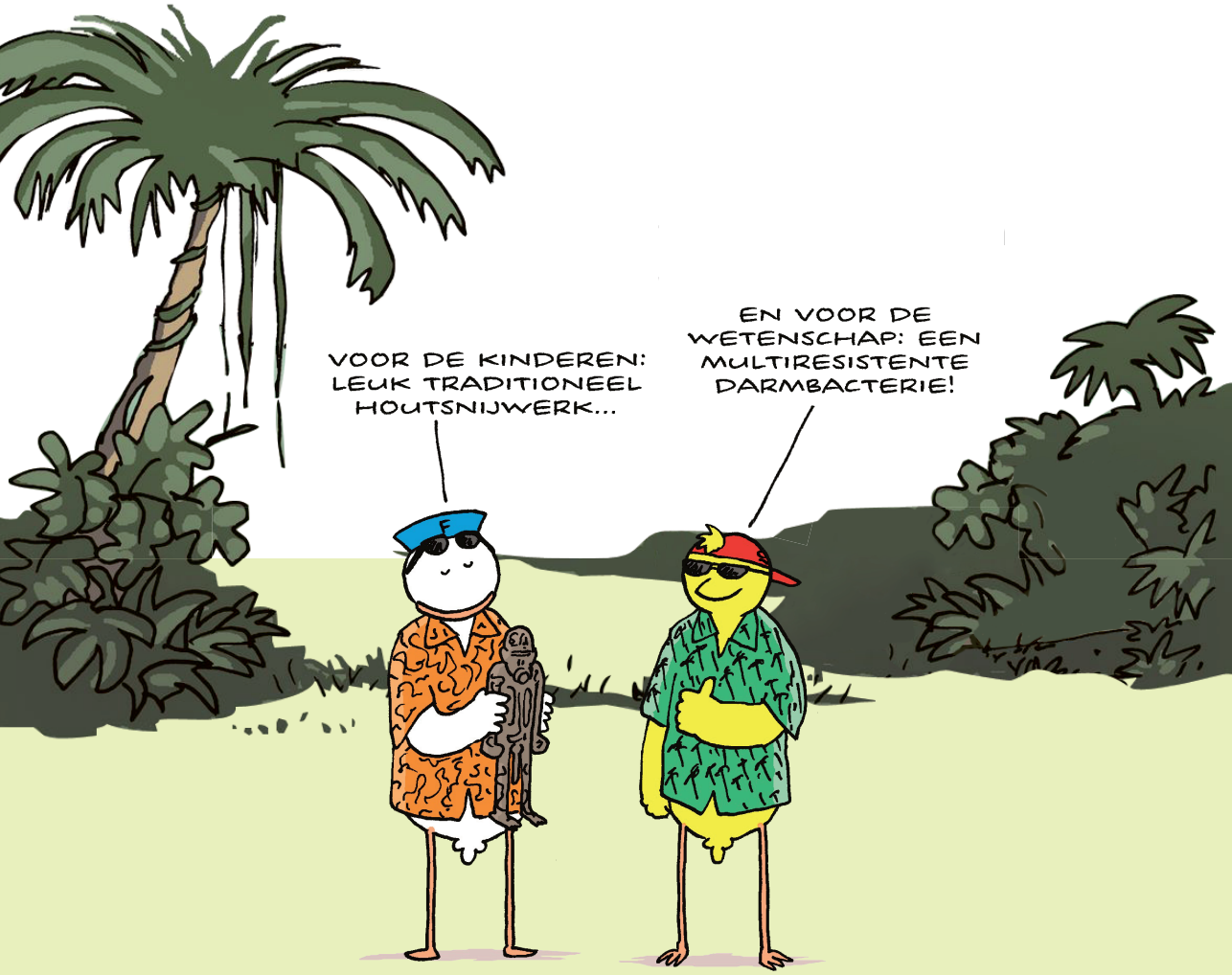
The Import and Spread of Antibiotic-Resistant Enterobacteriaceae by Healthy Travellers

Maris Arcilla



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ISBN: 978-94-6361-303-3
Lay-out and printing by Optima Grafische Communicatie

Printing of this thesis was financially supported by the Erasmus University Medical Center, the Netherlands Society of Medical Microbiology (NVMM) and the Royal Netherlands Society for Microbiology (KNVM), Copan Italia S.p.A., Check-Points and Chipsoft.

The Import and Spread of Antibiotic-Resistant Enterobacteriaceae by Healthy Travellers

De import en verspreiding van Enterobacteriaceae resistent voor antibiotica door gezonde reizigers

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

prof. dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 27 november om 9:30 uur

door

Maria Silangan Arcilla
geboren te Utrecht

Erasmus University Rotterdam

PROMOTIECOMMISSIE:

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CHAPTER 1

General introduction and outline of this thesis

INTRODUCTION

A major breakthrough in medicine was the discovery of penicillin in 1929 by Alexander Fleming (1). In 1940 the world celebrated as its clinical efficacy was demonstrated for the first time. In the same year however, a study was published which showed bacteria could produce an enzyme that inactivated penicillin (2), but this early warning was largely ignored.

Following a renewed warning by Calvin Kunin in 1993 (3) antimicrobial resistance has increasingly become recognized as a major global health problem over the past 25 years. Overuse of antimicrobials and lack of sanitation and infection control has led to rapidly increasing rates of antimicrobial resistance due to selection of resistant clones and their spread locally, regionally and often times worldwide. Recent world-wide estimates are that there are already 700.000 deaths annually due to antimicrobial resistance, and it is predicted that, without effective interventions, this number will further increase to 10 million deaths annually by the year 2050. The strongest impact of antimicrobial resistance emergence is predicted to occur in Asia and Africa where annual death rates are estimated to approach 5 million and 4 million by the year 2050, respectively. However, also Europe is already affected and may experience an estimated number of 400.000 deaths yearly by 2050 due to diseases caused by antimicrobial resistant micro-organisms (4). Although these estimates have been criticized for overestimating mortality resulting from antimicrobial resistance (5), there is no doubt that there is a large and increasing burden of antimicrobial resistance on clinical and public health that needs to be addressed.

Antibiotic-resistant Enterobacteriaceae

Among bacteria belonging to the family of Enterobacteriaceae resistance to beta-lactam antibiotics has been emerging worldwide. The family of Enterobacteriaceae consists of multiple species of Gram-negative bacilli, several of which are part of the normal human microbiota, especially in the gut. These same species are also important causes of community-acquired and nosocomial infections. Enterobacteriaceae can acquire resistance genes through horizontal transfer of mobile genetic elements like plasmids. Among the genes thus transferred may be those that encode for the production of enzymes called extended-spectrum beta-lactamases (ESBLs). ESBLs have broad-spectrum activity against penicillins, cephalosporins and monobactams, which they degrade by hydrolyzing the beta-lactam ring of these antibiotics, leading to inactivation. During the 1990s the first recognized ESBLs were the so called TEM and SHV genes, which were mostly carried by *K. pneumoniae* strains causing hospital infection. Over time, the epidemiology of ESBLs shifted towards CTX-M genes which were carried by *E. coli* originating from the community. Due to their rapid spread and increase around

the world the phenomenon has been referred as the "CTX-M pandemic" (6). CTX-M-15 (part of CTX-M group 1) and CTX-M-14 (part of CTX-M group 9) are the most prevalent ESBL genotypes (7). CTX-M-15 has spread worldwide and is the dominant ESBL-gene in most regions. CTX-M-14 is dominant in China, South-Korea, Japan and Spain (8). The only class of beta-lactam antibiotics that are relatively resistant to degradation by ESBLs are the carbapenems, including imipenem and meropenem; they are often used to treat infections due to ESBL-producing Gram-negative bacteria. However, Enterobacteriaceae can acquire resistance genes encoding for enzymes called carbapenemases. These carbapenemase-producing Enterobacteriaceae (CPE's) are resistant to most betalactam antibiotics, including the carbapenems. There are different groups of enzymes with the capability to inactivate carbapenems. The carbapenemases which have now spread worldwide are KPC (*Klebsiella pneumoniae* carbapenemases), NDM (New Delhi metallo-beta-lactamase), OXA-48 (oxacillinases), IMP (imipenemase) metallo-β-lactamase and VIM (Verona integron–encoded metallo-β-lactamase). Geographically, the primary reservoirs or sites of emergence of the carbapenemases have been the USA, Israel, Greece and Italy for KPC, the Indian subcontinent for NDM, Turkey and North Africa for OXA-48 and Greece, Taiwan and Japan for VIM, and the Asia Pacific for IMP (9, 10).

In addition, multiple genes encoding for resistance to other antibiotic classes such as quinolones and aminoglycosides are often located on the same plasmid (11, 12). Therefore, ESBL-E and CPE-E are often resistant to multiple antibiotic classes. Multidrug resistant Enterobacteriaceae leave doctors with few to no effective antimicrobial agents for the prevention and treatment of infections with these bacteria. Consequently, older drugs including polymyxin class antibiotics (polymyxin B and polymyxin E [colistin]), which were largely disregarded in the past due to their (nephro)toxic side effects, have made a comeback and are now prescribed as a last resort treatments for severe infections with multidrug resistant Enterobacteriaceae. Although it was long thought that only chromosomal mutations could code for colistin resistance, the 2015 discovery in China of a plasmid based colistine resistance gene, designated mrc-1, raised serious concerns (13). Soon after its discovery, many reports described the presence of the mcr-1 gene in isolates from animals, animal food products, humans and environmental samples from around the world. Reports on carbapenemase-producing Enterobacteriaceae that have acquired the mcr-1 gene worries the scientific and medical community as it could lead to the emergence of untreatable so called pandrug resistant Enterobacteriaceae (14). Unfortunately, innovative antimicrobial treatment options are few and the current rate of development of new antibiotics seems insufficient to keep up with the emergence and spread of antimicrobial resistance (15).

Antibiotic-resistant Enterobacteriaceae and travel

The overuse of antibiotics in animals and humans can lead to high endemic levels of ESBL-E locally, through selective pressure (16-18). Community carriage rates are high in regions like South-East Asia, Western Pacific and Africa (Figure 1). In addition to overuse, limited access to proper sanitation facilities and contamination of surface waters used for irrigation of crops, and of drinking water supplies facilitate the spread of ESBL-E and contribute to the level of endemicity of ESBL-E in these countries (Figure 2) (8, 19). Moreover, the overuse of antibiotics of multiple classes leads to accumulation of multiple resistance genes in the environment, which can subsequently be acquired by people (20). In such a situation, the use of just a single antibiotic may be sufficient to select for multidrug resistant isolates, a process called co-selection which drives the emergence of multidrug resistant isolates (21, 22).

Another potential mode of international spread of antimicrobial resistance is through the transport of contaminated foods, livestock and, last but not least, by national and international travel of people. Travellers visiting countries with high, endemic, levels of antimicrobial resistance can acquire bacteria or plasmids carrying resistance genes through contact with indigenous people, food or the environment and import them to their home country. As the human gut microbiota act as a reservoir for antimicrobial resistance genes, international travellers may substantially contribute to the emergence and spread of ESBL-E in their home countries. Given the enormous growth in the number of international travellers, from 25 million in 1950 to 1.326 billion in 2017 (23), it is important to asses to what extent foreign travel poses a risk for the acquisition and spread of antimicrobial resistance. More insights into the rates and determinants of acquisition, persistence and transmission of travel-associated antibiotic-resistant Enterobacteriaceae are needed. These new insights may lead to adjustments of infection prevention guidelines and empiric antibiotic treatment policies to prevent spread and optimize clinical care for the individual patient.

Antibiotic-resistant Enterobacteriaceae in the Dutch community

Since the 1980s cephalosporins have been widely used to treat a wide range of infections including those caused by members of the Enterobacteriaceae. Through selective pressure the ESBL carrying pathogens, resistant to the third generation cephalosporins often prescribed in hospitals, emerged. At first ESBL-infections were limited to hospital acquired infections, but nowadays ESBL genes have accumulated in community pathogens as well, most notably in the species *E. coli* (24). Community-acquired urinary tract and bloodstream infections caused by ESBL-*E. coli* have emerged in the past decade (25-27). Advancing age, urban living, health care contacts and international travel have been among the first risk factors identified for community acquired ESBL-E infections in a Canadian setting (28).

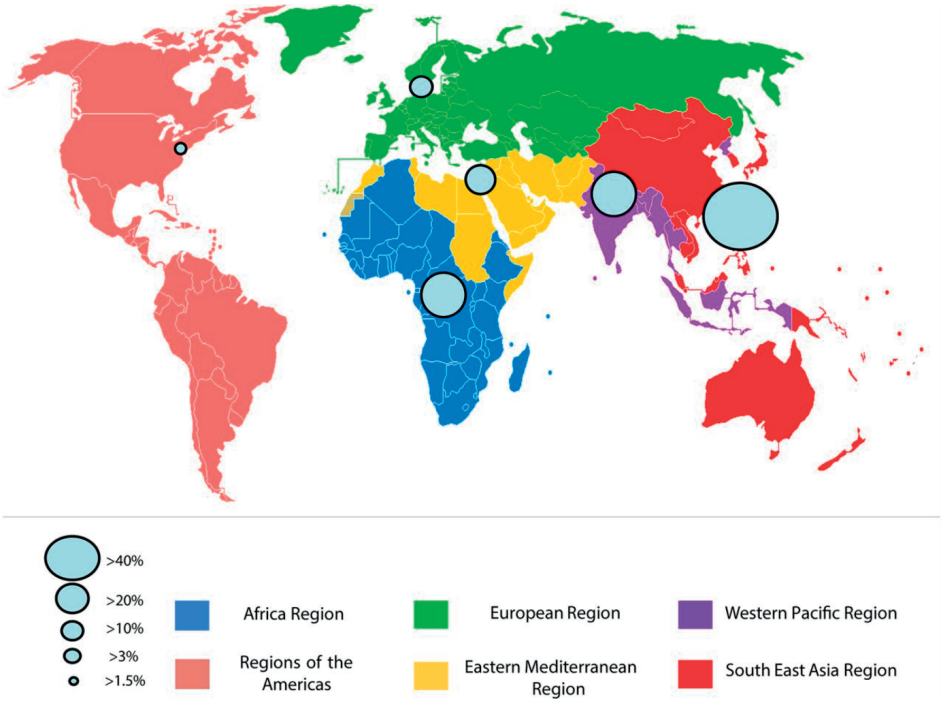

Figure 1. Pooled prevalence of fecal colonization of healthy individuals with extended-spectrum beta-lactamase (ESBL)–producing organisms per World Health Organization region. Circle size represents the ESBL colonization rates (19).

The increasing prevalence of ESBL carriage in the community at large, even in countries with restricted use of antibiotics like the Netherlands, is of great concern, as ESBL-E carriage has been associated with an increased risk of subsequent ESBL-E infections (29, 30). As most studies focus on risk-factors for ESBL-infection in hospitalized patients or outpatients and only few studies have investigated risk factors for ESBLs in healthy adults, predictive factors for ESBL-E carriage in the community are not well defined (19, 31-38). Identifying individuals at risk of ESBL-E acquisition and carriage enables to identify the origin for ESBL-E carriage in the community and enables to foresee public health risks and act accordingly.

AIM OF THE RESEARCH AND OUTLINE OF THIS THESIS

The main aim of the research described in this thesis was to determine the impact of intercontinental travel on the prevalence of antibiotic-resistant Enterobacteriaceae, especially ESBL-producing strains, in the gut of healthy citizens living in the Netherlands.

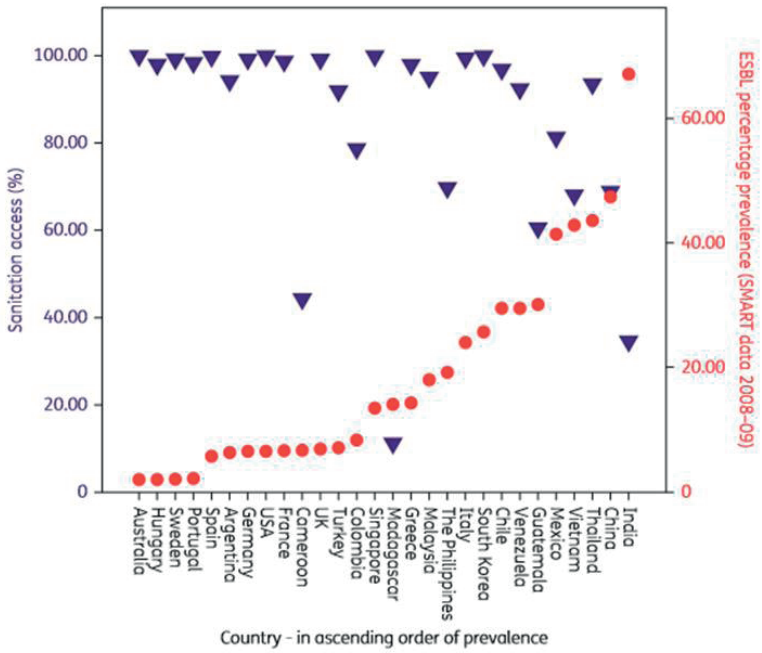

Figure 2. Sanitation access and ESBL prevalence (8).

Moreover, we aimed to determine the subsequent persistence of travel-acquired antibiotic-resistant Enterobacteriaceae after returning home from travel, and the likelihood of their spread from returning travellers to other members of the Dutch population. Potential risk factors for the acquisition during travel and for the persistence after travel of such strains were studied as well.

Travel and acquisition of antibiotic-resistant Enterobacteriaceae

Travel and acquisition of Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E)

In **chapter 2** we summarize findings from previous small to medium sized prospective cohort studies among returning travellers which investigated ESBL acquisition and associated risk factors. These previous studies have reported frequent acquisition of ESBL-E associated with various predictors and sporadic acquisition of CPE among international travellers. However, data on duration of ESBL-E colonisation after travel and assessment of associated predictors for sustained carriage and onward transmission within households were, partly due to the size of many of these studies, very limited.

Therefore, we performed a more definitive large scale study addressing these issues, a study that was named the COMBAT (Carriage of Multiresistant Bacteria After Travel)-

study. In **chapter 3,** we describe the methodology of this large-scale longitudinal cohort study of travellers, the aims of which were:

1. to determine the acquisition rate of ESBL- and carbapenemase-producing Enterobacteriaceae during foreign travel by comparing their prevalence in pre- and post-travel faecal samples
2. to ascertain the duration of carriage of these microorganisms (or their resistance genes/mobile genetic elements) by studying faecal specimens at regular intervals up to 1 year after the travellers had returned to the Netherlands.
3. to mathematically model the decolonization and transmission rates of these imported Enterobacteriaceae (or their resistance genes/mobile elements) within households by prospectively studying consecutive specimens from household members
4. to identify the risk factors for acquisition and persistence of carriage

In **chapter 4** we describe the main results of the COMBAT-study, focusing on ESBL-E acquisition by healthy international travellers and predicitive factors associated with acquisition of ESBL-E, the duration of travel-acquired ESBL-E colonization of travellers once back home and risk factors associated with persistent carriage, and the rate of onward transmission of travel-acquired ESBL-E to household members of these travellers.

Pre-travel carriage of ESBL-E by study participants

In **chapter 5** we investigated the prevalence of ESBL-E and the predictive factors for ESBL-E carriage in our cohort of travellers (and their household members) prior to their travel abroad. To gain more insight in the molecular epidemiology and their resistance phenotype prior to travel, we determined and compared the genotypes and the co-resistance profiles of ESBL-E isolated from pre- and post-travel faecal samples. The hypothesis was that carriage of ESBL-E strains before travel could, to a substantial degree, be attributed to prior international travel of the participants enrolled in the COMBAT study.

Travel and Carbapenemase-producing Enterobacteriaceae (CPE)

In **chapter 6** we focus on the acquisition, persistence and potential transmission of CPE in the same cohort of travellers. Although CPE prevalence in the gut microbiota of healthy community dwellers is much lower, the hypothesis was that, due to the large size of the COMBAT study, at least some participants in the COMBAT study would acquire a CPE during their intercontinental travel, especially if to regions of the world where such strains have emerged in the past, and import CPE strains into the Netherlands.

Travel and acquisition of Plasmid mediated colistin-resistant Enterobacteriaceae

In chapter 7 we describe the acquisition of the mcr-1 gene by a few participants in our study cohort. In order to understand the dynamics behind the worldwide spread of the mcr-1 gene, we subsequently determined the population structure of *E. coli* and the mobile genetic elements carrying the mcr-1 gene by reviewing and comparing whole-genome sequences and MLST profiles from our own travel-acquired mcr-1 carrying isolates and those available from publicly databases and the literature (**chapter 8**).

Travel and acquisition of diarrhoeagenic bacteria, enteral viruses and parasites

Limited prospective data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by healthy individuals during travel. We, therefore, exploited our cohort to study this issue and expand our knowledge in this respect. In **chapter 9** we describe the frequency of travel associated acquisition of eight viral pathogens, six bacterial enteric pathogens and five parasite species in a random selection of travellers by using sensitive PCR-based assays.

In **chapter 10** we present a Summarizing general discussion and present our future perspectives on the topic of emerging antimicrobial resistance in relation to international travel.

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CHAPTER 2

International travel and acquisition of multidrug-resistant *Enterobacteriaceae*: a systematic review

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Eurosurveillance. 2015; 20 (47)

ABSTRACT

International travel is considered to be an important risk factor for acquisition of multidrug-resistant *Enterobacteriaceae* (MRE). The aim of this systematic review was to determine the effect of international travel on the risk of post-travel faecal carriage of MRE. Secondary outcomes were risk factors for acquisition of MRE. A systematic search for relevant literature in seven international databases was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Articles needed to report on (i) foreign travel, (ii) screening in asymptomatic participants, (iii) antimicrobial susceptibility data and (iv) faecal *Enterobacteriaceae* carriage. Two researchers independently screened the abstracts, assessed the full article texts for eligibility and selected or rejected them for inclusion in the systematic review. In case of disagreement, a third researcher decided on inclusion. Eleven studies were identified. In all studies, a high prevalence (>20%) of carriage of MRE after international travel was found. The highest prevalence was observed in travellers returning from Southern Asia. Foreign travel was associated with an increased risk of carriage of MRE. Further research is needed to assess if this leads to an increase in the number of infections with MRE. Systematic review registration number: PROSPERO CRD42015024973.

INTRODUCTION

Rationale

Worldwide, the number of international travellers has grown from 25 million in 1950 to 1087 million in 2013 (1). According to the World Tourism Organization, this number is expected to increase by an average of 3.3% a year(1). Of the international travellers visiting the developing countries, 22-64% have self-reported health problems, and about 8% require medical care during or after travel (2, 3). Healthy travellers may be exposed to a broad range of microorganisms while travelling, including drug-resistant *Enterobacteriaceae,* which may subsequently be introduced into their home country (4, 5).

Enterobacteriaceae are Gram-negative bacteria that are part of the human body's normal commensal flora, called microbiota. *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella* species, are capable of causing both healthcare-associated, and community-acquired infections (6). Multidrug-resistant *Enterobacteriaceae* (MRE), including extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* (ESBL-E) and plasmid-mediated Amp-C producing *Enterobacteriaceae* (pAmp C-E) are emerging worldwide (7). Cases of carbapenemase-producing *Enterobacteriaceae* (CPE) are also reported more frequently (8).

Since 2003, community carriage rates of MRE have increased dramatically in various regions, such as South-east Asia, the Western Pacific and the Eastern Mediterranean (7). During visits to such areas, travellers might acquire MRE and become asymptomatic carriers of MRE. In their home country, they may cause spread in the community and contribute to worldwide emerging antimicrobial resistance (6,9,10). Acquired MRE in the digestive tract are considered apathogenic, however carriage of such *Enterobacteriaceae* have resulted in clinically relevant infections (8). International travel has been reported as a risk factor for urinary tract infections caused by ESBL-E (11,12). The question arises if these observations warrant clinicians being aware for MRE in recently returned otherwise healthy, international travellers who seek medical attention even for unrelated conditions.

Objectives

The aim of this systematic review was to determine the effect of international travel on the risk of acquisition of faecal carriage of MRE. A secondary objective was to determine risk factors for acquisition of drug resistance.

METHODS

Protocol and registration

A specific protocol was designed and used to conduct the study. The study is registered in the International prospective register of systematic reviews (PROSPERO) under registration number CRD42015024973.

Search strategy and selection criteria

The systematic review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (13). The following databases were searched, attempting to identify all relevant studies: Embase, MEDLINE, Web of Science, Scopus, Cochrane Library, PubMed publisher and Google Scholar. The latest search was conducted on 17 August 2015.

The topic search terms used for searching the databases were "Gram negative bacteria", "Gram negative bacterial infections", "Enterobacteriaceae", "Escherichia", "Klebsiella", "Campylobacter", "Salmonella", "Shigella", "Yersinia", "travel", "traveller", "tourist", "tourism", "turista", "aviation", "air transport", "airport", "colonisation", "carriage", "carrier", "susceptibility" and "(multiple) drug resistance".

The queries differed per database searched and were developed with help of a biomedical information specialist (Supplement 1). Articles written in English, German, French and Dutch were included.

For inclusion the article needed to fulfil the following criteria: (1) it needed to be related to foreign travel (2), report on screening in asymptomatic participants (3), present antimicrobial susceptibility data and (4) report on faecal *Enterobacteriaceae* carriage. We used the following exclusion criteria: case reports, reviews, meta-analysis, veterinary medicine, in vitro studies, and studies regarding symptomatic patients. The reference list of reviews were screened to identify studies possibly missed by the search.

Two researchers (R.H. and J.A.) independently performed the screening of the abstracts. Any discordant result was discussed in consensus meetings. After screening the abstracts, the full text of the articles was assessed for eligibility by the same two researchers and selected or rejected for inclusion in the systematic review. In case of disagreement a third researcher (A.V.) decided on inclusion.

Data collection process

The following data (if available) were extracted from each article: year of publication, country of the study, study period, study design, microorganism studied, study population, study size, age, sex, sample time before and after travel, duration of travel, travelling in pairs or groups, symptoms during travel, countries visited, MRE prevalence before travel, MRE prevalence after travel, MRE resistance acquired during travel, resistance to

other antibiotic drugs of acquired MRE, risk factors for acquisition (among which travel to predefined United Nations geographical region: southern Asia , Asia except southern Asia, Africa, South and Central America, North America, Europe and Oceania(14)), method of MRE susceptibility determination, phenotypic approaches, genotypic characterization of post-travel MRE isolates, molecular typing of post-travel MRE isolates, duration of MRE colonisation and MRE transmission to household contacts. To obtain missing data, authors of the article were contacted.

Quality assessment

We assessed the methodological quality and the risk of bias in individual studies that may affect the cumulative evidence, using tools for assessing quality and susceptibility to bias in observational studies as recommended in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (15, 16).

Data synthesis and analysis

As a result of the design of the studies (cohort studies) and the heterogeneity in patient populations (e.g. travellers, healthcare workers and healthcare students) a formal meta-analysis was not possible. Therefore, the study results were summarised to describe the main outcomes of interest. The principle summary measure was percentage of MRE acquisition during travel, defined as ESBL-E or pAmp C-E. Furthermore, risk factors for acquisition of drug resistance were assessed. If possible, percentages not presented in the articles were calculated from available data.

RESULTS

Study selection

A total of 2398 studies were identified through database searching after duplicates had been removed (Figure 1). After screening of titles and summaries, 36 articles were selected for full-text assessment. Eleven articles were included in qualitative synthesis of the systematic review (see Figure 1 for reasons for exclusion)(17-27).

Study characteristics

Eleven prospective cohort studies, conducted in northern and western Europe, Australia and the United States (US) were included (17-27). Characteristics of these studies are shown in Table 1. Nine studies investigated travellers visiting a travel or vaccination clinic, one study hospital staff and contacts, and one study healthcare students working or studying abroad. The number of study participants ranged from 28 to 574. The median age of travellers in the individual studies varied between 25 and 66 years, with

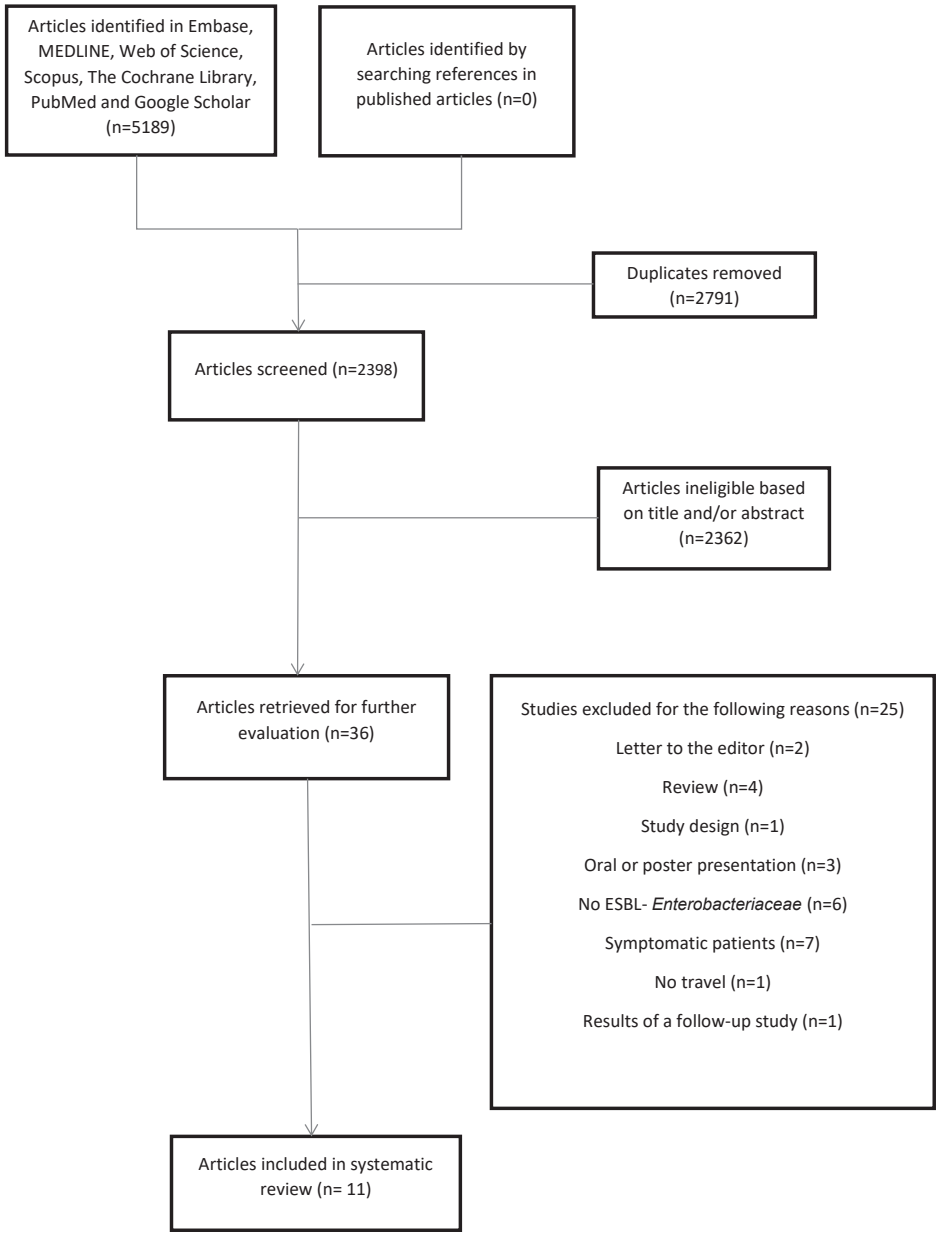

Figure 1. Flowchart for literature search on the acquisition of multidrug-resistant Enterobacteriaceae in international travel (n = 4,989)

the youngest group being healthcare students. In all studies, the majority of travellers were female (range: 55-78%). The proportion of participants who were lost to follow up varied from 3.8% (4/106) (18) to 30% (12/40)(21). The mean duration of travel was similar

in all studies (14 – 21 days). In the study of Angelin et al. on healthcare students, median length of stay was 45 days (range: 13-365 days)(22). In four studies, follow-up samples of MRE carriers were collected at six months after returning from travel, and in one of these studies, samples were collected monthly in the first three months with further follow-up until 12 months after return (25). Ten studies used a phenotypic method for susceptibility testing, with genotypic confirmation of ESBL positivity by PCR (17-22, 24-27). One study used a PCR-based approach(23). In one study, only isolated *E. coli* were included, whereas the other studies included all isolated *Enterobacteriaceae,* which mainly consisted of *E. coli* (17-27).

Acquisition of multidrug-resistant Enterobacteriaceae

Faecal carriage of MRE varied from 1 to 12 % before travel and acquisition of MRE from 21 % to 51 % (Table 2) (17-21, 23-27).

In the study of Kuenzli et al. on travellers to the Indian subcontinent only, a much higher MRE acquisition rate of 69% was demonstrated (26). The risk of acquisition of MRE varied with geographical region (Table 3) (17-21, 23-27). Travel to southern Asia posed the highest risk (range: 29 – 88%), followed by other Asian countries (18 – 67%) and Northern Africa (range: 31-57%). Acquisition of MRE after travelling to sub-Saharan Africa (range: 0 – 49%) or South and Central America (range: 0 – 33%) was less frequent, and three studies did not observe any acquisition MRE after travel to South or Central America (Table 3). Acquisition of MRE after travel to North America, Europe and Oceania was rare. Results of the genotypic characterisation of MRE isolated after travel are presented in Table 2, the majority of the genes belonged to the CTX-M type.

Risk factor for acquisition of multidrug-resistant Enterobacteriaceae

Besides travel destinations, other risk factors for acquiring MRE were age, use of antibiotics during travel (beta-lactam use) and gastroenteritis or other gastro-intestinal symptoms (Table 2). The study of Kantele et al., designed to study these risk factors as primary outcome, showed that travel diarrhoea (adjusted odds ratio (AOR) 31.0; 95% confidence interval (CI): 2.7-358.1)) and antibiotic therapy for travel diarrhoea (AOR = 3.0; 95% CI: 1.4-6.7) proved to be the most important risk factors for acquiring MRE (20). In the study of Kuenzli et al. in which only travellers to southern Asia were included, risk factors for MRE acquisition were length of stay, visit to family or friend and consumption of ice cream or pastry (Table 2)(26). Angelin et al. found a significant association for travel to the South-East Asia region (OR = 30; 95% CI: 6.3 - 147.2), and antibiotic treatment during travel (OR = 5; 95% CI: 1.1 - 26.2), but found no association with travellers' diarrhoea or patient-related healthcare work (22).

Table 1. Characteristics of prospective cohort studies included for systematic review of the acquisition of multidrug-resistant Enterobacteriaceae in international travel (n = 11)

Study	*Country*	*Study period*	*Population Characteristics*	*Study size*[a]	*Median Age in years (range or SD)*	*Proportion of woman in %*	*Identification of MRE-positive organisms in post-travel isolates*	*Sample method used*	*Sample time (range) before/after travel*	*Mean duration of travel in days (range)*	*Total number of co-travellers participating in study*	*Follow-up of resistant isolates*
Tängdén(39)	Sweden	November 2007 - 31 January 2009	Travel clinic	100	43 (2-84)	55	*Enterobacteriaceae* 100 % (24/24) *E.coli*	Stool	Unknown	14 (1-26)	23	6 months
Kennedy(40)	Australia	January 2008 – April 2009	Hospital staff and contacts	102	45 (17-77)	62	*E.coli*	Rectal or perianal swab	Within 2 weeks before and after	21 (9-135)	Unknown	6 months
Östholm-Balkhed(41)	Sweden	September 2008 - April 2009	Vaccination clinic	231	54 (18-76)	59	*Enterobacteriaceae* 90% (104/116) *E.coli*[b]	Stool sample	15 (1-114) days / 3 (0-191) days	16 (4-119)	Unknown	None
Kantele(42)	Finland	March 2009-February 2010	Travel clinic	430	40 (0-77)	61	*Enterobacteriaceae* 97% (94/97) *E. coli*[b]	Stool sample	Before and first (or second) stool after	19 (4-133)	83	None
Weisenberg(43)	United States	July 2009 - February 2010	Travel clinic	28	66 (41-83)	68	*Enterobacteriaceae* 100% (7/7) *E. coli*[b]	Stool sample	1 week before/ 1 week after	16 (8-24)	Unknown	None
Angelin(44)	Sweden	April 2010 - January 2014	Healthcare students	99	25 (20-15)	78	*Enterobacteriaceae* 100% (36/36)) *E. coli*[c]	Stool sample	Close to departure/ 1 to 2 weeks after returning	45 (13-365)[d]	Unknown	None
von Wintersdorff(45)	The Netherlands	November 2010-August 2012	Travel clinic	122	43 (18-72)	58	Not done	Stool sample	Before and immediately after	21 (5-240)	Unknown	None

Table 1. Characteristics of prospective cohort studies included for systematic review of the acquisition of multidrug-resistant Enterobacteriaceae in international travel (n = 11) (continued)

Study	*Country*	*Study period*	*Population Characteristics*	*Study size*[a]	*Median Age in years (range or SD)*	*Proportion of woman in %*	*Identification of MRE-positive organisms in post-travel isolates*	*Sample method used*	*Sample time (range) before/after travel*	*Mean duration of travel in days (range)*	*Total number of co-travellers participating in study*	*Follow-up of resistant isolates*
Paltansing(46)	The Netherlands	March 2011 - September 2011	Travel clinic	370	33 (19-82)	63	*Enterobacteriaceae* 92% (146/158) *E. coli*[c]	Rectal swab	Immediately before and after	21 (6-90)	None	6 months
Ruppé(47)	France	February 2012 - April 2013	Vaccination Centres	574	36 (SD 13)	61	*Enterobacteriaceae* 93% (491/526) *E. coli*[b]	Stool sample	Within 1 week before and after	20 (15-30)	None	12 months
Kuenzli(48)	Switzerland	December 2012-October 2013	Travel clinic	170	41 (30-53)	56	*Enterobacteriaceae* 98% (157/161) *E. coli*[b]	Rectal swab	1 week before/ directly after	18 (5-35)	Unknown	None
Lübbert(49)	Germany	May 2013 – April 2014	Travel clinic	205	34 (3-76)	57	*Enterobacteriaceae* 92% (58/63) *E. coli*[b]	Stool sample	Before/ within 1 week after	21 (3-218)	22	6 months

E.coli: Escherichia coli; MRE: multidrug-resistant *Enterobacteriaceae*; SD: standard deviation.

[a] Number of travellers who provided pre- and post-travel swab.

[b] Data of MRE-positive isolates newly acquired during travel.

[c] Data of MRE-positive isolates post-travel.

[d] Healthcare students, median duration of stay.

Table 2. Risk of multi-drug-resistant Enterobacteriaceae in travellers (n = 11 studies)

Study	*Method of MRE determination*	*Phenotypic approaches*	*Results genotypic characterisation post-travel MRE isolates*	*Results molecular typing of post-travel MRE isolates*	*MRE prevalence pre-travel % (ratio)*	*MRE prevalence post-travel % (ratio)*	*New MRE acquisition during travel % (ratio)[a]*	*Persistent newly acquired MRE carriage 6 months after travel % (ratio)*	*Results univariate /multivariable risk factor analysis for MRE acquisition*	*MRE in non-travelling household contacts % (ratio)*
Tängdén (39)	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: Etest, MRE confirmation: disc diffusion	TEM (n=11) , SHV (n=3), CTX-M group 1 (n=14) of which CTX-M-15 (n=13), CTX-M-1 (n=1), CTX-M group 4 (n=10) of which CTX-M-9 (n=3), CTX-M-14 (n=5), CTX-M-27 (n=2)[b]	No data	1 (1/105)	No data -[ll]	24 (24/100)	24 (5/21)	Gastroenteritis travel to India[c]	No data
Kennedy (40)	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: Vitek2, MRE confirmation: disc diffusion	TEM or SHV (n=4), CTX-M group 1 (n=12), CTX-M group 9 (n=6), and pAmp C genes (n=4)[d]	No data	2 (2/106)	22 (22/102)	21 (21/100)	6 (1/18)	Gastroenteritis Use of antibiotics travelling to Asia, South America and/or Middle East/Africa[e,c]	No data
Östholm-Balkhed (41)	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Etest, MRE confirmation: Etest	TEM-19 (n=1), SHV (n=6), CTX-M-15-like (n=36), CTX-M-14-like (n=36), CTX-M-27-like (n=5), CTX-M-53-like (n=5), CTX-M-1/61 like (n=3), CTX-M-2 like (n=2), CTX-M-3-like (n=1), pAmpC genes (n=15), no genes detected (n=13)[b]	No data	2 (6/251)	31 (72/231)	30 (68/226)	No data	Age Diarrhoea or other gastrointestinal symptoms, travel to Asia, Africa (north of equator), Indian subcontinent[f]	No data

Table 2. Risk of multi-drug-resistant Enterobacteriaceae in travellers (n = 11 studies) (continued)

Study	*Method of MRE determination*	*Phenotypic approaches*	*Results genotypic characterisation post-travel MRE isolates*	*Results molecular typing of post-travel MRE isolates*	*MRE prevalence pre-travel % (ratio)*	*MRE prevalence post-travel % (ratio)*	*New MRE acquisition during travel % (ratio)[a]*	*Persistent newly acquired MRE carriage 6 months after travel % (ratio)*	*Results univariate /multivariable risk factor analysis for MRE acquisition*	*MRE in non-travelling household contacts % (ratio)*
Kantele (42)	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Vitek2, MRE confirmation: disc diffusion	79% CTX-M-type (CTX-M-1 and CTX-M-9 most prevalent), other common strains TEM and OXA (data not published)[b]	No data	1 (5/430)	22 (93/430)	21 (90/430)	No data	Traveller's diarrhoea, age, use of antibiotics for traveller's diarrhoea[f]	No data
Weisenberg (43)	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Vitek2, MRE confirmation: disc diffusion	SHV-12 (n=1), CTX-M-14 (n=3), CTX-M-15 (n=2), no gene detected (n=1)[b]	MLST typing 7 multidrug-resistant *E. coli* isolates: ST 39, 8 (n=2), 37, 399, 437, 83	4 (1/28)	25 (7/28)	26 (7/27)	No data	No data	No data
Angelin (44)	Phenotypic approach for detection of ESBL, pAmp C and phenotypic approach with genotypic characterization for detection of OXA-48/ OXA-181	Selective media, AST: disc diffusion, MRE confirmation: Etest (ESBL), disc diffusion (pAmpC)	No data	No data	7 (7/99)	36 (36/99)	35 (35/99)	No data	Travel to the South-East Asia region (India, Nepal, Vietnam , Indonesia, Sri Lanka), antibiotic treatment during travel[g]	No data
von Wintersdorff (45)	Metagenomic approach (detection bla_{CTX-M})	No data	bla_{CTX-M} (n=41)[d]	No data	9 (11/122)	34 (41/122)	32 (36/111)	No data	Travel to Indian subcontinent[f]	No data

Table 2. Risk of multi-drug-resistant Enterobacteriaceae in travellers (n = 11 studies) (continued)

Study	*Method of MRE determination*	*Phenotypic approaches*	*Results genotypic characterisation post-travel MRE isolates*	*Results molecular typing of post-travel MRE isolates*	*MRE prevalence pre-travel % (ratio)*	*MRE prevalence post-travel % (ratio)*	*New MRE acquisition during travel % (ratio)[a]*	*Persistent newly acquired MRE carriage 6 months after travel % (ratio)*	*Results univariate /multivariable risk factor analysis for MRE acquisition*	*MRE in non-travelling household contacts % (ratio)*
Paltansing (46)	Phenotypic approach with genotypic characterisation by microarray	Enrichment broth, selective media, AST: Vitek2, MRE confirmation: disc diffusion	SHV (n=1), CTX-M group 1 (n=110) of which CTX-M-1-like (n=4), CTX-M-3-like (n=1), CTX-M-15-like (n=85), CTX-M-32-like (n=20), CTX-M-group 9 (n=42), CTX-M-group 2 (n=2), CTX-M-group 8/25 (n=1), pAmpC genes (n=3)[d]	MLST typing: 146 multidrug-resistant *E. coli* isolates: most prevalent ST 38 (n=17), ST10 (n=10), ST131 (n=9)	9 (32/370)	36 (133/370)	33 (113/338)	17 (19/113)	Travel to South or East Asia[f]	18 (2/11)
Ruppé(47)[h]	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: disc diffusion	Predominant CTX-M-type (95.4%) among which CTX-M-group 1 predominated (83.7% of all CTX-M), OXA-181 (N=2), NDM-1 (n=1)[b]	No data	12 (81/700)	No data	51 (292/574)	After 1 month 34 (83/245), after 2 months 19 (45/236), after 3 months 10 (24/233), after 6 months 5 (11/230), after 12 months 2 (5/227)	Travel to Asia or sub-Saharan Africa, beta-lactam use during travel, diarrhoea during travel, type of travel[f]	No data
Kuenzli(48)	Phenotypic approach with genotypic screening by microarray and confirmation by PCR/DNA sequence analysis	Enrichment broth, selective media, AST: Vitek2, MIC for meropenem and ertapenem: Etest, MRE confirmation: disc diffusion, modified Hodge test	TEM-1-like (n=33), SHV238S/240K (n=7), SHV238S (n=1), SHV-5/12-like (n=1), SHV-2/3-like (n=1), CTX-M-15-like (n=48), CTX-M group 9 (n=1), CTX-M group 1 (n=24), predominant ESBL gene was CTX-M-15 (80 representative *E. coli* isolates analysed), NDM-1 (n=1)[b]	80 representative *E. coli* isolates analysed by rep-PCR: not clonally related. MLST performed on 34 randomly selected *E. coli* isolates: only 3 pandemic strains found (ST131 n=2; ST648 n=1)	3 (5/175)	No data	70 (118/170)	No data	Travel to India, Bhutan, or Nepal, visiting friends and relatives, consumption of ice cream and pastry, Length of stay[f]	No data

Table 2. Risk of multi-drug-resistant Enterobacteriaceae in travellers (n = 11 studies) (continued)

Study	*Method of MRE determination*	*Phenotypic approaches*	*Results genotypic characterisation post-travel MRE isolates*	*Results molecular typing of post-travel MRE isolates*	*MRE prevalence pre-travel % (ratio)*	*MRE prevalence post-travel % (ratio)*	*New MRE acquisition during travel % (ratio)[a]*	*Persistent newly acquired MRE carriage 6 months after travel % (ratio)*	*Results univariate /multivariable risk factor analysis for MRE acquisition*	*MRE in non-travelling household contacts % (ratio)*
Lübbert(49)	Phenotypic approach with genotypic confirmation by PCR	Selective media,AST: microbroth dilution method, MRE confirmation:E-test	SHV-12 (n=1), CTX-M group 1 (n=37), of which CTX-M-15 (N=33), CTX-M-55 (n=4), CTX-M group 9 (n=19) of which CTX-M-14 (n=9), CTX-M-27 (n=1), CTX-M-65 (n=1)[b]		7 (14/205)	31 (63/205)	30 (58/191)	9 (3/35)	Travel to India or South-East Asia, Gastroenteritis[c]	No data

AST: antibiotic susceptibility testing; bla: beta-lactamase; CTX-M: cefotaximase; *E. coli: Escherichia coli*; ESBL: extended-spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; MLST: Multilocus sequence typing; MRE multidrug-resistant *Enterobacteriaceae*; NDM: New Delhi metallo-beta-lactamase; OXA: oxacillinase; pAmp C: plasmid-borne AMpC; PCR: polymerase chain reaction; PFGE: pulsed-field gel electrophoresis; rep-PCR: repetitive extragenic palindromic PCR; SHV: Sulphydryl variable; TEM: Temoniera.

[a] Percentage of MRE- positive post-travel samples in those travellers whose pre-travel sample was MRE-negative.

[b] Acquired genes detected in post-travel MRE isolates.

[c] Univariate statistics.

[d] Prevalent genes detected in post-travel MRE isolates.

[e] Risk factors for resistance to gentamicin, ciprofloxacin and/or third generation cephalosporins.

[f] Multivariable logistic regression analysis; participants ESBL-positive before travel were excluded.

[g] Binary regression analysis.

[h] Carbapenemase-positive isolates were included in the definition MRE.

Table 3. Proportion of travellers who acquired multidrug-resistant Enterobacteriaceae, by travel destination (n = 11 studies)

Study	*Southern Asia % (ratio)*	*Asia except southern Asia % (ratio)*	*Northern Africa % (ratio)*	*Sub-Saharan Africa % (ratio)*	*South and Central America % (ratio)*	*North America % (ratio)*	*Europe % (ratio)*	*Oceania % (ratio)*
Tängdén(39)[a,b]	78 (7/9)	29 (10/34)	33 (4/12)	4 (1/23)	0 (0/7)	0 (0/2)	13 (2/16)	No data
Kennedy(40)[a,c]	57 (8/14)	25 (21/85)	33 (1/3)	0 (0/2)	20 (1/5)	20 (2/10)	14 (3/21)	0 (0/2)
Östholm-Balkhed(41)[a,b]	71 (10/14)	43 (26/60)	57 (17/30)	21 (15/71)	16 (5/31)	0 (0/15)	0 (0/15)	No data
Kantele(42)[b,d]	46 (28/61)	32 (37/116)	67 (2/3)	12 (23/193)	0 (0/40)	0 (0/2)	0 (0/15)	No data
Weisenberg(43)[b]	28.6 (2/7)	25.0 (1/4)	33.3 (1/3)	12.5 (1/8)	33.3 (2/6)	No data	No data	No data
Angelin(44)	62.5 (25/40)	66.7 (6/9)	-	10 (4/40)	0 (0/5)	0 (0/4)	No data	No data
von Wintersdorff(45)[c]	58.1 (18/31)	20 (6/29)	31.3 (5/16)	29.4 (5/17)	0 (0/10)	No data	16.7 (1/6)	No data
Paltansing(46)[b,e]	72.0 (18/25)	41.1 (62/161)	40 (4/10)	24.4 (20/82)	15.0 (9/60)	No data	No data	No data
Ruppé(47)[f]	88.3 (53/60)	65.6 (61/93)	No data	48.9 (89/182)	31.0 (48/155)	No data	No data	0 (0/2)
Kuenzli(48)[b]	69.4 (118/170)	No data	No data	No data	No data	No data	No data	No data
Lübbert(49)[a,b]	72.2 (13/18)[g]	32.9 (24/73)[g]	No data	24.4 (19/78)	7.7 (6/78)	0 (0/2)	20.0 (2/10)	No data

MRE: multidrug-resistant *Enterobacteriaceae*.

[a] Travellers visiting more than one region are categorised in all the visited geographical regions.

[b] Study reports data on MRE acquisition in travellers.

[c] Study reports data on MRE prevalence in travellers.

[d] Travellers visiting more than one region are categorised in the geographical region with the longest stay for this study.

[e] One traveller who visited Iran is categorised in Asia instead of Southern Asia.

[f] 42 travellers visited more than one country in Asia and may be represented in more than one column in the Table; 28 of them acquired MRE.

[g] Exact numbers unpublished.

Southern Asia: Afghanistan, Bangladesh, Bhutan, India, Islamic Republic of Iran, Maldives, Nepal, Pakistan, Sri Lanka.
Asia (without southern Asia) Armenia, Azerbaijan, Bahrain, Brunei, Cambodia, China, Cyprus, Darussalam, Democratic People's Republic of Korea, Georgia, Hong Kong, Indonesia, Iraq, Israel, Jordan, Japan, Kazakhstan, Kuwait, Kyrgyzstan, Laos, Lebanon, Mongolia, Malaysia, Myanmar, North Korea, Oman, Philippines, Qatar, Republic of Korea, Saudi Arabia, South Korea, Singapore, Palestine, Syrian, Tajikistan, Thailand, Timor-Leste, Turkey, Turkmenistan, United Arab Emirates, Uzbekistan, Viet Nam, Yemen.
Northern Africa: Algeria, Egypt, Libya, Morocco, Sudan, Tunisia, Western Sahara.

Sub-Saharan Africa: Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo (Brazzaville), Côte d'Ivoire, Democratic Republic of the Congo, Djibouti, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Ghana, Guinea, Guinea-Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Namibia, Niger, Nigeria, Réunion, Rwanda, Sao Tomé and Principe, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Sudan, Swaziland, Tanzania, The Gambia, Togo, Uganda, Western Sahara, Zambia, Zimbabwe.
South and Central America: Anguilla, Antigua and Barbuda, Argentina, Aruba, Bahamas, Barbados, Belize, Bolivia, Bonaire, Sint Eustatius and Saba, Brazil, British Virgin Islands, Cayman Islands, Chile, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Ecuador, El Salvador, Falkland Islands, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat, Nicaragua, Panama, Paraguay, Peru, Puerto, Rico, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Saint-Barthélemy, Sint Maarten, Suriname, Trinidad and Tobago, Turks and Caicos Islands, US Virgin Islands, Uruguay, Venezuela
North America: Bermuda, Canada, Greenland, Saint Pierre and Miquelon, United States.
Europe: Åland Islands, Albania, Andorra, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Channel Islands, Croatia, Czech Republic, Denmark, Estonia, Faeroe Islands, Finland, the former Yugoslav Republic of Macedonia, France, Germany, Gibraltar, Greece, the Holy See, Hungary, Iceland, Ireland, Isle of Man, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Moldova, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Svalbard and Jan Mayen, Sweden, Switzerland, Ukraine, United Kingdom.
Oceania: American Samoa, Australia, Cook Islands, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia, Nauru, New Caledonia, New Zealand, Niue, Norfolk Island, Northern Mariana Islands, Palau, Papua New Guinea, Pitcairn Islands, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, Wallis and Futuna.

Resistance of multidrug-resistant Enterobacteriaceae to other antibiotic drugs

Resistance of post-travel MRE isolates to various antibiotics was determined in nine studies (Table 4) (17-19, 21-24, 26, 27). In the study of Wintersdorff et al., a PCR-based approach was used, therefore it was not possible to determine which microorganism carried the resistance genes (23). The resistance data to other antibiotic drugs in the study by Kennedy et al. were not part of the publication, but were provided on request (18). Antimicrobial resistance was high for ciprofloxacin, varying from 31% to 57%, and for cotrimoxazole, varying from 49% to 86% (17-19, 21-24, 26, 27). Aminoglycoside resistance was high for gentamicin (range: 17-50%) and tobramycin (range: 18-59%) and low for amikacin (range: 2-5%) (17-19, 21-24, 26, 27). Carbapenemase-producing *Enterobacteriaceae* were observed in four travellers who had all visited India (in the study by Ruppé et al., two OXA-181 and one New Delhi metallo-beta-lactamase 1 (NDM-1), and in the study of Kuenzli et al., one NDM-1, but this strain was not included in the resistance results) (25, 26). Resistance to nitrofurantoin, colistin and fosfomycin was only analysed in some of the studies (Table 4) (18, 19, 21-23, 26).

Duration of multidrug-resistant Enterobacteriaceae carriage after return, risk factors for a long duration and rate of infection after travel

Five studies analysed MRE carriage six months after travel, and the persistence rate of acquired MRE after six months was 6-24% of travellers (Table 2)(17, 18, 24, 25, 27). Ruppé et al. analysed MRE carriage one, two, three, six and twelve months after travel, showing persistence of carriage of an acquired MRE in 34, 19, 10, 5 and 2%, respectively (25). Travellers to Asia showed longer carriage of MRE compared with other travel destinations. Carriage of multidrug-resistant *E. coli* had a lower risk for prolonged carriage than other multidrug-resistant species. No other risk factors were found for prolonged carriage of MRE. Eight travellers in this study reported an episode of urinary tract infection after their return, but no microbiological data were available (25). In the study of Tängdén et al., five of 21 travellers remained carriers of MRE after six months. However, none of these participants reported clinical infections (17). In the study of Kennedy et al., one person developed a urinary tract infection with a travel-related organism (18). Kantele et al. performed a one-year laboratory-based follow-up and did not find any clinical samples with MRE (20).

Rate of transmission to household members

Only one study screened household contacts for MRE after return of the index traveller. Household contacts were defined as persons who shared the same household with a participant on a regular basis. Two of 11 contacts were found MRE-positive (24). Both carried a different ESBL-producing *E. coli* based on multilocus sequence typing (MLST) than the associated traveller.

Table 4. Antibiotic drug resistance of newly acquired multidrug-resistant Enterobacteriaceae in travellers (n = 11 studies)

Study	*Ciprofloxacin % (ratio)*	*Cotrimoxazole % (ratio)*	*Gentamicin % (ratio)*	*Amikacin % (ratio)*	*Tobramycin % (ratio)*	*Carbapenem % (ratio)*	*Nitrofurantoin % (ratio)*	*Colistin % (ratio)*	*Fosfomycin % (ratio)*
Tängdén(39)[a]	50[b]	79 (19/24)	45[b]	No data	38[b]	0[b]	0[b]	No data	8[b]
Kennedy(40)*	55 (12/22)	No data	50 (11/22)	No data	59 (13/22)	No data	No data	No data	No data
Östholm-Balkhed(41)[†]	31 (36/116)	70 (81/116)	41 (48/116)	2 (2/116)	46 (53/116)	0 (0/116)	7 (8/116)	No data	3 (3/116)
Kantele(42)	No data	No data	No data	No data	No data	No data	No data	No data	No data
Weisenberg(43)[a]	43 (3/7)[d]	86 (6/7)	43 (3/7)	No data	No data	0 (0/7)	No data	No data	No data
Angelin(44)	57 (28/49)	75[b]	30[b]	No data	No data	0 (0/49)	2[b]	No data	No data
von Wintersdorff(45)[e]	36.9 (45/122) *qnrB* 55.7 (68/122) *qnrS*	No data	71 (86/122) *aac(6′)-aph(2″)*	71 (86/122) *aac(6′)-aph(2″)*	71 (86/122) *aac(6′)-aph(2″)*	0 (0/122) bla_{NDM}	No data	No data	No data
Paltansing(46)[f]	36	67	35	No data	37	0	29	0	No data
Ruppé(47)	No data	No data	No data	No data	No data	0.6 (3/526)[g]	No data	No data	No data
Kuenzli(48)[a]	41 (64/157)	49 (77/157)	No data	5 (7/157)	18 (28/157)	0 (0/157)	2 (3/157)	0 (0/157)	0.6 (1/157)
Lübbert(49)[a]	43 (25/58)	83 (48/58)	17 (10/58)	2[b]	22[b]	0[b]	No data	0[b]	16[b]

bla: beta-lactamase; CPE: carbapenemase-producing *Enterobacteriaceae*; ESBL: extended-spectrum beta-lactamase.

[a] Resistance among acquired ESBL-positive isolates detected in pot-travel samples

[b] Data extracted from bar chart, exact numbers of data unpublished.

[c] Resistance among prevalent ESBL-positive isolates detected in post-travel samples.

[d] Percentage of susceptibility to levofloxacin.

[e] Prevalent resistance genes in faecal samples post-travel.

[f] Resistance among prevalent ESBL-positive isolates detected in pre- and post-travel samples.

[g] Three acquired CPE detected in post-travel samples.

Limitations of the studies

The quality of the studies and the susceptibility of bias between the studies were assessed. In all but one study, participants constituted a non-random sample of the general travelling population (17-21, 23-27). However, Angelin et al. studied healthcare students working or studying abroad(22). Studies were performed on three different continents. Travel destinations and travel behaviour may differ considerably between different nationalities and age groups. Including co-travellers, as done in all studies except Paltansing et al. and Ruppé et al., can result in similar travel behaviour and therefore, similar risk factors. Overall, the main outcome was not influenced by recall or interviewer bias. For other outcomes such as risk factors, the risk of recall bias or interviewer bias was low because of the use of self-administered questionnaires.

Every study had participants lost to follow-up for post-travel stool samples and follow-up stool samples. Asymptomatic faecal carriage of MRE is probably not related to loss to follow up, therefore, the risk of information bias is small. Ruppé et al. calculated post-travel MRE carriage as those travellers with persisting MRE carriage divided by all travellers with MRE acquisition plus all travellers without MRE post-travel (25). However, travellers without MRE were not included in the follow-up. As a result, local MRE acquisition was not included in the calculated post-travel MRE carriage prevalence. Therefore the true prevalence can be assumed to be higher.

In five studies, travellers visited multiple regions or even continents during their trip (17-20, 27). In these travellers, it was not possible to attribute MRE prevalence or MRE acquisition to a certain geographical region. However, travellers in these studies were included in the MRE prevalence or MRE acquisition rates of more than one geographical regions, which may have introduced information bias.

Seven studies used stool samples for detection of MRE (17, 19-21, 23, 25, 27) and three studies used rectal or perianal swabs for detection of MRE (18, 24, 26). This might have influenced detection of MRE carriage.

DISCUSSION

In this systematic review we found a high prevalence of faecal carriage of MRE after international travel. The highest prevalence of MRE was observed in isolates from travellers returning from southern Asia, with up to 88% acquisition of MRE. In addition to the antibiotics not effective against MRE, an alarmingly high prevalence of resistance to other commonly used antibiotics such as cotrimoxazole (49-86%), ciprofloxacin (31-57%) and aminoglycosides (gentamicin 17-71%) was observed in ESBL-positive isolates in travellers in all studies (17-27).

Returning international travellers with MRE may introduce these microorganisms in their home countries. This may cause community-onset infections with MRE in patients without obvious risk factors transmitted by healthy carriers through food or person-to-person contact (9). Infections caused by MRE are associated with poorer outcome and a higher overall mortality rate than infections caused by susceptible bacteria (28). In this review, all studies showed an increased prevalence of faecal carriage of ESBL after international travel. It is not possible to evaluate the proportion of travellers who will develop infection with these resistant bacteria. However, studies have demonstrated that international travel is a risk factor associated with developing an infection with an MRE (11, 12, 29).

Many countries have infection prevention and control guidelines to detect and treat multidrug-resistant organisms (MDROs) including MRE (30). In countries with low prevalence of MRE, infection prevention and control guidelines mainly include strategies for early identification and isolation strategies for patients recently hospitalised in foreign hospitals (30, 31). Patients with a recent history of travel to MRE-endemic areas but not admitted to healthcare facilities abroad are not normally considered at risk for carriage of MDROs. However, in hospitalised patients with a recent history of travel, increased rates of carriage of MRE have been observed (10, 29, 30). Physicians should be aware of the risk that patients with recent travel to areas with high faecal carriage of MRE, as presented in this review, may introduce MRE to the hospital. Routine screening for MRE seems indicated in such patients. Furthermore, empiric antibiotic therapy may fail when an infection by MRE is not taken into account. Therefore, careful recording of travel history needs to be incorporated in each patient evaluation. As shown in this review, there is also an increased risk of resistance against other antibiotics in travellers with MRE carriage. It is likely that this is caused by multiple genes, each encoding for resistance to different classes of antibiotics, which are often found on the same bacterial mobile genetic element (e.g. a plasmid) (32). As a result, other antibiotics, such as aminoglycosides, will also fail in many MRE-positive patients.

Of the MDROs, emergence of CPE is most worrisome because of the limited treatment options for these infections. NDM-1-producing *Enterobacteriaceae* have been found in environmental samples in endemic regions (33). CPE (NDM-1) in patients from the United Kingdom with a recent history of travelling or medical tourism to India are already an important public health problem (8). Case reports have also demonstrated acquisition of CPE in travellers without contact with medical healthcare facilities (34, 35). In this review, four travellers from India were carrying a carbapenemase-producing *E. coli* (25, 26). Preliminary results of the Carriage Of Multiresistant Bacteria After Travel (COMBAT) study, a large-scale multicentre longitudinal cohort study conducted in the Netherlands among 2001 travellers, show acquisition of CPE in four travellers (36).

There are, besides the destination of travel, additional risk factors for acquisition of MRE during travel. Antibiotic therapy was found to increase the risk (20, 22). In five studies, traveller's diarrhoea or gastroenteritis were associated with an increased risk of MRE acquisition during travel (17-20, 25). Also, in one study, meticulous hand hygiene or strict consumption of bottled water did not lower the risk of acquiring MRE (22). Therefore, it is not clear whether hygiene-related travel advice will decrease faecal carriage of MRE. Surprisingly, healthcare-related activities did not pose an increased risk of acquiring MRE in one study (22).

MRE and CPE could also be carried by food. International spread of these bacteria by food supply has been reported (37). In this review, only one study showed that food consumption (ice cream and pastry) was associated with MRE carriage in travellers to southern Asia, whereas most of the studies did not focus on dietary patterns during travel.

One of the limitations of this review is the recruitment of travellers from travel clinics only, resulting in inclusion of very few travellers with European destinations. European countries such as Greece and Cyprus also are endemic for MRE and popular travel destinations (34). In addition, travellers visiting their country of origin, especially Morocco and Turkey usually do not ask for a pre-travel consultation, although these countries are endemic for MRE and CPE (34). It is not clear whether not including these patients may have led to an under- or overestimation of MRE acquisition.

Another limitation is the lack of sufficient data regarding the duration of carriage and the transmission among non-travelling household members. The study by Ruppé et al. suggests that three months after return, MRE carriage is comparable with the baseline prevalence before travelling. However, the study did not include baseline prevalence in the follow-up. The COMBAT study will address some of these questions (36).

CONCLUSION

International travel is a major risk factor for acquisition of MRE. This risk is particularly high after travelling to (southern) Asia and in persons with travel-related diarrhoea and antibiotic use. Carriage of MRE-positive isolates is also associated with a high risk of resistance to ciprofloxacin, cotrimoxazole and aminoglycosides. Further research is needed to assess duration of carriage, spread to household contacts and whether introduction of MRE results in an increase of MRE infections. Our results, combined with the worldwide emergence of CPE, further stress the importance of infection prevention and control guidelines.

ACKNOWLEDGEMENTS

We thank Wichor M. Bramer of Erasmus MC Medical Library for performing the literature search. We thank Karina Kennedy, Scott Weisenberg, Anita Hällgren, Christian von Wintersdorff, Anu Kantele, Esther Kuenzli, Thomas Tängdén and Christoph Lübbert for providing additional data concerning their studies.

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Embase.com ('Gram negative bacterium'/exp OR 'Gram negative infection'/de OR Enterobacteriaceae/de OR Escherichia/exp OR Klebsiella/exp OR Salmonella/exp OR Shigella/exp OR Yersinia/exp OR 'Enterobacteriaceae infection'/exp OR ('Gram negative' OR Enterobacteri* OR (Enter* NEXT/1 bacteria*) OR Enterobacter* OR Escherichia* OR 'e coli' OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*):ab,ti) AND (travel/de OR 'traveller diarrhea'/de OR aviation/exp OR (travel* OR touris* OR turista OR aviation OR 'air transport' OR airport*):ab,ti) AND ('antibiotic resistance'/exp OR 'multidrug resistance'/de OR 'drug resistance'/de OR 'antibiotic sensitivity'/de OR 'bacterial colonization'/exp OR 'bacterium carrier'/de OR (resistan* OR coloni* OR ((antibiotic* OR antimicrob*) NEAR/3 sensitivit*) OR susceptib* OR carriage* OR carrier*):ab,ti) NOT ([animals]/lim NOT [humans]/lim)
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Google Scholar "Gram negative"|Enterobacteriaceae|Escherichia|Klebsiella|Salmonella|Shigella|Yersinia travel|traveller|tourist|tourism resistance|resistant|colonization|colonisation|susceptibility|carriage|carrier

Supplement 1. Queries per database

CHAPTER 3

The Carriage Of Multiresistant Bacteria After Travel (COMBAT) prospective cohort study: methodology and design.

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BMC Public Health. 2014 Apr 28;14:410.

ABSTRACT

Background

Antimicrobial resistance (AMR) is one of the major threats to public health around the world. Besides the intense use and misuse of antimicrobial agents as the major force behind the increase in antimicrobial resistance, the exponential increase of international travel may also substantially contribute to the emergence and spread of AMR. However, knowledge on the extent to which international travel contributes to this is still limited. The Carriage Of Multiresistant Bacteria After Travel (COMBAT) study aims to 1. determine the acquisition rate of multiresistant Enterobacteriaceae during foreign travel 2. ascertain the duration of carriage of these micro-organisms 3. determine the transmission rate within households 4. identify risk factors for acquisition, persistence of carriage and transmission of multiresistant Enterobacteriaceae.

Methods/design

The COMBAT-study is a large-scale multicenter longitudinal cohort study among travellers (n = 2001) and their non-travelling household members (n = 215). Faecal samples are collected before and immediately after travel and 1 month after return from all participants. Follow-up faecal samples are collected 3, 6 and 12 months after return from travellers (and their non-travelling household members) who acquired multiresistant Enterobacteriaceae. Questionnaires are collected from all participants at each time-point. Faecal samples are screened phenotypically for the presence of extended-spectrum beta-lactamase (ESBL) or carbapenemase-producing Enterobacteriaceae. Positive post-travel isolates from travellers with negative pre-travel samples are genotypically analysed for ESBL and carbapenemase genes with microarray and gene sequencing.

Discussion

The design and scale of the COMBAT-study will enable us to provide much needed detailed insights into the risks and dynamics of introduction and spread of ESBL- and carbapenemase-producing Enterobacteriaceae by healthy travellers and the potential need and measures to monitor or manage these risks.

Trial registration

The study is registered at clinicaltrials.gov under accession number NCT01676974.

BACKGROUND

The problem of antimicrobial resistance (AMR) is worldwide one of the foremost health issues that we face in the coming decades [1]. Bacterial AMR reduces clinical efficacy and increases treatment costs. Furthermore, AMR jeopardizes the achievements of modern medicine, since the success of interventions such as organ transplantation, cancer chemotherapy and major surgery depends on effective antimicrobial agents for prevention and treatment of infections. With a dearth of novel antibiotics in the pipeline, the conservation of existing ones is imperative [2].

Next to the well-established role of (inappropriate) antimicrobial use in humans and animals, the exponential increase of international travel may substantially contribute to the emergence and spread of AMR since it allows resistant bacteria or bacterial mobile gene elements carrying resistance genes (e.g. plasmids) to be rapidly transported between regions [3]. To what extent foreign travel poses a risk for the acquisition of AMR remains, however, largely unknown, as the presence of resistant bacteria in the normal human microbiota following travel usually remains undetected unless they cause manifest infection and disease. Yet, due to the high likelihood of contact and genetic exchange with potential pathogens, the human microbiota warrants special attention as perhaps the most accessible reservoir of resistance genes.

Besides being part of the normal human microbiota, Enterobacteriaceae are also important causes of community-acquired and nosocomial infections. Enterobacteriaceae can acquire resistance genes through horizontal gene transfer. Genes encoding for resistance to different classes of antibiotics, such as beta-lactams, quinolones and aminoglycosides are often located on plasmids. Multiple genes, each encoding for resistance to different classes of antibiotics, can be found on the same plasmid [4]. Selective pressure of one antibiotic can therefore lead to resistance to several classes of antibiotics.

Plasmid borne resistance to beta-lactam antibiotics in Enterobacteriaceae is emerging worldwide, due to the production of enzymes called extended-spectrum beta-lactamases (ESBLs). ESBLs have broad-spectrum activity against penicillins, cephalosporins and monobactams by hydrolyzing the beta-lactam ring of these antibiotics, leading to inactivation. Even more worrisome, Enterobacteriaceae can acquire resistance genes encoding for enzymes called carbapenemases. These carbapenemase-producing Enterobacteriaceae (CPE's) are "extreme drug resistant". Their enzymes are active against our last resort class of antibiotics: the carbapenems. Up to now, only case-reports have shown acquisition or infection with CPE's among travellers upon visit or hospitalization in endemic areas [5].

Besides horizontal gene transfer, AMR bacteria can spread from the traveller to other family members and beyond, through the faeco-oral route [6]. The traveller can therefore be seen as an interactive biological unit who picks up, processes, carries and drops

off microbial genetic material [7]. Consequently, local emergence of AMR can rapidly result in worldwide spread.

So far, five small to medium-sized prospective studies (n = 40–370) have investigated acquisition of AMR Enterobacteriaceae during international travel. These studies reported acquisition rates of ESBL-producing Enterobacteriaceae (ESBL-E) in faeces ranging from 24% to 33% among Swedish, Australian, American and Dutch travellers, with acquisition rates up to 88% depending on destination [8-12]. No acquisition of carbapenemase-producing Enterobacteriaceae was found.

In an earlier prospective study among travellers, a rapid decline in carriage of resistant isolates was demonstrated. A relative small proportion (10%) of subjects had persistent carriage after 6 months [13].

While these studies identified international travel as an important risk factor for acquiring AMR microorganisms, several important questions still need to be fully addressed to understand the contribution of travel to AMR emergence and spread, to assess the risk for public health and to identify measures to manage this risk. These knowledge gaps include (1) identification of travel-associated risk factors (including destination) for acquisition and subsequent carriage of these resistant microorganisms, (2) duration of colonization with AMR strains acquired during travel, (3) probability and dynamics of subsequent transmission of AMR strains within households and (4) the proportion of colonized travellers who develop infections with these resistant bacteria.

Scope of research

The Carriage Of Multiresistant Bacteria After Travel (COMBAT) Study, aims to prospectively study the influence of international travel and travel-associated risk factors on the acquisition, persistence and transmission of AMR in the endogenous microbiota of healthy individuals. The specific aims are:

1. to determine the acquisition rate of ESBL- and carbapenemase-producing Enterobacteriaceae during foreign travel by comparing pre- and post-travel faecal samples;
2. to ascertain the duration of carriage of these microorganisms (or their resistance genes/mobile genetic elements) by studying faecal specimens at regular intervals up to 1 year after return;
3. to mathematically model the decolonization and transmission rates of these imported Enterobacteriaceae (or their resistance genes/mobile elements) within households by prospectively studying consecutive specimens from household members (who did not join the index case on his/her travel);
4. to identify the risk factors for acquisition, persistence of carriage and transmission of ESBL- and carbapenemase-producing Enterobacteriaceae;
5. to examine whether carriers of resistant Enterobacteriaceae have a higher risk of bacterial infections in the year after travel (compared to non-carriers).

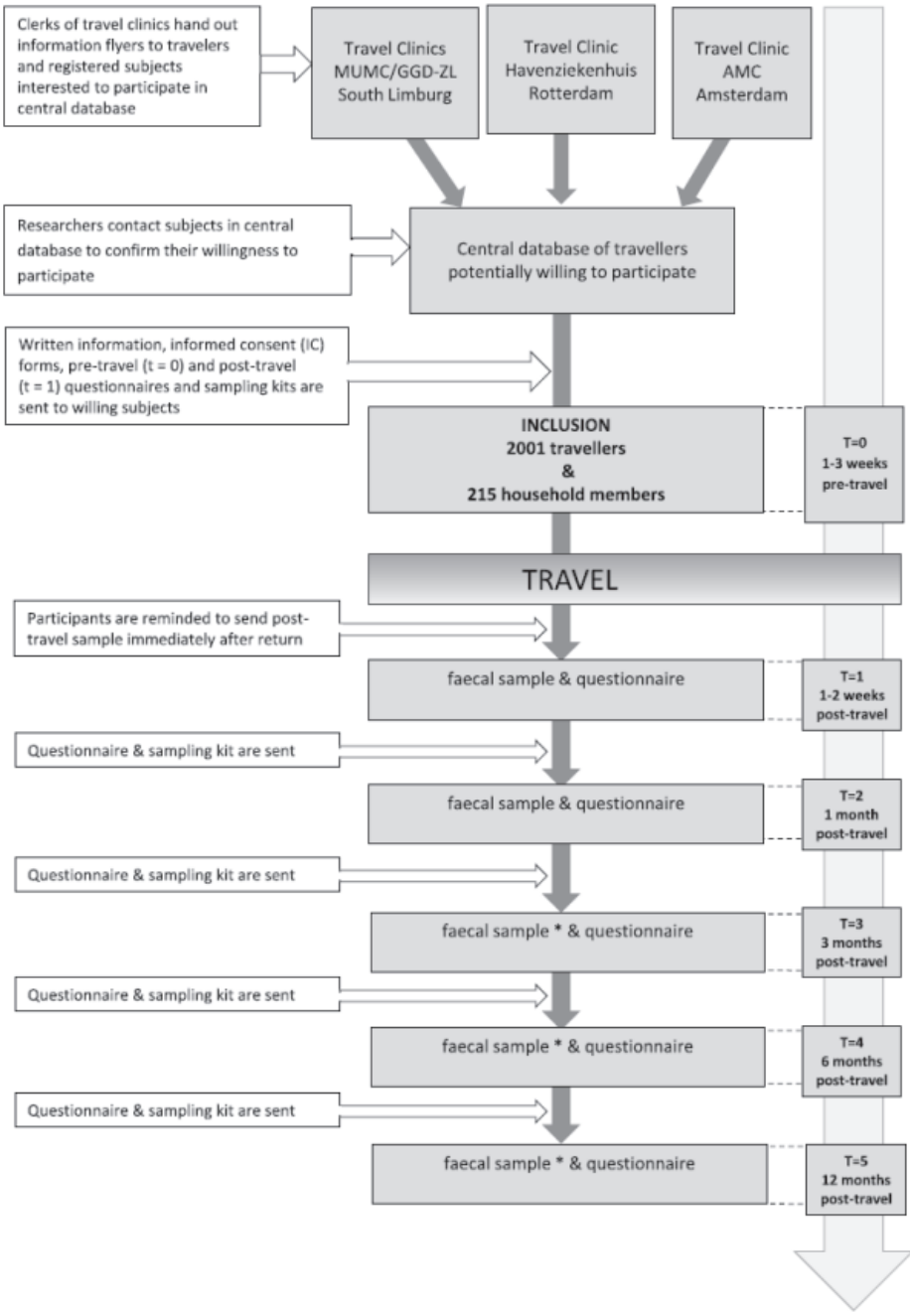

Figure 1. Flowchart of study design.

METHODS/DESIGN

Design

The design of the COMBAT-study is a multicenter longitudinal cohort study among travellers who are followed from one week prior to travel departure until 12 months after return. In order to study household transmission, non-travelling household members are also included and are followed over the same period as their travelling household members. Figure 1 depicts a flowchart of the study design and procedures.

Study area and recruiting centers

Participants are recruited at the outpatient clinics run by the Academic Medical Center (Amsterdam, the Netherlands), Havenziekenhuis (Rotterdam, the Netherlands) and Maastricht University Medical Center/Public Health Service South Limburg (Maastricht, the Netherlands), which together are visited by approximately 52.700 travellers each year for travel advice and vaccinations. Subjects are recruited within a period of one year, from November 2012 until November 2013.

Eligibility criteria

Travellers - Eligible subjects are adult (≥18 years) volunteers visiting one of the above stated travel clinics, travelling abroad for a minimum of one week to a maximum of three months. Minors (<18 years) and incapacitated subjects are excluded from this study.

Non-travelling household members - Non-travelling adult household members of participating travellers are also enrolled. A household contact is defined as an individual who lives in the same house as the traveller and shares the same kitchen and/or bathroom and/or toilet on a regular basis.

Sample size and power calculation

In order to determine the minimum number of travellers required to detect risk factors for acquisition of ESBL-producing Enterobacteriaceae with sufficient power, the following assumptions were made: 1. a 2% pre-travel prevalence of carriage of ESBL-producing Enterobacteriaceae; 2. an acquisition rate of ESBL-producing Enterobacteriaceae of 24% during travel; 3. a two-sided significance level (alpha) of 0.05; 4. a power (1-beta) of 80%; 5. a minimum odds ratio of 2.0; 6. a minimum prevalence of a travel-associated risk factor of 5%. Based upon these assumptions a sample size of 1541 analyzable subjects is required. Accounting for an estimated attrition rate of 20% immediately after travel (t = 1), a total of 1926 travellers need to be recruited.

After one year of follow-up, 2001 travellers were included fulfilling the requested sample size. To minimize the drop-out and non-response levels, participants are reminded through several channels in case questionnaires or samples are not received

in time. Participants are sent reminders initially by emails, followed by text messages to their mobile phones and, in case of no responses are received, are finally contacted by telephone by one of the researchers. This resulted in an attrition rate immediately after travel (t = 1) of 1,6%, being far lower than expected. Table 1 shows minimal effect sizes that can be detected within the final cohort according to a prevalence of a risk factor ranging from 5 to 50%.

Table 1. Effect sizes that minimally can be detected according to the prevalence of the exposure in the final cohort of 2001 travellers

Proportion exposed (%)	Odds ratio
50%	1,36
25%	1,41
10%	1,64
5%	1,92

Study procedures

Eligibility screening activities

All clerks of the participating outpatient travel clinics are instructed to hand out an information flyer on the COMBAT-study to all travellers visiting the clinics during the recruitment period. If travellers are interested to participate they are instructed to fill in the flyer with their contact details, date of departure and return, and number of non-travelling household members. Travellers who meet the eligible criteria are provided with additional information on the study procedure and subsequently contacted by phone to confirm their willingness to participate. Travellers not fulfilling the eligible criteria receive an email informing them on the reason for exclusion.

Ethical approval and informed consent

Subjects willing to participate are subsequently sent written information on the study procedures along with an informed consent form. Only participants providing written informed consent are enrolled. Ethical approval was obtained by the Medical Ethical Committee of Maastricht University Medical Center (study number: METC 12-4-093).

Data collection

Faecal sampling

Travellers and if applicable their participating non-travelling household members are instructed to self-collect a faecal sample before travel (t = 0) as well as immediately (t = 1) and one month (t = 2) after return. In case any of these samples from a traveller or his/her household member(s) is positive for ESBL- or carbapenemase-producing Enterobacteriaceae, both the traveller and the household member(s) are asked to provide

additional samples at each subsequent follow-up moment (3, 6 and 12 months after travel, t = 3-5). Sample collection and shipment kits are sent to participants before travel (for the collection of samples at t = 0 and t = 1), 2 weeks prior to the subsequent follow-up timepoint (t = 2) and if applicable 2 weeks prior to each of the follow-up time points (t = 3-5). A sample collection and shipment kit consists of an instruction form, a safety bag, a bibulous tissue, a postage paid airbag envelope and a faeces collection swab with modified Cary Blair transport medium (Fecal Swab®; Copan, Brescia, Italy). Participants are instructed to sample fresh stools by turning the swab into faeces without touching the toilet or water, package the sample according to the instructions and send to the laboratory immediately.

At the laboratory, samples are processed upon arrival. Residuals are aliquoted and stored at −80°C for future research.

Questionnaires

Questionnaires (in Dutch language) are sent to all participants at each timepoint. (t = 0-5). All questionnaires collect information on the date of sample collection and gastro-intestinal symptoms, including the ROME III IBS diagnostic questionnaire [14]. The pre-travel questionnaire (t = 0) comprises detailed information on demographic parameters (e.g. ethnicity, gender, age, household composition), travel history in the past years, pre-existing morbidity and medication use, hospital admissions and antibiotic use during the past year, as well as dietary preferences. The first post-travel questionnaire (t = 1) mainly collects information on travel details, such as duration; destination(s); urban/rural travel; type of travel (e.g. business, family visit, holiday); lodging (e.g. hotel, tent, family, locals); ailments or illnesses during travel (i.e. gastroenteritis); hospital admission; medical interventions and use of medication (in particular antibiotic use); place of meal consumption (e.g. at hotel, local restaurants, food stalls); unboiled/unbottled water consumption. The questionnaires at each subsequent follow-up collect data on intercurrent travel, medication use (including antibiotic use), hospital admissions and occurrence of illnesses/infections.

If applicable, travellers are asked to provide data on their relationship to the household members who also participate in the study. This includes data on the type of relationship (roommate, partner, parent, child, sibling, other), forms of contact (e.g. sharing of bed, towel, toothbrush, balms/lotions) and on household characteristics (e.g. household size). Participating non-travelling household members also receive questionnaires at each timepoint (t = 0-5) on demographic parameters, travel history, travel during the study period, medication use, hospital admission and occurrences of illness/infections.

Microbiological methods

Bacterial culture and antibiotic susceptibility testing

Faecal samples are processed immediately upon arrival at the laboratory. The samples are selectively enriched: 100 microliter of the liquid medium with faeces is pipetted into 5 ml of tryptic soy broth (TSB) supplemented with vancomycine (50 mg/l), followed by overnight incubation at 35°C [15]. The next day, volumes of 10 microliters are inoculated on chromID® ESBL (bioMérieux, Marcy l'Etoile, France), a selective agar plate to screen for ESBL- and carbapenemase-producing Enterobacteriaceae. These agar plates are incubated overnight at 35°C. All colonies growing on chromID® ESBL agar are further characterized to the species level using MALDI-TOF (Bruker, London, United Kingdom). Minimum inhibitory concentrations (MIC) are measured for all Enterobacteriaceae by the use of the automated susceptibility testing system Vitek 2 (bioMérieux, Marcy l'Etoile, France). The susceptibility testing results are interpreted by the clinical breakpoints recommended by EUCAST (the European Committee on Antimicrobial Susceptibility Testing). Phenotypic confirmation of ESBL-producing Enterobacteriaceae is performed by the combination disk diffusion test according to current national Dutch guidelines. Enterobacteriaceae with an MIC for imipenem and/or meropenem above the recommended screening breakpoint(s) measured by the Vitek 2 will be confirmed by Etest (bioMérieux, Marcy l'Etoile, France) [16].

Genotypic characterization

ESBL- and carbapenemase-producing post-travel isolates (t = 1) from travellers with negative pre-travel samples (t = 0) are screened for the presence of multiple classes of ESBL and carbapenemase genes using microarray (Identibac® AMR08; Alere Technologies GmbH, Jena, Germany). This platform is a miniaturized DNA-hybridization array in a strip based system for the detection of >120 antimicrobial resistance genes in Gram-negative bacteria, including those conferring resistance to aminoglycosides, trimethoprim, sulphonamides, tetracyclines, quinolones, and beta-lactams, including ESBLs and carbapenemases. In case of positive microarray signals, targeted PCR and DNA sequencing will be used to further genetically characterize the specific type of ESBL or carbapenemase in t = 1 isolates. DNA sequences will be analyzed using existing DNA databases (NCBI GenBank and Lahey beta-lactamase classification and amino acid sequences for TEM, SHV and OXA-Extended-Spectrum and Inhibitor Resistant Enzymes) which are updated regularly.

In case of negative microarray results of phenotypically resistant isolates, additional screening will be performed by PCR. To confirm persistence of colonization and/or transmission, phenotypically confirmed ESBL- or carbapenemase-producing isolates from follow-up samples of travellers and household members will be tested by targeted PCR and DNA sequencing (based on results from t = 1). Clonal bacterial spread within households will be confirmed or excluded by molecular (plasmid-) typing.

Table 2. Baseline characteristics of travellers and non-travelling household members according to study center

	Rotterdam		Amsterdam		Maastricht		Total	
	Travelers (n = 1109)	Household members (n = 129)	Travelers (n = 497)	Household members (n = 43)	Travelers (n = 395)	Household members (n = 43)	Travelers (n = 2001)	Household members (n = 215)
Sex								
Male	540 (48,7%)	39 (30,2%)	208 (41,9%)	18 (41,9%)	171 (43,3%)	23 (53,5%)	919 (45,9%)	80 (37,2%)
Female	569 (51,3%)	90 (69,8%)	289 (58,1%)	25 (58,1%)	224 (56,7%)	20 (46,5%)	1082 (54,1%)	135 (62,8%)
Age in years (median, range)	52,0 (18,1-81,7)	46,3 (18,4-82,0)	44,8 (19,8-74,6)	41,1 (18,9-78,0)	50,4 (18,2-71,9)	50,6 (18,4-71,6)	50,4 (18,1-81,7)	46,9 (18,4-82,0)
Continents visited by traveller								
Asia	556 (50,1%)		259 (52,1%)		200 (50,6%)		1015 (50,7%)	
Africa	362 (32,6%)		149 (30,0%)		123 (31,1%)		634 (31,7%)	
America	177 (16,0%)		81 (16,3%)		68 (17,2%)		326 (16,3%)	
Europe	11 (1,0%)		6 (1,2%)		4 (1,0%)		21 (1,0%)	
Oceania	3 (0,3%)		2 (0,4%)		0 (0,0%)		5 (0,2%)	

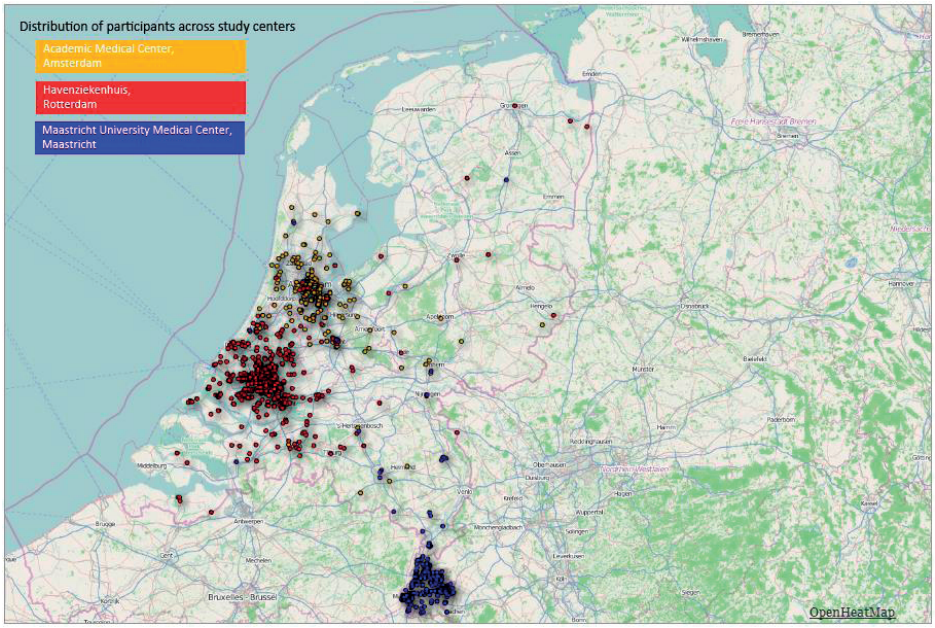

Figure 2. Geographic distribution of residences of participating travellers (n = 2001) throughout the Netherlands according to study center. i. Yellow circles represent participants from Tropencentrum AMC, Amsterdam. ii. Red circles represent participants from Travel Clinic Havenziekenhuis, Rotterdam. iii. Blue circles represent participants from Maastricht University Medical Center, Maastricht.

RESULTS

2001 travellers and 215 non-travelling household members were included. The median age of travellers and household members is respectively 50.5 years (range 18.1-81.7) and 46.9 years (range 18.4-82.0), 54.0% of travellers and 62.8% of household members are female (center-specific characteristics are presented in Table 2). The distribution of the participants throughout the Netherlands and across study centers is depicted in Figure 2. The regions most frequently visited were South-Eastern Asia, Eastern Africa, Southern Asia and South America (Figure 3).

DISCUSSION

The design and scale of the COMBAT-study are optimal to study the influence of international travel and travel-associated risk factors on the acquisition, persistence of carriage and transmission of AMR Enterobacteriaceae. A limited number of previous studies have suggested high acquisition rates of AMR Enterobacteriaceae during international travel, but most did not examine the duration of colonization and none looked at lo-

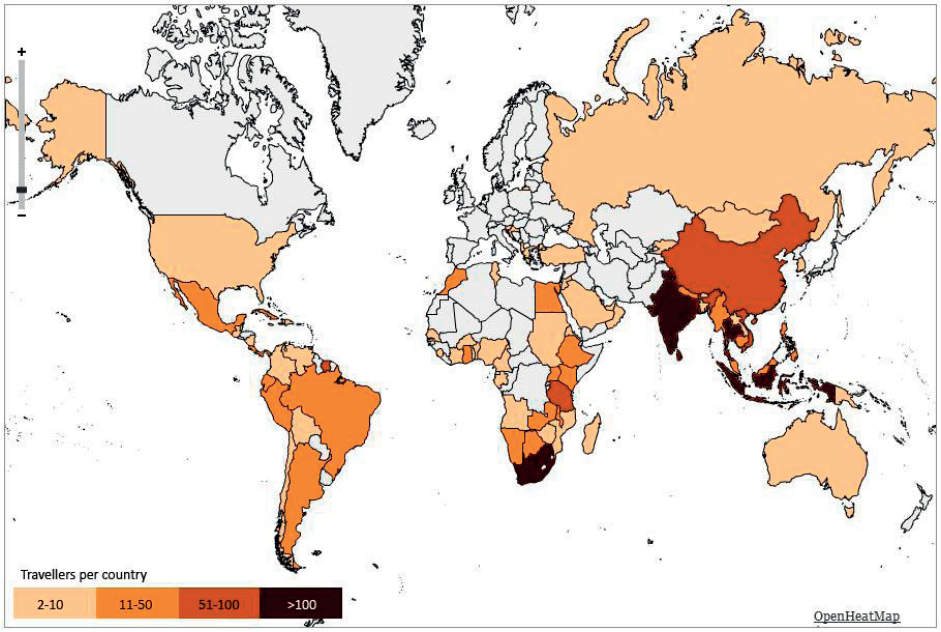

Figure 3. Heatmap showing the countries visited by the participating travellers (n = 2001). i. Grey color indicates 0 travellers visited country. ii. Light yellow color indicates 0-10 travellers visited country. iii. Orange color indicates 11-50 travellers visited country. iv. Light brown color indicates 50-100 travellers visited country. v. Dark brown color indicates > 100 travellers visited country

cal transmission of imported AMR. Our larger scale longitudinal studies will not only assess the probability of colonization by AMR Enterobacteriaceae during international travel along with associated risk factors, but will also determine the duration of such colonization as well as the probability and dynamics of subsequent transmission of AMR within households. In addition, while the main focus of the project will be on ESBL- and carbapenemase-producing Enterobacteriaceae, innovative molecular approaches (microarray) will be used to provide a more comprehensive and complete picture of associated resistance genes acquired during travel. Our extensive data from questionnaires will identify travel-associated risk factors for acquisition, persistence and transmission of AMR Enterobacteriaceae.

Selection towards a more affluent and healthy study population is a common phenomenon in epidemiological studies and has likely also occurred in our study. This potential selection may be related to some determinants and outcomes separately (non-differential selection), affecting the frequency rates and, as a consequence, the statistical power and generalizability of the results [17]. However, since we have access to the demographic data of all visitors of the travel clinics during the recruitment period, we will be able to perform detailed non-response analysis and examine to what extent the study population deviates from its source population. The incidence rates of AMR

acquisition found in the study will be interpreted accordingly. Moreover, this selection would only lead to bias in etiological association studies if the selection mechanisms are related to both the determinant and the outcome (differential selection), which is, in contrast to retrospective and cross-sectional studies, unlikely in the present prospective study.

Major efforts have been made to keep the follow-up rates as high as possible and to prevent (selective) loss to follow-up. This has resulted in follow-up rates as high as 98.4% immediately after travel (t = 1). Taken together, results from this study will provide much needed detailed insights into the risks and dynamics of introduction and spread of AMR by healthy travellers and the potential need and measures to monitor or manage these risks.

COMPETING INTERESTS

The authors declare they have no competing interests.

AUTHORS' CONTRIBUTIONS

MSA and JMH conduct the study and contributed to the design. MSA and JP drafted the manuscript. MDdJ, CS, DCM, HAV, JP designed the study and they are members of the supervising board. MCJB, PJG, EES, AG were involved in the design of the study. MSA, JMH, and JP revised several early drafts of the paper and MDdJ and DM commented on the final draft. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We are grateful to all clerks and nurses from the outpatient travel clinics run by the Academic Medical Center (Amsterdam, the Netherlands), Havenziekenhuis (Rotterdam, the Netherlands) and Maastricht University Medical Center/Public Health Service South Limburg (Maastricht, the Netherlands) for their immense support and productive collaboration in the implementation of this project and the recruitment of participants.

This study was funded by the Netherlands Organization for Health, Research and Development, ZonMw programme Priority Medicine Antimicrobial Resistance (project number: 50-51700-98-120).

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CHAPTER 4

Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study

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The Lancet Infectious Diseases. 2017 Jan;17(1):78-85.

SUMMARY

Background

International travel contributes to the dissemination of antimicrobial resistance. We investigated the acquisition of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) during international travel, with a focus on predictive factors for acquisition, duration of colonisation, and probability of onward transmission.

Methods

Within the prospective, multicentre COMBAT study, 2001 Dutch travellers and 215 non-travelling household members were enrolled. Faecal samples and questionnaires on demographics, illnesses, and behaviour were collected before travel and immediately and 1, 3, 6, and 12 months after return. Samples were screened for the presence of ESBL-E. In post-travel samples, ESBL genes were sequenced and PCR with specific primers for plasmid-encoded β-lactamase enzymes TEM, SHV, and CTX-M group 1, 2, 8, 9, and 25 was used to confirm the presence of ESBL genes in follow-up samples. Multivariable regression analyses and mathematical modelling were used to identify predictors for acquisition and sustained carriage, and to determine household transmission rates. This study is registered with ClinicalTrials.gov, number NCT01676974.

Findings

633 (34·3%) of 1847 travellers who were ESBL negative before travel and had available samples after return had acquired ESBL-E during international travel (95% CI 32·1–36·5), with the highest number of acquisitions being among those who travelled to southern Asia in 136 of 181 (75·1%, 95% CI 68·4–80·9). Important predictors for acquisition of ESBL-E were antibiotic use during travel (adjusted odds ratio 2·69, 95% CI 1·79–4·05), traveller's diarrhoea that persisted after return (2·31, 1·42–3·76), and pre-existing chronic bowel disease (2·10, 1·13–3·90). The median duration of colonisation after travel was 30 days (95% CI 29–33). 65 (11·3%) of 577 remained colonised at 12 months. CTX-M enzyme group 9 ESBLs were associated with a significantly increased risk of sustained carriage (median duration 75 days, 95% CI 48–102, p=0·0001). Onward transmission was found in 13 (7·7%) of 168 household members. The probability of transmitting ESBL-E to another household member was 12% (95% CI 5–18).

Interpretation

Acquisition and spread of ESBL-E during and after international travel was substantial and worrisome. Travellers to areas with a high risk of ESBL-E acquisition should be viewed as potential carriers of ESBL-E for up to 12 months after return.

Funding

Netherlands Organisation for Health Research and Development (ZonMw).

INTRODUCTION

Antimicrobial resistance constitutes an increasingly important human health hazard worldwide.[1] The use of antibiotics in human beings and food animals is a well established driving force behind increasing resistance.[2] Given the enormous growth of international tourism, from 25 million travellers in 1950 to 1·133 billion in 2014,[3] international travel might also contribute substantially to the rise in resistance because resistant bacteria or bacterial mobile genetic elements carrying resistance genes (eg, plasmids) may be rapidly transported between regions.[4] An important part of antimicrobial resistance genes is found on plasmids and codes for extended-spectrum β lactamase enzymes ([ESBLs] eg, TEM, SHV, and CTX-M) and carbapenemases that confer resistance to most β-lactam antibiotics.[2,4] Additionally, ESBL-producing Enterobacteriaceae (ESBL-E) and carbapenemase-producing Enterobacteriaceae (CPE) are typically resistant to multiple other antibiotic classes, which leaves few to no effective antimicrobial agents for prevention and treatment of infections.[4,5]

Previous studies have reported frequent acquisition of ESBL-E associated with various predictors and sporadic acquisition of CPE among international travellers.[6–10] However, data on ESBL-E colonisation after travel and assessment of associated predictors for sustained carriage and onward transmission within households are very limited. Such data are needed to establish the public health risk of the introduction and spread of antimicrobial resistance by travellers, and the potential needs and measures to monitor or manage these risks. Identifying individuals at risk of ESBL-E carriage enables appropriate measures to be taken to prevent introduction and spread of ESBL-E or CPE and for empirical adjustment of antibiotic treatment in individuals to optimise clinical care. We investigated the acquisition of ESBL-E during international travel, the associated predictive factors for acquisition, duration of colonisation, and onward transmission to household members.

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed on Aug 17, 2015, with the search terms "Gram negative bacteria", "Enterobacteriaceae", "Escherichia", "Klebsiella", "Salmonella", "Shigella", "Yersinia", "travel", "tourist", "tourism", "turista", "aviation", "air transport", "airport", "resistance", "colonisation", "antibiotic", "susceptibility", "carriage", and "carrier". We did a systematic review and identified 11 eligible studies. We updated this search on April 14, 2016, and found no new prospective studies. The results of the 11 prospective cohort studies showed high acquisition rates of extended-spectrum β-lactamase-producing Enterobacteriaceae

(ESBL-E) among travellers who had returned from southern Asia and northern Africa. Four travellers who visited India acquired carbapenemase-producing Enterobacteriaceae (CPE). However, whether antibiotic use and traveller's diarrhoea are predictors for ESBL-E acquisition was unclear. Moreover, these studies did not sufficiently address duration of ESBL-E carriage among travellers or onward transmission within households. One study asked travellers to provide stool samples up to 12 months after return, but duration of carriage was defined by ESBL phenotype. One other study looked at household transmission, but because only 11 household contacts were included, no reliable conclusion could be inferred about the risk of household transmission.

Added value of this study

In this large-scale, longitudinal cohort study, we followed up travellers and their non-travelling household members for up to 12 months after travel. The large sample size meant that we could investigate ESBL-E acquisition among travellers who had returned from a large number of countries across the world, including those such as Uganda, for which community carriage rates of ESBL-E were previously unknown. We identified several predictors (some new) for ESBL-E acquisition, including factors specific to subregions. Moreover, we were able to ascertain duration of ESBL-E carriage and associated resistance genes, identify predictors for sustained colonisation, and to model transmission rates mathematically within households.

Implications of all the available evidence

High frequencies of ESBL-E acquisition during travel, subsequent sustained carriage, and evidence of onward transmission within households show that travellers contribute to the emergence and spread of ESBL-E on a global scale. Active screening for ESBL-E and CPE and adjustment of empirical antimicrobial therapy should be considered for returning travellers at increased risk of ESBL-E carriage. However, implications for infection prevention and antibiotic treatment policies will differ locally because the degree of consequence of acquisition and spread of ESBL-E by travellers is highly dependent on local ESBL-E prevalence in the country of origin.

METHODS

Study design and participants

The study design and methods have been described in detail elsewhere.[11] Briefly, we did a multicentre, longitudinal, prospective cohort study involving travellers who were followed up from 1–3 weeks before travel departure until 12 months after return. To

study household transmission, we also assessed non-travelling household members in the same period.

Eligible participants were adults (age ≥18 years) planning to travel for at least 1 week and up to 3 months. They were recruited at three outpatient travel clinics across the Netherlands from November, 2012, to November, 2013. The study was approved by the Medical Research Ethics Committee, Maastricht University Medical Centre (METC 12-4-093). All participants provided written informed consent.

Procedures

Participants were provided with faeces collection kits and instructed to self-collect faecal swabs (appendix) before and immediately and 1 month after travel. If any of these samples contained ESBL-E, the traveller and his or her household members were asked to provide further samples at 3, 6, and 12 months after travel. If no samples were positive for ESBL-E, no additional samples were collected. Questionnaires were also collected at all timepoints to obtain information on potential risk factors for ESBL-E acquisition, including demographics, illnesses, and behaviour before, during, and after travel.

Samples were processed immediately after receipt. They were inoculated in tryptic soy broth supplemented with vancomycin (50 mg/L) to select for Enterobacteriaceae. The broth was then subcultured on chromID ESBL (bioMérieux, Marcy l'Etoile, France). All morphologically distinct colonies were characterised to the species level with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Bruker Microflex LT, Bruker, London, UK). Antibiotic minimum inhibitory concentrations were measured with the automated susceptibility testing system Vitek 2 (bioMérieux) for all Enterobacteriaceae. ESBL production was phenotypically confirmed by the combination disc diffusion test, according to current national Dutch guidelines.[12]

All phenotypically confirmed ESBL-E isolates acquired during travel were screened for the presence of ESBL genes with microarray, as described previously (appendix). The presence of ESBL genes was confirmed by PCR with primers specific for CTX-M enzyme groups 1, 2, 8, 9, and 25 and in-house primer sets. Further characterisation by sequencing was done for the most prevalent and largest CTX-M groups, 1 and 9. PCR confirmation and sequencing of genes for TEM and SHV ESBLs were limited to isolates that had negative microarray results for all CTX-M genes. A generic CTX-M PCR was done if no ESBL genes were detected by microarray, and, if positive, was followed by specific PCR and sequence confirmation for the different CTX-M groups (appendix). Sequences were compared with those in the NCBI GenBank and Lahey databases.

Acquisition was defined as the absence of ESBL-E in faecal samples before travel and the presence of ESBL-E in those obtained immediately after travel, as identified by phenotypic tests. Duration of carriage was defined by the last positive sample harbouring an ESBL of the same group (TEM, SHV, or CTX-M group 1, 2, 8, 9, or 25, or a combination)

as detected immediately after travel. Participants with consecutive samples positive for ESBL-E were classified as being persistent carriers and those with ESBL-E-positive samples interspersed with at least one negative sample were classified as being intermittent carriers.

Statistical analysis

Incidence proportions and incidence per 100 person-days of travel and accompanying 95% CIs for ESBL-E acquisition were calculated for each subregion (appendix) and country of destination. Incidence per 100 person-days of travel was calculated with a maximum likelihood method that was based on a constant acquisition rate with right-censored and interval-censored data.

Predictors for ESBL-E acquisition were determined by logistic regression models that were based on the method proposed by Bursac and colleagues[13] (appendix) and analysed with IBM SPSS Statistics (version 21.0). Results are presented as odds ratios (ORs) and 95% CIs. We did separate analyses for the subregions of southeast Asia, southern Asia, and eastern Africa, as several dietary variables (eg, consumption of chicken, barbecue meat, or pork) interacted with specific travel destination subregions.

Time to decolonisation was assessed with Kaplan-Meier survival analyses with right censoring for participants whose last provided sample was ESBL-E positive. Univariable and multivariable Cox's regression analyses were done to identify predictors associated with decolonisation (appendix). Results are presented as hazard ratios (HRs) and 95% CIs (HRs <1·00 indicate decreased risk of decolonisation and, therefore, increased duration of carriage).

A Markov model was used to calculate the probability of transmission within households. For computational reasons, this model was based on ESBL-E as defined by phenotypic confirmation, and only data from households consisting of at most five people were included, but these accounted for 98% of households. The model took into account false-negative results, missing culture results, and unobserved colonisation times. The method of calculation was as follows. ESBL-E-positive people (travellers or non-travelling household members) transmit ESBL-E to household members with rate β. Transmission from other sources was incorporated by the background transmission parameter α. Decolonisation of ESBL-E occurred with rate γ. Negative cultures could be false negative and affect the estimate of the sensitivity (ϕ). The specificity of culture was assumed to be 100%. Thus, the probability of transmission from an ESBL-E-positive to an ESBL-E-negative person, given that the ESBL-E-negative household member did not acquire ESBL-E via another route, could be calculated as $\beta/(\beta+\gamma)$. Model parameters were simultaneously estimated with a maximum likelihood method in Mathematica version 9.0. This study is registered with ClinicalTrials.gov, number NCT01676974.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

2737 travellers were screened for eligibility, of whom 2001 were included in the study (appendix), with median age 50·5 years (IQR 32·8–60·7) and good health before travelling in most (table 1). 49 travellers were lost to follow-up.

The main purpose for travel was tourism (1655 [84·2%] of 1965 travellers) and the median travel duration was 20 days (IQR 15·0–25·0; table 1). The subregions most frequently visited were southeast Asia (n=650), eastern Africa (n=287), South America (n=228), and southern Asia (n=217). 122 (6·1%) of 2001 travellers were carrying ESBL-E before travel, leaving 1879 at risk of ESBL-E acquisition. 1847 (98·3%) of these submitted faecal samples after travel, among whom 633 had acquired at least one ESBL-E during travel (table 2), giving an acquisition rate of 34·3% (95% CI 32·1–36·5). From these 633 travellers, 859 morphologically different ESBL-E strains were isolated (759 Escherichia coli, 67 Klebsiella pneumoniae, and 33 other species). CTX-M-15 was the most frequently acquired ESBL gene, being found in 338 (53·4%) of 633 travellers (appendix).

ESBL-E were most frequently acquired in southern Asia (75·1%, 95% CI 68·4–80·9), followed by central and eastern Asia (48·8%, 38·4–59·3; table 2, figure 1), but the frequency of acquisition varied widely between countries. Among the 22 most frequently visited countries, acquisition was highest in India (88·6%, 95% CI 79·8–93·9) and lowest in Suriname (3·6%, 1·0–12·1; appendix). Acquisition was also common after travel to eastern African countries, such as Uganda (44·4%, 27·6–62·7, appendix).

In the multivariable logistic regression, antibiotic use during travel was the strongest independent predictor for ESBL-E acquisition (table 3). To assess the effects of different antibiotic classes in the model, we exchanged the variable antibiotic use during travel (no *vs* yes) for a variable indicating antibiotic class (no antibiotics *vs* β-lactam, or quinolone, or other). Quinolone use was most strongly associated with ESBL acquisition (adjusted OR 6·0, 95% CI 2·9–12·4), whereas associations were non-significant for use of β-lactam (2·2, 0·95–5·14) or other antibiotics (1·7, 0·59–2·35). We also detected strong associations between ESBL-E acquisition and diarrhoea during travel and, particularly, traveller's diarrhoea that persisted on return (table 3). Travellers who had occasionally consumed food from street vendors were at increased risk of acquiring ESBL-E compared with those who had avoided street food vendors, and the risk increased further in travellers who consumed food from street vendors daily (table 3). Self-reported pre-existing chronic bowel disease was another notable risk factor for ESBL-E acquisition (table 3).

Table 1. Baseline characteristics of travellers and non-travelling household members

	Travellers (n=2001)*	Non-travelling household members (n=215)†
Sex		
Male	920 (46·0%)	80 (37·2%)
Female	1081 (54·0%)	135 (62·8%)
Age (years)	50·5(32·8–60·7)	46·9(25·7–55·8)
Education level		
No education, elementary school, or prevocational secondary education	243 (12·4%)	78 (36·4%)
Vocational secondary education	280 (14·2%)	37 (17·3%)
Senior general secondary education or education up to university	200 (10·2%)	45 (21·0%)
Higher professional education	642 (32·7%)	53 (24·7%)
Academic (university) education	595 (30·3%)	38 (17·8%)
Antibiotic use in previous 3 months		
No	1760 (90·1%)	189 (88·3%)
Yes	194 (9·9%)	25 (11·7%)
Travel in past year		
None	185 (9·5%)	27 (12·6%)
In Europe	915 (46·9%)	124 (57·7%)
Outside Europe	852 (43·6%)	64 (29·8%)
Chronic disease‡		
No	1500 (77·2%)	173 (82·0%)
Yes	443 (22·8%)	38 (18·0%)
Chronic bowel disease‡		
No	1912 (97·4%)	212 (99·1%)
Yes	51 (2·6%)	2 (0·9%)
Continent visited during travel§		
Asia	1016 (50·8%)	NA
Africa	633 (31·6%)	NA
America	326 (16·3%)	NA
Europe	21 (1·0%)	NA
Oceania	5 (0·2%)	NA
Duration of index travel (days)	20 (15·0–25·0)	NA
Purpose of index travel		
Holiday	1655 (84·2%)	NA
Work or internship	161 (8·2%)	NA
Visiting family or relatives	82 (4·2%)	NA
Other reason	66 (3·4%)	NA

Data are number (%) or median (IQR). NA=not applicable. *Some numbers do not add up to 2001 because of missing data. †Some numbers do not add up to 215 because of missing data. ‡Self-reported by traveller or household member. §If travellers visited multiple continents, only the main continent visited is presented in this table.

Table 2. Incidence proportion and incidence per 100 person-days of travel for ESBL-E acquisition in Dutch travellers, by subregion

	Number of travellers (n=1847)*	Number of travellers who acquired ESBL-E (n=633)†	ESBL-E incidence proportion (95% CI)‡	Number of travel-days	Mean (SD) duration of travel (days)	ESBL-E incidence per 100 person-days of travel (95% CI)§
Southern Asia	181 (9·8%)	136 (21·5%)	75·1 (68·4–80·9)	3727	20·6 (11·0)	7·2 (5·9–8·6)
Central and eastern Asia	84 (4·5%)	41 (6·5%)	48·8 (38·4–59·3)	1712	20·4 (10·8)	3·5 (2·5–4·7)
Western Asia	28 (1·5%)	12 (1·9%)	42·9 (26·5–60·9)	305	10·9 (7·5)	5·8 (3·0–9·9)
Northern Africa	81 (4·4%)	34 (5·4%)	42·0 (31·8–52·9)	981	12·1 (5·7)	4·5 (3·1–6·2)
Southeastern Asia	540 (29·2%)	200 (31.6%)	37·0 (33·1–41·2)	12 493	23·1 (11·6)	2·1 (1·8–2·4)
Caribbean and Central America	86 (4·7%)	24 (3·8%)	27·9 (19·5–38·2)	1653	19·2 (12·4)	1·7 (1·1–2·5)
Middle and eastern Africa	205 (11·1%)	57 (9·0%)	27·8 (22·1–34·3)	4060	19·8 (14·3)	1·6 (1·2–2·1)
Western Africa	106 (5·7%)	20 (3·2%)	18·9 (12·6–27·4)	1638	15·5 (11·1)	1·4 (0·8–2·0)
South America	180 (9·7%)	33 (5·2%)	18·3 (13·4–24·6)	4778	26·5 (14·7)	0·8 (0·5–1·1)
Southern Africa	116 (6.3%)	7 (1·1%)	6·0 (2·5–12·0)	2522	21·7 (8·6)	0·3 (0·1–0·6)
Northern America, Europe, and Oceania	17 (1·0%)	1 (<1·0%)	5·9 (1·1–27·0)	292	17·2 (11·3)	0·4 (0–1·6)

ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae. *Numbers do not add up to 1847 because 221 travellers visited more than one subregion (66 with ESBL-E acquisition) and destination information was missing for two. †Numbers do not add up to 633 because 66 travellers visited multiple subregions and destination information was missing for two. ‡Based on binomial distribution (Wilson's score interval). §Calculated with the maximum likelihood estimation method based on a constant acquisition rate with right-censored and interval-censored data.

In the multivariable logistic regression, antibiotic use during travel was the strongest independent predictor for ESBL-E acquisition (table 3). To assess the effects of different antibiotic classes in the model, we exchanged the variable antibiotic use during travel (no *vs* yes) for a variable indicating antibiotic class (no antibiotics *vs* β-lactam, or quinolone, or other). Quinolone use was most strongly associated with ESBL acquisition (adjusted OR 6·0, 95% CI 2·9–12·4), whereas associations were non-significant for use of β-lactam (2·2, 0·95–5·14) or other antibiotics (1·7, 0·59–2·35). We also detected strong associations between ESBL-E acquisition and diarrhoea during travel and, particularly, traveller's diarrhoea that persisted on return (table 3). Travellers who had occasionally consumed food from street vendors were at increased risk of acquiring ESBL-E compared with those who had avoided street food vendors, and the risk increased further in travellers who consumed food from street vendors daily (table 3). Self-reported pre-existing chronic bowel disease was another notable risk factor for ESBL-E acquisition (table 3).

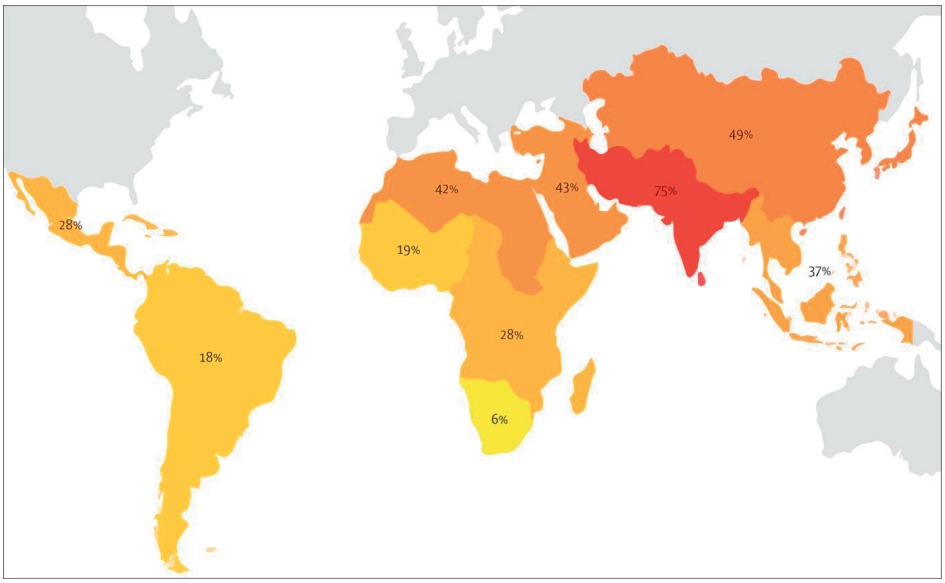

Figure 1. Percentages of travellers that acquired β-lactamase-producing Enterobacteriaceae per subregion, according to the United Nations geoscheme

In the separate analyses for three of the visited subregions, the consumption of raw vegetables and antibiotic use were predictors of ESBL-E acquisition in southeastern Asia. In southern Asia, the strongest predictors were contact with orphan children and daily food consumption at a hostel or guesthouse. In eastern Africa, the strongest associations were daily visits to the local markets and staying in rural areas (appendix).

Sustained ESBL-E carriage (persistent and intermittent) after acquisition was seen in 42·9%, 25·1%, 14·3%, and 11·3% of travellers at 1, 3, 6, and 12 months after return, respectively. Most of these participants were continuously colonised (appendix). The median duration of post-travel colonisation was 30·0 days (95% CI 28·9–33·1, figure 2). ESBL-producing *K pneumoniae* and travel to western Asia were associated with the shortest times to decolonisation. Travellers who acquired a CTX-M group 9 ESBL had a significantly increased risk of sustained carriage compared with travellers who acquired a CTX-M group 1 ESBL (appendix).

Of 215 non-travelling household members included in the study, 63 were ESBL-E negative at baseline and shared households with people who acquired ESBL-E while travelling. Additionally, 105 co-travellers who were ESBL-E negative immediately after return shared households with travellers who acquired ESBL-E. Thus, 168 household members (in 152 households) were at risk of ESBL-E transmission. Evidence of onward transmission within households was found in 13 (7·7%) of these 168 household members (ten co-travellers and three non-travelling household members, appendix), who

Table 3. Predictors for ESBL-E acquisition among travellers in the final adjusted logistic regression model

	Number of travellers at risk (n=1847)*	Number of travellers who acquired ESBL-E (n=633)†	Odds ratio (95% CI)‡	p value	Adjusted odds ratio (95% CI)§	p value
Pre-existing bowel disease						
No	1793 (97·3%)	606 (33·8%)	1·00	..	1·00	..
Yes	50 (2·7%)	24 (48·0%)	2·34 (1·26–4·34)	0·007	2·10 (1·13–3·90)	0·019
Beach holiday						
No	1404 (76·1%)	504 (35·9%)	1·00	..	1·00	..
Yes	441 (23·9%)	127 (28·8%)	0·72 (0·55–0·93)	0·010	0·73 (0·56–0·95)	0·021
Traveller's diarrhoea¶						
No	1085 (60·1%)	329 (30·3%)	1·00	..	1·00	..
During travel	593 (32·8%)	235 (39·6%)	1·56 (1·24–1·96)	<0·001	1·42 (1·12–1·80)	0·003
Immediately after travel	41 (2·3%)	14 (34·1%)	1·19 (0·58–2·44)	0·640	1·3 (0·63–2·68)	0·477
During travel and immediately after travel	87 (4·8%)	44 (50·6%)	2·42 (1·50–3·91)	<0·001	2·31 (1·42–3·76)	0·001
Antibiotic use during travel‖						
No	1697 (92·8%)	553(32·6%)	1·00	..	1·00	..
Yes	132 (7·2%)	73 (55·3%)	2·65 (1·80–3·91)	<0·001	2·69 (1·79–4·05)	<0·001
Attendance of large (religious) gathering						
No	1744 (94·6%)	595 (34·1%)	1·00	..	1·00	..
Yes	100 (5·4%)	36 (36·0%)	0·56 (0·34–0·92)	0·020	0·57 (0·34–0·94)	0·028
Daily hand hygiene before meals						
None	782 (42·4%)	265 (33·9%)	1·00	..	1·00	..
Clean with alcohol	161 (8·7%)	69 (42·9%)	1·03 (0·71–1·51)	0·870	0·97 (0·66–1·44)	0·885
Clean with soap	666 (36·1%)	200 (30·0%)	0·82 (0·64–1·04)	0·100	0·77 (0·60–0·99)	0·044
Clean with alcohol and soap	235 (12·7%)	97 (41·3%)	1·03 (0·74–1·44)	0·860	1·12 (0·79–1·59)	0·518
Meal at street food stalls during travel						
Never	1248 (67·7%)	386 (30·9%)	1·00	..	1·00	..
Occasionally	513 (27·8%)	205 (40·0%)	1·37 (1·08–1·73)	0·010	1·33 (1·04–1·71)	0·022
Daily	83 (4·5%)	40 (48·2%)	2·09 (1·30–3·38)	0·003	1·78 (1·07–2·95)	0·025

ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae. *Numbers do not add up to 1847 because of missing values. Valid percentages are reported after removal of missing values, which were assumed to be random. †Numbers do not add up to 633 because of missing values. The demoninators for percentages are the numbers of travellers at risk given in the previous column. ‡Only adjusted for travel destination subregion, defined according to the United Nations geoscheme: Caribbean and Central America, middle and eastern Africa, central and eastern Asia, North America, Europe, and Oceania, southern Asia, southeastern Asia, western Asia, northern Africa, southern Africa, western Africa, and South America. §Adjusted for travel destination and travel variables shown in table. ¶Defined as ≥3 unformed stools within 24 h, with or without accompanying symptoms. ‖Most frequently used to treat gastroenteritis (41 [31·1%] of 132 travellers), of whom 17 (41·5%) took them without consulting a doctor.

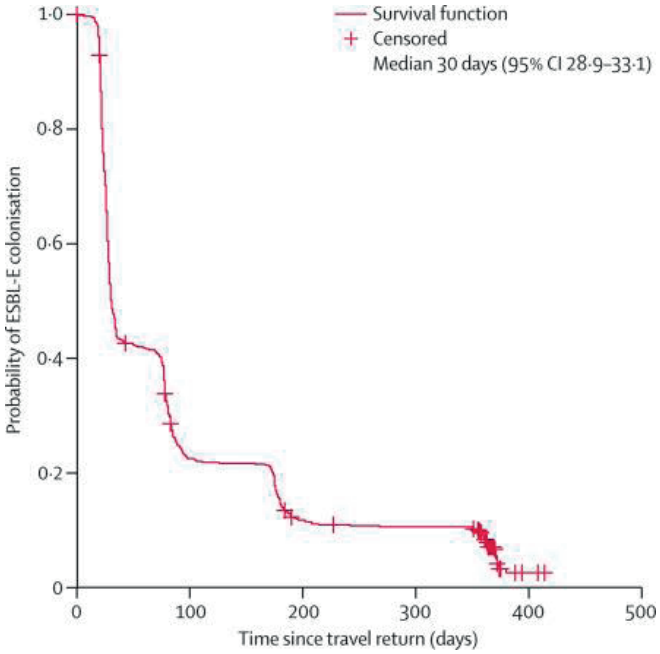

Figure 2. Kaplan-Meier estimate of time to decolonisation of ESBL-E in travellers
ESBL-E=extended-spectrum β-lactamase-producing Enterobacteriaceae.

had one or more follow-up isolates with the same ESBL group (TEM, SHV, CTX-M group 1, 2, 8, 9, or 25) as had been acquired by the index traveller.

We subsequently used a Markov model to estimate the transmission rate of ESBL-E after introduction into a household. We included 3330 people from 1542 households in the estimation of probability of transmission of ESBL-E after introduction. 381 households consisted of one person, 774 of two people, 187 of three, 160 of four, and 40 of five. Person-to-person transmission was estimated to occur at a rate of 0·0013 (95% CI 0·0005–0·0024) per colonised person per day, with background transmission occurring at a rate of 0·00073 (0·00054–0·0009) per day. The decolonisation rate was 0·010 (0·0092–0·011) per day. The sensitivity of the screening process was 90% (86–93). Thus, the probability of transmission from an ESBL-E-positive to an ESBL-E-negative person in the household was 12% (5–18).

DISCUSSION

Results from this large cohort study of travellers indicated that the risk of ESBL-E acquisition during travel is high, especially during travel to Asia and northern Africa. 11·3% of travellers who acquired EBSL-E remained colonised at 12 months after return, and

the estimated probability of onward transmission within households was 12%. Other important predictors for ESBL-E acquisition during travel were antibiotic use, traveller's diarrhoea that persisted after return, and pre-existing chronic bowel disease.

The frequency with which ESBL-E was imported by travellers is worrisome. 75·1% of travellers to southern Asia and 40–50% of those to central or eastern Asia, western Asia, and northern Africa acquired ESBL-E while travelling. Additionally, in central and eastern Africa, frequency of ESBL-E acquisition was substantial in some countries, particularly Uganda (44·4%). So far, data on acquisition among travellers to countries in central and eastern Africa have been very limited. Additionally, we have previously shown acquisition of carbapenemases and plasmid-mediated *mcr-1* colistin-resistance genes in, respectively, five and six travellers in this study cohort.[14,15]

Only two of six studies that previously did multivariable risk factor analysis identified antibiotic use and traveller's diarrhoea as significant travel-associated predictors for ESBL-E acquisition,[6–8] which probably reflects limited power to do extensive risk factor analysis. Self-reported pre-existing chronic bowel disease (mainly inflammatory bowel disease, irritable bowel syndrome, and coeliac disease) was a new predictor for ESBL-E acquisition in this study. Antibiotic use, traveller's diarrhoea, and chronic bowel disease have well established associations with dysbiosis of the gut microbiota.[16–18] A dysbiosis-induced reduction in colonisation resistance being the underlying biological mechanism through which these factors predispose to ESBL-E acquisition is, therefore, conceivable. Antimicrobial agents have substantial effects on the gut microbiota, which mainly manifest as decreased colonisation resistance resulting in consequent emergence of pathogenic or antibiotic-resistant strains.[19] In this study we found that, second to travel destination, antibiotic use was the strongest predictor for ESBL-E acquisition, particularly quinolone use during travel. Pervasive disturbance in the human microbiota has been reported after ciprofloxacin treatment.[16,20] For amoxicillin, although the effect on the human microbiota is moderate, an increase in the abundance of resistant Enterobacteriaceae has been reported after its use.[16,19] Similar to other studies,[21] antibiotics were mostly used to treat gastroenteritis. Counselling before travel to refrain from the use of antibiotics to treat self-limiting infections could reduce the import of ESBL-E by travellers. Kantele and colleagues,[22] for example, showed that use of loperamide alone to treat mild traveller's diarrhoea was not associated with an increased risk of ESBL-E colonisation.

The significantly higher frequency of ESBL-E acquisition among travellers to Asia than other regions is probably due to the widespread dissemination of ESBL-E in these regions and high risk of food contamination. Diet-associated predictors, therefore, might differ by travel destination, and might have been missed in previous studies that did not stratify data by destination. In the overall analysis, food consumption from street vendors was associated with an increased risk of ESBL-E acquisition, but in the strati-

fied analysis in southern Asia daily food consumption at a hostel or guesthouse and in southeastern Asia consumption of raw vegetables were predictive factors.

While the frequency of acquisition of ESBL-E by travellers is fairly consistent across studies, duration of carriage has varied from 5% to 24% at 6 months after return.[6] In our study, we found that 65 (11·3%) of 577 travellers who acquired ESBL-E during travel had sustained colonisation (persistent or intermittent) 12 months after return. Although our study focused on asymptomatic carriage of ESBL-E, international travel has also been associated with ESBL-E infection among patients in the community and in hospital.[23,24] Depending on the local policies, therefore, empirical adjustment of antimicrobial therapy should be considered in patients recently returned from international travel.

Our findings suggest that strains or plasmids carrying CTX-M group 9 ESBL genes have a colonisation advantage that results in sustained carriage. This finding agrees with those from other studies showing sustained carriage associated with these genes in travellers returningfrom Asia, in the community and in hospital.[25,26] Moreover, colonisation in this study was longer in travellers who acquired ESBL-producing *E coli* than in those with ESBL-producing *K pneumoniae*. These observations might be explained by accessory colonisation factors, such as P-fimbriae or aerobactin, or differences in fitness costs and plasmid stability between *E coli* and *K pneumoniae*.[27,28]

Our mathematical model of onward transmission of ESBL-E in households of travellers, which took into account factors such as total number of household members, estimated 12% probability of transmission. In households of recently discharged patients, Hilty and colleagues[29] reported transmission of ESBL-E to 20 (22·7%) of 88 household contacts. This higher risk might be due to more frequent and longer exposure times of caregiving household members to discharged patients. Practising hand hygiene at home might lessen the risk of household transmission of ESBL-E.[30]

Our study has some potential limitations. First, as in most epidemiological studies, our study population was probably more affluent and healthy than the average for the general population, which could have led to selection that affected the frequency of ESBL-E acquisition and the statistical power and generalisability of the results. However, for bias to occur, selection would have to affect both the exposure and the outcome, which is unlikely in prospective cohort studies. Inferences drawn from our study are also unlikely to be affected by (selective) attrition, since loss to follow-up was minimal and 12-month follow-up was achieved in 92·2% of participants after travellers returned. Second, faecal cross-contamination during collection of stool samples could theoretically have affected the estimations of colonisation and transmission. We aimed to keep the risk of cross-contamination to a minimum by providing participants with clear instructions for sample collection, including graphics. Lastly, although our results showed very low background transmission rates, in the absence of molecular typing of strains or mobile genetic elements harbouring ESBL genes, some overestimation of the duration of

colonisation and household transmission due to novel ESBL-E acquisition from outside the household cannot be completely excluded.

320 million people visit Asia, northern Africa, and the Middle East per year[3] and, therefore, international travel is expected to contribute substantially to the emergence and spread of ESBL-E in travellers' countries of origin. Taking into account the total number of Dutch travellers visiting these regions annually, we estimate that each year between 3·0% and 7·1% of the Dutch population acquires an ESBL-E during travel to destinations outside Europe, northern America, and Oceania (appendix). Overall, with acquisition of 34·3% and sustained carriage after acquisition seen in 11·3% of travellers 12 months after return, plus a 12% probability of household transmissions, our findings support the substantial contribution of international travel to the spread of ESBL-E and antimicrobial resistance worldwide. The degree of consequence of the emergence and spread of antimicrobial resistance by travellers, however, differs by region, and is highly dependent on local prevalence of antimicrobial resistance in the country of origin.

CONTRIBUTORS

MSA and JMvH did the study, collected the data, and contributed to the study design. PJJvG, CS, HAV, MDdJ, DCM, and JP designed the study and are members of the supervising board. MRH, MCJB, AG, MPG, AMOL, NM, and EES contributed to the study design, data collection, or both. MSA, JMvH, MRH, MCJB, PJJvG, CS, HAV, MDdJ, DCM, and JP contributed to the data analysis and interpretation. MSA, JMvH, MRH, and JP drafted the Article with help from all authors. MSA, JMvH, MRH, MCJB, PJJvG, AG, MPG, CS, EES, HAV, MDdJ, DCM, and JP contributed to the critical revision of the drafts for important intellectual content. All authors read and approved the final version of the paper.

DECLARATION OF INTERESTS

We declare no competing interests.

ACKNOWLEDGMENTS

This work was supported by Netherlands Organisation for Health Research and Development (ZonMw, grant number 205200003). We thank all the employees of the Travel Clinics (Institute for Tropical Diseases, Havenziekenhuis; Centre of Tropical Medicine and

Travel Medicine, Academic Medical Centre; EASE Travel Health & Support) for their help in the recruitment of participants.

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SUPPLEMENTARY APPENDIX

MATERIALS AND METHODS

Collection of fecal samples
Genotypic characterization of ESBL-E
Classification of travel destinations into subregions according to according to United Nations geoscheme
Logistic regression analyses
Cox regression analyses

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Table E8: Estimation of annual travel-related ESBL-E acquisition in Dutch population in 2013 (excluding North America, Europe & Oceania).

SUPPLEMENTARY FIGURES

Figure E1. Flowchart of study.

Figure E2. Acquisition of unique ESBL genes (n = 692) by travellers (n = 633) with ESBL-E acquisition.

Figure E3. Duration of ESBL-E carriage among travellers with ESBL-E acquisition (n = 633).

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Figure E5. Flow chart of non-travelling and co-travelling household members at risk for acquisition of ESBL-E through onward transmission from household members that acquired ESBL-E during travel.

Figure E6. Model to estimate the transmission rate within households with 2 household members.

Figure E7. Model to estimate the transmission rate within households with 3 household members.

MATERIALS AND METHODS

Collection of fecal samples

Travellers and if applicable their participating non-travelling household members were instructed to self-collect fecal samples using the provided sample collection and shipment kits. The sample collection and shipment kit consisted of an instruction form, a safety bag, a bibulous tissue, a postage paid airbag envelope and a feces collection swab with modified Cary Blair transport medium (Fecal Swab®; Copan, Brescia, Italy).

In order to avoid potential cross-contamination and to ensure collection of sufficient fecal matter, participants were provided the following instructions on how to sample their stools: i. place plenty of toilet paper in the toilet before defecation to avoid stools to slide into the water; ii after defecation, stick the entire tip of the fecal swab in the stool to ensure that the entire tip is covered with fecal matter, and; iii. prevent at any cost that the fecal swab comes into contact with anything else that the stool itself (e.g. the toilet or toilet water). Subsequently, participants were instructed to package the sample according to the instructions and send to the laboratory immediately.

Genotypic characterization of ESBL-E

All phenotypically confirmed ESBL-E isolates acquired during travel were screened for the presence of ESBL genes using microarray as described previously.[1-3]

In short, bacterial DNA was extracted after overnight cultivation, followed by biotin labelling and amplification in a linear multiplex reaction using 184 primer and probe sets targeted at 124 resistance genes, and microarray hybridization in duplicate or triplicate of the resulting labelled mix (Identibac® AMR08; Alere Technologies GmbH, Jena, Germany). Mean signal intensities, as measured by calculating the quantitative staining value using IconoClust software installed on the Alere ArrayMate Reader, of the replicate spots per probe were used for analysis. Intensities of ≥0.4 were considered positive as established previously.[3]

The presence of ESBL genes was confirmed by PCR using primers specific for CTX-M groups 1, 2, 8, 9 and 25[1,4-6] and in-house primer sets. Further characterization by sequencing was performed for CTX-M groups 1 and 9. PCR confirmation and sequencing of detected TEM and SHV genes was limited to isolates with negative microarray results for all CTX-M group genes. A generic CTX-M PCR[7] was performed in case no ESBL genes were detected by microarray, and if positive, followed by specific PCR and sequence confirmation for the different CTX-M groups. Sequences were analysed using the NCBI GenBank (www.ncbi.nlm.nih.gov/) and Lahey (www.lahey.org/studies/web/html) databases.

Continent	UN subregion	countries
Africa	Eastern Africa	Burundi, Comoros, Djibouti, Eritrea, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Mayotte, Mozambique, Réunion, Rwanda, Seychelles, Somalia, Uganda United Republic of Tanzania, Zambia, Zimbabwe
	Middle Africa	Angola, Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Sao Tome and Principe
	Northern Africa	Algeria, Egypt, Libya, Morocco, South Sudan, Sudan, Tunisia Western Sahara
	Southern Africa	Botswana, Lesotho, Namibia, South Africa, Swaziland
	Western Africa	Benin, Burkina Faso, Cape Verde, Cote d'Ivoire, Gambia Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria Saint Helena, Senegal, Sierra Leone, Togo
Americas	Carribean	Anguilla, Antigua and Barbuda, Aruba, Bahamas, Barbados, Bonaire, Saint Eustatius and Saba, British Virgin Islands, Cayman Islands, Cuba, Curaçao, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Jamaica, Martinique, Montserrat, Puerto Rico, Saint-Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin (French part), Saint Vincent and the Grenadines, Sint Maarten (Dutch part), Trinidad and Tobago, Turks and Caicos Islands, United States Virgin Islands
	Central America	Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama
	South America	Argentina, Bolivia (Plurinational State of), Brazil, Chile, Colombia, Ecuador, Falkland Islands (Malvinas), French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay, Venezuela (Bolivarian Republic of)
	Northern America	Bermuda, Canada, Greenland, Saint Pierre and Miquelon, United States
Asia	Central Asia	Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan
	Eastern Asia	China, China, Hong Kong Special Administrative Region China, Macao Special Administrative Region, Democratic People's Republic of, Korea, Japan, Mongolia, Republic of Korea
	Southern Asia	Afghanistan, Bangladesh, Bhutan, India, Iran (Islamic Republic of), Maldives, Nepal, Pakistan, Sri Lanka
	South-Eastern Asia	Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Philippines, Singapore, Thailand, Timor-Leste, Viet Nam
	Western Asia	Armenia, Azerbaijan, Bahrain, Cyprus, Georgia, Iraq, Israel, Jordan, Kuwait, Lebanon, Occupied Palestinian Territory, Oman, Qatar, Saudi Arabia, Syrian Arab Republic, Turkey, United Arab Emirates, Yemen

Europe	Eastern Europe	Bulgaria, Czech Republic, Hungary, Poland, Republic of Moldova, Romania, Russian Federation, Slovakia, Ukraine
	Northern Europe	Åland Islands, Channel Islands, Denmark, Estonia, Faeroe Islands, Finland, Guernsey, Iceland, Ireland, Isle of Man, Jersey, Latvia, Lithuania, Norway, Sark, Svalbard and Jan Mayen Islands, Sweden, United Kingdom of Great Britain and Northern Ireland
	Southern Europe	Andorra, Bosnia and Herzegovina, Croatia, Gibraltar, Greece, Holy See, Italy, Malta, Montenegro, Portugal, San Marino, Serbia, Slovenia, Spain, The former Yugoslav Republic of Macedonia
	Western Europe	Belgium, France, Germany, Liechtenstein, Luxembourg, Monaco, Netherlands, Switzerland
Oceania	Australia & New Zealand	Australia, New Zealand, Norfolk Island
	Melanesia	Fiji, New Caledonia, Papua New Guinea, Solomon Islands Vanuatu
	Micronesia	Guam, Kiribati, Marshall Islands, Micronesia (Federated States of), Nauru, Northern Mariana Islands, Palau
	Polynesia	American Samoa, Cook Islands, French Polynesia, Niue Pitcairn, Samoa, Tokelau, Tonga, Tuvalu, Wallis and Futuna Islands

Classification of travel destinations into subregions according to United Nations geoscheme

Logistic regression analyses

To identify predictors associated with ESBL-acquisition during travel we used the purposeful selection method as proposed by Hosmer and Lemeshow. The selection process began by a bivariable logistic regression analysis for each individual predictor as independent variable and ESBL-E acquisition as dependent variable, while adjusting for travel destination. As travel destination appeared a strong confounder, this approach was used instead of a univariable regression as originally proposed by Hosmer and Lemeshow. Before starting the multivariable analysis, variables were tested for multicollinearity and for interaction with travel destination (subregion). Variables that showed interaction with travel destination were removed. Subsequently, all potential predictors with $p<0.25$ in the bivariable regression were entered together in a multivariable logistic regression model. This multivariable model was reduced by removing variables one at the time if they were neither statistically significant ($p< 0.05$, starting with the variable with the highest p-value) nor a confounder (change in one of the remaining parameter estimates greater than 20% compared to the full model). At the end of this step the model contained only significant variables and confounders. Thereafter all potential predictors that were initially not selected for the multivariable regression model (i.e. those with $p>0.25$ in the bivariable regression models) were added separately to the model with significant variables and confounders retained earlier. This step can be helpful in identifying predictors that, by themselves, are not significantly related to the outcome but make an important contribution in the presence of other variables. All variables that appear significant at $p<0.15$ were put in the model and the model is iteratively reduced as before. The final model only included significant variables ($p<0.05$) and confounders, presented with odds ratio's and accompanying 95% confidence intervals. Because several dietary variables (consumption chicken, BBQ meat, pork) interacted with travel destination in association to ESBL-E acquisition (effect modification), we decided to perform separate bivariable and multivariable analyses for the subregions South-Eastern Asia, Southern Asia and Eastern Africa. The logistic regression analyses per subregion were conducted following the same approach, with the exception that analyses were now adjusted for country of travel destination instead of subregion. See online supplementary material table E1 for all the potential predictors considered.

Cox regression analyses

Univariable Cox regression analyses were conducted to identify predictors associated with decolonization. Variables associated with $p< 0.05$ were subsequently entered into a multivariable Cox regression model. See the online supplementary material table E5 for all the potential predictors considered.

SUPPLEMENTARY TABLES

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847).

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Median age in years [IQR]						
	50·76 [33·04:60·73]	50·52 [32·33:59·95]	1·00 (0·99-1·00)	0·220		
Gender						
Male	847 (46·03%)	295/847 (34·83%)	1·00			
Female	993 (54·97%)	336/993 (33·84%)	0·97 (0·78-1·19)	0·748		
Median weight in kg [IQR]						
	75·00 [66·00:85·00]	76·00 [66·00:85·00]	1·00 (1·00-1·01)	0·521		
Country of birth						
Netherlands†	1719 (93·37%)	590/1719 (34·32%)	1·00			
Other country	122 (6·63%)	40/122 (32·79%)	0·83 (0·54-1·27)	0·391		
Education level						
No education, elementary school or pre-vocational secondary education †	234 (12·72%)	78/234 (33·33%)	1·00			
Vocational secondary education	266 (14·46%)	91/266 (34·21%)	1·08 (0·72-1·61)	0·707		
Senior general secondary education or pre-university education	181 (9·84%)	62/181 (34·25%)	0·90 (0·58-1·42)	0·657		
Higher professional education	599 (32·55%)	204/599 (34·06%)	1·04 (0·73-1·47)	0·846		
Academic education	560 (30·43%)	195/560 (34·82%)	1·04 (0·73-1·49)	0·817		
Housing						
Flat or apartment†	659 (35·82%)	223/659 (33·84%)	1·00			
Terraced house	621 (33·75%)	207/621 (33·33%)	1·00 (0·78-1·28)	0·985		
Semi-detached house	207 (11·25%)	74/207 (35·75%)	1·25 (0·88-1·78)	0·220		
Detached house	271 (14·73%)	93/271 (34·32%)	0·99 (0·71-1·37)	0·944		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Other	82 (4·46%)	32/82 (39·02%)	1·04 (0·62-1·76)	0·886		
Cigarette smoking						
No†	1329 (77·09%)	473/1329 (35·59%)	1·00			
Yes	395 (22·91%)	115/395 (29·11%)	0·75 (0·575-0·979)	0·034		
Alcohol use (median, number of glasses per week [IQR]						
	5·00 [2·00:10·00]	5·00 [2·00-10·00]	1·01 (0·99-1·02)	0·381		
Diarrhoea before travel§						
No diarrhoea	1447 (78·73%)	482/1447 (33·31%)	1·00			
Diarrhoea in the past three months	349 (18·99%)	130/349 (37·25%)	1·19 (0·92-1·56)	0·192		
Diarrhoea at this moment only	0 (0·00%)	0 (0·00%)				
Diarrhoea in the past three months and at this moment	42 (2·29%)	16/42 (38·10%)	1·03 (0·52-2·06)	0·926		
Fever within three months prior to travel						
No†	1637 (88·82%)	561/1637 (34·27%)	1·00			
Yes	206 (11·18%)	69/206 (33·50%)	0·92 (0·66-1·28)	0·611		
Antibiotic use within three months prior to travel						
No†	1662 (90·62%)	568/1662 (34·18%)	1·00			
Yes	172 (9·38%)	59/172 (34·30%)	1·11 (0·78-1·57)	0·578		
Chronic disease¶						
No†	1404 (76·89%)	480/1404 (34·19%)				
Yes	422 (23·11%)	142/422 (33·65%)	1·03 (0·80-1·33)	0·802		
Chronic bowel disease¶						
No†	1793 (97·29%)	606/1793 (33·80%)	1·00		1·00	

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Yes	50 (2·71%)	24/50 (48·00%)	2·34 (1·26-4·34)	0·007	2·10 (1·13-3·90)	0·019
Antacid use						
No†	1607 (87·38%)	545/1607 (33·91%)	1·00			
Yes	232 (12·62%)	84/232 (36·21%)	1·17 (0·85-1·60)	0·344		
Corticosteroid use						
No†	1706 (93·17%)	594/1706 (34·82%)	1·00			
Topical use	78 (4·26%)	19/78 (·24·36%)	0·62 (0·35-1·10)	0·100		
Oral use	47 (2·57%)	10/47 (21·28%)	0·60 (0·28-1·27)	0·178		
Immunosuppressant use						
No†	1812 (99·3%)	618/1812 (34·11%)	1·00			
Yes	13 (0·7%)	6/13 (46·15%)	2·31 (0·68-7·89)	0·181		
Diet						
No diet†	1669 (90·56%)	563/1669 (33·73%)	1·00			
Vegetarian or vegan diet	93 (5·05%)	44/93 (47·31%)	1·54 (0·98-2·44)	0·063		
Other diet (Islamic, Jewish, other)	81 (4·40%)	23/81 (28·40%)	0·63 (0·36-1·08)	0·093		
Probiotics use						
Never†	1059 (57·93%)	357/1059 (33·71%)	1·00			
Rare	321 (17·56%)	126/321 (39·25%)	1·20 (0·91-1·58)	0·205		
Occasionally	233 (12·75%)	73/233 (31·33%)	0·93 (0·67-1·30)	0·673		
Frequent	102 (5·58%)	31/102 (30·39%)	0·80 (0·49-1·29)	0·357		
Daily	113 (6·18%)	36/113 (31·86%)	0·93 (0·59-1·46)	0·739		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Daily patient contact						
No profession in healthcare†	1421 (77·95%)	482/1421 (33·92%)	1·00			
Profession in healthcare without daily patient contact	99 (5·43%)	30/99 (30·30%)	0·88 (0·55-1·41)	0·583		
Medical profession in healthcare with daily patient contact	160 (8·78%)	67/160 (41·88%)	1·43 (0·99-2·06)	0·055		
Other profession in healthcare with daily patient contact	143 (7·84%)	44/143 (30·77%)	0·95 (0·64-1·43)	0·814		
Accommodation during travel[ll]						
Luxury†	396 (21·46%)	136/396 (34·34%)	1·00			
Hotel or apartment	457 (24·77%)	169/457 (36·98%)	1·20 (0·88-1·63)	0·240		
Low budget	205 (11·11%)	77/205 (37·56%)	1·29 (0·88-1·90)	0·200		
Tent	51 (2·76%)	10/51 (19·61%)	0·79 (0·35-1·81)	0·580		
Family or local people	91 (4·93%)	27/91 (29·67%)	1·03 (0·60-1·77)	0·920		
Ship	31 (1·68%)	8/31 (25·81%)	0·93 (0·38-2·26)	0·870		
Other	72 (3·90%)	17/72 (23·61%)	0·96 (0·50-1·84)	0·910		
Several	542 (29·38%)	187/542 (34·50%)	1·14 (0·85-1·54)	0·380		
Purpose of travel						
Holiday†	1553 (84·17%)	540/1553 (34·77%)	1·00			
Work/internship	154 (8·35%)	47/154 (30·52%)	0·84 (0·56-1·25)	0·390		
Visit to family or friends	77 (4·17%)	24/77 (31·17%)	0·98 (0·58-1·66)	0·950		
Other	61 (3·31%)	20/61 (32·79%)	0·82 (0·45-1·51)	0·530		
Type of holiday						
Luxury						
No†	1698 (92·03%)	580/1698 (34·16%)	1·00			

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Yes	147 (7·97%)	51/147 (34·69%)	1·01 (0·69-1·48)	0·950		
Beach						
No†	1404 (76·10%)	504/1404 (35·90%)	1·00		1·00	
Yes	441 (23·90%)	127/441 (28·80%)	0·72 (0·55-0·93)	0·010	0·73 (0·56-0·95)	0·021
Active in nature						
No†	1172 (63·52%)	416/1172 (35·49%)	1·00			
Yes	673 (36·48%)	215/673 (31·95%)	0·90 (0·72-1·13)	0·370		
Backpacking						
No†	1442 (78·16%)	453/1442 (31·41%)	1·00			
Yes	403 (21·84%)	178/403 (44·17%)	1·55 (1·20-1·99)	<0·001		
Safari						
No†	1522 (82·49%)	554/1522 (36·40%)	1·00			
Yes	323 (17·51%)	77/323 (23·84%)	1·01 (0·67-1·52)	0·960		
Citytrip						
No†	1446 (78·37%)	481/1446 (33·26%)	1·00			
Yes	399 (21·63%)	150/399 (37·59%)	1·06 (0·82-1·38)	0·640		
Spiritual						
No†	1821 (98·70%)	620/1821 (34·05%)	1·00			
Yes	24 (1·30%)	11/24 (45·83%)	0·91 (0·36-2·30)	0·840		
Other						
No†	1489 (80·70%)	493/1489 (33·11%)	1·00			
Yes	356 (19·30%)	138/356 (38·76%)	1·09 (0·84-1·42)	0·510		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Median duration of travel in days [IQR]						
	20·00 [15·00:25·00]	20·00 [15·00:24·00]	1·00 (0·99-1·01)	0·790		
Traveller's diarrhoea§						
No traveller's diarrhoea†	1085 (60·08%)	329/1085 (30·32%)	1·00		1·00	
Diarrhoea during travel	593 (32·83%)	235/593 (39·63%)	1·56 (1·24-1·96)	<0·001	1·42 (1·12-1·80)	0·003
Diarrhoea immediately after travel	41 (2·27%)	14/41 (34·15%)	1·19 (0·58-2·44)	0·640	1·30 (0·63-2·68)	0·477
Diarrhoea during and immediately after travel	87 (4·82%)	44/87 (50·57%)	2·42 (1·50-3·91)	<0·001	2·31 (1·42-3·76)	0·001
Fever during travel						
No†	1728 (93·76%)	575/1728 (33·28%)	1·00			
Yes	115 (6·24%)	55/115 (47·83%)	1·57 (1·04-2·39)	0·030		
Medical care during travel						
None†	1767 (95·93%)	591/1767 (33·45%)	1·00			
Visit to doctor or hospital	75 (4·07%)	39/75 (52·00%)	2·10 (1·27-3·47)	<0·001		
Antibiotic use during travel						
No†	1697 (92·78%)	553/1697 (32·59%)	1·00		1·00	
Yes	132 (7·22%)	73/132 (55·30%)	2·65 (1·80-3·91)	<0·001	2·69 (1·79-4·05)	<0·001
Use anti-diarrhoeal drugs during travel						
No†	1460 (79·13%)	475/1460 (32·53%)				
Yes	385 (20·87%)	156/385 (40·52%)	1·36 (1·06-1·74)	0·020		
Use analgesics during travel						
No†	1510 (81·84%)	511/1510 (33·84%)	1·00			
Yes	335 (18·16%)	120/335 (35·82%)	1·02 (0·78-1·33)	0·900		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Use antacids during travel						
No†	1752 (94·96%)	598/1752 (34·13%)	1·00			
Yes	93 (5·04%)	33/93 (35·48%)	1·11 (0·69-1·80)	0·670		
Use other medication without prescription during travel						
No†	1631 (88·40%)	556/1631 (34·09%)	1·00			
Yes	214 (11·60%)	75/214 (35·05%)	1·14 (0·82-1·58)	0·440		
Activities during travel						
Attendance large (religious) gathering						
No†	1744 (94·58%)	595/1744 (34·12%)	1·00		1·00	
Yes	100 (5·42%)	36/100 (36·00%)	0·56 (0·34-0·92)	0·020	0·57 (0·34-0·94)	0·028
Visit to local market						
Never†	234 (12·69%)	65/234 (27·78%)	1·00			
Occasionally	1406 (76·25%)	462/1406 (32·86%)	1·00 (0·71-1·41)	0·990		
Daily	204 (11·06%)	104/204 (50·98%)	2·04 (1·32-3·15)	<0·001		
Visit to local population daily						
No†	1488 (80·69%)	499/1488 (33·53%)	1·00			
Yes	356 (19·31%)	132/356 (37·08%)	1·12 (0·86-1·46)	0·390		
Contact with orphan children						
No†	1566 (84·92%)	532/1566 (33·97%)	1·00			
Yes	278 (15·08%)	99/278 (35·61%)	1·15 (0·84-1·56)	0·380		
Contact with patients						
No†	1773 (96·15%)	609/1773 (34·35%)	1·00			

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Yes	71 (3·85%)	22/71 (30·99%)	1·07 (0·60-1·89)	0·830		
Daily contact with animals						
No†	1743 (94·52%)	601/1743 (34·48%)	1·00			
Yes	101 (5·48%)	30/101 (29·70%)	0·97 (0·60-1·56)	0·890		
Stay in rural area daily						
No†	1376 (74·62%)	496/1376 (36·05%)	1·00			
Yes	468 (25·38%)	135/468 (28·85%)	0·88 (0·68-1·15)	0·350		
Trek through jungle						
No†	1026 (55·64%)	370/1026 (36·06%)	1·00			
Yes	818 (44·36%)	261/818 (31·91%)	0·95 (0·76-1·18)	0·620		
Swim in sea daily						
No†	1689 (91·59%)	581/1689 (34·40%)	1·00			
Yes	155 (8·41%)	50/155 (32·26%)	0·90 (0·62-1·30)	0·570		
Swim in swimming pool daily						
No†	1622 (87·96%)	565/1622 (34·83%)	1·00			
Yes	222 (12·04%)	66/222 (29·73%)	0·81 (0·58-1·12)	0·200		
Swim in other waters						
No†	1313 (71·20%)	463/1313 (35·26%)	1·00			
Yes	531 (28·80%)	168/531 (31·64%)	1·01 (0·79-1·28)	0·950		
Daily hand hygiene before meals during travel						
No daily hand hygiene before meals†	782 (42·41%)	265/782 (33·89%)	1·00		1·00	
Daily hand washing before meals with alcohol	161 (8·73%)	69/161 (42·86%)	1·03 (0·71-1·51)	0·870	0·97 (0·66-1·44)	0·885

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Daily hand washing before meals with soap	666 (36·12%)	200/666 (30·03%)	0·82 (0·64-1·04)	0·100	0·77 (0·60-0·99)	0·044
Daily hand washing before meals with alcohol and soap	235 (12·74%)	97/235 (41·28%)	1·03 (0·74-1·44)	0·860	1·12 (0·79-1·59)	0·518
Daily hand hygiene after toilet use during travel						
No daily hand hygiene after toilet use†	372 (20·17%)	128/372 (34·41%)	1·00			
Daily hand washing after toilet use with alcohol	115 (6·24%)	49/115 (42·61%)	1·02 (0·64-1·63)	0·930		
Daily hand washing after toilet use with soap	1085 (58·84%)	341/1085 (31·43%)	0·91 (0·69-1·19)	0·470		
Daily hand washing after toilet use with alcohol and soap	272 (14·75%)	113/272 (41·54%)	1·05 (0·74-1·50)	0·790		
Location of meals during travel						
Daily meal at hotel						
No†	1244 (67·46%)	420/1244 (33·76%)	1·00			
Yes	600 (32·54%)	211/600 (35·17%)	0·90 (0·71-1·13)	0·370		
Daily meal at hostel/guesthouse						
No†	1708 (92·62%)	585/1708 (34·25%)	1·00			
Yes	136 (7·38%)	46/136 (33·82%)	1·03 (0·68-1·56)	0·900		
Daily meal at luxurious/ star restaurant						
No†	1725 (93·55%)	587/1725 (34·03%)	1·00			
Yes	119 (6·45%)	44/119 (36·97%)	1·16 (0·76-1·77)	0·490		
Daily meal at local restaurant						
No†	1211 (65·67%)	389/1211 (32·12%)	1·00			
Yes	633 (34·33%)	242/633 (38·23%)	1·28 (1·02-1·60)	0·030		
Meal at food stalls along the road						
Never†	1248 (67·68%)	386/1248 (30·93%)	1·00		1·00	

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Occasionally	513 (27·82%)	205/513 (39·96%)	1·37 (1·08-1·73)	0·010	1·33 (1·04-1·71)	0·022
Daily	83 (4·50%)	40/83 (48·19%)	2·09 (1·30-3·38)	0·003	1·78 (1·07-2·95)	0·025
Daily meal at home with family or local population during travel						
No†	1719 (93·22%)	596/1719 (34·67%)	1·00			
Yes	125 (6·78%)	35/125 (28·00%)	0·87 (0·56-1·35)	0·530		
Food consumption during travel						
Tap water¶						
Hardly ever†	1631 (88·55%)	597/1631 (36·60%)	1·00			
Occasionally	86 (4·67%)	17/86 (19·77%)	0·69 (0·38-1·24)	0·220		
Often	125 (6·79%)	17/125 (13·60%)	0·54 (0·31-0·95)	0·030		
Ice in soda¶						
Hardly ever†	967 (52·50%)	363/967 (37·54%)	1·00			
Occasionally	454 (24·65%)	136/454 (29·96%)	0·88 (0·67-1·15)	0·340		
Often	421 (22·86%)	132/421 (31·35%)	1·03 (0·78-1·37)	0·830		
Beef¶						
Hardly ever†	581 (31·54%)	247/581 (42·51%)	1·00			
Occasionally	803 (43·59%)	234/803 (29·14%)	0·80 (0·62-1·02)	0·080		
Often	458 (24·86%)	150/458 (32·75%)	1·04 (0·78-1·39)	0·780		
Pork¶						
Hardly ever†	967 (52·50%)	352/967 (36·40%)	1·00			
Occasionally	637 (34·58%)	179/637 (28·10%)	0·89 (0·69-1·13)	0·330		
Often	238 (12·92%)	100/238 (42·02%)	1·42 (1·03-1·96)	0·030		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Chicken¶						
Hardly ever†	290 (15·74%)	125/290 (43·10%)	1·00			
Occasionally	643 (34·91%)	198/643 (30·79%)	0·67 (0·49-0·92)	0·010		
Often	909 (49·35%)	308/909 (33·88%)	0·67 (0·49-0·90)	0·010		
Other meat¶						
Hardly ever†	1235 (67·05%)	453/1235 (36·68%)	1·00			
Occasionally	480 (26·06%)	146/480 (30·42%)	0·96 (0·75-1·23)	0·770		
Often	127 (6·89%)	32/127 (25·20%)	0·74 (0·47-1·16)	0·190		
BBQ meat¶						
Hardly ever†	1224 (66·45%)	453/1224 (37·01%)	1·00			
Occasionally	499 (27·09%)	153/499 (30·66%)	1·00 (0·78-1·27)	0·980		
Often	119 (6·46%)	25/119 (21·01%)	0·73 (0·45-1·18)	0·200		
Undercooked meat¶						
Hardly ever†	1712 (92·94%)	597/1712 (34·87%)	1·00			
Occasionally or often	130 (7·06%)	34/130 (26·15%)	1·21 (0·78-1·87)	0·400		
Raw meat¶						
Never†	1766 (95·87%)	608/1766 (34·43%)	1·00			
Ever	76 (4·13%)	23/76 (30·26%)	1·11 (0·64-1·92)	0·720		
Eggs¶						
Hardly ever†	222 (12·05%)	71/222 (31·98%)	1·00			
Occasionally	593 (32·19%)	183/593 (·30·86%)	1·08 (0·75-1·55)	0·680		
Often	1027 (55·75%)	377/1027 (36·71%)	1·20 (0·86-1·68)	0·290		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Raw vegetables¶						
Hardly ever†	478 (25·95%)	169/478 (35·36%)	1·00			
Occasionally	665 (36·10%)	247/665 (37·14%)	1·32 (1·01-1·73)	0·040		
Often	699 (37·95%)	215/699 (30·76%)	1·21 (0·92-1·59)	0·180		
Salad prepared by others¶						
Hardly ever†	639 (34·69%)	243/639 (38·03%)	1·00			
Occasionally	659 (35·78%)	217/659 (32·93%)	1·00 (0·78-1·28)	1·000		
Often	544 (29·53%)	171/544 (31·43%)	1·11 (0·84-1·45)	0·470		
Unpeeled fruit¶						
Hardly ever†	1238 (67·21%)	436/1238 (35·22%)	1·00			
Occasionally	373 (20·25%)	119/373 (31·90%)	0·96 (0·73-1·26)	0·760		
Often	231 (12·54%)	76/231 (32·90%)	0·93 (0·68-1·29)	0·680		
Fruit prepared by others¶						
Hardly ever†	553 (30·02%)	181/553 (32·73%)	1·00			
Occasionally	598 (32·46%)	215/598 (35·95%)	1·22 (0·93-1·60)	0·150		
Often	691 (37·51%)	235/691 (34·01%)	1·28 (0·98-1·68)	0·070		
Shellfish¶						
Hardly ever†	1536 (83·39%)	525/1536 (34·18%)	1·00			
Occasionally or often	306 (16·61%)	106/306 (34·64%)	1·27 (0·96-1·69)	0·100		
Raw fish¶						
Hardly ever†	1683 (91·37%)	575/1683 (34·17%)	1·00			
Occasionally or often	159 (8·63%)	56/159 (35·22%)	1·30 (0·90-1·88)	0·160		

Table E1. Bivariable and multivariable logistic regression analyses on potential predictors for ESBL-E acquisition among at risk travellers (n = 1847). (continued)

	All travellers n (%)	Travellers with ESBL acquisition n/N (%)	Odds ratio (95%CI)*	p-value	Adjusted Odds ratio (95% CI)‡	p-value
Unpasteurized milk/cheese¶						
Hardly ever†	1690 (91·75%)	570/1690 (33·73%)	1·00			
Occasionally or often	152 (8·25%)	61/152 (40·13%)	1·50 (1·03-2·18)	0·040		
Fresh milk from bovine, sheep or goat[						
Never†	1781 (96·69%)	609/1781 (34·19%)	1·00			
Ever	61 (3·31%)	22/61 (36·07%)	0·83 (0·45-1·51)	0·540		

†reference category

*Only adjusted for travel destination defined as UN subregions: Caribbean/Central America, Middle/Eastern Africa, Central/Eastern Asia, Northern America/Europe/Oceania, Southern Asia, South-Eastern Asia, Western Asia, Northern Africa, Southern Africa, Western Africa, South America

‡Adjusted for travel destination as well as age, gender, weight, country of birth, education level, housing, alcohol use, diarrhoea, fever, antibiotic use, chronic disease, bowel disease, antacid/corticosteroid/immunosuppressant use, diet, probiotics use, daily patient contact, accommodation, purpose of travel, type of holiday, duration of travel (in days), traveller's diarrhoea, fever, medical care during travel, antibiotic use, use of medication without prescription (anti-diarrhoeal drugs;analgesics;antacids), activities during travel (attendance large (religious) gathering; visiting local markets, population; contact with orphan children/patients/animals, stay in rural area, trek through jungle, swim in sea/swimming pool/other waters, daily washing hands before eating/after toilet use, daily meal at hotel/ hostel, guesthouse/luxurious restaurant/local restaurant/, meal at food stalls along the road, daily meal at home with family or local population, consumption of tap water, ice in soda, beef, pork, chicken, other meat, BBQ meat, undercooked meat, raw meat, eggs, raw vegetables, salad prepared by others, unpeeled fruit, fruit prepared by others, shellfish, raw fish, unpasteurized milk/cheese, fresh milk from bovine, sheep or goat

§(Traveller's) diarrhoea was defined as three or more unformed stools per 24 hour period with or without accompanying symptoms

[Chronic disease and chronic bowel disease was considered to be present when self-reported to be present by traveller

||Luxury=all inclusive resort/4 or 5 star hotel, hotel or apartment= no star, 1,2, or 3 star hotel or apartment, low budget=low budget, sleep in, guesthouse, (youth) hostel

¶Hardly ever= never or less than once a week, occasionally= on average once a week, often= several times a week or daily

[Never=never, ever=less than once a week, on average once a week, several times a week or daily

Table E2. Incidence proportion and incidence rates (per 100 person-days of travel) for extended-spectrum beta-lactamase producing Enterobacteriaceae acquisition in Dutch travellers according to the most visited countries (n=1047).

Destination†	Travellers (n)	Travellers (n) with ESBL acquisition	ESBL incidence proportion		Travel-days all travellers	Mean duration of travel (SD)	ESBL incidence rate/100 pdt	
			%	95% CI‡			IR§	95% CI§
India	79	70	88·61	79·75-93·89	1647	20·85 (14·31)	12·18	9·05-16·25
Egypt	30	24	80·00	62·69-90·50	355	11·83 (3·70)	13·98	8·73-21·54
Nepal	29	23	79·31	61·61-90·15	623	21·48 (10·83)	7·49	4·64-11·57
Vietnam	36	26	72·22	56·01-84·15	833	23·14 (10·94)	6·10	3·91-9·14
Peru	20	12	60·00	38·66-78·12	430	21·50 (5·30)	4·28	2·26-7·37
China	67	36	53·73	41·92-65·14	1333	19·90 (11·28)	4·19	2·93-5·81
Myanmar	15	8	53·33	30·12-75·19	288	19·20 (3·95)	3·91	1·76-7·42
Thailand	89	46	51·69	41·45-61·78	1715	19·27 (5·77)	3·78	2·77-5·02
Sri Lanka	43	22	51·16	36·75-65·38	850	19·77 (6·34)	3·66	2·31-5·47
Uganda	27	12	44·44	27·59-62·69	586	21·70 (20·45)	2·65	1·41-4·49
Turkey	16	7	43·75	23·10-66·82	150	9·38 (2·85)	6·03	2·57-11·81
Ghana	20	8	40·00	21·88-61·34	372	18·60 (12·14)	2·65	1·20-4·97
Kenya	30	10	33·33	19·23-51·22	581	19·37 (16·47)	1·95	0·97-3·42
Malaysia	28	7	25·00	12·68-43·36	543	19·39 (6·18)	1·50	0·64-2·91
Tanzania	57	14	24·56	15·23-37·10	1010	17·72 (11·49)	1·73	0·99-2·76
Morocco	36	8	22·22	11·72-38·09	494	13·72 (7·21)	1·81	0·83-3·38
Mexico	18	4	22·22	9·00-45·22	271	15·06 (5·77)	1·73	0·53-4·03
Indonesia	211	40	18·96	14·24-24·78	4823	22·86 (8·95)	0·92	0·66-1·24
Gambia	49	8	16·33	8·51-29·04	669	13·65 (8·70)	1·30	0·59-2·43
Brazil	25	2	8·00	2·22-24·97	500	20·00 (7·64)	0·42	0·07-1·30
South Africa	66	3	4·55	1·56-12·53	1409	21·35 (8·31)	0·22	0·05-0·56
Suriname	56	2	3·57	0·99-12·12	1498	26·75 (15·54)	0·14	0·02-0·42

*ESBL, extended spectrum beta-lactamase; SD, standard deviation; 95% CI, 95% confidence interval; IR, incidence rate; pdt, person-days of travel

†data are shown for 22 countries with 15 or more visitors

‡based on binomial distribution (Wilson Score interval)

§calculated with maximum likelihood estimation method based on a constant acquisition rate with right-censored and interval-censored data

Table E3. Predictors for ESBL acquisition among at risk travellers to South-Eastern Asia (n = 540) in the final adjusted logistic regression model after manual stepwise elimination.

	Travellers at risk (%)‡	Travellers with ESBL acquisition n/N (%)	Adjusted odds ratio (95% CI)*	p-value
Antibiotic use during travel				
No†	485 (90·65%)	166/485 (34·23%)		
Yes	50 (9·35%)	32/50 (64·00%)	3·90 (1·90-7·98)	<0·001
Activities during travel				
Attendance large (religious) gathering				
No†	511 (94·81%)	193/511 (37·77%)		
Yes	28 (5·20%)	7/28 (25·00%)	0·29 (0·09-0·89)	0·031
Food consumption during travel				
Raw vegetables				
Hardly ever†	168 (31·17%)	43/168 (25·60%)		0·008
Occasionally	201 (37·29%)	79/201 (39·30%)	2·18 (1·29-3·68)	0·004
Often	170 (31·54%)	78/170 (45·88%)	1·97 (1·15-3·40)	0·014
Diet				
No diet†	490 (90·74%)	188/490 (38·37%)		0·007
Vegetarian or vegan diet	28 (5·19%)	10/28 (35·71%)	0·84 (0·28-2·53)	0·762
Other diet (Islamic, Jewish, other)	22 (4·07%)	2/22 (9·09%)	0·08 (0·02-0·38)	0·002

*Adjusted for travel destination and variables shown in bold
‡Numbers do not add up to 540 travellers because of missing data

Table E4. Predictors for ESBL acquisition among at risk travellers to Southern Asia (n = 181) in the final adjusted logistic regression model after manual stepwise elimination.

	Travellers at risk n (%)‡	Travellers with ESBL acquisition n/N (%)	Adjusted odds ratio (95% CI)*	p-value
Accommodation				
Luxury†	34 (18·78%)	28/34 (82·35%)		
Hotel or apartment	51 (28·18%)	39/51 (76·47%)	0·23 (0·05-0·96)	0·044
Low budget	26 (14·36%)	17/26 (65·38%)	0·09 (0·02-0·55)	0·008
Other	70 (38·67%)	52/70 (74·29%)	0·24 (0·06-1·00)	0·05
Contact with orphan children				
No†	141 (77·90%)	101/141 (71·63%)		
Yes	40 (22·10%)	35/40 (87·50%)	7·26 (1·74-30·26)	0·007
Daily meal at hostel/guesthouse daily during travel				
No†	159 (87·85%)	117/159 (73·58%)		
Yes	22 (12·15%)	19/22 (86·36%)	15·40 (2·62-90·56)	0·002
Daily meal at home with family or local population				
No†	171 (94·48%)	130/171 (76·02%)		
Yes	10 (5·52%)	6/10 (60·00%)	0·06 (0·01-0·42)	0·004
Frequency of consumption food				
Raw vegetables				
Hardly ever†	67 (37·02%)	57/67 (85·07%)		
Occasionally	73 (40·33%)	50/73 (68·49%)	0·34 (0·12-0·93)	0·036
Often	41 (22·65%)	29/41 (70·73%)	0·26 (0·08-0·87)	0·028
Shellfish				
Hardly ever†	171 (94·48%)	131/171 (76·61%)		
Occasionally or often	10 (5·52%)	5/10 (50·00%)	0·15 (0·03-0·78)	0·024

*Adjusted for travel destination and variables shown in bold

‡Numbers do not add up to 181 travellers because of missing data

Table E5. Predictors for ESBL acquisition among at risk travellers to Eastern Africa (n = 190) in the final adjusted logistic regression model after manual stepwise elimination.

	Travellers at risk n (%)‡	Travellers with ESBL acquisition n/N (%)	Adjusted odds ratio (95% CI)*	p-value
Accommodation				
Luxury†	32 (16·84%)	11/32 (34·38%)		
Hotel or apartment	35 (18·42%)	10/35 (28·57%)	0·63 (0·18-2·18)	0·466
Low budget	24 (12·63%)	6/24 (25·00%)	0·46 (0·11-2·01)	0·304
Other	99 (52·11%)	27/99 (27·27%)	0·54 (0·20-1·49)	0·235
Reason travel				
Holiday†	129 (67·89%)	37/129 (28·68%)		
Work/internship	31 (16·32%)	9/31 (29·03%)	0·76 (0·27-2·16)	0·607
Visit to family or friends	11 (5·79%)	5/11 (45·45%)	1·58 (0·36-6·86)	0·546
Other	19 (10·00%)	3/19 (15·79%)	0·38 (0·08-1·77)	0·215
Duration stay abroad (in days)	190	54	0·98 (0·94-1·02)	0·215
Visit to local market				
Never†	36 (18·95%)	12/36 (33·33%)		
Occasionally	132 (69·47%)	31/132 (23·48%)	0·62 (0·24-1·56)	0·307
Daily	22 (11·58%)	11/22 (50·00%)	4·89 (1·15-20·81)	0·032
Stay in rural area daily				
No†	87 (45·79%)	20/87 (22·99%)		
Yes	103 (54·21%)	34/103 (33·01%)	2·63 (1·20-5·79)	0·016
Swim in waters other than sea or swimming pool				
No†	159 (83·68%)	49/159 (30·82%)		
Yes	31 (16·32%)	5/31 (16·13%)	0·27 (0·07-1·02)	0·054
Location of meals during travel				
Meal at food stalls along the road during travel				
No†	187 (98·42%)	52/187 (27·81%)		
Yes	3 (1·58%)	2/3 (66·67%)	5·53 (0·26-119·66)	0·276
Gender				
Male	88 (46·56%)	29/88 (32·95%)		
Female	101 (53·44%)	25/101 (24·75%)	0·49 (0·23-1·06)	0·070

*Adjusted for travel destination and variables shown in bold
‡Numbers do not add up to 190 travellers because of missing data

Table E6. Univariable and multivariable Cox regression analyses on potential predictors for prolonged ESBL-E carriage upon acquisition during travel (n = 633).

	Travellers with ESBL acquisition n* (%)	Hazard ratio (95% CI)†	p-value	Adjusted Hazard ratio (95% CI)†‡	p-value
Genotype group					
CTX-M group 1 ESBL	399 (64·04%)				
CTX-M group 9 ESBL	175 (28·09%)	0·67 (0·56-0·81)	0·000	0·66 (0·53-0·81)	<0·001
CTX-M group 1 and CTX-M group 9 ESBL	10 (1·61%)	0·89 (0·44-1·79)	0·740	0·96 (0·47-1·94)	0·900
Other ESBL group	39 (6·26%)	1·12 (0·79-1·58)	0·532	0·95 (0·67-1·37)	0·797
ESBL species					
E. coli	537 (86·20%)				
K. pneumoniae	27 (4·33%)	2·23 (1·51-3·30)	0·000	2·17 (1·45-3·26)	<0·001
E. coli and *K. pneumoniae*	38 (6·10%)	1·28 (0·91-1·78)	0·155	1·27 (0·90-1·78)	0·176
Other species	21 (3·37%)	1·19 (0·75-1·89)	0·455	1·14 (0·71-1·82)	0·595
Age	617	1 (1·00-1·00)	0·234		
Gender					
Male	292 (47·02%)				
Female	329 (53·32%)	1·04 (0·88-1·23)	0·641		
Duration stay abroad (in days)	617	0·992 (0·986-0·999)	0·021	1·00 (0·99-1·00)	0·180
Bowel disease					
No†	596 (96·13%)				
Yes	24 (3·87%)	0·93 (0·62-1·42)	0·748		
Travel destination					
Central/Eastern Asia					
No†	580 (93·40%)				
Yes	41 (6·60%)	0·78 (0·56-1·11)	0·165	1·04 (0·68-1·59)	0·864
South-Eastern Asia					
No†	426 (68·60%)				
Yes	195 (31·40%)	0·90 (0·75-1·07)	0·222	1·07 (0·79-1·44)	0·671
Southern Asia					
No†	485 (78·10%)				
Yes	136 (21·90%)	0·87 (0·72-1·06)	0·171	0·87 (0·63-1·19)	0·376
Western Asia					
No†	609 (98·07%)				
Yes	12 (1·93%)	2·25 (1·27-4·00)	0·006	2·25 (1·20-4·22)	0·011
Northern Africa					
No†	587 (94·52%)				

Table E6. Univariable and multivariable Cox regression analyses on potential predictors for prolonged ESBL-E carriage upon acquisition during travel (n = 633). (continued)

	Travellers with ESBL acquisition n* (%)	Hazard ratio (95% CI)†	p-value	Adjusted Hazard ratio (95% CI)†‡	p-value
Yes	34 (5·48%)	1·35 (0·94-1·93)	0·101	1·29 (0·83-2·00)	0·264
Middle/Eastern Africa					
No†	564 (90·82%)				
Yes	57 (9·18%)	1·53 (1·16-2·02)	0·003	1·42 (0·98-2·07)	0·066
Southern Africa					
No†	616 (99·19%)				
Yes	5 (0·81%)	1·60 (0·66-3·86)	0·297	2·10 (0·84-5·26)	0·115
Western Africa					
No†	602 (96·94%)				
Yes	19 (3·06%)	1·09 (0·68-1·75)	0·712	0·90 (0·53-1·54)	0·707
Central America/ Caribbean					
No†	597 (96·14%)				
Yes	24 (3·86%)	1·14 (0·75-1·74)	0·537	1·21 (0·74-1·97)	0·449
South America					
No†	588 (94·69%)				
Yes	33 (5·31%)	1·06 (0·74-1·53)	0·743	1·04 (0·67-1·62)	0·846
Northern America/Europe/Oceania					
No†	620 (99·84%)				
Yes	1 (0·16%)	1·01 (0·14-7·19)	0·992	1·06 (0·15-7·66)	0·957
Traveller's diarrhoea					
No traveller's diarrhoea†	327 (53·34%)				
Diarrhoea during travel	229 (37·36%)	0·86 (0·72-1·02)	0·087		
Diarrhoea immediately after travel	14 (2·28%)	1·68 (0·98-2·87)	0·060		
Diarrhoea during travel and immediately after travel	43 (7·01%)	1·00 (0·73-1·37)	0·988		
Antibiotic use during travel					
No†	543 (88·15%)				
Yes	73 (11·85%)	0·82 (0·63-1·06)	0·124		
Antibiotic use in period since return (up to 1 month after return)					
No†	572 (94·70%)				
Yes	32 (5·30%)	1·01 (0·70-1·47)	0·941		

*Number of travellers with ESBL acquisition do not add up to 633 because of missing data
†Hazard ratio <1 indicated a decreased risk for decolonization
‡Adjusted for travel destination, ESBL genotype, ESBL-E species, age, gender, chronic disease, bowel disease, duration stay abroad
(in days), traveller's diarrhoea (yes/no), antibiotic use (yes/no), antibiotic use in period since return (up to 1 month after return)

Table E7. Predictors for prolonged ESBL-E carriage upon acquisition (n = 633) during travel in the final adjusted Cox regression model.

	Travellers with ESBL acquisition n*	Hazard ratio (95%CI)	p-value
Genotype group			
CTX-M group 1 ESBL	399 (64·04%)		
CTX-M group 9 ESBL	175 (28·09%)	0·66 (0·53-0·81)	<0·001
CTX-M group 1 and CTX-M group 9 ESBL	10 (1·61%)	0·96 (0·47-1·94)	0·900
Other ESBL genotype	39 (6·26%)	0·95 (0·67-1·37)	0·797
ESBL species			
E. coli	537 (86·20%)		
K. pneumoniae	27 (4·33%)	2·17 (1·45-3·26)	<0·001
E. coli and *K. pneumoniae*	38 (6·10%)	1·27 (0·90-1·78)	0·176
Other species	21 (3·37%)	1·14 (0·71-1·82)	0·595
Travel destination			
Central/Eastern Asia			
No†	580 (93·40%)		
Yes	41 (6·60%)	1·04 (0·68-1·59)	0·864
South-Eastern Asia			
No†	426 (68·60%)		
Yes	195 (31·40%)	1·07 (0,79-1,44)	0·671
Southern Asia			
No†	485 (78·10%)		
Yes	136 (21·90%)	0·87 (0,63-1,19)	0·376
Western Asia			
No†	609 (98·07%)		
Yes	12 (1·93%)	2·25 (1·20-4·22)	0·011
Northern Africa			
No†	587 (94·52%)		
Yes	34 (5·48%)	1·29 (0·83-2·00)	0·264
Middle/Eastern Africa			
No†	564 (90·82%)		
Yes	57 (9·18%)	1·42 (0·98-2·07)	0·066
Southern Africa			
No†	616 (99·19%)		
Yes	5 (0·81%)	2·10 (0·84-5·26)	0·115
Western Africa			
No†	602 (96·94%)		
Yes	19 (3·06%)	0·90 (0·53-1·54)	0·707
Central America/ Caribbean			

Table E7. Predictors for prolonged ESBL-E carriage upon acquisition (n = 633) during travel in the final adjusted Cox regression model. (continued)

	Travellers with ESBL acquisition n*	**Hazard ratio (95%CI)**	**p-value**
No†	597 (96·14%)		
Yes	24 (3·86%)	1·21 (0·74-1·97)	0·449
South America			
No†	588 (94·69%)		
Yes	33 (5·31%)	1·04 (0·67-1·62)	0·846
Northern America/Europe/Oceania			
No†	620 (99·84%)		
Yes	1 (0·16%)	1·06 (0·15-7·66)	0·957
Duration stay abroad (in days)	617	0·996 (0·989-1·002)	0·180

*Number of travellers with ESBL acquisition do not add up to 633 because of missing data
†Hazard ratio <1 indicated a decreased risk for decolonization

Table E8. Estimation of annual travel-related ESBL-E acquisition in Dutch population in 2013 (excluding Northern America, Europe & Oceania).

Region	acquisition rate		Dutch travellers per year¶	% of Dutch population		
	%	95% CI		(n = 16.781.000)§	Risk*	95% CI Risk*
Southern Asia	75·14	63·51-88·89	48·000	0·29	0·00214929	0·00181663-0·00254259
Central and Eastern Asia	48·81	35·94-66·29	78·000	0·46	0·00226874	0·00167053-0·00308123
Western Asia	42·86	24·34-75·46	895·000	5·33	0·02285901	0·01298153-0·04024593
Northern Africa	41·98	29·99-58·75	338·000	2·01	0·00845554	0·00604053-0·01183332
South-Eastern Asia	37·04	32·24-42·54	203·000	1·21	0·00448073	0·00390008-0·00514607
Caribbean and Central America	27·91	18·71-41·64	186·000	1·11	0·00309353	0·00207381-0·00461536
Middle and Eastern Africa	27·80	21·45-36·05	51·000	0·30	0·00084488	0·00065190-0·00109561
Western Africa	18·87	12·17-29·25	67·000	0·40	0·00075341	0·00048590-0·00116784
South America	18·33	13·03-25·79	61·000	0·36	0·00066631	0·00047365-0·00093748
Southern Africa	6·03	2·88-12·66	45·000	0·27	0·00016170	0·00007723-0·00033949
Yearly risk					**0·04573314**	**0·03017178-0·07100494**
Daily Risk					**0·00012530**	**0·00008266-0·00019453**

¶Based on Centraal Bureau voor de Statistiek (CBS): Long holidays per country and continent 2002-2013[8] Travellers visiting the same regions multiple times or visiting multiple regions will be counted multiple times

§ Number of travellers visiting a region divided by the size of the total Dutch population according to the Centraal Bureau voor de Statistiek at 1 January 2013

*Acquisition proportion multiplied by proportion of Dutch population annually visiting this region

SUPPLEMENTARY FIGURES

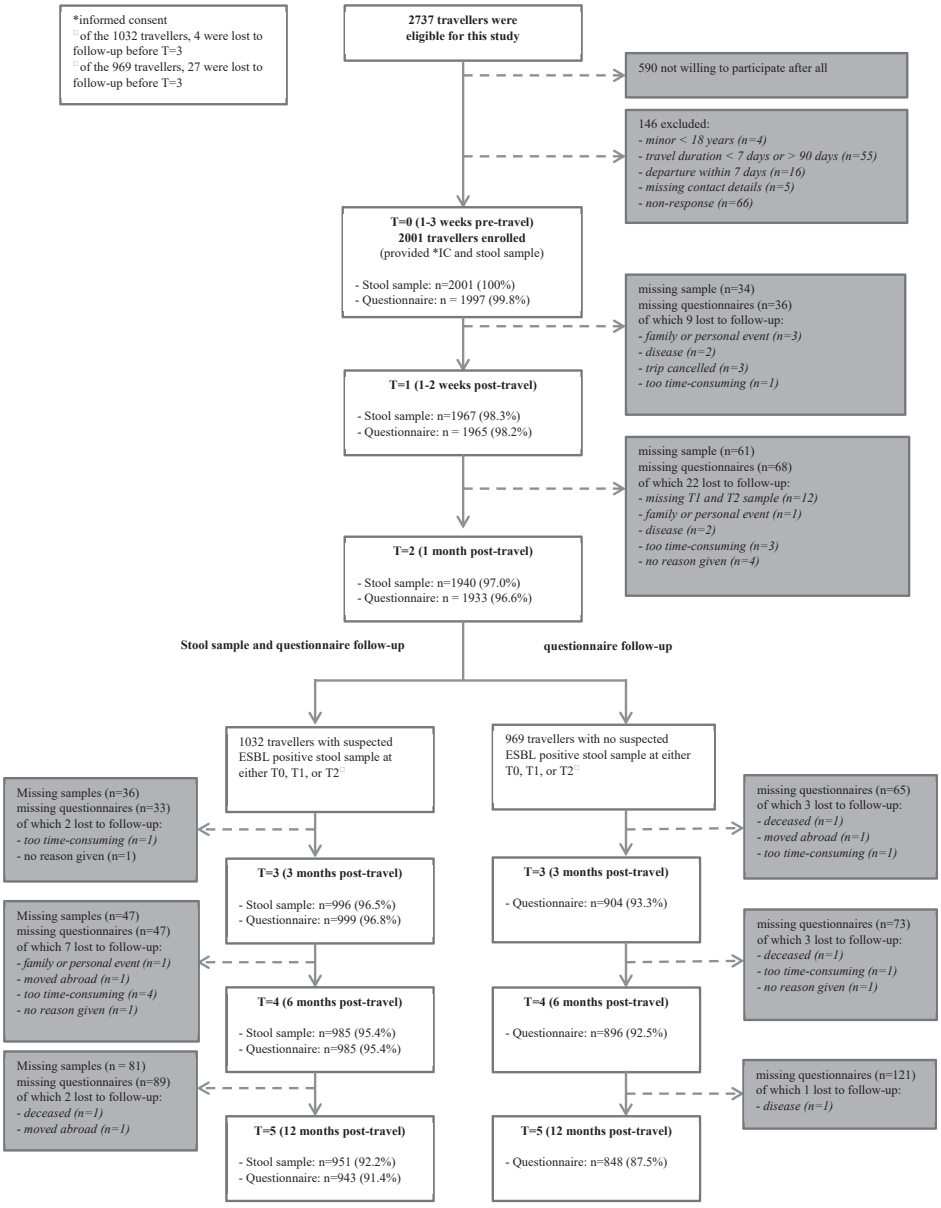


Figure E1. Flowchart of study.

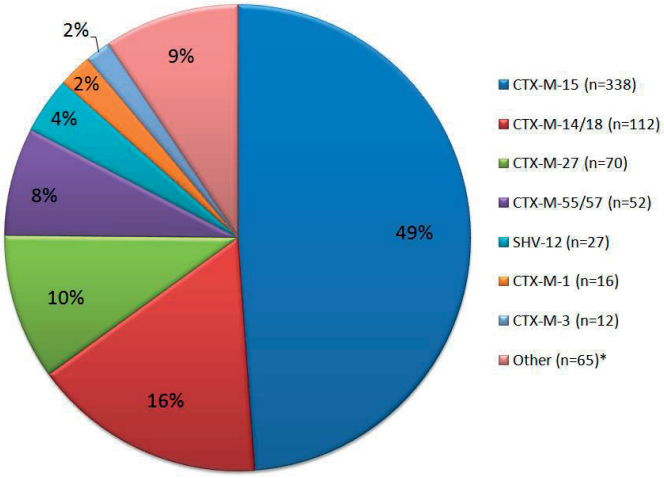
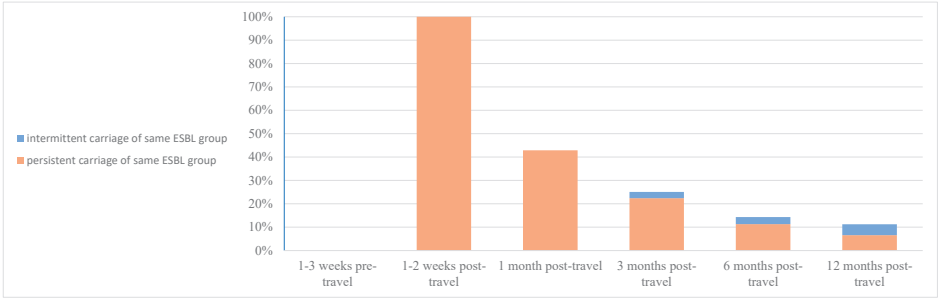


Figure E2. Acquisition of unique ESBL genes (n = 692) by travellers (n = 633) with ESBL-E acquisition. *Other: CTX-M-14-like (n=10), CTX-M group 8 (n=9), CTX-M-65 (n=8), CTX-M-32 (n=7), CTX-M group 2 (n=7), CTX-M-24b (n=6), TEM-52c (n=4), SHV-2a (n=3), CTX-M-24 (n=2), TEM-176 (n=2), CTX-M-15 like (n=2), CTX-M-38 (n=1), SHV-2 (n=1), SHV-28 (n=1),VEB (n=1), CTX-M group 1 not specified(n=1)



persistent carriage of same ESBL group	0% (0/633)	100% (623/623)	42.9% (263/613)	22.4% (136/606)	11.3% (68/601)	6.6% (38/577)
intermittent carriage of same ESBL group				2.6% (16/606)	3.0% (18/601)	4.7% (27/577)

Figure E3. Duration of ESBL-E carriage among travellers with ESBL-E acquisition (n = 633).

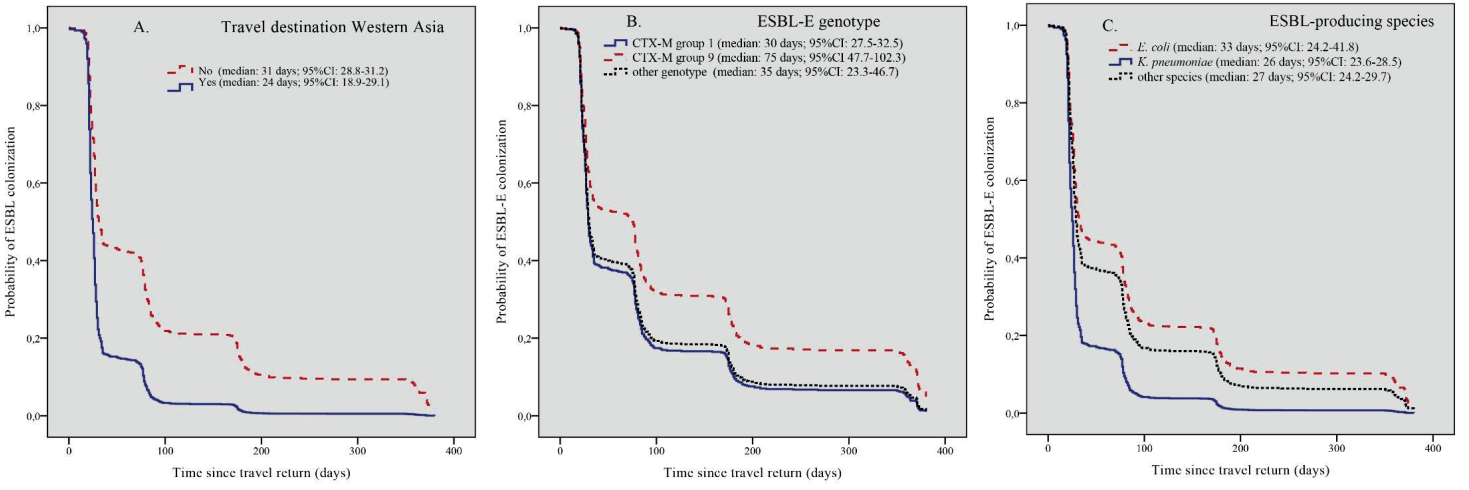


Figure E4. Stratified Cox regression plots time to decolonization of ESBL-E in travellers according to travel destination Western Asia (A), ESBL genotype (B) and ESBL-producing species (C).

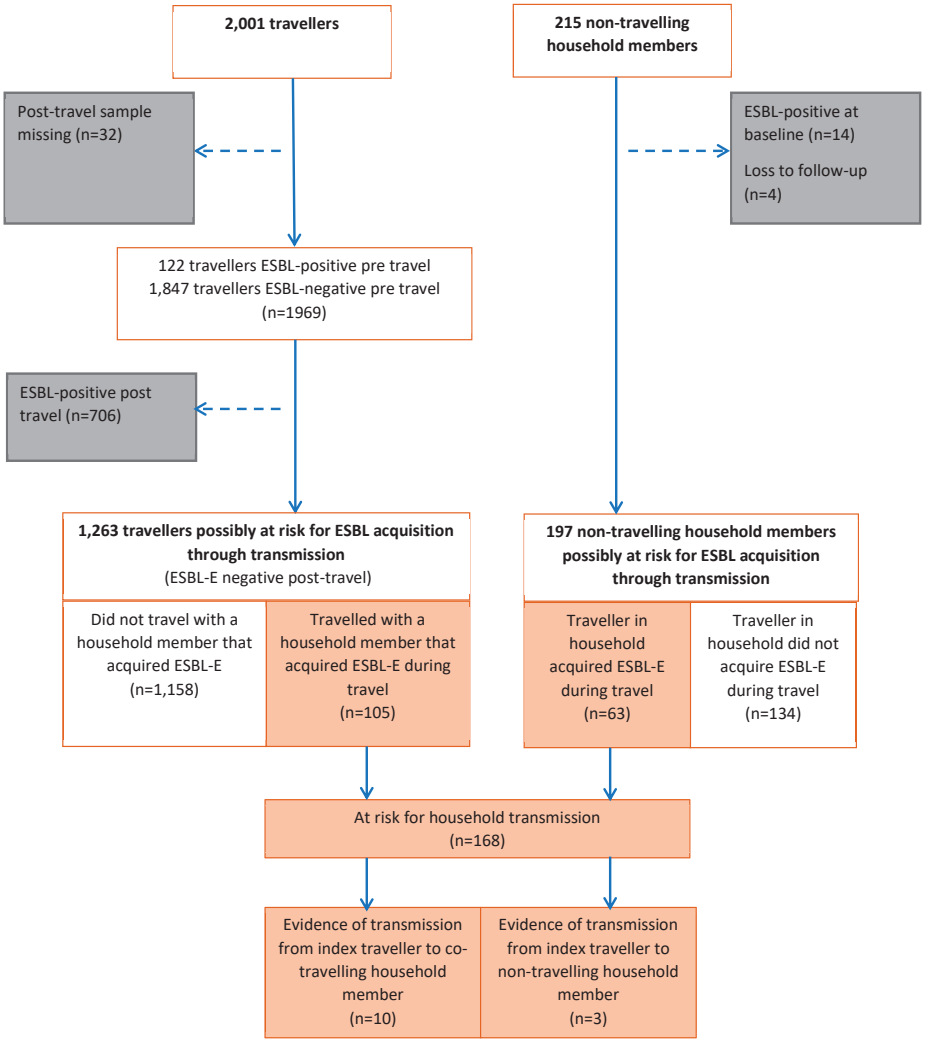


Figure E5. Flow chart of non-travelling and co-travelling household members at risk for acquisition of ESBL-E through transmission from household members that acquired ESBL-E during travel.

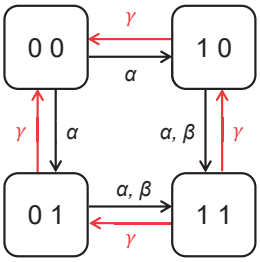


Figure E6. Model to estimate the transmission rate within households with 2 household members. Every square is a household, with an ESBL-negative (0) and/or positive (1) household member. Rates in red correspond to decolonization of an individual, rates in black correspond to acquisition. Alpha (α) = background acquisition rate. Beta (β) = within-household transmission rate. Gamma (γ) = decolonization rate.

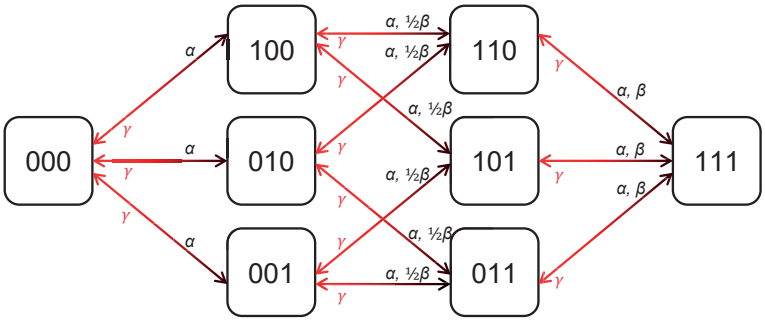


Figure E7. Model to estimate the transmission rate within households with 3 household members. Every square is a household, with ESBL-negative (0) and/or positive (1) household members. Rates in red (below the arrow) correspond to decolonization of an individual (from right to left), rates in black (above the arrow) correspond to acquisition (from left to right). Alpha (α) = background acquisition rate. Beta (β) = within-household transmission rate. Gamma (γ) = decolonization rate.

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CHAPTER 6

Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travellers

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Future Microbiology. 2016 Jul;11:857-64.

ABSTRACT

Aim

The aim was to study acquisition and persistence of carbapenemase-producing Enterobacteriaceae (CPE) among travellers.

Materials & methods

Stools from 2001 travellers and 215 nontraveling household members, collected before and immediately post-travel as well as 1, 3, 6 and 12 months upon return, were screened for CPE.

Results

Five travellers, all visiting Asia outside the Indian subcontinent, acquired CPE. One traveler persistently carried the same OXA-244 CPE up to 6 months post-travel. Three months after travel, her co-traveling spouse also became positive for this OXA-244 CPE strain, suggesting clonal transmission within this household.

Conclusion

Acquisition of CPE is not restricted to travellers to the Indian subcontinent and/or to travellers seeking healthcare during travel and can persist up to at least 6 months post-travel.

INTRODUCTION

The Indian subcontinent has been identified as an important reservoir of carbapenemase-producing Enterobacteriaceae (CPE). Indeed, acquisition of CPE during travel to India has recently been reported, illustrating the risk of further global spread by travellers to this CPE-endemic region [1]. However, acquisition of CPE in healthy travellers to Asian regions other than the Indian subcontinent is suggested but has not been reported from prospective studies thus far. There are a number of prospective studies on acquisition of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) and CPE in healthy travellers [1–10]. Except for the above-mentioned study on CPE acquisition in travellers to India [1], none of these studies identified subjects with CPE acquisition during travel.

Within the context of a large-scale prospective cohort of healthy travellers, the COMBAT study, we aimed to determine CPE acquisition, persistence of colonization and potential onward transmission.

MATERIALS & METHODS

Design & data collection

As part of the COMBAT study (ClinicalTrials.gov identifier: NCT01676974), a multicenter longitudinal cohort of healthy travellers (n = 2001) and their nontraveling household members (n = 215) was followed. Participants were recruited within a period of 1 year, from November 2012 until November 2013 at the outpatient clinics run by the Academic Medical Center (Amsterdam, The Netherlands), Havenziekenhuis (Rotterdam, The Netherlands) and Maastricht University Medical Center/Public Health Service South Limburg (Maastricht, The Netherlands). Adult (≥18 years) travellers visiting one of the above-stated travel clinics, traveling abroad for a minimum of 1 week to a maximum of 3 months were eligible for participation. Minors (<18 years) and incapacitated subjects are excluded from this study. No restriction was applied with respect to travel destination.

Fecal samples (Fecal Swab®; Copan, Brescia, Italy) were collected before, immediately after (within 1–2 weeks) and 1 month after return from all participants. Follow-up fecal samples were collected 3, 6 and 12 months after return from travellers (and their household members) who acquired extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) and/or CPE. Ethical approval was obtained by the Medical Ethical Committee of Maastricht University Medical Center (study number: METC 12-4-093). A full description of the study design has been published elsewhere [11].

Microbiological methods

Fecal samples were enriched overnight at 35°C in TSB with vancomycin (50 mg/l) to prevent overgrowth of Gram-positive bacteria [12]. Ten microliters were inoculated on chromID® ESBL agar (bioMérieux, Marcy l'Etoile, France) and incubated overnight at 35°C. Screening with this cefpodoxime-containing agar aims at maximum detection of ESBL-E. However some CPE, such as those producing OXA-48-like β-lactamases not co-producing an ESBL, remain susceptible and can be missed using this agar [13]. To see what the proportion of EBSL-negative, OXA-48-positive CPE was in our cohort, a substudy was held from August until November 2013. In total, 500 consecutive post-travel samples were additionally screened for OXA-48 CPE using chromID OXA-48 agar. All colonies of different morphologies growing on any of these two agars were characterized to the species level using MALDI-TOF (Bruker, London, UK). Minimum inhibitory concentrations (MICs) were measured for all Enterobacteriaceae by the use of the Vitek 2 system (bioMérieux, Marcy l'Etoile, France).

Phenotypic confirmation of ESBL production was performed by the combination disk diffusion test according to current national Dutch guidelines [14]. Isolates with MICs for imipenem >1 mg/l or for meropenem >0.25 mg/l, confirmed with E-test (bioMérieux), were considered possible carbapenemase producers [14] and genotypically characterized. In case a CPE was cultured from the post-travel sample (either from the ESBL or OXA-48 agar) specific PCR for the detected carbapenemase gene was performed on fecal metagenomic DNA of the pretravel sample. All CPE suspected isolates were screened for the presence of multiple classes of ESBL and carbapenemase genes using microarray (Identibac® AMR08; Alere Technologies GmbH, Jena, Germany) [15,16]. Targeted PCR and DNA sequencing of the PCR-amplicons was performed with primers as described previously [15,17–23] and in-house primers. The underlined nucleotide from the IMI primer 5'-CAAAGCAAATGAAC-GATTTC-3' was modified from [23]. DNA was extracted as described by Anjum *et al*. [15]. The lysate containing the crude DNA was used for biotin labeling and PCR.

All acquired carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from post-travel samples were further analyzed with multilocus sequence typing (MLST) [24,25] and sequence types (ST) were assigned by querying the respective MLST databases for *E. coli* [26] and *K. pneumoniae* [27].

To determine persistence of carriage and clonal transmission, amplification fragment length polymorphism (AFLP) [28] was assayed for all consecutive CPE isolates of the travellers and their household members.

Results

More than half of all included travellers visited destinations in Asia (n = 1016/2001, 50.8%), while Africa was visited by 633 travellers (31.6%). America (mainly South and Central America), Europe and Oceania were visited by 326 (16.3%), 21 (1.0%) and 5 (0.2%) travellers, respectively.

Prior to travel, one participant was carrying an OXA-48-producing *E. coli* and as such excluded from subsequent analysis. This 47-year-old healthy male subject had no travel history in the previous 12 months, but had been admitted to a Dutch hospital and treated with azithromycin within the preceding 3 months.

In five travellers, CPE was detected in specimens collected immediately upon return. Specific PCR for the detected carbapenemase-encoding gene on fecal metagenomic DNA of the pretravel sample was negative in all five cases. Subject- and travel-related characteristics of the travellers who acquired CPE during travel, as well as the molecular characteristics and dynamics over time of acquired CPE isolates, are summarized in Table 1.

Out of the five travellers who acquired CPE, none had sought medical care during their travel, all but one (subject 3) reported diarrhoea during travel and one traveler (subject 5) had used broad-spectrum antibiotics (Table 1). This traveler used an oral drug called *Disento* for complaints of watery diarrhoea during travel (a mix of quinoline, aminoglycoside, nitrofuran and sulfonamide).

Out of the acquired CPEs, three isolates were *E. coli* of various sequence types and encoding different carbapenemase genes, one was an *bla* $_{OXA-48}$-encoding ST363 *K. pneumoniae* and one an *E. cloacae* complex harboring an *bla* $_{IMI-2}$ gene (Table 1).

Similar to the previous report by Ruppé *et al.* [1], carriage of acquired CPE was of limited duration in three of our travellers, in whom CPE could not be detected 1 month after return from travel (Table 1). However, in one traveler, CPE carriage persisted at least 1 month and in another traveler (subject 2a), an OXA-244-producing *E. coli* isolate persisted for at least 6 months after return, as evidenced by similar AFLP patterns of isolates in follow-up specimens (Figure 1). Three months after travel, an OXA-244 *E. coli* isolate with a similar AFLP pattern was isolated from a fecal sample collected from her spouse and travel companion (subject 2b; Table 1 & Figure 1). As all other fecal specimens from this subject were CPE negative, this strongly suggests post-travel acquisition of the same bacterium through transmission from his wife.

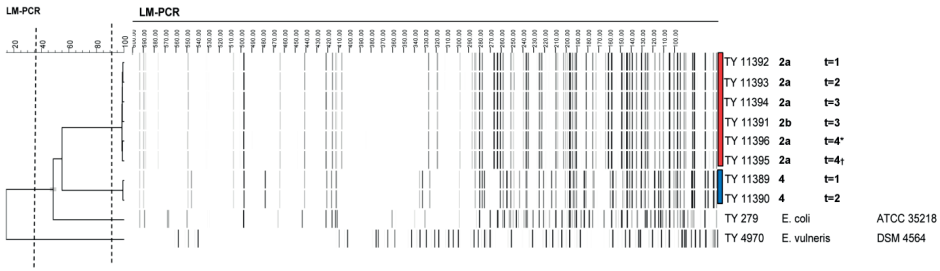

Figure 1. Genetic relatedness among travel-acquired carbapenemase-producing Escherichia coli.
Red and blue bars indicate isolates with identical amplification fragment length polymorphism pattern. Cut-off for identical strains is set at 90% as indicated by the right dashed line.
†Isolated from chromID® ESBL agar. ‡Isolated from chromID OXA-48 agar. 2a: Subject 2a; 2b: Subject 2b; 4: Subject 4; t = 1: Within 1 week after travel; t = 2: 1 month after travel; t = 3: 3 months after travel; t = 4: 6 months after travel.

Table 1. Characteristics of travellers that acquired carbapenemase-producing Enterobacteriaceae during travel, characteristics of journeys made, characteristics of acquired carbapenemase-producing Enterobacteriaceae isolates and dynamics of acquired carbapenemase-producing Enterobacteriaceae over time.

Characteristics of travellers				**Characteristics of journeys**			
Traveler	**Age**	**Sex**	**Chronic diseases**	**Countries visited**	**Duration (days)**	**Period**	**Purpose of visit**
1	64	F	Type II diabetes	Myanmar	16	July 2013	Maritime study trip
2a	58	F		Indonesia	22	August 2013	Backpack holiday
2b	59	M	'Cardiac arrythmia'	Indonesia	22	August 2013	Backpack holiday
3	41	M		Turkey, Greece	14	September 2013	Active/backpack holiday
4	37	F	Asthma, hypothyroidism	China, Thailand, Vietnam, Japan, Hong Kong and Singapore	22	October 2013	Luxury/wellness holiday
5	64	F	Seborrheic eczema	Myanmar	22	October 2013	Active/backpack holiday

Travellers 2a and 2b belong to the same household. None of the travellers had sought medical care during their travel, all but one (subject 3) reported diarrhoea during travel and one traveler (subject 5) had used broad-spectrum antibiotics.

[†]Screening with chromID® ESBL agar.

[‡]Isolated from chromID ESBL agar.

[§]Screening with both chromID ESBL agar and chromID OXA-48 agar.

[¶]Isolated from both chromID ESBL and chromID OXA-48 agar.

[#]Isolated from chromID OXA-48 agar.

–: No CPE isolated; CPE: Carbapenemase-producing Enterobacteriaceae; ESBL: Extended-spectrum β-lactamase-producing Enterobacteriaceae; ND: Not determined; MLST: Multilocus sequence typing

DISCUSSION

Our report underscores that CPE are indeed acquired during travel by healthy travellers in the absence of exposure to local healthcare during travel. Importantly, our observations also indicate that a risk of such acquisition during travel is not limited to travellers to the Indian subcontinent. In fact, none of 119 travellers in our cohort who traveled to India had acquired CPE. Instead, CPE acquisition was observed in five study participants who traveled to Europe and countries in south-eastern, eastern and western Asian, including two out of 23 visitors to Myanmar.

The low prevalence of CPE in the pretravel samples from our study (one of 2001 subjects was positive for CPE) is consistent with the very low background carriage of CPE in the Dutch community as described previously. In two studies on the prevalence and molecular characteristics of ESBL-E in the Dutch community, conducted in 2010

Characteristics of acquired CPE			Dynamics of CPE over time					
Species	**MLST**	**ESBL gene(s)**	**Before travel**	**On return**	**1 month after travel**	**3 months after travel**	**6 months after travel**	**12 months after travel**
Enterobacter cloacae complex	ND	None	–[†]	*IMI-2* [†,‡]	–[†]	–[†]	–[†]	–[†]
Escherichia coli	ST38	*CTX-M-14*	–[†]	*OXA-244* [†,‡]	*OXA-244* [†,‡]	*OXA-244* [†,‡]	*OXA-244* [§¶]	–[§]
E. coli	ST38	*CTX-M-14*	–[†]	–[†]	–[†]	*OXA-244* [†,‡]	–[§]	–[§]
Klebsiella pneumoniae	ST363	None	–[†]	*OXA-48* [§,#]	–[§]	–[§]	–[§]	–[§]
E. coli	ST2914	*CTX-M-15* and *CTX-M-55*	–[†]	*NDM*-1/2 [†,‡]	*NDM*-1/2 [†,‡]	–[†]	–[†]	–[†]
E. coli	ST162	*CTX-M-15*	–[†]	*NDM-7* [†,‡]	–[†]	–[†]	–[†]	–[†]

and 2011 and including 720 and 1033 subjects, respectively, no CPE or carbapenemase-encoding genes were found [29,30]. Moreover, the prevalence of carbapenemase resistance in clinical isolates in The Netherlands was only 0.01% for *E. coli* and 0.15% for *K. pneumoniae* in 2013–2014 [31].

One traveler to Myanmar acquired an *E. cloacae* complex isolate harboring an *bla* $_{\text{IMI-2}}$ gene. The IMI β-lactamases are a relatively uncommon group of carbapenemases. They are sporadically found in clinical isolates and environmental isolates from rivers in the USA [20] and in clinical isolates from China [32] and France [33]. Acquisition of IMI carbapenemases in travellers has not been described yet. This acquisition shows that travel might not only play a role in the spread of more common OXA-48-like and NDM-carbapenemases, but also of rarer plasmid-encoded carbapenemases such as IMI-2.

Another traveler to Myanmar acquired an ST162 *E. coli* isolate carrying an *bla* $_{\text{NDM-7}}$ gene. Although data on the prevalence of antimicrobial resistance in Myanmar are very limited, it is interesting to note that the first NDM-7 *E. coli* was recovered in France from urine of a female patient who also traveled to Myanmar [34]. Concordantly with the isolate retrieved in our study, this isolate also harbored a *bla* $_{\text{CTX-M-15}}$ ESBL gene, but belonged to a different sequence type (ST167).

A second *bla* $_{\text{NDM}}$ gene was acquired by a traveler when traveling throughout south-eastern and eastern Asia. NDM-producers are considered to be endemic in India and Pakistan [35], but likely have spread from the Indian subcontinent to neighboring countries and throughout Asia as reflected by the acquisition of *bla* $_{\text{NDM}}$ genes in the two

travellers that traveled outside these countries and confirmed by publications on the emergence of NDM-producing Enterobacteriaceae in Thailand [36].

One of the 270 travellers that visited Indonesia acquired a CPE: an OXA-244 positive *E. coli* isolate belonging to ST38. OXA-244 is an OXA-48-like β-lactamase that exhibits weak carbapenemase activity and which differs by a single amino acid substitution from classical OXA-48 [37]. ST38-type *E. coli* isolates harboring a classical *bla* $_{\text{OXA-48}}$ gene have previously been recovered from Lebanon, Egypt, Turkey, Switzerland and France [38–41]. More recently, the OXA-48-like variant OXA-244 was also identified in an ST38 *E. coli* isolated from a hospitalized patient in France without any travel history [42]. To our knowledge, there are no reports of OXA-48-like harboring Enterobacteriaceae from Indonesia.

An OXA-48 harboring *K. pneumoniae* isolate was acquired by a traveler that visited Turkey and Greece. For many years, almost all the reports of OXA-48-producers remained from patients hospitalized in Turkey or from patients with a link to Turkey [21]. The endemicity of OXA-48 in this country has most likely resulted to the acquisition in this traveler, as Greece is known as an important reservoir for KPC, but not for OXA-48 [35].

Household transmission of travel-acquired CPE between healthy subjects 2a and 2b was strongly suspected, although the possibility cannot be fully excluded that subject 2b also acquired CPE during travel but that this remained undetected in initial specimens. Possible household transmission from a documented carbapenemase-producing *K. pneumoniae* carrier – a female with amyotrophic lateral sclerosis that required mechanical ventilation and had been hospitalized in a tertiary hospital in the Tel Aviv area – to her spouse has previously been reported [43]. Another paper describes possible vertical or horizontal transmission of an NDM-1-producing *Enterobacter cloacae* in an Australian newborn that did not travel overseas [44].

The COMBAT-study aimed at maximum detection of ESBL-E, which might have led to an underestimation of acquisition of OXA-48-like β-lactamase producers. In our subset of 500 travellers, one additional OXA-48 acquisition was found (subject 3). This indicates that likely only a few additional OXA-48 acquisitions would have been detected when all 2001 subjects would have been screened with OXA-48 agar.

Literature on antimicrobial resistance in resource-limited settings is infrequent thereby hampering the comparison between CPEs acquired by travellers and the local prevalence and molecular characteristics of CPEs at the travel destination. However, travellers may act as a sentinel for emerging local resistance in developing countries like Indonesia and Myanmar as illustrated in this paper.

CONCLUSION & FUTURE PERSPECTIVE

In conclusion, the risk of acquisition of CPE during travel is not restricted to travellers to the Indian subcontinent and/or to travellers seeking healthcare during travel and carriage of travel-acquired CPE can persist up to at least 6 months after return from travel. Prolonged carriage obviously increases the risk of onward transmission and further spread of CPE. These observations deserve consideration by healthcare providers and public health professionals worldwide. Particularly in countries with low-level prevalence of CPE, screening for CPE in patients who are admitted to healthcare facilities should be considered, not only after recent travel, but even several months after returning from high-risk countries.

EXECUTIVE SUMMARY

Background

Acquisition of carbapenemase-producing Enterobacteriaceae (CPE) in healthy travellers without local healthcare contact during travel has recently been described for travellers to India.

Results

- This study reports acquisition of CPE in five travellers to Asia that did not travel across the Indian subcontinent.
- None of these travellers had sought medical care during their travel, all but one reported diarrhoea during travel and one traveler had used broad-spectrum antibiotics.
- Persistence of colonization up to at least 6 months after return from travel was found for one traveler.
- In one of the CPE-positive travellers evidence was found for clonal transmission of OXA-244 Escherichia coli to her spouse.

Conclusion

- Acquisition of CPE during travel is neither restricted to travellers to the Indian subcontinent nor to travellers seeking healthcare during travel.
- Screening for CPE in patients who are admitted to healthcare facilities should be considered, not only after recent travel, but even several months after returning from high-risk countries.

ACKNOWLEDGEMENTS

The authors would like to thank Muna Anjum and Roderick Card from the Animal Health and Veterinary Laboratories Agency (AHVLA), Weybridge, UK, for their help with the microarray.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

This study was funded by The Netherlands Organization for Health, Research and Development (ZonMw) programme Priority Medicine Antimicrobial Resistance (project number: 50-51700-98-120). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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CHAPTER 7

Dissemination of the mcr-1 colistin resistance gene

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The Lancet Infectious Diseases. 2016 Feb;16(2):147-9

Plasmid-mediated transferable colistin resistance encoded by the *mcr-1* gene was described in *Escherichia coli* and *Klebsiella pneumoniae* isolates from pigs and chicken at a prevalence of around 20%, and in clinical isolates from human beings at a prevalence of around 1% in China.[1] The prevalence of the *mcr-1* gene in Enterobacteriaceae in other countries and in the community is unknown.

We did a prospective study of acquisition of fecal colonisation and carriage with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in 2001 Dutch travellers (the COMBAT study),[2] from November, 2012, to November, 2013. Acquisition was defined as the absence of ESBL-producing Enterobacteriaceae in a fecal swab sample taken immediately before travel and detection of ESBL-producing Enterobacteriaceae in a sample taken within 1–2 weeks after return to the Netherlands. Of 1847 travellers at risk, 633 (34%) acquired ESBL-producing Enterobacteriaceae. Nine of these 633 travellers acquired ESBL-producing *Escherichia coli* with a colistin minimum inhibitory concentration of 4–8 mg/L (EUCAST clinical breakpoint for resistance >2 mg/L) as detected using Vitek-2 and confirmed by E-test. After publication of the report by Yi-Yun Liu and colleagues,[1] these nine isolates were tested by PCR[1] for the presence of the *mcr-1* gene. The gene was detected in six of nine isolates and sequencing of the amplicons showed a 100% homology over the length of the fragments with the published sequence.[1] Three ESBL-producing *E coli* were *mcr-1* PCR negative, suggesting colistin resistance due to other mechanisms.[3] Analysis of ESBL genes by microarray,[4] PCR, and sequencing showed that the *mcr-1* positive ESBL-producing *E coli*carried ESBL genes belonging to multiple groups (table).

Of the six travellers who acquired ESBL-producing *E coli* carrying the *mcr-1* gene, two unrelated travellers visited Peru and Bolivia, two unrelated travellers visited China, one visited Tunisia, and one visited multiple countries in southeast Asia (Thailand, Vietnam, Laos, and Cambodia). The duration of travel ranged between 8 and 40 days (mean 21·3 days). None of the travellers had accessed medical care and none had used antimicrobial drugs during travel, while five had experienced traveller's diarrhoea. Analysis of subsequent fecal samples collected at 1, 3, 6, and 12 months after return to the Netherlands did not show ESBL-producing *E coli*, suggesting short-term colonisation with colistin resistant ESBL-producing *E coli* or loss of plasmids carrying ESBL and potentially *mcr-1* genes.

Colistin is used as an ultimate refuge antimicrobial drug in the treatment of infections caused by multidrug resistant Gram-negative microorganisms.[5] Our data suggest a worrisome spread of the *mcr-1* gene in *E coli* in the community across at least three continents. The diversity of ESBL genes present in *mcr-1* positive isolates suggests that the *mcr-1* gene might be carried on multiple plasmid backbones.

This research was funded by Netherlands Organization for Health, Research and Development (ZonMw; 50-51700-98-120) and EU-H2020 programme (COMPARE, 643476). We declare no competing interests.

Table. Characteristics of travellers and acquired fecal Escherichia coli isolates carrying the mcr-1 gene

	Traveller with isolate 1	**Traveller with isolate 2**	**Traveller with isolate 3**	**Traveller with isolate 4**	**Traveller with isolate 5**	**Traveller with isolate 6**
Travel destination	Thailand, Vietnam, Cambodia, Laos	Tunisia	Peru, Bolivia, Colombia	China	China	Peru, Bolivia
Travel duration (days)	21	8	40	14	23	22
Age (years)	56	55	25	54	62	26
Sex	Female	Female	Female	Male	Female	Male
ESBL gene (ESBL group)	CTX-M-14 (CTX-M group 9)	CTX-M-1 (CTX-M group 1)	CTX-M-15 (CTX-M group 1)	CTX-M-65 (CTX-M group 9)	CTX-M-55 (CTX-M group 1)	CTX-M-55 (CTX-M group 1)
Minimum inhibitory concentration of antimicrobial drug (mg/L)*						
Amoxicillin-clavulanic acid	16	8	>16	16	8	4
Piperacillin-tazobactam	8	≤4	8	≤4	≤4	≤4
Cefotaxime	16	8	32	16	>32	>32
Cefoxitin	16	≤4	8	≤4	≤4	≤4
Ceftazidime	≤1	≤1	16	≤1	4	4
Cefepime	2	2	2	≤1	2	2
Imipenem	≤0·25	≤0·25	≤0·25	≤0·25	≤0·25	≤0·25
Meropenem	≤0·25	≤0·25	≤0·25	≤0·25	≤0·25	≤0·25
Gentamicin	>8	≤1	>8	≤1	>8	≤1
Tobramycin	8	≤1	>8	8	8	≤1
Nitrofurantoin	256	≤16	128	32	64	≤16
Co-trimoxazole	>8	>8	>8	≤1	>8	>8
Norfloxacin	>8	8	>8	2	>8	>8
Ciprofloxacin	>2	>2	>2	1	>2	>2
Colistin	4	4	4	4	4	8

ESBL=extended-spectrum β-lactamase. *Determined using Vitek-2, except for colistin for which E-test results are provided.

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CHAPTER 8

Global phylogenetic analysis of *Escherichia coli* and plasmids carrying the *mcr-1* gene indicates bacterial diversity but plasmid restriction

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Scientific Reports. 2017 Nov 10;7(1):15364

ABSTRACT

To understand the dynamics behind the worldwide spread of the *mcr-1* gene, we determined the population structure of *Escherichia coli* and of mobile genetic elements (MGEs) carrying the *mcr-1* gene. After a systematic review of the literature we included 65 *E. coli* whole genome sequences (WGS), adding 6 recently sequenced travel related isolates, and 312 MLST profiles. We included 219 MGEs described in 7 Enterobacteriaceae species isolated from human, animal and environmental samples. Despite a high overall diversity, 2 lineages were observed in the *E. coli* population that may function as reservoirs of the *mcr-1* gene, the largest of which was linked to ST10, a sequence type known for its ubiquity in human faecal samples and in food samples. No genotypic clustering by geographical origin or isolation source was observed. Amongst a total of 13 plasmid incompatibility types, the IncI2, IncX4 and IncHI2 plasmids accounted for more than 90% of MGEs carrying the *mcr-1* gene. We observed significant geographical clustering with regional spread of IncHI2 plasmids in Europe and IncI2 in Asia. These findings point towards promiscuous spread of the *mcr-1* gene by efficient horizontal gene transfer dominated by a limited number of plasmid incompatibility types.

INTRODUCTION

Antimicrobial resistance (AMR) represents a growing threat to global health[1]. With barely any new antimicrobial drugs in development[2], limiting the spread of AMR is key in order to maintain current treatment options[3].

Colistin is an antibiotic of the polymyxin class, discovered in 1950 and effective against Gram-negative bacteria[4]. The emergence of multidrug-resistant Gram-negative bacteria, especially those producing carbapenemases, has reintroduced colistin as a last resort antibiotic for the treatment of severe infections[5]. In contrast to its limited use in humans, colistin is widely used in food-producing animals[6]. While colistin resistance was long thought to be caused by chromosomal mutations only[7], the emergence of plasmid-mediated resistance, conferred by the mobilized colistin resistance (*mcr-1*) gene, was recently reported[8]. This gene encodes for a protein of the phosphoethanolamine transferase enzyme family, and its expression results in the addition of a phosphoethanolamine to lipid A, the target of colistin, decreasing the interaction between colistin and the bacterial lipopolysaccharide[8]. Since its discovery in 2015 in China, this gene has been described in several bacterial species that were isolated from animals, animal food products, humans and environmental samples from around the world[9-13]. Our previous study in travellers indicated acquisition of *mcr-1* carrying bacteria by healthy individuals during travel to destinations around the world, potentially related to food exposure, as well as rapid clearance after return[14]. It has been suggested that *mcr-1* has spread from food animals to humans[8,15-17], but there is a lack of comparison of *mcr-1* carrying isolates on a global level to support this hypothesis.

We studied the global population structure as well as the geographic and host distribution of *mcr-1*-carrying *Escherichia coli*, and mobile genetic elements (MGEs), to establish the population structure and to assess whether the spread of the *mcr-1* gene is linked to clonal dissemination or transmission of MGEs from animal, human, or environmental sources within geographic regions.

RESULTS

Literature search

A systematic review of the literature on *mcr-1*, published until 1 January 2017 resulted in the inclusion of 95 articles, representing a total of 410 entries (whole genome sequences, MLST profiles, and/or plasmid types) for analysis (See detailed methods and results in Supplementary data, Supplementary Figure 1 and Supplementary Table 1).

Population structure

Whole genome sequencing (WGS)

The genomes of 65 *mcr-1*-carrying *E. coli* were analysed, including 6 genomes from *E. coli* isolated from travellers that were sequenced for the purpose of the present study. Isolates originated from Asia (n = 36; 55.4%), Europe (n = 20; 30.8%), North-America (n = 4; 6.2%), South-America (n = 4; 6.2%) and Africa (n = 1; 1.5%). 45 were of animal origin (69.2%), 19 of human origin (29.2%) and one strain (1.5%) was isolated from water (Supplementary Table 1).

The average size of the genomes (all contigs in each assembly, representing chromosomes and plasmids) of these 65 isolates was 4.9 Mbp, with a median number of genes identified of 4785 (ranging from 4266 to 7083), representing a pangenome of 23248 genes and a core genome (defined by genes present in at least 99% of the isolates) of 2216 genes. An unbiased analysis of the population structure was performed using a Bayesian approach with the BAPS software[18], based on the nucleotide alignment of the core genome sequences. It revealed the presence of 5 distinct phylogenetic clusters (Fig. 1; Supplementary Figure 2; Supplementary Table 1). The largest cluster (cluster 1) consisted of 26 isolates from 16 different STs (26/65; 40.0%) and the second cluster consisted of 24 isolates from 15 different STs (36.9%). No significant relationship between clustering (BAPS) and geographical origin or isolation source was observed (χ^2-test) (Fig. 1) except that all 5 isolates that belong to BAPS cluster 3 are from Europe. Twenty isolates showed less than 10 SNPs/Mbp difference with at least 1 other isolate and were considered clonally related (Supplementary Tables 2 and 3).

Multilocus sequence typing (MLST)

For 312 *E. coli* isolates originating from 69 studies, a MLST profile was published or could be deduced from the corresponding WGS. Of these, 206 were isolated from animals or animal products (66.0%), 101 were isolated from humans (32.4%), including the 6 travel acquired isolates, and 5 from the environment (1.6%). 141 Isolates from 25 studies (141/312; 45.2%) originated from Asia and 125 isolates from 25 studies (40.1%) from Europe, together accounting for 85.3% of all included isolates. The isolates represented 112 unique sequence types (STs) with ST10 being most common, comprising 40/312 (12.8%) isolates originating from Africa, Asia, Europe and South-America.

eBURST analysis[19] was performed on all isolates included in the study, to identify their genetic relatedness based on their MLST profiles. Three main clusters were identified, for which the predicted founders, i.e. the ST in a cluster from which all other SLVs and DLVs in the cluster have most likely diversified[19], were ST10, ST1114 and ST410. The largest cluster contained all 40 ST10 isolates and an additional 46 isolates in 21 STs that were single (SLV) or double locus variants (DLV) of ST10 (86/312; 27.6%) (Supplementary Figure 3). The predicted founder of the second largest cluster was ST1114, a SLV of ST165

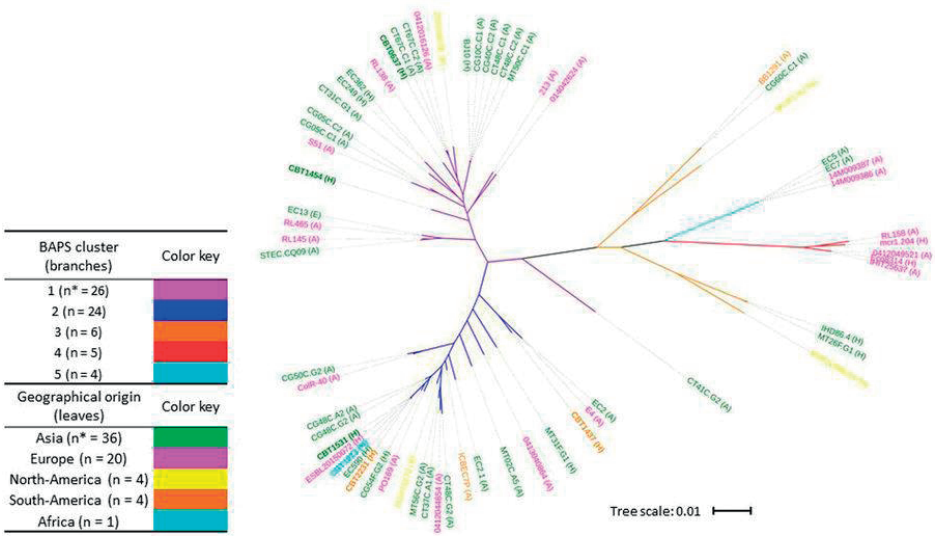

Figure 1. Maximum-likelihood tree based on concatenated core genome sequences of 65 *mcr-1*-carrying *E. coli* isolates. Branch colours indicate phylogenetic clusters as determined by BAPS. Isolates from ST10, ST165 and closely related isolates are all grouped in the BAPS cluster 2 (dark blue). Leaf (isolates identifiers) colours indicate geographical region of origin. Isolation source is indicated in brackets: A = animal or meat; H = human; E = environment. The 6 travellers' isolates that were sequenced for this study are highlighted in bold and names start with CBT. Tree scale in number of substitutions per site. *Number of isolates.

and ST100, and included 19 isolates belonging to 7 different STs (5.4%), while the third cluster was centred on ST410 and included 14 isolates from 3 different STs (4.5%).

A maximum-likelihood tree based on concatenated MLST gene sequences showed a main clade of 128 isolates (represented by blue branches in Fig. 2 and Supplementary Figure 4; bootstrap value of the main branch = 0.98), including most, but not all, isolates from the eBURST clusters of ST10 and ST1114 (Supplementary Figure 4A). All isolates from these 2 eBURST clusters for which a WGS was available were grouped in BAPS cluster 2. Similarly, all the isolates from the eBURST cluster ST410 grouped into BAPS cluster 1, along with 6 isolates from ST155. Seven isolates belonged to the globally successful extra-intestinal pathogenic *E. coli* clone ST131 (Supplementary Figure 4A).

As observed in the WGS analysis, animal isolates were interspersed with isolates from humans and the environment throughout the tree, as were isolates from different continents indicating a lack of clustering by isolation source or geographical origin (Fig. 2). Similarly, no clustering by health status of the host was observed (Supplementary Figure 4B).

Mobile genetic elements

The plasmid incompatibility group of the *mcr-1*-carrying plasmids could be determined for 217 Enterobacteriaceae isolates from 7 different species (*Escherichia sp., Salmonella* sp., *Klebsiella* sp., *Cronobacter* sp., *Enterobacter* sp., *Kluyvera* sp. and *Shigella* sp.), representing a total of 219 plasmids since 2 isolates carried 2 different plasmids (Table 1). These plasmids were described in 71 studies (1 to 33 plasmids per study, average = 3.1). In addition, the gene was integrated in the chromosome of 6 isolates. The incompatibility group could not be determined for 27 of the 65 isolates for which WGS was available. Similarly the plasmid type was not available for 182 of the 312 isolates included in the MLST analysis. A total of 14 different plasmid incompatibility groups were identified. 198/219 (90.4%) of the identified plasmids belonged to one of 3 incompatibility groups: IncX4 (77/219 plasmids, 35.2%), IncI2 (76/219 plasmids, 34.7%) and IncHI2 (45/219 plasmids, 20.5%). 50/76 IncI2 plasmids (65.8%) originated from Asia and 33/45 IncHI2 plasmids (73.3%) from Europe. IncX4 plasmids were more evenly distributed: 44/77 (57.1%) were recovered from Europe, 29 from Asia (37.7%) and 4 from other regions (5.2%). Observed proportions were significantly different from expected for IncI2 (χ^2-test, $p < 0.001$) and IncHI2 ($p < 0.001$) but not for IncX4. The distribution of these 3 plasmid types was not significantly different from expected between animal (χ^2-test, $p = 0.24$), human ($p = 0.88$) and environmental sources ($p = 0.38$). Isolates from the BAPS groups 1 and 2 carried plasmids from the 3 major types in similar proportions (Supplementary Figure 5A; Supplementary Table 1). Isolates from the eBURST clusters of ST10 carried plasmids belonging to 7 different incompatibility groups, including IncHI2, IncI2 and IncX4. No clustering of plasmid type with MLST phylogeny was observed either (Supplementary Figure 5B; Supplementary Table 1).

Figure 3 shows the alignment of the complete sequences or contigs from IncI2 (panel A), IncX4 (B) and IncHI2 (C) incompatibility group plasmids. IncHI2 plasmids had the largest size, with sequence lengths up to 267486 bp.

The ISA*pl1* transposon element situated upstream of the *mcr-1* gene was present in 7/9 (77.8%) IncHI2 plasmids, but only in 11/29 (37.9%) of IncI2 plasmids and completely missing in all of the 24 reported IncX4 plasmids (Fig. 3). In the isolates from travellers the ISA*pl1* transposon was identified in 3 out of our 6 *mcr-1*-carrying contigs including one isolate from a traveler to Asia (ST101, IncI2 incompatibility group), one to Africa (ST744, IncHI2) and one to South-America (ST744, incompatibility group not identified).

Antimicrobial resistance genes

All sequences of the *mcr-1* gene collected in the present study were 100% identical to the original sequence described by Liu *et al*.[8].

Multiple resistance genes were detected in most of the studied isolates (Supplementary Results). The florfenicol resistance gene *floR* was present in 32 (49.2%) isolates; in

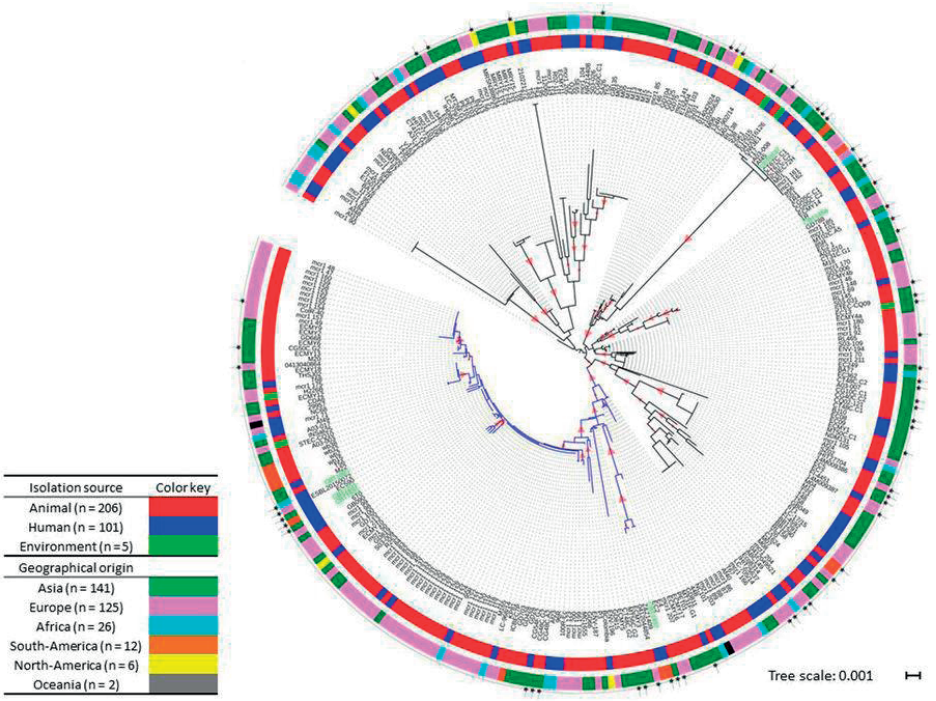

Figure 2. Phylogeny of the *mcr-1*-carrying *E. coli* isolates. Maximum-likelihood tree based on concatenated MLST gene sequences, mid-point rooted. Inner coloured circle: isolation source; outer circle: region of origin. Stars indicate the isolates from which a whole genome sequence was available. The 6 travellers' isolates that were sequenced for this study are highlighted in green. Bootstrap values between 0.9 and 1 are indicated by red triangles (size proportional to bootstrap value). The blue branches represent the main clade of 128 isolates including most isolates from ST10. Tree scale in number of substitutions per site. See Supplementary Figure 4A for additional information on the relationship between STs, eBURST clustering and WGS BAPS clustering.

22 of 45 isolates from animals (48.9%) and 10 of 19 isolates from humans (52.6%). The *baeR* and *baeS* genes, encoding novobiocin resistance, were found in 64 (98.5%) and 65 (100%) isolates, respectively.

Plasmid analysis from the WGS data showed that 4 of the 29 *mcr-1*-carrying IncI2 plasmids (13.8%) contained an additional ESBL gene. IncHI2 plasmids (n = 9) carried between 0 and 12 additional AMR genes. In particular, 4 plasmids carried CTX-M ESBL genes and 2 carried the *floR* gene. In 4 out of the 9 IncHI2 plasmids analysed in this study, the *mcr-1* gene was shown to be integrated alongside a large multi-drug resistance (MDR) gene cassette (Fig. 3C). None of the IncX4 plasmids carried additional AMR genes.

Table 1. Incompatibility types of mcr-1 carrying plasmids and distribution by geographical regions.

	IncF	IncFI	IncFIB	IncFII	IncHI1	IncHI1A/ IncHI1B/ IncFIA	IncHI2	IncI2	IncI2-IncX4	IncP	IncX3-X4	IncX4	IncY	repB (p0111)	**Total plasmids**		Chromosome
Africa	0	0	1	0	0	0	4	8	0	0	0	1	0	0	**14**	**6.4%**	0
Asia	1	3	0	0	0	2	7	50	0	1	1	29	2	0	**96**	**43.8%**	2
Europe	0	0	2	1	1	0	33	5	0	3	0	44	0	1	**90**	**41.1%**	4
North-America	1	0	0	0	0	0	1	4	0	0	0	1	0	0	**7**	**3.2%**	0
Oceania	0	0	0	0	0	0	0	2	0	0	0	0	0	0	**2**	**0.9%**	0
South-America	0	0	0	0	0	0	0	7	1	0	0	2	0	0	**10**	**4.6%**	0
Total (% of total)	**2 (0.9)**	**3 (1.4)**	**3 (1.4)**	**1 (0.5)**	**1 (0.5)**	**2 (0.9)**	**45 (20.5)**	**76 (34.7)**	**1 (0.5)**	**4 (1.8)**	**1 (0.5)**	**77 (35.2)**	**2 (0.9)**	**1 (0.5)**	**219 (100)**	**100.0%**	**6**

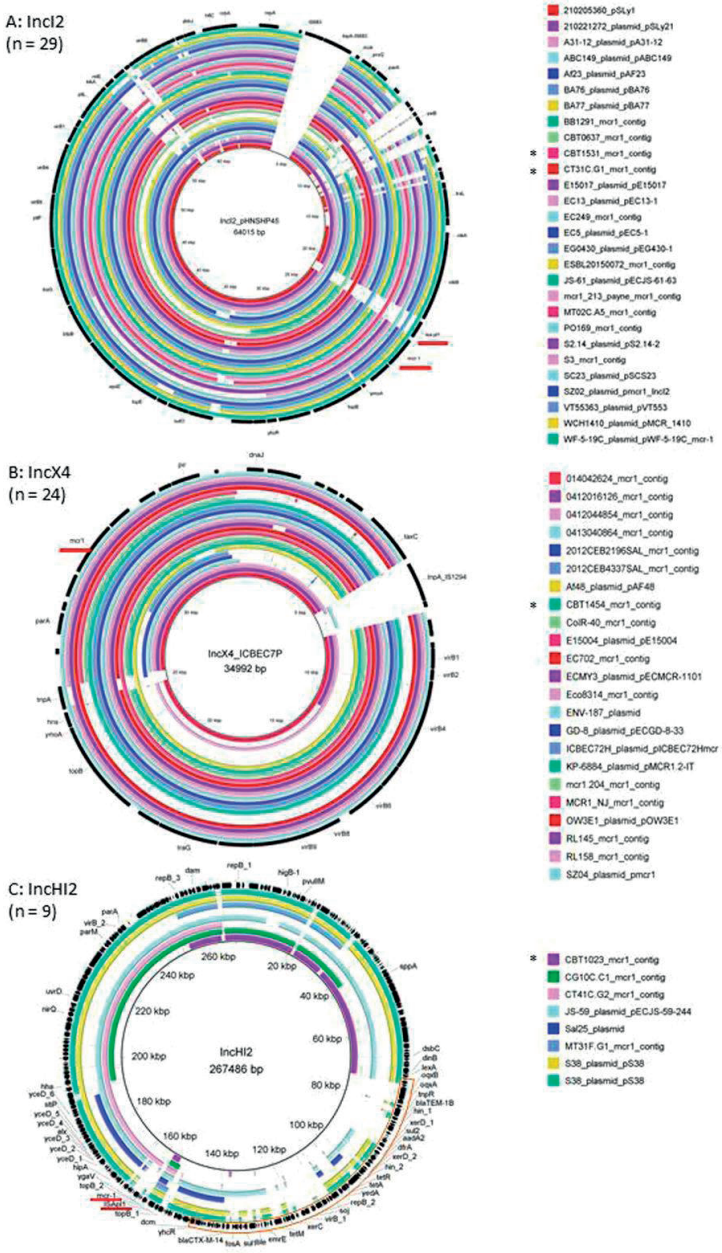

Figure 3. Alignment of *mcr-1*-containing plasmids and contigs. Panel A: IncI2 plasmids (n = 29); panel B: IncX4 (n = 24); panel C: IncHI2 (n = 9). Black outer ring: plasmid used as reference for the alignment; name and size of the reference indicated in the middle of each panel. Plasmid names followed by "_mcr1_contig" refer to assembled contigs from whole genome sequences. Other names refer to plasmid sequences deposited in online databases. The *mcr-1* gene and ISA-pl1 location are underlined in red. Plasmids indicated with an asterisk are from the 6 travellers' isolates that were sequenced for this study. Panel C: Putative MDR cassette is highlighted in orange.

DISCUSSION

Analysis of all reported WGS of *mcr-1*-carrying isolates shows that the population of *E. coli* is highly diverse, but is dominated by 2 large groups of related isolates. Most of the isolates from BAPS group 2 grouped into a MLST cluster centred on ST10. An over-representation of isolates related to ST10 and ST165 (a SLV of ST1114) in *mcr-1*-carrying *E. coli* isolates was previously reported at a smaller scale in isolates from European farm animals[20]. *E. coli* ST10 and closely related STs are frequently recovered from food and human intestinal samples and studies have shown a higher prevalence of plasmid-carried AMR genes in ST10, including CTX-M ESBL genes, compared to other STs[21-24].

The other BAPS group of interest in our study (group 1) included isolates belonging to ST155. This ST has been described as a major vector of spread of ESBL genes from animals to humans[25]. It is thus possible that zoonotic transmission leads to the spread of the *mcr-1* gene, as has been suggested in studies from China and Vietnam[16,17], notably through the 2 main phylogenetic clusters identified in this study.

Additionally, we found clonally related isolates, including some belonging to ST744, a SLV of ST10, carrying the *mcr-1* gene on different plasmid backbones and recovered from different continents (see Supplementary Results). These results point towards a worldwide dissemination *of mcr-1* driven mainly by highly promiscuous plasmids rather than the worldwide spread of one or more *mcr-1*-carrying clones. We hypothesize that several populations of *E. coli* isolates, notably those related to ST10 or ST155, acquired the *mcr-1* gene due to their intrinsic ability of acquiring AMR genes and their high prevalence in humans and food animals. These populations of commensal isolates then may play a crucial role as a reservoir for this gene, which can explain their over-representation in the present study.

In the timeframe of our literature search, 3 *E. coli* strains carrying the *mcr-2* gene were isolated from animals in Belgium. These isolates belonged to ST10 (2 isolates) and ST167 which is a SLV of ST10 and carried the gene on an IncX4-type plasmid. No WGS data was available from these isolates[26]. More data about the *mcr-2* gene is needed to assess its spread and determine if *E. coli* ST10 plays a similar role in its dissemination as it does for *mcr-1*.

More than 90% of published plasmid types carrying *mcr-1* genes belonged to either IncI2, IncX4 or IncHI2. Almost 75% of the isolates carrying an IncHI2 plasmid originated from Europe: 26 from animals and 7 from humans (Table 1 and Supplementary Table 1). In a traveller's isolate acquired in Tunisia, the *mcr-1*-carrying plasmid was identified as an IncHI2-type backbone of the ST4 pMLST subtype which co-carried a CTX-M-1 ESBL gene (Fig. 3C). This traveller reported consumption of beef, chicken and eggs during travel to Tunisia which can potentially be the source for the acquisition of the *mcr-1* positive isolate. When investigating the presence of the *mcr-1* gene in cephalosporin resistant

E. coli isolates from chicken farms in Tunisia, Grami *et al*.[27] found that all *mcr-1*-carrying plasmids from their study (n = 37) also belonged to the IncHI2-type, ST4 subtype and harboured CTX-M-1 genes. PFGE typing of the isolates harbouring this plasmid showed various bacterial genetic backgrounds. Interestingly, these chickens were all imported from France, either as adults or chicks. Other studies showed the presence of this IncHI2, CTX-M-1 and *mcr-1* combination in *Salmonella enterica* Typhimurium isolates from meat samples in Portugal from 2011[28,29] and diarrhoeic veal calves in France[30]. The IncHI2 subtype ST4 was also detected in an *E. coli* isolate from retail chicken breast in Germany[31] and the faecal sample of a veal calve from the Netherlands[32], suggesting widespread dissemination of this particular plasmid in European farm animals and possible transmission to humans.

The high prevalence of novobiocin *baeR* and *baeS* and florfenicol *floR* resistance genes[33,34] in the genomes of isolates of human and animal origin together with the fact that florfenicol and novobiocin are used almost exclusively in veterinary medicine further supports the potential role of food animals as an important reservoir of *mcr-1* containing bacteria and MGEs[15].

In contrast with the IncHI2 plasmids, 65.8% of all IncI2 plasmids recovered so far originated from Asia, with a much lower prevalence in *mcr-1* carrying Enterobacteriaceae from other regions. Taken together, these elements point toward a more regional circulation and dissemination of the *mcr-1*-carrying plasmids IncI2 and IncHI2.

We found the ISA*pl1* transposon element associated with the *mcr-1* gene, as originally described by Liu *et al*.[8] to be present in a minority of studied plasmids and contigs. However, since some of the *mcr-1*-carrying contigs were obtained by assembly of Illumina short reads from WGS data, we cannot exclude that some of these gaps are explained by an incomplete assembly of (plasmid) sequences. The ISA*pl1* transposon element is considered to be the main driver of horizontal gene transfer of the *mcr-1* gene and has been shown to be highly unstable in IncI2 plasmids[35-37]. The absence of the ISA*pl1* transposon element in *mcr-1*-carrying IncX4 plasmids as described here has recently been proposed to be essential for the maintenance of the *mcr-1* gene in this particular backbone, but the exact mechanism still requires further investigation[38].

WGS analysis provided in-depth information about the *mcr-1*-carrying *E. coli* isolates and their phylogenetic relationship, but the number of available genomes was limited. On the other hand, whilst MLST data have a lower resolution, the higher number of available profiles allowed analysis of the isolates' origin (geographical, source of isolation, diseased status of the host, etc.).

A limitation of our study is the potential for bias. The overrepresentation of isolates originating from Asia and Europe could be explained by a higher prevalence of *mcr-1* genes on these continents, but the effect of publication bias cannot be excluded. Isolates from North-America only represented 2.2% of the collection. Noteworthy, colistin,

except for ophthalmic ointment, has never been marketed for use in animals in the United States[39,40].

Sampling bias should also be considered when several isolates with an identical ST are presented from a single study, as is the case for ST100 and ST752. Additionally, in the absence of a control population of *mcr-1*-negative isolates obtained from similar sources as the *mcr-1* positive isolates, results of analysis of population structures should be interpreted with caution. Because many studies screened existing collections of (resistant) isolates for colistin resistance or presence of *mcr-1*, selection bias has probably been introduced.

The findings in this study suggests that the *mcr-1* gene has locally and globally disseminated through MGEs that are mainly IncHI2, IncI2 and IncX4 plasmids and provides additional support for the hypothesis of the animal reservoir, that is driven by the use of colistin in livestock, as a source of *mcr-1* in humans. A global ban of colistin use in animals to preserve colistin for use in human medicine seems therefore justified.

MATERIAL AND METHODS

Literature search

Relevant papers that published on *mcr-1* and *mcr-2* were identified in PubMed, Web of Science, Scopus, ScienceDirect and Google Scholar using the query 'mcr-1 OR mcr1 OR mcr-2 OR mcr2 OR (mcr AND colistin)' (see Supplementary Material for full search strategies). To be able to study the associations between phylogeny, geographic distribution and isolation source we only included sequences from papers that provided sufficient metadata. As a consequence, plasmids and genomes sequences that were deposited in online databases without metadata were not included in the analysis.

Selection of isolates for whole genome sequencing

We subjected 6 *mcr-1* positive isolates that were collected as part of a prospective study (COMBAT study) aimed at studying acquisition of extended-spectrum β-lactamase (ESBL) -producing Enterobacteriaceae during travel to whole-genome sequencing[14,41]. Additionally, we included 22 whole genome sequences of isolates from Vietnamese chickens and humans that were still unpublished when performing our literature search[17].

Whole genome sequencing of *mcr-1*-positive *E. coli* isolates

Bacterial DNA was extracted from fresh pure cultures using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Library preparation was done according to manufacturer's instruction (Illumina, San Diego, CA, USA) and sequenced using Illumina MiSeq technology with 150nt paired-end settings. Sequences have been deposited in

the European Nucleotide Archive under the accession numbers SAME104030441 to SAME104030446.

Bio-informatic analysis

MLST analysis

For each *mcr-1*-carrying *E. coli* isolate for which the ST or the whole genome sequence was available, the sequences of the corresponding alleles were downloaded from the *E. coli* MLST genes repository of the University of Warwick (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/handlers/getFileData/home/cbailster/mlst/zope/Extensions/gadfly/Ecoli/DB/) and concatenated. When STs of isolates were not described in literature, the ST was determined from available whole genomes using the online service provided by the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/MLST/) according to the Achtman MLST scheme[42,43]. MLST clusters (STs and their single locus or double locus variants) were defined using e-burst V3 (http://eburst.mlst.net/v3/enter_data/single/)[19] and goeBURST v1.2.1[44] using only profiles from this study.

WGS and plasmid analysis

Raw sequence reads in fastq format or pre-assembled sequences in fasta format were downloaded from online databases for all available isolates (Supplementary Table 1). Additional sequences not yet deposited in online databases were requested from their respective authors. The quality of the raw sequence reads was checked using fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), quast[45] and KmerFinder 2.0 (https://cge.cbs.dtu.dk/services/KmerFinder/) (see Supplementary Methods for more details). Reads were trimmed using Trimmomatic V0.33[46]. *De-novo* genome assembly was performed with SPAdes 3.9[47] for Illumina short reads and with Canu v1.3 for PacBio long reads[48]. Contigs of less than 500 bp long were removed from the genomes to improve the overall quality of the assembly. Size of the genomes was calculated by adding the length of all remaining contigs. Identification of open reading frames (ORFs) and gene contents in the assembled genomes (*de-novo* assemblies and pre-assembled sequences) was performed using Prokka v1.11[49]. Core genome analysis was performed with Roary v3.6.8[50]. Clustering of isolates was performed using the hierBAPS module of the Bayesian Analysis of Population Structure (BAPS) software v6.0[18]. The core genome alignment output provided by Roary was used as input for BAPS with 2 levels of hierarchy and a maximum number of cluster (K) of 10. The estimated number of clusters was 5 for both levels of hierarchy.

Sequences (concatenated MLST loci or concatenated core genes) were aligned using mafft v6.864b[51]. The resulting alignment was used as input for calculation of distances

and tree building using RAxML v8.1.6[52]. MLST and WGS trees were visualized using iTOL v3.3.1 (http://itol.embl.de/)[53].

Identification of plasmid incompatibility group and typing of IncHI2 plasmids were performed on assembled sequences (*de-novo* or pre-assembled) via the CGE online services PlasmidFinder v1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and pMLST v1.4 (https://cge.cbs.dtu.dk/services/pMLST/)[54]. Alignment and visualization of plasmids was performed with BRIG v0.95[55]. The majority of the isolates and plasmids described in this study were sequenced using a short read technology (Illumina). This technology does not allow for a high quality assembly of the plasmids due to the high number of repeat regions present in these MGEs. Therefore no phylogenetic analysis of the *mcr-1*-carrying plasmids was conducted in this study.

Two different databases were used for identification of other antibiotic resistance genes: ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/[56]) was used to detect acquired resistance genes commonly located on mobile genetic elements (MGEs) and CARD Resistance Gene Identifier (https://card.mcmaster.ca/analyze/rgi[57]) was used to detect chromosomal genes.

Statistics

The distribution of isolates and plasmids from different geographical origins and isolation sources was determined by a χ^2-test comparing the expected distribution (proportions of the total studied population) to the observed proportions using GraphPad Prism6 (La Jolla, CA, USA).

ACKNOWLEDGEMENTS

The authors would like to thank the team of the Department of Genome analysis of the AMC (in particular Dr. F. Baas and L. Koster) for their support in the sequencing bacterial genomes. Financial support for this study was provided by the European COMPARE project (http://www.compare-europe.eu/) under the European Union's Horizon 2020 research and innovation programme, grant agreement No. 643476. The VIBRE and COMBAT studies were supported by The Netherlands Organisation for Health Research and Development/The Netherlands Organisation for Scientific Research (ZonMw) under grants number 205100012 and 50–51700–98–120 respectively.

AUTHOR CONTRIBUTIONS

S.M. and J.v.H. contributed equally to this work. S.M and J.v.H. performed the systematic review, performed the experiments, interpreted the results and wrote the manuscript. N.W. contributed to bio-informatics analyses. M.A., D.M., J.P., M.d.J., the COMBAT consortium members, N.V., N.T.H. and C.S. contributed isolates and genome sequence data and associated meta data. M.d.J. and C.S. designed the study. All authors contributed to the writing of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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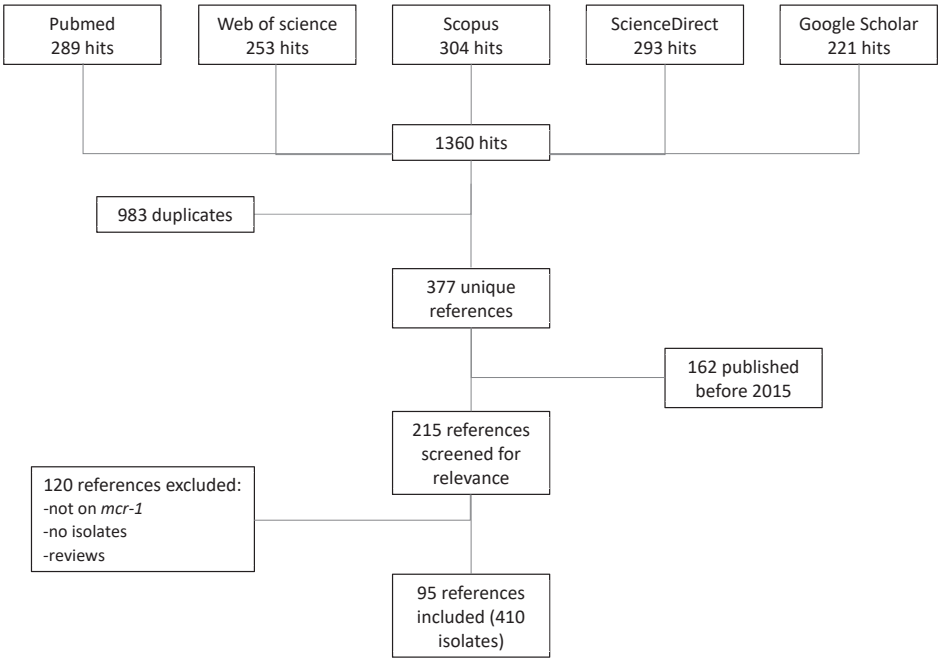
SUPPLEMENTARY METHODS AND RESULTS



Supplementary Figure 1. Flow chart of the systematic literature review according to the PRISMA guidelines.

Systematic literature review

Relevant papers that had published on *mcr-1* and *mcr-2* were identified in Pubmed, Web of Science, Scopus, ScienceDirect and Google Scholar using the query 'mcr-1 OR mcr1 OR mcr-2 OR mcr2 OR (mcr AND colistin)'. The database was accessed on 4 January 2017.

Criteria for considering studies for this study

Eligible for inclusion were all studies publishing:

- a multilocus sequence typing (MLST) profile (sequence type; ST) of at least one *mcr-1* positive *E. coli* isolate, or
- a whole genome sequence (WGS) of any species. For *E. coli* species the WGS was used for both phylogeny and plasmid analysis. For other species the WGS was used only for plasmid analysis, or
- the incompatibility group of a plasmid carrying a *mcr-1* or *mcr-2* gene.

All studies which did not satisfy these criteria were excluded. Studies presenting data on the same isolate were included only once.

Selection of studies

Two authors (SM and JvH) independently assessed the titles and abstracts of studies identified in terms of their relevance. Full versions of articles were obtained if the initial assessment of these met the inclusion criteria.

A flow chart of the search strategy is presented in Supplementary Figure 1. A complete list of included isolates and plasmids and their references can be found in Supplementary Table 1.

The number of entries (WGS, MLST profile, plasmid sequence or plasmid type) per included study ranged from 1 to 42 (average = 4.3; median = 2).

Search strategies

Pubmed, 289 hits

mcr1[All Fields] OR
mcr-1[All Fields] OR
mcr2[All Fields] OR
mcr-2[All Fields]) OR
("mcr"[All Fields] AND ("colistin"[MeSH Terms] OR "colistin"[All Fields])

Web of Science [v.5.23], 253 hits

TOPIC: (mcr1 OR mcr-1 OR mcr2 OR mcr-2 OR (colistin AND mcr))
Timespan: All years. Indexes: SCI-EXPANDED, SSCI, A&HCI, ESCI.

Scopus, 304 hits

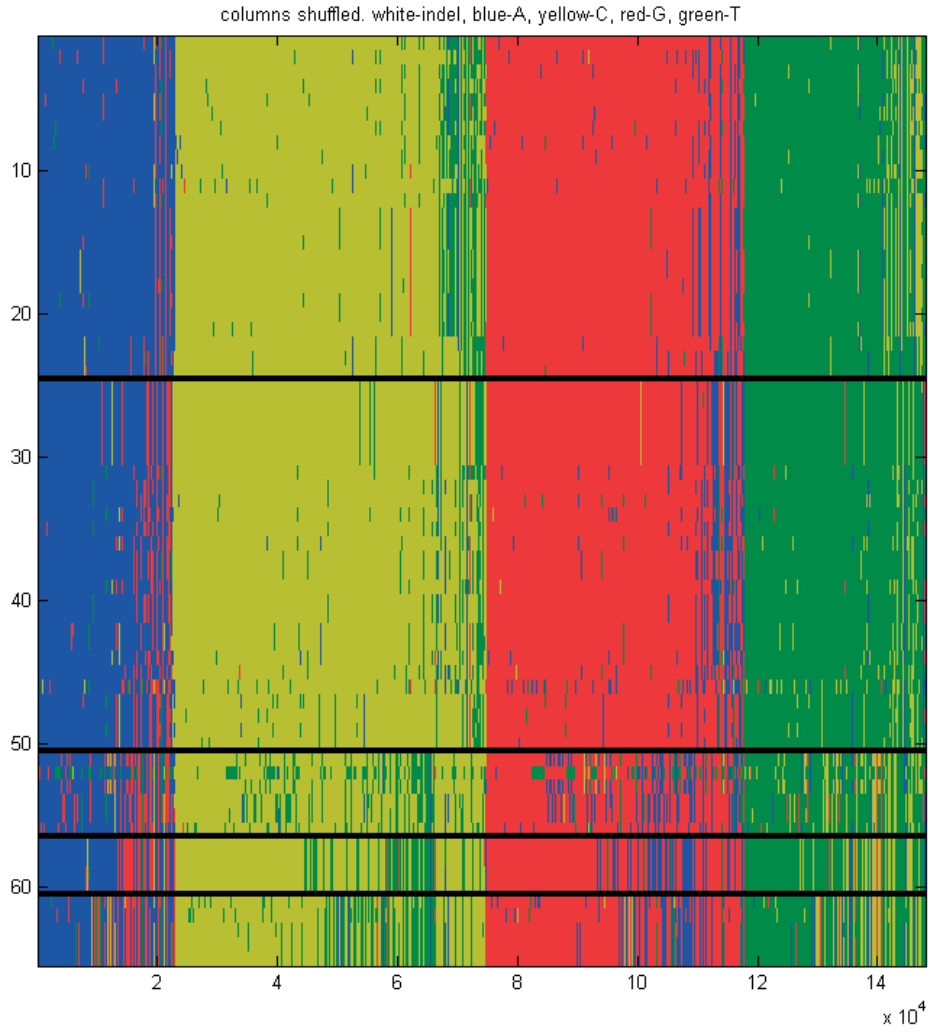
(TITLE-ABS-KEY (mcr1)) OR (TITLE-ABS-KEY (mcr-1)) OR (TITLE-ABS-KEY (mcr2)) OR (TITLE-ABS-KEY (mcr-2)) OR (TITLE-ABS-KEY (colistin AND mcr))

Science direct, 293 hits

"mcr1" OR "mcr-1" OR "mcr2" OR "mcr-2" OR (colistin AND mcr) AND LIMIT-TO(yearnav, "2017,2016,2015").

Google scolar, 221 hits

mcr1 OR mcr-1 OR mcr2 OR mcr-2 OR (colistin AND mcr)



Supplementary Figure 2. Output alignment of the BAPS clustering. A shuffled alignment of the core genome SNPs is divided by black bars to indicate five BAPS groups. Each row represents an isolate and the colors in every column represent a different nucleotide. On the left-side X-axis the number of nucleotides is indicated. Clusters were numbered from 1 to 5 according to the number of isolates they contain and cluster numbers are indicated on the right-side X-axis.
White-indel = insertion or deletion; blue-A = adenine; yellow-C = cytosine; red-G = guanine; green-T = thymine

SNP counts and clonal relationships of the isolates

SNP count was performed using kSNP v3.021[1] using a k-mer size of 19 bp and retaining only core SNPs (SNPs present in the core genome). The calculation of the genetic distance was performed using the average size of the genomes observed (4.9 Mbp). A

Hamming distance matrix was created comparing the SNP/Mbp distance between each pair of isolates in the study (Supplementary Table 3).

Among the 2080 pairs of isolates studied, 20 showed between 0 and 10 SNPs/Mbp differences and were considered closely related clones (Supplementary Table 4).

Among these, 13 pairs came from live chicken from southern Vietnam and were distributed across 5 farms (Supplementary Table 4). These results suggest regional circulation of *mcr-1* carrying clones. A pair of isolates originating from the Netherlands showed 0 SNP/Mbp difference and was isolated from the same batch of chicken meat. Another pair, originating from Malaysia, was isolated from the same chicken. These two pairs of isolates most probably represent duplicates. In only one of these isolates (EC5, from Malaysia) could the plasmid incompatibility group be identified (IncI2). Thus removing the pairs of isolates from the analysis did not modify the statistical outcome of the plasmid geographical distribution. Two closely related isolates sequenced for the present study (5.5 SNPs/Mbp difference) were recovered from travellers to China and Tunisia respectively. However, they carried the *mcr-1* gene on different plasmids (IncI2 and IncHI2 respectively). These 2 isolates also differed by less than 10 SNPs/Mbp with an isolate recovered from a patient in Denmark that carried an IncI2 plasmid. An isolate recovered from a traveller to South-America, formed a pair with an isolate from a patient in Malaysia. The plasmid type could not be identified for these 2 isolates. Finally a pair of isolates belonging to ST131 and showing 5.5 SNPs/Mbp difference between them were recovered from a patient in Spain and a chicken in Germany. These two isolates carried the *mcr-1* gene on two different plasmid types, IncX4 and IncHI2 respectively.

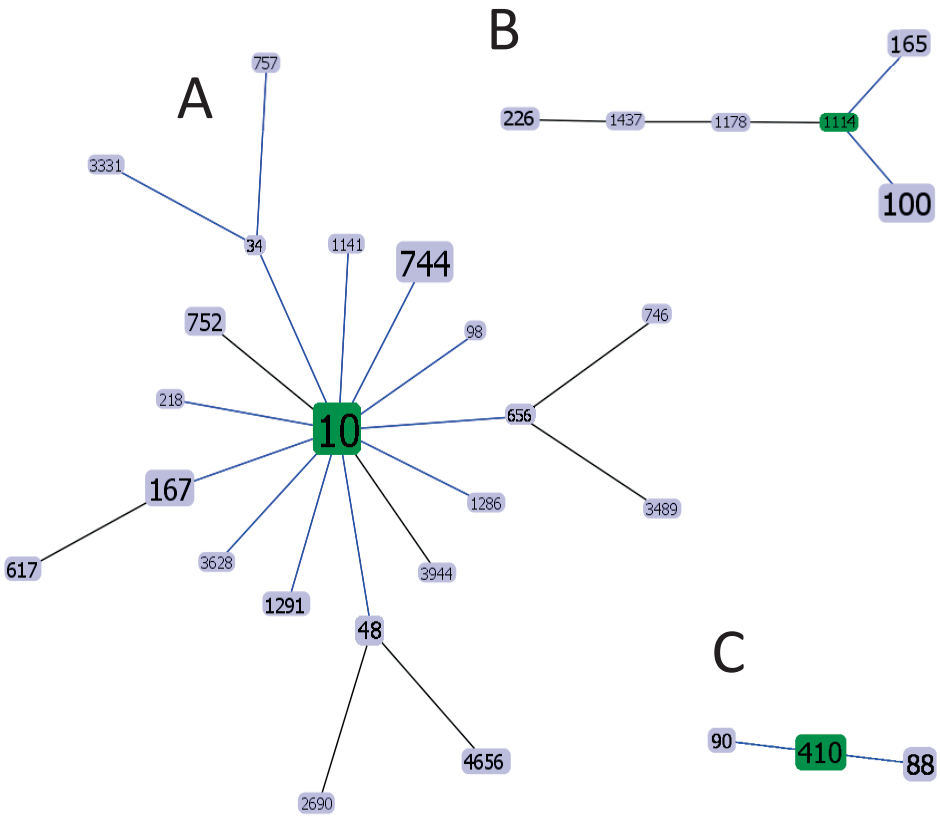
These findings implicate that, despite the spread of certain *E. coli* clones to different continents, the *mcr-1*-carrying plasmids are acquired separately in most cases.

WGS – quality check

A total of 68 *mcr-1*-carrying *E. coli* genomes were analysed, including those 6 from travellers that were sequenced for the purpose of the present study. WGS datasets of 3 previously published isolates did not pass quality check and were discarded from the analysis. Two of these isolates (BB1290 [11.5 Mbp] and PO155 [8.5 Mbp]) had an unusually large genome. When tested using KmerFinder 2.0, the WGS fastq files of BB1290 and PO155 exhibited k-mer scores suggesting the presence of sequences from a different species in addition to the expected *E. coli*: *Enterobacter cloacae* and *Salmonella enterica* respectively. For isolate STEC-CQ10, only the sequences of the identified genes were available rather than the complete genome sequence or scaffolds. This resulted in a very fragmented genome (n = 6903 contigs), lack of intergenic regions and ultimately a serious decrease in quality of the population core genome determination.

N50 values, a statistic that defines assembly quality, for these isolates were 1096 for STEQ-CQ10; 14079 for PO155 and 31281 for BB1290. These values were much lower than values observed for the other genomes of the collection (N50 > 100000).

All these results taken together resulted in the exclusion of the WGS of STEQ-CQ10, BB1290 and PO155 from the phylogenetic analysis.

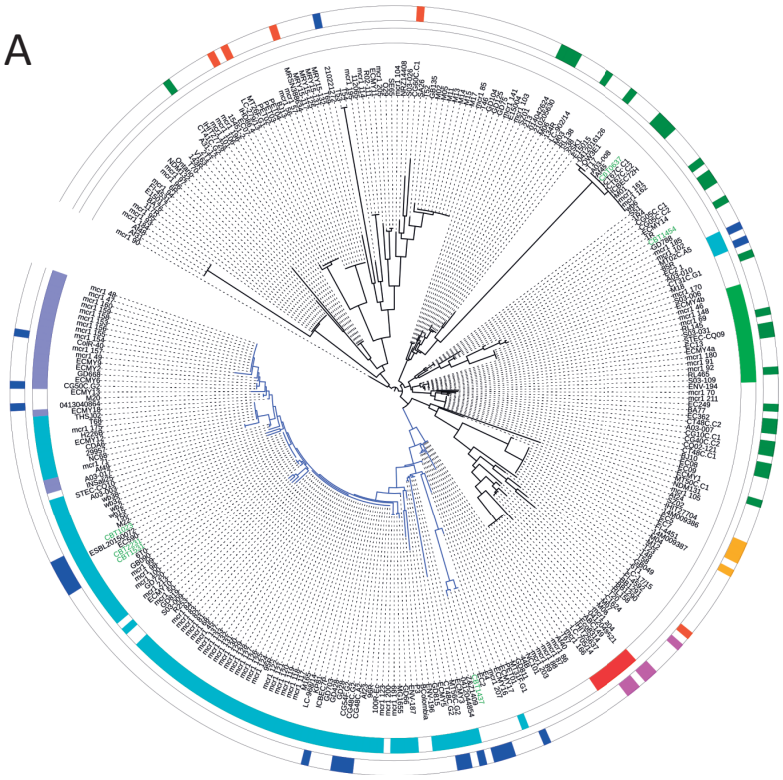


Supplementary Figure 3. Representation of the eBURST analysis showing the MLST clonal relationships of the 3 largest clusters containing 120 of the 312 included *mcr-1*-carrying *E. coli* isolates. Each rectangle represents a distinct sequence type (ST), with the number inside the rectangle referring to the ST's nomenclature according to the University of Warwick MLST database. The predicted founder ST of each group is indicated in green. Black lines indicate links drawn without recourse to tiebreak rules by goeBURST [2]; Blue lines indicate links drawn using tiebreak rule. The size of the rectangles that indicate the STs are proportional to the number of isolates they contain.

A: eBURST cluster 1 (n = 86 isolates).

B: eBURST cluster 2 (n = 20 isolates).

C: eBURST cluster 3 (n = 14 isolates).

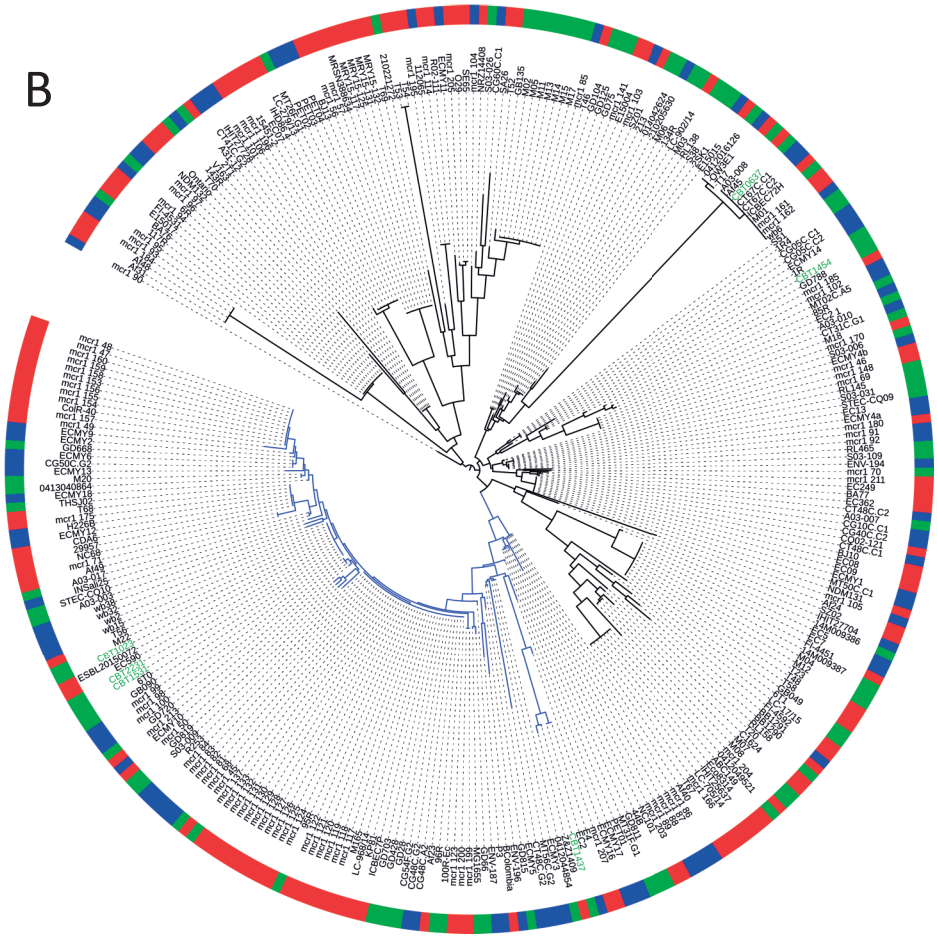


eburst_clusters*	Color Key
1 (ST10)	
2 (ST1114)	
3 (ST410)	
ST131	

BAPS cluster	Color key
1	
2	
3	
4	
5	

A: Colours of the inner circle indicate in which eBURST cluster (SLVs and DLVs clusters) the isolates belonged after MLST analysis; no colour indicates that the isolates were not part of one of the 3 indicated clusters. ST131 is not part of any eBURST cluster and is indicated separately. Colours of the outer circle indicate in which BAPS cluster the isolates belonged after whole genome sequence (WGS) analysis. No colour indicates that no WGS was available.

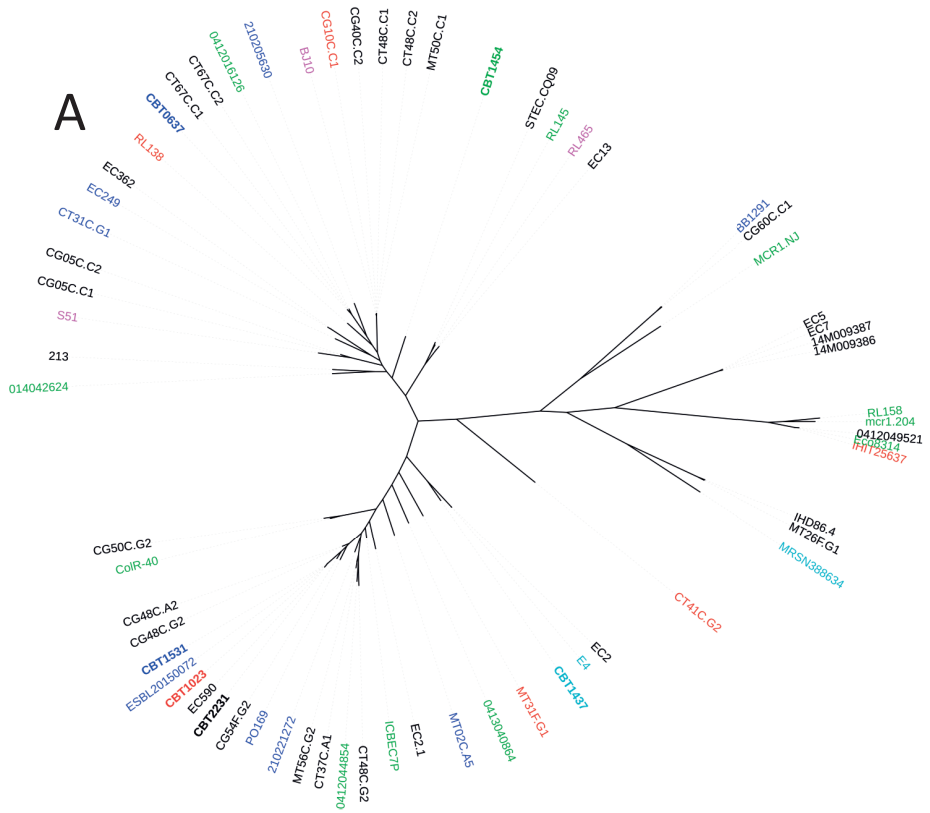
Supplementary Figure 4. Phylogeny of 312 *mcr-1*-carrying *E. coli* isolates. Maximum-likelihood trees based on concatenated MLST gene sequences, mid-point rooted.
The 6 travellers' isolates that were sequenced for this study are highlighted in green. The branch of the main clade containing most isolates from ST10 is coloured in blue. Tree scale in number of substitutions per site.
*The predicted founder ST of each cluster is indicated in brackets.



B: Colours of the circle indicate the health status of the host from which the isolate was recovered, if available.

MLST analysis

Four different STs consisting of more than 2 isolates that were possibly clonally related were found in a single region: ST100 (10 isolates) and ST752 (3 isolates) in Europe; ST156 (12 isolates) and ST165 (3 isolates) in Asia. However all 3 isolates from ST752 were reported in a single study [3], as were 8 out of 10 isolates from ST100 [4].

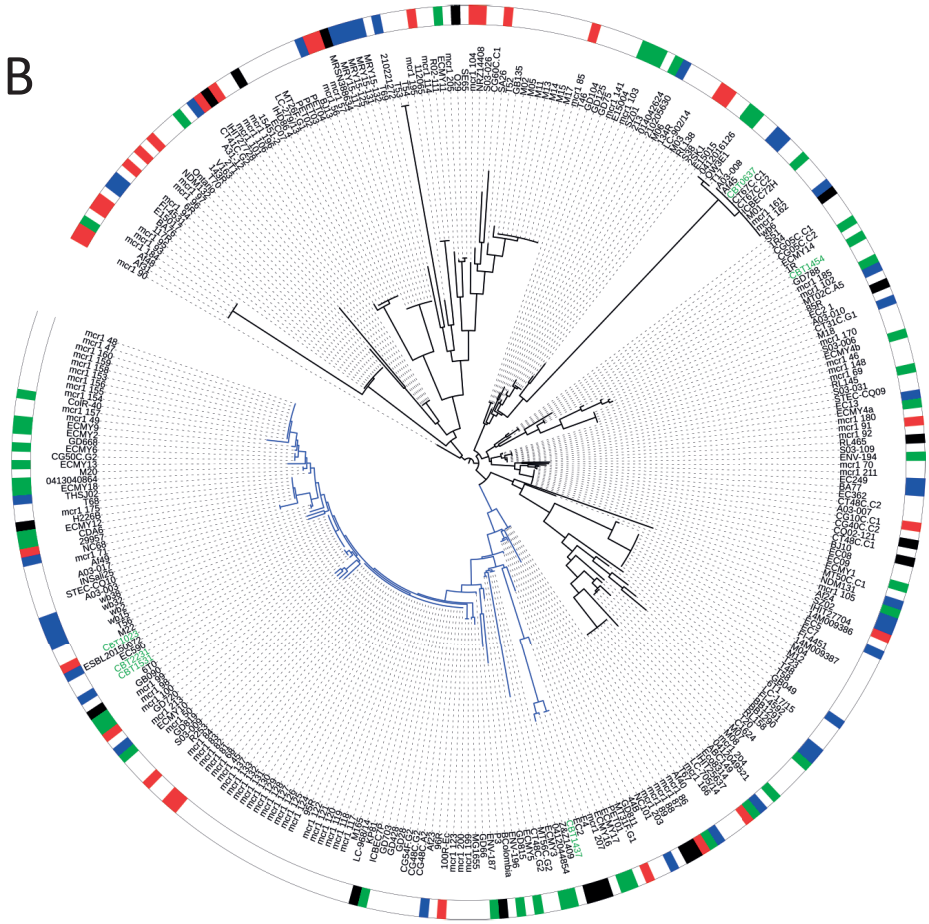


mcr-1 location	Color key
IncHI2	
IncI2	
IncX4	
Chromosome	
Others	

Tree scale: 0.01

A: Maximum-likelihood tree based on concatenated core genome sequences of the 65 *mcr-1*-carrying *E. coli* isolates. Isolate names are coloured according to the location of the *mcr-1* gene, either on an identified plasmid, chromosome of the isolate or unknown location. The 6 travellers' isolates that were sequenced for this study are highlighted in bold.

Supplementary Figure 5. Association between location of the *mcr-1* gene and WGS and MLST phylogeny of population of studied *mcr-1*-carrying *E. coli* isolates.
Only plasmids IncHI2, IncI2 and IncX4 are indicated by separate colours, all other plasmid types are indicated as "other". Trees scales in number of substitutions per site.



mcr-1 location	Colour key
IncHI2	
IncI2	
IncX4	
Chromosome	
Other	
Unknown	

B: Maximum-likelihood tree based on concatenated MLST gene sequences, mid-point rooted. Colours indicate the location of the *mcr-1* gene. The branch of the main clade containing most isolates from ST10 is coloured in blue. The 6 travellers' isolates that were sequenced for this study are highlighted in green.

Antimicrobial resistance genes

Using the ResFinder tool, a total of 65 different unique acquired antimicrobial resistance (AMR) genes were identified in the *E. coli* WGS dataset. Many additional chromosomally encoded AMR genes were found using the CARD database. The most prevalent acquired gene (after *mcr-1*) was *tetA*, found in 52/65 (80%) of isolates followed by *sul2* (39/65; 60%) and the beta-lactamase gene TEM-1b in 38 isolates (58%). One isolate only carried just one additional acquired gene, the AmpC beta-lactamase gene *blaCMY-2*, and one isolate carried no other acquired AMR gene apart from *mcr-1*. In addition to *mcr-1*, each of the 65 isolates carried 4 or 5 genes of the *pmr* family (A, B, C, E and F variants). These genes are involved in the resistance to polymyxin, but are present in all *E. coli* strains [5]. Genetic alignments showed SNPs differences between the *pmrA* and *B* genes found in this study and both the sensitive and resistant templates previously described [5], thus preventing from drawing any conclusion relative to the involvement of these genes in the colistin resistance mechanisms. Carbapenemase encoding genes were found in the genomes of 4 isolates; NDM-5 (in isolates BJ10 and MCR1.NJ from China and USA respectively) and KPC-2 (EC362 and EC249, both from Singapore). 29/65 isolates (44.6%) carried plasmid-encoded CTX-M ESBL genes with CTX-M-55 being most prevalent in 18/29 (62.1%) of these isolates. Ten (15.4%) isolates were found to carry plasmid mediated *Qnr* quinolone resistance genes, 9 isolates carried *qnrS1* and one both *qnrS1* and *qnrS2*. In addition, 36 (55.4%) isolates displayed chromosomal mutations in both *parC* and *gyrA* genes associated with resistance to (fluoro)quinolones [6]. 34 (52.3%) isolates carried the *strA* and *strB* streptomycin resistance genes. The florfenicol resistance gene *floR* was present in 32 (49.2%) isolates; in 22 of 45 isolates from animals (48.9%) and 10 of 19 from humans (52.6%). The *baeR* and *baeS* genes are regulators of resistance mechanisms to novobiocin [7,8] and were both found in 64 (98.5%) and 65 (100%) isolates, respectively.

Supplementary data references

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SUPPLEMENTARY TABLES

Supplementary tables are too extensive to be printed and can be downloaded as an Excel file from:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5681592/bin/41598_2017_15539_MOESM2_ESM.xlsx

CHAPTER 9

Travel-related acquisition of diarrhoeagenic bacteria, enteral viruses and parasites in a prospective cohort of 98 Dutch travellers

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Travel Medicine and Infectious Disease. 2017 Sep;19:33-36.

ABSTRACT

Background

Limited prospective data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by healthy individuals during travel.

Methods

To determine the frequency of travel associated acquisition of 19 pathogens in 98 intercontinental travellers, qPCR was used to detect 8 viral pathogens, 6 bacterial enteric pathogens and 5 parasite species in faecal samples collected immediately before and after travel.

Results

We found high pre-travel carriage rates of *Blastocystis* spp. and *Dientamoeba fragilis* of 32% and 19% respectively. Pre-travel prevalences of all other tested pathogens were below 3%. *Blastocystis* spp. (10%), *Plesiomonas shigelloides* (7%), *D. fragilis* (6%) and *Shigella* spp. (5%) were the most frequently acquired pathogens and acquisition of enteral viruses and hepatitis E virus in this relatively small group of travellers was rare or non-existent.

Conclusions

Our findings suggest that the role of viruses as the cause of persisting traveller's diarrhoea is limited and bacterial pathogens are more likely as a cause of traveller's diarrhoea. The substantial proportion of travellers carrying *Blastocystis* spp. and *D. fragilis* before travel warrants cautious interpretation of positive samples in returning ravellers with gastrointestinal complaints.

INTRODUCTION

Although diarrhoea ranges in the top of the most frequently occurring travel associated conditions [1], limited data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by previously healthy individuals during travel. In addition to the epidemiological relevance, such data may help to understand the clinical relevance of detected pathogens in the era of extremely sensitive diagnostic testing with real-time PCR (qPCR). This study was initiated to determine the frequency of travel associated acquisition of 19 (entero)pathogens by qPCR.

MATERIALS & METHODS

Study population

The COMBAT-study is a multicenter longitudinal cohort study primarily focussing on the acquisition of ESBL- and carbapenemase-producing *Enterobacteriaceae* [2,3]. A total of 2001 Dutch adults travelling intercontinentally for 1–12 weeks were included from November 2012 until November 2013. All subjects received a faeces collection swab (Fecal Swab; Copan, Brescia, Italy) with transport medium and a questionnaire before and after travel. The questionnaires comprised information about previous travel, health, travel destination and behaviour during travel. Stool samples were stored at −80 °C for future analysis. For this study, 100 travellers were randomly selected from the complete study population of 2001 travellers.

Extraction and PCR

Automated nucleic acid extraction was performed using the MagNA Pure 96 instrument (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) with the inclusion of internal controls for generic RNA and DNA. Reverse-transcriptase PCR was applied immediately after extraction to form cDNA. qPCR was performed using the LightCycler480 (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) to detect 8 viral pathogens, 6 bacterial enteric pathogens and 5 parasite species in faecal samples collected immediately before and after travel (see Table 1 for targeted micro-organisms and Supplementary Table for primer and probe sequences). A positive (Phocine Herpesvirus, PhoHV) and negative extraction control (STAR-buffer) were included in every qPCR run.

Descriptive and statistical analysis

Acquisition was defined as a negative pre-travel and a positive post-travel test result for the examined micro-organism, irrespective of signs and symptoms of illness. Acquisition rates were calculated for travellers with a negative pre-travel test: the population at risk.

Traveller's diarrhoea (TD) was defined as three or more unformed stools within a 24-h period during travel.

Table 1. Micro-organisms studied by qPCR in the cohort of travellers.

Viruses	Bacteria	Parasites
Adenovirus (40, 41, 52)	*Campylobacter* spp.	*Blastocystis* spp.
Astrovirus	*Clostridium difficile*	*Cryptosporidium* spp.
Enterovirus	*Plesiomonas shigelloides*	*Dientamoeba fragilis*
Hepatitis E virus	*Salmonella* spp.	*Entamoeba histolytica*
Norovirus	*Shigella* spp.	*Giardia lamblia*
Parechovirus	*Yersinia enterocolitica*	
Rotavirus (A, C)		
Sapovirus		

Table 2. Baseline characteristics of current study group and the full study population.

	current study, n/N (n = 98[a])	full study population, n/N (n = 2001[a])
Female	51/97 (53%)	1079/1996 (54%)
Median age, years (range)	48 (19–76)	51 (18–82)
Median travel duration, days (range)	20 (7–72)	20 (3–105)
Chronic illness	17/97 (18%)	450/1977 (23%)
Antibiotic use last 3 months	11/98 (11%)	197/1988 (10%)
Diarrhoea pre-travel	10/97 (10%)	242/1993 (12%)
Travel reason: vacation	84/97 (87%)	1655/1964 (84%)
Travellers' diarrhoea	34/97 (35%)	734/1962 (37%)
Travel destination:		
South-Eastern Asia	30/97 (31%)	578/1965 (29%)
South America	13/97 (13%)	192/1965 (10%)
Eastern Africa	10/97 (10%)	206/1965 (10%)
Northern Africa	7/97 (7%)	83/1965 (4%)
Multiple sub-regions in Asia	6/97 (6%)	76/1965 (4%)
Western Africa	4/97 (4%)	114/1965 (4%)
Other sub-regions	27/97 (28%)	716/1965 (36%)

a. Numbers do not always add up to 98 and 2001 because of missing data.

RESULTS

For one of the 100 randomly selected travellers a sample was missing, and for another one, DNA extraction failed. Therefore, 98 travellers were analysed. Distributions of the population characteristics were similar to the distributions in the full study population

(Table 2). The median age was 48 years (range 19–75) and 53% was female. Median travel duration was 20 (7–72) days. Most frequently visited sub-regions were South-Eastern Asia (n = 30), South America (n = 13) and Eastern Africa (n = 10). Thirty-five percent of travellers (34/97) reported TD.

Pre-travel carriage rates for *Blastocystis* spp. and *Dientamoeba fragilis* were 31/98 (32%) and 19/98 (19%), respectively of which seven participants carried both species (Table 3). Pre-travel prevalences of the other tested species were much lower and ranged from 0 to 3%. A total of six travellers carried one or more pathogenic bacteria before travel. None of these six travellers had diarrhoeal complaints before travel.

Table 3. Pre-travel carriage and acquisition rates of diarrhoeagenic bacteria, parasites and viruses.

Pathogen	Pre travel (n-98)		Acquisition[a]				
			TD[b]	%	No TD[b]	%	Total
Bacteria							
Campylobacter spp.	0	0%	3/34	9%	1/63	2%	4/98 (4%)
Clostridium difficile	0	0%	1/34	3%	0/63	0%	1/98 (1%)
Plesiomonas shigelloides	1	1%	1/34	3%	6/62	10%	7/97 (7%)
Salmonella spp.	0	0%	2/34	6%	1/63	2%	3/98 (3%)
Shigella spp.	2	2%	4/34	12%	1/61	2%	5/96 (5%)
Yersinia enterocolitica	3	3%	2/33	6%	0/61	0%	2/95 (2%)
Parasites							
Blastocystis spp.	31	32%	3/25	12%	4/41	10%	7/67 (10%)
Cryptosporidium spp.	0	0%					0
Dientamoeba fragilis	19	19%	1/24	4%	4/54	7%	5/79 (6%)
Entamoeba histolytica	0	0%					0
Giardia lamblia	0	0%	0/34	0%	1/63	2%	1/98 (1%)
Viruses							
Adenovirus (40, 41, 52)	0	0%					0
Astrovirus	0	0%					0
Enterovirus	0	0%	1/34	3%	0/63	0%	1/98 (1%)
Hepatitis E virus	2	2%					0
Norovirus	1	1%	2/34	6%	0/62	0%	2/97 (2%)
Parechovirus	0	0%	0/34	0%	2/63	3%	2/98 (2%)
Rotavirus (A, C)	0	0%					0
Sapovirus	0	0%					0

TD: traveller's diarrhoea.

Numbers add up to 97 instead of 98 because of one missing post travel questionnaire.

a. Acquisition rates were calculated for travellers with a negative pre-travel test: the 'population at risk'.

b. Denominator is the number travellers with and without TD.

Blastocystis spp. were acquired most frequently, followed by *Plesiomonas shigelloides*, Dientamoeba fragilis and *Shigella* spp. (Table 3). Pathogenic bacteria were acquired by 19/98 (19%) of travellers, of whom 7 acquired *P. shigelloides*.

Entamoeba histolytica, *Cryptosporidium* spp., Rotavirus (A, C), Adenovirus (40, 41, 52), Astrovirus and Sapovirus were neither detected in any of the pre-travel samples nor in the post-travel samples. Hepatitis E virus (HEV) was detected in 2 pre-travel samples only. Norovirus and Parechovirus were both acquired by two travellers and Enterovirus by one traveller, respectively. Of note, the traveller who acquired an Enterovirus travelled to China only and did not visit a polio-endemic area.

4/5 travellers that acquired *Shigella* spp. and 3/4 that acquired *Campylobacter* spp. reported TD (OR 8.0; p = 0.054 by Fisher's exact test and OR 6.0; p = 0.12 respectively). Of the 7 travellers that acquired *P. shigelloides* only one reported TD (OR 0.3; p = 0.42) (Table 3).

DISCUSSION

This study shows that *Dientamoeba fragilis* and *Blastocystis* spp. were highly prevalent before travel. *Blastocystis* spp., *Plesiomonas shigelloides*, Dientamoeba fragilis and *Shigella* spp. were the most frequently acquired pathogens and acquisition of enteral viruses and HEV in this relatively small group of travellers was rare or non-existent.

To our knowledge, travel associated acquisition of a wide spectrum of nineteen viral, bacterial and parasite species has not been reported before. The PCR's used for this study are validated and used for routine diagnostics and are considered very sensitive. However, as with all molecular tests, the detection of DNA does not necessarily mean that detected pathogens are viable. Also, the possibility exists that pathogens acquired during travel were already cleared by time the traveller returned and did the post travel sampling, and some brief intermittent acquisitions may therefore have been missed in this analysis. However, post-travel samples were provided within a mean of 3 days (range 0–19 days; median 2 days) and the mean duration between sample collection and processing in the laboratory was 2 days (range 1–23 days) so we do not regard this possibility as a significant source of bias. A major limitation of this study is the sample size that is probably too small to detect rarely acquired enteropathogens. Therefore, no firm conclusions on the acquisition of HEV and other rare pathogens can be drawn from this study. Also, an association between the acquisition of studied microorganisms and TD was not found, but because a limited number of travellers were tested, the statistical power to study this association was low. Unfortunately we were not able to study *Cyclospora cayetanensis*, which is considered an important parasitic cause of traveller's diarrhoea in some regions [4].

Lääveri and co-workers performed a prospective study on bacterial TD pathogens in 382 Finnish travellers [5] and found acquisition of *Salmonella* spp. and *Shigella*/EIEC to be slightly lower than in the present study; namely 2.4% and 0.8% respectively; and *Campylobacter* was acquired by slightly more (6.8%) travellers. However, they did not test for Clostridium, *Yersinia*, *Plesiomonas*, parasites and viruses.

A non-prospective case-control study in 114 returning travellers with diarrhoea and 56 travellers without diarrhoea [6] found 19/170 (11.2%) to be positive by microscopy for *Blastocystis* spp. after travel and only one positive for *Dientamoeba fragilis*. Probably, these lower post-travel prevalences are the result of lower diagnostic sensitivity of microscopy compared to PCR. Interestingly, they found 10 cases of *Giardia lamblia* and 3 cases of *Cryptosporidium* spp., whereas in the present study only one acquisition of *Giardia lamblia*and no *Cryptosporidium* spp. was found. In their study, PCR for Norovirus was positive in 10.5% of the cases and 3.6% of the controls, whereas we found only 2.1% acquisition in the total studied population. The observed differences might be explained by a selection towards a more symptomatic population in the study by Paschke [6], because all of their participants presented as patients to the Department of Infectious Diseases of which two-third with diarrhoea [6].

Travel associated acquisition of HEV is a potential threat to blood safety [7], but no acquisitions were found in the present study. Accordingly, in a Dutch study, no seroconversions to anti-HEV were found in 1206 travellers to (sub)tropical countries [8]. An Australian study estimated the risk of acquiring HEV to be 0.01 to 18 per 10,000 travellers [7] indicating that our limited sample size likely is too small to reliably determine travel-associated acquisition of HEV. Furthermore, the incubation period of symptomatic HEV infections is approximately 40 days (range 2–10 weeks) [9], hence infections may be missed by analysis of samples that were collected shortly after return.

The low acquisition rates of enteric viruses during travel could indicate that the role of viruses as the cause of persisting TD is limited or that viral RNA or DNA is rapidly cleared before returning. For Norovirus however, virus is shed for a median of 28 days after inoculation in previously healthy persons [10] and for Rotavirus, median duration of shedding is approximately 3 weeks [11]. Since bacterial pathogens were acquired more often, they are likely to be more important as a cause of traveller's diarrhoea. For *Blastocystis* and *Dientamoeba*, pathogenicity of these parasites is under debate. To that end, a positive test in a symptomatic returning traveller must be interpreted with caution, keeping in mind that a substantial proportion probably would already have been carrier before travel.

Since *Blastocystis* spp were acquired relatively often, it would be interesting to study if acquisition could be assigned to specific travel destinations.

ACKNOWLEDGEMENTS

The COMBAT-study was funded by Netherlands Organisation for Health Research and Development (ZonMw, grant number 205200003).

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Appendix A. Supplementary data

	Species	Gene targeted	Primer-F	Primer-R	Probe
Viruses	Adenovirus (40, 41)		TTC CAG CAT AAT AAC TCW GGC TTT G	AAT TTT TCT GWG TCA GGC TTG G	CCW TA+CCC+C+CTT ATT+GG
	Adenovirus (52)		AAC AGA TAC CGC AAC CAC CC	CCT GCC ACT TTA TCA TTA GTG CCT A	TAT CAA+CCT+GAA+C+CA+CAA G
	Astrovirus		GAC TGC WAA GCA GCT TCG T	GCC ATC ACA CTT CTT TGG T	TCA CAG AAG AGC AAC TCC ATC GCA TTT G
	Enterovirus		GGC CCT GAA TGC GGC TAA T	GGG ATT GTC ACC ATA AGC AGC C	GCG GAA CCG ACT ACT TTG GGT
	Hepatitis E virus		CGG TGG TTT CTG GGG TGA	GCR AAG GGR TTG GTT GG	ATT CTC AGC CCT TCG C
	Norovirus G1		ATG TTC CGC TGG ATG CG	CGT CCT TAG ACG CCA TCA TC	TGG ACA GGA GAT CGC
	Norovirus G2		CAA GAI CCI ATG TTY AGI TGG ATG AG	TCG ACG CCA TCT TCA TTC AC	TGG GAG GGC GAT CG
	Parechovirus		CTG GGG CCA AAA GCC A	GGT ACC TTC TGG GCA TCC TTC	AAA CAC TAG TTG TAW GGC CC
	Rotavirus A		ACC ATC TWC ACR TRA CCC TC	GGT CAC ATA ACG CCC C	ATG AGC ACA ATA GTT AAA AGC TAA CAC TG+T+CAA
	Rotavirus C		CTA CAA GTA ATG GAA TCG GAT G	TGG GTG TCA TTT GAT ACA ACT TCA	ACC AGC TAG TA+C+A+G+A+AAC
	Sapovirus		F1: GAC CAG GCT CTC GCY ACC TAC F2: TTG GCC CTC GCC ACC TAC	CCC TCC ATY TCA AAC ACT AWT TTG	TGG TT+C ATA+G+GT+G+GT AC
Bacteria	*Campylobacter* spp.*	16S			
	Clostridium difficile	toxB	CAAAYGAGTATTCAAARGAKATAGATGAA	TCTTTCTACYAACTCTTGTTCATATAADTTGAAT	AATAGTGGRAATGATGTTAGAAA
	*Plesiomonas shigelloides**	gyrB			
	Salmonella spp.*	ttr			
	Shigella spp.*	ipaH			
	*Yersinia enterocolitica**	gyrB			
Parasites	*Blastocystis* spp.	18S rRNA	CGTTGTTGCAGTTAAAAAGCTCGT	GATTAATGAAAACATCCTTGGTAAATGC	CAGTTGGGGGTATTCATATTC
	Cryptosporidium spp.	DNAJ-like protein	CGCTTCTCTAGCCTTTCATGA	CTTCACGTGTGTTTGCCAAT	CCAATCACAGAATCATCAGAATCGACTGGTATC
	Dientamoeba fragilis	18S rRNA	CAA CGG ATG TCT TGG CTC TTT A	TGC ATT CAA AGA TCG AAC TTA TCA C	CAA TTC TAG CCG CTT AT
	Entamoeba histolytica	18S rRNA	ATTgTCgTggCATCCTAACTCA	gCggACggCTCATTATAACA	UCAUUGAAUGAAUUGGCCAUUU
	Giardia lamblia	18S rRNA	GAC GGC TCA GGA CAA CGG TT	TTG CCA GCG GTG TCC G	CCC GCG GCG GTC CCT GCT AG

* Detection of these bacteria was performed using The LightMix® Modular Assay (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) for which no primer and probe sequences are available.

CHAPTER 10

General discussion and summary

INTRODUCTION

Healthy travellers can pick up micro-organisms carrying antimicrobial resistance genes when visiting regions of the world where antimicrobial resistance among common pathogens has reached high endemic levels, and import these to their home countries. The microbiota of healthy travellers may, thus, become reservoirs of resistant strains and their antimicrobial resistance genes. However, because acquisition of antimicrobial resistant pathogens is only noticed in travellers when they cause an infection, little is known about carriage of these resistant pathogens by healthy travellers. As it is estimated that the number of international tourists to endemic regions in Asia and Africa will continue to grow at the rate of 4.4% a year (1), it is prudent to investigate the risks of introduction and possible spread of antimicrobial resistance (in particular antibiotic-resistant Enterobacteriaceae) by healthy travellers from the Netherlands, a country known for its restricted antibiotic use and correspondingly low levels of resistant pathogens in its community and health care system.

MAIN FINDINGS AND DISCUSSION

Impact of international travel on the endemicity of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) in the Netherlands

Previous small to medium sized studies of international travellers had reported high acquisition rates of ESBL-E, with observed frequencies varying between 21-51% **(Chapter 2)**. To more definitively assess the impact of international travel on carriage of antimicrobial resistant pathogens we investigated the acquisition, persistence of carriage and transmission of ESBL-E by healthy travellers prospectively enrolled in a large, multicenter longitudinal cohort. We found that overall, 34.3% (633/1847) of travellers acquired ESBL-E during their international travel **(Chapter 4)**. The median duration of carriage after travel was 30 days and approximately 11% remained a carrier of ESBL-E for more than one year after travel. Also, transmission to close household contacts was observed. Acquisition rates varied per subregion from 5.9% in travellers returning from Northern America/ Europe/Oceania to 75.1% in travellers returning from Southern Asia. Moreover, the frequency of acquisition also varied widely per country within these regions.

We determined the contribution of the import of ESBL-E by healthy travellers on a population level by using the acquisition rates we found per subregion in our study and by taking into account the total number of Dutch travellers visiting these subregions annually (2). From these calculations we estimate that each year between 3.0% and 7.1% of the Dutch population acquire an ESBL-E during travel to Asia, Africa and Latin-America.

In absolute numbers that is a yearly influx of 500.000 to 1.2 million Dutch travellers with ESBL-E. So far no other factor in the Netherlands has been identified accounting for so much ESBL-E influx in to the Netherlands. According the Central Bureau for Statistics of the Netherlands the two most popular regions visited for long holidays are Western Asia and Northern Africa. The large number of visitors to Western Asia can be explained by the preference of Dutch tourists for all-inclusive hotels in Turkey (personal data Travel Clinic Harbor Hospital), and by first and second generation immigrant inhabitants with Turkey as their migration background, as this is the largest group in the Netherlands with a non-western migration background. The large number of visitors to Northern Africa can be explained by the preference of Dutch tourists for all-inclusive hotels in Egypt (personal data Travel Clinic Harbor Hospital) and by first and second generation immigrant inhabitants of the Netherlands that have Morocco as their migration background, as this is the second largest group in the Netherlands with a non-western migration background. Our study found high acquisition rates (42.0-42.9 %) by travellers to these two regions. With our calculations we estimate that, Dutch travellers to Western Asia and Northern Africa together account for an annual influx of as much as 320.000 to 870.000 travellers importing ESBL-E into the Netherlands. Thus, although travellers to Southern Asia have the highest risk (75.1%) to acquire ESBL-E, travellers to Western Asia and Northern Africa contribute most to ESBL-E endemicity at the level of the Dutch population. Since the Dutch population has a long history in international travel and global trade, including visits to regions with high endemic levels of antimicrobial resistance, the yearly influx of ESBL-E yielded by healthy travellers is a major determinant of the ESBL-E endemic level in the Netherlands.

A limitation of our study is that our study population included mostly native Dutch travellers who came to the travel clinics for vaccinations and travel advice. By using data of the Central Bureau of Statistics of long holidays among the Dutch population, we were able to include the Dutch inhabitants of Turkish or Moroccan origin who go on holiday to their country of origin in our calculation of ESBL-E influx by Dutch travellers contributing to ESBL-E endemicity in the Netherlands. We were not able to take into account the contribution to ESBL-E endemicity in the Netherlands of recently arrived refugees. In 2018 there were almost 26.000 asylum requests in the Netherlands most of which were made by Syrian refugees (3). Research in Germany showed that 35% of Syrian refugees were carrier of ESBL-E. Since the number of refugees is much lower than that of Dutch inhabitants travelling internationally, the contribution of refugees is expected to be much smaller than that of Dutch travellers (4).

Another limitation of our study is that we were not able to include travellers to countries within Europe or other countries for which no vaccinations or travel advice is needed. Data show ESBL prevalence rates among hospital *E. coli* isolates in the range of 10-25% for popular summer holiday destinations including Spain, Portugal, Greece

and Israel (5-7). As resistance genes can be acquired in the first days after arrival in high endemic countries (John Penders, not published), also short holidays to these popular destination within Europe are likely to contribute to the ESBL-E endemic level in the Netherlands.

Although little more than half of travellers who acquired ESBL-E during their travel lost their ESBL-E within one month after return, 11.3% of travellers remained a carrier of ESBL-E 12 months after return. These sustained carriers also contribute to the ESBL-E endemic level in the Netherlands, since 11.3% of 500.000 to 1.2 million healthy travellers will yield 56.000 to 135.000 long term carriers with imported ESBL-E carriage up to twelve months after return. Our review showed that other traveller studies found varied duration of carriage rates from 5% to 24% at 6 months after return. Our Cox regression analysis showed travellers who carried *E. coli* (vs *K. pneumoniae*) or an ESBL-gene from CTX-M group 9 (vs CTX-M group 1) were at increased risk for sustained carriage. These results suggest plasmids carrying CTX-M group 9 provide a colonization advantage over plasmids carrying CTX-M group 1. CTX-M group 9 ESBL genes are known to be dominant in China. In line with this is the observation that travellers to Central- and Eastern Asia in our study cohort were at increased risk for acquisition of CTX-M group 9 (OR 3.3, CI_{95} 1.4-7.5) and ESBL-gene CTX-M-14/18 (OR 3.5, CI_{95} 1.5-7.9), when compared to travellers who did not visit this subregion **(Chapter 5)**. Although our Cox regression analysis did not demonstrate that travel to Central and Eastern Asia posed an increased risk for sustained carriage, our data do suggest that these travellers are more at risk for sustained carriage of ESBL-E, because of the greater chance to acquire ESBL CTX-M group 9 when compared to travellers to other destinations. Another study found travellers returning from Asia had significantly longer sustained carriage compared to travellers from sub-Saharan Africa or Latin America (8). Other studies also found CTX-M group 9 to be associated with sustained carriage in travellers returning from Asia, and in patients from hospital, long-term care facilities and primary care practices (9, 10). A recent study which investigated long-term ESBL-E carriage in the adult Dutch community suggested that in half of persons who continued to carry CTX-M group 9 ESBL genes for up to 8 months, persistence of successful clones rather than horizontal gene transfer was explanatory for the persistence of these ESBL-E. They found that those with antibiotic use during the last 6 months, proton pump inhibitor use or living within 1,000 meters of a pig farm are at increased risk for sustained ESBL-E carriage (11).

With our mathematical model which took factors into account like actual total household size, information which was derived from our questionnaires, we estimated that there was a 12% probability of transmission from ESBL positive persons to ESBL negative persons in households. Perhaps an epidemiologically more popular way to describe transmission rates is the basic reproductive ratio (R_0), which is the total number of secondary ESBL-E cases generated from the total number of ESBL index persons introduced

into a susceptible population of household contacts. A basic reproductive ratio >1 is considered as the threshold for ESBL-E to spread in the susceptible population (12). In our mathematical model of onward transmission of ESBL-E in households of travellers, we estimated person-to-person transmission to occur at a rate of 0.0013 per colonized person a day and the average duration of carriage to be 100 days. R_0 is calculated to be 0.20, by multiplying the transmission rate (0.0013/day) with the average duration of carriage (100 days) and the average number of household numbers (1.54). As this R_0 of 0.20 is far below 1.0, we conclude that transmission to household members does not lead to significant spread within the susceptible population. To put our finding in perspective, a higher R_0 of 0.90 was found for MRSA transmission from patients to household contacts (13). Another Dutch study using the same mathemathical model as ours for onward transmission of ESBL-E in households of recently discharged patients, estimated a similar average duration of carriage of 111 days. However, they estimated a higher person-to-person transmission rate of 0.0053 per colonized person a day, which is most likely explained by the more frequent and longer exposure times between caregiving household members to discharged patients (14). Although transmission of ESBL-E in households of travellers unlikely leads to sustained spread, a transmission rate of 12% does contribute to the endemic level of ESBL-E in the Netherlands, as 12% of 500.000 to 1.2 million healthy travellers implies 60.000 to 144.000 travellers who transmit ESBL-E to their household members yearly.

Travel-associated risk factors

Travel destination is the dominant determinant for acquisition of ESBL-E in our cohort. Our travellers to Asia had a higher frequency of ESBL-E acquisition, which is probably due to the widely spread distribution of ESBL-E in these regions and, therefore, a high risk of food-contamination. In our overall analysis, occasional food consumption from street vendors was associated with an increased risk for ESBL-E acquisition, whereby this risk further increased when food from street vendors was consumed daily. As diet-associated predictors might differ per subregion, we performed a stratified analysis per subregion, which identified daily food consumption at a hostel or guesthouse in southern Asia and consumption of raw vegetables in southeastern Asia as predictive factors. The highest ESBL-E acquisition rate of 88.6% was found in travellers returning from India. Apart from the overuse of antibiotics in humans and animals, the high level of ESBL-E endemicity in India can, at least in part, be explained by its lack of access to proper sanitation facilities, with less than 40% of its population having access to sanitation (15). Also, several large and small pharmaceutical factories producing antibiotics are located in India. Research in the vicinity of those factories have shown that tons of antibiotics such as ciprofloxacin are released in the environment every year (16). In such setting, the selective pressure of just one antibiotic likely leads to the co-selection of multi-drug resistant isolates and

drive their spread (17). One study demonstrated that the use of fluoroquinolones during travel selected for ESBL-E acquisition during travel (18). Moreover, a recent meta-analysis showed 12.4% of antibiotics were substandard or falsified in low,- and middle-income countries, which is also thought to contribute to increasing antimicrobial resistance in these countries (19).

In our multivariable analysis we found that antibiotic use (in particular the use of quinolones) during travel, traveller's diarrhoea, and pre-existent chronic bowel disease were independently associated with an increased risk of ESBL-E acquisition. These conditions are all known to be associated with a dysbiosis of the human gut microbiota (20-22). Therefore, dysbiosis of the human microbiota with subsequent decreased colonization resistance might be a biologically plausible common precondition or mechanism for increased susceptibility to the acquisition of ESBL producing micro-organisms. Dutch national guidelines for the use of antibiotics advise the prescription of ciprofloxacin or azithromycin for moderately severe traveller's diarrhoea in patients with chronic conditions like diabetes mellitus or use of immunosuppressive drugs. Both ciprofloxacin and azithromycin are known to induce a dysbiosis of the human microbiota (20, 23, 24). The traveller study of Kantele *et al.* demonstrated ESBL-E acquisition rates in travellers to South-East Asia without traveller's diarrhoea and without antibiotic use, in those with traveller's diarrhoea but without antibiotic use, and in those with traveller's diarrhoea and antibiotic use to be 19%, 32%, and 69%, respectively. For travellers to South Asia similar rates were reported, i.e. 23%, 47% and 80%, respectively. These results suggest that the resistance of travellers to become colonized by ESBL-E is synergistically decreased by diarrhoea in combination with the use of antibiotics (25, 26). In our study we found travel-related and non-travel related risk factors for ESBL-E acquisition, all known to be associated with gut dysbiosis. However, the effect of travel itself on the composition and function of the human gut microbiota remains unknown, as only a single study has examined the gut microbiota of diarrhoea-free travellers. This study found the gut microbiome in healthy, diarrhoea-free, travellers to be dysbiotic, suggesting that travel itself could be associated with temporary dysbiosis of the gut microbiota. However, this was merely an assumption or hypothesis, since pre-travel faecal samples of these healthy travellers were not analysed (27).

ESBL-E carriage in the community

In **chapter 5** we investigated ESBL prevalence and associated risk factors in travellers prior to their index travel. These results are, to some extent, reflective for ESBL-E carriage and predictors in the Dutch general adult population. We found a prevalence for ESBL-E carriage of 6.1% (136/2216) prior to travel, which was slightly higher (versus 4.5% and 5.1%) and lower (versus 8.6%) compared to previous Dutch studies among healthy individuals (28-30). From the multivariable risk factor analysis, previous antibiotic use in

the past three months and previous travel outside of Europe in the past twelve months were the major risk factors for ESBL-E carriage prior to travel.

The association between carriage of ESBL-E prior to travel and recent antibiotic use found in this study, may reflect reduced colonization resistance to ESBL-E acquisition from external sources as well as selection of ESBL-E present in the gut prior to exposure, albeit at densities below the detection threshold of routine microbiological culture. Especially the use of beta-lactam antibiotics was associated with ESBL-E carriage prior to travel. Beta-lactams are the class of antibiotics most commonly prescribed by general practitioners, by doctors from outpatient clinics and by dentists in the Netherlands (31). Quinolones are less often prescribed, which may help to explain why we did not find a significant association between its use and ESBL-E carriage prior to travel. In the Netherlands the use of amoxicillin and amoxicillin clavulanic acid in outpatients has been declining over the past decade (31). Future research has to determine whether, and if so, to what extent, this decline in the use of beta-lactam antibiotics will be accompanied by a decrease in ESBL-E carriage in the community.

The association between carriage of ESBL-E prior to travel and travel outside of Europe was particularly strong for travel to Asia (Eastern Asia), Africa (Northern Africa) and Oceania (Australia and New Zealand). Other studies on ESBL-E carriage in the community of countries with prudent use of antibiotics also found that those who travelled to Asia and Africa in the past twelve months are at increased risk for ESBL carriage (29, 30, 32, 33). The finding that travel outside of Europa is a major risk factor for ESBL-E carriage prior to travel confirms our finding that travellers returning from high endemic regions for ESBL-E substantially contribute to the ESBL-E endemicity in their country of origin. Moreover, in our population of travellers, the population attributable risk was greatest for travel outside of Europe; it's attributable risk was two times higher than that for antibiotic use. In our cohort of travellers the prevalences of CTX-M-15 and CTX-M-14/18 prior to travel were 31.3% (40/128) and 21.9% (28/128), respectively. Previous other Dutch studies found lower or similar proportions (13-19%) of *bla*CTX-M-14 in the community. Although our study did not have the statistical power to investigate if CTX-M-14 carriage prior to travel was associated with previous travel to CTX-M 14 endemic regions, it is probable that these CTX-M-14 genes were acquired during previous travel to CTX-M-14 endemic regions. Since CTX-M-14 is not prevalent in the Netherlands it is less likely that CTX-M-14 carrying Enterobacteriaceae were acquired in the Netherlands (29, 30, 34). With the unabated growth in international travel by tourists, business travel, but also by migrants and refugees, it is expected that the impact of international travel on ESBL-E carriage and infection in the community will keep growing the coming years.

We found that participants working in healthcare with daily patient contact tended to be at increased risk for ESBL-E carriage prior to travel, although the association was not statistically significant. The number of participants working in healthcare may have

been too low to reach statistical significance. Just a few studies reported ESBL-E carriage rates among healthcare workers, which ranged from 3 to 21% (35-37).

We did not find an association between consumption of chicken meat or other food products and ESBL-E carriage prior to travel. In accordance with our findings, none of the studies investigating potential predictors for ESBL-E carriage in countries with prudent antibiotic use did identify meat products as a predictor for ESBL-E carriage, except for one study (38). Recent studies using whole genome sequencing show that there is no evidence of clonal spread of ESBL *E. coli* through the food chain, rather they suggest that distinct plasmids contribute to the spread of antibiotic resistance between different reservoirs (39). However, a recent study failed to demonstrate a epidemiological link of ESBL genes and plasmid types between isolates from livestock or food reservoirs and human isolates from the community (40).

We also did not find the use of antacids as a risk factor for ESBL-E carriage in our study population before travel. In addition, antacid use during travel was also not identified as a risk factor for ESBL-E acquisition during travel. The use of prescribed antacids has been identified as risk factor for ESBL-E carriage in the Dutch population, and the use of proton pump inhibitors, the dominant class of antacids prescribed here, has been identified as a risk factor for ESBL-E carriage at hospital admission (29, 41). Moreover, the use of proton pump inhibitors was recently reported to be associated with sustained carriage of ESBL-E in the Dutch community (11). Proton pump inhibitors have been associated with a dysbiosis of the gut microbiota (42-44). We did not make a distinction between proton pump inhibitors (which are prescribed antacids) and neutralizing antacids (that can be obtained over the counter medicine without prescription). Thus, it could be that our participants were mostly using neutralizing antacids, which have so far not been associated with ESBL-E carriage.

As we have shown that returning international travellers are a dominant source for influx of ESBL-E (mostly ESBL-*E.coli*) in the Netherlands, contributing to ESBL-E endemicity in the community, it is assumed that travellers also contribute to the prevalence of community and hospital acquired infections caused by ESBL-E. A recent Dutch study provided evidence that ESBL *E. coli* strains causing hospital infections most likely originated from the community. These authors analysed trends of ESBL *E. coli* and *K. pneumoniae* infections among patients attending a general practitioner and in hospital patients from 2008-2012. They found an increase in the proportion of ESBL among *E. coli* isolates from urine and blood samples in both general practitioner patients and hospital patients, including ICU patients. However, the proportion of ESBL among *K. pneumoniae* isolates from urine and blood samples did not increase in hospital patients, including ICU patients. Therefore, the rise of ESBL-*E. coli* in the hospital is most likely explained by increased influx from the community, as *E. coli* is a common community pathogen and, therefore, little affected by infection prevention policies in hospitals. In contrast,

the prevalence of ESBL- *K. pneumoniae* in the hospital did not increase, suggesting that infection prevention policies restricted the spread of this hospital pathogen (45). However, from 2013 to 2018 the percentages of both ESBL- *E. coli* and *K.pneumoniae* have risen to 2-6% for *E. coli* and 4-9% for *K. pneumoniae*, with the lowest percentages in GP patients/outpatients and the highest percentages in inpatients/ ICU patients (31). The reasons behind the overall rise of ESBL- *K. pneumoniae* need to be further investigated. Interestingly, 19% of bean sprouts samples in the Netherlands were found to be contaminated with mostly ESBL-*K.pneumoniae* in the absence of ESBL-*E. coli* (46).

Implications of our findings: who is at risk for carriage of antibiotic resistant Enterobacteriaceae

To control MRSA in hospital, current standard care, at least in many Western countries including the Netherlands, include risk assesments for MRSA carriage by questionnaires to be taken for every patient at the time of their admission. When patients have one or more risk factors for MRSA carriage they are isolated and first screened for MRSA. For carriage of ESBL-E and carbapenemase producing Enterobacteriaceae (CPE), hospitalization abroad, especially if treated on the ICU, is likewise associated with an increased risk (4). Moreover, infections with CPE in countries with very low CPE prevalence have been associated with prior hospitalization in CPE endemic regions (47). Currently, to control antibiotic resistant Enterobacteriaceae, patients are asked whether they have been examined, treated or admitted to a hospital abroad in the preceding two months, or if longer than two months ago whether they have been operated in a hospital abroad or have wounds. If so they are screened for antibiotic resistant Enterobacteriaceae and other (multidrug) resistant bacteria. However, this approach does not encompass the latest insights into the risk for ESBL-E carriage associated with recent international travel. Several recent studies, including our large cohort study, clearly identified travel to certain regions in Asia and Africa to be associated with a high risk for ESBL-E carriage, even without hospitalization abroad. These findings would argue that patients who have recently travelled to ESBL-E endemic regions need to be screened for ESBL-E carriage at the time of admission to hospital. As it is not feasible and effective to screen all patients with recent travel abroad, screening should focus on those patients with a clearly increased risk for ESBL-E. The first question is which regions or countries can be considered as high ESBL-E endemic regions? As reliable surveillance data on ESBL-E carriage rates are lacking for most low to middle income countries in Asia and Africa (48), data on ESBL-E acquisition in returning travellers might be used as a surrogate marker for ESBL-E prevalence in the community in these countries. As acquisition rates vary widely per country within subregions, for example acquisition in South-Eastern Asia varied from 19.0% in Indonesia to 72.2% in Vietnam, it would be prudent to ascertain ESBL-E endemicity per country. If we would define high ESBL-E endemic countries as

those from which >40% of travellers return with ESBL-E, current data suggests that patients returning from India, Egypt, Nepal, Vietnam, Peru, China, Myanmar, Thailand, Sri Lanka, Uganda and Turkey should be screened for ESBL-E on admission to hospital. Unfortunately, this list is not complete since it only includes countries which are popular, intercontinental, travel destinations. This lack of completeness is also illustrated by data showing ESBL prevalence rates among hospital *E. coli* isolates in the range of 25-50% in Italy, Morocco, Cyprus, Slovakia and Bulgaria (5, 49, 50). These high ESBL *E. coli* hospital prevalence rates suggest that the ESBL carriage in the community of these countries is also high, as *E. coli* is a true community pathogen. Therefore, admitted patients recently returning from these countries may also have to be considered for admission screening.

The second question to consider is how long after return should patients be screened on admission to hospitals. Our COMBAT study, which is the largest traveller study so far, revealed that 43%, 25%, 14%, and 11% of travellers with ESBL-E acquisition during travel still carried their ESBL-E at 1, 3, 6, and 12 months after their return, respectively. The second largest traveller study, the VOYAG-R study reported somewhat lower sustained carriage rates of 34%, 10%, 5% and 2% at 1, 3, 6, and 12 months after return, respectively (8). They concluded that ESBL-E acquisition during travel was relatively short-lived afterwards. However, their rates are not comparable with the rates we reported because, for unclear reasons, when calculating carriage rates they included missing samples in the denominator, thus assuming that these would be negative. For example, at 12 months after return only 8 of 227 travellers provided a stool sample, of whom 5/8 (62%) still carried ESBL-E. The stated carriage rate of 2.2% (5/227) 12 months after return is, thus, a gross underestimation as 219 samples were missing. Therefore, we suggest that the sustained carriage rates from the COMBAT study are more accurate. I would, therefore, suggest that patients with travel to ESBL-E high endemic regions (as specified above) in the preceding 1- 3 months, whether they traveled as tourists, migrants or refugees should be screened for ESBL-E at the time of their admission to a hospital, as 25-43% of travel acquired ESBL-E persisted in the gut beyond that timepoint.

Our findings also suggest that patients with recent international travel and antibiotic use during travel or pre-existent chronic bowel disease should be screened for ESBL-E carriage when admitted to the hospital.

In our study, we did not analyse the subsequent risk for infection with ESBL-E in travellers with ESBL-E acquisition during travel. There is not much data on travellers colonized with ESBL-E or CPE and their subsequent risk for developing an infection with these antibiotic resistant Enterobacteriaceae. One traveller study followed 90 travellers with ESBL-E carriage up to one year after travel and reported no laboratory evidence of pyelonephritis or other infections with ESBL-E (25). A French study prospectively screened patients who were admitted to the Infectious Diseases ward and had travelled abroad in the past twelve months. 5/191 travellers had a clinical infection by multidrug

resistant Gram-negative bacteria and 18/191 only carried such strains, of which 22/23 were ESBL-E. Of the five travellers with infection, four had an urinary tract infection with ESBL producing *E. coli*. Infection or carriage with ESBL-E in patients was ten times higher in those who travelled abroad in the past twelve months compared those wo did not travel abroad (51). Moreover, other studies identified international travel as a risk factor for ESBL-E community acquired urinary tract infections more than a decade ago (52-54). Overall, these studies indicate travellers who acquired ESBL-E during travel can develop an infection with the ESBL-E strain imported from abroad.

Specific attention is warranted for patients admitted for transrectal ultrasonography-guided prostate biopsy, elective colorectal surgery and liver transplantation as ESBL-E colonization in patients undergoing these procedures have a higher risk for post procedure ESBL-E infection (55-58). A recent study among patients admitted for elective colorectal surgery showed the risk for deep surgical site infections was doubled in patients who carried ESBL-E versus non-carriers, and the causative pathogen of the surgical site infection was more likely to be ESBL-E versus in non-carriers (57). The higher risk for surgical site infections in patients carrying ESBL-E is most likely explained by the failure of the routinely recommended prophylaxis with cefazolin and metronidazole to cover for ESBL-E wound contamination during intra-abdominal surgery (59). These findings suggest patients admitted for elective colorectal surgery who have recently travelled to high endemic ESBL regions should be screened for carriage of ESBL-E, so that either the prophylaxis regimen is adjusted or adjustment of empirical therapy will be considered, in case surgical site infection follows after the procedure. More research is needed to weigh the (dis)advantages of targeted surgical prophylaxis in ESBL-E carriers.

The high rates of co-resistance to other classes of antibiotics are a further worry. We observed resistance to gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin and multidrug resistance in 30.8%, 64.4%, 44.8%, 44.3%, respectively, among ESBL *E. coli* strains isolated from returning travellers. Our finding of high co-resistance rates among travel-acquired ESBL *E. coli* strains requires doctors to consider adjusting their empirical therapeutic regimens in patients who recently travelled to a high ESBL-E endemic country. In line with antibiotic stewardship, the risk factors identified for ESBL-E carriage in returning travellers can be used to make a more informed assessment of the personal risk of ESBL-E infection, thereby limiting the prescription of second tier antibiotics to those with an evident increased risk for ESBL-E infection. Recently, risk scoring methods based on case-control studies have been developed to predict bloodstream infection caused by ESBL-E in patients with suspected community sepsis. The so-called Stockholm-score (60) includes healthcare contact abroad within 6 months in addition to a history of prior ESBL-E positive culture or prostate biopsy when predicting ESBL-E community bloodstream infection. If one of these risk factors is present, the authors suggest empirical treatment has to be adjusted. Although the severity of disease and prior antibiotic use

increased the risk for ESBL-E infection, these risk factors were not useful as a tool for guiding empirical therapy as these were present in both cases and controls. However, the severity of illness should be considered in the decision to adjust empirical therapy, with the aim to limit unnecessary use of carbapenems (61).

From a prevention perspective, travellers to endemic areas should be informed about possible exposure to multidrug resistant micro-organisms, especially ESBL-E and CPE, while travelling and how best to limit their risk for ESBL-E acquisition. They should be advised by travel clinics to take the usual hygiene measures to prevent traveller's diarrhoea, to refrain from antibiotic use when diagnosed with self-limiting gastroenteritis, and to avoid eating food from street vendors. These are, so far, the only factors which can be acted upon, as our multivariable risk factor analysis showed that additional actions including regular hand hygiene did not protect against ESBL-E acquisition. Also, we did not find that probiotics had a protective effect on ESBL-E acquisition among travellers. Similarly, a randomized controlled trial showed travellers using probiotics had similar ESBL-E carriage rates compared to the control group of travellers not using probiotics (62).

Impact of international travel on carbapenemase-producing Enterobacteriaceae (CPE) in the Netherlands

In 2010 the first case of infection with a KPC-2 producing *Klebsiella pneumoniae* was reported in a patient after hospitalization in Greece, who later died of pneumoniae caused by this CPE. In the same year, two patients with carriage of NDM-1 producing *K. pneumoniae* were reported after they had travelled in India (63). The danger of CPE was demonstrated in 2010 when a large outbreak of OXA-48 carbapenemase producing *K. pneumoniae* occurred in the Maasstad Ziekenhuis in Rotterdam, a medium to large sized hospital in the Netherlands. Molecular typing showed the OXA-48 producing *K. pneumoniae* strain to be identical or very closely related to other OXA-48-positive *K. pneumoniae* isolates identified in France and Morocco, suggesting the strain originated from Morocco (64). The prevalence of CPE among clinical isolates in the Netherlands has up to now been low (<1%). Medical microbiologists and health care politicians are very much aware of the danger of CPE for which, an action plan designated "Netherlands CPE green in 2025" was developed in 2015. This is a campaign that aims to keep the Dutch CPE statistics in the "green" zone, i.e. at levels below <1% up to the year 2025.

In our cohort of 2,001 international travellers we found 5 travellers (0.25%) who acquired CPE during their travel **(Chapter 6)**. The CPE genes acquired by these travellers were NDM-1/2, NDM-7, OXA-48, OXA-244 and IMI-2 that were acquired in South-East Asia/Eastern Asia, Myanmar, Turkey/Greece, Indonesia and Myanmar, respectively. Persistence of CPE colonization up to 1 month after return was found in two travellers, and up to 6 months after return in one traveller. Moreover, we found evidence of transmis-

sion of CPE from a traveller who had acquired OXA-244 in Indonesia to his household member, after returning home.

Only three previous studies have reported CPE acquisition in returning travellers who were not hospitalized abroad. These included one French traveller with an OXA-181 producing *E. coli* returning from India, a French traveller and Swiss traveller with NDM-1 producing *E. coli* acquisition from India and an OXA-48 producing *E. coli* acquired by a Dutch traveller returning from Egypt (26, 65, 66). Again, these observations provide evidence that travellers can acquire CPE during travel without being hospitalized abroad.

In countries in Asia with high CPE endemicity, the percentage of CPE infections that have its origin in the community have been reported to be as high as 29.5%. However, in Europe and the United States the percentage of CPE infections that are community acquired can be high as well, with reported proportions up to 18.2% (67).

In low endemic CPE countries, like France, preceding travel to Asia has been identified as a risk factor for CPE infection in hospitalized patients (68).

Although CPE acquisition rates in travellers are low, screening for CPE may be considered as possible spread of CPE can lead to large outbreaks in the hospital. Such admission screening for CPE should take into account the most popular travel destinations of Dutch inhabitants (2, 7) (personal data Travel Clinic Harbor Hospital). High endemic CPE regions in which Dutch travellers are most likely to be exposed to CPE are Turkey and Egypt (OXA-48), Italy (KPC), Spain (OXA-48) and Greece (KPC, VIM) (69-71). However, one should also consider to include India, Pakistan, Bangladesh, and Myanmar in its admission screening, countries which are less frequently visited by Dutch inhabitants, but are highly endemic for NDM-producing Enterobacteriaceae, (72-74).

Impact of international travel on mcr-1 in the Netherlands

In 2015, the scientific community was stunned by a report describing the discovery in China of a plasmid based gene, designated *mcr-1*, that encoded for colistin resistance (75). Following this new report, we analysed our collection of ESBL positive samples from returning travellers for *mcr*-1 presence. Six travellers returning from different geographic regions were identified to have acquired the mcr-1 gene, providing clear evidence that the mcr-1 gene had already spread worldwide at the time of enrolment in 2013-2014 of participants in our cohort study **(Chapter 7)**. Indeed, soon after its discovery, many reports described the mcr-1 gene in isolates from animals, animal food products, humans and environmental samples from around the world. In China a hospital outbreak (n=6) of a *mcr-1*-producing *K. pneumoniae* strain among children with acute leukaemia was described (76).

In our review we aimed to analyze the dynamics behind this worldwide spread of *mcr-1* **(Chapter 8)**. By reviewing whole-genome sequences and MLST profiles from 65 published *mcr-1* carrying *E. coli* human, animal and environmental isolates we found

the worldwide dissemination of *mcr-1* is driven mainly by highly promiscuous plasmids rather than *mcr-1*-carrying clones. More regional circulation and dissemination of the *mcr-1*-carrying plasmid IncHI2 was found in Europe and IncI2 in Asia.

Of the 65 *E. coli* isolates, 4 carried carbapenemase encoding genes (NDM-5 from China and USA) and KPC-2 (from Singapore). Moreover, 29/65 (44.6%) carried plasmid-encoded CTX-M ESBL genes, and 10/65 (15.4%) carried plasmid mediated quinolone resistance (qnr) genes. However, the co-resistance rates may not be representative, as there is a significant selection bias of the published *mcr-1* carrying *E. coli* isolates, since most studies searched for *mcr-1* in their existing ESBL-E collections. Interestingly, we found a high percentage of florfenicol (48.9%) and novobiocin (100%) resistance, antibiotics that are only used in food animals, strengthening the current hypothesis that *mcr-1* emerged first in animals.

Although we found the population of *mcr-1*-carrying *E. coli* to be highly diverse, it was dominated by two large groups of genetically related isolates. One of the groups was centered on ST10, which has a high prevalence among the microbiota of humans and food animals and possesses a range of antimicrobial resistance genes (among which plasmid-carried CTX-M ESBL genes) that is more extensive compared to other ST types, suggesting it has an intrinsic propensity for acquiring AMR genes. Therefore, we hypothesized that certain commensal *E. coli* populations, including ST10, may act as a reservoir for the *mcr-1* gene, which likely explains their over-representation in our study.

In the Netherlands a very low prevalence of *mcr-1* was recently reported in only 2/576 (0.35%) fecal samples of patients attending the Leiden University Medical Center, a tertiary hospital in the Netherlands, this gene was detected by real-time PCR. These patients had no history of recent travel nor of recent colistin use (77). Another Dutch study found 2/18 phenotypically colistin resistant *E.coli* isolates, as determined by Vitek susceptibility testing, to harbour the *mcr-1* gene (78). In the meantime, other plasmid mediated genes encoding for colistin resistance have been identified (labeled *mcr-2* to *mcr-8*). The low prevalence of *mcr-1* in combination with a low prevalence of CPE found in the Netherlands, suggests health care in this country is currently not facing a substantial threat of extremely resistant or even pan-drug resistant Gram-negative bacteria in the near future. A low prevalence of *mrc-1* (1.5%) was detected in collections of ESBL *E.coli* cultured from retail chicken meat in the Netherlands in 2009 and 2014 (79), suggesting *mrc-1* reservoirs have been present in Dutch animal husbandry for at least a decade. Also, the rate of *mrc-1* positive *E.coli* isolated from animals (including chicken) coming to slaughter in European countries is low (0.7%) (80). Interestingly, a recent study found a large variation in the prevalence rate of *mcr-1* (2-39%) in retail chicken meat bought from different supermarket chains throughout the Netherlands (81). As the country of origin of the chicken meat could not be traced back, the variation might be explained by the fact that the chicken meat originated from different farms.

In Tunesia, *mcr-1* prevalence in chicken from different farms varied from 20-83% (82). In one of our traveller's *E. coli* isolate acquired in Tunesia, the *mcr-1* carrying plasmid was identified as an INcHI2-type backbone of the ST4 pMLST subtype co-carrying a CTX-M-1 ESBL gene, which was also described in all *mcr-1* carrying plasmids from *E. coli* isolates from chicken farms in Tunesia.This suggests that transmission from chicken meat to the traveller might have occurred, as the traveller reported consumption of beef, chicken and eggs (82).

Since our review revealed *mcr-1* carrying *E. coli* isolates along with carbapenemase encoding genes, one should be aware of the possibility of the emergence of untreatable pandrug resistant Enterobacteriaceae and the possible danger of import of these pandrug resistant Enterobacteriaceae by travellers to their home country or from the veterinary sector of society.

FUTURE RESEARCH

For the future, first of all it is important to recognize antimicrobial resistance is a problem that affects all regions, therefore a global solution to tackle the problem of antimicrobial resistance is needed. Indeed, the WHO has launched such a program, a global action plan on antimicrobial resistance (48). To be able to solve the problem of antimicrobial resistance at its roots, awareness and political will to address the problem in countries with high rates of antibiotic resistance, including resistant strains belonging to the family of Enterobacteriaceae, is needed (83). The finding that neonatal sepsis caused by ESBL-E is a risk factor for neonatal death in low income countries with high level ESBL-E endemicity, highlights the severity of the problem (84). One of the key actions needed is to gather more surveillance data on antimicrobial resistance, as data on ESBL and CPE prevalence rates are lacking in many low- and middle income countries (49). The implementation of this action plan is underway, and, predictably, meets difficulties since it involves funding issues, public awareness, multisectoral collaboration and political will to combat antimicrobial resistance (85, 86).

Secondly, new antibiotics are needed to combat antimicrobial resistance in gram negative bacteria. If one thing is sure, it is that antimicrobial resistance will pose new challenges for treatment. Development of new antibiotics, like new combinations of already-licensed beta-lactams and beta-lactamase inhibitors, and reappraisal of "old antibiotics" should be encouraged by the government and other stakeholders (87).

Thirdly, more detailed molecular characterization of strains from hospital, community and travellers are needed to better understand the dynamics of import, spread and sustained carriage of ESBL-E by travellers. Available data show that the most frequent ST-types found in *E. coli* from the Dutch community and in a Dutch traveller study were

similar, namely ST131, ST10 and ST38 (11, 29, 88). Another Dutch study in the community found persistent carriage of CTX-M group 9 by ST131 and ST10 *E. coli.* Persistent carriage in this study was most likely explained by successful clones, as CTX-M group 9 was carried by the same ST type *E. coli* and the same plasmid in the first and last positive sample (11).

Fourthly, more insights are needed in the dynamics and functioning of the human microbiota in relation to international travel to better understand what causes the increased susceptibility for the acquisition of ESBL producing organisms in the gut. As of now, no studies have analysed the human microbiota of travellers before and after travel, to answer the question if, independent of known risk factors, travel itself causes a dysbiosis in the human gut, and if so, which intermediate factors may be involved in this disturbance (27).

Lastly, the large impact of international travel on ESBL-E endemicity in the Netherlands is relevant for medical specialists, infection prevention specialists, general practitioners and public health practitioners. Implications for each group needs further thought and possibly more research to be able to formulate and manage the implications of international travel on ESBL-E endemicity through specific policies and guidelines.

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CHAPTER 11

Nederlandse samenvatting

Antimicrobiële resistentie vormt een steeds belangrijkere dreiging voor de gezondheidszorg wereldwijd. Het gebruik van antibiotica in mens en dier is een belangrijke risicofactor voor deze toenemende resistentie, het geeft verminderd gevoelige varianten of resistente mutanten een selectief voordeel. Daarnaast is de verspreiding van dergelijke resistente mutanten een belangrijke oorzaak van de opkomst van resistentie als medisch en maatschappelijk probleem. Gezien de enorme groei van internationaal toerisme, namelijk van 25 miljoen reizigers in 1950 naar 1,133 miljard reizigers in 2014 levert internationaal reizen mogelijk ook een substantiële bijdrage aan de opkomst van het resistentieprobleem. Immers resistente bacteriën of hun mobiele genetische elementen die resistentiegenen bevatten, kunnen zich dankzij allerlei vormen van transport makkelijk verspreiden tussen regio's. Een belangrijk deel van de antimicrobiele resistentiegenen is gelegen op plasmiden en codeert o.a. voor extended-spectrum beta-lactamases (ESBLs, zoals TEM, SHV en CTX-M) en carbapenemases, enzymen die resistentie veroorzaken tegen de meeste beta-lactam antibiotica zoals penicillines, cefalosporines en de carbapenems. Bovendien zijn ESBL-producerende Enterobacteriaceae (ESBL-E) en carbapenemase producerende Enterobacteriaceae (CPE) vaak resistent tegen meerdere andere antibiotica klassen, een fenomeen dat co-resistentie wordt genoemd, waardoor weinig tot geen effectieve antimicrobiële middelen overblijven voor de preventie en behandeling van infecties.

Eerdere studies hebben laten zien dat mensen tijdens internationale reizen vaak ESBL-E en soms CPE oppikken (hoofdstuk 2). Echter, er waren nauwelijks gegevens over ESBL-E kolonisatie en transmissie binnen het huishouden na afloop van dergelijke reizen, noch over de duur van de kolonisatie en de factoren die daar van invloed op zijn. Zulke gegevens zijn nodig om vast te stellen wat het risico is van internationaal reizen op de introductie en verspreiding van antimicrobiële resistentie onder de bevolking, en welke maatregelen eventueel nodig zijn om deze risico's te beperken. Om deze vragen te beantwoorden hebben wij een prospectieve multicenter cohort studie opgezet waarin de acquisitie van ESBL-E tijdens internationaal reizen, de risicofactoren voor deze acquisitie, de duur van kolonisatie en de transmissie naar huisgenoten zijn onderzocht (hoofdstuk 3).

De resultaten van dit onderzoek (hoofdstuk 4) geven aan dat het risico op ESBL-E acquisitie tijdens reizen naar met name Azië en Noord-Afrika erg hoog is. De waargenomen frequentie van ESBL acquisitie en import door reizigers, 34,3% gemiddeld onder alle deelnemers, is een reden tot zorg. 75,1% van de reizigers naar Zuid-Azië en 40-50% van reizigers naar Centraal, Oost of West-Azië, en Noord-Afrika liepen tijdens hun reis een ESBL-E op. Naast reisbestemming bleken antibioticagebruik, het optreden van reizigersdiarree (vooral wanneer deze aanhield bij terugkomst), en een voor de reis al bestaande chronische darmziekte de belangrijkstee risicofactoren voor de acquisitie van ESBL-E gedurende de reis. Van antibioticagebruik, reizigersdiarree en chronische darm-

ziekten is bekend dat deze geassocieerd zijn met een verstoring van de microbiële flora (microbiota) van de darm. Waarschijnlijk is verminderde kolonisatieresistentie vanwege een verstoring van de darm microbiota het onderliggende biologische mechanisme waardoor reizigers ontvankelijk zijn voor ESBL-E acquisitie.

11,3% van de reizigers die een ESBL-E hadden verworven waren 12 maanden na terugkomst nog steeds gekoloniseerd met deze resistente bacteriën. Onze bevindingen geven aan dat met name stammen die CTX-M groep 9 genen (dat zijn genen die coderen voor ESBL) bij zich dragen kennelijk een ecologisch voordeel hebben t.o.v. stammen die andere ESBL-genen hebben, want zij koloniseerden de reizigers het langst. Meer getailleerd moleculair en ecologisch onderzoek is nodig om te bepalen welke factoren verantwoordelijk zijn voor het persisterend dragerschap. Door onze acquisitiepercentages te combineren met gegevens van het Centraal Bureau voor de Statistiek met betrekking tot lange vakanties onder de Nederlandse bevolking hebben we de bijdrage van internationaal reizen aan het voorkomen van ESBL-E onder de Nederlandse bevolking (ESBL-E endemiciteit) kunnen schatten. De schatting is dat er jaarlijks een instroom van 500.000 tot 1.2 miljoen Nederlandse reizigers met ESBL-E is. Persisterend dragerschap draagt ook bij aan de ESBL-E endemiciteit, aangezien 11.3% van 500.000-1.2 miljoen betekent dat er 56.000-135.000 langdurige dragers van geimporteerde ESBL-E zijn tot 12 maanden na de reis.

De geschatte kans op transmissie van ESBL-E van een ESBL-E positief persoon naar een ESBL-E negatief persoon binnen het huishouden van de betreffende reizigers bleek 12%, voornamelijk naar hun directe partner in het huishouden. Hoewel de berekende transmissiekans te laag is om een verdere verspreiding naar andere leden van het huishouden te veroorzaken, draagt deze wel bij aan de ESBL-E endemiciteit in Nederland, aangezien 12% van 500.000-1.2 miljoen reizigers betekent dat er 60.000 tot 144.000 reizigers jaarlijks hun ESBL-E doorgeven aan een partner in hun huishouden.

Op basis van onze gegevens stellen wij voor om reizigers die in een Nederlands ziekenhuis moeten worden opgenomen, terwijl zij in de afgelopen 1-3 maanden naar een ESBL-E hoog-endemisch gebied zijn geweest (landen waar > 40% van de reizigers een ESBL-E oploopt) te screenen voor ESBL-E. Ook kunnen de door ons vastgestelde risicofactoren gebruikt worden om een goede inschatting te maken of een patiënt een verhoogd risico heeft op ESBL-E dragerschap of ESBL-E infectie. In het kader van Antibiotic Stewardship programma's van ziekenhuizen kan zo het empirisch voorschrijven van zogenoemde reserve-antibiotica worden beperkt tot patiënten die een evident verhoogd risico hebben op een ESBL-E infectie. Vanzelfsprekend moet bij die keuze ook rekening worden gehouden met de ernst van infectie.

In ons cohort was 6.1% (136/2216) van de deelnemers voor aanvang van hun index reis al drager van ESBL-E (hoofdstuk 5). Dit percentage was goed vergelijkbaar met de percentages (4,5-8,6%) vermeld in eerdere Nederlandse studies onder gezonde indivi-

duen. Onze risicofactor analyse identificeerde antibioticagebruik, met name beta-lactam antibiotica in de afgelopen drie maanden en reizen buiten Europa in de afgelopen 12 maanden als belangrijkste risicofactoren voor ESBL-E dragerschap onder de deelnemers voor aanvang van hun reis. In onze populatie, was het populatie-attributieve-risico voor reizen buiten Europa tweemaal zo groot dan dat voor antibioticagebruik. Een nieuwe bevinding was dat reizigers naar Australië, ook na correctie voor reisduur en andere reisbestemmingen, een verhoogd risico voor ESBL dragerschap hebben. Tot op heden zijn er geen gegevens over het voorkomen (de prevalentie) van ESBL-E in de open bevolking van Australië. Wel is er een hoge ESBL-E prevalentie, 18%, gevonden in instellingen voor langdurige zorg. Overigens, vonden wij geen verband tussen het eten van bepaalde voedingsmiddelen (inclusief kip en rauwe groenten) of gebruik van maagzuurremmers en ESBL-dragerschap voor aanvang van de reis.

ESBL-E stammen geïsoleerd bij deelnemers voor aanvang van hun reis bleken in 31.3% (40/128) het CTX-M-15 gen te bezitten en in 21.9% (28/128) het CTX-M-14/18 gen te bezitten. Onze hypothese is dat een groot deel van de ESBL-genen gevonden bij deelnemers voor hun reis zijn verworven tijdens een eerdere internationale reis. De gevonden prevalentie voor CTX-M-14/18 was hoger of vergelijkbaar met prevalenties (13-19%) gevonden in eerdere Nederlandse studies in de open bevolking. Hoewel onze studie statistisch onvoldoende omvang had om te kunnen vaststellen of CTX-M-14 dragerschap voor de reis geassocieerd was met een eerdere reis van de deelnemer naar een CTX-M-14 endemische regio in de wereld, is het zeer goed mogelijk dat deze CTX-M-14 genen oorspronkelijk zijn verworven tijdens een eerdere reis naar een CTX-M-14 endemische gebied, aangezien CTX-M-14 niet dominant aanwezig is in Nederland.

Co-resistentie tegen gentamicine, nitrofurantoine, co-trimoxazol en multipele resistentie was significant lager onder stammen geïsoleerd van deelnemers met ESBL-E dragerschap voor de reis in vergelijking tot reizigers met ESBL-E acquisitie tijdens de reis. Een mogelijke verklaring hiervoor is dat genen die verantwoordelijk zijn voor de co-resistenties relatief snel worden verloren na de reis, sneller dan een eventueel verlies van ESBL-genen zelf.

In ons reizigerscohort bleken 5 van de 2001 (0.25%) reizigers een CPE te hebben opgelopen tijdens hun reis. De verworven CPE-genen waren NDM-1/2, NDM-7, OXA-48, OXA-244 en IMI-2, en de reizigers waren in, respectievelijk, Zuid-Oost Azie/Oost-Azie, Myanmar, Turkije/Griekenland, Indonesie en Myanmar geweest (hoofdstuk 6). Bij een reiziger die een OXA-244 positieve CPE had opgelopen tijdens zijn reis trad na terugkomst van zijn reis transmissie van de CPE naar zijn huisgenoot op. Wanneer we rekening houden met de meest populaire vakantiebestemmingen van Nederlanders dan zullen Nederlanders het meest waarschijnlijk worden blootgesteld aan CPE in de hoog endemische regio's Turkije en Egypte (OXA-48), Italië (KPC), Spanje (OXA-48) en Griekenland (KPC, VIM).

Nadat in najaar 2015 in de literatuur werd gerapporteerd dat er in China een plasmide was geïsoleerd met daarop het voor colistine-resistentie coderende mcr-1 gen, hebben wij in onze collectie stammen gekeken naar de prevalentie van mcr-1. Zes reizigers uit ons studiecohort bleken het mcr-1 gen verworven te hebben tijdens hun reis naar verschillende regio's (Zuid-Oost Azie, Oost-Azie, West-Azie en Zuid-Amerika). Deze bevinding betekent dat het mcr-1 gen zich al voor de start van onze studie in 2013 wereldwijd had verspreid (hoofdstuk 7). Vlak na deze ontdekking werden vele studies uit vele delen van de wereld gepubliceerd die het mcr-1 gen beschreven in Enterobacteriaceae stammen geïsoleerd van dieren, dierlijke producten, mensen en uit omgevingsmonsters. In een overzichtsartikel hebben we de dynamiek van de wereldwijde verspreiding van het mcr-1 gen beschreven (hoofdstuk 8). Door vergelijking van de complete genoom sequenties van de 65 gepubliceerde mcr-1 positieve E. coli isolaten afkomstig van mensen, dieren en de omgeving, verzameld over de wereld, konden wij aannemelijk maken dat de wereldwijde verspreiding van het mcr-1 gen vooral het gevolg is geweest van de verspreiding van een beperkt aantal plasmiden en niet het gevolg was van klonale verspreiding van mcr-1 positieve E.coli stammen zelf.

Hoewel de populatie van mcr-1 dragende E. coli stammen zeer divers was, vonden wij dat de verzameling voornamelijk bestond uit twee grote groepen van aan elkaar genetische verwante isolaten. Een van die twee groepen bevatten stammen behorend bij het klonale type ST10, een veelvoorkomend E.coli ST type bij mensen en dieren dat vaak resistentie genen bij zich draagt. Omdat ST10 waarschijnlijk makkelijk resistentiegenen oppikt, is onze hypothese dat bepaalde commensale E. coli kloons, zoals ST10, kunnen fungeren als reservoir voor het mcr-1 gen.

Van de 65 mcr-1 positieve E. coli isolaten bevatten er vier genen die voor carbapenemase coderen, en 29/65 (44.6%) bevatten plasmiden met CTX-M ESBL genen. Hoewel studies laten zien dat de prevalentie van mcr-1 onder de Nederlandse bevolking vooralsnog erg laag is (0.35%), blijft het belangrijk alert te zijn op het opduiken van voor alle middelen-resistente (pan-drug resistant) Enterobacteriaceae, en op de import van dergelijke stammen door reizigers of transmissie vanuit de veterinaire naar de humane sector.

In hoofdstuk 9 hebben we de acquisitie van verscheidene bacteriële, virale en parasitaire darmpathogenen onderzocht bij 98 reizigers uit ons studiecohort. In deze studie vonden we dat een groot aantal reizigers al voor aanvang van hun reis drager waren van de parasietensoorten Dientamoeba fragilis (32%) en Blastocystis spp (19%). De meest frequent verworven parasitaire darmpathogenen tijdens de reis waren ook Blastocystis spp. (10%) en D. fragilis (6%). Hoewel deze laatste reizigers voor hun reis géén drager waren van deze darmparasieten, moet men een positieve test met Blastocystis spp. en/of D. fragilis bij een terugkerende reiziger met gastro-intestinale klachten nuanceren, aangezien er een kans bestaat dat de reiziger voor aanvang van de reis al drager hiervan

was. Van de bacteriële pathogenen werden Plesiomonas shigelloides (7%) en Shigella spp (5%) het vaakst opgelopen tijdens de reis. Gezien bacteriële darmpathogenen vaker werden verworven dan virale darmpathogenen, lijken bacteriële darmpathogenen een grotere rol te spelen bij reizigersdiarree.

In het laatste hoofdstuk worden onze belangrijkste resultaten samengevat en een aantal punten verder bediscussieerd (hoofdstuk 10). In dit hoofdstuk wordt onder andere dieper ingegaan op de risicofactoren voor ESBL-E acquisitie tijdens de reis. Reisbestemming is de allerbelangrijkste factor voor het oplopen van een ESBL-E tijdens reizen. Hoewel reizigers naar Zuid-Azië het hoogste acquisitiepercentage (75.1%) hadden, dragen reizigers naar West-Azië en Noord-Afrika waarschijnlijk het meest bij aan de endemiciteit van ESBL-E in Nederland. Dit komt doordat dit de twee meest populaire regio's zijn voor lange vakanties bij Nederlanders. In ons onderzoek vonden wij een acquisitiepercentage van 42.0-42.9% onder reizigers naar deze twee regio's. Onze inschatting is dat Nederlandse reizigers naar West-Azië (met name Turkije) en Noord-Afrika (met name Egypte en Marokko) samen verantwoordelijk zijn voor een jaarlijkse instroom van 320.000 tot 870.000 personen die ESBL-E invoeren in Nederland. Ook worden de impact en eventuele implicaties van internationaal reizen op ESBL-E, CPE en mcr-1 in Nederland besproken. Tenslotte worden kennishiaten aangekaart en suggesties voor toekomstig onderzoek gedaan.