capsule synthesis locus (ECSF_2771 to ECSF_2783). Several variant region 2 gene clusters were observed between region 1 (*kpsFEDUCS*) and region 3 (*kpsTM*) of ST131 genomes, consistent with multiple instances of replacement since divergence of ST131 clades A and C with corresponding differences in K-antigen serotype (46). As described above, differences in the LPS core biosynthesis locus within the 70.3-kb Rec_069 recombinant fragment suggest that the O25b serotype is also associated with divergence of clades B and C from clade A (13).

Several less-well characterized genomic regions that could differentiate clade C strains from other ST131 strains were also identified. Two regions with the most distinctive recombination profiles that clearly distinguished all three clades were Rec_131 (ECSF_4099 to ECSF_4159) upstream of GI-*leuX*, and part of Rec_137 (ECSF_4277 to ECSF_4338). The Rec_131 region contains the *tamAB* genes, which encode a recently described translocation and assembly module that contributes to the secretion of some AT proteins (47), whereas the Rec_137 region contains genes associated with salt resistance (*osmY*), siderophore-based iron transport (*fhuF*), and regulation (*creBC*). When the impact of recombination on major gene functions independent of virulence was considered, significant differences were observed in genes encoding transporters, fructose-mannose metabolism, histidine metabolism, and the pentose-phosphate pathway (*SI Appendix*, Fig. S9). The impact of these sequence changes remains to be determined.

Conclusion

Our whole-genome phylogenetic analysis indicates that ST131 has arisen from a single progenitor *E. coli* that diverged into three sublineages some time before the year 2000 with acquisition of multiple mobile genetic elements, associated recombination events, and point-mutations jointly responsible for the emergence of the most prevalent clade C strains. In addition to the known *fimH*, *fimB, parC*, and *gyrA* alleles that characterize ST131 clade C, we have defined several additional genes and regions that may be important for adaptive diversification in response to host or antibiotic resistance pressures. These results also provide a framework for future PCR-based assays to rapidly classify ST131 strains and monitor their evolution. Further molecular analysis of the clade defining variants and MGEs identified in this study will help to elucidate the mechanisms that have led to ST131 colonization of the urinary tract and other clinical sites, and the rapid global dispersal of this important group of ExPEC.

Materials and Methods

Genome Sequencing and Assembly. Draft genomes were generated by using 100-bp paired-end Illumina HiSeq 2000 reads and assembled with Velvet (48). Contigs ≥200 bp were ordered against the EC958 draft genome (BioProject: PRJEA61443) by using Mauve (49). Sequencing reads are available at the European Nucleotide Archive under study number ERP001354, accessions in Study ERP001354 (ERS126551–ERS126646) (see Dataset S1 for accession numbers) with draft genomes available at http://github.com/BeatsonLab-MicrobialGenomics/ST131_99. See also *SI Appendix, SI Materials and Methods*.

Genome Analysis. Alignment of the ST131 draft genome assemblies and three ST131 reference genomes (SE15, NA114, and EC958), plus completely sequenced non-ST131 genomes belonging to the *E. coli* B2 phylogenetic group (CFT073, UTI89, E2348/69, ED1a, 536, S88, APEC O1), was performed by using Mugsy (50) and GBLOCKS (51) with a minimum syntenic block of 5 kbp. Recombination in the ST131 sequences was estimated by using BratNextGen, which implements a Bayesian clustering algorithm for detection of recombinant fragments in closely related sequences (34). See also *SI Appendix, SI Materials and Methods*.

Read Mapping and SNP Analysis. Reads from each ST131 isolate and reads simulated *in silico* for the 10 complete genomes used in this study were mapped onto the reference genome SE15 (16) by using SHRIMP 2.0 (52). Nesoni (www.vicbioinformatics.com/software.nesoni.shtml) was used to perform SNP calling (conservative default parameters), small indel prediction, and coding effect SNP annotation. In addition, the Nesoni *n-way* pairwise comparison method was used to establish the list of all polymorphic positions conserved in all strains of the dataset. Polymorphic substitution-only sites were concatenated to produce an alignment that was used for phylogenetic tree construction. Analysis and visualization of SNP distribution across the collection were performed by using custom R scripts. See also *SI Appendix, SI Materials and Methods*.

Phylogeny. ML phylogenetic trees were estimated by using RAxML 7.2.8 (53) for the inferred core genome and the SNP alignments (prerecombination and postrecombination filtering) under the GTR nucleotide substitution model with a gamma correction for ASRV. Recombination filtering was performed by collapsing the recombinant segment boundaries predicted for each strain into a unique list of 137 nonoverlapping segments and subsequently

masking these regions from the alignment (Dataset S3). Support for nodes was assessed by using 1,000 random bootstrap replicates. See also *SI Appendix, SI Materials and Methods*.

Comparative Genomics. Virulence factor profiles, and the presence of other regions in the draft genomes, were visualized by using seqfindr (http://github.com/mscook/seqfindr). Query sequences and their source are listed in Dataset S1 and with sequences available at http://github.com/BeatsonLab-MicrobialGenomics/ST131_99/. Comparisons between individual genomes and verification of seqfindr results were performed by using BLAST (54), Artemis Comparison Tool (55), Easyfig (56), and BRIG (57). See also *SI Appendix, SI Materials and Methods*.

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