An investigation into the relationship between antimicrobial prescribing and antimicrobial resistance in urinary tract infections at a population level

By

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Abstract

Inappropriate use of antibiotics is a key factor in the development of antimicrobial resistance (AMR). UK national guidance has been ineffective in standardising the management of infections in the community. Many community prescribers are sceptical that their actions have an effect on AMR in their locality.

As part of this study, routine surveillance of AMR in a large regional population was established. To help interpret surveillance data, two surveys were undertaken: a survey of laboratory methods, and a survey of GP sampling and prescribing protocols. Using these survey results, surveillance tools were developed to provide hospital and community prescribers with data on antibiotic resistance in bacteria within their locality; and enable laboratories to compare methods for determining antibiotic susceptibility.

The results of this thesis demonstrated that routine AMR surveillance can be used to monitor key antibiotic resistance, detect emergence of new or unusual resistance mechanisms, and enable the bench-marking of laboratory methods. This study was also able to demonstrate that small increases in antibiotic prescribing by individual GPs increases the number of non-susceptible bacteria isolated from specimens taken from their practice population. Results from this thesis provides supporting evidence to those developing strategies to combat AMR in the community.

Dedication

To Harry and Margaret

Acknowledgements

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Abbreviations

Abbreviation	Definition
AMR	Antimicrobial Resistance
AMRHAI	Antimicrobial Resistance and Healthcare Associated Infections Unit
API	Analytical profile index
ASB	Asymptomatic bacteriuria
AST	Automated Susceptibility Test
BNF	British National Formulary
BSAC	British Society for Antimicrobial Chemotherapy
CCG	Clinical Commissioning Group
CDC	Centers for Disease Control and Prevention
CDR	Communicable Disease Report
CEL	Cultural, Ethnic and Linguistic groups
CI	Confidence Interval
CLED	Cystine Lactose Electrolyte-Deficient agar
CLSI	Clinical and Laboratory Standards Institute
CMO	Chief Medical Officer (England)
CPA	Clinical Pathology Accreditation
CPE	Carbapenemase-producing Enterobacteriaceae
CTX-M	Cefotaximase
DDD	Defined Daily Dose (WHO)
DHCP	Dihydropteroate synthetase
DNA	Deoxyribonucleic acid
EARS-NET	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
ERS	Electronic Reporting System
ESBL	Extended-spectrum beta-lactamase
ESPAUR	English Surveillance Programme for Antimicrobial Utilisation and Resistance
ETL	Extraction Translation and Loading processes
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GES	Guiana extended spectrum
GLMs	multilevel mixed-effects Generalised Linear Models
GNB	Gram-negative bacteria
GP	General Practitioner
GPC	Gram-positive bacteria
HPA	Health Protection Agency
IMD	Index of Multiple Deprivation
IMP	Imipenemase
IPR	Intellectual Property Right
IQR	interquartile range
IV	Intravenous
KPC	*K. pneumoniae* carbapenemase

LCTF	Long-term care facility
LIMS	Laboratory Information Management Systems
MALDI-TOF	Matrix assisted laser desorption ionization-time of flight mass spectrometry
MDR	Multi-drug resistance
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant Staphylococcus Aureus
NDM	New-Delhi metallo
NEQAS	National External Quality Assessment Service
NHS	National Health Service (UK)
ODS	Organisation Data Service (NHS)
OR	Odds ratio
OXA	Oxacillinase
PABA	*para*-aminobenzoic acid
PBP	Penicillin binding protein
PCT	Primary Care Trust
PHE	Public Health England
REU	Regional Epidemiology Unit (PHE)
RNA	Ribonucleic acid
SAPG	Scottish Antimicrobial Prescribing Group
SGSS	Second Generation Surveillance System (PHE)
SHV	Sulfhydryl variable
SMI	Standard for Microbiology Investigations (PHE)
SQL	Structured Query Language
SSIS	SQL Server Integration Services
ST	Strain Type
STAR-PU	Specific Therapeutic group Age-sex Related Prescribing Unit
TARGET	Treat Antibiotics Responsibly, Guidance, Education, Tools
TEM	Temoniera
UAT	User Acceptance Test
UK	United Kingdom
UKAS	United Kingdom Accreditation Service
UPEC	Urinary pathogenic *Escherichia coli*
USA	United States of America
UTI	Urinary Tract Infection
VIM	Verona integron encoded metallo
WHO	World Health Organisation

Publications Arising From This Thesis

Chapter 3

Ironmonger, D., Edeghere, O., Gossain, S., Bains, A., & Hawkey, P.M. 2013. AmWeb: a novel interactive web tool for antimicrobial resistance surveillance, applicable to both community and hospital patients. *J.Antimicrob.Chemother.*, 68, (10) 2406-2413

Chapter 4

Ironmonger, D., Edeghere, O., Gossain, S., & Hawkey, P.M. 2016. Use of antimicrobial resistance information and prescribing guidance for management of urinary tract infections: survey of general practitioners in the West Midlands. *BMC.Infect.Dis.*, 16, (1) 226

Chapter 5

Ironmonger, D., Edeghere, O., Bains, A., Loy, R., Woodford, N., & Hawkey, P.M. 2015. Surveillance of antibiotic susceptibility of urinary tract pathogens for a population of 5.6 million over 4 years. J.Antimicrob.Chemother., 70, (6) 1744-1750

Chapter 6

Ironmonger, D., Edeghere, O., Verlander, N.V., Gossain, S., Hopkins, S., Hilton, B., Hawkey, P.M. 2017. Effect of general practice characteristics and antibiotic prescribing on *Escherichia coli* antibiotic non-susceptibility in the West Midlands region of England: a 4 year ecological study, J.Antimicrob.Chemother. dkx465, https://doi.org/10.1093/jac/dkx465

Other publications

Ironmonger, D., Edeghere, O., Hunt, A.L., S., Hopkins, S., Johnson A., Hawkey, P.M.. 2014. Antibiotic resistance – a major public health issue. Guest editorial. Dental Update Nov 2014, 621-622.

Freeman, R., Ironmonger, D., Puleston, R., Hopkins, K.L., Welfare, W., Hope, R., Staves, P., Shemko, M., Hopkins, S., Cleary, P., Patel, B., Muller-Pebody, B., Li, X., Alvarez-Buylla, A., Hawkey, P.M., Johnson, A.P., Woodford, N., & Oliver, I. 2016. Enhanced surveillance of carbapenemase-producing Gram-negative bacteria to support national and international prevention and control efforts. Clin.Microbiol.Infect., 22, (10) 896-897

Rosello, A., Hayward, A.C., Hopkins, S., Horner, C., Ironmonger, D., Hawkey, P.M., & Deeny, S.R. 2017. Impact of long-term care facility residence on the antibiotic resistance of urinary tract *Escherichia coli* and *Klebsiella*. J.Antimicrob.Chemother., 72, (4) 1184-1192

1 Introduction

1.1 Background

Antimicrobial resistance (AMR) is a serious and growing public health problem that has been recognised as one of the greatest threats to human health (World Health Organisation, 2012). Since the introduction of penicillin, a little over 70 years ago, we are now faced with the prospect of a world with few effective antibiotics, where patient outcomes in specialties such as oncology, transplant and complex surgery may deteriorate as infections become untreatable (Chief Medical Officer, 2013). Such a prospect may result in higher mortality, longer duration of illness and increased healthcare costs, ultimately contributing to a depletion of the global economy (World Health Organisation, 2016b). AMR is a complex subject with many contributing factors. This introduction section will introduce the concept of antibiotics, the development of resistance, the epidemiology of urinary-tract infections, and will conclude with a summary of the surveillance of antimicrobial resistance.

In Europe, approximately 25,000 patients die annually from infections with multi-resistant bacteria, including: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate-resistant and vancomycin-resistant *S. aureus* (VISA/VRSA), vancomycin-resistant *Enterococcus* spp. (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), third-generation cephalosporin-resistant Enterobacteriaceae and carbapenem-resistant Enterobacteriaceae or non-fermentative Gram-negative bacteria (European Centre for Disease Prevention and Control (ECDC) and European Medicines Agency (EMEA), 2009). These bacteria are not only frequently responsible for bloodstream infections but are also associated with resistance to multiple antibiotics. The estimated annual associated costs of

these infections is around EUR 1.5 billion (European Centre for Disease Prevention and Control (ECDC) and European Medicines Agency (EMEA), 2009).

The estimated increase in cost in the United States for patients with infections caused by antimicrobial-resistant bacteria is between US$6,000 and $30,000 more than those infected with susceptible bacteria (Maragakis et al., 2008). Approximately 23,000 people die each year in the USA as a result of an infection with antimicrobial-resistant bacteria, at a cost as high as $20 billion in direct healthcare, with potential societal costs as high as $35 billion each year (CDC, 2014). A review commissioned by the UK government in 2014 estimated that by 2050 AMR would account for 10 million lives a year worldwide, with a cost of $100 trillion in lost productivity (Wellcome Trust and UK Department of Health, 2016).

The inappropriate use of antibiotics for human health, animal welfare and the production of food is a major factor leading to the development of AMR (CDC, 2014). The bulk of worldwide sales of antibiotics occur in animal health and food production sectors. It is estimated that 70% (by weight) of antibiotics defined as important for human health that are sold in the USA are for use in agriculture or farming (Wellcome Trust and UK Department of Health, 2016). It has been suggested that the increasing use of third-generation cephalosporins in food animal production is associated with the emergence and spread of MDR bacteria in poultry, cattle and pigs; which may be a threat to humans by transmitting resistant strains via the food chain (Department of Health, 2012). The use of antibiotics in food production, animal and human health has led to resistant genes being released to the environment in the form of waste products. Antibiotics released into the soil may be transported to surface or ground water, and cycled within the environment (Wellington et al., 2013).

Antibiotic selective pressure is exacerbated by inadequate prevention and control of bacterial infections, and a lack of new treatment options (World Health Organisation, 2012). In terms of human health, over-prescribing, easy access to over-the-counter drugs and internet sales are factors driving the increased use of antibiotics (Morgan et al., 2011).

In the last 20 years, increasing trade and people mobility has led to the spread of antibiotic genes across the world (Hawkey, 2015). The resulting global nature of AMR means that it is difficult for any single nation or organisation to manage the problem alone (World Economic Forum , 2013). In September 2016 a declaration was signed by all 193 United Nation members to endorse a WHO Global Action Plan (World Health Organisation, 2016b) which requires nation states to address AMR by developing national action plans, implementing antimicrobial stewardship and strengthening AMR surveillance (United Nations, 2016). The guiding principles of the Global Action Plan are:

1) Whole-of-society engagement including a one-health approach
2) Prevention first (involving sanitation, hygiene and other infection prevention measures)
3) Access – equitable access to and appropriate use of antibiotics
4) Sustainability - plan for required resources to implement surveillance, research, training etc.
5) Produce incremental targets for implementation

Several countries have influenced these guiding principles with national AMR initiatives. In the USA the Obama administration issued a plan for combating antibiotic resistance in 2015 that was founded on a ‘one-health’ approach to tackle both human and animal pathogens. This plan called for stronger partnerships with foreign governments and aggressive action to achieve significant reductions in antibiotic resistance (The White House, 2015).

In England the Chief Medical Officer, in her first annual report (Chief Medical Officer, 2013) and the subsequent UK five-year antimicrobial resistance strategy (Department of Health, 2013), made a series of recommendations aimed at conserving the effectiveness of existing antimicrobial treatments, improving the antimicrobial development pipeline and improving surveillance of both AMR and antimicrobial consumption.

The epidemiology of AMR has also changed in the last 20 years, with the increasing incidence of Gram-negative multi-drug resistant (MDR) pathogens, such as those producing extended-spectrum beta-lactamases (ESBLs). MDR has been defined by the European Centre for Disease Prevention and Control (ECDC) and the US Center for Disease Control and Prevention (CDC) as ‘acquired non-susceptibility to at least one agent in three or more antimicrobial categories’ (Magiorakos et al., 2011). Increasing numbers of MDR Gram-negative bacterial infections has led to a reliance on carbapenems as antibiotics of ‘last-resort’ (Nordmann et al., 2009). As a consequence, carbapenemase-producing bacteria have emerged which demonstrate high levels of resistance to carbapenem antibiotics (Nordmann et al., 2011). The situation is exacerbated by the fact that no new classes of antibiotics active against

Gram-negative bacteria have been discovered in the last 25 years (Department of Health, 2013).

In the following sections of this chapter, the discovery of antibiotics, their development and their various modes of action are described. This is followed by a review of the mechanisms of antibiotic resistance that have evolved to enable bacteria to combat the effects of different classes of antibiotics currently available for the treatment of bacterial infections.

1.2 Antibiotics

1.2.1 Discovery and development

Although the word antibiotic is formed from the classical Greek words anti (against) and bios (life), the essence of antibiotic action is that they act selectively against bacterial life (Gould, 2016). Bacteria are prokaryotes, being structurally and metabolically different from eukaryotic cells, and therefore can be killed or inhibited from growth by agents that do not affect animal cells (Skold, 2011). Although mechanisms of action were not understood, in ancient history treatment for infections included honey, herbs, soil and moulds (Gould, 2016). Traces of the antibiotic tetracycline have been detected in thousand-year-old old Nubian mummies (Levy, 2002). The occasional efficacy of these treatments may have been due to metabolites or chemicals harmful to bacteria, such as antibiotics from mould /soil extracts or the substantial levels of hydrogen peroxide in honey (Gould, 2016).

The age of antibiotics started in 1889 when Rudolf Emmerich and Oscar Loew performed clinical trials of a substance they named pyocyanase (Gould, 2016). This chemical was produced by *Pseudomonas aeruginosa* and was found to inhibit the growth of a range of bacteria. The trials of this compound at the time of discovery had some success against common infections; however, the instability of pyocyanase and its toxicity led to the agent being abandoned as a treatment option (Levy, 2002). Paul Ehrlich coined the term 'chemotherapy' after experimenting with chemical dyes (Levy, 2002). In 1909 he found a dye, salvarsan, that was successfully used to treat syphilis infections (Levy, 2002). Although toxicity issues limited its usefulness, this work led Gerhard Domagk to discover another dye, Prontosil rubrum in 1935, for which he was awarded the Noble prize (Ryan, 1992). Although the dye showed activity against bacterial infections in animals, it was the clear colourless metabolite of this chemical, sulphanilamide, that was the anti-bacterial substance (Ryan, 1992). Chemically synthesised sulphonamides became widely available in the 1940s as a treatment for Gram-positive and Gram-negative bacteria, and reportedly saved the life of Winston Churchill in 1943 when he contracted bacterial pneumonia (Skold, 2011)

Although the inhibiting effect of fungi on bacterial growth had been observed by Sir John Scott Burdon-Sanderson (1870), Joseph Lister (1871) and Dr John Tyndall (1875), it was not until Alexander Fleming returned from holiday in 1928 to observe this phenomenon on one of his agar plates that the significance of these observations were fully appreciated (Gould, 2016). Fleming showed that the mould contaminating his agar plate, *Penicillium notatum,* was producing a substance small enough to diffuse through agar and lyse the bacterium *Staphylococcus aureus*. He

called this substance penicillin and by extracting filtrates from the mould, demonstrated the powerful antibacterial properties against a range of bacteria. (Fleming, 1929). It is not clear why Fleming ended his research on penicillin after just six months, but the inability at the time to purify the antibiotic, along with an observed short half-life have been cited as factors (Gould, 2016;Skold, 2011). It was not until Howard Florey and Ernst Chain in 1940 purified sufficient quantities to treat infections caused by *Streptococcus pyogenes* in animal models that the therapeutic potential of penicillin was realised (Skold, 2011).

The following 20 years became the golden era for antibiotic discovery. The discovery of streptomycin in 1944, which is produced by a soil bacterium (*Streptomyces griseus*) led to a widespread search for other potential bacteria from the environment (Ryan, 1992). At this time, most of the new antibiotics discovered were those produced by other microorganisms; however, soon advances in chemistry led to modifications of existing antibiotics to improve their effectiveness, such as the development of the first penicillinase-resistant beta-lactam antibiotic, methicillin in 1959, the development of semi-synthetic penicillins in the 1950s and 1960s, and the chemical synthesis of new antibiotic molecules such as trimethoprim and quinolones in the 1960s (Wright et al., 2014) (Figure 1.1).

1.2.2 Mechanisms of action

Antibiotics can be classified by their chemical structure, which is related to their mode of action. Antibiotics can either kill the bacteria (bactericidal), for example beta-lactam antibiotics, or slow their growth or reproduction (bacteriostatic) for example macrolide antibiotics (Shanson, 1999). Table 1 lists the major groupings and their primary bacterial targets. The following subheadings summarise the mechanisms of antibiotic action and describes how different classes of antibiotics may target similar bacterial processes. The descriptions below particularly focus on antibiotics acting against Gram-negative bacteria, which are the focus of this study.

1.2.2.1 Cell wall biosynthesis

The cell wall plays a vital role in the survival of bacterial cells by protecting against changes in osmotic pressures that could potentially lyse cells (Shanson, 1999). The cell wall is made up of long polysaccharide chains formed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid (Walsh and Wencewicz, 2016a). These chains are cross-linked by peptides to form the structure peptidoglycan. This peptidoglycan layer is found in both Gram-positive and Gram-negative bacteria; although it is typically thicker in Gram-positive bacteria (Shanson, 1999). The peptide cross-links provide stability and are formed through a series of biochemical reactions.

Two classes of clinically significant antibiotics target the peptidoglycan cell wall; beta-lactams and glycopeptides. Beta-lactam antibiotics are used to treat both Gram-positive and Gram-negative bacterial infections; however glycopeptide antibiotics are primarily used against Gram-positive bacterial infections (Skold, 2011), and therefore will not be discussed further here.

Beta-lactam antibiotics cause disruption of the cell wall structure. The stability of the cell wall depends on the peptide cross-linkage of these polysaccharide chains to form peptidoglycan. The peptide cross-linking is catalysed by transpeptidase enzymes, which are also referred to as penicillin-binding proteins. The beta-lactam ring component of the molecule is a structural mimic of the D-alanyl-D-alanine dipeptide, which is found at the end of cross-linking peptides that form the peptidoglycan. Therefore beta-lactam antibiotics inhibit the transpeptidation reaction, which in turn prevents the formation of the cross links in the late stages of forming peptidoglycan, and thereby weakens the newly formed cell wall in growing bacteria (Walsh & Wencewicz, 2016a).

Table 1.1 Classification of a selection of antibiotics (based on BNF classification - https://bnf.nice.org.uk/)

Class (chemical structure)	*Mode of action*	*Example*
Beta-lactam antibiotics	Inhibit bacterial cell wall synthesis	**Penicillins**
Penicillins		Amoxicillin
Cephalosporins		**Cephalosporins**
Carbapenems		Cefotaxime
		Carbapenem
		Meropenem
Polymyxins	Disrupt bacterial cell membrane	Colistin
Glycylcyclines	Inhibit bacterial protein synthesis	Tigecycline
Epoxides	Inhibits bacterial cell wall synthesis	Fosfomycin
Glycopeptide	Inhibits bacterial cell wall synthesis	Vancomycin
Lipopeptides	Disrupt bacterial cell membrane	Daptomycin
Oxazolidinones	Inhibit bacterial protein synthesis	Linezolid
Macrolides	Inhibit bacterial protein synthesis	Erythromycin
Tetracyclines	Inhibit bacterial protein synthesis	Tetracycline
Quinolones	Inhibit bacterial DNA synthesis	Ciprofloxacin
Sulphonamides and trimethoprim	Blocks bacterial cell metabolism by inhibiting enzymes	Co-trimoxazole
Aminoglycosides	Inhibit bacterial protein synthesis	Gentamicin
Imidazoles	Inhibit bacterial DNA synthesis	Metronidazole
Peptides	Inhibit bacterial cell wall synthesis	Bacitracin
Lincosamides	Inhibit bacterial protein synthesis	Lincomycin
Other	Inhibit bacterial protein synthesis	Fusidic acid

1.2.2.2 Cell membrane integrity

Unlike the bacterial cell wall, cell membranes are found in both eukaryotic and prokaryotic cells (Shanson, 1999), therefore, it is a challenge to identify antibiotics that are selective and non-toxic for mammalian cells. Many antiseptics, for example those used in hand-washing disrupt the bacterial membranes; however the lack of selectivity for prokaryotic cells restricts their use to topical application. Antimicrobial peptides are part of the innate immune system and destroy bacterial membranes. Some of these are now candidates for novel therapeutic agents (Walsh & Wencewicz, 2016a). The traditional membrane acting antibiotics include polymyxins (e.g. colistin) and lipopeptides (e.g. daptomycin). Polymyxins were once deleted from formularies due to their toxicity; however due to their effectiveness against multi-drug resistant bacteria they are becoming increasingly used as antibiotics of last resort. They act on the outer membrane of Gram-negative bacteria by disrupting its integrity and gain access to the inner membrane, where they again disrupt the membrane barrier, possibly by pore formation (Velkov et al., 2014). Polymyxins are not as effective against Gram-positive bacteria as their action depends on the positive side-chains, which react electrostatically with the negatively charged lipopolysaccharide (LPS) of Gram-negative bacteria; however lipopeptides are effective against Gram-positive bacteria, but not Gram-negative bacteria due to an inability to penetrate the outer membrane barrier (Walsh & Wencewicz, 2016a).

1.2.2.3 Protein synthesis

Most of the cellular structures and enzymes that make up a bacterial cell are made from proteins, and therefore protein synthesis is an essential process for the cell's survival (Shanson, 1999). There are several classes of antibiotics that act on

bacterial protein synthesis by either binding to the 30S or 50S subunits of the 70S prokaryotic intracellular ribosomes (Skold, 2011), these include aminoglycosides, macrolides and oxazolidinones. From this group, aminoglycosides are antibiotics primarily used in the treatment of Gram-negative infections, whilst macrolides and oxazolidinones mainly target Gram-positive bacteria due to poor penetration of the cell-membrane in Enterobacteriaceae and non-fermenting Gram-negative bacteria (Skold, 2011).

Aminoglycosides bind to the 16S rRNA in the 30S subunit and interfere with the precision of the translation process that directs which amino acids are included in the formation of peptides (Skold, 2011). The resultant mutations are not compatible with normal functions of the bacterial cell. Mutations in critical proteins, such as membrane proteins, have a lethal effect on the bacteria as this leads to leakage of ions and larger molecules (Walsh & Wencewicz, 2016a). It has also been suggested that aminoglycosides damage the bacterial outer-membrane during their transition into the cell, increasing general permeability and leading to leakage of cellular content, which may explain the bactericidal action of this family of antibiotics (Schurek et al., 2008).

1.2.2.4 DNA and RNA metabolism

DNA and RNA have a vital role in cell replication (Shanson, 1999). Some antibiotics bind to components involved in DNA or RNA synthesis, and thereby have a bactericidal effect. These antibiotics belong to the quinolone and rifamycin antibiotic classes. Quinolones do not interfere with DNA synthesis but do interfere with conformation changes in DNA required for replication (Skold, 2011). To enable the long molecular length of DNA to be accommodated inside a bacterial cell the DNA

has to undergo a process of supercoiling (Walsh and Wencewicz, 2016b). This supercoiling is facilitated by the enzyme DNA gyrase, which cuts both strands of DNA to allow another part of the circular double stranded DNA molecule to pass through the break. Quinolones act by binding to the enzyme-DNA interface in a non-covalent manner, which results in the DNA strand breaks becoming permanent. This triggers DNA repair pathways and the ultimate degradation of the 'broken' DNA, leading to cell death (Walsh & Wencewicz, 2016a). Topoisomerase IV, responsible for releasing the coiling to enable DNA replication, is another cellular enzyme that is inhibited by quinolones (Aldred et al., 2014).

Rifamycin antibiotics, such as rifampicin, inhibit the growth of bacteria by inhibiting the transcription of DNA. These antibiotics bind to the active centre of the bacterial DNA transcribing enzyme RNA polymerase, inhibiting the early stage RNA chain elongation. This leads to the transcription process being severely restricted preventing bacterial growth (Skold, 2011).

1.2.2.5 Folate biosynthesis

Folic acid is an essential coenzyme for all living cells as it is involved in the synthesis of DNA precursors (Alberts et al., 2008). Bacterial cells depend on an enzyme pathway for the formation of folic acid, which differs from mammalian cells, as they do not have these enzymes, and therefore have to acquire this coenzyme from food sources (Alberts et al, 2008). Sulphonamides and folic acid inhibitors (e.g. trimethoprim) are the broad classes of antibiotics that interfere with the bacterial syntheses of folic acid. Sulphonamides are structural analogues of *para*-aminobenzoic acid (PABA), which is a substrate for the key enzyme dihydropteroate synthetase (DHCP). This enzyme acts on PABA to catalyse the formation of the

folate intermediate dihydropteroic acid, therefore the competitive inhibition of this enzyme by sulphonamides prevents folic acid production (Chopra et al., 2002).

Trimethoprim acts on another part of the folic acid enzyme pathway by competitively inhibiting the reduction of dihydrofolate to tetrahydrofolate by the enzyme dihydrofolate reductase. The functional nature of trimethoprim and sulphonamide in inhibiting folic acid synthesis has been exploited over many decades by these antibiotics being used in combined therapy (Walsh & Wencewicz, 2016a).

The mechanisms of antibiotic action described above provide examples of only some of the many types of antibiotics that are currently available for the treatment of bacterial infections. In the next section antimicrobial resistance will be discussed, again with a focus on the mechanisms used by Gram-negative bacteria to combat the action of antibiotics.

1.2.3 Antimicrobial resistance

1.2.3.1 Introduction

Antibiotics changed the face of modern medicine and are now indispensable for a range of medical procedures such as surgery, organ transplants and cancer therapies (Department of Health, 2013); however, with the discovery of each new antibiotic, resistance to the agent developed soon after (Figure 1.1).

There are three main ways in which a bacterium may be resistant to antibiotics. They may be naturally resistant to antibiotics (intrinsic resistance), develop resistance by mutation or acquire resistance through the transfer of deoxyribonucleic acid (DNA)

(Walsh & Wencewicz, 2016a). A species of bacteria may be naturally resistant to an antibiotic, for example *Proteus mirabilis* is resistant to nitrofurantoin or *Pseudomonas aeruginosa* resistant to cloxacillin, due to the impermeable nature of the outer membrane and the use of efflux pumps that remove antibiotics from the cell (Cox and Wright, 2013). A bacterial strain may also develop antibiotic resistance by a process of spontaneous mutation. Mutation can occur within the bacterial chromosome at a frequency of 10^{-6} to 10^{-12} per generation (Shanson, 1999). Some bacterial strains exhibit increased mutation frequency (hypermutability) due to loss of DNA mismatch repair systems, which is an important mechanism of acquired resistance (Jolivet-Gougeon et al., 2011).

Acquisition of antimicrobial resistance genes is the major cause of resistance amongst Gram-negative bacteria. Foreign DNA can be spread horizontally via plasmids or transposons. Plasmids are mobile genetic elements that can carry antibiotic resistance genes. Transposons are genetic elements that, unlike plasmids, are not able to replicate; however they are able to 'jump' from plasmids to the chromosome (and vice-versa) and also move between plasmids. Many transposons include antimicrobial resistance genes and therefore this is an important mechanism for creating multi-drug resistant plasmids (Skold, 2011).

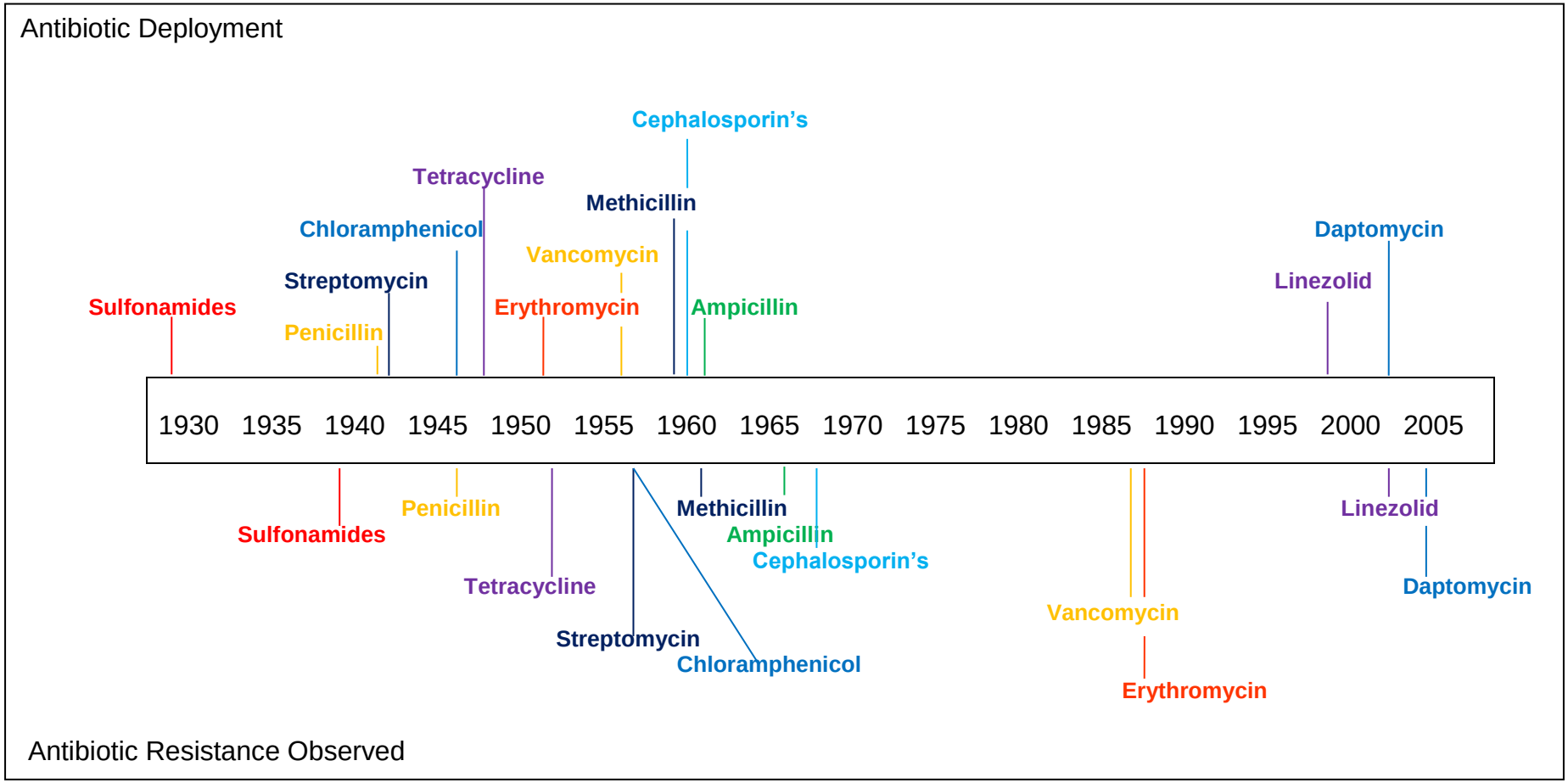
Plasmid and chromosomal DNA can be spread horizontally via conjugation (cell to cell contact with the same or unrelated species). A few species of bacteria (e.g. *Neisseria spp*. and *Streptococcus pneumoniae*) can take up extracellular DNA released by dead cells of related species (transformation) (Livermore, 2004a). Plasmid transfer differs in the Gram-positive bacterium *S. aureus*, with plasmid

genes being transferred by bacteriophages (transduction), rather than conjugation (Walsh & Wencewicz, 2016a).

Gram-negative bacteria are the focus of this study, in particular members of the family Enterobacteriaceae and the genus *Pseudomonas*. These bacteria have been associated with the recently observed rise in antibiotic resistance and its members are the major cause of urinary tract infections (UTI) in males and females from all age groups (Laupland et al., 2007).Therefore these bacteria and their resistance mechanisms will form the basis of the remainder of this section.

The bacterial mechanisms of resistance to antibiotics include: altering the antibiotic target; the use of alternative enzymatic pathways; production of antibiotic inactivating enzymes; reduced cell permeability and the ability to remove antibiotics that have entered the cell (efflux pumps). Examples of various mechanisms of antibiotic resistance in Gram-negative bacteria are described below.

Figure 1.1 Antibiotic resistance timeline (Clatworthy et al., 2007)



1.2.3.2 Beta-lactam inactivating enzymes

The production of enzymes (beta–lactamases) that hydrolyse antibiotics structured around a beta–lactam ring, rendering them ineffective as antimicrobial agents, is the most common mechanism of resistance in Gram-negative bacteria (Livermore, 2012a). The first beta-lactamase was discovered in 1940 in an isolate of *Escherichia coli* (Abraham and Chain, 1940). However it was the discovery of a beta–lactamase gene located on a mobile plasmid, shortly following the introduction of the first broad-spectrum beta-lactam, ampicillin, in 1961, that raised concerns regarding the spread of resistance to a range of clinically important Gram-negative bacteria (Datta and Kontomichalou, 1965). This mobile beta–lactamase, designated TEM-1(isolated from a patient named Temoniera), was soon to be found in other members of the Enterobacteriaceae and also other pathogens such as *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Brunton et al., 1986).

The range of beta–lactam antibiotics affected by resistance increased markedly with the emergence and spread of extended-spectrum beta–lactamases (ESBLs) in the early 1990s. ESBLs are enzymes that impart resistance to most beta-lactam antibiotics, including cephalosporins and penicillins. The earliest recognised ESBLs evolved from point mutations of known beta–lactamases (TEM-1, TEM-2 and SHV-1). Infections with these ESBLs were initially largely nosocomial from patients in specialist units and involving *Klebsiella spp.* (Shannon et al., 1998). A new class of ESBL called CTX-M appeared in the 1990's, named for their greater activity against cefotaxime. This new enzyme has been shown to originate from the chromosome of *Kluyvera sp* rather than from mutation of existing widely dispersed beta–lactamases (Poirel et al., 2002).

Another group of beta-lactamases, AmpC cephalosporinases, confer resistance to cephamycins, oxyimino- beta–lactams and are not inhibited by beta–lactamase inhibitors, such as clavulanic acid, which can be used to inhibit a range of beta-lactamases (Livermore and Hawkey, 2005a). A number of species of bacteria have inducible chromosomally coded AmpC beta-lactamases and have been given the acronym ESCAPPM (*Enterobacter* spp., *Serratia* spp., *Citrobacter freundii*, *Aeromonas* spp., *Proteus* spp., *Providencia* spp., and *Morganella morganii*) (Boyle et al., 2002). Although found encoded within the chromosome of these species, the genes have become mobilized by plasmids and are now found widely in bacteria lacking or poorly expressing the chromosomal gene, such as *K. pneumoniae*, and *P. mirabilis* (Jacoby, 2009).

Members of the carbapenem antibiotic family have been kept as reserve drugs for use against multi-resistant Gram-negative bacterial infections, such as those with bacteria producing CTX-M beta-lactamases. However resistance to this important group of antibiotics has emerged, either by combination of hyper-production of broad-spectrum beta–lactamases (i.e. ESBLs or AmpC) and porin loss (Tangden et al., 2013), or by acquiring the ability to produce carbapenemases. Carbapenemases are a diverse group of beta-lactamases that show broad-spectrum activity against beta–lactam antibiotics including carbapenem's (Table 2). They belong to three molecular classes and two distinct types are found among Gram-negative bacteria, those that have serine and those that have zinc (metallo beta–lactamases) at the active sites (Queenan and Bush, 2007).

Table 1.2 Carbapenemases by Ambler classification *(source UK Standards for Microbiological Investigation B60 issue 2.1)*

Enzyme type *	**Classification by Ambler class**	**Activity spectrum**	**Organism(s)**
KPC	A	All beta-lactams	Enterobacteriaceae *P. aeruginosa A. baumannii*
SME	A	Carbapenems and aztreonam, but not 3rd/4th generation cephalosporins	*S. marcescens*
NMC–A IMI	A	Carbapenems and aztreonam, but not 3rd/4th generation cephalosporins	*Enterobacter* species
GES	A	Depends on enzyme variant. Some are ESBLs, others eg GES-5 are carbapenemases	*P. aeruginosa* and Enterobacteriaceae
IMP **VIM** **NDM** AIM, GIM, SIM, (not detected in the UK yet) DIM, SPM	B (metallo-beta-lactamases)	All beta-lactams except monobactams (aztreonam)	*Pseudomonas* species *Acinetobacter* species Enterobacteriaceae
OXA	D	Carbapenems (note that many OXA enzymes are NOT carbapenemases)	*A. baumannii*, Enterobacteriaceae and rare *P. aeruginosa*

*Enzymes marked as bold are the enzymes most commonly found in the UK.

1.2.3.3 Aminoglycoside inactivating enzymes

High levels of resistance to aminoglycosides is commonly mediated in clinical infections by transferable genes that code for drug-inactivating enzymes that modify the antibiotic so that it is unable to bind to bacterial ribosome targets (Walsh & Wencewicz, 2016b). These inactivating substances are of three types: phosphorylating, adenylating, and acetylating enzymes. As they have differing substrate targets they can confer resistance to individual aminoglycosides or show extensive cross resistance to this group of antibiotics (Skold, 2011). Transferable aminoglycoside resistant genes are often found on plasmids that confer multi-drug resistance (MDR), for example the aac(6_)-Ib-cr aminoglycoside resistance gene is commonly found in a plasmid associated with CTX-M-15, TEM-1 and OXA-1 resistance genes (Carattoli, 2009).

1.2.3.4 Altering the antibiotic target

Fluoroquinolone resistance is increasingly found amongst Enterobacteriaceae (Hsueh et al., 2010). Resistance is achieved by stepwise mutations in the coding region of the gyrase subunits (gyrA and gyrB) and DNA topoisomerase IV (parC) (Drlica and Malik, 2003). Plasmid-mediated quinolone resistance (Qnr) is an increasing concern as it has been associated with plasmids encoding ESBLs conferring multi-drug resistance (Lavilla et al., 2008).

Polymyxins were first used in the 1950s and have broad spectrum activity against Gram-negative bacteria, including activity against the majority of Enterobacteriaceae. Due to nephrotoxicity and neurotoxicity, plus the availability of effective, less toxic antibiotics, its use has been very limited in recent decades. As this family of antibiotics remain active against most carbapenemase producing bacteria there has

been a renewed interest in these antibiotics. Polymyxin B and polymyxin E (colistin) have now become first-line therapy for the treatment of serious infections caused by multi-drug resistant bacteria (Schwarz and Johnson, 2016). Resistance to polymyxins is due to the modification of the target lipid A, which until recently was believed to be solely mediated by chromosomal genes. However a plasmid-mediated resistance mechanism, designated MCR-1, was discovered in China in late 2015. The *mcr*1 gene product aligns closely with phosphoethanolamine transferase which modifies the phospoethanolamine moiety of lipid A (Liu et al., 2016).

1.2.3.5 Reduced permeability and efflux pumps

The outer membrane of Gram-negative bacteria acts as a barrier to a number of antibiotics that are effective against Gram-positive organisms. This attribute, combined with large numbers of efflux pumps within the membrane that reduce the concentration antibiotics within the cell, provides some members of the Enterobacteriaceae family and many non-fermenting Gram-negative bacteria, the ability to resist the action of different classes of antibiotics (Cox & Wright, 2013).

Antimicrobial resistance in *Pseudomonas aeruginosa* is an increasing concern. As with members of Enterobacteriaceae, the acquisition of transferrable genetic elements, particularly class B carbapenemases can lead to multi-drug resistance. However *P. aeruginosa* is also noted for an ability of developing resistance to a range of antibiotics, including carbapenems, by the selection of chromosomal mutations. Although these can include mutations that result in the hyper-production of beta-lactam inactivating enzymes, they also often result in the upregulation of genes encoding efflux pumps that actively remove antibiotics from the cell, and

reduced antibiotic permeability by removal or inactivation of porins that allow antibiotic into the cell (Cabot et al., 2011).

1.3 Epidemiology of Gram-negative antimicrobial resistance

1.3.1 Background

In the 1990's the focus of concern for AMR was the emerging antibiotic resistance of Gram-positive bacteria causing invasive infections (Livermore, 2012b). In the early 2000's in the UK the proportion of *Staphylococcus aureus* resistant to methicillin (MRSA) isolated from blood had reached over 40%, and had become a major political issue for the UK government. This led to a national initiative to reduce MRSA bacteraemia by 60% over three years (Johnson et al., 2005). There has been a significant decline in the number of cases of MRSA bacteraemia and in the proportion of MRSA to methicillin-sensitive *Staphylococcus aureus* isolated from blood specimens between 2003-2010 (Livermore, 2012b). In the last ten years the focus of concern has changed from multi-resistant Gram-positive bacteria to the emergence of highly resistant Gram-negative bacteria.

1.3.2 Extended spectrum beta-lactamases

Transferable resistance to extended-spectrum cephalosporins was first described by Kliebe *et al*. in 1985 (Kliebe et al., 1985). Soon after, reports of transferable resistance to extended-spectrum beta-lactam antibiotics in outbreaks caused by Enterobacteriaceae were being reported in Europe (Sirot, 1995). These beta-

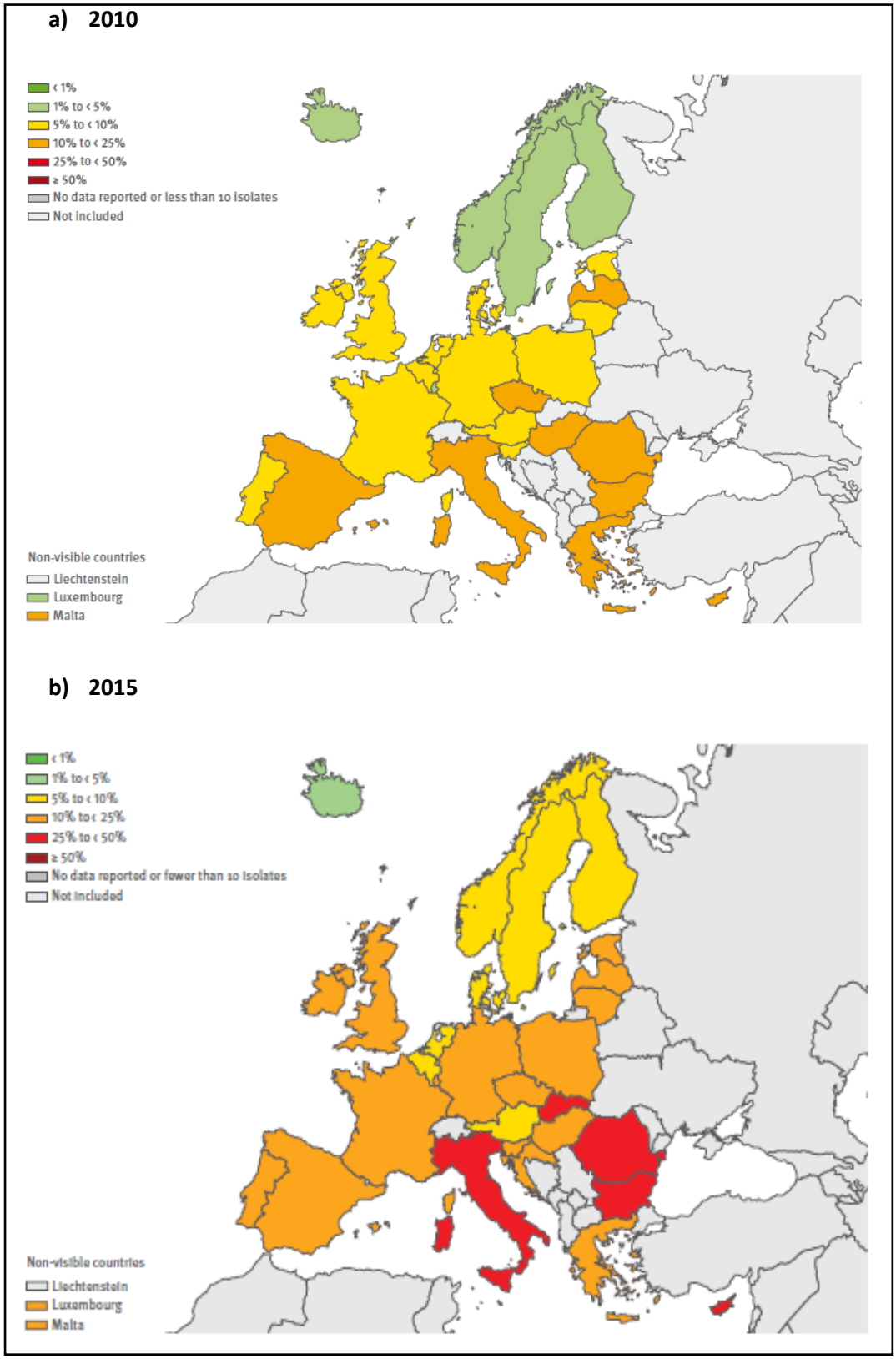
lactamases were found to be mutations of the classical TEM and SHV enzymes that were commonly found being produced by members of the Enterobacteriaceae family (Livermore and Hawkey, 2005b). These enzymes had activity against extended spectrum cephalosporins and by the late 1990s were reported from around the world (Livermore et al., 2007a). They were occasionally found in large outbreaks of infections in UK hospitals in the 1990s; however they remained uncommon in the UK, and mainly associated with nosocomial infections in specialist hospital units (Livermore & Hawkey, 2005b).

During 2003 the Health Protection Agency (HPA, now a part of Public Health England) began receiving isolates of *E. coli* with CTX-M type ESBL from laboratories around the UK. The significant difference with these reports was that they were not only being reported from hospital settings, but many of these isolates were from community patients with urinary tract infections (Woodford et al., 2004). The emergence of CTX-M enzymes has led to a significant change in the prevalence and epidemiology of ESBL-producing bacteria in the UK and Europe (Figure 1.2), with their widespread distribution representing a threat to the ability to treat infections in both hospital and community settings (Hawkey and Jones, 2009;Livermore & Hawkey, 2005a). These enzymes will be described in more detail in the following section.

A study in the Netherlands in 2010 reported that CTX-M, TEM and SHV extended-spectrum beta–lactamase (ESBL) genes were found in 79.8% of raw chicken meat, and that the predominant ESBL genes in chicken meat and human rectal swabs were identical (Overdevest et al., 2011). The widespread use of antibiotics in human and veterinary medicine, and the use of antibiotics as growth promoters in animal

feed, although now banned in the EU, are significant factors in the emergence and spread of antibiotic resistance (Wellcome Trust and UK Department of Health, 2016).

Figure 1.2 Percentage of invasive *E. coli* isolates with resistance to third-generation cephalosporins, by European country, a) 2010 b) 2015 (source ECDC)



1.3.2.1 CTX-M enzyme

Five major families of CTX-M genotypes have been recognised (Livermore, 2012a) and particular genotypes are associated with geographical regions. CTX-M-14 was associated with China and the Far East, and CTX-M-15 was the only genotype reported from India; however both of these genotypes are now spread widely across the world. High rates of ESBL-producing *E.coli* have been reported in India (61.2%), China (59.1%) and Thailand (53%) (Hsueh et al, 2010). CTX-M-15 is frequently carried by a very successful uropathogenic strain of *E. coli*, sequence type (ST) 131, which has led to it becoming the dominant genotype found in Western Europe (Livermore et al., 2007b).

In a survey in the West Midlands region of England in 2006, the majority of ESBL producing bacteria were found to be of the CTX-M-15 genotype. It was reported that a particular clonal group (025b-ST131) had become dominant in the region following its emergence in an outbreak only three years earlier (Xu et al., 2011). A further study in the West Midlands in 2012 demonstrated increased gut carriage of ESBL producing *E. coli* in residents with names associated with Middle East/South Asia compared with those with names of a European origin (22.8% compared with 8.1%). The authors suggest that frequent travel to areas of higher prevalence of ESBL by members of the West Midlands Middle East and South Asia community may account for the increased carriage (Wickramasinghe et al., 2012).

1.3.3 Carbapenemases

In 2004 it was reported that pan-resistance in nosocomial infections caused by Enterobacteriaceae was rare due to the continuing activity of carbapenems (Livermore, 2004b). Resistance to this important group of antibiotics has now emerged, with the acquisition of transferable genes coding for carbapenemase enzymes becoming a serious concern. Small numbers of carbapenem-resistant pseudomonads and other non-fermenting Gram-negative bacteria have been reported to the PHE Antimicrobial Resistance and Healthcare Infections (AMRHAI) reference laboratory since 2000; however, from 2008 a rising trend is observed in carbapenemase-producing Enterobacteriaceae (Figure 1.3).

1.3.3.1 Klebsiella pneumoniae carbapenemase (KPC)

Klebsiella pneumoniae carbapenemase (KPC) was first reported in the USA in 1996 (Yigit et al., 2001). KPC-producing bacteria have now been reported globally and have been associated with a successful clonal lineage of *K. pneumonia*e multi-locus sequence type (ST), ST258. This clone has caused large hospital outbreaks in several countries, including Israel, Greece and the USA (Nordmann et al, 2009). The success of this clone has led to a rapid change in the epidemiology of carbapenemase-producing bacteria across parts of southern Europe, with carbapenemase-producing *K. pneumoniae* becoming endemic in Greece and Italy (Figure 1.4).

Figure 1.3 Confirmed carbapenemase producing Enterobacteriaceae in the UK, 2003-2015 (source PHE Antimicrobial Resistance and Healthcare Infections reference unit)

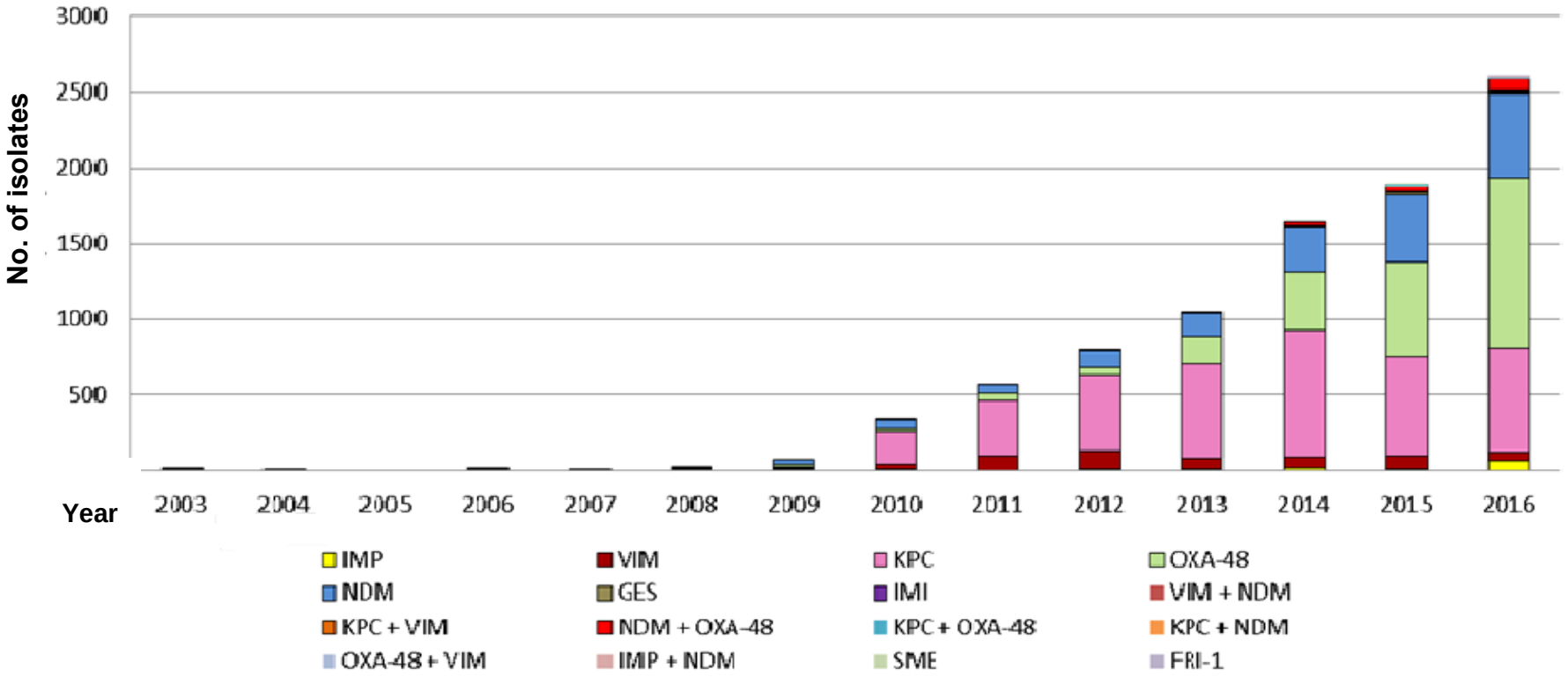


Figure 1.4 Percentage of *Klebsiella pneumoniae* invasive isolates resistant to carbapenems in Europe in a) 2009, b) 2014 (source ECDC)

a) 2009

b) 2014

The first *K. pneumoniae* KPC-producing organism in the UK was isolated from a blood specimen in Scotland in 2007 (Woodford et al., 2008). Up until 2010 there were sporadic geographically dispersed reports of KPC-producing *K. pneumoniae* in the UK, which were largely related to imported ST258 strains. This changed dramatically in 2010, with 231 bacteria, mostly from the Greater Manchester area, being identified as KPC-producers. This cluster of cases was the result of horizontal transmission of plasmids between species, rather than spread by a clonal KPC-producing strain (Livermore, 2012b). In contrast to the standard ST258 antibiogram, the KPC bacteria isolated in the North West region are mostly susceptible to fluoroquinolones and several aminoglycosides (Munoz-Price et al., 2013). Shown in Figure 1.3 increasing number of confirmed KPC-producing bacteria are observed up to 2014, and although the numbers fall slightly in 2015, there is a rising trend for the total number of confirmed carbapenemase producers. Although most of the confirmed KPC-producing bacteria were referred from the North West region; these referrals include bacteria from an extensive screening programme in this area.

1.3.3.2 New Delhi metallo (NDM) carbapenemase

New Delhi metallo (NDM) carbapenemases were first described in 2008 from a patient in Sweden who had recently returned from India (Yong et al., 2009). NDM-1 confers high level resistance to carbapenems and other beta-lactam antibiotics. The carriage of NDM-1 genes are also associated with genes conferring resistance to many antibiotic classes, including fluoroquinolones and aminoglycosides (Kumarasamy et al., 2010;Yong et al, 2009).

The emergence of NDM-1 is a concern as the gene is located in a mobile genetic element that enables it to be transferred easily to different strains of bacteria, rather

than be associated with a single strain (Nordmann et al, 2011;Yong et al, 2009). A study of NDM-1 producing isolates in India, Pakistan and the UK showed high-level resistance to all antibiotics except tigecycline and colistin (Kumarasamy et al, 2010). The NDM-1 UK isolates reported in this study were mostly associated with travel to India or Pakistan, with the Indian NDM-1 isolates being isolated from community-acquired infections (Kumarasamy et al 2010). A variant, designated NDM-2, was described in a strain of *Acinetobacter baumannii* from a patient transferred from an Egyptian hospital (Kaase et al., 2011) and now at least five other variants have been described (Jain et al., 2014).

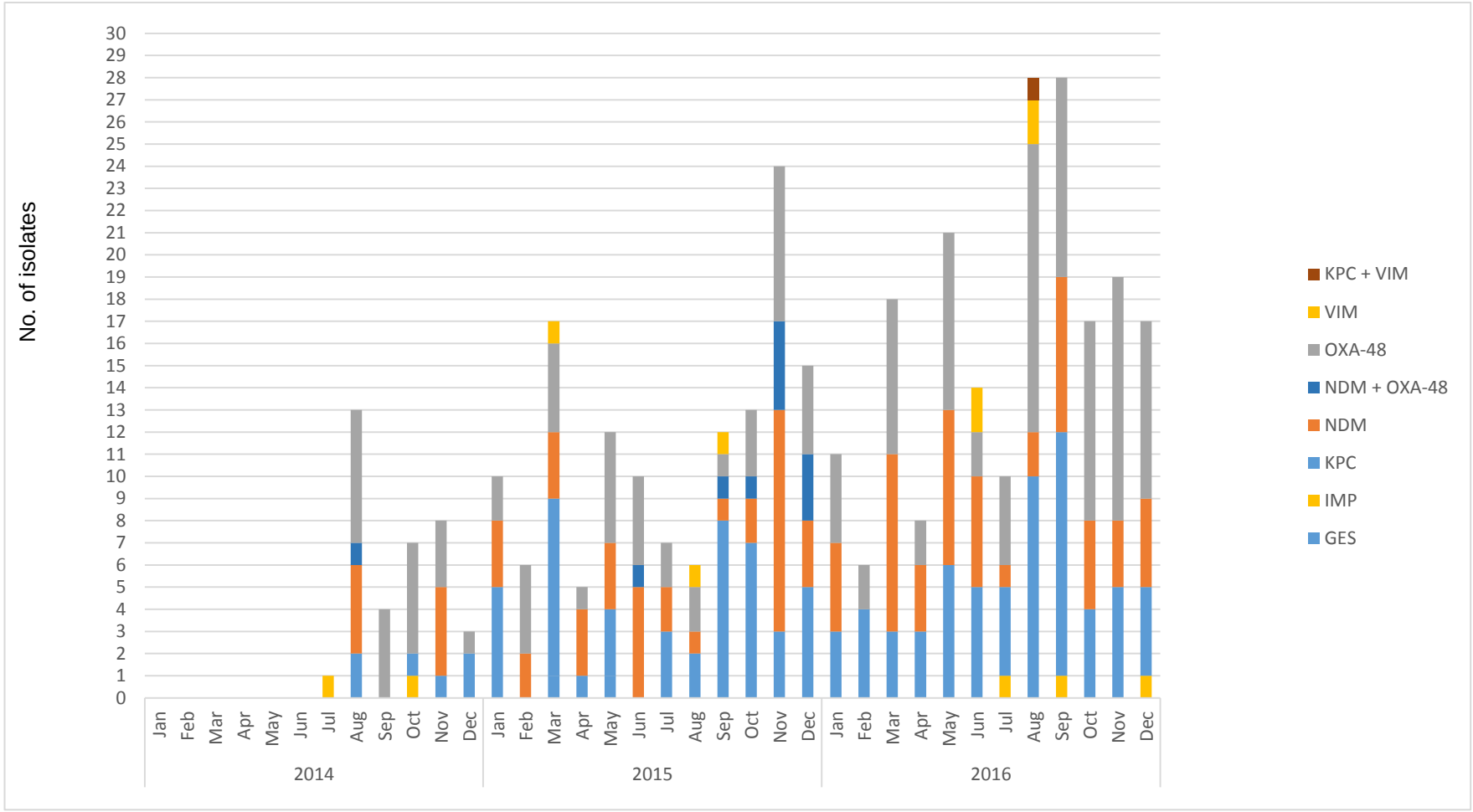
A review of the first 250 NDM cases in the UK reported that, although travel history was only available for 40% of cases, 41% (41/101) of patients with information on travel had not travelled outside the UK, suggesting a local UK reservoir of these bacteria (Jain et al, 2014). The majority of those (52%) with a travel history had travelled to, or received healthcare in the Indian subcontinent. The same study also reported that 12% of NDM cases were found in the community (Jain et al, 2014).

Previously metallo- and non-metallo carbapenemases were largely isolated from nosocomial infections caused by *K. pneumoniae* (Walsh et al., 2005). However increasing numbers of *E. coli* have been identified as having either an OXA-48 carbapenemase (Dimou et al., 2012) or New Delhi metallo- beta-lactamase (NDM-1) (Walsh and Toleman, 2012). The emergence of transmissible plasmids encoding carbapenemases in *E. coli* is a concern as this organism is widely found in the environment and is an important cause of infections in the community (Nordmann et al, 2011).

1.3.3.3 OXA-48 carbapenemase

OXA-48 was first identified in a strain of *K. pneumoniae* isolated in Turkey in 2001, being found on a mobile plasmid (Poirel et al., 2004). Reservoirs of the OXA-48 gene are now found in Middle East, North Africa and well as Turkey (Poirel et al., 2012). Although these enzymes have been isolated mainly from *K. pneumoniae*, they are increasingly being found in other Enterobacteriaceae, including *E. coli*. A recent study in Spain reported an increase in the prevalence of carbapenemase-producing *E. coli*, which was mainly due to the dissemination of OXA-48 producers (Ortega et al., 2016). Across the UK (Figure 1.3), and specifically in the West Midlands (Figure 1.5), increasing numbers of OXA-48 producers are being confirmed by the PHE reference laboratory.

Figure 1.5 Confirmed carbapenemase producers in Enterobacteriaceae. West Midlands July 2014-December 2016 (source PHE AMRHAI laboratory)



1.3.3.4 Mobilised colistin resistance (mcr-1)

The options for treating serious infections caused by carbapenemase-producing Enterobacteriaceae are limited (Livermore, 2012a). As many of these bacteria remain susceptible to colistin, the WHO has recently added colistin to the list of critically important antimicrobials (World Health Organisation, 2016a). Therefore the emergence of a plasmid-mediated mobilised colistin resistance (*mcr-1*) in bacteria isolated from animals and humans in China in 2016 is a serious concern (Liu et al, 2016). Polymyxins were not available for hospital use in China before the emergence of this plasmid-mediated resistance; however they were used heavily in agriculture. The prevalence of the *mcr-1* gene in bacteria carried by humans and food animals in south China suggests the possibility of this antibiotic resistance mechanism being driven by extensive use of colistin in agriculture and food production (Paterson and van, 2017). Although the *mcr-1* gene was originally thought to be confined to China, from sequencing archived bacterial DNA, it has now been found in countries on five continents and in many types of enterobacterial species dating back as far as the 1980s (Schwarz & Johnson, 2016).

1.4 Urinary Tract Infections

In this section the burden of urinary tract infections (UTI) will be put into context by describing the types of patients at risk of infection, aetiology (including host factors), the bacteria commonly responsible, diagnosis of UTI and treatment.

Urinary tract infections (UTI) are considered to be one the most common bacterial infections of humans, with acute uncomplicated cystitis affecting approximately 40% of women during the course of their lives (Sheerin, 2011). Although UTI has been associated with severe infections, including sepsis, most UTIs are not severe (Laupland et al, 2007). However, UTI can cause significant distress and discomfort and is one of the most commonly seen presentations in community health care settings. Patients suffering from UTI may present with one or more of the following symptoms: dysuria, frequency, suprapubic tenderness, urgency, polyuria and haematuria (Public Health England, 2014b). In the USA it has been estimated that seven million clinic visits per year are due to UTI at a cost exceeding $1.6 billion (Sheerin, 2011).

1.4.1 Definitions

Urinary tract infection (UTI) is caused by the presence of pathogenic bacteria within the urinary tract. These bacteria can be found infecting the bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria). UTI can be symptomatic with patients presenting with a range of symptoms, from mild irritation to sepsis (Foxman, 2003).

An uncomplicated UTI is an infection of an otherwise healthy individual, with normal structures and function of the urinary tract, whilst the term complicated UTI is assigned to those occurring in individuals with structural or functional abnormalities, those with indwelling catheters or other conditions including pregnancy. Patients with symptomatic renal infections that otherwise have a normal genitourinary tract are diagnosed with acute uncomplicated pyelonephritis (Foxman, 2010).

Asymptomatic bacteriuria is defined as the isolation of sufficient numbers of bacteria from urine to indicate an infection (>100,000 colony forming units/ml), yet the patient has no symptoms or signs of infection (Cormican et al., 2011).

1.4.2 Epidemiology of UTI

This section describes the distribution of UTI across various age groups and gender and describes associated risk factors that may lead to both uncomplicated and uncomplicated infections.

Asymptomatic bacteriuria (ASB) is common in women and the elderly from both sexes, being found in one to two percent of school-age girls, and five percent of adult women. In the >65 age-group asymptomatic bacteriuria has been reported in 21% of women and 12% men (Stamm and Hooton, 1993). The risk factors for ASB include sexual intercourse, diabetes, pregnancy and advancing age. The risk of developing symptomatic UTI is increased with ASB; however in most cases treatment is not recommended (Cormican et al, 2011). As ASB during pregnancy can progress to cause pyelonephritis, and is linked to premature delivery, hypertension and fetal mortality, treatment is always recommended in this group (Schieve et al., 1994).

Uncomplicated UTI is the most common form of symptomatic infection, affecting approximately 15% of women per year, with the incidence of infection highest in sexually active women (Sheerin, 2011). Recurrence is common with up to 50% of woman experiencing a recurrent infection, and around 33% experiencing frequent recurrences (Scholes et al., 2000;Stamm, 2002). Although uncomplicated UTI is often not a serious condition and the effects are normally short-lived, they can have significant short-term morbidity causing considerable discomfort and inconvenience. Recurrent UTI may also have an economic effect on individuals by disrupting a patient's working life (Foxman, 2003).

Catheter-associated UTI is a common healthcare-associated infection. In hospitals 25% of patients with catheters in place for over seven days develop UTIs (Tambyah and Maki, 2000). In the USA approximately one million cases of nosocomial UTI occur annually, of which 80% are associated with catheters (Tambyah & Maki, 2000) and these infections make up 40% of all hospital-associated infections (Foxman, 2010). As the bacteria causing catheter-associated UTI can only originate from either the patients rectal or perineal flora, or be carried on the hands of the healthcare professional, then good hygiene practices can reduce the number of infections (Meddings et al., 2014).

UTI is rare in young males that have a normal genitourinary tract. The risk groups include men who have sex with men or have a sexual partner that has vaginal colonisation with *E. coli* (Nicolle, 2008). UTI in older men is also uncommon until after the age of 50, when there is increasing risk of urinary flow being obstructed by

increasing prostatic hypertrophy (Stamm, 2002). As UTI in males is infrequent, it should always be managed as a complicated UTI (Nicolle, 2008).

UTI is common in children. These infections are commonly associated with renal tract abnormalities and are found most often in males in the first 3 months due to congenital abnormalities. In older children UTI is more common in females (Svanborg, 2013).

1.4.3 Host factors

Urine is a hostile environment for bacteria, having high osmolality and low pH, with frequent flushing helping to maintain a sterile environment (Sheerin, 2011). The epithelial lining of the urinary tract responds to bacteria by producing antibacterial peptides, which combined with the release of pro-inflammatory cytokines and chemokines initiate an innate immune response (Sheerin, 2011). Epithelial cells that are colonised with bacteria will be shed in to the urine through a process of apoptosis. The normal bacterial flora provides a degree of protection from colonisation by potential pathogens; however infections are more likely when this is disrupted by antibiotic therapy or post-menopausal oestrogen deficiency (Sheerin, 2011). Pathogen-specific immunoglobulin A (IgA) is found in urine following infection; however neutrophil killing of complement opsonised bacteria is instrumental for defence against UTI (Sheerin, 2011).

Women are more prone to UTI due to a shorter urethra and its proximity to large numbers of bacteria found in the rectum and vaginal cavity, which may be dispersed

during sexual activity (Foxman, 2003). The gender difference is less pronounced in the elderly with the rate of infections increasing in males over 50 years old (Foxman, 2010).

1.4.4 Bacterial uropathogens

Escherichia coli is responsible for 80% of uncomplicated UTI in women aged 18-39 years (Stamm & Hooton, 1993). A survey of community onset UTI in Canada found *E. coli* to be the cause in 70% across all patient groups (74.2% of ambulatory, 65.5% hospitalised). *Klebsiella pneumoniae* was responsible for 6.2% of the UTIs in ambulatory patients and eight percent in hospitalised patients (Laupland et al, 2007). A Gram-positive organism, *Staphylococcus saprophyticus* has the ability to adhere to epithelial cells lining the urinary tract and is responsible for around four percent of uncomplicated UTI (Public Health England, 2016).

Enterobacteriaceae (other than *E. coli*), *Staphylococcus aureus*, enterococci and *Streptococcus agalactiae* are more commonly found in complicated UTI; although *E. coli* is still the most common isolate in this group (Hooton, 1999). The wider range of bacteria found in complicated UTI is associated with a reduction in host defences due to anatomical or functional abnormalities of the renal tract (e.g. disruption of urine flow or a foreign body in the urinary tract) (Sheerin, 2011).

In addition to host factors, bacteria may have specific attributes that increase the likelihood of infection. Fimbriae are structures found on many uropathogenic bacteria and are involved in binding the bacteria to the epithelial cells (Nicolle, 2008). Uroplakin proteins that line the bladder are a target for Type 1 fimbriae, found on pathogens linked to uncomplicated UTI. Uropathogens, such as *E. coli*, also produce

toxins, such as haemolysin and colony-necrotising factor, which disrupt the epithelial cell walls and allow bacteria to enter the epithelial cells lining the urethra and bladder. Uropathogenic *E. coli* are able to replicate inside the host cells, which may provide a reservoir for recurrent infections (Sheerin, 2011).

Urinary pathogenic *Escherichia coli* (UPEC) are found in a restricted phylogenetic *E. coli* group and have additional virulence factors such as the ability to produce a biofilm to enable colonisation and protect the bacteria from the human immune system (Foxman, 2010). One sequence type (ST), *E. coli* ST131, is a successful uropathogenic clone that not only has an array of virulence factors, but is also commonly associated with multi-drug resistance (Rogers et al., 2011).

1.4.5 Diagnosis and treatment

The gold standard for the diagnosis of a UTI is the detection of a urinary bacterial pathogen in the presence of clinical symptoms; however current guidance for primary care diagnosis of UTI is not to submit urine samples for laboratory investigation in adult women under the age of 65 with urinary symptoms (Public Health England, 2014b). For adult women under the age of 65 where clinical symptoms do not clearly indicate a UTI, it is recommended that a urine sample should be tested locally using chemical dipsticks to determine presence of nitrite (a metabolic product of many bacteria causing UTI), leucocytes, protein and/or blood and a diagnostic algorithm followed to determine if treatment is required and if urine specimens should be referred to the laboratory (Public Health England, 2014). With diagnostic sensitivity using these algorithms being reported as high as 80%, urine samples are not

commonly sent to laboratories for confirmation and immediate therapy is provided following consultation (Bent and Saint, 2003).

The laboratory investigation of UTI involves methods to measure cellular components such as leucocytes combined with methods to quantify the number of bacteria in the urine. Traditionally this has involved microscopy and quantitative agar culture techniques; however new semi-automated technologies are being introduced such as particle detection and flow cytometry, that are able to differentiate between cell types and count bacteria (Public Health England, 2016). These new techniques are often used to screen for negative samples so that predicted positive urines can be cultured and antibiotic susceptibility tested. Urine chromogenic agar is increasingly used to enable the identification of common uropathogens (Public Health England, 2016). Quantitative culture results showing $\geq 10^5$ colony forming units /mL (cfu/mL) are indicative of a urinary tract infection; although pure cultures of 10^4-10^5 cfu/mL should be reviewed depending on clinical features (Public Health England, 2016).

In England the recommended first-line empirical treatment of uncomplicated UTI has changed in recent years. Nitrofurantoin has replaced trimethoprim as first-line treatment in recognition of the increasing levels of trimethoprim resistance in *E. coli* (Vellinga et al., 2012). Nitrofurantoin should not be prescribed for patients with renal impairment and trimethoprim is still recommended as first-line treatment for UTI in children, although susceptibility should be confirmed by laboratory analysis. Co-amoxiclav or ciprofloxacin are the recommended options for pyelonephritis (Public Health England, 2017).

1.5 Antibiotic Prescribing

1.5.1 Background

This section on antibiotic prescribing describes the volume and variation of antibiotic prescribing within the UK and internationally. A discussion on the relationship between antibiotic prescribing and antimicrobial resistance from a community perspective follows. Finally there is a description of interventions designed to reduce overall antibiotic prescribing in the UK.

There were 39.2 million antibiotic prescriptions dispensed in the community across England in 2007 (The Information Centre for Health and Social Care, 2013). In 2014 in the UK, 74% of antibiotic prescribing occurred in general practice (Public Health England, 2014a). The quantity of antibiotics prescribed between general practices varies considerably, with a large study across England finding a five-fold difference in prescribing rates between practices at the extremes of the studies dataset (Wang et al., 2009).

The use of antibiotics is acknowledged as the single most important factor leading to the development of antibiotic resistance (CDC, 2014). The association between antibiotic consumption and observed resistance has been well described in Europe and other parts of the world (Albrich et al., 2004a;Sande-Bruinsma et al., 2008b).

1.5.1.1 Community antibiotic prescribing and resistance

A study in Wales reviewed the prescribing of individual general practices and linked these data to the antibiotic susceptibility of isolates from routine urine specimens submitted by these practices. Prescribing rates were shown to vary more than four-

fold between general practices and rates of resistance between these practices also varied markedly. The authors reported that resistance to specific antibiotics was found to be associated with prescribing at the general practice level (Howard et al., 2001).

A study conducted in the South West and North West regions of England also published in 2001 found similar findings reported in Wales; however this study showed only modest correlation between antibiotic prescribing at practice level and observed resistance. The authors argued that the weak correlation observed does not support community prescribing being an important contributor to antibacterial resistance (Priest et al., 2001). More recently, two large systematic reviews concluded that antibiotic prescribing in the community is associated with the development of AMR and results in the increased use of second line antibiotics (Bell et al., 2014;Costelloe et al., 2010).

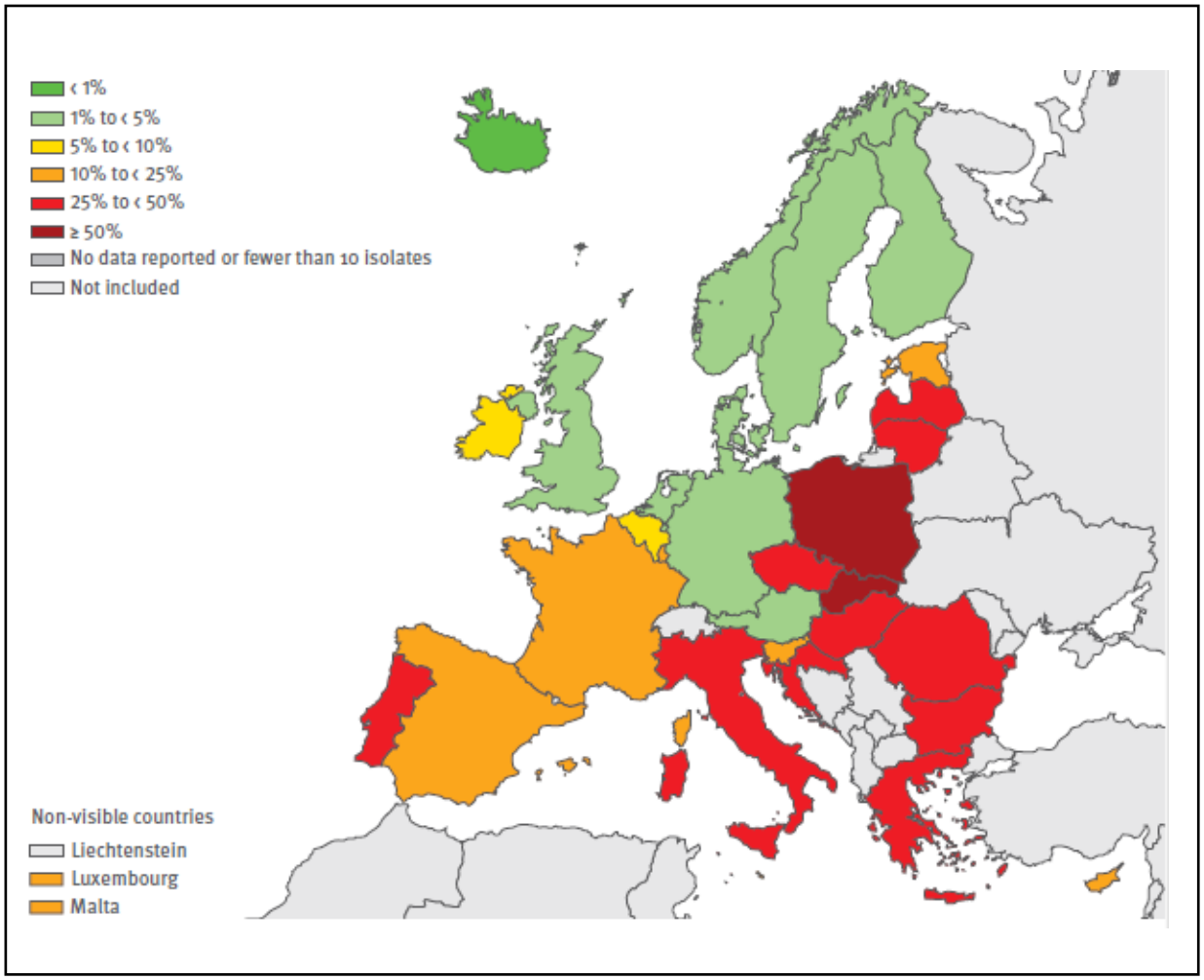
The overuse of antibiotics reserved for the treatment of MDR bacteria has led to these antibiotics now becoming ineffective against these bacteria. Fosfomycin was first developed in the 1960s but became unpopular due to issues associated with toxicity. Fosfomycin has now been re-introduced, along with other older antibiotics such as colistin and chloramphenicol to combat MDR Gram-negative infections due to the shortage of alternative therapeutic options (Theuretzbacher et al., 2015). A Spanish study in 2008 described a significant increase in ESBL-producing *E. coli* resistant to fosfomycin. The authors suggested that this could be accounted for by a large increase (340% over a period of ten years) in the use of this antibiotic in the community (Oteo et al., 2010).

1.5.1.2 International antibiotic prescribing

The World Health Assembly (1998) recognised the international dimension of the misuse of antibiotics and urged member states to develop measures to encourage appropriate and cost effective use of antimicrobials; to develop sustainable systems to detect resistant bacteria; to monitor use of antimicrobials; and to monitor the impact of control measures (WHO Report, 2000).

Large variations exist in resistance rates between individual countries. Figure 1.6 shows *K. pneumoniae* with combined resistance to the major antibiotic classes in 2015, demonstrating increased resistance proportions in central and southern Europe. High resistance rates have been linked to countries with high consumption of antibiotics, suggesting that selective pressures from higher consumption explain some of the observed geographical differences. It is a concern that non-prescribed, over-the-counter use of antibiotics is a significant factor in high consumption countries, for example it is estimated that 30% of antibiotics in Spain are obtained without prescriptions (Goossens et al., 2005). There is also a concern as to whether collating data at national level is sufficient to monitor subtle interactions between prescribing and resistance; however the strength of association has been shown to be strong between consumption and observed resistance of antibiotics across Europe, North America and Australia (Albrich et al., 2004b;Sande-Bruinsma et al., 2008a).

Figure 1.6 *K. pneumoniae* invasive isolates with combined resistance (%) to fluoroquinolones, third-generation cephalosporins and aminoglycosides by country, 2015 (source ECDC).



1.5.2 Antimicrobial stewardship

1.5.2.1 AMR and reduced antibiotic prescribing

A key intervention in the strategy to slow down the development of AMR is the reduction of antibiotics prescribed in both hospital and community settings (Department of Health, 2013). The reversal of resistance by reducing the use of antibiotics is not fully understood; however, due to fitness costs associated with acquiring resistance mechanisms, it is plausible that reducing antibiotic exposure leads to increased numbers of susceptible wild-type strains (Andersson, 2006); however there is evidence that resistance remains after exposure is removed. For example, persistence in the level of resistance to sulphonamide in *E. coli* has been observed despite a sharp decrease in use of this drug in the community (Vernaz et al., 2011); although this may partly be explained by co-selection of resistance by the use of other antibiotics (Bean et al., 2009).

A national intervention that was reported to be effective in reducing resistance was reported from Finland, where high rates of erythromycin resistance to group A streptococci was reversed by a national reduction in the use of this antibiotic (Seppala et al., 1997). Following a reduction in prescribing in the UK there has been a fall in the resistance to penicillin in pneumococci although it is difficult to assign causality (Livermore, 2004c). However unlike group A streptococci and pneumococci, the primary isolates found in UTI (i.e. Gram-negative bacilli) are found in a range of environments and hosts, including the normal flora of farm and domestic animals. Therefore Gram-negative bacteria are more exposed to other selective pressures, such as the widespread use of antibiotics in veterinary medicine (Gaze et al., 2008).

Interventions designed to reduce the burden of *Clostridium difficile* infections included a significant reduction in prescribing cephalosporins and quinolones in UK hospitals. This fall in prescribing of cephalosporins and quinolones from 2005 to 2009 was associated with a fall in the non-susceptibility of Enterobacteriaceae to these antibiotics (Livermore et al., 2013).

1.5.2.2 Community interventions

With the majority of antibiotic prescribing taking place in community settings, adherence to antimicrobial stewardship strategies are being encouraged in general practices (McNulty and Francis, 2010). Patients with upper respiratory tract infections have been shown to receive the most community prescriptions, closely followed by (in descending order): lower respiratory tract infections, sore throat, urinary tract infection and otitis media (Petersen and Hayward, 2007).

A significant factor in community prescribing is the patient's expectation to receive an antibiotic prescription when consulting a general practitioner. In a survey in India almost 50% of those questioned reported that they would change their doctor if they were not prescribed antibiotics for a common cold (WHO Community Survey India, 2011). In the UK a number of educational campaigns have aimed to educate the general public regarding the appropriateness of antibiotic prescribing. A campaign launched in 1999 in England and Wales attempted to reduce the expectation for antibiotics being prescribed for upper respiratory tract infections (McNulty, 2001).

The Department of Health in England launched an antibiotic campaign featuring posters aimed at general practice surgeries and pharmacies plus newspaper advertisements on how antibiotics do not work for upper respiratory infections

(Department of Health, 2008). A survey of the effectiveness of this campaign reported that there was little evidence that the campaign raised awareness in the English general public (McNulty et al., 2010). However an educational pack about the prudent use of antibiotics (e-Bug) aimed at school children across Czech Republic, France and England was seen as a success (Lecky et al., 2010). Supporting materials are now part of the Department of Health Antibiotic Awareness Campaign (Department of Health Antibiotic Awareness Campaign, 2011) and the European Antibiotic Awareness Day (EAAD) is held annually in November with individual web resources tool kits targeted for use by the general public, primary care prescribers and hospital prescribers (EAAD, 2011).

In the UK, the success of interventions aimed at reducing antibiotic usage is often measured by the NHS Prescription Service's Prescribing Analysis and Cost (PACT) data set (Lovejoy and Savage, 2001). This is a measure of dispensed prescriptions for all conditions and therefore specific illness episodes cannot be separately identified. There are initiatives to use primary care databases such as the General Practice Research Database (GPRD) to monitor antibiotic prescribing for specific conditions in the community (Petersen & Hayward, 2007). An ideal surveillance system for measuring the effect of a reduction in antibiotic use will be the use of patient level primary care prescribing data to link to antibiotic resistance data for the same population (McNulty, 2001).

Theories of behaviour have been used to help understand the difficulties in achieving change in the prescriber's habits of general practitioners. A simple model has been suggested, which involves understanding an individual's perception of 'why' they

should change prescribing practice and ‘how’ change can realistically be achieved. The authors suggest that a change in prescribing will not be achieved unless the general practitioners believe it is important for them to do so (McNulty & Francis, 2010). A study in Sweden reported that GPs held a range of views on antimicrobial resistance in the treatment of UTI. Their views were assigned to the following categories: a) there is not a problem, b) the problem is found elsewhere or c) that AMR is a serious issue. The authors reported that only the GPs who believed that AMR is a serious problem followed prescribing guidance (Björkman et al., 2013).

Initiatives to change prescribing practice have focused on the productions of a range of evidence based national and local guides being made available to primary care prescribers. In the UK these include the PHE Management of Infection Guidance for Primary Care (Public Health England, 2014a), which is designed to be adapted by local primary care teams. A UK study using a large database of primary care consultations between 1995 and 2011 found large variations in prescribing between practices following the introduction of the national PHE guidance aimed at promoting a consistent approach to treatment of infectious disease (Hawker et al., 2014). Antimicrobial results reported to primary care by the local diagnostic laboratory have also been shown to influence prescribing, and therefore can be used as a mechanism to encourage the use of preferred antibiotics (McNulty et al., 2011).

1.6 Surveillance of Antimicrobial Resistance

1.6.1 Surveillance systems

1.6.1.1 Background

Antimicrobial resistance surveillance has been defined by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) as “a systematic, ongoing data collection, analysis and reporting process that quantitatively monitors temporal trends in the occurrence and distribution of susceptibility and resistance to antimicrobial agents, and provides information useful as a guide to medical practice, including therapeutics and disease control activities” (Cornaglia et al., 2004). This is a variation of the classic CDC definition of disease surveillance which states: “epidemiologic surveillance is the ongoing and systematic collection, analysis, and interpretation of health data in the process of describing and monitoring a health event. This information is used for planning, implementing, and evaluating public health interventions and programs. Surveillance data are used both to determine the need for public health action and to assess the effectiveness of programs”. (Klaucke et al., 1988).

An important action in the global strategy to contain antimicrobial resistance is the establishment of effective surveillance systems at local, sub-national and national levels (Commission to the European Parliament and the Council, 2011;World Health Organisation, 2001). Such surveillance systems should be designed to meet clearly defined objectives that address the requirements of key health partners. These objectives may include defining the extent of the problem and changes over time, detecting the emergence of new mechanisms of resistance and outbreaks, providing

local information to inform the development of formularies, guiding the development of effective strategies and interventions, and evaluating the effectiveness of implemented control measures (Bax et al., 2001;Felmingham, 2002;Johnson, 2015;O'Brien and Stelling, 2011). Surveillance information from these systems is only useful when it triggers an intervention. To this end, surveillance outputs from AMR surveillance systems must be timely, present data unambiguously and meet the needs of a range of users, including physicians, general practitioners, microbiologists, commissioners and providers of healthcare, national and international health organisations (Johnson, 2015).

International and national AMR surveillance schemes have been recently introduced, including schemes in the UK devolved countries of Scotland and Wales. These AMR surveillance systems are described in section 3.1.4 of Chapter 3.

1.6.1.2 AMR surveillance in England prior to 2009

In England, before the introduction of AmSurv (described in Chapter 3), antimicrobial resistance surveillance has been mostly undertaken by Public Health England reference laboratories and the British Society for Antimicrobial Chemotherapy (BSAC). These are voluntary targeted sentinel surveillance systems that monitor AMR trends in specific infections, for example gonorrhoeae, or isolates from respiratory and blood specimens. These bacteria are sent by participating laboratories to PHE reference laboratories for antibiotic susceptibility testing and characterisation (White, 2008).

A further potential source of antimicrobial resistance data in England is a surveillance system, operated by PHE, known as CoSurv when it was introduced in 1996, but is

now incorporated as part of the Second Generation Surveillance System (SGSS). This surveillance system collates notifiable 'Communicable Disease Reports' (CDR) from diagnostic laboratories, and therefore is described in this section as CDR SGSS to distinguish from the AMR SGSS data collection described later (Health Protection Agency, 2012). CDR SGSS also collects data from Wales and Northern Ireland, although these countries have developed independent national AMR surveillance systems and therefore do not participate in the AMR SGSS.

Together, the notification and targeted systems provide a mechanism for monitoring antimicrobial resistance for specific bacteria and infections. As CoSurv only collects antibiotic susceptibility data that is reported by the laboratory to clinicians (that is, it does not collect all tested antibiotics) and the bacteria included are mostly from more serious or invasive infections, there has been a significant gap in monitoring resistance from isolates acquired from routine diagnostic microbiology, particularly those isolated from community specimens. Specifically, there has not been a system to collate resistance data from bacteria responsible for urinary tract infections, for which plasmid-mediated multi-resistance is increasingly being reported (Hayward et al., 2007).

1.6.1.3 The AmSurv system

In order to complement existing UK systems and address the current gaps in AMR surveillance in England, the Health Protection Agency (HPA) developed antimicrobial surveillance software (AmSurv) to facilitate the collection of antimicrobial susceptibility reports from all bacterial isolates tested against antibiotics, including those from routine community samples. Implementation of this system across the

nine NHS organisational English regions began in 2009 (Public Health England, 2014a). The dataset collected includes patient demographics, specimen details, sending organisation, organism, antibiotic and susceptibility result. The minimum inhibitory concentration (MIC) for each antibiotic test is also collected if this is available on the laboratory information Management system (LIMS).

A comparison of the AmSurv and Cosurv systems is given in Table 3. The AmSurv system was incorporated into the SGSS PHE laboratory surveillance application in 2014. SGSS collates AmSurv files from laboratories and collates these in a centralised data repository (Hopkins, 2016). An advantage of using the SGSS process is that the collection of data from laboratories may be fully automated, which will reduce the burden of reporting for laboratories, improve timeliness and ensure the system is sustainable over time (Johnson, 2015).

Table 1.3 AmSurv and CoSurv comparison(based on PHE reporting guide https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/545183/PHE_Laboratory_Reporting_Guidelines.pdf)

CoSurv	**AmSurv**
Implemented 1996	Implemented 2009
Surveillance system for communicable disease reports (CDR)	Antimicrobial resistance (AMR) surveillance system
Mandatory reporting for designated organisms since 2010 (Health Protection Regulations 2010)	Voluntary reporting system
Only receives antibiotic susceptibility results reported to the clinician	Collects all antibiotic susceptibility results tested in laboratory
Only receives organisms of public health interest (5-7% of bacterial isolates from a laboratory)	Collects all organisms isolated that have antibiotic results
Antibiotic results only reliably received from sterile fluids (e.g. blood cultures)	Collates antibiotic susceptibility test results from all specimen types
Predominantly antibiotic reports received from more serious infections within hospital environments	Includes results from community and hospital isolates

1.6.2 Interpretation of surveillance data

A challenge for surveillance systems based on routine reporting by microbiology laboratories is in understanding how observed results relate to the general population. There are a number of factors that may influence interpretation of routine laboratory surveillance data, which are discussed below.

1.6.2.1 Submission of specimens

A source of potential bias is the variation in submission of specimens for microbiological examination. The frequency at which urine specimens are sent for microbiological examination varies greatly between practices (Howard et al, 2001;McNulty et al., 2004). Selection bias requires consideration when interpreting AMR data from the community, as it is likely that specimens are sent for microbiological examination from initial treatment failures, those with more complicated medical histories and those suffering severe infections (Hay et al., 2005a;Hillier et al., 2006). To mitigate for this type of bias in antibiotic resistance, studies would require specimens being taken systematically prior to antibiotic exposure. A study in the South West region of England assessing the relationship between prescribing and resistance in primary care examined *E. coli* contaminating urine samples from asymptomatic adult patients. Evidence of antibiotic exposure was captured for individuals in the preceding 12 months. The authors reported greater resistance in patients exposed to antibiotics within two months of sampling (Hay et al., 2005b). This short term increased resistance following community prescribing has also been reported in studies of respiratory and urine infections in children (Chung et al., 2007;Paschke et al., 2010).

1.6.2.2 Duplicate data

The inclusion of duplicate data has been a flaw in a number of AMR surveillance reports (Morris and Masterton, 2002). Guidelines from the US Clinical and Laboratory Standards Institute (CLSI, previously called the National Committee for Clinical Laboratory Standards) recommended that only results from the first isolate of a species from a patient should be included in calculating the percentage susceptibility to an antibiotic (Clinical and Laboratory Standards Institute, 2014). Shannon et al agreed with this approach for its simplicity, although their data only showed significant advantages for defined organism and antibiotic combinations (Shannon and French, 2002). Selecting only the first isolate, however, limits the ability to monitor changes in individual resistance, perhaps as the result of antimicrobial therapy (Morris & Masterton, 2002).

A study reviewing exact duplicates (i.e. same organism, patient and antimicrobial susceptibility test results) found that exclusion of duplicates did not make a significant difference in regional resistance estimates, with the exception of screening for MRSA (Magee, 2004). A further study specifically examining the effect of duplicates when calculating prevalence and antimicrobial susceptibility of isolates from urinary specimens found that most duplicates appeared within seven days and that there were more ‘repeat’ isolates from patients admitted to hospital than those in the community. The study concluded that although the effect of duplicates was relatively minor when calculating susceptibility levels in the community, using the first isolate per episode may minimize any bias (Cebrian et al., 2005).

1.6.2.3 Standard laboratory methodology

Interpretation of AMR surveillance data are dependent on standard methodology being adopted by the testing laboratories (Johnson, 2015). Surveillance of antimicrobial resistance using routine laboratory reports is subject to a number of potential factors that may introduce bias that are inherent within the methods and protocols used by individual laboratories. There are a number of different methods used to test susceptibility to antibiotics. In the UK these have been mostly disc diffusion methods (such as modified Stokes and BSAC) or breakpoint methods (see Chapter 2 section 2.1) (Wootton et al., 2017). The results from these methods do sometimes vary, with potentially significant errors reported as a result (Gosden et al., 1998;Potz et al., 2004). Laboratories have also adapted or changed standard methods, for example performing susceptibility testing direct from urine specimens in order to improve timeliness and reduce costs (Oakes et al., 1994).

In recent years the introduction of automated systems, such as the VITEK2® (bioMerieux, Lyon, France), has had a significant impact on antibiotic testing within laboratories (Livermore et al., 2002). These automated devices provide an element of standardisation and in combination with interpretative software systems allow the detection and interpretation of resistance mechanisms; however confirmation by other methods is sometimes required, for example with ESBL detection (Espinar et al., 2011;Thomson et al., 2007;Valenza et al., 2011).

1.6.2.4 Expert rules

It is not rational to report each individual antibiotic result as if they were independent of other antibiotic results, due to the fact that multi-resistance often depends on a single mechanism (Livermore et al, 2002). Many laboratories, therefore, derive particular antibiotic results based on the organism isolated or other antibiotic susceptibility results. A software rule, for example, may change 'susceptible' antibiotic susceptibility test results to 'resistant' for beta-lactam antibiotics such as aminopenicillins and cephalosporins for Gram-negative bacteria suspected of producing an ESBL. This is achieved by automated rules within the Laboratory Information System (LIS) or 'expert' rules (Leclercq et al., 2013) developed within the automated testing device.

1.6.2.5 Identification of bacteria

Another source of variation between results submitted to the surveillance systems relates to the variation in identification methods and protocols for bacterial isolates used by different laboratories. Some laboratories may report all lactose-fermenting bacteria from urine specimens as 'coliforms', whilst others will identify all isolates using a combination of colonial morphology and enzyme or biochemical tests. The pooling of bacteria from different species has important implications for surveillance as it will mask the detection of emerging or new resistance in species isolated less frequently (Hayward et al, 2007).

In the last 5 years Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry has been introduced into diagnostic microbiology laboratories to rapidly and cost-effectively identify bacteria, including directly from

clinical specimens (Croxatto et al., 2012). The introduction of this technology has improved the quality and timeliness of bacterial identification and enable a wider range of bacteria to be characterised (Carbonnelle et al., 2011).

1.7 Study population

In 2015 the West Midlands was one of nine English PHE regions (NHS, 2015), with a population of 5.6 million (2011 census) and contains the City of Birmingham, the second most populous city in the UK. It is the second most ethnically diverse region of the UK (after London), with 10.8% of the population being Asian or British Asian (Office for National Statistics).

At the start of this study in 2010 the region was divided into 17 Primary Care Trusts (PCTs), and these bodies acted as commissioners of health services for their local populations. A reorganisation of the National Health Service (NHS) in England led to PCTs being abolished on 31 March 2013, with 22 newly established Clinical Commissioning Groups (CCGs) taking on their commissioning role.

In 2012 there were 950 general practices with a total of 3635 general practitioners responsible for 5.8 million registered patients (Health and Social Care Information Centre). Each practice had an average of four GPs with an average practice list size of just over 6,000 patients and 73% of practices were located in Local Authority (English local administrative unit) areas designated as urban (Health and Social Care Information Centre).

During the study period (2010-2014) there were 15 diagnostic microbiology laboratories in the West Midlands serving both community-based centres and hospitals. The daily average for occupied hospital beds in the West Midlands for 2013 was 10,626 (NHS England).

1.8 Hypothesis

Surveillance data collected routinely from diagnostic microbiology laboratories in the West Midlands region of England will be able to demonstrate an association between antibiotic prescribing in the community and antibiotic resistance in bacteria causing urinary tract infections.

1.9 Aim and objectives

The overarching aim of this study was to determine if routine antimicrobial surveillance data may be utilised to influence local antibiotic prescribing habits by demonstrating an association between prescribing and resistance at the general practice level.

To achieve this aim a number of objectives needed to be achieved:

- The establishment of routine AMR surveillance in the West Midlands
- To develop an understanding of the methods used by diagnostic laboratories in the West Midland to identify bacteria from urine specimens and perform antibiotic susceptibility tests.
- To understand how and when protocols are used in the community for 1) sending urine specimens for microbiological analysis and 2) prescribing antibiotics for urinary tract infections (UTI).
- To review AMR in bacteria isolated from urine specimens across the West Midlands
- To examine the effect of general practice characteristics and antibiotic prescribing on antibiotic resistance in bacteria isolated from community urine specimens.

1.10 Ethics

PHE has approval under Section 60 of the Health and Social Care Act 2001 (now subsumed into the National Information Governance Board for Health and Social Care with Section 60, now Section 251 of the NHS Act 2006) to process confidential

patient information for public health surveillance (http://www.legislation.hmso.gov.uk/si/si2002-20021438.htm). Following PHE Research Ethics and Governance Group (REGG) policies and with reference to the NHS Research Ethics Committee decision tool (http://www.hra-decisiontools.org.uk/ethics/) it was determined that the studies reported in the following chapters did not require specific ethical approval.

The AMR surveillance data extracted for the studies reported in chapters three, five and six did not include patient identifiers. Individual GP identifiers were not collected in the survey reported in chapter 4. Laboratory and general practice identifiers were anonymised throughout the thesis.

2 A survey of methodologies for the identification and antibiotic susceptibility testing of bacterial isolates from urine samples submitted to laboratories based in the West Midlands

2.1 Background

2.1.1 Laboratory testing protocols for urine specimens

Diagnostic microbiology laboratories offer a range of tests to help in the diagnosis and treatment of patients. Diagnostic methods and techniques are often selected based on the reliability and reproducibility of results; however the speed in which results are delivered and the overall costs associated with specific tests also have to be justified by the clinical usefulness of the results provided (World Health Organisation, 2003). Laboratory analysis of urine may comprise four stages: chemical tests, usually in the form of dipsticks, to detect the presence of leucocytes, nitrite, protein, and blood; microscopy to detect the presence of cellular components, such as white blood cells, red blood cells casts, and bacteria; culture is used for the quantification of bacteria present, and isolation of the suspected causative organism; and finally tests may be completed to identify the bacteria present and determine antibiotic susceptibility.

2.1.2 Initial examination

PHE guidance suggests that a urine specimen is taken if clinical symptoms suggest a possible UTI. If the urine is cloudy on visible examination, then a biochemical dipstick test should be considered (Public Health England, 2014). Dipstick tests are commonly used to aid diagnosis and determine if a specimen should be sent to the laboratory for analysis, or if empirical antibiotic treatment is required (Public Health England, 2014). For urine specimens sent to the laboratory initial microscopy is now

being replaced by new technologies such as flow-cytometry and particle recognition systems. These systems are being used to screen-out negatives and thereby reducing the number of samples sent for culture (Public Health England, 2016).

2.1.3 Urine culture

Urine specimens are selected for culture based on laboratory protocols. The most common culture techniques used for determining the number of bacteria in urine are the use of calibrated loops, sterile paper strips or multi-point inoculators to deliver a standard inoculum onto either Cystine Lactose Electrolyte-Deficient (CLED) agar or chromogenic agar (Public Health England, 2016). CLED agar has been the standard media used for urine culture in the UK as it is able to support the growth of most urinary pathogens, prevents the swarming spread of *Proteus* spp. and allows some colonial morphology to be determined (Munoz et al., 1992).

Chromogenic agar has been developed to enable the presumptive identification of urinary pathogens and enable the differentiation of bacteria in mixed cultures. Chromogenic agar media for culture of bacteria from urine samples combines the ingredients of CLED agar with a range of chromogenic substrates (Fallon et al., 2003a). Bacteria growing on this media show either a distinctive pigmentation or change the colour of the media, allowing the presumptive identification of a number of common urinary pathogens (Fallon et al., 2003b).

2.1.4 Identification of bacteria isolated from urine specimens

Laboratories undertaking further identification of bacteria isolated from urine use a mixture of automated and non-automated systems. One of the most popular non-automated identification system for Enterobacteriaceae in Europe and the USA is the API® 20E system (bioMérieux) (O'Hara, 2005). It consists of a strip of 20 plastic wells that contain substrates and indicators that are inoculated with bacteria and incubated for 24hr-48hrs. Results are given a numerical value based on reactions in each plastic well, which is referenced in the API® database to provide identification. The API® 20E identification system provides high levels of accuracy for the identification of Enterobacteriaceae commonly isolated from clinical specimens and became a gold-standard for the assessment of new methods (O'Hara, 2005). Other manual identification systems available at the time of the this study included: the BBL Crystal® identification system (Becton Dickinson), which includes a miniaturised plastic panel of 30 biochemical tests, and Enterotube® II (Becton Dickenson), which consists of a tube with 12 different media with indicators (O'Hara, 2005). Automated susceptibility testing devices, discussed below, also include the ability carry out identification tests.

2.1.5 Antibiotic susceptibility tests

2.1.5.1 Manual susceptibility tests

Antimicrobial susceptibility testing is a key function of diagnostic microbiology laboratories. The aim of antimicrobial susceptibility testing is to provide an indication

of whether the bacteria present in a sample, and thus potentially causing an infection, will respond to treatment by an antibiotic using the normal dosage for the type of infection and organism isolated (Andrews et al., 1996). A ‘susceptible’ result indicates that the antibiotic will be effective whereas a ‘resistant’ result indicates that at the normal dosage the antibiotic will not inhibit the bacteria (Jorgensen and Ferraro, 2009). Laboratories usually test bacteria against a standard set of antibiotics based on initial identification and / or the site the organism was isolated from (known as first-line testing). If the organism is found to be resistant to ‘first-line’ antibiotics then a further set of antibiotics are tested (known as second-line testing) (Public Health England, 2016). Automated susceptibility testing (AST) systems often test up to 20 antibiotics, compared with the six antibiotics tested first-line by laboratories using the BSAC method. Therefore laboratories using AST systems do not need to undertake second-line testing for most of their isolates (Jorgensen & Ferraro, 2009).

A number of methods are available for determining antibiotic susceptibility and methods may vary within a laboratory depending on the identification of the bacteria and the specimen type from which it was isolated. These are discussed further in section 2.4.

One of the first methods for measuring antibiotic susceptibility was the broth dilution technique, which was later miniaturised using ‘microdilution’ trays (Jorgensen & Ferraro, 2009). This technique is quantitative and provides the Minimum Inhibitory Concentration (MIC), which is the lowest concentration of the antibiotic that prevents bacterial growth. This method required manual reading and interpretation of growth within the dilution wells; however automated readers have been developed that

allow plates to be electronically scanned and results linked to laboratory computer systems, which enables standardised result reporting (Jorgensen & Ferraro, 2009).

The breakpoint method of susceptibility testing involves seeding an agar plate with a concentration of an antibiotic that is close to its breakpoint value (Waterworth, 1981a). The breakpoint value is the concentration (mg/L) of an antibiotic used to determine whether a bacterial isolate is susceptible or resistant to that antibiotic (BSAC, 2017). In the breakpoint method a diluted suspension of bacteria is spotted on to the agar plate to determine if the bacteria are able to grow in the presence of the specific concentration of antibiotic. Using multipoint inoculators this method was scalable for large volume testing (Waterworth, 1981a).

Disc diffusion susceptibility testing methods are popular due to their simplicity to complete, control over the bacterial inoculum and the provision of categorical results. In the 1990s the Modified Stokes disc susceptibility method was the most popular method in the UK (Andrews et al, 1996). This method involved comparing the test bacteria with a susceptible control organism on the same agar plate; however, the method was not standardised between laboratories and was found to have a number of serious quality issues (Gosden et al., 1998).

Using standardised methods, disc diffusion has been shown to be a reproducible and accurate method for determining antibiotic susceptibility (Woods, 1995). Various national bodies introduced standardised disc susceptibility testing methods and interpretive guidelines, including, in 2001, in the UK the British Society for Antimicrobial Chemotherapy (BSAC) (Andrews, 2001). The BSAC standardised disc diffusion method largely replaced the Modified Stokes method as the most popular

technique in the UK over the following ten years (Wootton et al., 2017). In 2009, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provided breakpoint standards and introduced a standardised disc diffusion method which is calibrated to harmonised European MIC breakpoint standards (Matuschek et al., 2014). To help standardise susceptibility testing methods in Europe, BSAC announced that from January 2016 it will no longer support the BSAC disc diffusion method and will encourage UK laboratories to use the EUCAST standardised method (http://bsac.org.uk). The EUCAST disc diffusion method uses Mueller Hinton agar rather than Isosensitest Agar used in the BSAC method (British Society for Antimicrobial Chemotherapy (BSAC), 2016), and covers a greater range of antibiotics and bacteria (Brown et al., 2016).

A variation of the disc diffusion method is the antimicrobial gradient diffusion method (Jorgensen & Ferraro, 2009). This method uses an antimicrobial gradient in an agar medium to determine the MIC of an organism. A commercial version of the gradient strip is a system called Etest® (bioMérieux), which is a strip impregnated with a gradient concentration of an antibiotic with a scale inscribed on the reverse side. Following incubation of the agar plate, the Etest® strips are read and the MIC determined by the intersection of the bacterial growth with the strip. Etest® strips are relatively expensive when compared with disc diffusion or break point techniques; however they have been found to be useful for determining the MIC for fastidious bacteria or where standard methods are unreliable for particular antibiotics (Huang et al., 1992).

In the 1990's direct antibiotic susceptibility testing of urines was introduced by some laboratories in the UK. This involved inoculating the urine directly onto disc or breakpoint agar plates and therefore results were available after overnight incubation (Oakes et al., 1994). The merits of this technique are discussed further in section 2.4.

2.1.5.2 Automated susceptibility tests

Following the automation of other pathology services, automated systems are being introduced into diagnostic microbiology laboratories. Automated systems standardise the reading of susceptibility results and sensitive detection devices provide faster results by detecting small changes in bacterial growth (Jorgensen & Ferraro, 2009). Four commercial systems were available at the start of this study in 2010: three of which: the MicroScan® Walkaway (Siemens Healthcare Diagnostics), the Phoenix® Automated Microbiology System (BD Diagnostics) and the VITEK 2® (bioMérieux) are able to generate rapid susceptibility test results (3.5hrs-16hrs). The fourth system, the Sensititre® ARIS 2X (Trek Diagnostic systems) requires overnight incubation (Jorgensen & Ferraro, 2009).

With the introduction of AmSurv in 2009 (see Chapter 3), laboratories in the West Midlands were asked during the configuration visit, if they used automated susceptibility testing (AST) devices, as it is possible to interface these so that minimum inhibitory concentration (MIC) values can be captured by the surveillance system. The MIC is the lowest concentration of an antibiotic that inhibits the growth of a bacterium (BSAC, 2017), and MIC values are sometimes output by these automated susceptibility testing devices (O'Hara, 2005). The only AST system being

used by laboratories in the West Midlands at that time was VITEK 2®, which does assign susceptibility results based on predictive MIC values.

The VITEK® system was originally part of a McDonnell Douglas program to identify bacteria in space and was acquired by bioMérieux in 1988 (O'Hara, 2005). VITEK 2®, using fluorescence-based technology to identify bacteria and test susceptibility, using separate identification test substrates or antibiotics in microliter quantities within plastic cards, was introduced into clinical practice in 1997. VITEK 2® is a closed system designed to process 60 or 120 cards at a time and may provide results within 4-8hrs by repeated turbidimetric monitoring of bacterial growth (Jorgensen & Ferraro, 2009). The need for improvement in laboratory efficiency, rapid turnaround times and reliable results have led to the widespread introduction of VITEK 2® by diagnostic microbiology laboratories (Ling et al., 2001).

2.2 Objectives

- To determine how many urine specimens are tested by West Midland microbiology laboratories
- To understand how bacteria isolated from urine specimens are identified in West Midland microbiology laboratories
- To determine which antibiotic susceptibility test methods are being used and which antibiotics are tested for a range of bacteria identified from urine specimens in West Midland microbiology laboratories
- To document 'expert rules' used by West Midland laboratories to determine antibiotic susceptibility test results or change existing results
- To document any changes in susceptibility testing methods or breakpoint standards

2.3 Methods

2.3.1 Survey protocol

In 2011 there were 15 diagnostic microbiology laboratories in the West Midlands (14 NHS laboratories and one public health laboratory). In March 2011 all laboratories in the West Midlands were contacted by email and informed about the aims of the forthcoming voluntary survey of methods. At the same time they were asked to provide the total number of urines processed by their laboratory in 2010 and provide the proportion of urines received from community patients compared with those received from hospitalised patients. The email request was followed-up after a two week period with a phone call to laboratories that had not responded.

A survey of laboratory methods was developed using the web Select Survey v4 application (Classapps, KS, USA). All participating laboratories were sent an email with links inviting them to complete the on-line survey in April 2011. The email requested that the survey be completed by a member of laboratory staff with knowledge of current methods and protocols for the analysis of urine samples. Laboratory staff were required to register on-line to gain access to the survey. Registration on the survey site allows users to save partially completed questionnaires for later completion, and also ensures details of the participant are recorded. Two weeks following the initial request, non-responding laboratories were contacted by telephone to request enrolment on the survey site and completion of the survey. Following analysis of the survey and request for numbers of urines tested, laboratories were anonymised for the remainder of the analysis.

The electronic survey (Appendix 1) consisted of 19 questions, in a format of 'drop-down' selections and textual response boxes. The survey focused on methods used to identify bacteria isolated from urine specimens and the techniques employed to ascertain the antibiotic susceptibility of these bacteria.

2.3.2 Survey format

The survey was designed to obtain the following information from each of the diagnostic microbiology laboratories in the West Midlands (Survey Appendix 1):

- The current methodologies for identifying bacterial isolates from urine samples
- The techniques used in the laboratory to determine antimicrobial susceptibilities for bacterial isolates from urine
- The antibiotics routinely tested against urinary isolates by the laboratory
- The protocols employed for determining when methods are used for identifying bacteria from urine specimens
- The protocols for determining which antibiotic susceptibility testing method is used for bacteria isolated from urine samples
- The protocols employed to specify which antibiotics are tested as first line or second line for bacterial groups
- If reporting rules are used to determine antibiotic results based on other test results or the bacteria isolated
- Which antibiotics are reported to general practices for urinary isolates
- If protocols or methods have changed in the recent past and if there are any plans to make changes in the future

2.3.3 Survey follow-up questions

Initial analysis of the results from the on-line survey identified areas where the answers to specific questions were unclear and required clarification. A follow up

email was sent to each respondent of the electronic survey. These follow-up emails were tailored for each laboratory based on their response to the questions on the web survey. The aim of the follow up email was to provide an opportunity for the respondent to clarify and expand on ambiguous answers, and if necessary, complete gaps in their responses. The follow-up questions were also an opportunity to improve the data by asking for further detail on some of the techniques (Table 2.1).

Table 2.1 Supplementary questions to laboratories in follow-up survey

1	What criteria are used to determine if second-line antibiotic panel testing is used against urinary isolates?
2	If you use VITEK 2® for testing urinary isolates please could you provide the names of the VITEK 2® cards used?
3	What version of clinical breakpoint standards are in current use?
4	Do you use standard media or chromogenic agar for direct culture of urine specimens?
5	Are the specified urine antibiotic panels used against both Gram-positive and Gram-negative isolates?
6	Please can you specify the individual rules employed by the laboratory to change the reporting of antibiotic susceptibility results (e.g. based on the isolate and/or other antibiotic results)?

2.4 Results

2.4.1 Response

All laboratories in the West Midlands responded to the request for urine numbers, the electronic survey and follow up questionnaires (n=15). The majority of the survey respondents were senior Biomedical Scientists (12), with two Consultant Microbiologists and one Information Manager responding. All respondents confirmed that they were familiar with their laboratory protocols for the microbiological analysis of urine specimens. A response to certain questions was not provided by all laboratories.

2.4.2 Number of urines tested by West Midlands laboratories

The number of urine specimens submitted for microbiology analysis by West Midland laboratories in 2010 was 1.1 million. Laboratories varied considerably in the number of urine specimens tested, ranging from 8,000 to 190,000 (Figure 2.1). Two of the laboratories served specialist Trusts and did not provide a diagnostic service for the community. The remaining 13 general diagnostic laboratories received approximately equal numbers of urine samples from the community and hospital patients in 2010 (55% of urines received from the community over the whole region).

Figure 2.1 Number of urine samples tested in 2010 by West Midland laboratories (anonymised as 1 to 15)

2.4.3 Identification of bacterial species isolates from urine

Laboratories in the West Midlands reported using a number of different methods for the identification of bacteria isolated from urine specimens, including: microscopy (detection of bacteria and to determine cell morphology), colonial morphology, biochemical tests, single enzyme tests (e.g. oxidase, coagulase) and automated testing devices (e.g. VITEK 2®). These tests were often used in various combinations depending on the initial presumptive identification from colonial morphology and/or chromogenic indicators (Figure 2.2), for example laboratories reported using an oxidase test to confirm presumptive *Pseudomonas* spp. identification following chromogenic indicator results.

Laboratories in the West Midlands varied in the level of identification for different bacterial groups. For Gram-negative bacilli (GNB) three laboratories indicated that non-lactose fermenting bacteria may be reported at the 'bacterial group' or 'family' level (e.g. coliform or Enterobacteriaceae). Only two laboratories indicated that lactose-fermenting bacteria are reported at the 'group' level (e.g. 'coliform'). The majority of laboratories identified Gram-positive bacteria to at least the genus level (Figure 2.3). All laboratories reported that they would fully identify bacteria exhibiting multi-drug resistance.

The majority of West Midland laboratories used a chromogenic agar as their primary urine culture medium (10/15). CLED medium was used by the remaining five laboratories for primary culture, although three of these laboratories also used chromogenic agar as an aid to identification.

Figure 2.2 Number of different test methods used by each laboratory to identify bacterial isolates from urine

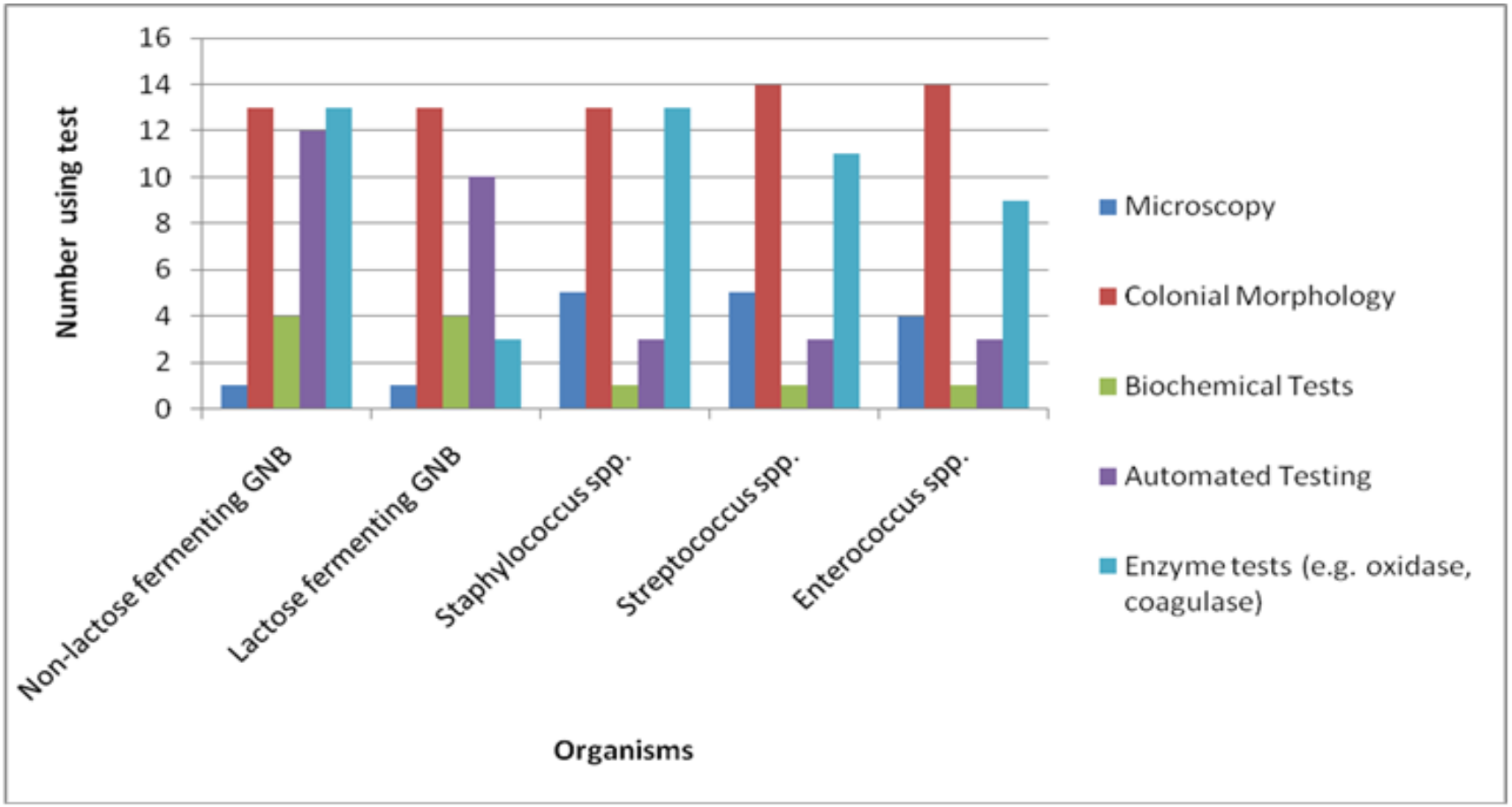
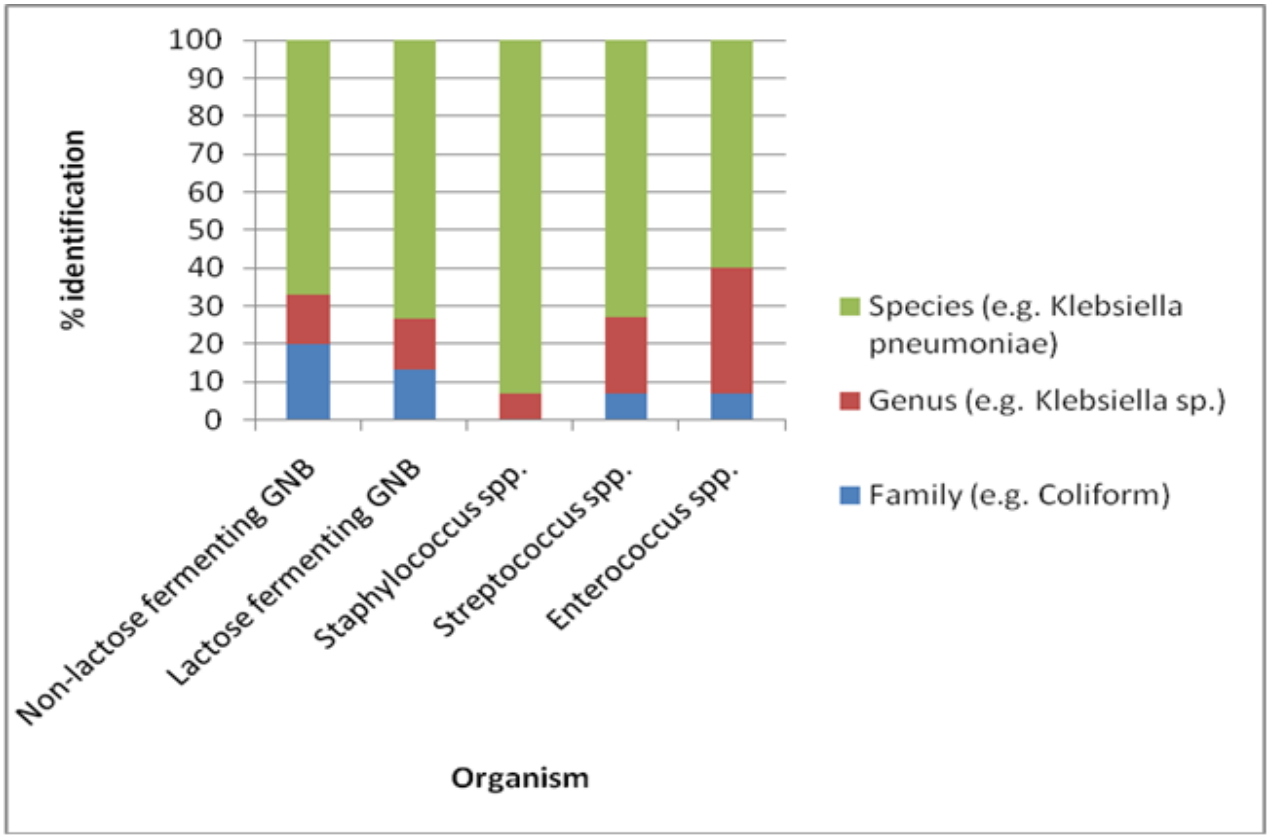


Figure 2.3 Identification of bacteria isolated from urine specimens by laboratories in the West Midlands (n=15)



2.4.3.1 Gram-negative bacilli (GNB)

Identification of all Gram-negative bacilli isolated from urine specimens to species level was performed by nine laboratories (Table 2.3). This was achieved by using chromogenic agar as the first line test followed by either the use of biochemical test strips (bioMérieux API® test system) or the automated VITEK 2® system (bioMérieux). Laboratories varied in the way they used chromogenic agar to identify bacteria isolated from urines, with five of the 13 laboratories using this medium to only identify *E. coli*. Seven laboratories used chromogenic agar to identify *E. coli*, *Proteus spp*., *Pseudomonas spp*. and *Klebsiella spp*.. Two of the laboratories using chromogenic agar for initial identification did not further identify bacteria that were not identified by the chromogenic agar and reported these as coliform bacteria.

Of the two laboratories culturing urine specimens on CLED agar and not subsequently using chromogenic agar for genus or species identification, one laboratory used colonial morphology on the CLED agar to identify a limited set of bacteria (e.g. *E . coli* and *Proteus sp*.) with non-identified bacteria reported as coliform (Table 2.2). The other laboratory using CLED agar only for the culture of uropathogens reported that they did not identify GNB routinely from urines and reported these bacteria as ‘coliform’.

Table 2.2 Identification of Gram-negative bacilli isolated from urines

Lab	Culture media	Chromogenic agar for ID	Identify isolates to species level?	Method(s) used for Identification
1	CLED	Yes	All	Chromogenic agar to ID *E.coli*, *Proteus sp*.. Biochemical test strip to ID others
2	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli*, VITEK 2®to ID others
3	CLED	Yes	All	Chromogenic agar to ID *E.coli*. VITEK 2® or biochemical strip to ID others
4	Chromogenic	Yes	Some	Chromogenic agar to ID *E.coli*, *Proteus sp*. and *Pseudomonas sp*. Other bacteria reported as coliform
5	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli*, *Proteus sp*. and *Pseudomonas sp*. Biochemical strip to ID others
6	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli*, *Proteus sp*.. VITEK 2® to ID others
7	CLED	No	None	Bacteria reported as coliforms. Only resistant bacteria are tested by VITEK 2®
8	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli,* VITEK 2® to ID others
9	Chromogenic	Yes	All	Chromogenic agar to ID E.coli, VITEK 2® to ID others
10	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli*, *Proteus mirabilis*. VITEK 2® to ID others
11	CLED	No	Some	Some bacteria reported at genus level by colonial morphology. Others reported as coliform. Only resistant bacteria sent for VITEK 2®
12	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli*, *Proteus sp*., *Klebsiella sp*.. VITEK 2® to ID others
13	CLED	Yes	All	Chromogenic agar to ID *E.coli*. C390 disc* to ID *P.aeruginosa*. VITEK 2® to ID others
14	Chromogenic	Yes	Some	Chromogenic agar to ID *E.coli*, *Proteus sp*.. Others reported as coliform unless resistant then VITEK 2® test for ID
15	Chromogenic	Yes	All	Chromogenic agar to ID *E.coli and Proteus sp.,* VITEK 2® to ID others

*C-390 disc (9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan) for identification of *P. aeruginosa*

2.4.3.2 Gram-positive cocci (GPC)

Members of the genus *Enterococcus*, *Staphylococcus* and *Streptococcus* are the most common Gram-positive cocci isolated from urine specimens submitted for microbial analysis (Laupland et al., 2007), and these were identified in West Midland laboratories by a combination of colonial morphology and enzyme tests (e.g. coagulase, DNAase etc.). Fourteen of the 15 laboratories indicated that members of the genus *Staphylococcus* were reported to species level. The majority of laboratories (11/15) indicated that members of the genus *Streptococcus* were reported to species level. Members of the genus *Enterococcus* were identified to species level by nine of 15 laboratories from urine culture in the region (Figure 2.2).

2.4.4 Antibiotic susceptibility testing methods for urinary isolates

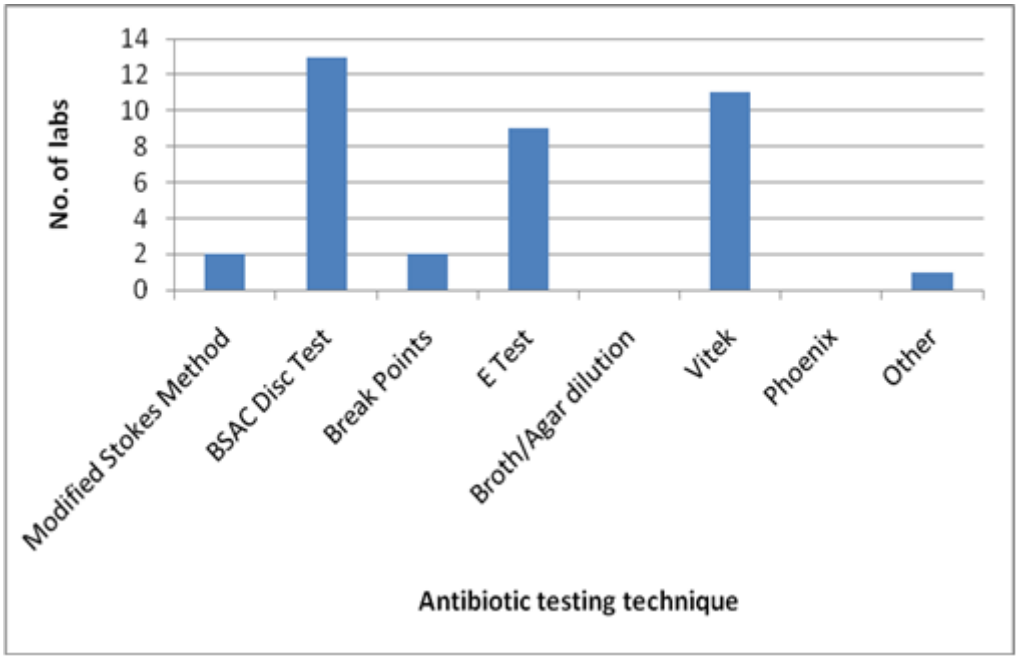
All 15 West Midland laboratories responded to questions related to antibiotic testing methods and panels of antibiotics used. Thirteen of the 15 laboratories reported using the standardised BSAC disc diffusion method for assessing the susceptibility of urinary isolates to antibiotics (Figure 2.4). Nine (9/13) of these laboratories used this method routinely for all or selected bacterial isolates from urine specimens. The BSAC method was used by 4/13 laboratories only in specific circumstances (e.g. out-of-hours working) or for specific organism / antibiotic combinations which may be problematic for other methods (Table 2.2).

As described previously, the VITEK 2® system was introduced into diagnostic microbiology the late 1990s. At the time of the survey 11 laboratories in the West

Midlands were in possession of one of these automated antibiotic susceptibility and bacterial identification testing devices (Figure 2.5).

The VITEK 2® device was used by 7/11 laboratories for testing the antibiotic susceptibility of bacteria isolated from urine specimens. Four of these laboratories processed the majority of bacteria isolated from urine specimens using the VITEK 2® device, with the only exceptions being the testing of the antibiotic piperacillin / tazobactam or for performing susceptibility tests on more fastidious bacteria isolated from urine, (e.g. alpha-haemolytic Streptococci) as VITEK 2® is not recommended for these tests or bacteria (Sader et al., 2006). The remaining 3 laboratories using VITEK 2® for urinary isolates selected bacteria for testing on this device based on a preliminary identification (Table 2.3). One laboratory routinely processed only GNB from urine samples on VITEK 2®, and one laboratory routinely processed only Staphylococci and Enterococci on VITEK 2®, with one laboratory using the VITEK 2® for bacteria provisionally identified as *Staphylococcus* spp., *Pseudomonas* spp. and coliform bacteria. The VITEK 2® system was not used for routine antibiotic susceptibility testing of urinary isolates in 4 of the 11 laboratories. These laboratories used the VITEK 2® system for specific isolates (e.g. those demonstrating resistance with other methods or those not identified by routine identification tests).

Figure 2.4 Methods(s) used to assess antibiotic susceptibility of bacterial pathogens isolated from urine specimens by laboratories in the West Midlands



The breakpoint susceptibility testing method was used by 2 laboratories in the West Midlands region. This technique was used for the majority of bacterial isolates from urine specimens in one laboratory, although this laboratory also reported using the Modified Stokes technique, E Tests or the VITEK 2® if additional antibiotics were required. The second laboratory using the breakpoint method also used the Modified Stokes technique as first line for *Enterococcus* spp., *Streptococci* spp. and *Pseudomonas aeruginosa*. Direct sensitivity testing on urine specimens was used routinely by 2 laboratories in the West Midlands region. One laboratory performed direct testing using the breakpoint technique and the other used the BSAC disc diffusion method. Etests® (bioMérieux) were used by 9 laboratories to verify abnormalities or test highly resistant bacteria. Some of the laboratories using the VITEK 2® device for bacterial isolates from urine, routinely used Etests® for testing piperacillin / tazobactam as VITEK 2® was not recommended for testing this antibiotic, as previously mentioned (Table 2.3).

Table 2.3 Antibiotic testing methods used to test bacteria isolated from urine samples in the West Midlands region

Laboratory	Direct sensitivity testing?	Primary Antibiotic testing techniques	E-tests used for bacteria isolates from urine
1	Yes	BSAC*	No
2	No	VITEK 2® (BSAC* for piperacillin / tazobactam and fastidious bacteria)	Yes (vancomycin and daptomycin)
3	No	VITEK 2® (BSAC* weekends only)	No
4	No	BSAC*	Yes (ertapenem, meropenem, vancomycin and/or teicoplanin)
5	No	BSAC*	Yes (mupirocin, ciprofloxacin, teicoplanin, vancomycin, ceftriaxone, penicillin)
6	No	VITEK 2® (BSAC* for piperacillin / tazobactam)	No
7	Yes	Breakpoint (occasionally Modified Stokes or VITEK 2® if additional antibiotics are requested)	Yes (piperacillin / tazobactam, but only occasionally on bacterial isolates from urine)
8	No	VITEK 2® for Gram negative bacteria, BSAC* Gram positive bacteria	Yes (piperacillin / tazobactam on ESBL producing bacteria)
9	No	VITEK 2® (occasionally BSAC*)	Yes (mostly carbapenems)
10	No	BSAC*	Yes (vancomycin and ertapenem)
11	No	Breakpoint for coliform bacteria, Modified Stokes for Enterococci, Streptococci, Pseudomonas spp., VITEK 2® for resistant coliforms / Enterococci and all Staphylococci	No
12	No	BSAC* (coliforms resistant to cefpodoxime or only one antibiotic reported as sensitive are tested on VITEK 2®)	No
13	No	VITEK 2® for all Staphylococci, Pseudomonads, and coliforms. BSAC* for Streptococci and Enterococci	Yes (piperacillin / tazobactam and ESBL confirmation)
14	No	BSAC (coliforms resistant to cefpodoxime are tested on VITEK 2®)	No
15	No	BSAC (non-*E.coli* or *Proteus sp*. or cefpodoxime /cefotaxime resistant are tested on VITEK 2®)	Yes (piperacillin / tazobactam if requested)

*British Society for Antimicrobial Chemotherapy (BSAC) disc susceptibility testing method

2.4.5 Rule based reporting

All laboratories responded to the question regarding whether the laboratory modified antibiotic susceptibility test results or reported antibiotic susceptibility based on defined 'expert' rules rather than using the results obtained from testing (Leclercq et al., 2013). The majority of West Midland laboratories (13/15) used logic rules defined in their Laboratory Information System (LIS) or within an automated testing device (i.e. VITEK 2®) to alter tested results or report untested antibiotics. These logic rules were based on; bacteria isolated (e.g. nitrofurantoin always reported resistant for *Proteus spp*.), results of other antibiotic tests (e.g. bacteria resistant to co-amoxiclav are also reported resistant to ampicillin), detection of resistance mechanisms (e.g. all bacteria suspected of producing intrinsic AmpC beta-lactamase, based on the results of other antibiotic susceptibility results, were reported as resistant to first, second and third-generation cephalosporins).

Of the two laboratories that indicated that they did not alter the reported antibiotic results by specific rules, one reported that their laboratory used their LIMS reporting tools to release, or suppress antibiotic results based on the organism isolated, or other antibiotic results. The second laboratory not using 'expert' rules indicated that results were potentially changed manually by medical microbiologists during the vetting of reports, based on organism or other antibiotic results.

2.5 Discussion

Ideally an antimicrobial surveillance scheme will obtain data that have been derived using standard methods (preferably a single methodology) to enable direct comparison of susceptibility testing results. It is evident from the survey of West Midland laboratories that a range of techniques and protocols are used for the microbiological analysis of urine specimens and the subsequent identification and antibiotic susceptibility testing of isolates.

The PHE publish UK Standard for Microbiology Investigations (SMIs) (Public Health England), which are a collection of recommended algorithms and procedures for laboratories to follow. These guidelines are not prescriptive; and some of the SMIs, such as the 'Investigation of urine', provide detail about a range of methods for undertaking various aspects of the analysis (Public Health England, 2016). One of the stated aims of the SMI's is to standardise the diagnostic process by helping to assure the equivalence of diagnostic investigations in UK laboratories (Public Health England); however, within these standard procedures there is still scope for laboratories to use different approaches. For example the 'Investigation of urine' SMI does not stipulate that species level identification should be undertaken for Enterobacteriaceae; although it does recommend the use of standardised susceptibility testing methods, which do recommend species identification (British Society for Antimicrobial Chemotherapy (BSAC)).

The results of this survey of laboratory methods show that although national guidance and protocols aimed at standardising methods were available in this period, they were not applied by all laboratories. Two West Midlands laboratories routinely

used the Modified Stokes method for testing bacteria isolated from urine, although guidelines available at the time of the survey (Health Protection Agency, 2008) and published research (Gosden et al, 1998) state that the Modified Stokes method is not recommended due to poor standardisation between laboratories. Standard method protocols for antibiotic susceptibility testing have to be strictly followed to maintain reliability and reproducibility; however 2 laboratories in the West Midlands report performing direct susceptibility testing on urine specimens, using BSAC disc diffusion or breakpoint methods. Susceptibility testing of antibiotics by inoculating agar plates directly with urine, rather than with bacteria grown overnight, was a popular technique in the 1990's as it improved turnaround times and was even suggested as a method for use in primary care settings (Scully et al., 1990). However as it is not possible to control the organism inoculum, these techniques are not recommended by standardised susceptibility testing method protocols.

A potential method for encouraging the use of standardised microbiological methods is through the laboratory accreditation process. All West Midlands laboratories are accredited by the Clinical Pathology Accreditation (CPA) service which is now part of the UK Accreditation Service (UKAS) (https://www.ukas.com/services/accreditation-services/clinical-pathology-accreditation/). Unfortunately for those involved in surveillance, CPA standards focus on the safe management of diagnostic laboratories and do not specifically prescribe the use of 'recommended' or 'standardised' laboratory methods.

All West Midlands laboratories take part in the national external quality control scheme managed by the UK National External Quality Assessment Service (NEQAS)

(http://www.ukneqas.org.uk/content/PageServer.asp?S=377166&C=1252&AID=16&IID=8). This quality control scheme sends 2 bacteria a month to laboratories for antibiotic susceptibility testing and scores laboratories based on the identification and susceptibility results. The aim of the scheme is to improve local and national testing standards and reveal areas of difficulty. It is possible that the processing of quality control (QC) specimens are prioritised by laboratories; however this scheme provides assurance that each laboratory in the region is reporting similar categorical susceptibility results for a range of bacteria and resistance mechanisms, and any potential variation is identified and notified to the laboratory. Although the NEQAS scheme does not promote 'recommended' testing methods, it does record the methods used by the laboratory and monitors the performance of these methods against the 'test' bacteria. In 2016 Public Health England approached UKAS and NEQAS to discuss the possible inclusion of PHE SMIs as benchmark methods when assessing laboratories (request by PHE AMR team leaders).

As intrinsic resistance varies between genera and species of bacteria, the identification of urinary isolates is necessary to enable surveillance systems to monitor emerging resistance at this level. Twenty years ago in the UK a significant proportion of laboratories did not identify Enterobacteriaceae accurately to species level, which led to difficulties in interpreting susceptibility test results (Livermore et al., 2001). Two factors seemed to have reversed this trend: 1) the adoption of standardised antibiotic susceptibility testing methods, including automated methods and 2) the introduction of chromogenic agar. Standardised antibiotic susceptibility testing methods such as the BSAC disc diffusion method requires laboratories to identify bacteria at species level to enable the interpretation of susceptibility test

results (British Society for Antimicrobial Chemotherapy (BSAC)). Automated devices such as the VITEK 2® include bacterial identification modules, and the 'expert' rules interpret antibiotic susceptibility results based partly on the bacterial identification (Ling et al, 2001).

Chromogenic agar has been shown to be effective for the identification of the majority of routine isolates from urine specimens (Fallon et al, 2003b). Although chromogenic agar is more expensive than standard media, such as CLED agar, it has been suggested that the additional cost can be off-set by the reduced requirement for additional tests and reduced labour time processing suspected pathogens (Perry and Freydiere, 2007).

In the West Midlands chromogenic agar is used by all but 2 of the 15 laboratories to aid identification of urinary isolates, with 10 laboratories using chromogenic agar as the primary urine culture medium. However one of the laboratories using chromogenic agar for primary urine culture reported that they may stop using this media due to increased cost pressures. It is encouraging that 9 of the 15 laboratories identified all GNB urinary isolates to species level and only 1 laboratory reported all of their Enterobacteriaceae isolates from urine specimens as coliform bacteria.

Thirteen of the 15 laboratories in the West Midlands reported that they use the BSAC standardised disc diffusion method for some or all of their susceptibility testing. Although this method has been reported as being a reliable and reproducible technique for determining antibiotic susceptibility, it is mostly dependent on manual laboratory procedures. Biological variation such as the genetic background or metabolic state of the bacteria can influence results; however inoculum preparation

and manual plate streaking account for 6.8%-24.8 and 6.6%-24.3%, respectively, of the total imprecision of the test (Hombach et al., 2016).

Removing manual processes with automation will improve the precision and reproducibility of susceptibility testing. The introduction of the VITEK 2® automated identification and antimicrobial testing device has had an impact on laboratory antibiotic susceptibility testing practice in the West Midlands. For the laboratories that have acquired these devices in the West Midlands, there is variation between laboratories in the way they are used. Four of the 11 laboratories with a VITEK 2® do not use this device for routine bacterial isolates from urine. The higher cost of VITEK 2® tests and some technical limitations, such as difficulties with fastidious bacteria and testing some antibiotics, leads laboratories to employ other techniques for the determination of antibiotic susceptibility for high volume specimen types such as urine, or for particular classes of bacteria.

The introduction of automated testing systems provides an opportunity to improve the standardisation of methods between laboratories and can greatly increase the range of tested antibiotics. Greater standardisation of methods between laboratories will improve the quality of routine surveillance data and the increased range of antibiotics tested first-line will reduce the bias introduced by only testing second-line antibiotics against more resistant bacteria. Automated testing systems also often determine minimum inhibitory concentrations (MICs) which can be captured by routine surveillance systems to provide a measure of emerging antibiotic resistance and indicate potential resistance mechanisms (Jorgensen & Ferraro, 2009).

The number of urines tested by individual West Midland laboratories varies considerably, which is related to the size of the hospital/s served, the case-mix of patients and the geographical area covered. Considering the different urban/rural mix, size of the hospital Trusts served and varying community populations for the 13 general diagnostic laboratories, the proportion of their urine specimen requests received from community settings was consistently around half of their total requests for each year of the study.

To be able to interpret AMR surveillance data and monitor changes in resistance to specific antibiotics, results that are reported by 'expert' rules rather than actual laboratory tests needs to be identified within the dataset. Expert rules describe how results should be interpreted and reported based on the results of other specified antibiotics. They are based on a mixture of clinical and microbiological evidence (Leclercq et al, 2013). Rules that change results or report untested antibiotics are employed by most laboratories in the West Midlands (13/15). These are managed either by the automated testing device (e.g. VITEK 2®) or by the Laboratory Information Management System (LIMS). As part of this survey of laboratory methods we obtained details of these rules from West Midland laboratories and these will inform the analysis and interpretation of AMR surveillance data later in this thesis (Chapters 3, 5 and 6). For example, most West Midlands laboratories reported having a rule to change nitrofurantoin susceptible results for *Proteus* spp. to non-susceptible due to the intrinsic resistance of this organism. As a result of this finding, this antibiotic can be excluded from the analysis of *Proteus* spp. non-susceptibility trends.

In order to interpret antibiotic susceptibility tests reported by different laboratories, it is important that clinically relevant and up-to-date clinical breakpoints are applied. The original breakpoint standards provided to laboratories in the 1970s were based on frequency distributions to separate resistant and susceptible phenotypes. In 1991 BSAC published more accurate breakpoints based on the pharmacokinetics of the individual antibiotics rather than microbiological characterisation (British Society for Antimicrobial Chemotherapy (BSAC), 1991). Between 2002 and 2008 there were incremental steps to harmonise European breakpoints standards, resulting in the first comprehensive EUCAST published standards in 2008 being incorporated in the annually updated BSAC breakpoints (Wootton et al, 2017). Thirteen of the 15 West Midlands laboratories reported using the latest published EUCAST or BSAC breakpoint standards, with 2 laboratories using the previous year's version of the BSAC breakpoint standards. None of the West Midlands laboratories reported using Clinical and Laboratory Standards Institute (CLSI) breakpoint standards developed in the USA. Although antibiotic breakpoint standards have now been harmonised across Europe, there is still work on-going to harmonise with other international standards. CLSI and EUCAST breakpoints have differed for some antibiotics which has led to discrepancies in interpreting antibiotics such as co-amoxiclav, and cephalosporins (Delgado-Valverde et al., 2017;Hombach et al., 2012).

There are a number of limitations within this survey. The design of the initial on-line survey was an attempt to achieve a balance between 1) obtaining a detailed picture of the methods and protocols employed by individual laboratories in the West Midlands for identifying bacteria from urine and performing antibiotic susceptibility tests, and 2) keeping the survey short to reduce the burden on busy laboratory staff

and encourage completion. Therefore detailed methodologies for each individual laboratory were not captured by the on-line survey. However, the relationships formed with laboratories during this period enabled a dialogue regarding the survey results and provided an opportunity to follow-up the on-line survey with questions to complete missing information and enrich the data. For example the initial survey did not specifically ask about methods used to culture bacteria from urines; however the responses indicated that some laboratories may use media that is able to identify common urinary pathogens (chromogenic media) as their primary urine culture agar.

Following completion of this survey of laboratories in 2011, the introduction of Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometers have provided laboratories with a cost-effective, fast and accurate method of identifying most bacteria isolated from clinical specimens (Carbonnelle et al., 2011). The introduction of these devices will aid the interpretation of antibiotic test results within the laboratory by providing species level identification and strengthen the ability of routine surveillance to monitor changes in resistance between species.

In summary, this survey has shown that diagnostic microbiology laboratories in the West Midlands vary considerably in the methods used to identify bacteria isolated from urine specimens, the techniques to determine antibiotic susceptibility and the range of antibiotics tested. All West Midlands laboratories are CPA (UKAS) accredited and partake in an internationally recognised quality control scheme. Although these schemes assess safe practice and accuracy of results, they do not stipulate specific methods employed by laboratories. With new technologies emerging, such as the MALDI-TOF for bacterial identification, and new breakpoint

standards being released annually, the protocols employed by laboratories are subject to constant change or updates. This survey of laboratory methods represents a snapshot of the methods used in 2011. Therefore, although an understanding of the variation in laboratory practice in this period will help inform the interpretation of routine antimicrobial resistance surveillance data and enable comparison of AMR between laboratories for this study, it is important that regular surveys of laboratory methods are undertaken to support the on-going interpretation of routine AMR surveillance data.

3 Implementation of AmSurv and development of web-based reporting tools for the surveillance of antimicrobial resistance

3.1 Background

3.1.1 AmSurv

The concept for a routine antimicrobial surveillance system was first suggested in the late 1990s by the Public Health Laboratory Service, which was incorporated into the Health Protection Agency (HPA) in 2005. A prototype modular database was developed and given the name AmSurv. In the following years a national AmSurv database was specified and a software company was tasked to build the application. In 2005 during the final stage of development, the software company involved went in to liquidation and it was not possible for PHE to acquire the intellectual property rights (IPR) for the application. A PHE project team was established, including representatives from the West Midlands, and a new AmSurv functional specification was developed. A new software company was appointed to develop the system in 2006.

AmSurv was launched by the HPA in 2009 to facilitate the collection of antimicrobial susceptibility reports for all bacterial isolates tested in participating laboratories, including those from community samples. The surveillance system was made available to all nine HPA Regional Epidemiology Units (REUs) in 2009. As part of this PhD study, the implementation of AmSurv was prioritised and piloted in the West Midlands region.

To commence this study in 2009, all 15 West Midland laboratories were visited and the plan to implement AMR surveillance was discussed. All laboratories in the West Midlands agreed to send data to the system; although some of their Laboratory Information Management Systems (LIMS) would require modification in order to

produce the required outputs. Laboratory codes for data entities such as: organism, antibiotic, specimen type, hospital site and GP were collected from each laboratory and were mapped to standard national codes provided for use by the AmSurv system. During these discussions, the West Midland Consultant Microbiologists expressed their interest in AMR surveillance and requested that the data collated from their laboratories be made available for analysis by laboratory and Trust information specialists. The AmSurv application has in-built defined reports; however these were viewed as inadequate to meet the laboratories surveillance requirements as they were only available to designated HPA information specialists with access to the regional database server.

3.1.2 AmWeb

West Midland microbiologists and Trust infection control teams required access to AMR surveillance data to enable the monitoring of AMR in their populations and allow them to benchmark AMR between laboratories and hospitals. LIMS primary functions were to facilitate entering laboratory results and for sending reports back to test requesters. The technology used to build these applications has changed little since the 1980s, with most LIMS built on non-relational database platforms. The reporting tools provided within LIMS are often limited or complex, and therefore laboratory scientists and microbiologists expressed that they had experienced difficulty viewing data for their own hospitals /Trusts. Following discussions with laboratories in the region it became apparent that to ensure participation in a regional routine AMR surveillance system, a mechanism was required to allow microbiologists

easy access to regional AMR data. It was therefore decided to initiate the development of AmWeb, a web-enabled reporting tool to allow laboratories and other health professionals in the region to analyse and review local data which is electronically submitted to the regional AmSurv server and compare this with AMR data from laboratories across the region.

3.1.3 Community AMR web bulletin

AmWeb was primarily focused on providing access to AMR surveillance data to laboratories and NHS Trusts and therefore it was not made directly available to prescribers in the community. The majority of antimicrobial prescribing occurs in community settings (Public Health England, 2014), with much of this being empirical prescribing, as microbiological data are often not available at the time of consultation (McNulty and Francis, 2010).

AmSurv collates AMR data from both community and hospital patients, and therefore providing access to local AMR data for healthcare professionals in the community may help inform prudent antibiotic prescribing. As part of this study, a regional AMR bulletin was developed for primary care antibiotic prescribers. This bulletin aimed to raise awareness of AMR in the community, and provide antibiotic susceptibility data at local geographical areas.

3.1.4 AMR surveillance systems outside England

When planning the implementation of routine AMR surveillance in the West Midlands, a review of systems in the UK, Europe and the USA was undertaken to compare techniques and technologies used.

In 1999 NHS Wales initiated a project to collate routine microbiology data from all laboratories in Wales using a database application called Datastore (NHS Wales, 2002). This application has been used to produce annual AMR national reports. A plan to build a web application by 2018 has been published, which will enable hospital and community health professionals to interrogate local AMR data using the data collated by Datastore (NHS Wales, 2016). In Scotland each laboratory was provided with a VITEK® automated susceptibility testing system to standardise susceptibility testing and data collated for the EARS-Net European surveillance network (https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net). Each laboratory in Scotland was also asked to send AMR data to a central database for 400 isolates from urine specimens each month (Health Protection Scotland, 2011). The analysis from these data are included in the SAPG Annual report on Antimicrobial Use and Resistance (http://www.isdscotland.org/Health-topics/Prescribing-and-Medicines/SAPG/AMR-Annual-Report/).

A number of countries have sentinel surveillance schemes for AMR. In Germany a sentinel laboratory-based Antibiotic Resistance Surveillance (ARS) system collects electronic reports of all clinically relevant bacterial pathogens from healthcare providers in 9 of the 16 federal states in Germany (as of 2011) with access to reports

via a web portal (Schweickert et al., 2011). In France the focus of AMR surveillance is based on a sentinel scheme for infections from an animal origin. (https://www.anses.fr/en/content/resapath-french-surveillance-network-antimicrobial-resistance-pathogenic-bacteria-animal-0). Similarly, in the USA a national scheme for AMR focuses on foodborne bacterial infections (Karp et al., 2017).

3.2 Objectives

The objective of the work detailed in this chapter was to complete the implementation of the AmSurv AMR surveillance system within the West Midlands. Simultaneously web-based reporting tools will be developed to provide epidemiologists, microbiologists, infection control teams and community prescriber's access to regional AMR surveillance data. These surveillance tools should be designed to allow users to monitor the emergence and spread of AMR within local and regional settings, inform the development of local prescribing formularies and to provide an incentive for continued participation in the voluntary surveillance scheme.

3.3 Methods

3.3.1 Population studied

The study included all residents receiving healthcare in the West Midlands region of England, which is described in Chapter 1.

3.3.2 AmSurv system and data sources

There were 15 diagnostic microbiology laboratories in the West Midlands region serving primary and secondary healthcare settings during the study period. Six different LIMS were in operation across the region, with each individual laboratory using a range of bespoke codes for recording data items including antibiotic susceptibility test results. In the 12 months prior to receiving the release version of the AmSurv software, code tables for bacteria, antibiotics, GPs and requesting locations were requested from each of the 15 laboratories. Code mappings were created for each of the individual code tables to translate bespoke laboratory codes to NHS Organisation Data Service (ODS) codes (NHS Connecting for Health, 2012) where available, or if not available then translations were made to standard HPA codes. In 2016 ODS was incorporated into the new NHS Digital service and this body is now responsible for maintaining these codes. These translation tables were inserted into HPA software called LabLink+ which managed the standard AMR text files generated by each laboratory using LIMS reporting tools. LabLink+ software reformatted these files and applied the created translation tables to produce nationally standardised AMR report files.

The HPA CoSurv database application installed in every laboratory (LabMod3) for the delivery of communicable disease reports (CDR) was adapted to also deliver the AMR files to the REU. These files were encrypted and emailed weekly using semi-automated batch routines to the AmSurv database at the REU (Figure 3.1).

The AmSurv files included the following: the organism isolated, antibiotic susceptibility interpretation (i.e. Susceptible/Resistant/Intermediate), MIC value

(where available), patient identifier, date of birth, gender, patient postcode, requesting source (community or hospital), specimen type, specimen date and the medical specialty of the doctor who submitted the specimen to the laboratory.

3.3.3 Data validation

Each file produced by LabLink+ software was assigned a unique rolling check-digit. This check-digit was used to ensure files were received in sequence from each individual laboratory. Laboratories were informed of missing files and asked to resend. Laboratory reports received were checked for completeness of data items and correct coding using the AmSurv import/validation processing. These were a set of ETL (extraction translation and loading) processes developed using Microsoft SQL Server Integration Services (SSIS). Reports failing data validation were held in 'quarantine' until the sending laboratory was contacted to attain the missing data items/codes translations (Figure 3.2). The loading processes included de-duplication routines that removed exact duplicates (i.e. same patient, same specimen number with matching results) and appended any changes in results to existing records in the database. The AmSurv module included some reporting tools; however, the reports were limited in scope and could only be accessed by HPA information managers. They were also designed in the 1990s for a prototype AMR surveillance application, and therefore did not include changes in NHS boundaries and organisational entities.

3.3.4 Development of AmWeb

A functional specification was developed for a web-based AMR reporting tool based on consultations with microbiologists and NHS Trust infection control teams. The specification was delivered to a software development company (RADAS Ltd.) in April 2011. A technical specification was agreed and indicative costs provided. A business case was developed to obtain funding and this was presented to the HPA Regional Management Team. Funding was formally agreed in July 2011. The application was delivered in October 2011 and historic AMR data collated by the AmSurv application since its launch in 2009 were migrated into the AmWeb database.

On completion of the application, User Acceptance Testing (UAT) was undertaken. The UAT process involved systematically querying the AmSurv database directly using the Transact SQL language. A wide range of drug/bug and tabular queries were applied directly to the AmSurv database. These queries were then recreated in the reporting application of AmWeb. The results from each of the query tools were compared for consistency and accuracy. Following successful completion of the UAT, a four week pilot was initiated in two West Midland laboratories. Eight microbiologists from the two laboratories were enrolled to undertake the pilot. On completion of the pilot the web application was signed-off and rolled-out to all laboratories in the region in January 2012.

3.3.4.1 AmWeb design objectives

- To develop a web based database application that is capable of hosting regional AmSurv data
- To automate the extraction of data from the regional AmSurv database, apply a 14 day episode length and remove all personal identifiable information (PII)
- To automate the secure delivery of processed AmSurv data to the AmWeb application hosted on the HPA West Midlands web server
- To enable the management of user privileges and log-in via maintenance screens accessible only to designated system administrators
- To provide reporting tools that enable users to define drug/bug combinations and produce graphical reports over a defined period of time
- To enable users to specify the content of antibiotic panels, and produce tabular reports using these panels against named bacteria over a defined period of time
- To enable all reports to be aggregated and viewed by Local Authority/ Primary Care Trust geographical boundaries or by reporting laboratories

Figure 3.1 AmSurv Data collection flow chart

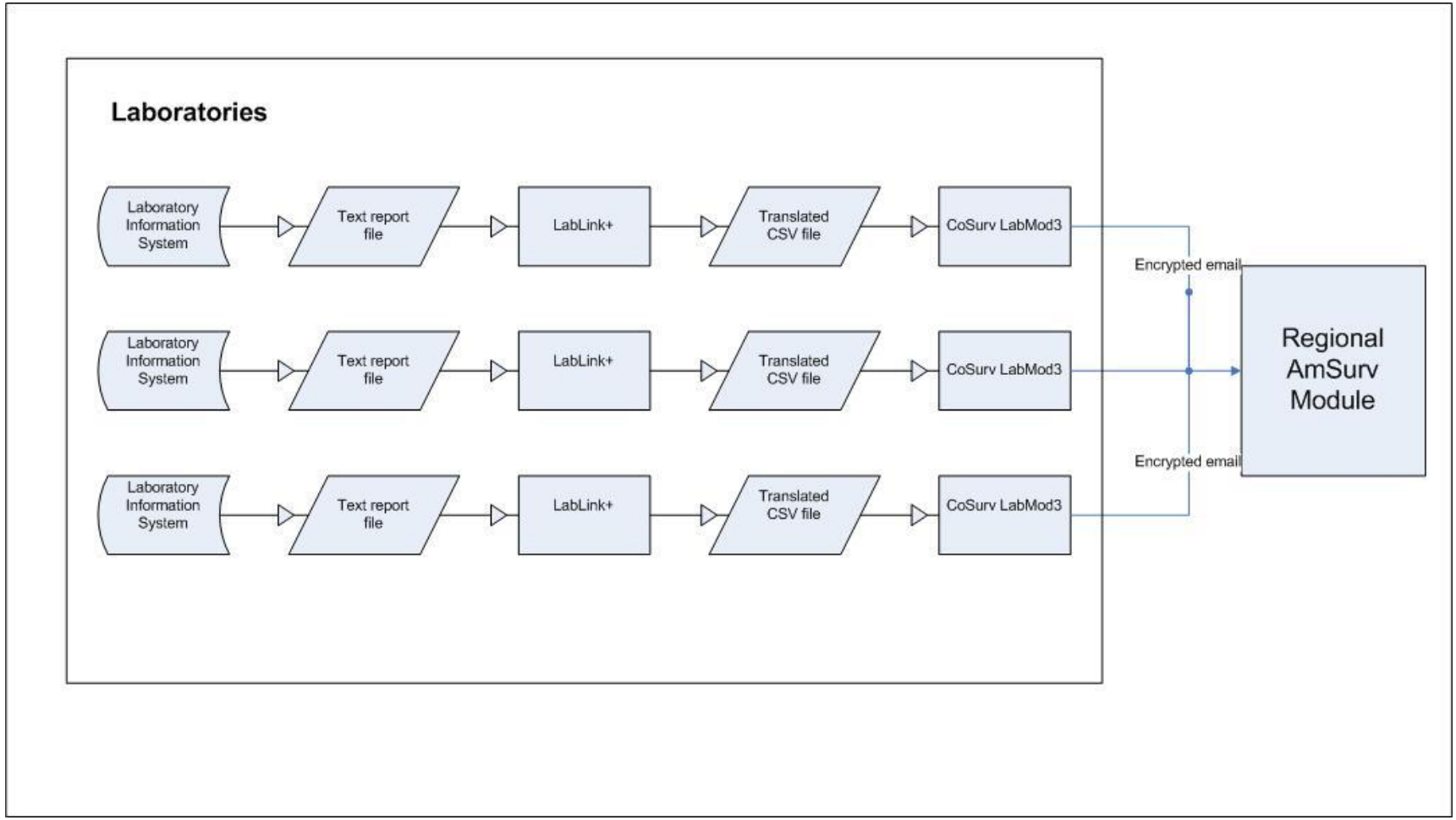
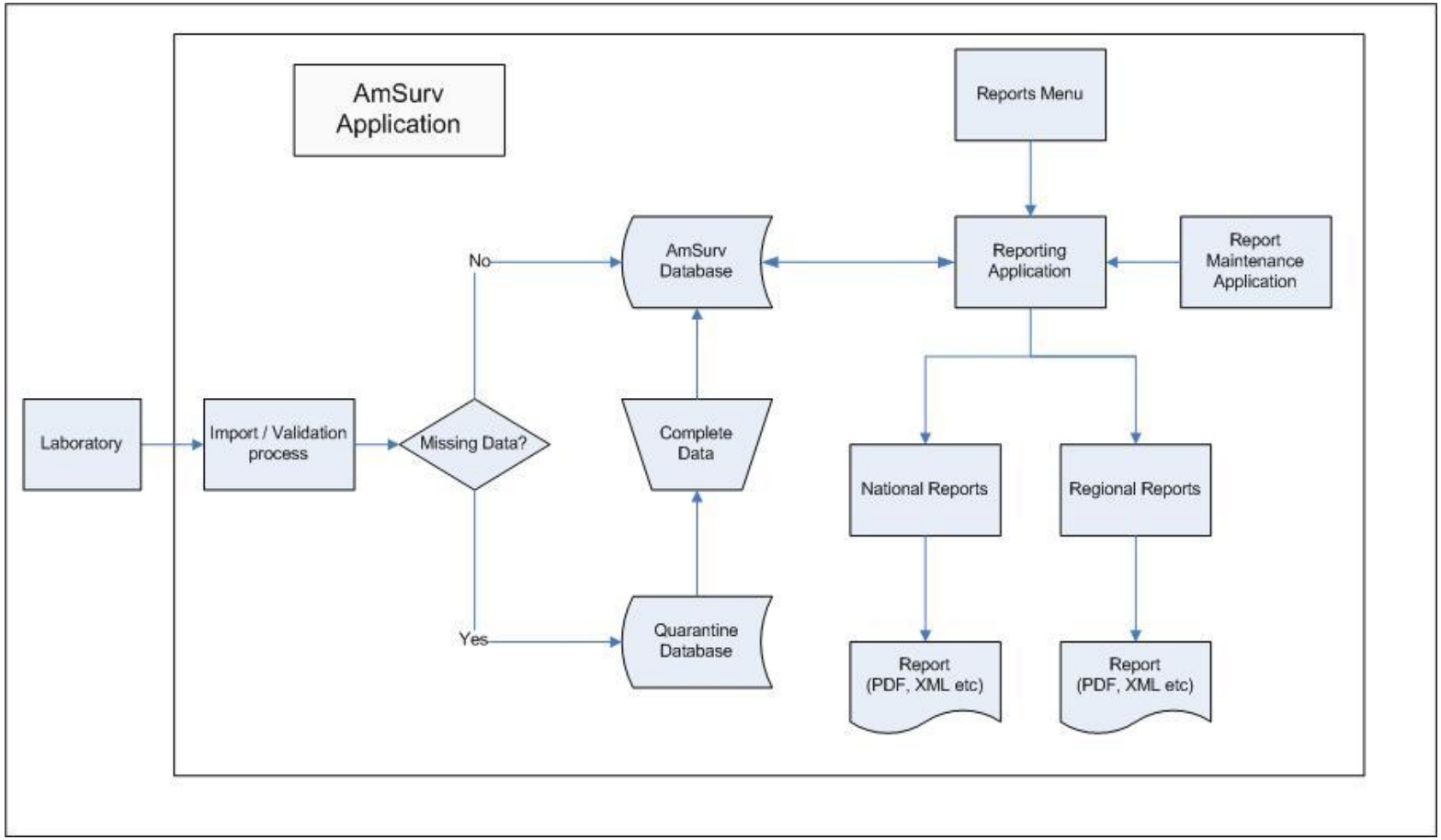


Figure 3.2 AmSurv process map

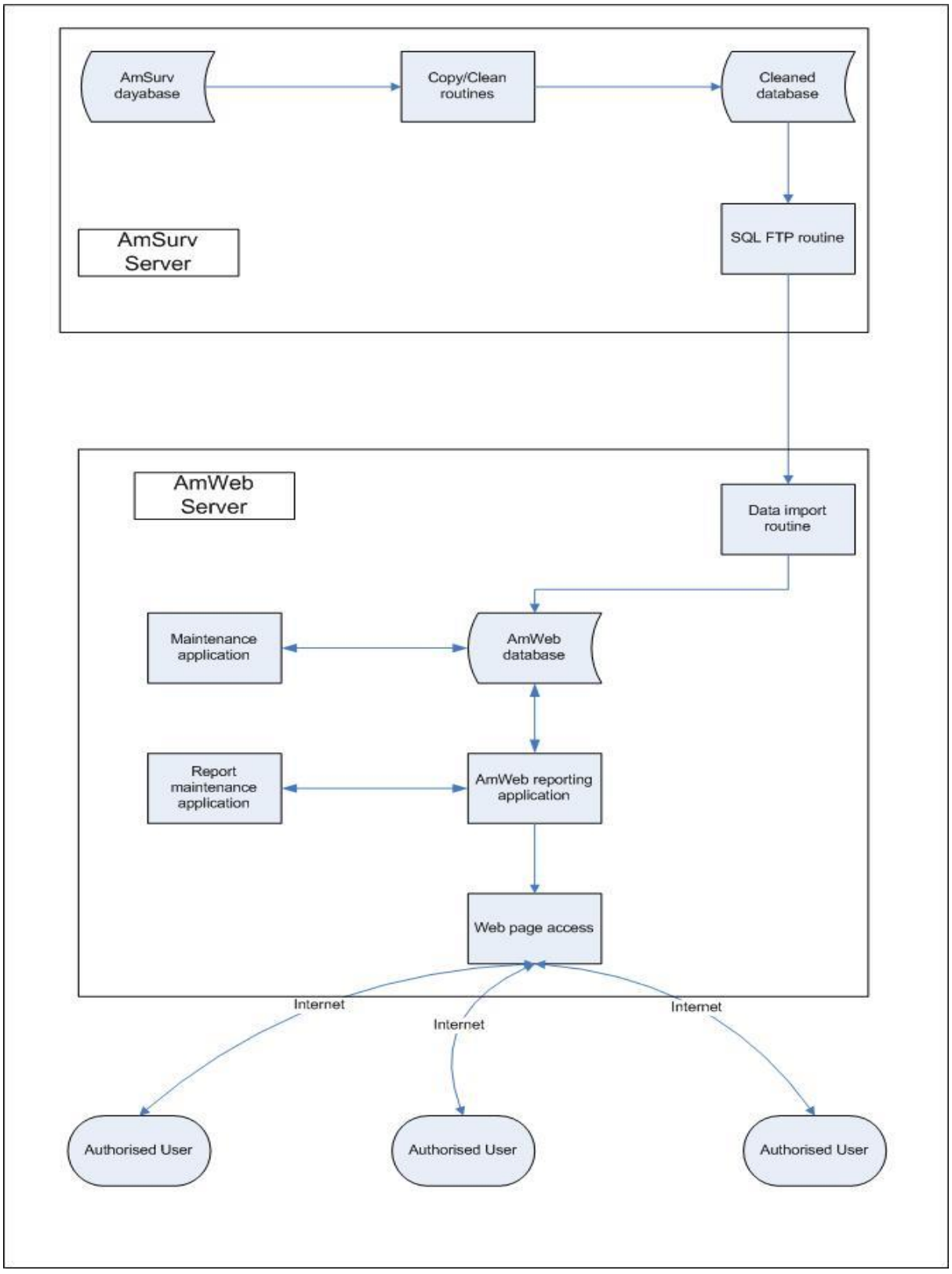


3.3.4.2 AmWeb Architecture and Processes

The extraction, cleaning and secure transfer of AMR data from the AmSurv database to the AmWeb application was automated on a weekly schedule. The application included management tools that controlled internet access via set user permissions and log-ins. The AmWeb application ran on Microsoft.Net Framework 2.0 and its database was driven using SQL Server 2005. The AmWeb application was built using Microsoft's Visual Studio 2008 in VB.NET and ASP.NET.

The processing commenced with a copy of the regional AmSurv database being created using scheduled SQL Server routines that removed subsequent specimens from the same patient and specimen type, with matching results, within a 14 day episode length (based on specimen date) from the copied database (Figure 3.3). The fields used for matching and de-duplicating records were as follows; Laboratory ID, Patient ID, Patient NHS No, Patient date of birth, Patient Postcode, Organism, Antibiotic, Antibiotic Result, Specimen, Specimen date, Specimen Source Location and Medical Speciality. Once duplicate episodes had been excluded, the records were anonymised by removing Patient ID, NHS number, Date of Birth and Postcode data from the copied database. A scheduled output file was created using Microsoft SQL Server Integration Services (SSIS). The export application polled for a suitable file every 15 minutes and when found used secure File Transfer Protocol (FTP) to transmit the file to a designated directory on the AmWeb server. In the AmWeb application an import routine built in VB.net ran continually as a Microsoft Windows service. The import directory was polled every 15 minutes for new files. When a new file was found the application inserted the data into the AmWeb database using SQL database transaction routines.

Figure 3.3 AmWeb Process Map



3.3.4.3 User defined reports

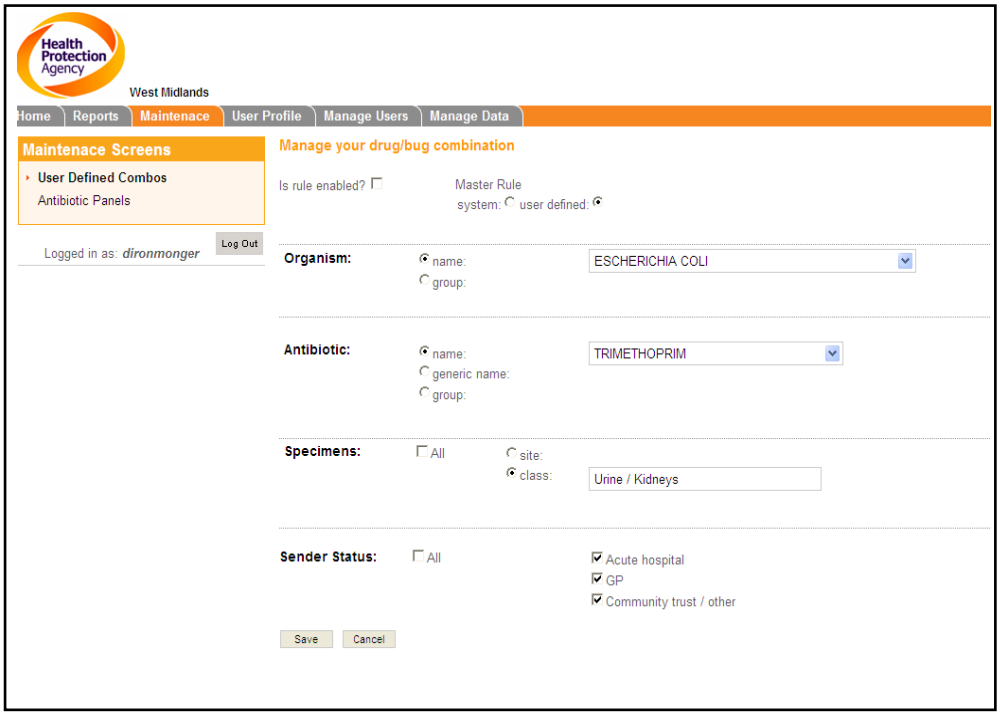
Microsoft SQL Server 2005 Reporting Services (SSRS) were used to extract data from the SQL Server database and provide graphical and tabular outputs. Reports were viewed using a Report Viewer Control which is embedded into the web application.

Two report types were developed: drug/bug combinations and tabular reports. For drug/bug reports a maintenance screen allowed users to select antibiotic or antibiotic group versus an organism or organism group. Additionally the reports could be filtered on specimen types or groups of specimens (e.g. all lower respiratory specimens) and the status of the sender, that is acute hospital, GP or community hospital (Figure 3.4 (a)). The reports could be saved to the account of the user and an option was provided for the report to be included or excluded when reports were next run.

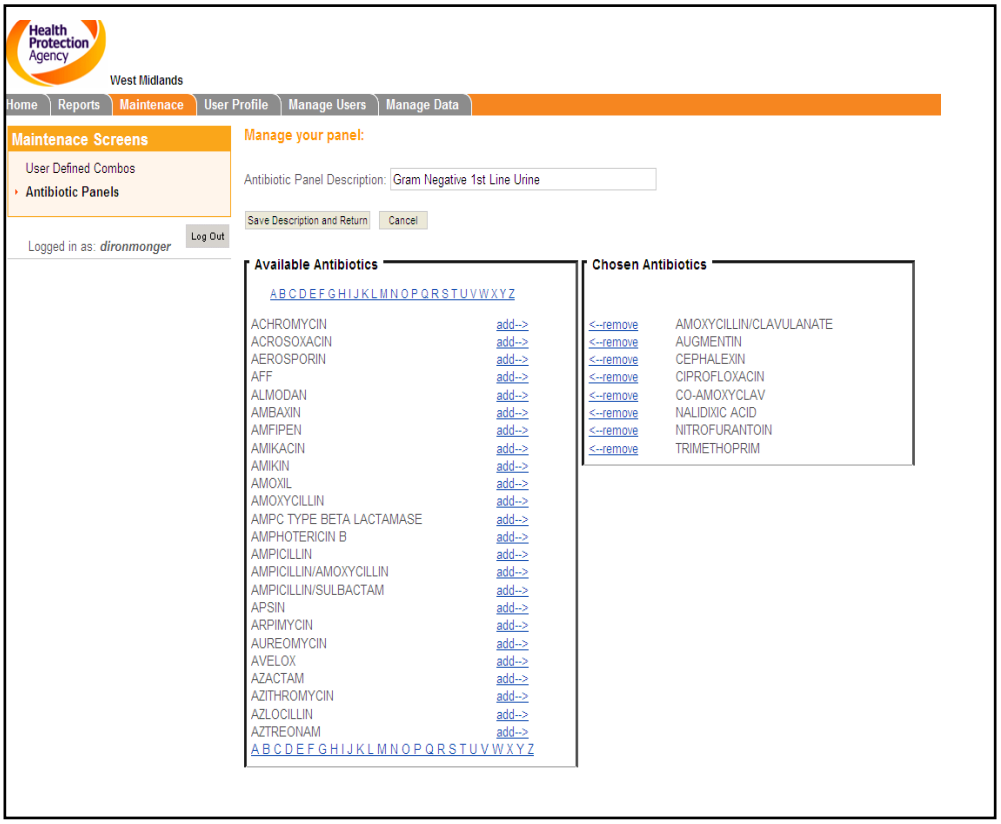
For tabular reports another maintenance screen allowed users to create an antibiotic panel to be included in the report (Figure 3.4 (b)). These antibiotic panels could be created by selecting from a complete list of available antibiotics and allowed users to create antibiotic panels appropriate for the treatment of specific bacteria (e.g. MRSA panels) or infection types (e.g. urinary tract infection panels). These antibiotic panels were saved to the users' account and could be retrieved at any time for editing or deletion. When running a tabular report the user selected the antibiotic panel to include (which could be user-defined or standard system panels), the organism or organism group, the specimen type or specimen group, the gender of the patients, patient age ranges and the date range for the report.

Figure 3.4 AmWeb report maintenance screens*(screen shots from application)*

a) Drug / bug reports



b) *Tabular reports*



The ‘run reports menu’ enabled users to select either a tabular or drug/bug report type and allowed these reports to be viewed by; Hospital Trust, Reporting Laboratory, Local Government Authority or Primary Care Trust (replaced by Clinical Commissioning Group, CCG, geographical boundaries in April 2013). Within each of these categories individual or groups of organisations (e.g. laboratories or NHS Trusts) or geographical boundaries (e.g. Local Authorities) were available for selection by application users.

3.3.5 Community AMR web bulletin

With the objective of increasing the availability of local AMR information to clinicians in primary care settings in the West Midlands, a regional AMR Focus Group was established, comprising microbiologists, pharmacists and epidemiologists within the West Midlands. The group met just once and was tasked to guide the development of surveillance outputs to meet the needs of the local community. It was agreed that a Community AMR bulletin should be developed, and the regional focus group advised on the antimicrobial resistance trends that should be incorporated in the bulletin (see section 3.4.6).

A small number of randomly selected GPs were approached in November 2012 to pilot the first community AMR bulletin. Meetings were held in two practices to discuss the desired format and content required by local antibiotic prescribers. From the verbal feedback received it was agreed that the bulletin should be produced quarterly and provide headline summaries for common bacteria and antibiotics seen in the community, with links from the front page to provide more detailed analysis by local

geographies. It was decided that to deliver these requirements a web formatted bulletin should be built, hosted by the HPA regional epidemiology unit web server.

Data for the bulletin was obtained using the Microsoft Structured Query Language (T-SQL), to extract information from the regional AMR database (AmSurv) on a quarterly basis. Templates were created in Microsoft Excel to create the charts for each of the antibiotic and organism combinations. The web pages were developed using the Dreamweaver (Adobe, California USA) web development application.

To minimise selective testing bias, only antibiotic susceptibility test results were included in the analysis from a local laboratory where at least 70% of the bacteria are tested against the antibiotic being assessed. Initially susceptibility results were viewed by Local Authority geographical boundaries; however with the introduction of Clinical Commissioning Groups (CCGs) the bulletin was changed to show AMR by West Midland CCG boundaries. Reports were assigned to local area boundaries using an algorithm that assigned location using the postcode of the GP practice requesting the specimen. For the infrequent occasions where a report was missing a GP practice code, then patient postcode, or as a last resort the reporting laboratory postcode would be assigned.

During pilot sessions with GP practices, requests were received to include a printable version of the bulletin. A PDF version was made available and this was linked to a button on the home page of the web bulletin that printed this version. Also following feedback from these sessions, links to other internet community prescribing guidance resources and a warning regarding interpreting complex AMR surveillance data was added on the front page of the bulletin.

The inaugural ‘West Midlands Community Antimicrobial Resistance Bulletin’ was distributed in February 2013 by incorporating a link to the bulletin within an introductory email. Emails were sent to GP practice managers, microbiologists and pharmacists across the region. The first bulletin contained a message for GP prescribers from the head of the national Antimicrobial Resistance and Healthcare Associated Infection (AMRHAI) reference unit, Professor Neil Woodford, describing the dangers of AMR and how prudent antibiotic prescribing in the community can help reduce the selection and spread of resistant bacteria.

3.3.5.1 Community bulletin user survey

A short survey of users of the bulletin was undertaken in September 2016 using the on-line survey tool Select Survey (Classapps, KS, USA). The purpose of the survey was to assess the following: if users found the bulletin relevant to their practice, if the content and format was appropriate to their needs, and if it had influenced their prescribing habits.

The request to complete the survey, with a link to the web page, was sent with the email notification to GP practices informing that a new quarterly bulletin was available for viewing. Free text comments were analysed thematically.

3.4 Results

3.4.1 AMR reporting volumes

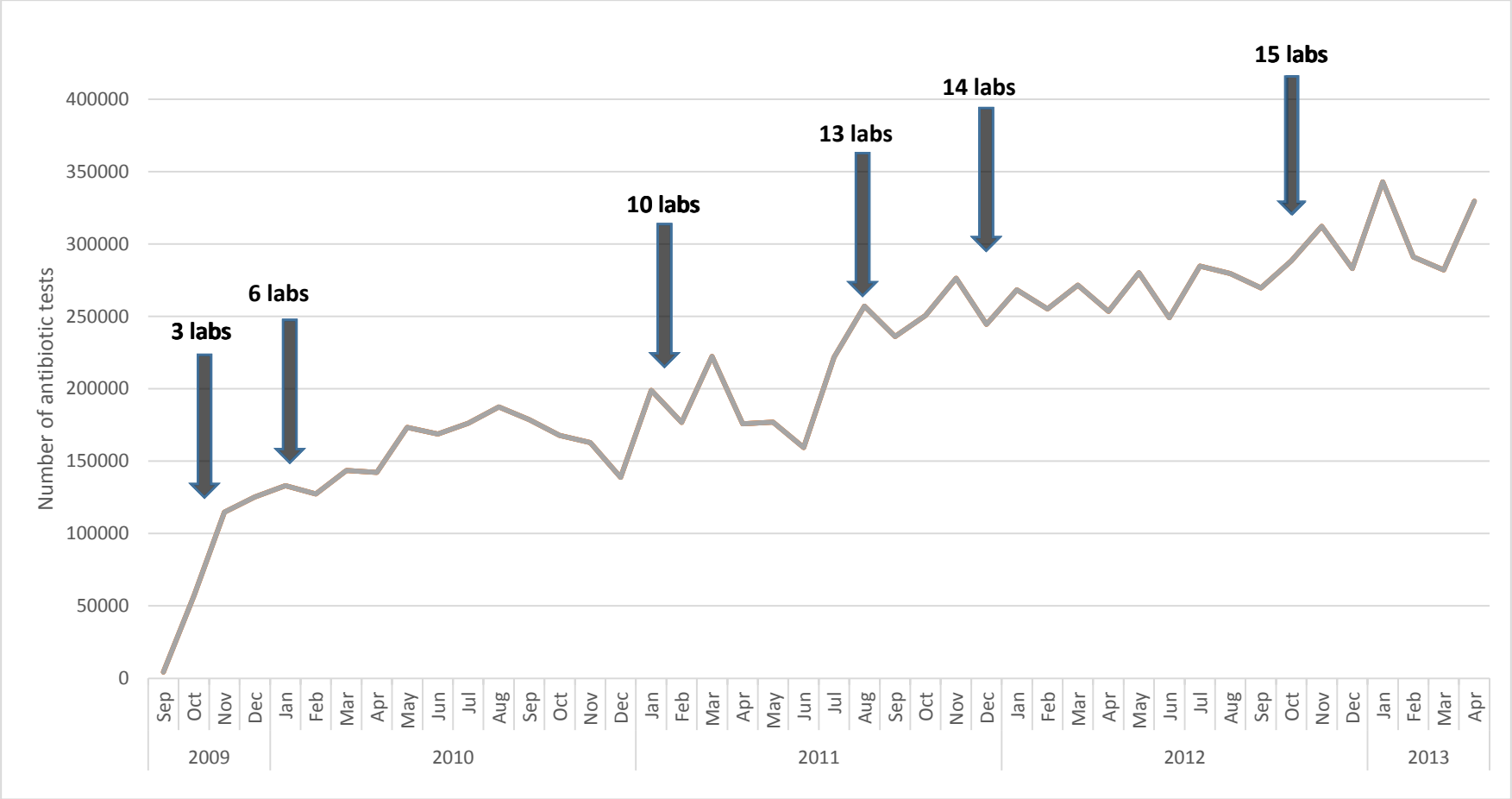
The first laboratory was configured for reporting to AmSurv in October 2009, with all laboratories reporting by 2012. The larger regional laboratories were prioritised for configuration. Monthly reports of individual antimicrobial susceptibility tests (ASTs) rose from 120,000 per month in November 2009, when three laboratories were reporting, to approximately 320,000 per month November 2012 when all fifteen laboratories were reporting. In January 2013, there were 10 million individual records of antimicrobial susceptibility tests captured in the database (Figure 3.5).

With all laboratories in the region reporting in 2012, an average of 40,000 bacterial isolate reports were received each month by the REU, ranging from 40 isolates / month from smaller specialist laboratories to 4,000 isolates / month from the larger laboratories.

Although the AmSurv database application was released across England in 2009, in 2012 AMR surveillance was not a high priority within the HPA, and with resourcing issues in some PHE regions, the national AmSurv reporting levels from laboratories in England (excluding the West Midlands) was less than 30%. Therefore it was decided that there were not sufficient levels of coverage to allow valid and representative national comparisons at the time of this study.

In the following section the usefulness of the AmWeb web surveillance application will be described by using specific case studies.

Figure 3.5 Number of antibiotic tests reported to AmSurv in the West Midlands, with arrows indicating the total number of laboratories enrolled by specific dates (n=15). September 2009 – April 2013



3.4.2 AmWeb case study A

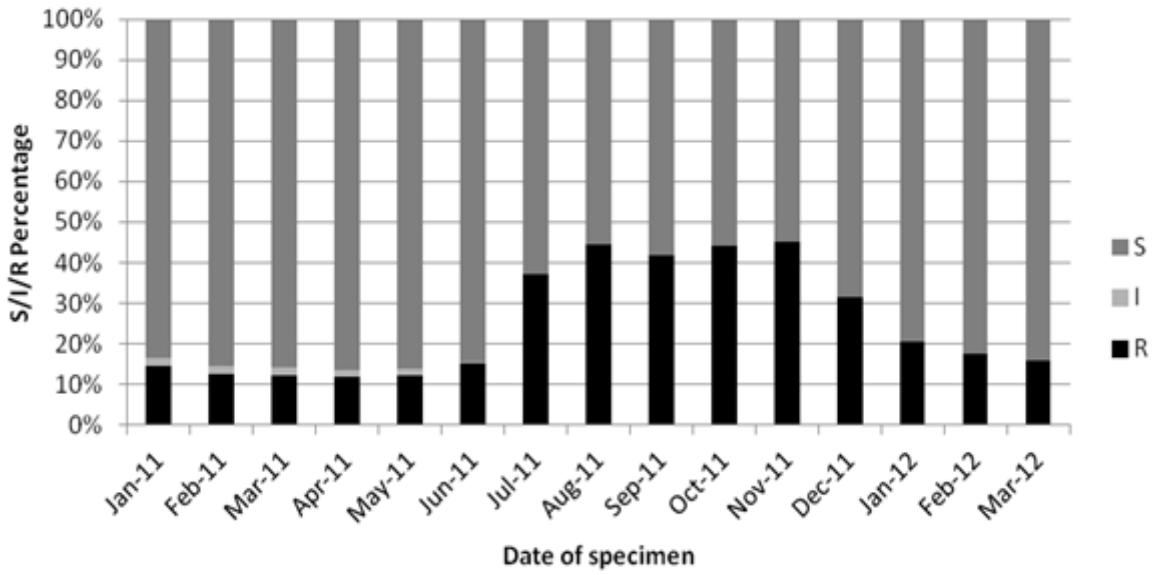
Figures 3.6 and 3.7 are the AmWeb graphical representations of a time series of drug / bug combination reports at the local and regional setting. Figure 3.6a shows the trends in proportion of *E. coli* isolates reported as susceptible, intermediate or resistant to co-amoxiclav by a local laboratory, and Figure 3.6b shows the number of *E. coli* isolates tested against co-amoxiclav by the same laboratory over the same period. These charts show that over a 14 month period, testing of *E.coli* isolates against co-amoxiclav remained relatively stable, but the proportion of isolates reported as resistant to co-amoxiclav increased steeply in July 2011 from approximately 15% to 40% and remained at this level for six months before decreasing to the levels observed in the first half of 2011. On investigation this observed pattern was found to be due to a change to BSAC breakpoint guidelines,(Andrews and Howe, 2011) recommending an increase in the zone diameter for interpreting *E. coli* susceptibility to co-amoxiclav when testing urine samples, which the laboratory instituted in July 2011. The breakpoint was subsequently reversed by the laboratory for isolates from patients with a UTI, consequently resistance proportions returned to the previous level. Concern was raised by laboratories regarding reporting increased resistance to co-amoxiclav for isolate from urine specimens resulting from the implementation of new guidelines. The BSAC, therefore, introduced an increased MIC breakpoint specifically for UTIs.(Howe and Andrews, 2012). Figure 3.7 shows the regional trends for the same drug-bug combination during this period for comparison and clearly shows a stable trend over time, suggesting that most laboratories ignored the change in recommendation.

3.4.3 AmWeb case study B

Table 3.1 shows an AmWeb tabular report for numbers of isolates and proportions of *E. coli* urinary isolates reported as resistant in selected Primary Care Trusts (PCTs) in the West Midlands in 2011. The table shows wide variation in reported resistance proportions between the PCT areas, with the proportion of *E. coli* isolates resistant to cephalexin ranging between 4% to 10%, and co-amoxiclav resistance ranging between 9% and 24%. The number of tests performed for each antibiotic against the specific organism is also displayed. It can be observed that in some areas local laboratories were performing selective testing for certain antibiotics by not testing all *E. coli* isolates against specific antibiotics, which may lead to higher apparent rates of resistance due to selection bias. This is particularly true for PCT 4 where the local laboratory only tested isolates against ciprofloxacin when resistance to first-line antibiotics was detected. The corresponding resistance proportion to ciprofloxacin in this area was 27% compared with the regional average of 10%.

Figure 3.6 (a): Distribution of resistance profile of *E. coli* isolates from all specimens, tested against co-amoxiclav in laboratory A between January 2011 and March 2012. (b): Number of *E. coli* isolates from all specimens, tested against co-amoxiclav in laboratory A between January 2011 and March 2012 (charts from report prepared for publication).

a)



b)

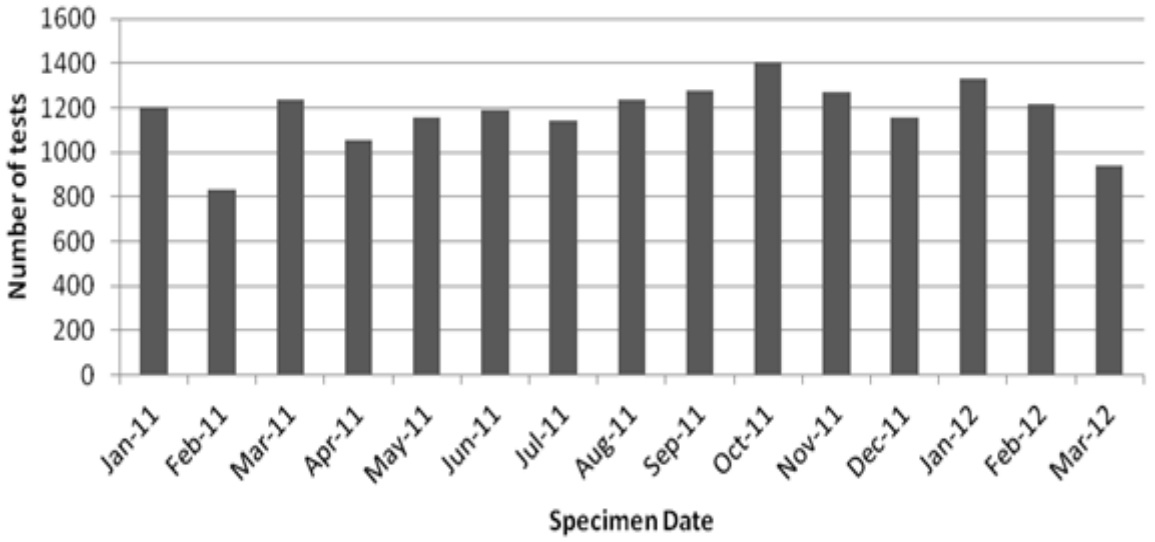


Figure 3.7 Drug/Bug example regional reports for *E. coli* isolates from all specimens tested against co-amoxiclav by laboratories in the West Midlands region between January 2011 and March 2012 (charts from report prepared for publication).

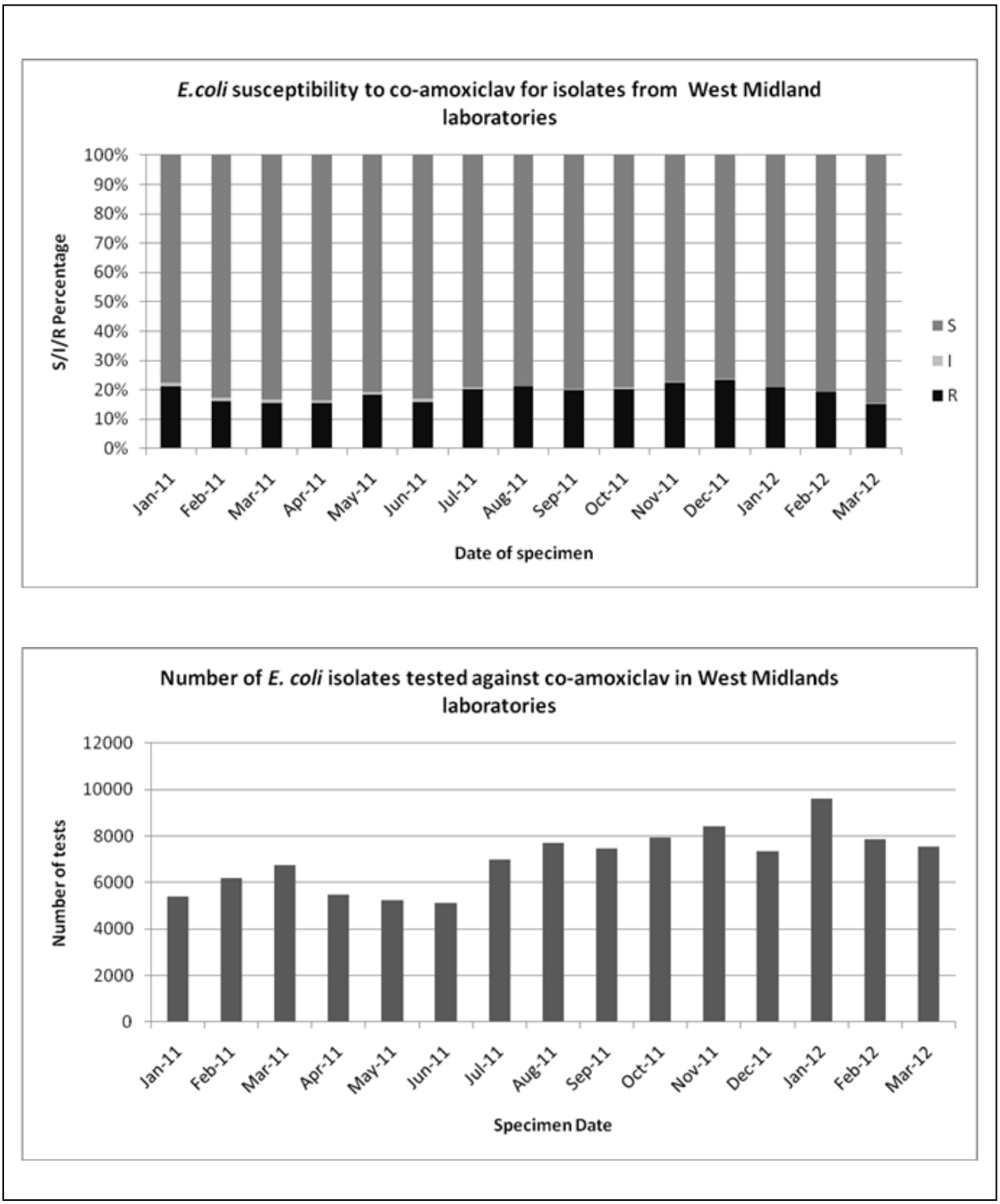


Table 3.1 Susceptibility of *E. coli* isolates from urine samples to co-amoxiclav, ciprofloxacin and cephalexin in selected PCT areas in 2011.

		Co-amoxiclav		Ciprofloxacin		Cephalexin	
	No. of *E. coli* UTI isolates	**No. isolates tested**	**% resistant**	**No. isolates tested**	**% resistant**	**No. isolates tested**	**% resistant**
PCT1	2523	2523	21%	2519	7%	2516	6%
PCT2	1685	1685	24%	1681	15%	1685	10%
PCT3	3165	3162	10%	411	14%	132	4%
PCT4	9830	9067	9%	923	27%	8557	5%
Regional Totals [a]	**54287**	**50339**	**18%**	**44493**	**10%**	**48068**	**7%**

[a] Includes isolates from all 17 Primary Care Trusts areas within the region.

3.4.4 AmWeb case study C

A review of AmWeb data for the susceptibility of *Pseudomonas aeruginosa* to piperacillin / tazobactam in 2016 revealed variation in the proportions reported as non-susceptible by laboratories in the West Midlands. On further investigation it appeared that the variation seemed to be associated with the susceptibility testing method used by the laboratory. Laboratories using disc diffusion methods reported comparatively low proportions of their *P. aeruginosa* as non-susceptible to piperacillin / tazobactam (Figure 3.8); however, laboratories using VITEK 2® devices (described in Chapter 2) to test this organism (n=5) reported a higher proportion non-susceptible to piperacillin / tazobactam (Figure 3.9). The results from laboratories using VITEK 2® reported a large proportion of their piperacillin / tazobactam results as having intermediate resistance; although the EUCAST breakpoint standards used by these devices does not include a definition for intermediate susceptibility for *P. aeruginosa* tested against any antibiotics, including piperacillin / tazobactam (European Committee on Antimicrobial Susceptibility Testing, 2017). A review of the 'predictive' MIC results from a laboratory using VITEK 2® to perform susceptibility testing on *P. aeruginosa* for January-May 2017 found that many of the tests reported with 'intermediate' susceptibility had 'predictive' MIC values of <16 mg/L, which following EUCAST guidelines would be the 'susceptible' range (Table 3.2).

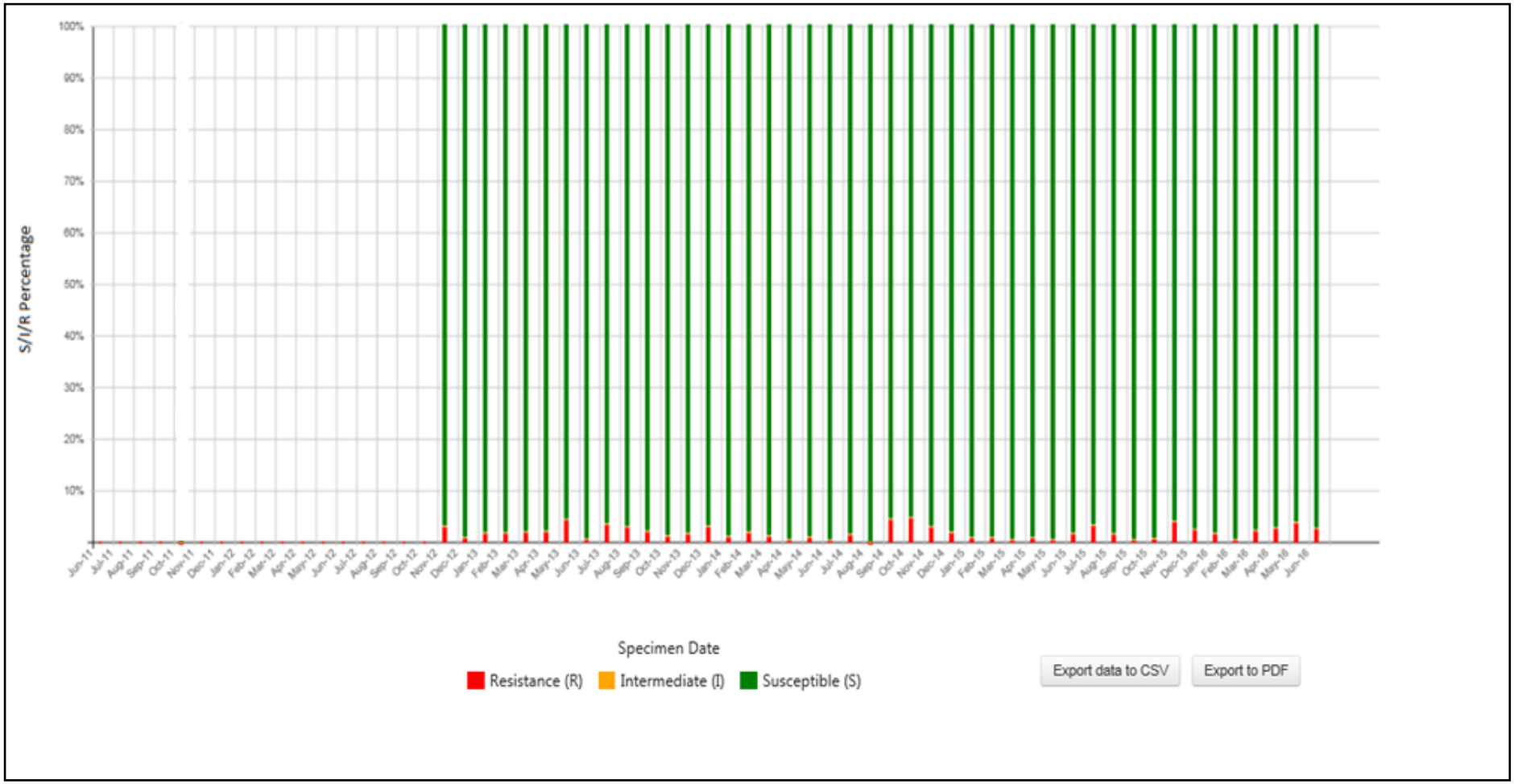
As a result of reporting these findings, the laboratories using VITEK 2® to perform susceptibility testing of *P. aeruginosa* to piperacillin / tazobactam in the West Midlands contacted the manufacturer (bioMérieux) in September 2016 for information on how the results are assigned for this antibiotic, and two laboratories changed to a disc diffusion method for testing *P. aeruginosa*. A new automated sensitivity test card

for *Pseudomonas* spp. was deployed by bioMérieux in January 2017 and results from laboratories using the new card are being monitored using AmWeb.

Table 3.2 Piperacillin/tazobactam VITEK 2® susceptibility results for *P. aeruginosa* tested by a West Midlands laboratory, January – May 2017.

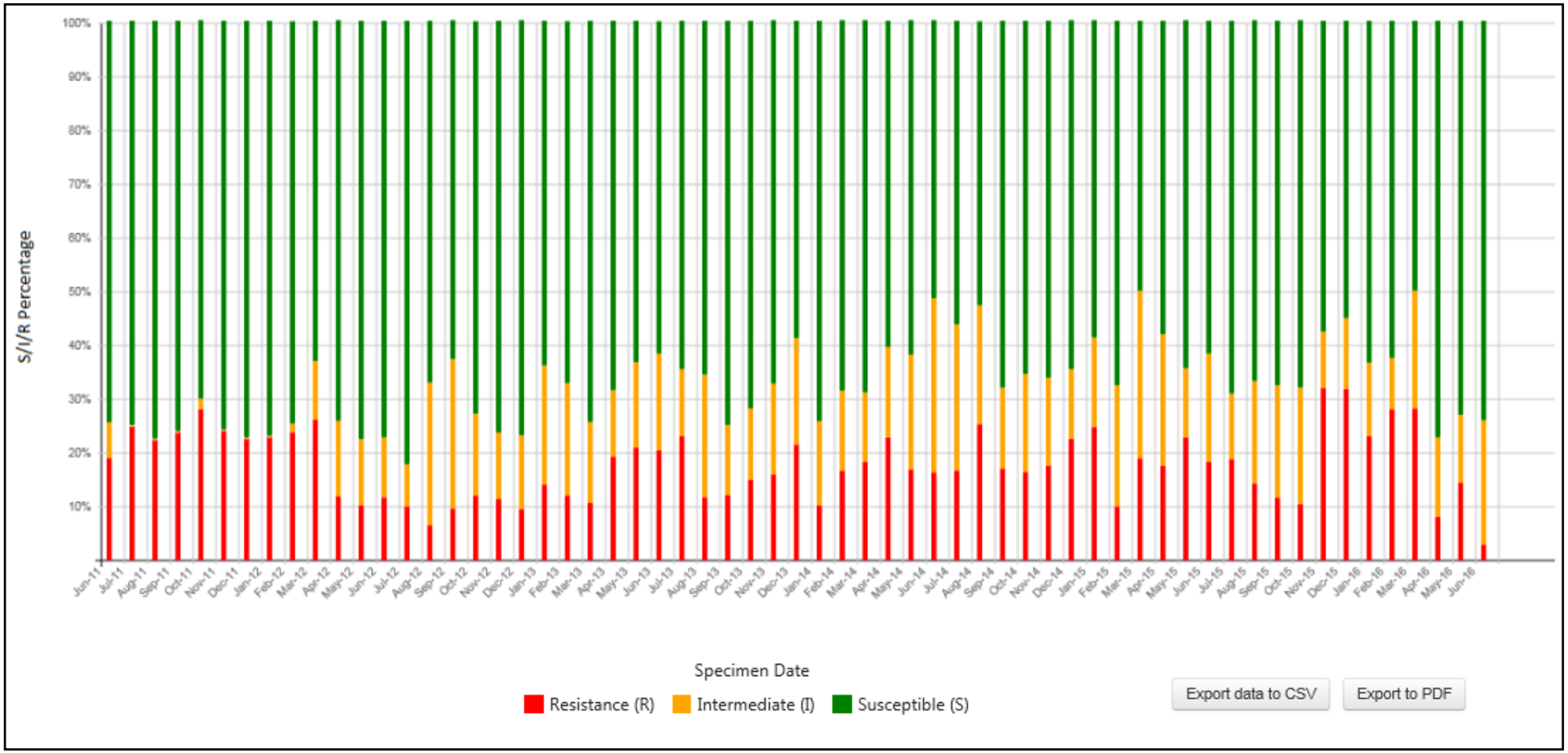
Susceptibility result reported	**MIC test results reported (mg/L)**				**Total susceptibility results**
	≤4	**8**	**16**	**≥32**	
Sensitive	83	32	8	0	123
Resistant	0	1	1	24	26
Intermediate	158	302	21	0	481
Total	**241**	**335**	**30**	**24**	**630**

Figure 3.8 AmWeb drug/bug report for *P. aeruginosa* isolates from all specimens tested against piperacillin / tazobactam by a West Midland laboratory using a disc diffusion method, June 2011-June 2016* (screen shot from AmWeb application).



*Laboratory commenced reporting to AmSurv in November 2012

Figure 3.9 AmWeb drug/bug report for *P. aeruginosa* isolates from all specimens tested against piperacillin / tazobactam by a West Midland laboratory using a VITEK 2® automated testing system,June 2011-June 2016 (screen shot from AmWeb application).



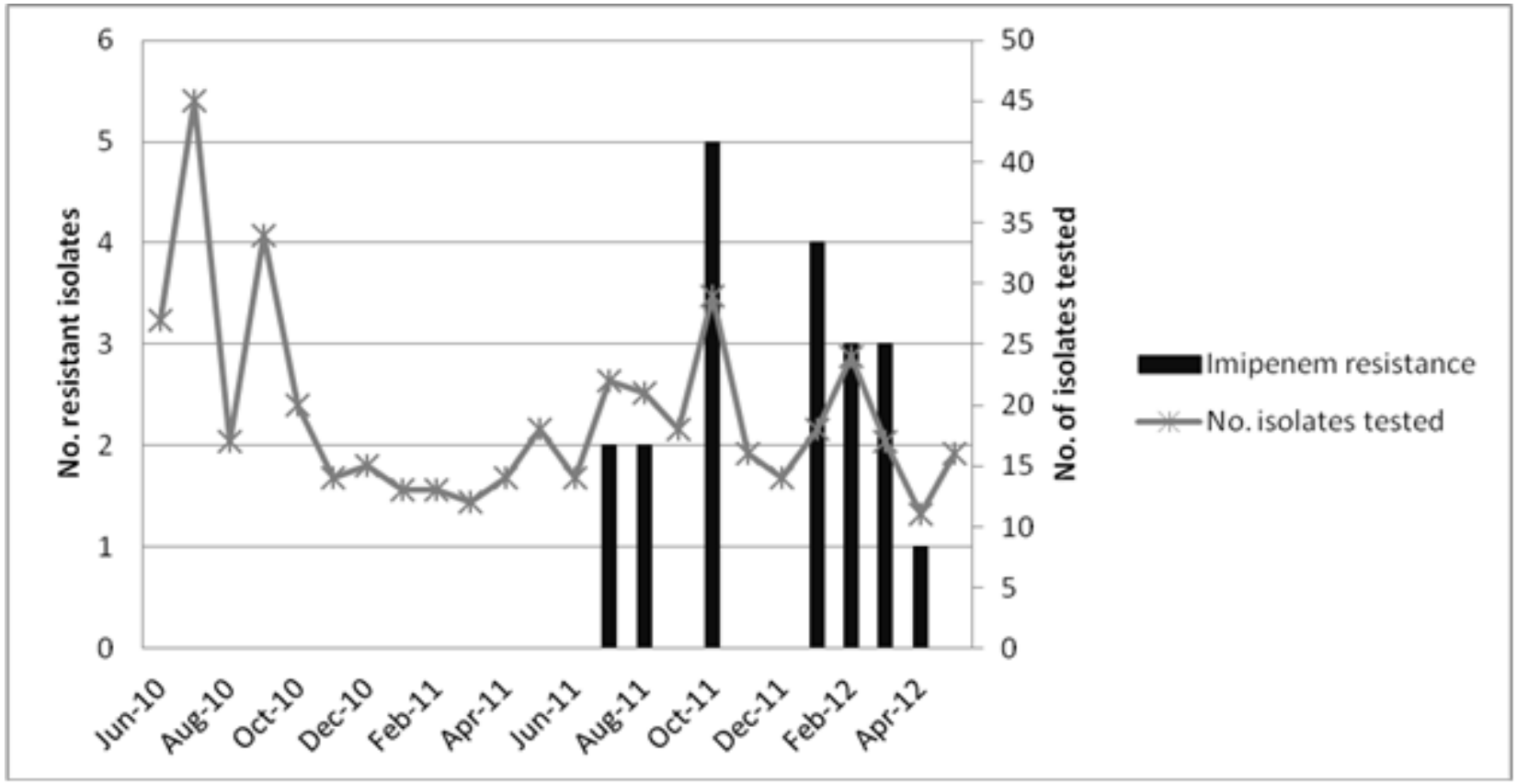
3.4.5 Regional surveillance of AMR

Following the launch of AmWeb in England in January 2012, the AmWeb application was used by the West Midlands REU to detect and monitor unusual resistance profiles, such as outbreaks of multi-drug resistant Gram-negative bacteria in local hospitals. Following small outbreaks of carbapenemase-producing Enterobacteriaceae in local hospitals, AmWeb was used to monitor the occurrence of new, potentially linked cases, through the use of distinctive antimicrobial profiles set up as alerts on the system so as to detect potential local spread of these resistant bacteria.

As an example Figure 3.10 shows a time series chart marking the appearance of a *Klebsiella pneumoniae* resistant to imipenem in a local hospital. The number of *K. pneumoniae* isolates tested against imipenem by the laboratory is also shown.

This hospital experienced an outbreak of ESBL-producing Enterobacteriaceae during the summer of 2010, and implemented a screening programme for patients on affected wards and all new admissions. This may account for the increased testing of imipenem observed during this period, as more isolates detected in the screening programme would be tested for carbapenem resistance. We investigated the spike in imipenem resistance with the local microbiologist and found that a strain of *K. pneumoniae* producing a Verona Integron-encoded Metallo-β-lactamase (VIM) had spread between patients in the hospital.

Figure 3.10 Results of susceptibility testing to imipenem for *K. pneumoniae* isolates from all specimens, reported by laboratory B, together with total numbers of isolates of *K. pneumoniae* tested against imipenem by laboratory B (chart from report prepared for publication).



3.4.6 Community AMR web bulletin

The quarterly community AMR bulletin provides temporal antibiotic resistance trends for *E. coli* isolated from community urine specimens against trimethoprim, co-amoxiclav, cephalexin and nitrofurantoin. Figure 3.11 shows a chart taken from the community AMR bulletin released in July 2016. This chart displays AMR resistance in *E. coli* community isolates from urine specimens from January 2012 to June 2016. The proportion of *E. coli* resistant to nitrofurantoin and trimethoprim appears to be stable during this period at approximately 2.5% and 35.0%, respectively; although in Chapter 6 trimethoprim is shown to have a gradual rising trend in non-susceptibility in the period 2010-20112, which then levels out between 2012-2014. There was variation in the resistance proportion of co-amoxiclav during this period (range 11.0% - 19.7%). There also appeared to be a gradual linear increase in resistance to cephalexin between 2012 and 2016, with the resistance proportion at 8.7% in June 2016 compared with 6.8% in January 2012. A separate table on the community bulletin home page provides the resistance proportions of pathogens commonly isolated from community specimens for a particular calendar quarter (Figure 3.12).

Figure 3.11 Trends in regional antimicrobial resistance in *E. coli* isolated from community urine specimens, West Midlands, Jan 2012 - May 2016 (screen shot from web bulletin).

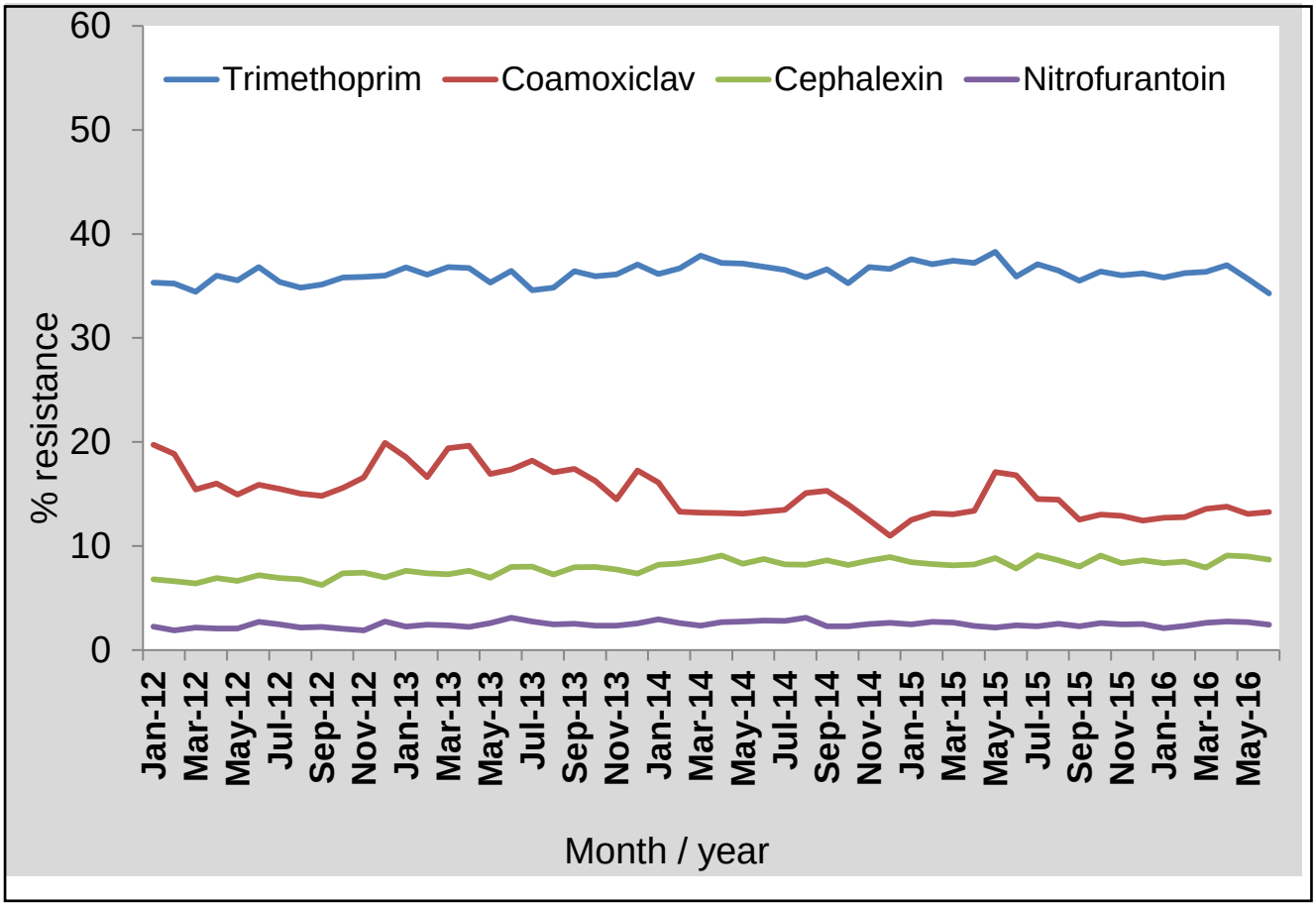
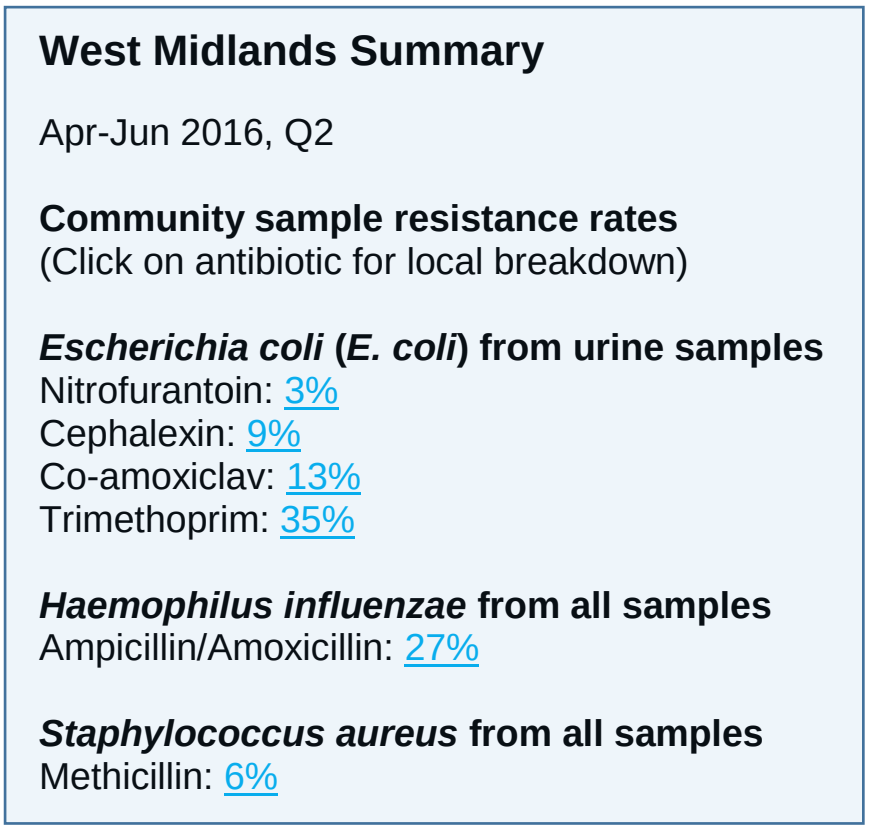


Figure 3.12 Summary table from the Quarter 2 2016 Community AMR bulletin



West Midlands Summary

Apr-Jun 2016, Q2

Community sample resistance rates
(Click on antibiotic for local breakdown)

***Escherichia coli* (*E. coli*) from urine samples**
Nitrofurantoin: 3%
Cephalexin: 9%
Co-amoxiclav: 13%
Trimethoprim: 35%

***Haemophilus influenzae* from all samples**
Ampicillin/Amoxicillin: 27%

***Staphylococcus aureus* from all samples**
Methicillin: 6%

Each of the quarterly resistance proportions for the various bacteria and antibiotics provided on the homepage of the Bulletin (Figure 3.12) was set as a hyperlink that on clicking would open a new web page that showed resistance proportion by local geographies (initially Local Health Authorities and replaced by CCGs in 2013). Figures 3.13 and 3.14 illustrate examples of the local breakdown charts displayed for co-amoxiclav and trimethoprim respectively for the Quarter 2 2016 bulletin.

In Figure 3.13 it can be observed that the proportion of *E. coli* isolates resistant to co-amoxiclav in CCG areas during April-May 2016 ranged from 5% to 24%, with a regional average proportion of 13% for this quarter. In Figure 3.14 the resistance proportion for trimethoprim to *E. coli* in CCG areas shows less variance and ranges from 30% to 43% with a regional average resistant proportion of 35% for this quarter.

The pages showing resistance in *E. coli* to each of the selected antibiotics by local commissioning groups also included a chart to describe the age and sex breakdown for patients with *E. coli* urinary infections that were tested against the selected antibiotic. Figure 3.15 and Figure 3.16 show examples for cephalexin and nitrofurantoin respectively from the quarter 2 2016 bulletin.

Figure 3.13 Chart from bulletin showing number of *E. coli* isolates from community urine samples tested against co-amoxiclav (blue columns), regional average resistance (green line) and CCG average (red squares), West Midlands April-June 2016 (screen shot from community AMR bulletin).

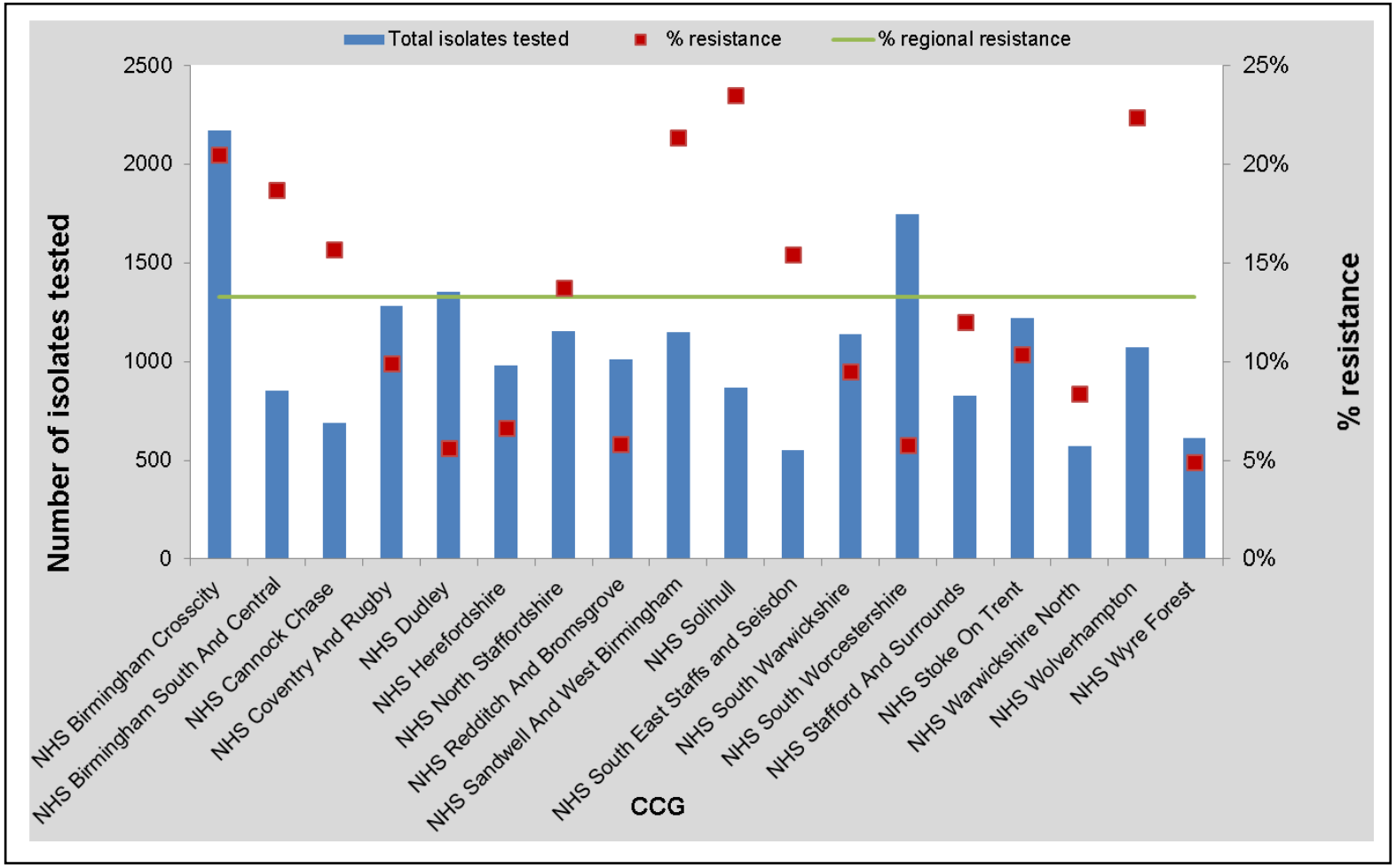
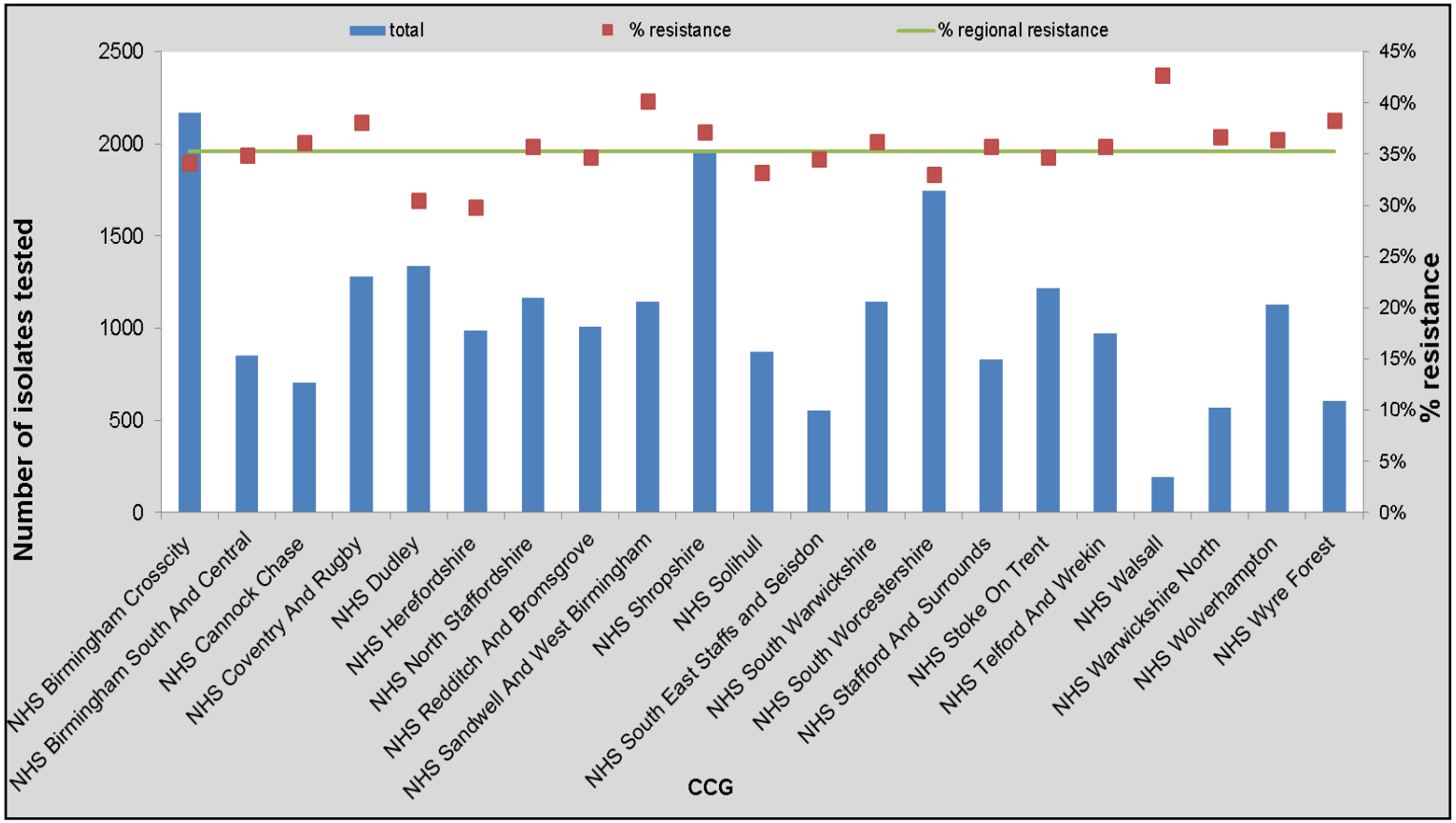


Figure 3.14 Chart from bulletin showing number of *E. coli* isolates from community urine samples tested against trimethoprim (blue columns), regional average resistance (green line) and CCG average (red squares). West Midlands, April - June 2016 (screen shot from community AMR bulletin).



The age and sex breakdown for patients with *E. coli* isolates resistant to cephalexin for quarter 2 in 2016 (Figure 3.15) shows higher proportions of resistance in the younger and older age groups for both male and female patients. The chart shows the much higher number of tests performed on *E. coli* isolated from female patients, particular the over 65 age group. The bulletin commentary for this page informs that 85% of the *E. coli* tested against trimethoprim were from female patients and that as a proportion of all *E. coli* isolates tested against trimethoprim the overall proportion of resistant isolates in females was slightly higher than that reported in males (36% vs. 33%).

Figure 3.16 shows the age and sex breakdown for nitrofurantoin testing released in the quarter 2 2016 bulletin. A marked increase in resistance to nitrofurantoin is observed in the older age groups, with a resistance proportion of over 5% for the male 65+ age group. Increased resistance is also observed in the female under 5 age group. The number of tests performed again also rises sharply in the female 65+ age group. The bulletin commentary for this page informs that while the majority (85%) of susceptibility reports were from females, when viewed as a proportion of all *E. coli* isolates tested against nitrofurantoin, the overall proportion of resistant isolates in females was lower than that reported in males (2% vs.4%). This is possibly explained by a higher proportion of urines received from male patients in the 65+ age group, in which higher proportions of resistance are found (Figure 3.16).

Figure 3.15 Community bulletin chart showing age and sex breakdown for *E. coli* resistance to cephalexin in the West Midlands, quarter 2 2016 (screen shot from community AMR bulletin).

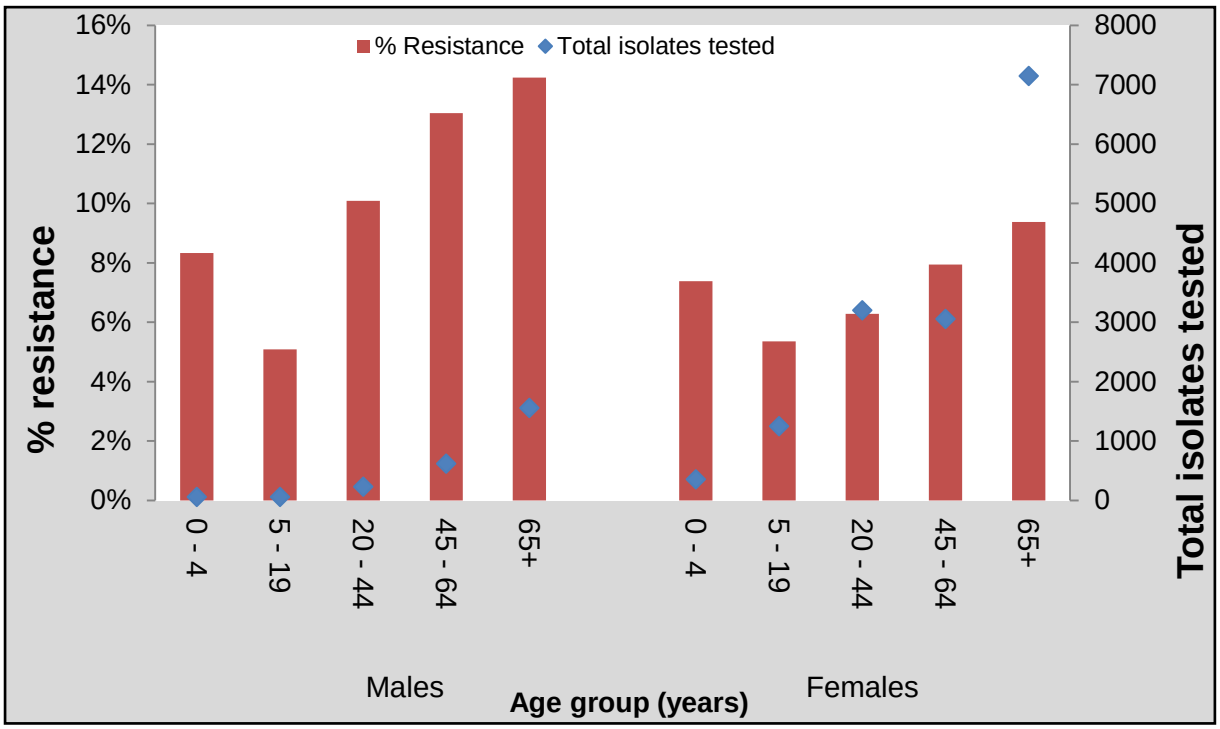
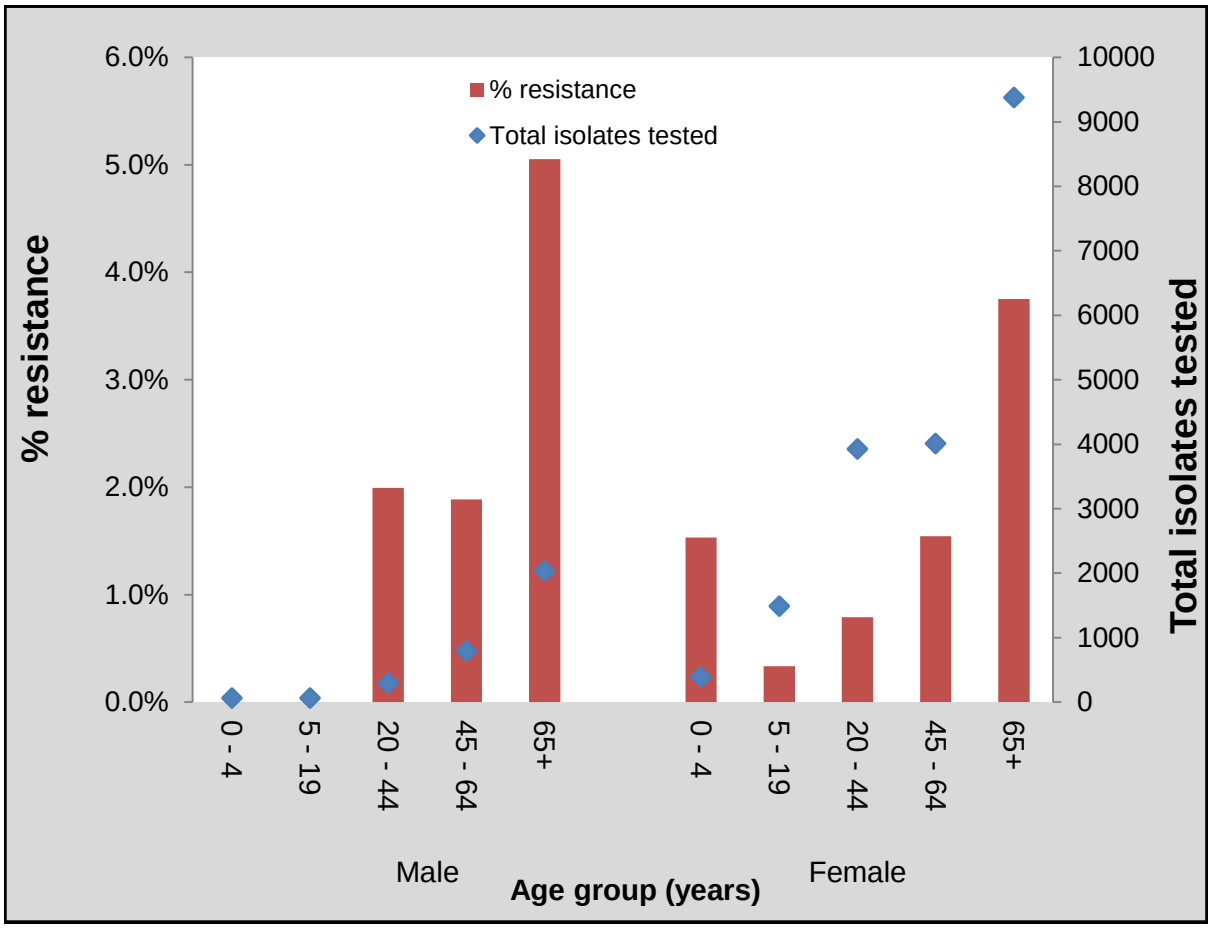


Figure 3.16 Community bulletin chart showing age and sex breakdown for *E. coli* resistance to nitrofurantoin in the West Midlands, quarter 2 2016 (screen shot from community AMR bulletin).



3.4.7 Community AMR bulletin survey

The on-line survey of GP practices received 90 responses, representing 80 GP practices (8% of practices in the West Midlands). Forty-one (46%) of the respondents were GPs, 38 (42%) practice managers, 5 (6%) practice nurses and 6 (7%) were other members of the practice team.

There were 54 responses to the question whether the bulletin was useful / relevant to the practice with 81% (44/54) answering ‘yes’, 2% (1/54) ‘no’ and 17% (9/54) ‘unsure’. There were 53 reponses to the question asking whether the bulletin had influenced prescribing or prescribing policy, with 51% (27/53) answering ‘yes’, 9% (5/53) ‘no’ and 40% (21/53) ‘unsure’.

The survey invited recipients of the bulletin to agree or disagree with statements regarding the format, content and frequency of publication (Table 3.3). Sixty-four percent (34/53) ‘agreed’ or ‘completely agreed’ that ‘the content is appropriate’ and ‘quarterly reporting of the bulletin is appropriate’. Sixty-two percent (33/53) ‘agreed’ or ‘completely agreed’ that the ‘format is appropriate’ and ‘the level of detail is just right’.

Table 3.3 Community bulletin survey question results for content and format (53 responses)

	Completely disagree	Disagree	Neutral	Agree	Completely agree
It is simple to use.	0 (0%)	0 (0%)	20 (38%)	25 (47%)	8 (15%)
The antibiotics and organisms included are appropriate.	0 (0%)	0 (0%)	19 (36%)	27 (51%)	7 (13%)
The format is appropriate to my needs.	0 (0%)	0 (0%)	20 (38%)	27 (51%)	6 (11%)
The level of detail is just right.	0 (0%)	0 (0%)	20 (38%)	29 (55%)	4 (8%)
Quarterly reporting is appropriate	0 (0%)	1 (2%)	18 (34%)	27 (51%)	7 (13%)

The last two questions in the survey requested additional free-text responses. The first of these asked for examples of where the bulletin had influenced antibiotic prescribing or prescribing policy. There were 18 free-text comments to this question with two themes emerging within the group; the practice had changed to prescribing nitrofurantoin rather than trimethoprim as a result of reading the bulletin (n=4) and the bulletin had reaffirmed or prompted the following of national prescribing guidelines for UTI in the practice (n=8). One response stated that the bulletin was used to show to patients to explain practice prescribing policy.

The second free-text question asked for general comments regarding the bulletin. There were 15 individual comments in this section with one common theme emerging, which was that practices are overwhelmed by emails and therefore notification of the AMR community bulletin can be easily overlooked (n=9).

3.5 Discussion

3.5.1 AmSurv and AmWeb

3.5.1.1 Advances in informatics

The development of surveillance systems to monitor trends in antimicrobial resistance at the local, regional and national levels is an important element in controlling the emergence and spread of antibiotic resistant bacteria (O'Brien and Stelling, 2011a). In the UK, a lot of healthcare information is available in electronic format (Johnson, 2015). Modern relational database management systems, such as Microsoft SQL Server, are able to store large amounts of data, can retrieve information rapidly and have in-built advanced security features (Wisniewski et al., 2003). Advances in informatics reduce the burden on laboratories of reporting timely routine surveillance data by using automated routines, and web-enabled database tools are now able to process large datasets in real-time. The use of new technologies such as the secure web based data capture and processing provide an opportunity to improve data quality, obtain near real-time data and provide a mechanism of automating the generation of alerts for users of the system (Hayward et al., 2007;O'Brien and Stelling, 2011b).

3.5.1.2 Standard laboratory methods

As discussed in Chapter 2, an AMR surveillance system based on routine laboratory reporting ideally requires standard methods to be used by laboratories and a consistent approach to the interpretation of antibiotic resistance. The survey of laboratories in the West Midlands, undertaken in 2011 and described in chapter 2, showed variation in laboratory methods and protocols for both the identification of

bacterial isolates and the determination of antimicrobial susceptibility. This is underscored in this chapter by the observed shift in resistance trend in laboratory A following adoption of different guidelines for the interpretation of susceptibility to co-amoxiclav (Figure 3.6). However the laboratory survey reported in Chapter 2 also demonstrated a recent growing trend towards using Automated Susceptibility Testing (AST) systems, with 11 of the 15 diagnostic laboratories now using the bioMérieux VITEK 2® system for some or all of their antimicrobial susceptibility testing. There has also been a progressive move to greater International Standardisation with the adoption of much more similar breakpoints within Europe and internationally (Hombach et al., 2012). This increasing standardisation will improve the quality of routine AMR surveillance and enable direct comparison between laboratories, hospitals and geographical areas (O'Brien & Stelling, 2011a).

3.5.1.3 De-duplication of records

AMR surveillance systems need to incorporate a process for identifying and handling duplicate entries. Inadequate de-duplication risks affecting the validity of AMR surveillance information through the introduction of measurement bias. Although as mentioned in Chapter 1, guidelines from the CLSI recommended that results from only the first isolate of a species from a patient should be included in calculating the percentage susceptibility to an antibiotic (National Committee for Clinical Laboratory Standards, 2000), selecting only the first isolate limits the ability to monitor and identify any changes in antimicrobial susceptibility at the individual level, perhaps as the result of antimicrobial therapy (Morris and Masterton, 2002). We found a 14 day repeat exclusion rule removed on average less than 5% of AmSurv reports, and this did not increase significantly if the repeat exclusion episode length was extended

beyond 14 days. To this end, we were confident in implementing a 14 day comprehensive repeat exclusion rule in AmWeb, which is also the period used to determine episodes of infection in the HPA CoSurv system (now incorporated into SGSS).

3.5.1.4 Laboratory information management systems and coding

The number and variety of Laboratory Information Management Systems (LIMS) in use across England has always posed a problem for those designing laboratory-based surveillance systems (Hayward et al, 2007). In parts of Europe one laboratory can serve over 60 hospitals (Schweickert et al, 2011); however, in the UK during this study period, each NHS laboratory usually provides services for a single or small group of hospitals and their local community healthcare providers. In 2012 there were nearly 200 NHS diagnostic laboratories in England and 14 different varieties of LIMS. There is not a universal national system in England for coding clinical microbiological data items (Zhao et al., 2014). Each laboratory has therefore developed their own bespoke codes or have been provided with a set of hard-coded data items by their LIMS manufacturer, who are often not based in the UK. This poses a real challenge in extracting and collating healthcare information from the disparate information systems, and an even greater challenge in trying to impose new standard codes on historic patient care data (O'Brien & Stelling, 2011a).

AmSurv was designed to manage this diversity of LIMS systems by simplifying the output requirements and translating local codes to nationally recognised formats. However the solution for dealing with bespoke local coding was still a significant obstacle for the implementation of AmSurv. The requirement to obtain a range of code directories from each laboratory and individually translate the several thousand

codes obtained to standardised HPA/PHE national codes required significant resource and political will, which may explain why national reporting of AMR data from local laboratories, excluding the West Midlands, was only around 30% in 2012. As implementing AMR surveillance was an objective of this study, code mapping was prioritised in the West Midlands and regional HPA management agreed to provide some additional resource to help build translation tables in preparation for the launch of AmSurv. Personal visits to laboratories to discuss regional AMR surveillance plans and the development of tools to allow microbiologist's access to regional AMR data (via AmWeb), helped secure participation of all West Midland laboratories in this voluntary surveillance scheme.

3.5.1.5 AmWeb case studies

The reported AmWeb case studies show that the application can be used to monitor the emergence of new resistance mechanisms (case study B) and can also be used to act as a benchmark to improve the quality of susceptibility testing and reporting by laboratories. The laboratory that was using systemic breakpoints to interpret susceptibility testing results of isolates from urine (case study A) changed their practice following reports received from the system.

Piperacillin / tazobactam is an antibiotic used to treat infections caused by MDR bacteria. To limit the use of drugs of last resort, such as carbapenems, piperacillin / tazobactam is often first-line treatment for serious infections, such as sepsis, in many hospitals (Lodise, Jr. et al., 2007). Therefore it was concerning to discover the range of non-susceptibility being reported between laboratories in the West Midlands. With non-susceptibility being reported at levels of 30%-40% by some West Midland laboratories, clinicians may switch to 'reserve' antibiotics for first-line treatment. As

described in the previous chapter, piperacillin / tazobactam testing was not recommended on earlier versions of VITEK 2® systems as a study of direct susceptibility testing of blood using VITEK 2® found major discrepancies for piperacillin/ taxobactam (Ling et al., 2001). The high proportion of intermediate susceptibility reported by laboratories using VITEK 2® systems is a significant factor in the overall higher non-susceptibility reported by laboratories using VITEK 2® devices. These intermediate test results are unexplained as the breakpoint standards used by VITEK 2® (EUCAST) do not contain a definition for intermediate susceptibility for piperacillin / tazobactam tested against *Pseudomonas* spp..

A review of the predictive MIC results provided by a VITEK 2® device used by a West Midland laboratory (Table 3.2) showed that a high proportion of the results determined as having 'intermediate' susceptibility would have been reported as susceptible (i.e. MIC <16mg/L) using EUCAST breakpoints (European Committee on Antimicrobial Susceptibility Testing, 2017). Although these are only predictive MIC values based on a limited range of antibiotic concentrations, they are presumably being overwritten by the VITEK 2® 'expert rules' when determining the final susceptibility results.

3.5.1.6 National AmWeb application

The HPA Healthcare Associated Infections and Antimicrobial Resistance (HAIAMR) programme board and the government Advisory Committee on Antimicrobial Resistance and Healthcare Infections (ARHAI) requested that AmWeb be demonstrated in 2012 to their respective group members. These bodies supported the development of the AmWeb application and endorsed it as a suitable surveillance tool for interrogating AMR data across England. It was therefore requested that the

West Midlands AmWeb application be adapted to enable the collation of AMR data from all nine regional AmSurv modules in England. A functional specification was agreed in August 2012 and the ‘national’ AmWeb application was launched in November 2012. This application, for the first time in England, created a national repository of AMR surveillance data, and gave regional HPA colleagues the ability to setup their laboratories so that they could access AMR data, and benchmark against regional / national data. The HPA was incorporated within Public Health England (PHE) in April 2013 and AMR was assigned one of the top priority areas for the new organisation. The AmSurv system of distributed databases was replaced by the Second Generation Surveillance System (SGSS) in 2014, with the AmWeb application incorporated into the new SGSS national reporting tools. With increased PHE resource in 2016 to support the translation of laboratory AMR report codes, combined with the incentive of regions and laboratories in England having the AmWeb surveillance applications to access local and national data, reporting of AMR by English laboratories improved dramatically. By April 2017 98% of laboratories were reporting AMR data to PHE.

The next section discusses the information output and some of the findings reported in the community AMR bulletin. It also considers the results from a survey of general practice users.

3.5.2 Community AMR bulletin

3.5.2.1 Bulletin report

The quarterly community AMR bulletins have shown a relatively stable trend in resistance proportions for *E. coli* isolated from urine between January 2012 to June 2016 against trimethoprim, cephalexin and nitrofurantoin (Figure 3.11). The trend line for co-amoxiclav resistance, however, is less stable. As described above, changes to the BSAC breakpoint guidelines for interpreting co-amoxiclav susceptibility test results in 2011, which were reversed later that year, may be responsible for the higher resistance proportion observed in early 2012. The variability in co-amoxiclav resistance proportions is also observed in the bulletin chart for quarter 2 in 2016, showing resistance proportions by local geographic areas (Figure 3.13). The range for resistance to co-amoxiclav between CCGs is 5% to 24% in this period. Testing for co-amoxiclav susceptibility has been problematic for laboratories, with the action of two drugs (amoxicillin and clavulanic acid), being assessed simultaneously (Barrett et al., 1999). The NEQAS external quality assessment service issued a test sample containing *E. coli* to laboratories in 2012 that was susceptible to co-amoxiclav but had an MIC that was close to the breakpoint for non-susceptibility. BSAC, EUCAST and CLSI had the same breakpoint guidelines for co-amoxiclav (S ≤ 8mg/L); however, 41% of laboratories using BSAC guidelines reported the organism as resistant compared with 3.5%, 4.7% using EUCAST and CLSI, respectively (Brown, 2012). A reason for this discrepancy may be the use of a 2:1 ratio for amoxicillin and clavulanic acid in the BSAC method compared with the fixed 2mg/L clavulanic acid used in the EUCAST method (Diez-Aguilar et al., 2015). It is therefore difficult to measure actual variability in resistance proportions between geographical areas for co-amoxiclav using the charts provided in the community bulletin.

Trimethoprim is often used to treat UTI empirically and was previously recommended for first-line empirical treatment in national formularies (McNulty et al., 2006a). However the proportion of *E. coli* from urinary specimens that are resistant to trimethoprim in the region is approximately 35% (Figure 3.11). It is accepted that this resistance rate may be higher than the actual non-susceptibility rates within the population due to specimen selection bias described previously; however studies using data from practices sending all urine specimens for suspected UTI have found similar high levels of resistance to trimethoprim, with a sampling study from 22 practices in Ireland showing the mean proportion resistant to trimethoprim as 31.5% (Vellinga et al., 2012). It has been suggested that antibiotics should be prescribed empirically provided the local resistance rate does not exceed 10-20% (Naber et al., 2001;Warren et al., 1999); however, some suggest antibiotics should be used even at higher rates of local resistance rates in order to avoid increased use of broad-spectrum antibiotics such as ciprofloxacin (Gupta et al., 2001;McNulty et al., 2006b). An argument put forward to justify this approach is that resistance rates have been traditionally based on hospital patients and therefore likely to be higher than those found in the community (Hooton, 2012). Although the definition for 'community patients' is sometimes ambiguous, as many patients seen in primary care will have come into contact with secondary care facilities, this study shows that resistance rates for *E. coli* versus trimethoprim from urine specimens sent by GPs are at a level that should lead to a re-assessment of empirical prescribing choices. Recently, based partly on data provided via the AmSurv surveillance system and published research, there has been change in the national formulary, with nitrofurantoin now recommended, rather than trimethoprim for first-line treatment of lower UTI (Public Health England, 2017). Prior to this change in national formulary a number of

anecdotal reports from GPs in the West Midlands were received, informing they had changed to prescribing nitrofurantoin following receiving the community AMR bulletin, with some of these comments being replicated in the results from the user survey reported above.

The risk of acquiring a UTI is higher in females compared with males; although this increased risk for female patients is cancelled-out in the elderly population (Laupland et al., 2007). The West Midland community bulletin reflects the higher proportions of UTI in females for the various antibiotics tested (Figure 3.15 and Figure 3.16). The bulletin age and gender charts also show increased levels of antibiotic resistance in the older generations, with for example nitrofurantoin resistance being 5% for *E. coli* in males over 65 years compared with 2% or less resistance in the younger age groups (Figure 3.16). It is plausible that the older generation are more likely to come into contact with hospitals or be institutionalised in the community. Catheter-associated UTI is a common nosocomial infection in community nursing homes (Foxman, 2003). A recent study in the West Midlands region found that patients in long-term care facilities (LTCFs), compared with similar age groups living in the community, are more than twice as likely to have a laboratory confirmed UTI and the bacteria isolated from these patients are more resistant to commonly prescribed antibiotics (Rosello et al., 2017). The increased incidence of infection in this age group will inevitably lead to greater exposure to antibiotics.

3.5.2.2 Survey of community bulletin users

The low response rate from the survey of recipients of the community AMR bulletin is a limitation on the validity of any findings due to non-response bias. From the received responses, 81% (44/54) stated that the bulletin was useful / relevant, with 51% (27/53) stating that it had influenced prescribing or prescribing policy. A majority of responses agreed that the bulletin was simple to use, provided appropriate drug/bug results, the format meets user requirements and that the quarterly interval was adequate. It is plausible, however, that that community prescribers who did not find the bulletin helpful may be less inclined to respond to the survey (non-response bias).

Again the number of free text comments received is not sufficient to be analysed as a representative sample of GP practices in the West Midlands. However the emerging themes regarding moves towards using nitrofurantoin and reaffirming / promoting correct prescribing for UTI based on the bulletin data is encouraging. The dominant theme in the general comments section regarding email notification of the bulletin being lost due to the high volume of emails received by practices may account for the low survey response rate. The design of future community surveillance outputs will need to consider alternative methods of engaging with general practices.

3.6 Summary

The AmSurv system collated routine reports of all bacterial isolates tested against antimicrobials, rather than the small proportion of bacteria that laboratories have a statutory requirement to report under the Health Protection (Notifications)

Regulations 2010 (UK Government Legislation, 2010).The development of AmWeb therefore provided a tool for health professionals to interrogate a complete range of AMR surveillance data, produce reports relevant to their geographic area, and identify the first appearance of new or emerging resistance. It also provided an opportunity, for the first time in England, to review variation in laboratory to laboratory antimicrobial susceptibility testing as a first step to identifying and understanding the reasons behind the observed differences.

Culture-based susceptibility testing information is rarely available to the community clinician at the time of therapeutic decision-making, and there can be geographical differences in susceptibility to specific antimicrobials, (Felmingham, 2002;Gupta et al, 2001;Howard et al., 2001). Therefore timely antibiotic susceptibility data, filtered by hospital or community samples, and viewed by local geographies has the potential to inform local prescribing.

The majority of responders to the survey of recipients of the West Midlands Community AMR Bulletin found the bulletin was useful and relevant for their practice; however the low response rate and comments received suggest alternative methods are required to engage with community healthcare professionals.

4 Antimicrobial resistance information and prescribing guidance used in the management of urinary tract infections: a survey of general practitioners in the West Midlands

4.1 Background

The relation of observed susceptibility testing results to the population being studied is a challenge for surveillance systems that are based on routine reporting by microbiology laboratories. The interpretation of these data are dependent on an understanding of the frequency of sending specimens for microbiological examination and how the various presentations of potential UTI are managed in the community. Although national guidelines for the management of UTI are provided in many European countries, there is a paucity of information on adherence to these policies by general practitioners (Hummers-Pradier et al., 2005).

As described in Chapter 3, antimicrobial susceptibility data from diagnostic microbiology laboratories can be used to monitor temporal trends and emerging antibiotic resistance. AMR data are gathered on bacteria isolated from specimens submitted to laboratories by clinicians in hospitals and the community, and therefore may be subject to selection bias due to over sampling of patients with initial treatment failures, complicated clinical histories or severe infections (Hillier et al., 2006a;McNulty et al., 2004). There is evidence to suggest that there is substantial variability in local sampling policies. For instance, an English study in 2004 found differences in taking urine specimens between practices, ranging from 29 to 266 urine specimens/1000 registered patients/year (McNulty et al, 2004). A Welsh study found a similar range, with specimen submission rates varying from 0.6 to 237.2 urine specimens/1000 registered patients/year (Howard et al., 2001a).

A linear relationship between trends in antibiotic consumption and antibiotic resistance, for many antibiotic and organism combinations, has been described in the

literature (Bell et al., 2014;Costelloe et al., 2010). Therefore it is plausible that practices that prescribe greater quantities of antibiotics will select higher levels of antibiotic resistance in their practice population, and this hypothesis is explored in Chapter 6. The amount of antibiotics prescribed in the community varies between practices. A study in England in 2009 reported a fivefold difference in antibiotic prescribing volume between general practices, with the authors reporting that the strongest predictor of higher antibiotic prescribing was being located in the north of England (Wang et al., 2009a). In 2014, 74% of antibiotic prescribing occurred in community settings (Public Health England, 2014a). Variation in antibiotic prescribing rates in general practices have been shown to be negatively associated with variation in observed antibiotic resistance in the local population (Howard et al., 2001b;Vellinga et al., 2012). National guidance for the management of infections and prescribing in the community has not reduced the variation in antibiotic prescribing across general practices in the UK, particularly in the management of upper respiratory and urinary tract infections (Hawker et al., 2014).

UTI is one of the most common infections found in community settings, with associated medical and financial implications for patients contracting these infections and those providing healthcare (Foxman, 2003). The management of UTI in the community should focus on patient safety and efficacy of treatments by considering factors such as: local *in vitro* susceptibility of bacterial pathogens, adverse effects of treatment (or non-treatment) and cost-effectiveness (Gupta et al., 2001a). Widespread variation in the management of UTI in the community has been reported. A survey in the US found that out of 137 responses, there were 82 different management strategies for the management of uncomplicated UTI (Berg, 1991).

In the West Midlands, a survey of local antibiotic prescribing by the West Midlands Strategic Health Authority was undertaken in 2010. This survey demonstrated significant variation between PCTs for commonly prescribed antibiotics in the community, showing a two-fold variation in prescribing quinolones and co-amoxiclav, with a four-fold difference between PCTs prescribing cephalosporin in the period 2007-2011 (R. Seal, personal communication). The reported variation in antibiotic prescribing across the West Midlands region, and the observed variance in the proportion of urinary isolates non-susceptible to antibiotics in the community in AmWeb reports (Chapter 3), prompted a review of variables such as local prescribing formularies and local microbiology sampling policies used by GPs in order to identify and quantify potential confounders and bias within the AMR surveillance dataset. This chapter describes a survey of GPs in the West Midlands to help determine the role of prescribing formularies and practice protocols in the management of UTI.

4.2 Objective

To conduct a survey among general practitioners (GPs) in the West Midlands to better understand some of the organisational and behavioural factors driving variation in both antibiotic prescribing and the taking of urine specimens for diagnostic microbiology, and thereby aid the interpretation of routine AMR surveillance data.

4.3 Methods

4.3.1 Setting / population

The survey was designed for GPs working within practices in the West Midlands region of England. As described previously, in 2012 there were 950 general practices with a total of 3635 general practitioners responsible for 5.8 million registered patients. Each practice had an average of four GPs with an average practice list size of just over 6,000 patients (NHS Digital, 2014).

4.3.2 Survey of GPs in the West Midlands

A cross-sectional survey was conducted during November 2012 to February 2013 among GPs providing community healthcare in the West Midlands. Community healthcare was defined as ambulatory primary healthcare delivered by registered GPs working within practices in the West Midlands.

The survey was developed using a template from an earlier Welsh study (Hillier et al, 2006a) and consisted of 17 questions divided into four sections (Appendix 2). Section one collected demographic data related to the practice and GPs. Section 2 elicited information on policies for the management of UTI, comprising questions on the use and source of prescribing formularies, existence of practice policies for urine sampling; how microbiological results influenced antibiotic prescribing; and an estimate of the proportion of patients clinically suspected as having a UTI for which urine specimens were requested. Section 3 described five hypothetical clinical scenarios (A to E) involving potential UTI presentations and GPs were asked whether

they would request a specimen and/or prescribe antibiotics empirically (Table 4.1). Section 4 captured free text comments from the respondents.

Table 4.1 Clinical scenarios presented in the survey

Scenario A: Treatment failure in a young woman	A 20 year old lady re-attends surgery and complains that the loin pain and frequent urination symptoms reported to you the previous week had worsened despite finishing a complete course of trimethoprim (no sample was taken previously).
Scenario B: Probable uncomplicated UTI	A 43 year old woman complains of pain passing urine and frequency. She feels well otherwise and has not previously been treated for a UTI.
Scenario C: Probable UTI in an adult male	A 51 year-old man attends your surgery complaining of pain passing urine and perineal tenderness. On examination you find suprapubic tenderness and a temperature of 38.5 C is measured.
Scenario D: Possible asymptomatic UTI in pregnancy	During a routine antenatal clinic an 18 year old girl who is 20 weeks pregnant produces a cloudy urine sample. She reports no symptoms or discomfort. The urine dipstick tests positive for nitrite but negative for leukocytes and protein.
Scenario E: Catheterised asymptomatic elderly female	You visit an 82 year old female in a nursing home. She is catheterised, afebrile and has no symptoms but the staff inform you that the urine is cloudy.

In October 2012, five GP practices were randomly selected from the sampling frame of all GP practices in the West Midlands, and invited to pilot the questionnaire. Two of these practices, consisting of 20 registered GPs participated in the pilot and the feedback received was used to improve the questionnaire.

The final questionnaire was produced and hosted online using SelectSurvey.net (ClassApps, USA). No sample size calculation was undertaken as all eligible practices were invited to complete the survey via email during November 2012. One email reminder was sent out to practices in January 2013 and the survey closed in February 2013. Not all of the questions were answered by all the responding GPs. Therefore response proportions detailed in the following result sections are based on the number of responses (n) to the individual questions.

4.3.3 Statistical analysis

The survey data were collated using Microsoft Excel (Microsoft Redmond, WA). Categorical variables were summarised as counts and proportions with differences between male and female GPs tested using a two-proportion Z test with $p < 0.05$ considered statistically significant. All statistical analyses were performed using STATA v12 (StataCorp, USA). All free text comments were analysed by thematic analysis.

4.4 Results

4.4.1 Survey response

The response rate was 11.3% (409/3635 GPs) equivalent to a practice response rate of 26% (248/950). The age group distribution of respondents were 10% aged under 35 years, 31% aged 35-45 years, 44% were aged 46-55 years and 16% were over 55 years old. The gender distribution of the responders was similar, with 54% of the GPs being female (222/409), which compares to 44% of all GPs in the West Midlands being female. Eleven percent of GP responders had been qualified for less than 10 years; however, a majority (62%) of responders had been qualified for 20 or more years. The age range of the responders was comparable with the demographic profile of all GPs in the West Midlands (NHS Digital, 2014).

4.4.2 Use of prescribing formularies

Eighty-six percent (314/366) of respondents reported that they used antibiotic prescribing formularies to guide prescribing decisions. The majority of these respondents (73%; 269/366) stated that they used a formulary provided by their PCT; with 45 (12%) reporting using more than one formulary (Table 4.2). Thirty four percent (123/366) had reviewed compliance with the existing policy for the management of UTI in the last 12 months.

4.4.3 Influence of laboratory AMR results on antibiotic prescribing

Two hundred and fifty (70%) respondents indicated that susceptibility results always or frequently influenced their antibiotic prescribing decisions for UTI. There was a significant difference (79% vs. 68%; p=0.016) between female and male GPs in the use of laboratory results to guide prescribing following treatment failure (Table 4.3). Only 6/362 (2%) GPs reported that laboratory results infrequently or never influenced their prescribing in the case of reported resistance to initial therapy.

The proportion of GPs that indicated that laboratory reports always influenced their prescribing habits was slightly higher in the <35 years age group for each scenario, with 95% (35/37) of the <35 age group reporting that laboratory results always influenced their prescribing when resistance is reported by the laboratory to the initial agent (Table 4.4).

Table 4.2 Reported source of antibiotic prescribing formularies/ prescribing guidance used by survey respondents (N=352).

Source of antibiotic formulary	Number using source ǂ
Primary Care Trust +	269
British National Formulary	46
Local area prescribing committee	17
Practice formulary	13
Local NHS Microbiology department	6
NHS Hospital/Trust	4
Health Protection Agency (now part of Public Health England)	3
NICE	1

+ On April 2013, PCTs were replaced by Clinical Commissioning Groups

ǂ Note some respondents listed more than one source

Table 4.3 Influence of laboratory results on antibiotic prescribing decision (number answering category / number of respondents)

	Male				Female			
	Always	**Frequently**	**Infrequently**	**Never**	**Always**	**Frequently**	**Infrequently**	**Never**
General prescribing	22% (37/167)	46% (77/167)	25% (41/167)	7% (11/167)	21% (40/190)	51% (97/190)	22% (42/190)	6% (12/190)
In the case of a treatment failure	68% (114/168)*	29% (49/168)	2% (3/168)	1% (1/168)	79% (154/195)*	19% (38/195)	1% (2/195)	1% (1/195)
When resistance is reported to initial prescribed agent	81% (136/168)	16% (27/168)	2% (4/168)	1% (1/168)	86% (168/195)	13% (26/195)	1% (1/195)	0% (0/195)

*Significant statistical difference between male and female response (p=0.016)

Table 4.4 Number of GPs indicating that laboratory results always influence empirical prescribing by age group

	Age of GP respondents			
Always influenced by laboratory results	**<35 years**	**35-45 years**	**46-55 years**	**>55 years**
For general empirical prescribing:	28% (10/36)	21% (23/109)	21% (35/163)	18% (9/49)
In the case of a treatment failure:	81% (30/37)	72% (79/110)	72% (119/165)	80% (40/50)
When resistance is reported to initial prescribed agent:	95% (35/37)	84% (92/110)	82% (136/166)	82% (41/50)

4.4.4 Factors influencing GPs decision to send urine specimens for analysis

Half (183/366) of the respondents reported that their surgery had a policy to inform on the criteria for taking urine samples to send for microbiological examination. There was considerable variation among respondents regarding the approximate proportion of clinical consultations for suspected UTI that resulted in a urine specimen being sent for diagnostic microbiology (median 50%, IQR 30% to 75%). Fourteen percent (50/365) of respondents suggested that they sampled 20% or less urines from their patients, whereas 82/365 (23%) of respondent GPs reported they would sample urine specimens from 80% or more of their patients (Table 4.5).

Table 4.5 Estimates by GPs of proportion of patients with clinically suspected UTI on whom the practitioner would submit specimens to the laboratory

Range of urine specimens collected (%)	No of GPs within range (n=365)	Proportion of GPs within range
0-9	14	4%
10-19	36	10%
20-29	40	11%
30-39	57	16%
40-49	78	21%
50-59	1	0%
60-69	23	6%
70-79	34	9%
80-89	25	7%
90-100	57	16%

4.4.5 Clinical Scenarios

In scenarios A, C and D (Table 4.6) the majority of GPs would submit a urine specimen for diagnostic microbiology (98%, 98% and 97% respectively), which is in-line with Public Health England (PHE) national guidance (Public Health England, 2014a). In scenario B, 40% of GPs indicated that they would submit a urine specimen for microbiological testing, even though PHE guidance recommends samples should not be sent for examination routinely for uncomplicated UTI in female adults <65 years of age. In scenario E, 38% of GPs reported that they would submit a urine sample, which is contrary to PHE guidance, which recommends that urine specimens should only be sent for examination in catheterized patients when features of systemic infection are observed (Public Health England, 2014a).

A higher proportion of female GPs (46% compared with 36% males, p=0.057) indicated that they would collect a urine specimen in scenario B, probable uncomplicated UTI (Table 4.7). The majority of GPs follow PHE guidance (Public Health England, 2014a) by prescribing an antibiotic empirically for probable treatment failure (scenario A, 80%), suspected uncomplicated UTI (scenario B, 78%) and probable UTI in a male adult (scenario C, 98%) (Table 4.6). There was significant variation between male and female GPs for prescribing antibiotics in the suspected UTI in pregnancy scenario (scenario D) where 43% of female GPs would prescribe compared with 30% of male GPs(p=0.0123) (Table 4.8). There was also a difference in urine sampling between genders for the catheterised asymptomatic elderly female scenario (scenario E) with 32% of male GPs indicating they would submit a sample, compared with 43% of female GPs (p=0.034) (Table 4.9).

Table 4.6 Count and percentage of GPs requesting urine samples and prescribing antibiotics for each clinical scenario

Clinical scenarios	**Number (%) of GPs requesting a specimen**	**Number (%) of GPs that would prescribe an antibiotic**
A. Treatment failure in a young women	344/352 (98%)	284/353 (80%)
B. Probable uncomplicated UTI	144/359 (40%)	270/345 (78%)
C. Probable UTI in an adult male	348/354 (98%)	344/352 (98%)
D. Possible asymptomatic UTI in pregnancy	341/353 (97%)	129/352 (37%)
E. Catheterised asymptomatic elderly female	134/354 (38%)	5/348 (1%)

One hundred and four (104/409, 25%) GPs entered additional free text comments. The main themes emerging from the analyses were the use of urinary dipstick test to investigate UTI in some of the scenarios presented, particularly scenario A (55/104, 53%); the need to gather additional clinical information (15/104, 14%); inclination to send urine specimens by default (14/104, 13%), and influence of the timing of the consultation in determining whether to take a specimen due to specimen transport issues (8/104, 8%).

Table 4.7 Responses for probable uncomplicated UTI, Clinical Scenario B

Scenario B. A 43 year old woman complains of pain passing urine and frequency. She feels well otherwise and has not previously been treated for a UTI.	**Male**			**Female**			**Difference in 'Yes' returns (male/female)**
	Yes	**No**	**Response Total**	**Yes**	**No**	**Response Total**	
Would you collect a urine sample for microbiological examination?	36% (59)	64% (105)	**164**	46% (86)	54% (101)	**187**	z= -1.901 (p= 0.0573)
Would you prescribe an antibiotic?	79% (127)	21% (34)	**161**	77% (143)	22% (42)	**185**	z= 0.3551 (p= 0.7225)

Table 4.8 Responses for possible UTI in pregnancy scenario, Clinical Scenario D

Scenario D. During a routine antenatal clinic an 18 year old girl who is 20 weeks pregnant produces a cloudy urine sample. She reports no symptoms or discomfort. The urine dipstick tests positive for nitrite but negative for leukocytes and protein.							
	Male			**Female**			**Difference in 'Yes' returns (male/female)**
	Yes	**No**	**Response Total**	**Yes**	**No**	**Response Total**	
Would you collect a urine sample for microbiological examination?	94% (155)	6% (10)	**165**	99% (187)	1% (2)	**189**	z = -2.5945 (p = 0.0095)
Would you prescribe an antibiotic?	30% (49)	70% (116)	**165**	43% (80)	57% (108)	**188**	z = -2.5027 (p = 0.0123)

Table 4.9 Responses for catheterised asymptomatic elderly female, Clinical Scenario E

Scenario E. You visit an 82 year old female in a nursing home. She is catheterised, afebrile and has no symptoms but the staff inform you that the urine is cloudy.							
	Male			**Female**			**Difference in 'Yes' returns (male/female)**
	Yes	**No**	**Response Total**	**Yes**	**No**	**Response Total**	
Would you collect a urine sample for microbiological examination?	32% (53)	68% (113)	**166**	43% (81)	57% (108)	**189**	z = -2.1196 (p = 0.0340)
Would you prescribe an antibiotic?	2% (3)	98% (161)	**164**	1% (2)	99% (183)	**185**	z = 0.5870 (p = 0.5572)

4.5 Discussion

This study examined the role of specific organisational and behavioral factors in the observed variation of urine sampling for diagnostic microbiology, and antibiotic prescribing for patients with UTIs among GPs in the West Midlands region of England. Although the response overall rate was low, the responders were representative in terms of age and gender to the GP population in the West Midlands.

The response rate of 11.3% of West Midland GPs, covering 26% of practices was similar to a Welsh study (16% of GPs covering 20% of practices); although the Welsh approach involved recruiting targeted practices, offering financial incentives and following-up with phone calls (Hillier et al, 2006a).

4.5.1 Specimen collection

A commonly cited issue in interpreting routinely reported AMR data from community settings is sampling bias (McNulty et al., 2006a), which may lead to observed levels of resistance that overestimate the burden of AMR in the general population. Only half of the GPs who responded reported having a practice policy to guide clinical sampling for diagnostic microbiology. Ideally, surveillance systems for UTI and AMR require the standardised submission of urine specimens by GP practices to laboratories for microbiological analysis (McNulty et al, 2004). National guidelines do provide recommendations on when to submit urine for analysis (Public Health England, 2014b); however, it appears that these are not being universally adopted,

with a study in the South West of England in 2004 reporting 10-fold differences in urine submission rates between general practices (McNulty et al, 2004).

As the volume of urine samples makes up a large proportion of a laboratory's workload, laboratories have a role, alongside commissioning bodies, in influencing the specimen submission policies for primary care healthcare providers (Morency-Potvin et al., 2017). Testing methods, and their associated costs, vary between laboratories (as described in Chapter 2), which may introduce bespoke commissioning of services and therefore influence submission protocols. Projected laboratory cost-savings introduced following a major review of NHS pathology services may dictate the services that local laboratories are able to offer in the near future, and thereby have an impact on community sampling policies (Department of Health, 2008).

In the present survey there was considerable variation between GPs in the estimated proportion of clinical consultations for suspected UTI in which a urine specimen is sent for diagnostic microbiology. However it was found that by using scenario questions, the response was broadly consistent for the scenarios involving: treatment failure, probable UTI in an adult male and possible UTI in pregnancy, and therefore using clinical scenarios may provide a more reliable insight into GP sampling practice than relying on a general view of GPs prescribing habits (Hillier et al, 2006a).

A survey of females in England in 2014 reported that 76% of those reporting symptoms of UTI had urine samples taken (with 52% receiving immediate local testing results), and 25% reporting that their urine was sent to the laboratory (Butler et al., 2015). The present survey showed that 40% of GPs would submit a sample

for diagnosis of the most commonly encountered presentation of uncomplicated UTIs, although PHE guidance recommends not sending urine samples for this presentation (Public Health England, 2014b). These high levels of sampling and local testing are also contrary to evidence of poor negative predictive value from dipstick analysis (Little et al., 2010). If the finding of 40% of urines being sent to laboratories from uncomplicated UTI is representative of GPs in the West Midlands then this type of sampling would be responsible for a considerable proportion of the 500,000 / year urine specimens sent for microbiological examination. This is a similar finding to a study in Wales in 2006 that found 56% of randomly selected GPs would submit a urine specimen for probable uncomplicated UTI (Hillier et al., 2006b). Also PHE guidance for management of UTIs in catheterised patients recommends that a urine sample should only be sent if there are signs of systemic infection (Public Health England, 2014a), however 38% of respondents would send a urine specimen in the catheterised asymptomatic elderly female scenario (Table 4.6). In the thematic analysis of free-text comments, 14 of the 104 responders in this section indicated that they would send urine specimens for all suspected urinary infections.

A study of diagnosis of UTI in Germany in 2004 found that GPs clinical diagnostic accuracy of UTI was low, even with the use of dipstick indicators, suggesting that over-treatment, with inappropriate use of antibiotics, would be avoided by taking more specimens for culture; however the authors do acknowledge the increased costs of this approach (Hummers-Pradier et al, 2005). It has also been suggested that increased use of diagnostic services is more successful than targeted antimicrobial stewardship interventions in improving appropriate prescribing in the community (van Buul et al., 2015); however with the cost of empirical treatment often

being less than the cost of the microbiological analysis, not taking specimens can save valuable health resources (McNulty et al., 2006a).

4.5.2 Prescribing

The majority of respondents in the present survey used local prescribing formularies produced by their PCTs. As PCTs were abolished at the end of March 2013 and replaced with Clinical Commissioning Groups (CCGs), it is not known whether these formularies have been updated and are still being utilised. In November 2012 a national antimicrobial stewardship (AMS) toolkit was launched for primary care called Treat Antibiotics Responsibly, Guidance, Education, Tools (TARGET), which provides prescribing guidance, access to prescribing surveillance data and audit tools (Moore and McNulty, 2012). A study in 2016 on behalf of the English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) evaluated the uptake of the TARGET AMS toolkit by CCGs and found that 60% of the 82 responding CCGs had reviewed the toolkit; however only 13% had AMR action plans to implement the recommendations. The authors reported that in CCGs with a dedicated antimicrobial pharmacist leading the implementation of the toolkit, more time was dedicated to antimicrobial stewardship activities; however only 5% of CCGs had an antimicrobial pharmacist in post (Ashiru-Oredope et al., 2016).

In the present study a small proportion of respondents (14%) indicated that they did not use a prescribing formulary to guide treatment decisions. A study in Canada reviewed provincial prescribing formularies during 2010 and compared these to actual prescribing practice. This study reported a wide variation in prescribing rates, but found no significant correlation between prescribing rates for provinces that had strictly-regulated formularies and those that had more flexible approaches. The

authors suggested that educational programmes and treatment guidance had a greater effect on prescribing habits (Glass-Kaastra et al., 2014).

From the study described in this chapter, it is not possible to ascertain whether the non-utilisation of an antibiotic formulary by GPs results in inappropriate prescribing. Therefore it is recommended that use of formularies is routinely assessed through the regular auditing and feedback of individual prescribing patterns, combined with the implementation of other interventions to address inappropriate prescribing as part of a wider antimicrobial stewardship programme.

Only six GPs cited microbiology laboratories and three GPs cited the Health Protection Agency (HPA, to become part of PHE in 2013) as the source of their prescribing formularies. Microbiologists can play an important role in antimicrobial stewardship by providing local resistance profiles and actively participating in CCG stewardship committees (Morency-Potvin et al, 2017). It is likely that the PCTs based their formularies on national guidance or have input from local laboratories; however microbiologists and pharmacists wishing to influence local prescribing practice will need to engage with the new commissioning bodies to ensure the production of evidence based guidance. A number of studies have found that the selection of antibiotics reported on microbiology forms and interpretive comments, influence prescribing decisions. A study in the South West of England found that cephalexin prescribing for UTI increased when this was included on the laboratory report and co-amoxiclav prescribing decreased when this was removed from the report (McNulty et al., 2011). A recent Australian study found that withholding antibiotic results on microbiology forms for bacteria suspected of colonisation rather than causing infection reduces antibiotic use (Papanicolas et al., 2017).

In the present survey, seventy percent of GP responders stated that laboratory susceptibility results frequently or always influenced their prescribing, with the proportion being slightly higher for female GPs and GPs under the age of 35. However, based on their response to the scenario questions, most GPs would prescribe empirically, suggesting that previous laboratory results may influence their choice of empirical agents.

The symptoms of UTI are often distressing to the patient, requiring immediate empirical therapy (Gupta et al., 2001b). In the clinical scenarios most GPs would prescribe an antibiotic empirically for scenarios A, B and C (Table 4.6), which is in-line with national PHE guidance (Public Health England, 2014b); although finding that a fifth of GP respondents would not prescribe an antibiotic in the treatment failure scenario (scenario A) given the presence of worsening symptoms was unexpected.

National guidance for the management of UTI in the community recommends that antibiotic treatment should not be given for suspected UTI in pregnancy unless bacteruria is confirmed by laboratory culture (Public Health England, 2014b); although the survey reported in this chapter found over a third of GP respondents do not follow this guidance and would prescribe antibiotics empirically in these cases.

4.5.3 Gender of prescriber

There was a slightly higher proportion of female GP responders (54%) than male GPs in this survey, which is a higher proportion of female GPs than that found in all West Midland GPs (44%), suggesting that response rate was higher amongst this gender group. The survey did show that the gender of the GP was a factor in the

responses to some of the survey questions, with a greater proportion of female GPs reporting being influenced by laboratory results, taking specimens and prescribing in scenarios D and E.

A possible explanation for this variation may be differences in patient empathy with particular patients groups or difference in the desire to meet patient expectations (Coenen et al., 2006). A large English study in 2009 found a higher proportion of male GPs prescribing antibiotics in the community, and suggested male GPs perceive a greater pressure from patients to prescribe (Wang et al., 2009b). A Belgium study reviewing prescribing in 2002-2009 reported that the gender of the prescriber may influence the type of antibiotic given, as the authors found male prescribers were more likely to prescribe broader spectrum antibiotics (Blommaert et al., 2013). It is therefore suggested that further behavioural studies are required to better understand variation in prescribing between genders and help inform the design of interventions aimed at changing prescribing habits. Another area for further study, which was not possible to investigate in the survey reported in this chapter, was gender difference in patients receiving antibiotics. A study in Germany in 2016 reported that females between 16 and 34 years old were prescribed 36% more antibiotics than males, and this increased to 40% more for females between 35 and 54 years old (Schroder et al., 2016)

4.5.4 Primary care guidance

The results of this survey indicate GP non-compliance with guidance for certain clinical scenarios and a degree of inappropriate microbiological testing. A German

study in 2005 concluded that most patients in their study were not treated according to current guidelines and for half the patients the decision to prescribe an antibiotic or the antibiotic prescribed was inappropriate, with a quarter of the patients having a bacterial infection that was resistant to the prescribed antibiotic (Hummers-Pradier et al, 2005).

It is plausible that this non-compliance with the guidance may be driven by ambiguity in the advice provided by existing national guidance. The National Institute for Health and Care Excellence (NICE) Clinical Knowledge Summaries advise that a urine sample should be sent to the laboratory for all women with suspected UTI associated with visible or non-visible haematuria (NICE guidelines, 2015); however PHE guidance advises that urine samples should not be routinely submitted from women <65 of age, and if there are signs of UTI, including haematuria, then only empirical treatment should be given (Public Health England, 2014a). Both guidelines need to be reviewed so that unambiguous evidence based guidance is made available to GPs.

Prescribing in general practice is not only influenced by the availability of national or local guidance but also factors such as prescriber's gender, socio-economic deprivation, geographical area and clinical autonomy (Mason, 2008;Wang et al, 2009a). Prescribing in secondary care occurs in a much more controlled environment, with doctors working in teams which include pharmacists and infection control physicians. Prescribing by individual doctors in secondary care is often the subject of frequent reviews and may be changed by other members of the healthcare team. GPs have considerable clinical autonomy in prescribing and have much less diagnostic support; however community prescribers are increasingly aware of

national / local guidance and the monitoring of both prescribing habits and adherence to guidance (Mason, 2008).

Whilst acknowledging the importance of autonomy in clinical decision making, there is value in developing and utilising standardised, evidence-based sampling policies to ensure that diagnostic and treatment decisions are both clinically effective and cost-effective (McNulty et al, 2004). Increasingly limited healthcare resources make a compelling case for standardising sampling policies, but this will only be achieved with consensus between microbiologists, community clinicians and policy makers.

4.5.5 Study limitations and next steps

There were some limitations to the present study. The low response rate raises the possibility of non-response bias and its potential effect on the external validity of the study. It is believed that any effect on these estimates and the generalisability of these findings is low given that the demographic profile of our respondents is similar to that of all GPs in the West Midlands. In the free text comments, three GP respondents indicated that they may delay prescribing in some of the clinical scenarios; however the ‘yes’ or ‘no’ response options to these questions prevented the capture of this information.

Our analyses and interpretation of the free text comments may not be representative of the cohort of respondents as the number of comments was relatively small. However emerging themes from the analysis of these comments suggests that some GPs may be more inclined to send urine specimens by default. This needs to be

explored further using alternative qualitative research methods such as focus groups of GPs.

The next steps include a survey of CCGs to determine whether antibiotic prescribing formularies developed by the PCTs are still being used and updated since the abolition of PCTs. We are also currently exploring the use of mobile device technologies to deliver timely localised AMR surveillance data and national prescribing guidance directly to clinicians in community settings and healthcare commissioners to support the management of UTI.

4.6 Summary

Understanding the knowledge and attitude of GPs towards the management of UTI within a healthcare region will help understand bias within routine surveillance data and aid the interpretation of AMR in the community. This survey showed that national guidelines for the management of UTI are not followed consistently by GPs in the West Midlands. It is reasonable to assume that specimens will only be taken in primary care in treatment failures or in more complicated etiologies, leading to a sampling bias within routine surveillance systems; however this survey found that half of the responders did not have sampling policies and the answers to clinical scenario questions suggest a significant proportion of urines are sent for microbiological examination from the most common forms of UTI. The survey also found significant differences between male and female GPs in both the management

of UTI and decisions to prescribe, which may inform those designing local antibiotic stewardship interventions.

The delivery of clinical care of consistent high quality will benefit from the implementation of antimicrobial stewardship programmes in community settings that include prescribing formularies based on local AMR surveillance and unambiguous national guidance on the management of infections. Most prescribers in the West Midlands used formularies developed by their PCT. With the reduction in the number of community pharmacists and the formation of new commissioning bodies, on-going audit and feedback are required to ensure consistent policies are provided to local healthcare providers within the region. Evidence-based prescribing formularies and policies to guide clinical specimen sampling will also facilitate the cost-effective use of available laboratory, and other finite healthcare resources.

5 Surveillance of the antibiotic susceptibility of bacteria found in the urinary tract in the West Midlands over a four year period

5.1 Introduction

In 2002 the Chief Medical Officer (CMO) for England published a strategy document for combating infectious disease, which concluded that the surveillance systems available at the time were not adequate to protect public health as they were not able to determine the size and nature of the threat from infectious disease (Department of Health, 2002). At this time, infectious disease surveillance systems were focused on a select number of diseases and tended to be influenced by media coverage or political interventions (Boyce et al., 2009). Many of the systems in operation were focused on regional or national trends in infections and were often not adequate to detect local outbreaks of disease (Huang et al., 2010). In 2013 the current CMO for England published a five year AMR strategy and action plan calling for strengthened AMR surveillance, and a new focus on the Gram-negative bacteria that were linked with outbreaks of MDR infections occurring at the time. The strategy suggested key drug bug combinations that should be monitored in the UK (Table 5.1) (Department of Health, 2013).

As discussed in Chapter 3 (section 3.1.1), to address some of these gaps in AMR surveillance in England, particularly the surveillance of resistant bacteria in the community, Public Health England (PHE) implemented the AmSurv system to facilitate collection of all AMR reports from diagnostic laboratories in England. The development of a web-enabled reporting tool (AmWeb) in 2012 to allow laboratories and infection prevention and control teams timely access to AMR surveillance data was described in Chapter 3 (section 3.3.4).

Table 5.1 Antibiotic and bacteria combinations recommended for monitoring in the UK (Department of Health, 2013).

Multi-Drug Resistant Bacteria	**Metric**
Klebsiella spp - carbapenem	% non-susceptible to imipenem and/or meropenem
E. coli - carbapenem	% non-susceptible to imipenem and/or meropenem
E. coli - cephalosporin	% non-susceptible to cefotaxime and/or ceftazidime
E. coli – fluoroquinolone	% non-susceptible to ciprofloxacin
Pseudomonas - carbapenem	% non-susceptible to imipenem and/or meropenem
N. gonorrhoeae – ceftriaxone	% non-susceptible
Klebsiella spp - cephalosporin	% non-susceptible to cefotaxime and/or ceftazidime
Pseudomonas – cephalosporin	% non-susceptible to ceftazidime
E. coli – gentamicin	% non-susceptible
S. pneumoniae – penicillin	% non-susceptible

A key component in the response to the threats of increasing resistance amongst Gram-negative bacteria, and in particular resistance to third-generation cephalosporins and carbapenems in *Escherichia coli* and *Klebsiella pneumoniae*, is the routine reporting and monitoring of surveillance information on resistance patterns of isolates from various settings and specimens including those from urine samples.

International travel and population migration has a significant role in the spread of AMR (Hawkey, 2015). Computer software is now available to categorize populations into cultural, ethnic and linguistic (CEL) groups based on family names (Webber, 2007). This type of categorization has been used to associate lineage of bacterial strains with different population groups from around the world (Evans et al., 2010). Origins software (Experian, Nottingham, UK) is used in the study reported in this chapter to examine the association between CEL and multidrug resistant Gram-negative bacteria reported by laboratories in the West Midlands.

In the following sections in this chapter, the results from an analysis of routine laboratory-based surveillance data on bacteria isolated form urine specimens, collated from the West Midland laboratories over a four-year period, are presented.

5.2 Objective

To describe baseline antibiotic resistance levels among *E. coli*, *K. pneumoniae* and *Pseudomonas aeruginosa* isolated from urine samples submitted to all laboratories in the West Midlands, in order to support the monitoring of key organism / antibiotic

combinations as described in the UK Five Year AMR Strategy and to inform ongoing public health action.

5.3 Methods

5.3.1 Population and data sources

The West Midlands population has been described previously in Chapter 1, section 1.7. During the period of this study there were 15 diagnostic microbiology laboratories in the West Midlands serving both community-based centres and hospitals. The daily average of occupied hospital beds in the West Midlands during 2013 was 10,626 (NHS England). AMR surveillance data are captured via the AmSurv system, which collects all laboratory identification and antimicrobial susceptibility testing data directly from each laboratory information system. AmSurv designates all specimens sent from general practice surgeries and primary care clinics as community requests, and distinguishes these from specimens requested in hospitals by collecting data on the hospital sites for inpatients and requesting GP for community specimens. Nine of the West Midland laboratories were reporting data regularly to AmSurv at the start of our study in 2010, and complete coverage of all 15 laboratories was achieved in December 2012 (Figure 3.5).

It is requested that all Gram-negative bacteria suspected of producing a carbapenemase by diagnostic microbiology laboratories in England be referred to the national PHE Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for molecular confirmation and further characterisation

(Public Health England, 2014a). The AMRHAI Reference Unit provided this study with molecular carbapenemase detection test results for all isolates of *E. coli*, *Klebsiella* spp. or *Pseudomonas* spp. referred for confirmation of suspected carbapenemase production by West Midland laboratories during the four-year period (2010-2013).

5.3.2 Data extraction

The processing of laboratory files in the AmSurv database and de-duplication routines are described in Chapter 3 (section 3.3.3). Data were extracted from the AmSurv database using a combination of Microsoft SQL Server Management Studio and the AmWeb application (Chapter 3, section 3.3.4). A 14-day repeat exclusion rule was applied to the extracted data and to mitigate for selective testing, only records from laboratories testing ≥70% of each bacterial species against a particular antibiotic or antibiotic group were included (Table 5.2). Non-susceptibility to an antibiotic was defined as test results with a 'resistant' (R) or 'intermediate' (I) designation.

The bacteria and antibiotic combinations for clinical isolates recommended for monitoring in the UK Five Year AMR Strategy are listed in Table 5.1. Data were extracted based on these recommendations for the period 2010 to 2013, with the exception that *Klebsiella* spp. was replaced with *K. pneumoniae*, as recommended in a review of the strategy by the UK government Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infections (ARHAI, 2014); and a third-generation cephalosporin group was added, as initial data profiling showed that many

West Midlands laboratories follow UK guidance for detecting ESBLs, by testing cefpodoxime rather than cefotaxime for community isolates (Health Protection Agency, 2008).

Table 5.2 Number of West Midland laboratories consistently testing < 70% of isolates from urine specimens against specific antibiotics in 2010-2013 (n=15).

Organism	Antibiotic	Number of laboratories testing <70% of isolates
E. coli	Third-generation cephalosporin	2
	Ciprofloxacin	2
	Gentamicin	2
	Meropenem/imipenem	7
K. pneumoniae	Third-generation cephalosporin	2
	Meropenem/imipenem	2
P. aeruginosa	Third-generation cephalosporin	1
	Meropenem/imipenem	3

Denominator data were based on laboratory reports of the total number of urine specimens received during the study period from hospital and community settings. Information on the antimicrobial susceptibility testing methods employed in each laboratory was also obtained.

5.3.3 Determination of global origin and statistical methods

The proportions of West Midland urine specimens processed by laboratories and yielding *E. coli*, *K. pneumoniae* or *P. aeruginosa* were calculated by year. This calculation utilised an adjusted denominator that took into account the length of time that the laboratory had contributed to AmSurv during the qualifying year. Annual non-susceptibility proportions of each bacteria / antibiotic combination were calculated with trend analysis undertaken using chi-square statistic for trend to determine whether there was a statistically significant linear trend ($p<0.05$) over the study period. All statistical analysis was performed using STATA v12 (StataCorp, USA).

Origins software (Experian, Nottingham, UK) was used to determine the likely global origin of the names of patients with confirmed carbapenemase-producing isolates of *E. coli, Klebsiella* spp. or *Pseudomonas* spp.. Names were classified as belonging to one of 257 Cultural, Ethnic and Linguistic (CEL) codes representing the most likely cultural origin of the person's name. The CEL codes were grouped into international geographies used previously in this context.(Evans et al, 2010;Wickramasinghe et al., 2012)

5.4 Results

5.4.1 Routine surveillance data

During the four-year study period (2010-2013) there were 431,461 reports for *E. coli,* 23,786 for *K. pneumoniae*, and 6,985 for *P. aeruginosa* from urine specimens collected by laboratories in the West Midlands. These represented 61%, 3% and 1% respectively of the total isolates obtained from urine specimens sent from hospital patients and the community during the period.

The proportion of *E. coli* non-susceptible to antibiotics recommended for monitoring in the UK five year AMR Strategy is shown in Figure 5.1. During the period, there was a rising trend in reported non-susceptibility to third-generation cephalosporins for *E. coli* isolated from community and hospital sources, from 4.5% and 6.3%, respectively in 2010, to 5.5% and 7.7% in 2013 (*P for trend* < 0.001). Similarly, a rising trend was observed for non-susceptibility to ciprofloxacin in *E. coli* for both community and hospital isolates, from 9.4% and 13.5%, respectively in 2010, to 13.1% and 17.1% in 2013 (*P* < 0.01).

Only a small proportion of *E. coli* isolates were non-susceptible to meropenem and/or imipenem for community and hospital sources and this remained very low during the study period with no evidence of linear trend (*P*=0.09 and *P*=0.13 respectively). In *E. coli,* non-susceptibility to gentamicin fell between 2010 and 2011, from around 10% to 8% for hospital and 7% to 5% for community submitted specimens, and then remained stable for the remaining period (Figure 5.1).

During the study period, a rising trend in the proportion of *K. pneumoniae* non-susceptible to third-generation cephalosporins was also observed for both community and hospital isolates, from 3.8% and 8.5% respectively in 2010, to 10.1% and 15.9% in 2013 ($P < 0.001$). *K. pneumoniae* non-susceptibility to meropenem / imipenem remained very low from community and hospital sources, with no evidence of linear trend (P=0.12 and P=0.44 respectively) (Figure 5.2).

The proportion of *P. aeruginosa* exhibiting non-susceptibility to meropenem/imipenem fluctuated considerably, with values between 4.6% and 11.3%. Interestingly there appeared to be a mirroring of trend lines for non-susceptibility to meropenem and/or imipenem and ceftazidime for *P. aeruginosa*, which demonstrated significant correlation (P = 0.0013), with the ceftazidime range being 4.6% to 12.5% (Figure 5.3).

Among all isolates from urine specimens, *Pseudomonas* spp. had the highest number of reported non-susceptibility to carbapenems (n=786), followed by *E. coli* (n=254). The proportion non-susceptibility to carbapenems has remained relatively stable for target bacterial species during the study period. The only species with a marked difference in the proportion non-susceptible to carbapenems was *Acinetobacter* spp. with 41.12% non-susceptible in 2010 and non-susceptibility of 21.24%, 25.95% and 24.47% in the following 3 years (Table 5.3).

Figure 5.1 Non-susceptibility (%) of *E. coli* from West Midlands urine specimens to (a) third-generation cephalosporins, (b) meropenem and/or imipenem, c) gentamicin and (d) ciprofloxacin. Hospital source, dotted line; community source, solid line.

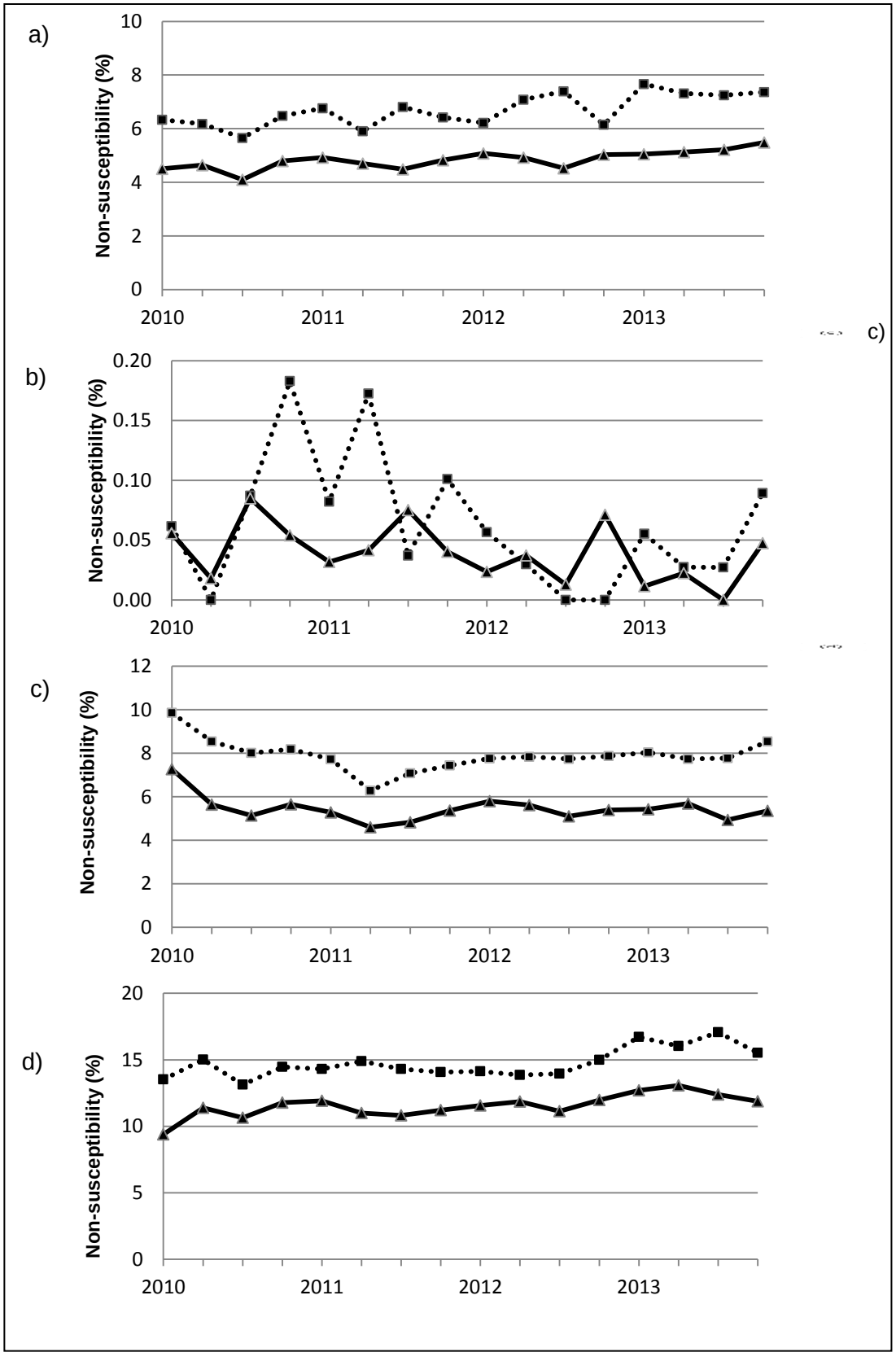


Figure 5.2 Non-susceptibility (%) of *K. pneumoniae* from West Midlands urine specimens to (a) third-generation cephalosporins, and (b) meropenem / imipenem. Hospital source, dotted line; community source, solid line.

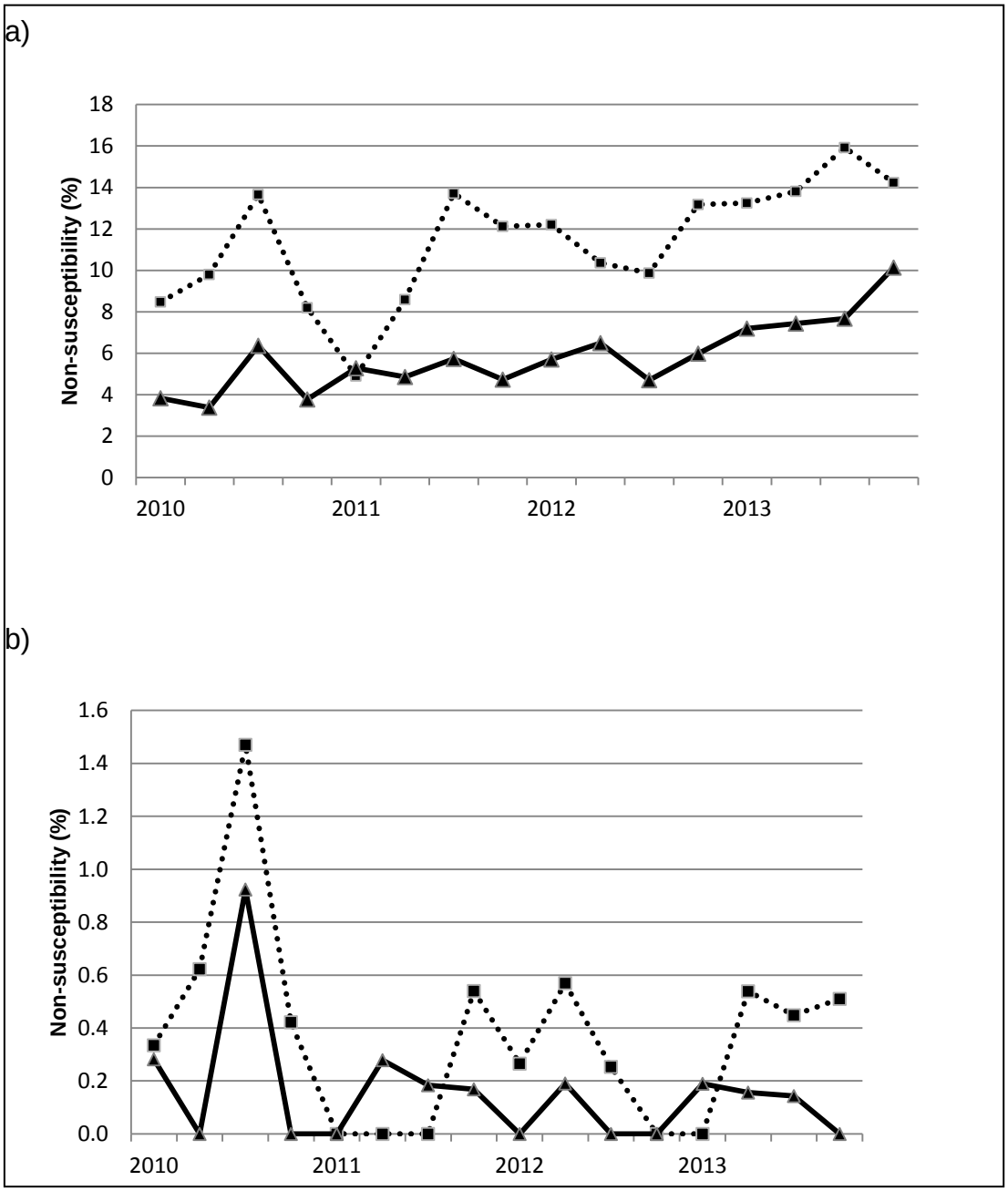


Figure 5.3 Non-susceptibility (%) of *P. aeruginosa* from West Midlands urine specimens to ceftazidime (dotted line) and meropenem / imipenem (solid line).

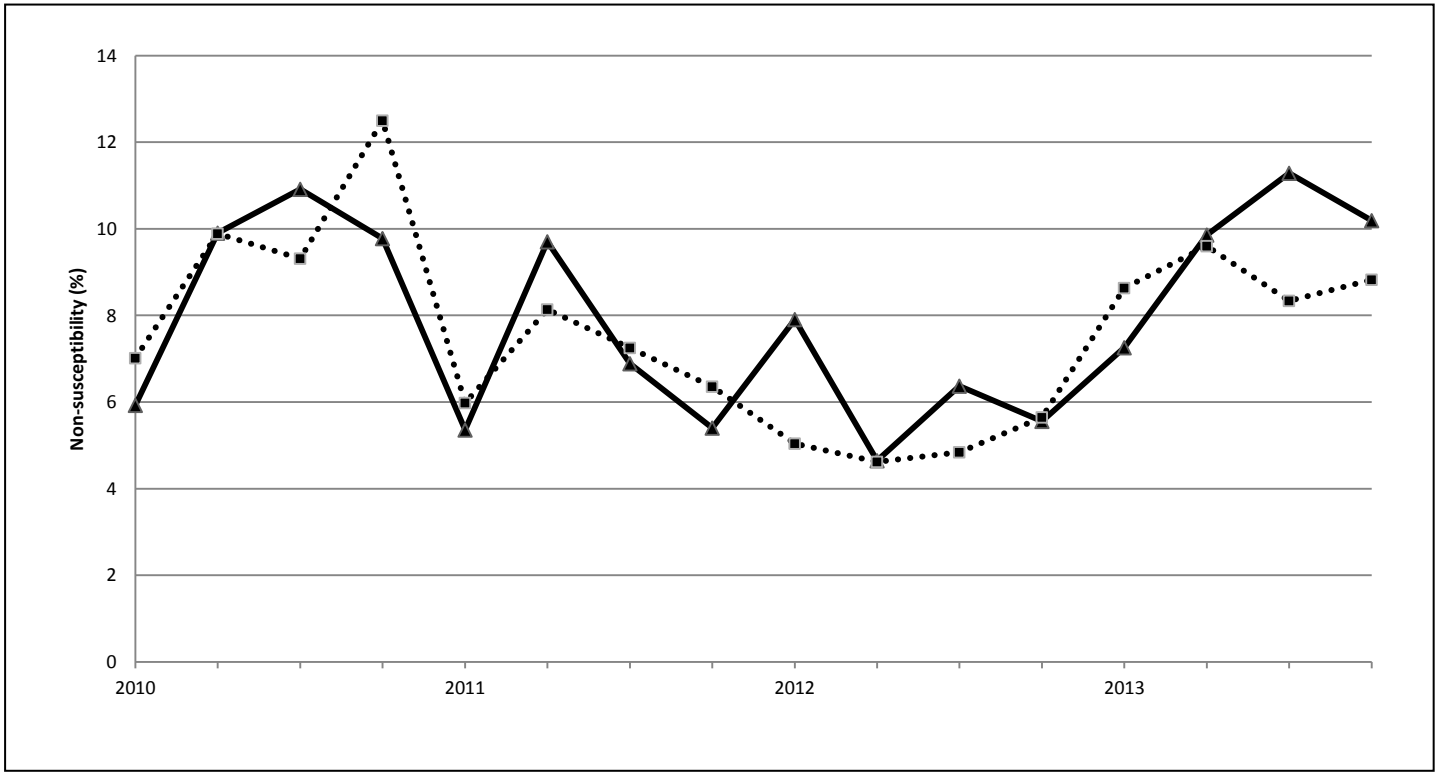


Table 5.3 Number of study target bacteria, (plus all other isolates below dotted-line) from urine specimens (% of total) that are non-susceptible to any carbapenem antibiotic, West Midlands, 2010 to 2013

Organism	2010	2011	2012	2013	Total	
Pseudomonas spp.	156 (6.18)	163 (3.57)	192 (3.38)	275 (4.22)	**786 (4.08)**	
E. coli	59 (0.10)	58 (0.06)	56 (0.05)	81 (0.05)	**254 (0.06)**	
K. pneumoniae	15 (0.40)	15 (0.32)	16 (0.22)	30 (0.37)	**76 (0.32)**	
Acinetobacter spp.	44 (41.12)	24 (21.24)	48 (25.95)	46 (24.47)	**162 (27.32)**	
Coliform	5 (0.37)	13 (0.13)	25 (0.14)	44 (0.19)	**87 (0.17)**	
S. maltophilia	7 (26.92)	11 (18.03)	14 (24.56)	16 (26.23)	**48 (23.41)**	
Klebsiella (other)	24 (1.42)	7 (0.21)	0 (0.00)	7 (0.23)	**38 (0.34)**	
Serratia spp.	11 (3.28)	8 (1.65)	6 (1.17)	8 (1.44)	**33 (1.75)**	
C. freundii	6 (1.84)	1 (0.22)	7 (1.17)	7 (1.24)	**21 (1.08)**	
All bacteria	**392 (0.52)**	**351 (0.28)**	**445 (0.26)**	**583 (0.28)**	**1771**	**31)**

5.4.2 Reference laboratory (AMRHAI) data

In this section results from the analysis of the data retrieved from the AMRHAI Reference Unit database is reported.

5.4.2.1 All referred bacterial isolates

In 2010 - 2013, 222 *E. coli* or *Klebsiella spp*. bacteria, isolated from any specimen type, were referred to AMRHAI from West Midland laboratories for confirmation of resistance and full characterisation of the bacteria (Table 5.4). The majority of these bacteria sent to the PHE AMRHAI reference unit were from urine specimens (45%), with 28 (13%) being recorded as unknown specimen types (Table 5.4).

There were 112 *E. coli* and 110 *Klebsiella* spp referred from all specimen types; with 9 (8%) and 41 (37%) respectively being confirmed as carbapenemase producers. The overall proportion of confirmed carbapenemase producers from *E. coli* or *K. pneumoniae* referred from all specimen types was 23% (50/222) (Table 5.4).

The specimen types designated as being of rectal or faecal origin are likely to be taken from patients as part of active screening programmes for MDR Gram-negative bacteria, rather than being taken from patients with clinical infections (Public Health England, 2014b). There were 36 *E. coli* or *Klebsiella* spp. sent to the AMRHAI reference unit from faecal or rectal specimens, which represented 16% of the total for these bacteria. The majority (25 of 36, 70%) of these bacteria referred from rectal or faecal specimens were sent in 2010-2011.

5.4.2.2 Isolates referred to AMRHAI from urine specimens

From all bacteria sent to AMRHAI Reference Unit in the study period for investigation of resistance mechanisms from both hospital and community settings, 174 isolates of

E. coli, *Klebsiella spp*. or *Pseudomonas spp.* were referred from urine specimens. The isolates were received from 147 unique patients; isolates from 28 patients (19%) were confirmed as carbapenemase-producing bacteria (n=11 *K. pneumoniae*, n=10 *Klebsiella sp*., n=4 *P. aeruginosa* and n=3 *E. coli*), with 16 (57%) identified as producing New Delhi metallo-beta-lactamase (NDM) (Table 5.5). Isolates from the remaining 119 patients did not have carbapenemase production confirmed.

Of the 119 bacterial isolates that were not confirmed as producing a carbapanemase, 50 (42%) of were determined as susceptible to carbapenems by BSAC MIC clinical breakpoints. The remainder were determined as non-susceptible to at least one carbapenem, but not producing a carbapenemase, and included 19 *E. coli* (all resistant to ertapenem, but susceptible to meropenem and imipenem), 13 *Klebsiella spp*. (all resistant to ertapenem, but susceptible to meropenem and imipenem) and 37 Pseudomonas spp. (non-susceptible to meropenem and/or imipenem).

Table 5.4 Number of requests (de-duplicated by specimen and patient) received by the AMRHAI reference laboratory from the West Midlands by specimen type 2010-2013 (AMRHAI laboratory information system data).

	Bacteria received by the AMRHAI unit (confirmed carbapenemase producers)								
	2010		2011		2012		2013		2010-2013
	E. coli	***Klebsiella spp.***	***E. coli***	***Klebsiella spp.***	***E. coli***	***Klebsiella spp.***	***E. coli***	***Klebsiella spp.***	
Urines	14 (1)	32 (14)	8 (0)	7 (1)	16 (0)	6 (1)	8 (2)	11 (5)	102 (24)
Not-stated	4 (0)	9 (3)	1 (0)	0 (0)	0 (0)	0 (0)	9 (0)	5 (2)	28 (5)
Faecal specimens	10 (1)	4 (0)	10 (0)	1 (1)	4 (0)	0 (0)	1 (0)	1 (1)	31 (3)
Blood culture	3 (2)	0 (0)	5 (0)	1 (0)	2 (0)	1 (1)	2 (0)	4 (2)	18 (5)
Swab (general)	3 (0)	1 (0)	1 (0)	2 (0)	4 (0)	0 (0)	2 (2)	4 (3)	17 (5)
Sputum	0 (0)	1 (0)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	4 (2)	7 (4)
Umbilicus	1 (0)	5 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (0)
Rectal swab	0 (0)	0 (0)	0 (0)	5 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (0)
Fluid	0 (0)	1 (0)	1 (0)	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	4 (2)
Peritoneum	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)
Placenta	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Other	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)
Total	35 (4)	53 (17)	28 (0)	19 (5)	26 (0)	7 (2)	23 (5)	31 (17)	222 (50)

Table 5.5 Number of isolates of *E. coli*, *Klebsiella spp*. and *Pseudomonas spp*. received (and carbapenemase confirmations) by the PHE AMRHAI Reference Unit from urine specimens received by West Midland laboratories, 2010-2013.

	E. coli	***Klebsiella* spp.**	***Pseudomonas* spp.**
2010	14 (1 NDM)	32 (11 NDM, 3 KPC)	18 (0)
2011	8 (0)	7 (1 NDM)	13 (2 VIM)
2012	16 (0)	6 (1 KPC)	6 (1 VIM)
2013	8 (1 KPC, 1 OXA-48)	11 (3 NDM, 2 OXA-48)	8 (1 VIM)

5.4.3 Assigning cultural, ethnic and linguistic origin

The names of patients with confirmed carbapenemase-producing bacteria isolated from urine specimens (Table 5.5) were categorised as being of Middle East/South Asia (n = 6) and Europe (n = 22) cultural, ethnic and linguistic (CEL) origin. Fourteen of the 16 (88%) patients with NDM carbapenemase-producing bacteria were grouped as having European CEL origin. The carbapenemase-producing isolates from the six names categorised as of Middle East/South Asia origin were 3 VIMs, 2 NDMs and 1 OXA-48.

5.4.4 Proportion of urines reported with a bacterial isolate

In 2010 to 2013, the annual number of urine samples submitted for microbiological examination in the West Midlands remained relatively constant at around 1.1 million, with approximately 55% of these being received from the community (Figure 5.4).

The proportion of *E. coli* isolates (15%) from urine specimens submitted from community settings was higher than those from hospital settings (6%) (Figure 5.4). The proportion of *K. pneumoniae* from community isolates was also slightly higher than those isolated from hospital (0.7% and 0.5% respectively), with the proportion of *P. aeruginosa* similar from both settings during the study period (0.2%). The total number of urine specimens received in 2010–2013 by individual laboratories varied considerably, with different catchment populations and sizes of hospitals served (Figure 5.5).

Figure 5.4 Total number of urine specimens received and proportion (%) positive for *E. coli*, West Midland laboratories, 2010-2013.

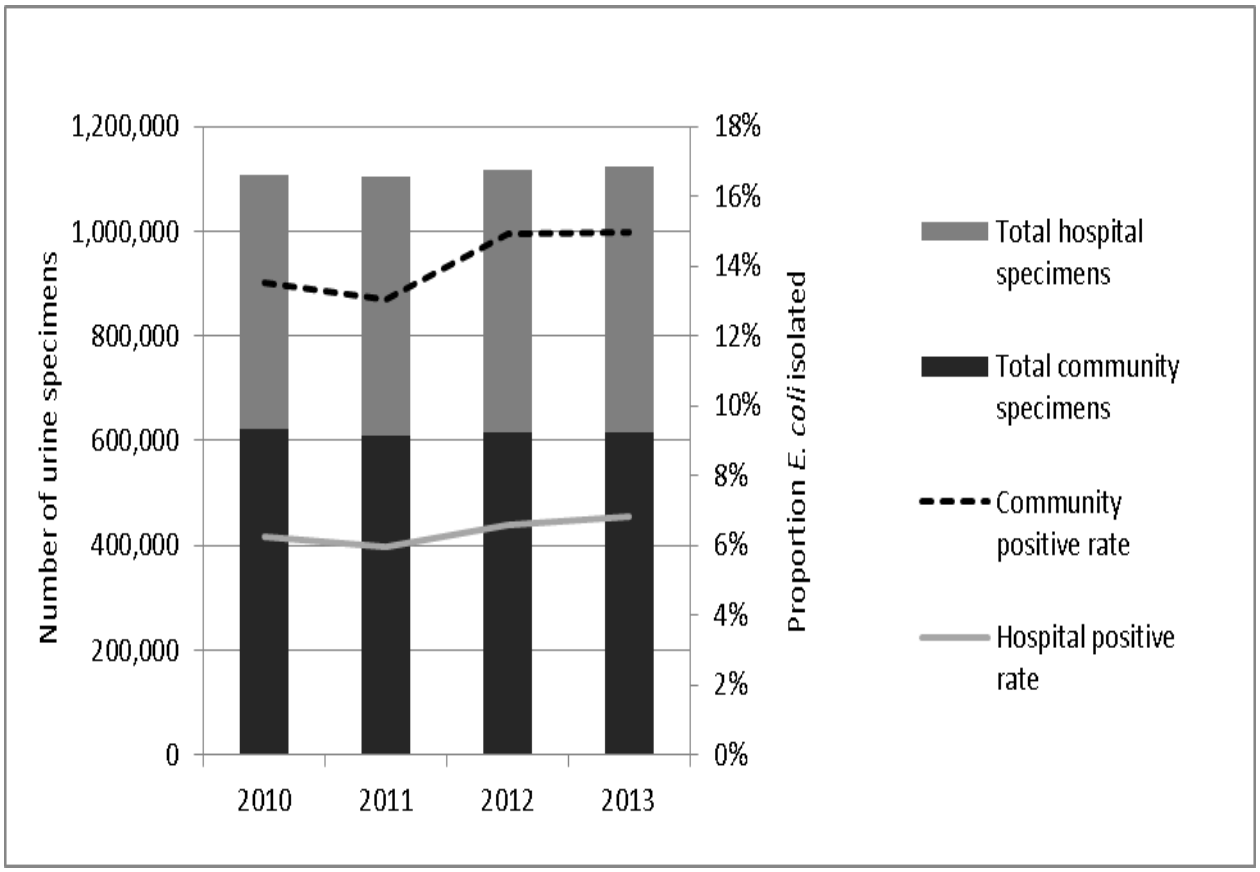
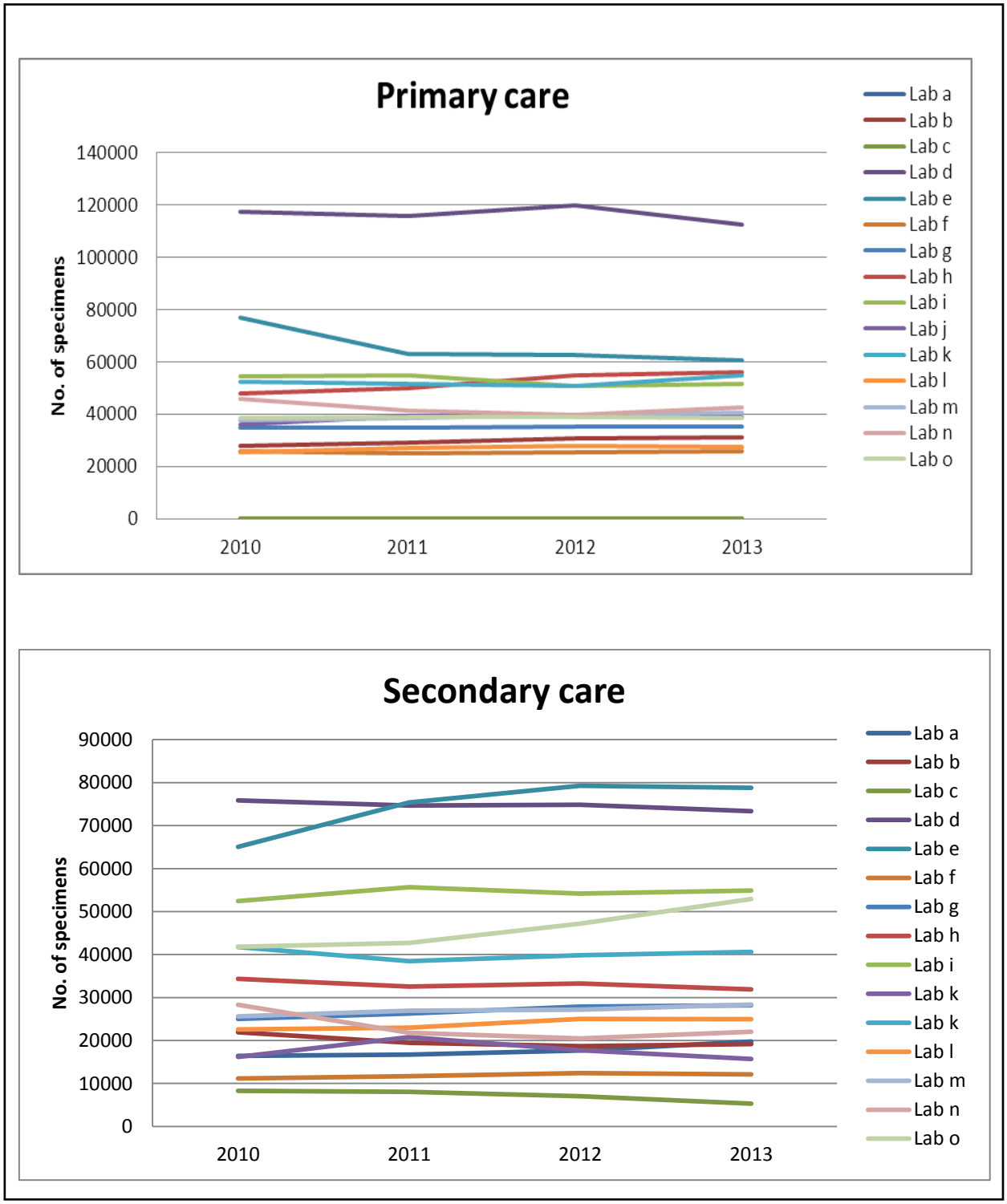


Figure 5.5 Total number of urines received from primary and secondary care by West Midland laboratories 2010-2013



5.4.5 Number of reports of bacteria received by AmSurv

The number of reports of *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolated from urine specimens in the West Midlands increased significantly during 2010-2013 (Figures 5.6 and 5.7). Although the majority of laboratories were reporting to AmSurv by 2010 (Figure 3.5), it was not until 2012 that all laboratories reported results by this mechanism, therefore some of the rise in number of reports in the years 2010 and 2011 may be accounted for by laboratories joining the surveillance scheme. Increases in the number of these named bacterial species may also be due to laboratories beginning to identify isolates from urine specimens to species level; however, the survey of methods described in Chapter 2 reported that only 3 of the 15 West Midland laboratories did not fully identify Gram-negative bacteria isolated from urine in 2011. Much of the increase in numbers of *E. coli* from urine specimens during the study period can be accounted for by the sharp rise in numbers isolated from specimens sent to laboratories by GPs (Figure 5.6).

Figure 5.6 Total no. of *E. coli* reports received from urine specimens submitted by GPs and Acute Trusts in the West Midlands 2010-2013

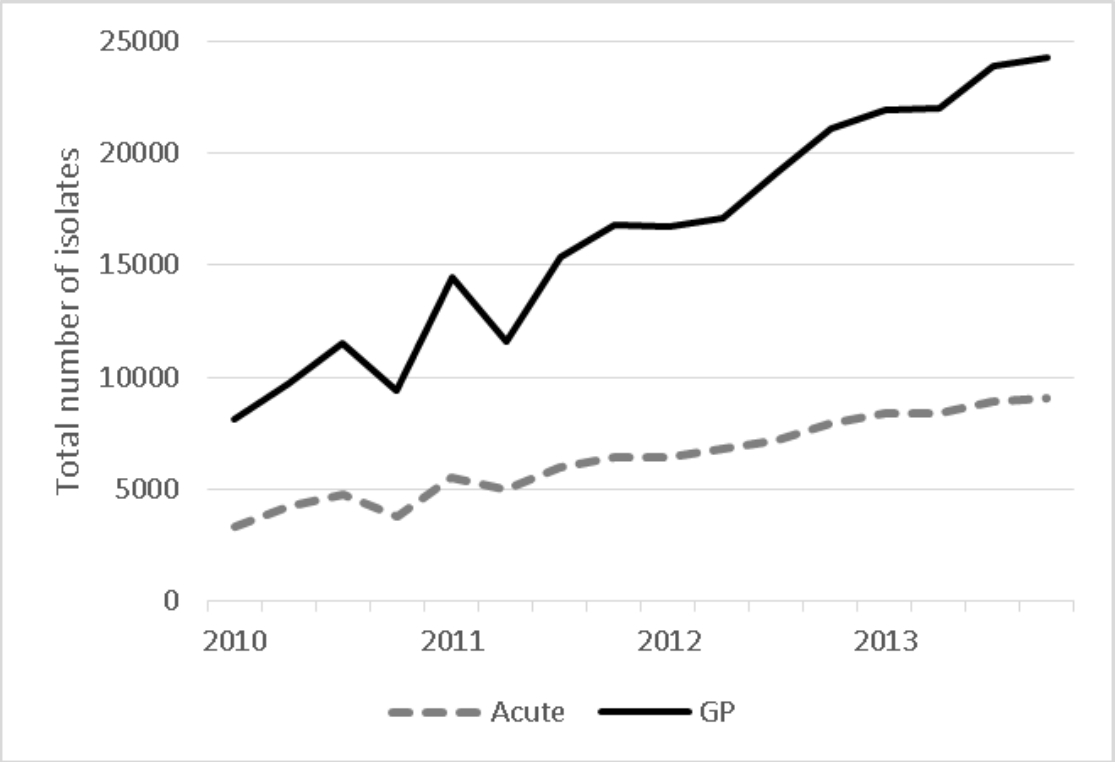
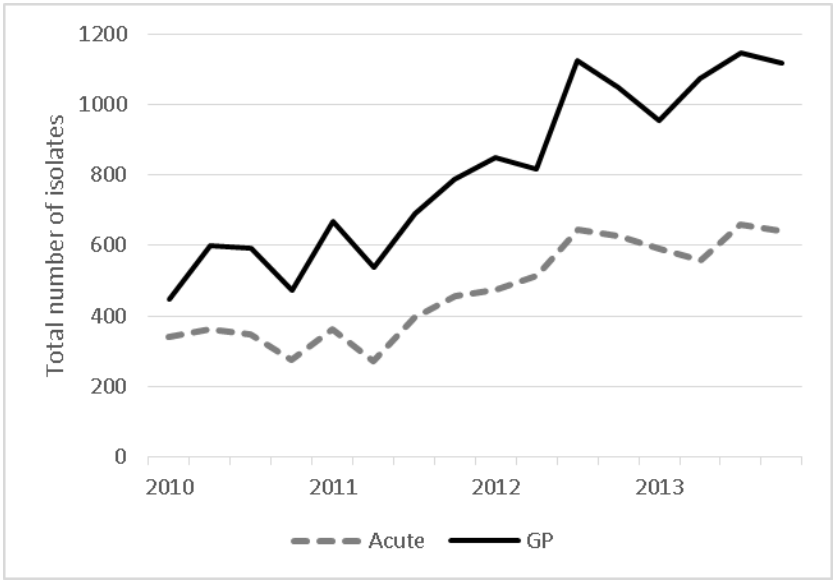
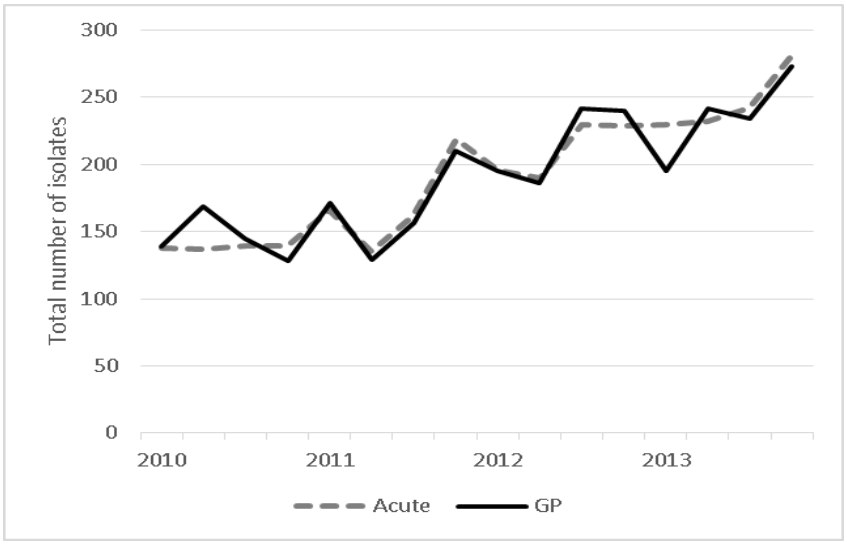


Figure 5.7 Number of a) *K. pneumoniae* and b) *P. aeruginosa* reports from urine specimens in the West Midlands 2010 – 2013

a)



b)



5.4.6 Antibiotic susceptibility testing

As reported in Chapter 2, there was variation in the reported antibiotic susceptibility test methods in use by West Midland laboratories during the study period for bacteria isolated from urine. The methods were BSAC disc diffusion (n=7), VITEK 2® (n=6) and breakpoint methods (n=2). One laboratory used a combination of VITEK 2® and BSAC disc diffusion depending on whether the tests were performed during or outside normal working hours. All but one of the seven laboratories using the BSAC method reported using the most recent available breakpoint standards during the study period, and are currently using the latest breakpoint standard (version 12) (British Society for Antimicrobial Chemotherapy (BSAC), 2013), with just one laboratory using an earlier version (version 10) (British Society for Antimicrobial Chemotherapy (BSAC), 2011). The standard VITEK 2® software included EUCAST v1.1 (2010) breakpoints during this period (European Committee on Antimicrobial Susceptibility Testing). During the study period, two laboratories changed from the BSAC method to a breakpoint technique. One of the breakpoint users reported using 'in-house' breakpoint standards.

5.5 Discussion

5.5.1 *E. coli* and *K. pneumoniae* isolates from urine specimens

This chapter describes the findings from the early implementation of the AmSurv system in the West Midlands. This system, for the first time in England, has enabled the routine analysis and reporting of antibiotic susceptibility tests results of all bacterial isolates from specimens submitted by hospital and community healthcare providers, for a defined population. Members of the family Enterobacteriaceae are the most common cause of UTI in hospitals and the community (Bean et al., 2008;Kahlmeter and Poulsen, 2012;Laupland et al., 2007), and they are implicated in many of the current problems of transferrable multi-antibiotic resistance. The ability to monitor AMR trends in infections most commonly caused by these bacteria, across all patient groups, provides valuable additional insight needed to inform public health action.

E. coli is the most frequent uropathogen responsible for community and nosocomial UTI (Bean et al, 2008;Laupland et al, 2007). The larger proportion of urine specimens yielding *E. coli* were observed from community sources (15% compared with 6% from hospital settings) and may reflect differences in urine sampling strategies in these settings combined with the modulating effect of urinary dipstick screening results in the community being used as a prerequisite for sending to the laboratory. It is also plausible that urine sampling is undertaken within a more systematic and stringent framework in hospital settings (Hayward et al., 2007). *K. pneumoniae* has been associated with hospital settings and complicated UTIs (Stamm, 2002); however a Canadian study reported that *K. pneumoniae* accounted for 7% of community-onset

UTI (Laupland et al, 2007). This study found that 3% of the total positive urine cultures in the West Midlands were *K. pneumoniae* and that there was a slightly higher positivity rate for *K. pneumoniae* in urines received from the community compared with those received from hospital settings (0.7% and 0.5% respectively).

5.5.2 Extended spectrum beta-lactamase

This study found increasing non-susceptibility in *E. coli* isolates from urine specimens tested against third-generation cephalosporins and ciprofloxacin in 2010 to 2013. As previously described, the successful uropathogenic *E. coli* sequence type 131 (ST131) is often associated with the CTX-M-15 beta-lactamase and also fluoroquinolone resistance (particularly the *H*30 subclone) (Johnson et al., 2016), and therefore the rise in non-susceptibility in the West Midlands may be the result of the continued spread of this sequence type. The horizontal transfer of the CTX-M gene in conjugative plasmids is pivotal to the spread of this ESBL, with plasmids of the IncF family being the common carrier (Novais et al., 2012).

As described in Chapter 1, bacteria carrying ESBL genes have a global prevalence (section 1.3.2). A recent review reported that the prevalence of bacteria carrying ESBLs is increasing in Europe and found a significant rise in community ESBL rates in all WHO geographical regions. The authors report that the *E. coli* ST131 is now the dominant global extraintestinal pathogenic strain, and that clonal spread of virulent strains has led to the widespread dissemination in Europe and North America of ESBL-producing ST131 sub clone *H*30-Rx, which often carries $bla_{CTX\text{-}M}$ (Bevan et al., 2017).

As discussed in Chapter 1 (section 3.1), international travel is a common mechanism for the dispersal of successful MDR bacterial clones. The successful replacement of other *E. coli* clones by ST131 in South America and Europe is probably due to human migration (Bevan et al, 2017). As also described in Chapter 1 the increased carriage of CTX-M ESBLs in community patients in the West Midlands with South Asian connections,(Wickramasinghe et al, 2012) may act as a reservoir for the increasing levels of resistance being detected in this surveillance study.

A rise in third-generation cephalosporin non-susceptibility for *E. coli* has not been detected for isolates from blood in England during 2012-2014; however, the on-going rise in the number of *E*. *coli* bacteraemia cases is leading to more non-susceptible isolates being detected (Bou-Antoun et al., 2016). A UK study reported that non-susceptibility to cephalosporins and quinolones amongst *E. coli* and *K. pneumoniae* isolates from bloodstream infections rose significantly from 2001 to 2006 and then plateaued or fell between 2007 and 2011. The authors suggested a link to a change in prescribing practices in the UK, which involved significant reductions in the use of cephalosporins and quinolones in the middle of the decade. However the data presented in their study suggests that the decline in non-susceptibility in 2007-2010 may be starting to be reversed again for *E. coli* in 2011, with a rise in non-susceptibility for both third-generation cephalosporins and quinolones (Livermore et al., 2013). Therefore it is possible that these rates may continue to rise following 2011, as was found in the study reported in this chapter for 2010-2013 West Midland isolates from urine specimens.

5.5.3 Carbapenemase producing bacteria

5.5.3.1 Enterobacteriaceae

A recent study reviewed the confirmed carbapenemase-producing Enterobacteriaceae (CPE) cases referred to the AMRHAI Reference Unit from the West Midland between 2007 and 2014. The authors of this study concluded that the number of CPE reports had increased in the 7 year period (Findlay et al., 2017). There has been an increase in the numbers of *E. coli* bacteria reported from invasive infections in England in 2012 to 2014 (Bou-Antoun et al, 2016). The increased number of isolates, combined with a greater awareness of CPE, may account for the increased referral of potential isolates to PHE reference laboratories, and the increased number of confirmed CPE reported in the AMRHAI study (Findlay et al, 2017). Figures provided by the AMRHAI reference unit show that from 2014 onwards the number of confirmed CPE are continuing to increase in the West Midlands (Figure 1.5). In the study reported in this chapter there was not any increase in the proportion of *E. coli* and *K. pneumoniae* isolated from urine specimens that were non-susceptible to carbapenems in 2010 – 2013; and monitoring of routine West Midland susceptibility data post 2013 has not detected an increase in the proportion of Enterobacteriaceae non-susceptible to carbapenems (PHE internal quarterly surveillance reports). The study described in this chapter has shown increasing numbers of *E. coli*, *K. pneumoniae* and *P. aeruginosa* being reported by West Midland laboratories (Figures 5.6 and 5.7) from urines, and this may lead to an increase in the number of reported non-susceptible to carbapenems, even if the proportion that are non-susceptible does not rise.

The situation in the West Midlands contrasts sharply with the situation in the North West of England, where much higher numbers of CPE are being detected (Findlay et al, 2017). A sentinel study of UK laboratories in 2013-2014 reported that the incidence of CPE in the North West region was 0.033 per 1000 patient days (95% CI=0.012-0.072) compared with an incidence of 0.007 per 1000 patient days (95% CI=0.005-0.010) across the UK (Trepanier et al., 2017).

The majority of confirmed carbapenemase-producing *E. coli* and *K. pneumoniae* in the West Midlands were NDM producers (Table 5.5) rather than the KPC producing strains that predominate in the North West region (Livermore, 2012;Munoz-Price et al., 2013). In the rest of the UK, the number of KPC-producing bacteria being detected have increased but have not been associated with the major outbreaks that have been observed in the North West region (Findlay et al., 2016). The on-going outbreak of KPC producing bacteria in the North West region is due to the horizontal spread of IncFIIK plasmid rather than the emergence of a successful clone, and this is being detected in a range of Enterobacteriaceae (Munoz-Price et al, 2013). The KPC-producing bacteria being reported in the UK outside the North West region are predominantly *K. pneumoniae* strain type (ST) 258, which has been responsible for many clonal outbreaks across Europe and the USA (Findlay et al, 2016).

There is a significant local community originating from South Asia in the West Midlands, who frequently travel to their countries of origin (Wickramasinghe et al, 2012). It has been suggested that this is a potential source of acquiring carbapenemase-producing pathogens for some UK residents (Kumarasamy et al., 2010). However, the analysis of cultural, ethnic and linguistic origin based on patient names revealed that the majority of CPE reported in the West Midlands during 2010-

2013 were from individuals with European origins. Although NDM-producing strains are endemic in parts of South Asia,(Walsh et al., 2011;Walsh and Toleman, 2012), in the study reported in this chapter only 2 of the 16 patient names with NDM-producing isolates were categorised as being of Middle East / South Asian origin. These findings are supported by a study of NDM in the UK which reports >40% of cases providing travel information had no history of foreign travel (Jain et al., 2014). A study of confirmed CPE cases in the West Midlands in 2007 to 2014 found 137 isolates from 108 patients. Travel history was available for 42 patients, with 23 patients indicating travel outside the UK. The most frequently visited countries reported to have been visited outside the UK in the previous 6 months were; India (14/23) and Pakistan (5/23). From the 14 patients with a confirmed CPE that had reported travel to India, 10 had isolates positive for NDM, two had isolates positive for OXA-48-like genes and two had isolates positive for both NDM and OXA-48-like genes. All five of the patients with a confirmed CPE that had visited Pakistan yielded bacteria with NDM genes (Findlay et al, 2017).

Currently most NDM isolates in South Asia are associated with hospital care (Kumarasamy et al, 2010), with time it is possible that bacteria carrying NDM may spread into the general community. It is therefore plausible that NDM-producing Enterobacteriaceae may follow the same pattern of dispersal in the UK as the CTX-M ESBL gene (Livermore, 2012).

In this study of bacteria isolated from urine in the West Midlands in 2010 to 2013, three cases of bacteria producing the OXA-48 carbapenemase in 2013 (one *E.coli* and two *K. pneumoniae*) were reported. As described in Chapter 1 (Figure 1.5) 2016 data from AMRHAI for the West Midlands shows OXA-48 producers are replacing

KPC and NDM as the predominant carbapenemase enzyme. OXA-48 enzymes hydrolyse carbapenems at a low level and have no effect on broad-spectrum cephalosporins (Public Health England, 2014b); and as bacteria expressing this enzyme do not often co-express an ESBL, their phenotypic susceptibility to third-generation cephalosporins complicates their laboratory detection (Poirel et al., 2012). The successful uropathogenic *E. coli* ST131 has been associated with production of the OXA-48 enzyme, leading to a concern that this may lead to the widespread dissemination of this resistance mechanism in the community (Dimou et al., 2012).

5.5.3.2 Pseudomonas aeruginosa

P. aeruginosa is a non-fermenting Gram-negative opportunistic pathogen, often associated with nosocomial pneumonia, blood stream infections and UTI (Mittal et al., 2009). In the study reported in this chapter, *P. aeruginosa* accounted for only 1% of the positive isolates from urine specimens; however the prevalence of MDR strains of *P. aeruginosa* has risen sharply in many parts of the world in the last 20 years, including the UK (Nathwani et al., 2014). The increase of *P. aeruginosa* MDR infections prompted the inclusion of this organism in the 'ESKAPE' (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*.) pathogens list which is published by the Infectious Disease Society of America to highlight bacteria posing a serious risk to human health (Boucher et al., 2009).

In this chapter it was reported that *P. aeruginosa* isolated from urine specimens was demonstrated to have an increased proportion of non-susceptibility to carbapenems,

compared with *E. coli* and *K. pneumoniae*. Imipenem resistance in *P. aeruginosa* is often due to the loss of OprD porin, however this does not affect susceptibility to other β-lactams (Public Health England, 2014b). Up-regulation of the MexAB-OprM efflux system can confer reduced susceptibility to meropenem and resistance to anti-pseudomonal cephalosporins (Bonomo and Szabo, 2006). A Spanish study investigating the overexpression of AmpC and efflux pumps in *P. aeruginosa* isolates from bloodstream infections provides insight into the observed correlation between trends in reported incidence of carbapenem and ceftazidime non-susceptibility reported in this chapter (Cabot et al., 2011). The study demonstrated a statistically significant correlation between overexpression of *ampC* and both *mexY* and *mexB* genes coding for efflux pumps, in isolates of this phenotype. This association between mechanisms of resistance in *P. aeruginosa* adds to its already formidable ability to resist a range of antibiotics, and further complicates treatment options (Cox and Wright, 2013). Further analysis is required to determine whether the isolates identified in the West Midlands exhibit the same pattern as those in Spain.

5.5.3.3 Acinetobacter spp. and Stenotrophomonas maltophilia

In Table 5.3 all bacteria non-susceptible to carbapenems in the West Midlands during the study period were listed. Two bacteria showed high levels of non-susceptibility to carbapenems; *Acinetobacter spp.* and *Stenotrophomonas maltophilia* (27% and 23% non-susceptibility respectively over the study period). The majority of infections involving the genus *Acinetobacter* are by members of the *A. baumanii* complex, which includes *A. baumanii*, *A. calcoaceticus* and *A. nosocomialis*, which cannot be distinguished by current routine diagnostic laboratory identification tests, and are

therefore mostly reported as *A. baumannii* (Camp and Tatum, 2010). *A. baumannii* is the most clinically important species and is included in the 'ESKAPE' pathogens list due to its increasing prevalence and high-levels of antibiotic resistance (Boucher et al, 2009). *A. baumannii* is a particular concern due to the increasing frequency of isolation in hospital settings and the extensive antibiotic resistance being found, including against antibiotics used as last-resort options (Wenzler et al., 2017). These bacteria are particularly resistant to desiccation and able to survive in the hospital environment for many days (Wenzler et al, 2017). *A. baumannii* have been found to be associated with a range of infections, including pneumonia, wound infections, bloodstream infections and UTI. A wide range of antibiotic resistance mechanisms are used by this organism, including production of enzymes, porin loss and efflux pumps. The frequent acquisition of metallo beta-lactamases such as IMP, VIM, SIM and NDM are of particular concern as these further restrict the available treatment options (Wenzler et al, 2017). In recent years *A. baumannii* has been associated with infections in wounded soldiers returning from Afghanistan and Iraq (Camp & Tatum, 2010). In the study described in this chapter, over a third of the reports of *A. baumannii* received in the 2010-2013 study period were from an Acute Trust in the West Midlands that includes a British military hospital unit; although this Trust also has a number of other specialist units, including a major transplant centre.

S. maltophilia (previously known as *Pseudomonas maltophilia*) is also a non-fermenting Gram-negative opportunistic pathogen that is intrinsically resistant to many antimicrobials. The mechanisms of resistance include decreased permeability, multi-drug efflux pumps, chromosomally and plasmid encoded beta-lactamases and biofilms (Brooke, 2014). L1, a chromosomally encoded metallo-beta-lactamase, is

found widely in *S. maltophilia* (Livermore and Woodford, 2000). Although the study described in this chapter reported a high proportion of these bacteria to be non-susceptible to carbapenems (23%), and the number of isolates increased during the study period, there were still only comparatively small numbers reported from urine specimens in the West Midlands (Table 5.3). *S. maltophilia* is implicated in infections of immuno-compromised patients and is associated with a high mortality rates (Gales et al., 2001). The increasing worldwide prevalence of *S. maltophilia* prompted the WHO to list this opportunistic pathogen as a serious public health concern (World Health Organisation, 2017).

5.5.4 Limitations

There are some limitations in this study. As discussed previously it is likely that urine specimens sent from the community for microbiological examination represent cases with initial treatment failures, more complicated medical histories and severe infections, (Hillier et al., 2006) and, therefore, the observed levels of resistance are liable to be an overestimate of the true levels of resistance in the population. The survey of GPs described in Chapter 4 showed that 40% of respondents reported sending urines for microbiological examination from patients with uncomplicated UTI, contrary to national guidelines (Public Health England, 2014b), and therefore the routine surveillance data in the West Midlands is likely to include a significant proportion of bacteria isolated from these 'uncomplicated' infections. A report of the Specialist Advisory Committee on Antimicrobial Resistance (SACAR) Surveillance Subgroup states that evidence is mixed on the extent and impact of sampling bias, and, as it is difficult to overcome, AMR surveillance should be mainly based on

routine laboratory reports (Hayward et al, 2007). It also reassuring that the observed proportion (15%) of primary care urine specimens positive for *E. coli* is similar to that reported in another UK based study of antibiotic resistance in isolates from urine examining all specimens from community patients with UTI symptoms.(Butler et al., 2006)

As described in Chapter 2, there is some variation in antibiotic susceptibility testing methods in West Midlands laboratories, although it was encouraging that 13 of the15 laboratories apply recent BSAC or EUCAST MIC breakpoint standards and all laboratories participate in the monthly internationally accredited external quality control assessment of susceptibility testing methods (NEQAS) (Chapter 2, section 2.4). The UK NEQAS scheme did show variation in 2011 between laboratories using BSAC or EUCAST breakpoint guidelines and those using CLSI guidelines (Brown, 2012); however no laboratories in the West Midlands report using CLSI in this period. No variation in antibiotic susceptibility proportions was noted from two laboratories following a change in their testing methods during the study period. There was only one significant change for this study within the BSAC and EUCAST breakpoint standards introduced during 2010-2013; a lowering of the breakpoint MIC for Enterobacteriaceae against ceftazidime in BSAC v10, released in January 2011 (British Society for Antimicrobial Chemotherapy (BSAC)). The majority of West Midland laboratories did not perform first line testing of ceftazidime against Enterobacteriaceae isolated from urine, and therefore it is not believed that this change had a significant effect on study findings presented here.

5.5.5 New developments

The growing problem of Enterobacteriaceae resistant to third-generation cephalosporins has led to increased use of carbapenems as 'last resort' antibiotics. As resistance is now emerging to these drugs, multifaceted interventions are required to preserve their effectiveness, including antimicrobial stewardship, rapid confirmation of potential CPE, meticulous infection control practices and enhanced surveillance. Following the surveillance study reported in this chapter, PHE in the West Midlands implemented, in 2014, a pilot rapid CPE confirmation service for local laboratories. To enable electronic reporting of these confirmation tests a web based Electronic Reporting System (ERS) was developed. Routine AmSurv reports are used to trigger automated alerts for potential CPE, which are sent to reporting laboratories to remind them to send bacteria to the reference laboratory. In response to the increased numbers of CPE in the North West region, the ERS was further developed as a national enhanced surveillance system for carbapenemase-producing Gram-negative bacteria (Freeman et al., 2016). Although this study shows low numbers of *E. coli* and *Klebsiella spp*. resistant to carbapenems in the West Midlands during 2010-2013, the experience of other regions of England and parts of Europe emphasises the requirement for vigilance and on-going monitoring of these bacteria.

5.5.6 Summary

Better access to and use of surveillance data constitute a key objective in the UK Five Year Antimicrobial Resistance Strategy (Department of Health, 2013). AmWeb has improved access to AMR data for a diverse group of health professionals,

with130 registered users across the region within twelve months of its implementation. Automated AMR surveillance is capable of providing a representative picture of the burden of resistance in Gram-negative uropathogens from both hospitals and the community.

In 2010 to 2013 the predominant organism isolated from urine specimens referred by hospitals and the community was *E. coli* (61%). Routine AMR surveillance data demonstrated an increasing trend in *E. coli* and *K. pneumoniae* non-susceptibility to third-generation cephalosporins, and *E. coli* non-susceptibility to ciprofloxacin. The proportion of *E. coli* and *K. pneumoniae* non-susceptible to carbapenems remains low in the West Midlands; however increasing numbers of isolates observed in this study will result in greater numbers of carbapenem resistant bacteria being reported in the region.

The observed increasing trends in antibiotic non-susceptibility reported in this study strengthens the recommendation in the UK 5 Year AMR Strategy for the on-going surveillance of these bacteria / antibiotics, combined with surveillance of antibiotic usage in hospitals and the community settings.

6 How General Practice characteristics and antibiotic prescribing effect the rates of non-susceptibility of *Escherichia coli* in the West Midlands region of England – a four-year ecological study

6.1 Background

In 2014 in England, 74% of antibiotic prescribing occurred in general practice (Public Health England, 2015a). Antibiotic prescribing is associated with the development of AMR and this linkage has been demonstrated in community settings at both individual patient level and within communities, regions and countries (Bell et al., 2014a; Costelloe et al., 2010a). It has been suggested that antibiotic prescribing at a population level may have greater significance than individual level consumption for determining the risk of an individual harbouring antibiotic-resistant bacteria (Bergman et al., 2009).

With increasing evidence of an association between antibiotic prescribing and AMR, the Chief Medical Officer (CMO) for England in her 2011 annual report promoted the use of antibiotic stewardship as a measure to control the development and spread of AMR (Chief Medical Officer, 2013). There are some antibiotic prescribers, however, that are sceptical that a reduction in their antibiotic prescribing will reduce the levels of AMR in their practice population (Björkman et al., 2013). PHE national prescribing guidelines were provided as a tool to promote consistent and prudent prescribing in primary care in England (Public Health England, 2017b); however a study in 2014 reported that these guidelines have not encouraged uniformity or reduced the volume of prescribing in the community (Hawker et al., 2014). The expectation of patients to receive an antibiotic is also driving the level of prescribing (Teixeira et al., 2013), and several initiatives have been introduced to modify patients expectations (see discussion section 6.5.1.4).

To understand antibiotic prescribing practice in the community, the characteristics of the general practice have to be taken into account (Wang et al., 2009a). A number of factors have been shown to influence the volume of prescribing within general practices in the UK, such as the practice location, length of appointment (Wang et al., 2009b), social deprivation (Covvey et al., 2014a) and being a single-handed practice (Wilson et al., 1999). A systematic review of studies reporting on the association between antibiotic prescribing and resistance reported that the control for practice or population characteristics and the inability to measure the time between prescribing and detection of resistance has been a limiting factor when interpreting results (Bell et al., 2014b). The period between prescribing an antibiotic and the development of resistance has been reported as soon as a month following consumption (Costelloe et al., 2010b), or up to 12 months for some antibiotic combinations (Bergman et al, 2009).

Antibiotics have been shown to have seasonal prescribing patterns, both in Europe and the USA (Goossens et al., 2005; Sun et al., 2012a). In England increases in the volume of antibiotics prescribed in the winter months is associated with the treatment of upper respiratory infections (Fleming et al., 2003a).

The pandemic *E. coli* ST131 has been responsible for community UTIs across the globe and is commonly resistant to a range of antibiotics, including beta-lactams, fluoroquinolones and trimethoprim (Rogers et al., 2011), therefore, the use of any of these antibiotics can potentially select for these MDR strains in the community (Petty et al., 2014). A number of studies have shown an association between prescribing a specific antibiotic, or structurally similar antibiotics, and non-susceptibility to the same antibiotic or antibiotic class (Goettsch et al., 2000; Leflon-Guibout et al., 2002);

however, a recent systematic study reported that there is a paucity of studies examining co-selection of antibiotic non-susceptibility in one antibiotic when prescribing a structurally different antibiotic with a different mechanism of action (Bell et al, 2014b).

Urinary tract infections (UTI), and in particular those caused by *Escherichia coli*, were chosen as the focus of the study described in this chapter as 1) UTIs are one of the most common conditions diagnosed in community settings in Europe and are an important clinical indication of prescribing in primary care, and 2) *E. coli* are the most common cause of UTIs in both primary and secondary care (Petersen and Hayward, 2007).

Statistical modelling has been defined as using data to explicit a mathematical model to enable data generation (Greenland, 1989). The process of selecting a model, and its precision, distinguishes statistical modelling from more basic statistical techniques. Statistical modelling has been used as a more efficient way of detecting and summarising data patterns (Greenland, 1989).

In this study multilevel mixed-effects Generalised Linear Models (GLMs) were used to measure the association between antibiotic prescribing and non-susceptibility to antibiotics in *E .coli* bacteria isolated from urine specimens. Multilevel mixed-effects GLMs allow for a range of response variable distributions, including binomial distributions, which are used when assessing antibiotic susceptibility data. Mixed-effect GLMs also allow both fixed effects and random effects, so that explanatory variables (fixed effects), such as the amount of prescribed antibiotic or the deprivation score, can be modelled alongside random effects, which allows for

variations among entities usually following a normal distribution, such as GP Practices (Bolker et al., 2009).

To address some of the limitations listed in previous studies described above, an ecological study was undertaken to examine the relationship between prescribing antibiotics commonly used in general practice and the number of non-susceptible *E. coli* isolates from urine samples taken in general practices in the West Midlands region of England over a four-year period.

6.2 Objectives

- To identify any associations between prescribing antibiotics in primary care and the non-susceptibility of *E. coli*, taking into account potential confounders, such as general practice characteristics.
- To describe seasonal antibiotic non-susceptibility of *E. coli* isolated from urine specimens and antibiotic prescribing in the West Midlands community
- To examine variation in antibiotic prescribing between GP practices in the West Midlands

6.3 Methods

6.3.1 Population and healthcare facilities

The West Midlands population has been described in Chapter 1. In 2012, the mid-point of this study, there were 950 general practices with 3635 general practitioners, serving a population of 5.8 million registered patients in the West Midlands Region (NHS Digital, 2014). During this study period, 2010-2014, there were 15 diagnostic microbiology laboratories serving both community-based healthcare centres and hospitals.

6.3.2 Data sources

Antibiotic prescribing data on items dispensed in each general practice during the period 2010-2014 was obtained from NHS Digital (previously known as the Health and Social Care Information Centre) (NHS Digital, 2016b). Antibiotic prescribing data are expressed as defined daily doses (DDD) per 1000 general practice population.

Data on antibiotic non-susceptibility for *E. coli* isolates from urine specimens submitted from general practices were obtained from the Public Health England (PHE) Second Generation Surveillance System (SGSS), previously known as the AmSurv system (see Chapter 3 for description of the AmSurv implementation). To detect emerging non-susceptibility, the dataset was de-duplicated by removing only duplicate *E. coli* reports from each patient having exactly matching antibiotic susceptibility results within the same year. Nine of the15 laboratories were reporting data regularly to SGSS/AmSurv at the start of our study period in 2010, and complete coverage of all 15 laboratories was achieved in 2012.

General practice characteristics were obtained from the National Health Service (NHS) Business Services Authority (http://www.nhsbsa.nhs.uk/). This included information on the total practice population, proportion of the practice population <15 years old and ≥65 years, and ratio of females to males in the practice population. The number of general practitioners (GPs) within each practice was obtained from NHS Digital, with single-handed practices defined as those practices with only one registered GP (NHS Digital, 2016a). A variable was created for the number of GPs per 100,000 practice population to include in the statistical modelling.

Social-economic deprivation was measured using data from the English Index of Multiple Deprivation 2010 (Department for communities and local goverment, 2016). A deprivation index was assigned to each general practice based on the deprivation index assigned to the Local Authority (English administrative area) in which the practice was located.

The general practices were categorised as 'urban' or 'rural' based on whether the majority of the population in the Local Authority in which the practice is situated live in a rural or urban setting, according to definitions in the Defra Classification of Local Authority Districts and Unitary Authorities in England (Department for Environment, 2016).

As defined previously in Chapter 3, non-susceptibility to an antibiotic is defined as test results with a 'resistant' (R) or 'intermediate' (I) designation.

6.3.3 Prescribing and AMR descriptive analysis

Previous prescribing studies have assigned seasons based on standard calendar quarters (Suda et al., 2014). In this study, to match seasonal periods in England, seasons were defined as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). Seasonal total DDD prescribing quantities and DDDs /1000 practice population for the period March 2010 to February 2014 were calculated. These were compared with non-susceptibility proportions for *E. coli* urinary isolates against the six antibiotics selected for analysis in order to describe prescribing and non-susceptibility trends during the study period.

6.3.4 Statistical analysis

Sixteen individual datasets were created. Each dataset consisted of practice level data on all reported *E .coli* isolates non-susceptible to one of the six selected antibiotics, alongside matching practice prescribing data for the same antibiotic or another commonly prescribed antibiotic that may select non-susceptibility (Table 6.1). Table 6.1 shows that for each antibiotic, non-susceptibility is compared with prescribing data for the same antibiotic, or a structurally similar antibiotic with the same action (models: 1, 2, 4, 8, 10, 14, and 15). Other antibiotic combinations were selected based on biological plausibility of an exposure and non-susceptibility relationship, and to enable comparisons with published international studies (models: 3, 5, 6, 7, 8, 11, 12, 13, and16) (Table 6.1).

Table 6.1 Antibiotic combinations evaluated to measure associations between antibiotic non-susceptibility and prescribing of the same antibiotic / antibiotic class, or an antibiotic that may co-select non-susceptibility

***Escherichia coli* non-susceptibility**	**Prescribed antibiotic**	**Statistical model no.**	**References that suggest potential associations**
Ampicillin / amoxicillin	ampicillin/amoxicillin	2	
	co-amoxiclav	1	
	fluoroquinolones	3	(Johnson et al., 2010a)
Cephalexin	cephalosporins	10	
	fluoroquinolones	11	(Rogers et al, 2011)
	trimethoprim	16	(Petty et al, 2014)
	nitrofurantoin	12	(Bergman et al, 2009)
Co-amoxiclav	co-amoxiclav	8	
	ampicillin/amoxicillin	4	
Ciprofloxacin	fluoroquinolones	8	(Rogers et al, 2011)
	ampicillin/amoxicillin	6	(Johnson et al, 2010a)
	co-amoxiclav	5	(Johnson et al, 2010a)
	cephalosporins	7	(Rogers et al, 2011)
Trimethoprim	trimethoprim	15	
Nitrofurantoin	nitrofurantoin	14	
	cephalosporins	13	(Bergman et al, 2009)

In each model, seasonal quarterly trends in non-susceptibility of *E. coli* isolates for each general practice from 01/03/2010 to 28/02/2014 were compared with trends in antibiotic prescribing data in the same quarter and antibiotic prescribing in previous quarters (up to four lagged quarters, with quarter 'minus four' being prescribing data from the same quarter in the previous year). General practice characteristics were included in the statistical models as potential explanatory variables (Table 6.2).

National community prescribing guidance recommends course lengths of between 3-7 days depending on the antibiotic and the clinical presentation (Public Health England, 2017b). A prescribing unit within the statistical models was therefore set as 50 DDDs, which represents approximately 10 prescriptions, taking an average of five days for each course.

Multilevel mixed-effects generalised linear models, using a binomial distribution for the outcome, were developed to examine the relationship between antibiotic use and *E.coli* non-susceptibility. Each statistical model (one for each prescribing / non-susceptibility combination) consisted of the number of *E. coli* isolates non-susceptible by general practice as the outcome variable, number tested as the denominator, as well as the various explanatory variables described (Table 6.2). A composite group variable was created using general practice and Local Authority area to allow modelling of variability between these hierarchical populations as random effects. The seasonal quarters were assigned as categorical variables within the models and spring (March-May) was chosen as the comparator variable. Likelihood ratio testing was used to determine significance and a *P* value of ≤0.05 was considered statistically significant. Adjusted odd ratios were used as a measure of association.

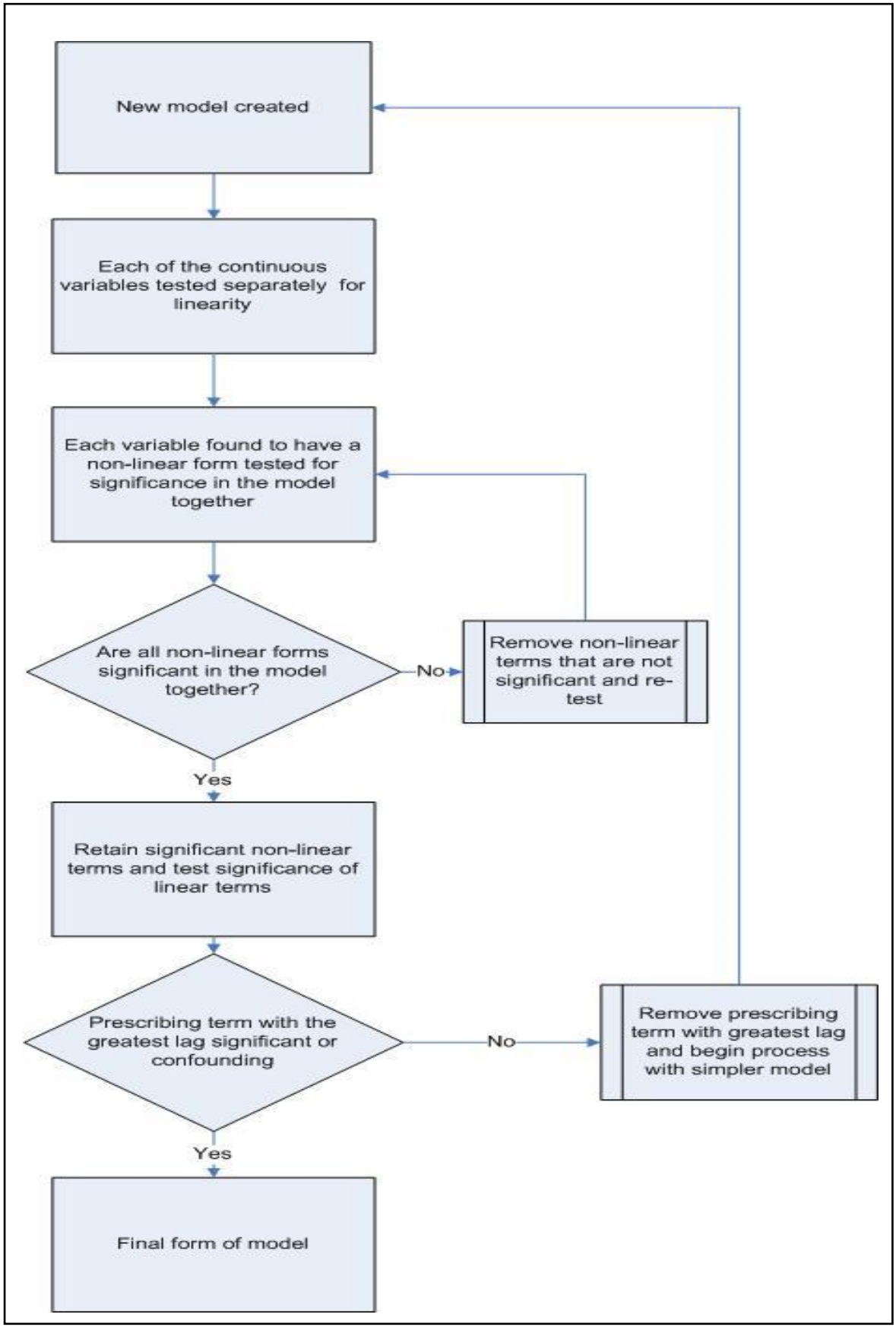
Table 6.2 Variables included in the multi-level mixed effects statistical model

Variable	**West Midlands data (where appropriate)**
Registered patient gender ratio (female/male)	0.98
Proportion of registered patients aged under age 15 years	18.21%
Proportion of registered patients aged 65 years and over	16.15%
Practices with one registered GP	15%
Average number of GPs per 100,000 registered patients in West Midlands	80.57
Location deprivation index (IMD2010) median for general practices in the West Midlands	30.50 (IQ range 29.23)
Rural practice location proportion	27%
General practice within Local Authorities composite variable	--
Time variable (time elapsed during study period)	--
Seasonal quarter (March-May, June-August, September-November, December - February)	--
Prescribing in the same quarter that non-susceptibility assessed (P 0)	--
Prescribing in the previous quarter that non-susceptibility assessed (P-1)	--
Prescribing in the quarter ‘minus 2’ that non-susceptibility assessed (P-2)	--
Prescribing in the quarter ‘minus 3’ that non-susceptibility assessed (P-3)	--
Prescribing in the quarter ‘minus 4’ that non-susceptibility assessed (P-4)	--

The statistical model building process involved constructing cubic functions of all continuous explanatory variables and then subsequently tested for linearity via a stepwise iterative process (Figure 6.1). Significant non-linear variables were retained and tested to determine if they were still significant when inserted into the model together. When satisfied that any remaining non-linear terms were still significant when tested together in the model, the significance of the linear covariates were tested. All lagged prescribing quarters were included in each of the statistical models. As prescribing data prior to 2010 was not available, to increase the number of complete observations, the DDD/1000 practice population variable with the greatest lag was removed if it was found to be not statistically significant, and was not a substantial confounder (i.e. its removal did not lead to a >10% change in the odds ratios of the linear variables). The model building process was then repeated with the increased number of comparable observations. All other explanatory variables were retained in a linear or non-linear form, depending on which form was found to best fit the data within each model (Figure 6.1).

All statistical analyses were performed using STATA v13 (StataCorp, USA).

Figure 6.1 Flow diagram of data modelling process used for each statistical model



6.4 Results

6.4.1 Descriptive analysis

6.4.1.1 Antibiotic prescribing

Data from all 948 general practices that prescribed antibiotics in the West Midlands during the study period were included. Two of the West Midland general practices may have merged or closed as they did not consistently report monthly prescribing during 2010-2014 and were therefore removed from the dataset. Fifteen percent (141/948) were single-handed general practices (Table 6.2), and 82% (116/141) of these were designated as being in rural locations. When comparing single-handed GPs as a group, the prescribing rate was consistently higher throughout the study period than the group consisting of non-single-handed GP practices (Figure 6.2).

In 2013, a total of 45 million antibiotic DDDs were prescribed in the West Midlands. Amongst the antibiotics included in the study, ampicillin / amoxicillin was the most commonly prescribed in 2013 with 13.6 million DDDs, followed by co-amoxiclav with 2.9 million DDDs and trimethoprim 2.8 million DDDs. The total antibiotic prescribing rate (DDD/1000 population) varied widely across general practices (Figure 6.3). In 2013, the 5th and 95th percentile for total antibiotics prescribed by individual West Midland general practices was 4431 DDD/1000 population and10076 DDD/1000 population.

Figure 6.2 Seasonal trends in total antibiotic prescribing rates by single GP and multiple GP practices, West Midlands, March 2010 – November 2013

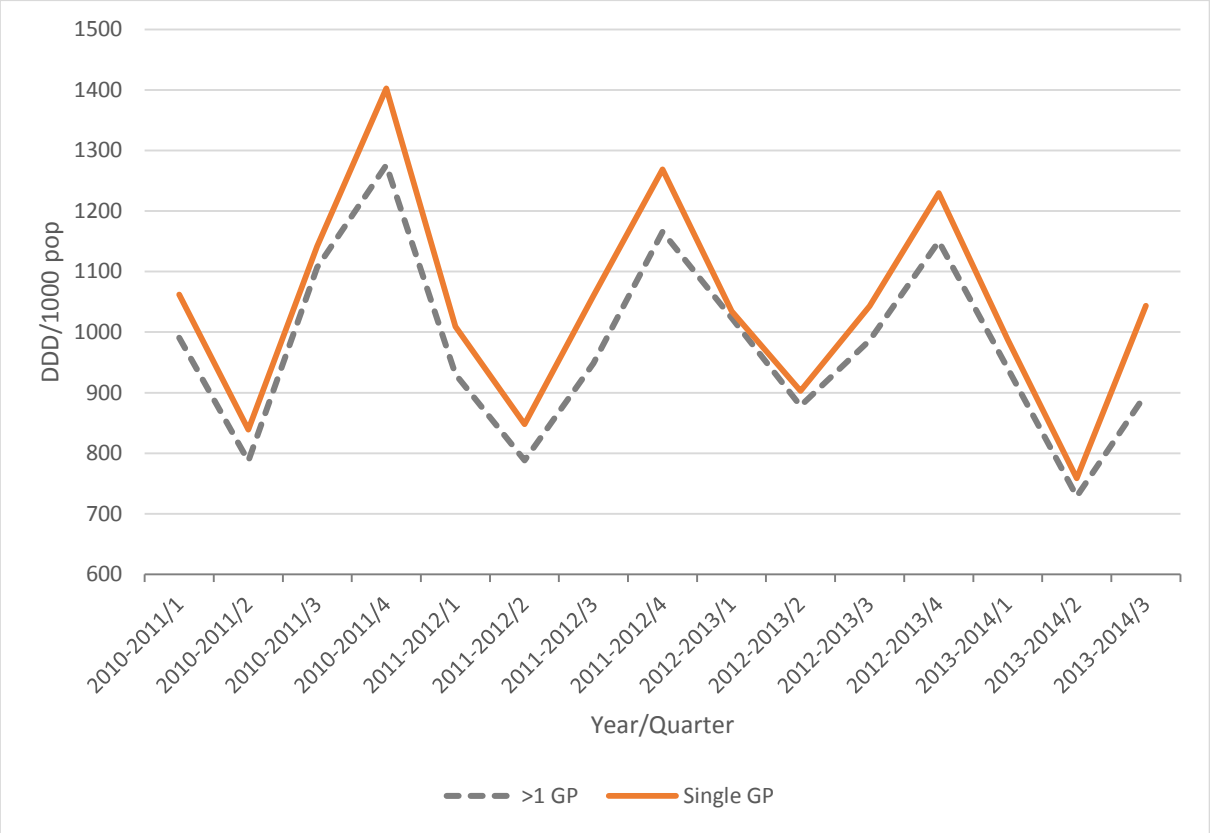
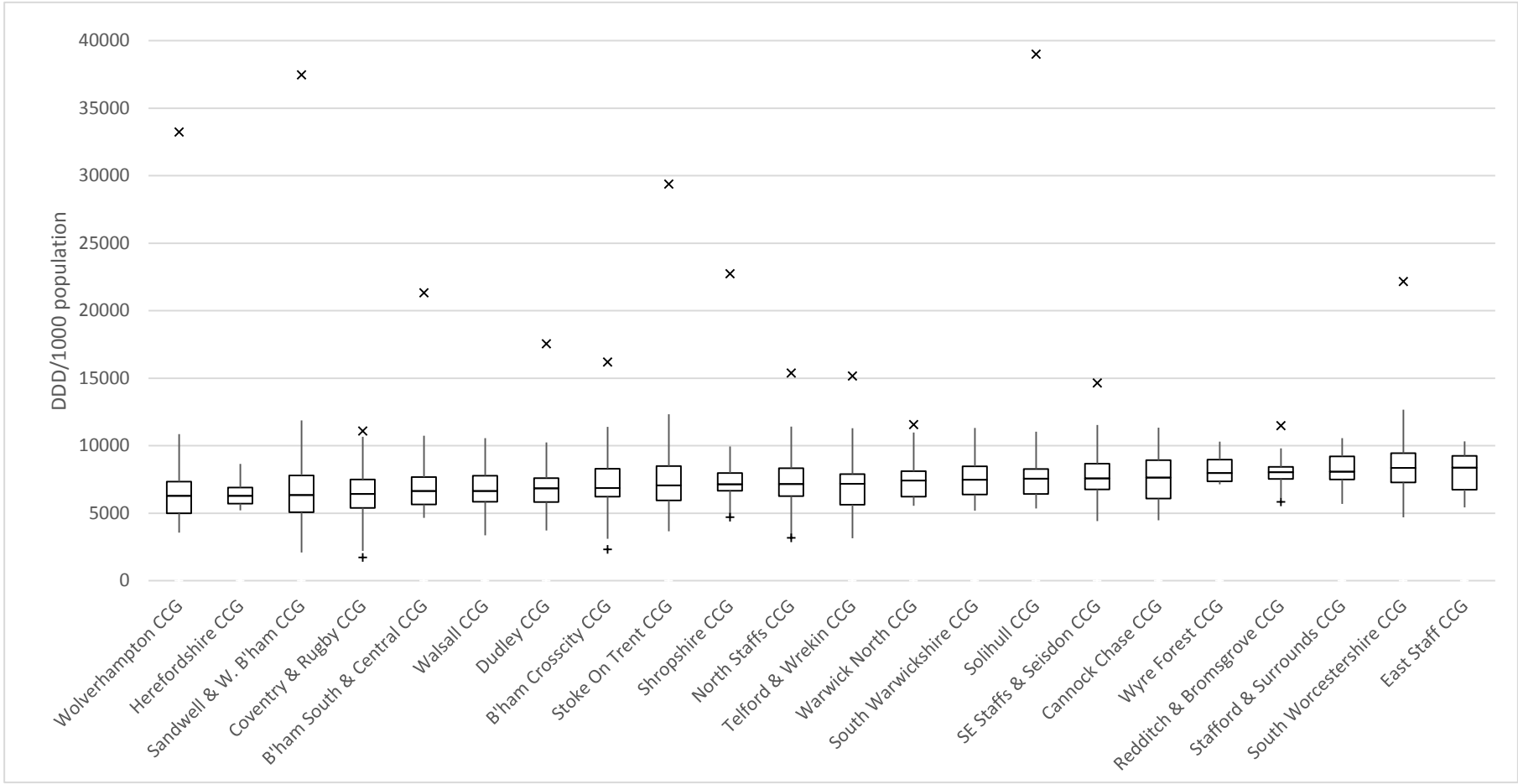


Figure 6.3 All antibiotics prescribing by CCGs in the West Midlands in 2013. Boxplot depiction of the mean (line through box), interquartile range (box), 1.5 * interquartile range (line), maximum outliers (x) and minimum outliers (+).



The prescribing rate (DDD/1000 population) for individual antibiotics also varied across the region. The 5th and 95th percentile prescribing rates by individual general practices in 2013 were: 1111 DDD/1000 population and 3884 DDD/1000 population for ampicillin/amoxicillin; 11 DDD/1000 population and 258 DDD/1000 population for cephalosporins; 93 DDD/1000 population and 1047 DDD/1000 population for co-amoxiclav; 70 DDD/1000 population and 524 DDD/1000 population for nitrofurantoin; 163 DDD/1000 population and 775 DDD/1000 population for trimethoprim and 22 DDD/1000 population and 262 DDD/1000 population for fluoroquinolones, respectively.

Trimethoprim and nitrofurantoin are recommended as first-line treatment for uncomplicated UTI in the UK. The updated PHE guidelines, published in 2014, recommended nitrofurantoin in place of trimethoprim for empirical treatment of uncomplicated UTI due to increasing community infections with community ESBL-producing bacteria and higher levels of trimethoprim resistance in *E. coli* (Public Health England, 2017a). The data presented in Figures 6.4 and 6.5 illustrate the prescribing rates for these antibiotics in the West Midlands prior to the change in the guidelines described above.

Most of the variation in prescribing rates cannot be explained by practice size (measured by registered population); although the highest level of prescribing by individual practices seems to be associated with practices that have smaller numbers of registered patients, compared with larger GP practices in the West Midlands (Figures 6.6 and 6.7).

Figure 6.4 Trimethoprim prescribing by CCG in the West Midlands in 2013. Boxplot depiction of the mean (line through box), interquartile range (box), 1.5 interquartile range (line), maximum outliers (x) and minimum outliers (+).

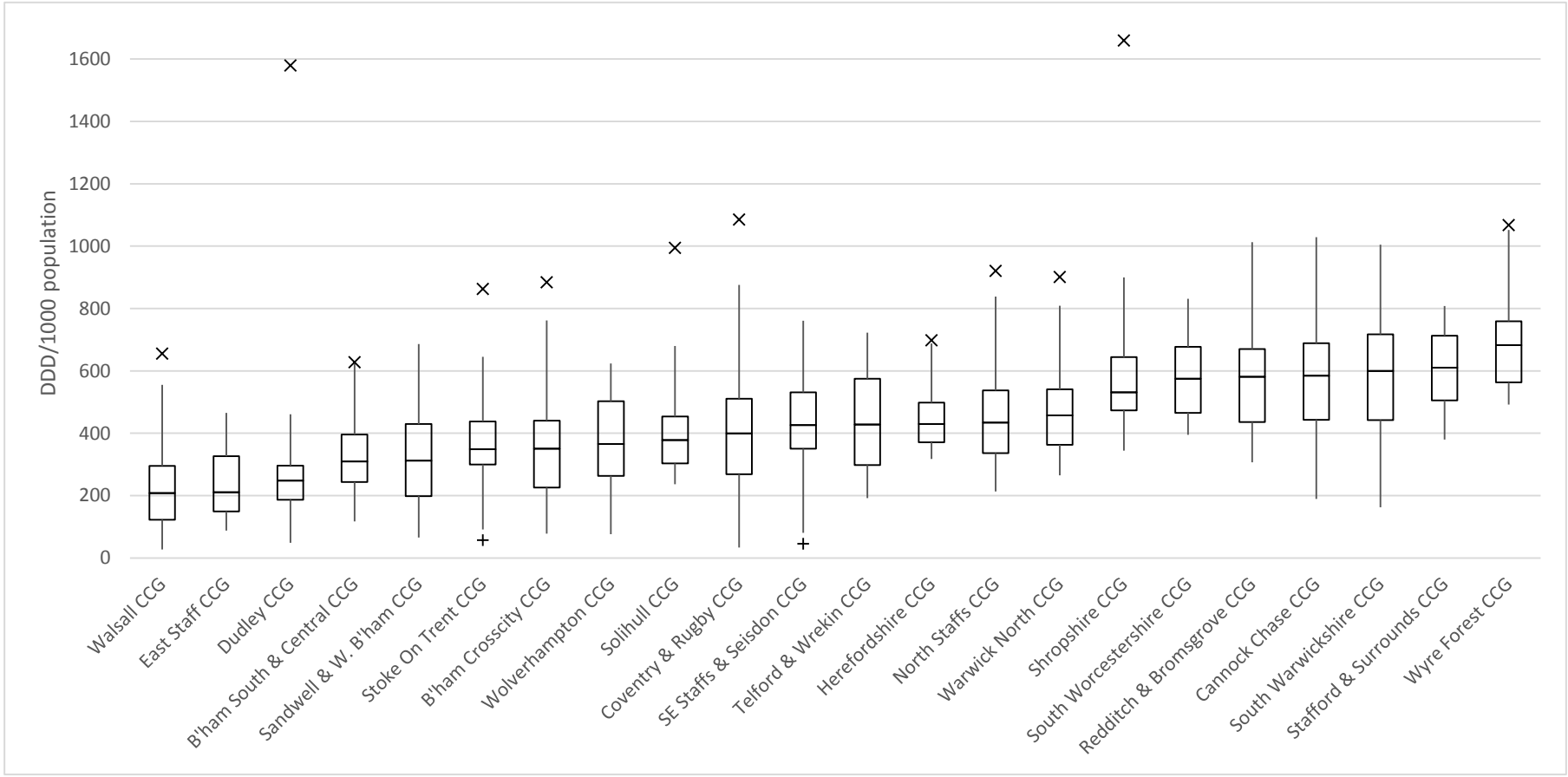


Figure 6.5 Nitrofurantoin prescribing by GP practices in the West Midlands in 2013. Boxplot depiction of the mean (line through box), interquartile range (box), 1.5 * interquartile range (line), maximum outliers (x) and minimum outliers (+).

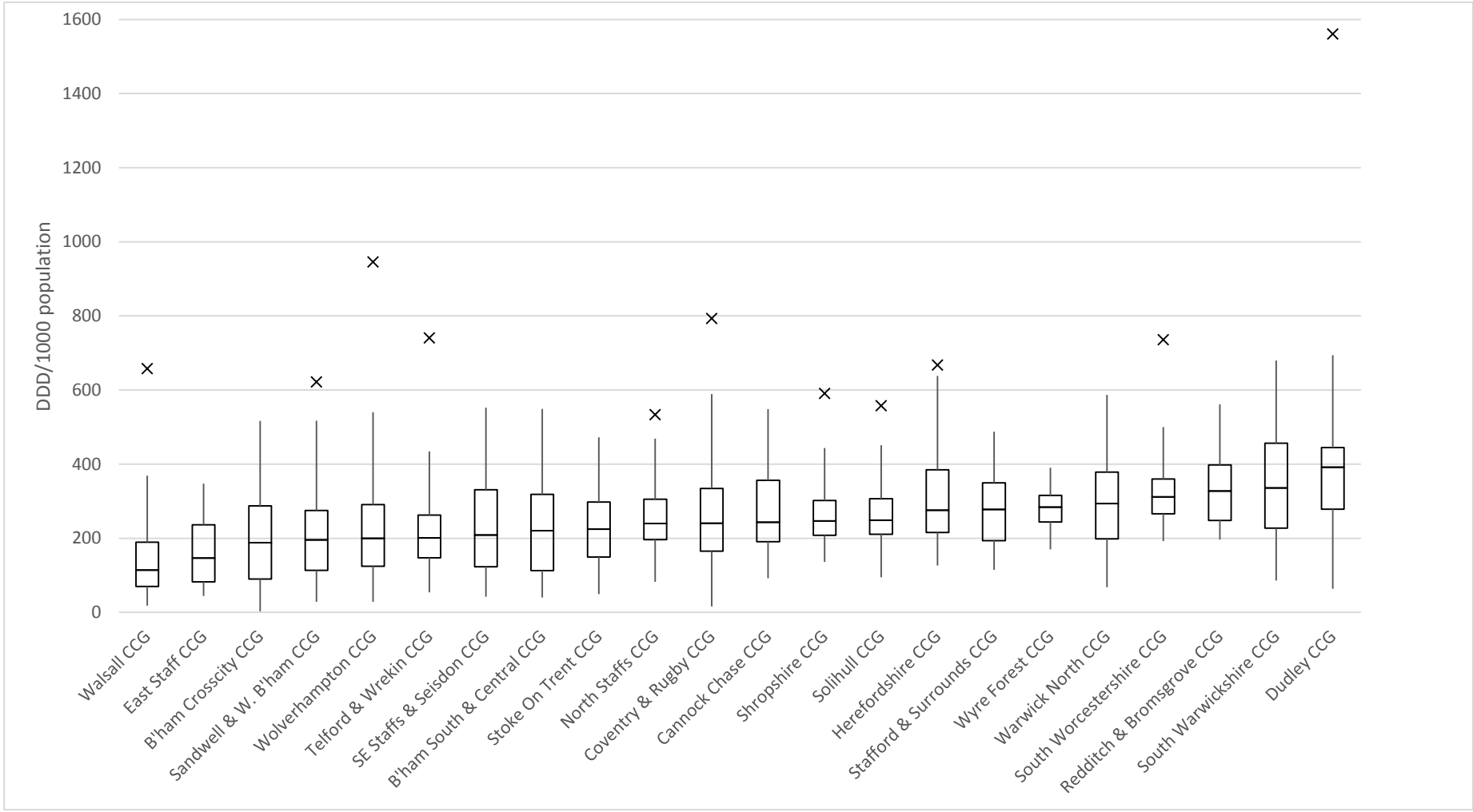


Figure 6.6 Co-amoxiclav prescribing by general practice versus the number of registered patients, West Mildands 2013.

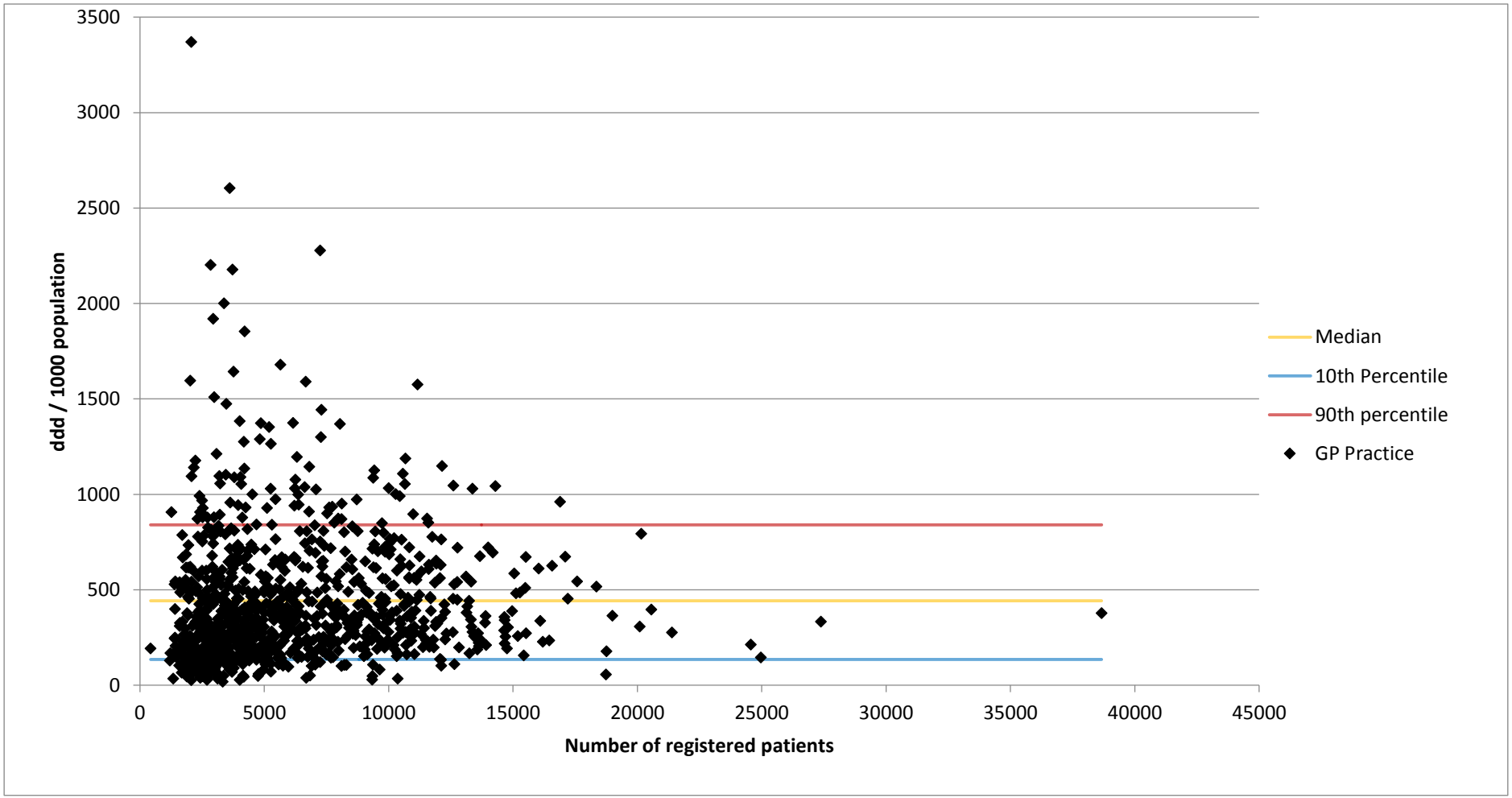
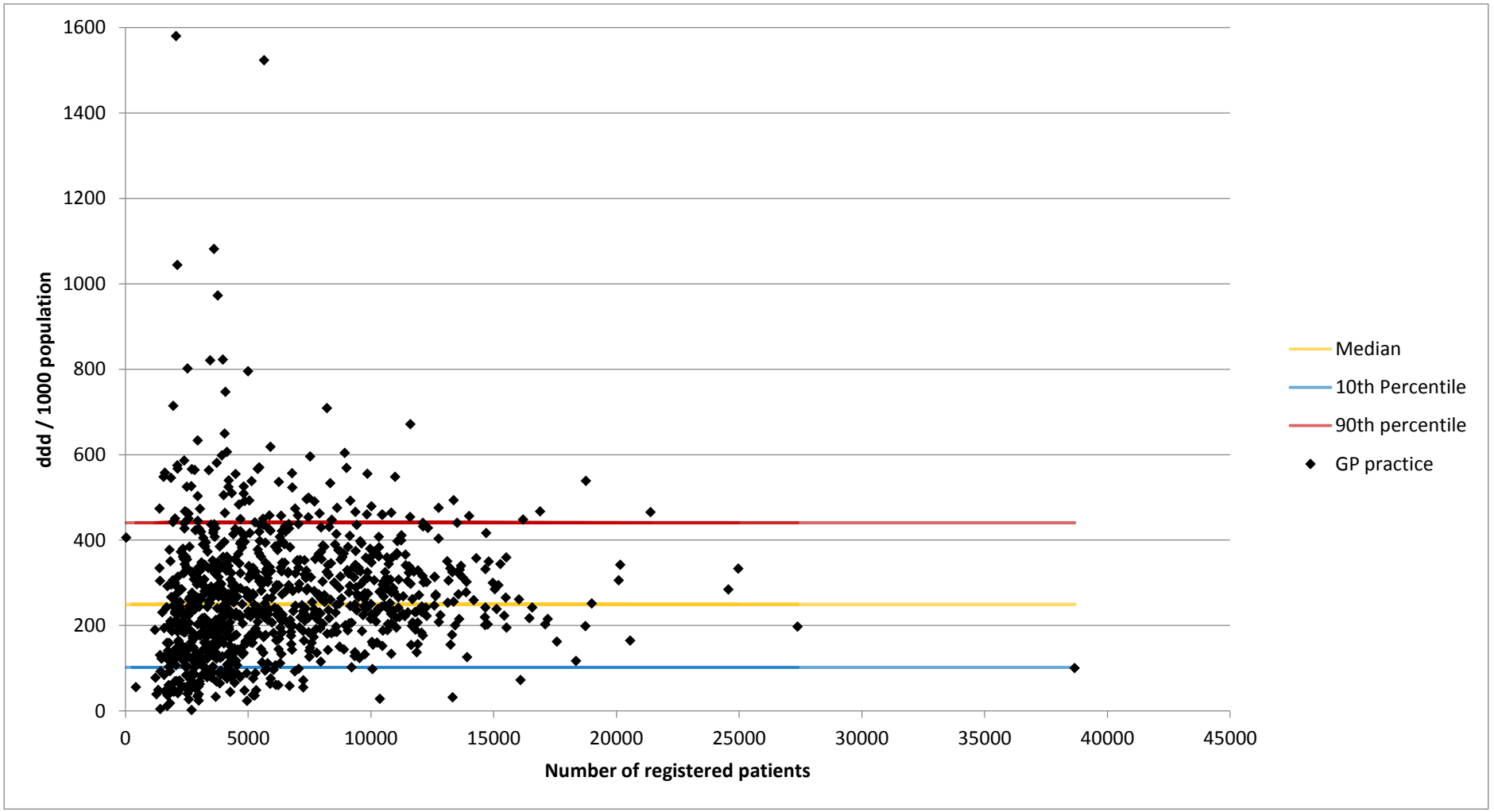


Figure 6.7 Nitrofurantoin prescribing by general practice versus the number of registered patients, West Mildands 2013.



6.4.1.2 Antibiotic susceptibility testing

During the study period there were 313,085 *E. coli* reports from urine specimens submitted by GPs situated in the West Midlands. These represented 247,971 de-duplicated laboratory reports of *E. coli*, from 181,764 patients, submitted by 911 of 948 (96%) general practices prescribing antibiotics in the West Midlands.

The proportion of *E.coli* isolates tested against the selected antibiotics and the proportion reported as non-susceptible during the study period are shown in Table 6.3. Trimethoprim and nitrofurantoin were the most consistently tested antibiotics, with essentially all *E. coli* isolates from urine specimens having susceptibility results for these antibiotics. The proportion of *E. coli* isolates tested against ciprofloxacin decreased during the period of the study, with over 90% tested in 2010 compared with <70% tested in 2013/2014. For the antibiotics selected in this study, ampicillin/amoxicillin had the highest proportions of non-susceptibility, averaging 52%, with nitrofurantoin having the lowest non-susceptibility, averaging 2.7% for the period 2010/11-2013/14 (Table 6.3).

Increased non-susceptibility to ampicillin/amoxicillin was observed in *E. coli* isolates from urine specimens in the winter periods. This appears to mirror observed winter peaks in the prescribing of ampicillin/amoxicillin (Figure 6.7). Pronounced seasonal changes in antibiotic prescribing combined with non-susceptibility was not observed for other antibiotics included in the study; although the prescribing of co-amoxiclav did appear to show increased prescribing during the winter periods. Non-susceptibility trends for defined antibiotics tested against isolates from urine specimens in the West Midlands were examined in Chapter 5. In that study a rising

trend in non-susceptibility was demonstrated for ciprofloxacin tested against West Midland *E. coli* isolates. For the additional antibiotics included in this part of the study, only trimethoprim demonstrated a rising linear trend for non-susceptibility during the study period (*p* for trend = <0.001) (Figure 6.8). Figure 6.8 also shows a gradual increase in total trimethoprim prescribing for the same time period.

Table 6.3 Quarterly count of E. coli isolates, proportion tested and proportion non-susceptible by antibiotic type, West Midlands, March 2010 – November 2013.

			Ampicillin/Amoxicillin		Cephalexin		Ciprofloxacin		Co-amoxiclav		Nitrofurantoin		Trimethoprim	
Year	Seasonal Quarter	*E. coli* isolates (n=247,971)	Tested (%)	Non-susceptible (%)	Tested (%)	Non-susceptible (%)	Tested (%)	Non-susceptible (%)	Tested (%)	Non-susceptible (%)	Tested (%)	Non-susceptible (%)	Tested (%)	Non-susceptible (%)
2010/11	1	8769	76.7	52.5	88.2	7	90.5	11.7	91.7	15.6	99.8	4.1	99.9	32.8
2010/11	2	10712	86.7	50.7	86.6	6.6	92.3	11.1	77.7	25.3	99.5	3.7	99.9	32.2
2010/11	3	10036	78.2	51.1	91.2	6	92.3	11.8	90.2	23.2	99.7	3.0	99.9	33.8
2010/11	4	11473	82.2	52.8	89.2	6.5	83	12.4	81.8	18.3	99.9	2.8	99.9	35.2
2011/12	1	13750	82.3	52.1	79.3	6.8	77.4	11.5	84.4	14.6	99.9	3.0	99.9	33.1
2011/12	2	13843	83.3	50.4	81.2	7	81	10.8	91.7	17.5	99.6	2.7	99.9	32.7
2011/12	3	16705	84.8	51	85.8	6.6	76.5	10.8	93.3	19.6	99.9	2.7	99.9	35.0
2011/12	4	16641	86	53.1	85.6	7	78.3	11.9	93.3	20.7	99.9	2.6	99.9	35.6
2012/13	1	17190	87.6	52.6	85.4	6.6	77.5	11.2	91.8	15.6	99.9	2.0	99.9	35.4
2012/13	2	18531	88.5	52.1	82.1	6.9	73.4	12.1	86	15.7	99.6	2.4	99.9	35.7
2012/13	3	20621	89.6	51.7	83.3	7	70.2	11.7	86.6	15.8	99.8	2.1	100.0	35.7
2012/13	4	21763	91.2	53.3	84.6	7.3	65.6	12.5	87.5	18.8	99.8	2.3	99.9	36.3
2013/14	1	21665	79.8	53.4	85.1	7.3	67.3	13	87.9	18.8	99.7	2.4	99.8	36.4
2013/14	2	22037	77.8	51.6	85.6	7.5	67.9	13.1	88.4	17.7	99.8	2.7	100.0	35.4
2013/14	3	24235	77.7	51.5	81.8	7.7	68.3	11.8	87.6	16.2	99.9	2.4	100.0	36.2

Figure 6.8 Ampicillin/amoxicillin prescribing and non-susceptibility of *E. coli* isolated from urine-specimens, West Midlands March 2010- December 2013

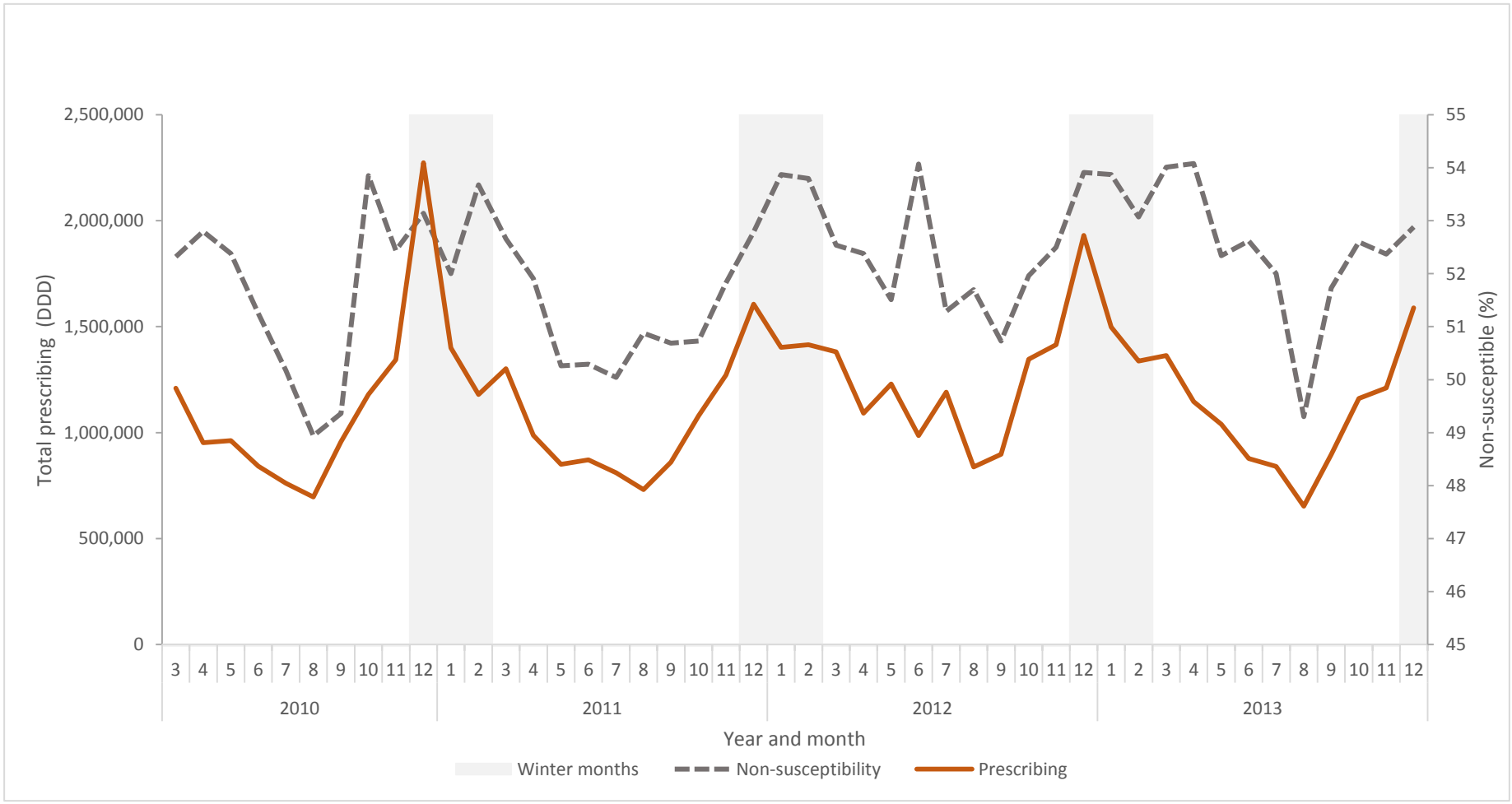
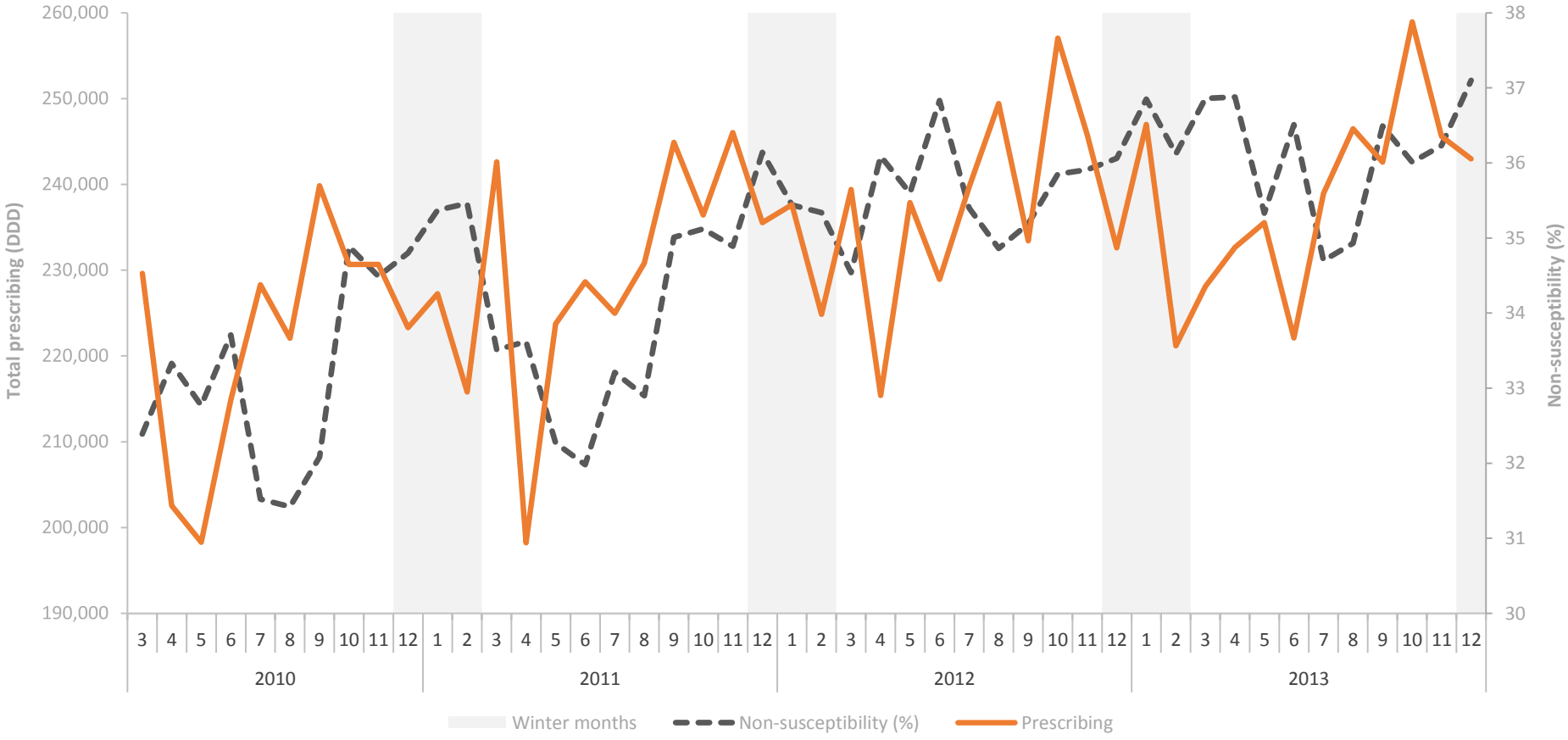


Figure 6.9 Trimethoprim prescribing and non-susceptibility of *E. coli* isolated from urine specimens, West Midlands March 2010 – December 2013



6.4.2 Statistical models

6.4.2.1 Antibiotic non-susceptibility and prescribing

Nine of the sixteen multi-level mixed effects statistical models showed a statistically significant linear relationship between *E.coli* non-susceptibility and the prescription of specific antibiotics during the same seasonal quarter or prescribing within the previous 12 months.

Ampicillin/amoxicillin was the only antibiotic for which the odds of increased *E.coli* non-susceptibility was associated with an increase in prescribing within the same quarter, when prescribing ampicillin/amoxicillin and co-amoxiclav (OR 1.003, 95% CI 1.001 - 1.006 and OR 1.006, 95% CI 1.002 - 1.009 respectively).

There was also an association between prescribing in previous quarters, and increased non-susceptibility of *E. coli* to co-amoxiclav (when prescribing ampicillin/amoxicillin), ciprofloxacin (when prescribing fluoroquinolones), nitrofurantoin (when prescribing cephalexin and nitrofurantoin) and trimethoprim (when prescribing trimethoprim) (Table 6.4).

The magnitude of the statistical associations varied, with the lowest being a 0.3% increase in the odds of non-susceptibility to ampicillin/amoxicillin for an increase in prescribing ampicillin/amoxicillin of 50 DDDs per 1000 practice population in the same quarter (95% CI 0.2% - 0.6%, p= 0.001), and the highest a 6.3% increase in the odds of non-susceptibility to nitrofurantoin for an increase in prescribing nitrofurantoin of 50 DDDs per 1000 practice population in the previous quarter (95% CI 1.3% -11.5%, p= 0.013) (Table 6.4).

There was a significant negative association in the same quarter with non-susceptibility in the following: co-amoxiclav when prescribing ampicillin/amoxicillin, ciprofloxacin when prescribing co-amoxiclav, trimethoprim when prescribing trimethoprim and in the same quarter and in the previous 12 months for nitrofurantoin when prescribing nitrofurantoin (Table 6.4), indicating increased prescribing in those periods are associated with lower numbers of non-susceptible *E. coli*.

In five of the statistical models (Models 9, 11, 12, 14 and 16) for one or more of the prescribing quarters the association was found to be a complex, non-linear form. These non-linear forms were found to be statistically significant within the models and therefore were retained.

Examining associations between antibiotic prescribing and antibiotic susceptibility was a key objective for this study. To asses these associations, a number of potential explanatory variables, including general practice characteristics, registered patients and seasons were included in the modelling process. Sections 6.3.2.2 to 6.3.2.9 provide the results from the statistical models for these other possible explanatory variables and details their relationship with non-susceptibility in *E. coli* isolated from urines specimens.

Table 6.4 Adjusted significant linear associations between antibiotic prescribing and non-susceptibility in E. coli, by current (0) or lagged (negative) quarter (models with non-linear or non-significant results not shown, see appendix 3 for full results)

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	Prescribing period	OR (50 DDD unit/1000 population)	95% CI (l)	95% CI (u)	*P* value
1	ampicillin / amoxicillin	co-amoxiclav	Quarter 0	1.006	1.002	1.009	**0.003**
2	ampicillin / amoxicillin	ampicillin / amoxicillin	Quarter 0	1.003	1.001	1.006	**0.001**
4	co-amoxiclav	ampicillin / amoxicillin	Quarter 0	0.994	0.991	0.998	**0.003**
			Quarter -3	1.006	1.002	1.009	**0.004**
			Quarter -4	1.006	1.002	1.009	**0.002**
5	ciprofloxacin	co-amoxiclav	Quarter 0	0.986	0.975	0.997	**0.015**
8	ciprofloxacin	fluoroquinolones	Quarter -4	1.033	1.003	1.066	**0.034**
9	co-amoxiclav	co-amoxiclav	Quarter -3	1.017	1.009	1.026	**<0.001**
13	nitrofurantoin	cephalexin	Quarter -3	1.041	1.009	1.075	**0.013**
14	nitrofurantoin	nitrofurantoin	Quarter 0	0.955	0.914	0.997	**0.036**
			Quarter -1	1.063	1.013	1.115	**0.013**
			Quarter -4	0.791	0.703	0.890	**<0.001**
15	trimethoprim	trimethoprim	Quarter 0	0.988	0.978	0.999	**0.031**
			Quarter -1	1.016	1.004	1.028	**0.008**
			Quarter -2	1.018	1.006	1.030	**0.003**
			Quarter -4	1.016	1.005	1.026	**0.005**

OR = adjusted odds ratio.
Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

6.4.2.2 Seasonal quarters

In all statistical models, the March to May (spring) period was used as the comparator for assessing seasonal association with antibiotic non-susceptibility. Seven of the 16 models had statistically significant associations for one or more seasonal periods (when compared with spring), with non-susceptibility in *E. coli* isolated from urine specimens (Table 6.5).

Models 1 and 3 have higher odds for reduced numbers of *E. coli* non-susceptible to ampicillin/amoxicillin in summer and autumn when prescribing co-amoxiclav and fluoroquinolones, but increased odds of higher numbers non-susceptible *E. coli* in the winter period when prescribing these antibiotics, compared with the spring period.

Models 4 and 9 (non-susceptibility to co-amoxiclav when prescribing ampicillin/amoxicillin and co-amoxiclav, respectively), showed increased odds of non-susceptibility to co-amoxiclav in the summer period; however the magnitude of the association was much higher for the number of *E. coli* non-susceptible to co-amoxiclav in the winter periods (model 4 adjusted OR=1.173, p=<0.001 and model 9 adjusted OR=1.179, p=<0.001) compared with spring.

The statistical models suggest odds for reduced numbers of *E. coli* non-susceptible to ciprofloxacin in the autumn period, compared with spring, when prescribing co-amoxiclav (Model 5), cephalexin (Model 7) and fluoroquinolones (Model 8).

6.4.2.3 Practice population age groups

The proportion of the practice population <15 years old showed significant statistical association with antibiotic non-susceptibility in *E. coli* isolates from urine specimens in 12 of the 16 antibiotic prescribing / antibiotic non-susceptibility combinations (Table 6.6). The statistical models suggest that for every one percent increase in the proportion of the population aged <15 years the percentage the odds of non-susceptibility increased, by 0.5% (Model 2) to 1.5% (Model 16).

Only model 4, co-amoxiclav non-susceptibility when prescribing ampicillin/amoxicillin, showed a significant linear association with the proportion of the practice population ≥65 years, with every 1% increase in proportion of registered practice patients ≥65 years the odds of fewer *E. coli* non-susceptible to co-amoxicillin increased by 1.4% (adjusted odd ratio=0.986, 95% CI=0.980-0.991, P=<0.001).

Table 6.5 Adjusted association (OR) of seasonal quarters, compared with March-May (spring), with non-susceptibility of E. coli isolated from urine specimens taken from patients in the community in the West Midland, March 2010-February 2014.

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	June-August (Summer)				September-November (Autumn)				December-February (Winter)			
			OR	95% CI (l)	95% CI (u)	*p* value	OR	95% CI (l)	95% CI (u)	*p* value	OR	95% CI (l)	95% CI (u)	*p* value
1	ampicillin / amoxicillin	co-amoxiclav	**0.953**	**0.929**	**0.977**	**<0.001**	**0.968**	**0.945**	**0.993**	**0.011**	**1.037**	**1.010**	**1.065**	**0.007**
2	ampicillin / amoxicillin	ampicillin / amoxicillin	0.994	0.966	1.023	0.680	1.005	0.966	1.046	0.809	1.033	0.990	1.078	0.130
3	ampicillin / amoxicillin	fluoroquinolones	**0.950**	**0.926**	**0.974**	**<0.001**	**0.968**	**0.944**	**0.992**	**0.010**	**1.040**	**1.013**	**1.068**	**0.004**
4	co-amoxiclav	ampicillin / amoxicillin	**1.082**	**1.014**	**1.156**	**0.018**	1.004	0.928	1.086	0.928	**1.173**	**1.096**	**1.255**	**<0.001**
5	ciprofloxacin	co-amoxiclav	0.991	0.944	1.041	0.730	**0.922**	**0.877**	**0.970**	**0.002**	0.992	0.939	1.047	0.758
6	ciprofloxacin	ampicillin / amoxicillin	1.020	0.942	1.104	0.626	0.979	0.889	1.078	0.662	1.018	0.936	1.107	0.681
7	ciprofloxacin	cephalexin	1.001	0.953	1.050	0.982	**0.923**	**0.878**	**0.969**	**0.001**	0.983	0.932	1.038	0.536
8	ciprofloxacin	fluoroquinolones	1.002	0.955	1.052	0.931	**0.915**	**0.871**	**0.962**	**<0.001**	0.982	0.930	1.036	0.497
9	co-amoxiclav	co-amoxiclav	**1.081**	**1.037**	**1.127**	**<0.001**	1.020	0.974	1.068	0.392	**1.179**	**1.128**	**1.233**	**<0.001**
10	cephalexin	cephalosporin	1.022	0.964	1.083	0.467	0.989	0.934	1.048	0.717	1.022	0.960	1.089	0.489
11	cephalexin	fluoroquinolones	1.011	0.955	1.069	0.708	0.989	0.935	1.045	0.691	1.057	0.997	1.121	0.062
12	cephalexin	nitrofurantoin	1.019	0.961	1.080	0.528	0.978	0.924	1.036	0.454	1.054	0.994	1.118	0.079
13	nitrofurantoin	cephalexin	1.021	0.940	1.109	0.618	0.928	0.853	1.009	0.079	0.966	0.887	1.051	0.422
14	nitrofurantoin	nitrofurantoin	1.041	0.957	1.131	0.350	0.939	0.862	1.024	0.153	0.961	0.872	1.058	0.415
15	trimethoprim	Trimethoprim	0.975	0.949	1.002	0.069	0.999	0.969	1.030	0.962	1.005	0.975	1.036	0.732
16	cephalexin	Trimethoprim	1.024	0.966	1.086	0.429	0.981	0.924	1.042	0.529	1.029	0.964	1.098	0.395

Bold values signify significant statistical association. OR = adjusted odds ratio. Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

Table 6.6 Adjusted significant associations (OR) between proportion of practice population <15 years old and non-susceptibility of E. coli isolated from urine specimens

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR (1%increase in proportion)	95% CI (l)	95% CI (u)	*p* value
1	ampicillin / amoxicillin	co-amoxiclav	**1.006**	**1.003**	**1.009**	**<0.001**
2	ampicillin / amoxicillin	ampicillin / amoxicillin	**1.005**	**1.002**	**1.008**	**0.001**
3	ampicillin / amoxicillin	fluoroquinolones	**1.006**	**1.003**	**1.009**	**<0.001**
4	co-amoxiclav	ampicillin / amoxicillin	1.005	0.997	1.013	0.243
5	ciprofloxacin	co-amoxiclav	Non-linear form			
6	ciprofloxacin	ampicillin / amoxicillin	**1.011**	**1.005**	**1.017**	**<0.001**
7	ciprofloxacin	cephalexin	Non-linear form			
8	ciprofloxacin	fluoroquinolones	**1.012**	**1.006**	**1.018**	**<0.001**
9	co-amoxiclav	co-amoxiclav	1.007	0.999	1.016	0.098
10	cephalexin	cephalosporin	**1.014**	**1.008**	**1.020**	**<0.001**
11	cephalexin	fluoroquinolones	**1.014**	**1.009**	**1.020**	**<0.001**
12	cephalexin	nitrofurantoin	**1.013**	**1.007**	**1.018**	**<0.001**
13	nitrofurantoin	cephalexin	**1.012**	**1.004**	**1.021**	**0.005**
14	nitrofurantoin	nitrofurantoin	**1.011**	**1.003**	**1.020**	**0.01**
15	trimethoprim	trimethoprim	**1.010**	**1.007**	**1.014**	**<0.001**
16	cephalexin	trimethoprim	**1.015**	**1.009**	**1.021**	**<0.001**

Bold values signify significant statistical association. OR = adjusted odds ratio. Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

6.4.2.4 General practices according to location (urban versus rural)

Five of the 16 statistical models had a significant association between general practices designated to be in rural locations and antibiotic non-susceptibility in *E. coli* isolated from urine specimens. The association in all five models was negative, indicating that a rural setting is associated with decreased numbers of non-susceptible *E. coli* (adjusted OR 0.866-0.588) (Table 6.7).

6.4.2.5 Antibiotic non-susceptibility and single-handed practices

A statistically significant association was found between *E. coli* non-susceptibility and single-handed practices in all 16 statistical prescribing / non-susceptibility models (Table 6.8). In all 16 models a single-handed practice was associated with increased numbers of non-susceptible *E. coli* isolates from urine specimens (adjusted ORs 1.083 -1.657).

Table 6.7 Adjusted association (OR) between rural practice location and antibiotic non-susceptibility of E. coli isolated from urine specimens

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR	95% CI (l)	95% CI (u)	*P* value
1	ampicillin / amoxicillin	co-amoxiclav	0.970	0.927	1.014	0.181
2	ampicillin / amoxicillin	ampicillin / amoxicillin	0.976	0.934	1.019	0.263
3	ampicillin / amoxicillin	fluoroquinolones	0.984	0.941	1.028	0.467
4	co-amoxiclav	ampicillin / amoxicillin	**0.617**	**0.530**	**0.718**	**<0.001**
5	ciprofloxacin	co-amoxiclav	1.012	0.912	1.124	0.816
6	ciprofloxacin	ampicillin / amoxicillin	0.979	0.878	1.091	0.701
7	ciprofloxacin	cephalexin	0.986	0.885	1.098	0.792
8	ciprofloxacin	fluoroquinolones	0.986	0.886	1.098	0.800
9	co-amoxiclav	co-amoxiclav	**0.588**	**0.502**	**0.689**	**<0.001**
10	cephalexin	cephalosporin	**0.876**	**0.794**	**0.967**	**0.008**
11	cephalexin	fluoroquinolones	0.923	0.839	1.015	0.097
12	cephalexin	nitrofurantoin	0.928	0.844	1.021	0.123
13	nirofurantoin	cephalexin	**0.866**	**0.771**	**0.974**	**0.016**
14	nitrofurantoin	nitrofurantoin	**0.866**	**0.773**	**0.972**	**0.014**
15	trimethoprim	trimethoprim	0.991	0.936	1.049	0.755
16	cephalexin	trimethoprim	0.909	0.817	1.011	0.079

Bold values signify significant statistical association. OR = adjusted odds ratio. Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

Table 6.8 Adjusted association (OR) between single-handed GP practices and antibiotic non-susceptibility of E. coli isolated from urine specimens

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR	95% CI (l)	95% CI (u)	*P* value
1	ampicillin / amoxicillin	co-amoxiclav	1.097	1.027	1.171	**0.006**
2	ampicillin / amoxicillin	ampicillin / amoxicillin	1.083	1.014	1.156	**0.018**
3	ampicillin / amoxicillin	fluoroquinolones	1.095	1.024	1.170	**0.008**
4	co-amoxiclav	ampicillin / amoxicillin	1.361	1.148	1.614	**<0.001**
5	ciprofloxacin	co-amoxiclav	1.458	1.267	1.676	**<0.001**
6	ciprofloxacin	ampicillin / amoxicillin	1.448	1.258	1.666	**<0.001**
7	ciprofloxacin	cephalexin	1.370	1.180	1.592	**<0.001**
8	ciprofloxacin	fluoroquinolones	1.371	1.182	1.590	**<0.001**
9	co-amoxiclav	co-amoxiclav	1.398	1.171	1.669	**<0.001**
10	cephalexin	cephalosporin	1.528	1.322	1.767	**<0.001**
11	cephalexin	fluoroquinolones	1.534	1.337	1.759	**<0.001**
12	cephalexin	nitrofurantoin	1.534	1.340	1.756	**<0.001**
13	nitrofurantoin	cephalexin	1.606	1.304	1.979	**<0.001**
14	nitrofurantoin	nitrofurantoin	1.657	1.352	2.031	**<0.001**
15	trimethoprim	trimethoprim	1.110	1.026	1.201	**0.009**
16	cephalexin	trimethoprim	1.603	1.395	1.841	**<0.001**

OR = adjusted odds ratio. Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

6.4.2.6 Antibiotic non-susceptibility and gender

Only Model 4, co-amoxiclav non-susceptibility with prescribing ampicillin / amoxicillin, demonstrated a significant linear association with population gender (adjusted OR 0.241, 95% CI 0.116 – 0.502, *p*= <0.001). All the other 15 models demonstrated significant but complex non-linear forms for the gender covariate.

6.4.2.7 Antibiotic non-susceptibility and deprivation

A significant association was found, between *E. coli* non-susceptibility and the IMD deprivation score derived for general practices, in 12 of the 16 statistical models. Five of these associations were significant complex non-linear forms, with seven non-susceptible / prescribing combinations found to have significant linear associations with deprivation scores (Table 6.9). However the adjusted ORs for the linear associations were small, with all being <1% increase in the odds of non-susceptibility for a unit increase in the deprivation score.

Table 6.9 Adjusted association (OR) between GP practice deprivation score and antibiotic non-susceptibility of E. coli isolated from urine specimens

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR (non-linear form)	95% CI (l)	95% CI (u)	*P* value
1	ampicillin / amoxicillin	Co-amoxiclav	(non-linear)			
2	ampicillin / amoxicillin	ampicillin / amoxicillin	(non-linear)			
3	ampicillin / amoxicillin	fluoroquinolones	(non-linear)			
4	co-amoxiclav	ampicillin / amoxicillin	(non-linear)			
5	ciprofloxacin	Co-amoxiclav	**1.005**	**1.001**	**1.010**	**0.023**
6	ciprofloxacin	ampicillin / amoxicillin	1.005	1.000	1.010	0.056
7	ciprofloxacin	cephalexin	1.005	1.000	1.009	0.064
8	ciprofloxacin	fluoroquinolones	**1.006**	**1.001**	**1.011**	**0.016**
9	co-amoxiclav	Co-amoxiclav	(non-linear)			
10	cephalexin	cephalosporin	1.004	1.000	1.009	0.073
11	cephalexin	fluoroquinolones	**1.006**	**1.002**	**1.011**	**0.005**
12	cephalexin	nitrofurantoin	**1.007**	**1.002**	**1.011**	**0.004**
13	nirofurantoin	cephalexin	**1.007**	**1.001**	**1.013**	**0.027**
14	nitrofurantoin	nitrofurantoin	**1.006**	**1.000**	**1.012**	**0.047**
15	trimethoprim	trimethoprim	1.002	1.000	1.005	0.080
16	cephalexin	trimethoprim	**1.005**	**1.000**	**1.010**	**0.037**

Bold values signify significant statistical association. OR = adjusted odds ratio.
Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

6.4.2.8 Antibiotic non-susceptibility and the number of GPs per population

Ten of the 16 statistical models had a significant association for the number of GPs per 100,000 population and antibiotic non-susceptibility in *E. coli* isolates from urine specimens (Table 6.10). The adjusted OR for each of the 10 significant linear associations was >1 suggesting that an increase in the number of GPs per 100,000 population increases the odds of increased numbers of non-susceptible *E. coli* in the practice population; however the magnitude of the increase was small for each increase in GP / 100,000 (adjusted ORs 0.001 to 0.002). The 10 models with significant associations comprised of only three antibiotics, which were assessed against all the prescribed antibiotic combinations used in the models; that is non-susceptibility to: ciprofloxacin (Models 5, 6, 7, 8), cephalexin (Models 10, 11, 12, 16) and nitrofurantoin (Models 13, 14) (Table 6.10).

6.4.2.9 Association of antibiotic non-susceptibility and time elapsed during study

In eight of the 16 models there was a significant linear association between the time elapsed during the entire study period and non-susceptibility of *E. coli* isolates from urine specimens (Table 6.11). For the three ampicillin/amoxicillin non-susceptibility models (Models 1-3) and cephalexin non-susceptibility (when prescribing cephalosporins), the association suggested an increase in non-susceptibility for increases in time; whereas all the models featuring non-susceptibility of ciprofloxacin (Models 5-8) suggested a decrease in non-susceptibility over time. The magnitude of increases or decreases in non-susceptibility in relation to time was small across all the models (<1% change in non-susceptibility).

Table 6.10 Adjusted association (OR) between the number of GPs per 100,000 practice population and antibiotic non-susceptibility of E. coli isolated from urine specimens

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR	95% CI (l)	95% CI (u)	*P* Value
1	ampicillin / amoxicillin	co-amoxiclav	1.000	0.999	1.000	0.843
2	ampicillin / amoxicillin	ampicillin / amoxicillin	1.000	0.999	1.000	0.438
3	ampicillin / amoxicillin	fluoroquinolones	1.000	1.000	1.001	0.842
4	co-amoxiclav	ampicillin / amoxicillin	1.000	0.999	1.002	0.693
5	ciprofloxacin	co-amoxiclav	**1.002**	**1.001**	**1.003**	**0.001**
6	ciprofloxacin	ampicillin / amoxicillin	**1.002**	**1.001**	**1.003**	**0.002**
7	ciprofloxacin	cephalexin	**1.002**	**1.001**	**1.003**	**0.003**
8	ciprofloxacin	fluoroquinolones	**1.002**	**1.000**	**1.003**	**0.007**
9	co-amoxiclav	co-amoxiclav	1.000	0.999	1.002	0.839
10	cephalexin	cephalosporin	**1.001**	**1.000**	**1.002**	**0.044**
11	cephalexin	fluoroquinolones	**1.002**	**1.000**	**1.003**	**0.010**
12	cephalexin	nitrofurantoin	**1.001**	**1.000**	**1.003**	**0.024**
13	nitrofurantoin	cephalexin	**1.002**	**1.000**	**1.004**	**0.026**
14	nitrofurantoin	nitrofurantoin	**1.002**	**1.000**	**1.004**	**0.024**
15	trimethoprim	trimethoprim	1.000	0.999	1.000	0.164
16	cephalexin	trimethoprim	**1.001**	**1.000**	**1.003**	**0.025**

Bold values signify significant statistical association. OR = adjusted odds ratio.
Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

Table 6.11 Significant associations (ORs) between time and antibiotic non-susceptibility of E. coli isolated from urine specimens (non-significant models not shown)

Model no.	Antibiotic non-susceptibility	Antibiotic prescribed	OR (non-linear form)	95% CI (l)	95% CI (u)	*P Value*
1	ampicillin / amoxicillin	Co-amoxiclav	**1.005**	**1.002**	**1.007**	**<0.001**
2	ampicillin / amoxicillin	ampicillin / amoxicillin	**1.005**	**1.002**	**1.007**	**<0.001**
3	ampicillin / amoxicillin	fluoroquinolones	**1.005**	**1.002**	**1.007**	**<0.001**
4	co-amoxiclav	ampicillin / amoxicillin	(Non-linear)			
5	ciprofloxacin	Co-amoxiclav	**0.992**	**0.986**	**0.998**	**0.006**
6	ciprofloxacin	ampicillin / amoxicillin	**0.992**	**0.986**	**0.998**	**0.006**
7	ciprofloxacin	cephalexin	**0.993**	**0.987**	**0.999**	**0.024**
8	ciprofloxacin	fluoroquinolones	**0.993**	**0.987**	**0.999**	**0.016**
9	co-amoxiclav	co-amoxiclav	(Non-linear)			
10	cephalexin	cephalosporin	**1.009**	**1.002**	**1.017**	**0.013**
11	cephalexin	fluoroquinolones	(Non-linear)			
12	cephalexin	nitrofurantoin	(Non-linear)			
13	nitrofurantoin	cephalexin	(Non-linear)			
14	nitrofurantoin	nitrofurantoin	(Non-linear)			
15	trimethoprim	trimethoprim	(Non-linear)			
16	cephalexin	trimethoprim	(Non-linear)			

Bold values signify significant statistical association. OR = adjusted odds ratio.
Lower and upper 95% confidence intervals = CI (l) and CI (u) respectively

6.5 Discussion

This section firstly discusses the findings from the descriptive analysis and then goes on to discuss the results from the statistical models. The main focus of the statistical study was to measure associations between antibiotic prescribing and non-susceptibility in *E. coli* isolates from urine specimens; however with the multifactorial nature of AMR, this section also discusses the effect of other potential explanatory variables included in the model building (Table 6.2).

6.5.1 Descriptive analysis

6.5.1.1 Seasonal prescribing

A temporal association was observed between prescribing ampicillin/amoxicillin and non-susceptibility to this antibiotic in *E. coli* isolated from urine specimens in this study, with peaks in the winter months (Figure 6.8). Seasonal increases in antibiotic prescribing in England and Wales has been shown to be associated with the increased number of winter respiratory infections diagnosed in the community (Fleming et al., 2003b). Increased antibiotic prescribing in winter months has been described in Europe (Goossens et al, 2005), including regional variation within countries (Achermann et al., 2011). A study in the USA in 2012 supports the findings reported in this study. The USA study used a dataset that covered 70% of all prescriptions, and seasonal relationships were demonstrated for a number of combinations of prescribed antibiotics and resistance, including a correlation of

prescribing aminopenicillins (lagged by 1 month) and resistance in all *E. coli* isolates (Sun et al., 2012b).

Ampicillin/amoxicillin prescribing represented 30% of the total quantity of antibiotics prescribed in the West Midlands in 2013; and although there is a seasonal trend in prescribing, the proportion has not changed significantly in this 2010-2013 study period (Figure 6.8). In the UK, amoxicillin is not first-line treatment for UTI, but it is first-line treatment for many community respiratory tract infections (Public Health England, 2017b). Most UK general practices over-prescribe for respiratory conditions, particularly in the winter period, with a study reporting that the median practice prescribed antibiotics in 38% of consultations for ‘colds and upper respiratory infections’, 48% for 'cough and bronchitis' and 60% for 'sore throat’ (Gulliford et al., 2014). It is therefore plausible that winter peaks in prescribing for respiratory conditions are contributing to the selection of non-susceptible *E. coli* in urine specimens from patients in the community.

Inappropriate seasonal antibiotic prescribing for respiratory infections in the community may also be driving antibiotic resistance in hospitals, as *E. coli* UTI in the community is an important risk factor for acquiring more serious infections, such a bacteraemia, requiring hospital care (Abernethy et al., 2017). A randomised controlled trial in 2005 showed that the use of narrow-spectrum antibiotics among hospitalised patients with community-acquired pneumonia, rather than a broad-spectrum antibiotic such as amoxicillin, resulted in comparable clinical outcomes (van der Eerden et al., 2005). The findings reported in this chapter suggest this approach may also result in reduced numbers of non-susceptible *E. coli* in the population.

6.5.1.2 Variation in antibiotic prescribing

The descriptive analysis part of this study describes the substantial variation in antibiotic prescribing by general practices in the West Midlands, with a greater than two-fold difference between the 5th and 95th percentile for total antibiotic prescribing between practices in 2013. This total variation included a four-fold difference in ampicillin / amoxicillin, eight-fold difference for nitrofurantoin and a 10-fold difference for co-amoxiclav amongst practices (section 6.4.1.1). These findings are supported by an English study using 2004-2005 prescribing data, with the authors reporting a two-fold difference in total prescribing between the 10th and 90th percentiles using the Specific Therapeutic group Age-sex Related Prescribing Unit (STAR-PU) standardised population measure (Wang et al, 2009b). The quantity of antibiotic prescribing also varies between countries, with more than three-fold difference in total antibiotic prescribing between European nations, with lower rates being reported in more northerly countries (Goossens et al, 2005).

Although total antibiotic prescribing declined in the UK between 1995 and 2000 it has since returned to similar levels observed in the 1990s (Ashiru-Oredope et al., 2012a; Wang et al, 2009a). The Quality, Innovation, Productivity and Prevention (QIPP) prescribing comparators were introduced in 2012 to reduce prescribing in primary care, and in particular reduce the proportion of cephalosporin and fluoroquinolone prescribing in general practice (Health and Social Care Information Centre, 2015). Cephalosporin and fluoroquinolone use has fallen markedly with the introduction of this initiative; however it appears that these antibiotics have been replaced by other antibiotics such as co-amoxiclav (Ashiru-Oredope et al., 2012b). The data presented in this chapter show comparatively lower proportions of cephalosporin and

fluoroquinolone prescribed by general practices in the West Midlands; however there is still a 20-fold and 10-fold difference respectively in prescribing these antibiotics between the 5th and 95th percentile in 2013.

There is a paucity of published literature explaining the large variation observed in antibiotic prescribing between practices in England. A study using 2004-2005 prescribing data showed small associations for higher prescribing in practices with higher population morbidity, shorter appointment times, non-training practices and practices with higher proportion of GPs who were male, >45 years old and qualified outside the UK. However these practice and population characteristics only explained 17% of the variance in prescribing between practices (Wang et al, 2009a).

6.5.1.3 Single-handed practices

Single-handed practices is one of the potential explanatory variables included in the statistical models. The descriptive analysis reported in this chapter demonstrates higher rates of antibiotic prescribing by single-handed practices (Figure 6.1). Although many single-handed practices are merging to form larger practices, 15% of practices have only one registered GP in the West Midlands (NHS Digital, 2016a). A study using English prescribing data for all drugs from 1994 to 1998 reported higher prescribing rates with all medicines amongst single-handed practices (Unsworth and Walley, 2001). A study from Norway suggests that higher prescribing, and prescribing broad spectrum antibiotics are associated with higher numbers of consultations per GP (Gjelstad et al., 2011), which may be a factor in the observed higher prescribing by single-handed practices reported in this study. Variation has also been reported in the prescribing habits of individual GPs (intra-physician variability), with a study in France suggesting that up to 70% of observed variation in

prescribing is due to prescribers not being consistent in their own approach (Mousques et al., 2010). It is conceivable that the inability to meet regularly with practice colleagues to discuss, review and audit the management of patients may be a factor in 'intra-physician' variability in a single-handed practice.

6.5.1.4 Patient-level factors influencing prescribing practice

As described in the background section of this chapter, understanding the expectations of patients in regards to antibiotic prescribing is important in understanding antibiotic prescribing variation in the community. Patient pressure may be responsible for much of the inappropriate prescribing by GPs (McNulty et al., 2007). A systematic review of prescribing behaviour concluded that prescribers perceive that patients want to be prescribed an antibiotic, and this combined with the fear of negative consequences if they do not provide antibiotics, is driving imprudent prescribing (Teixeira et al, 2013).

A 'situational analysis' by the WHO reported that knowledge of AMR was low in all WHO regions for both members of the public and healthcare workers (WHO, 2015), and although individual countries such as the UK have promoted national awareness campaigns, these had been largely ineffective (McNulty et al, 2007). A recent systematic international review of public knowledge and beliefs about antibiotic resistance concluded that the public has an incomplete understanding of AMR and that they do not believe they play any part in its development (McCullough et al., 2016). Since 1999 the Department of Health has run antibiotic awareness campaigns aimed at both the public and healthcare professionals, and since 2008 this has coincided with the European Antibiotic Awareness day (EAAD) on the 18th November (Ashiru-Oredope et al, 2012b). Using lessons learnt from previous

campaigns, in 2014 PHE has led an initiative called ‘Antibiotic Guardian’ to support people to take personal and collective action to use antibiotics wisely and overcome the ‘intention-behaviour’ gap. Within 3 months 11833 people had registered as antibiotic guardians, of which 31% were members of the public (Ashiru-Oredope and Hopkins, 2015). It is too early to assess the effectiveness of this campaign, but it is hoped that by increasing public knowledge of the misuse of antibiotics, not receiving a prescriptions or receiving a delayed prescriptions for antibiotics will be more acceptable to the general population (McNulty et al, 2007).

6.5.2 Statistical models

6.5.2.1 Prescribing and non-susceptibility

A key objective of this thesis was to determine if increased antibiotic prescribing in the community was associated with increased numbers of non-susceptible bacteria causing UTI in the general practice population. The multi-level modelling, described in this chapter, demonstrated that small increases in antibiotic prescribing by general practices in the West Midlands for a range of antibiotics increased the odds that *E. coli* isolated from urine specimens from the practice population would be non-susceptible to one or more antibiotics.

Excessive prescribing of antibiotics is associated with antibiotic resistance at national and regional levels (Goossens et al, 2005); however local prescribers are not always convinced that a reduction in their own prescribing will reduce resistance in their population (McNulty, 2001). The results reported in this chapter provide

evidence to show that small increases in prescribing at a practice-level significantly raises the odds of increased antimicrobial resistance. In order to change behaviour, prescribers also need to believe that the opposite behaviour, i.e. reduced prescribing, will reduce resistance within their practice (Björkman et al, 2013). It is plausible that in the absence of antibiotic selective pressure, the 'less-fit' antibiotic-resistant bacterial strains will be replaced by the 'fitter' wild-types that are susceptible to commonly prescribed antibiotics (Heinemann et al., 2000). A study in Wales found that a statistically significant decrease in ampicillin resistance of 1.03% for every decrease of 50 amoxicillin items dispensed per 1000 patients per annum and a decrease in trimethoprim resistance of 1.08% for every decrease of 20 trimethoprim items dispensed per 1000 patients per annum, (Butler et al., 2007). The Welsh study complements the findings reported in this chapter, suggesting that small increases, or decreases in prescribing, effect the numbers of resistant bacteria in the local practice population.

In the following sections the associations between exposure and non-susceptibility for specific antibiotic combinations found in the statistical models are discussed.

6.5.2.2 Ampicillin/amoxicillin, co-amoxiclav and trimethoprim prescribing

The findings reported in this chapter of an association between ampicillin/amoxicillin non-susceptibility in *E .coli* and prescribing levels during the same three month period at a population level are supported by a systematic international review of the effect of prescribing on AMR in individual patients. This study concluded that an association with resistance is strongest in the month immediately following treatment (Costelloe et al, 2010b). Individual patient-level studies in England in 2005 and Wales in 2007 also found that ampicillin/amoxicillin resistance was associated with

prescribing of ampicillin/amoxicillin within the previous 1-2 months (Hay et al., 2005a;Hillier et al., 2007). The Welsh study also supports the results presented in this study, by describing a temporal nature between exposure and resistance for ampicillin / amoxicillin; that is, although they found association in the first 1-2 months, they did not find an association with exposure 12 months previously (Hillier et al, 2007).

The immediate effect described above of increased numbers of *E. coli* non-susceptible to ampicillin/amoxicillin with increased prescribing of beta-lactam antibiotics (models 1 and 2) may be due to the selection and rapid multiplication of TEM beta-lactamase producing strains (Brismar et al., 1993). It is plausible that the selection of these strains would have a negative association with co-amoxiclav, as observed in Model 4, as this antibiotic remains active against common TEM beta-lactamases.

The successful *E. coli* urinary pathogenic clonal group ST131 is associated with combined non-susceptibility to beta-lactam antibiotics and fluoroquinolones, (Johnson et al., 2010b) and therefore the successful action of co-amoxiclav against these strains may also reduce the population non-susceptible to ciprofloxacin, as observed in Model 5 (Table 6.4). The negative association with non-susceptibility to co-amoxicillin (prescribing ampicillin/amoxicillin) and trimethoprim (prescribing trimethoprim) in the immediate quarter is reversed in previous prescribing quarters with a positive association, suggesting sufficient time had elapsed for a previously susceptible population to acquire resistance.

6.5.2.3 Nitrofurantoin, cephalosporin and fluoroquinolones prescribing

Nitrofurantoin remains active against multi-drug resistant (MDR) *E.coli*, which may explain the negative association with non-susceptibility and increased prescribing of nitrofurantoin in the same quarter observed in Model 14 (Sanchez et al., 2014). High-level non-susceptibility to nitrofurantoin (MIC > 32mg/L) is conferred by mutations in the *nsf*A and *nsf*B genes (Sandegren et al., 2008a). The mutation rate for developing resistance in a previously susceptible population is relatively high for *E. coli* at 10^{-7}/cell per generation (Sandegren et al, 2008a). Increased odds of non-susceptibility when prescribing nitrofurantoin in the previous quarter were observed in the present study, suggesting sufficient time had elapsed for these mutations to have occurred and become established. Longer term establishment of *E. coli* clones non-susceptible to nitrofurantoin is unlikely due to the severe fitness cost imposed by the mutations in the *nsf*A and *nsf*B genes (Poulsen et al., 2013). This biological cost imposed by acquiring resistance to nitrofurantoin plays a significant role in the extent a resistant mutant can spread within the community (Sandegren et al, 2008a). Therefore in the absence of the selective pressure resulting from exposure to nitrofurantoin, the resistant mutants will be outcompeted by the 'fitter' susceptible isolates. This applies particularly to bladder infections as an infecting organism needs to multiply quickly to establish itself in order to combat the flushing mechanism of the bladder / urinary systems (Sandegren et al., 2008b). The biological cost of resistance may explain the negative association observed in the statistical model for prescribing nitrofurantoin 12 months previously, which suggests that greater numbers of susceptible bacteria are found in the community after removal of the selective agent (Model 4).

In a similar ecological study based in Finland in 2009, 25 potential associations of antibiotic consumption and antibiotic resistance (*E. coli* resistance to 7 antibiotics was compared in different combinations with 12 antibiotics prescribed 12 months previously) were examined (Bergman et al, 2009). Only a few statistically significant associations were reported, of these nitrofurantoin use and nitrofurantoin resistance, and cephalosporin use and nitrofurantoin resistance correspond with the findings reported in this chapter for prescribing in lagged quarters. The Finnish study only included prescribing data from 12 months before measuring antibiotic resistance, which may explain why no association was found in their study between exposure and resistance for ampicillin/amoxicillin (see section 6.5.2.2). Unlike the Finnish study, the present study found an association between ciprofloxacin non-susceptibility and fluoroquinolone prescribing (Table 6.4); however the authors of the Finnish study speculate that not finding this association in their study may be due to high CLSI breakpoints used by Finnish laboratories for determining fluoroquinolone resistance (Bergman et al, 2009).

As stated previously the focus of this study was examining the association between antibiotic prescribing and non-susceptibility in *E. coli* isolated from community urine specimens. In the following sections other potential explanatory variables included in the statistical models and their association with non-susceptibility are discussed.

6.5.2.4 Single-handed practices and non-susceptibility

Whilst other studies report higher general prescribing by single-handed practices (discussed in section 6.5.1.3), the statistical models reported in this chapter suggest that single-handed practices in England are associated with higher levels of antibiotic non-susceptibility. It is possible that other factors may be involved in the higher-level

of non-susceptibility suggested by these models, such as links between these practices and other practice characteristics like rural location or deprivation status. As discussed previously higher general prescribing rates by single-handed practices may be due to higher workloads, shorter appointment times and the lack of opportunity to discuss prescribing protocols with colleagues (Damiani et al., 2013). Single-handed practices have been shown to prescribe greater quantities of broad-spectrum antibiotics inappropriately for community respiratory infections (Otters et al., 2004), which may be a factor in selecting increased numbers of non-susceptible isolates suggested by the association reported in this chapter. Although it was found that 15% of practices in the West Midlands were single-handed, in many parts of Europe single-handed practices still predominate (Damiani et al, 2013); therefore these findings of increased antibiotic non-susceptibility in these practices may have implications for countries with higher proportions of single-handed practices.

6.5.2.5 Seasons and non-susceptibility

The increased odds of reduced number of non-susceptible bacteria in the summer and autumn and higher numbers of non-susceptibility in the winter periods, compared with spring, found in Model 1 and Model 3 may possibly be explained by increased prescribing in the winter months. As mentioned in section 6.5.1.1 amoxicillin is recommended for the treatment of a number of respiratory infections usually prevalent in the winter months (Public Health England, 2017b), and in this chapter high levels of prescribing ampicillin/amoxicillin in winter months have been described (Figure 6.8). Although fluoroquinolones are not included in first-line recommendations for common winter respiratory conditions, the successful uropathogenic *E. coli* strain (ST131) commonly hosts a combination of beta-

lactamase and fluoroquinolone resistance genes (Rogers et al, 2011); therefore it is plausible that fluoroquinolone use may also select for ampicillin/amoxicillin non-susceptibility in *E.coli* isolated from urine specimens.

The increased odds of increased numbers of *E. coli* non-susceptible to co-amoxiclav in the winter months, compared with spring, when prescribing ampicillin/amoxicillin (Model 4) and co-amoxiclav (Model 9) again may be explained by the selective pressure of large amounts of winter prescribing. Although co-amoxiclav is not first-line for common winter respiratory infections, it is recommended as a treatment option if resistance is suspected, for example following treatment failure (Public Health England, 2017b).

Non-susceptibility of *E. coli* isolates from urine specimens to cephalexin, trimethoprim and nitrofurantoin (in Models 10-16) were not found to be associated with 'seasons' in the statistical modelling, which may be explained by findings in the descriptive analysis that these antibiotics do not have seasonal prescribing patterns, and the antibiotics prescribed in these models are not associated with the treatment of winter respiratory infections.

6.5.2.6 Population age and non-susceptibility

Twelve of the statistical models show increased linear antibiotic non-susceptibility in *E. coli* isolated from urine specimens with increases in the proportion of the practice population <15 years of age. Two of the models (5 and 7) had significant, but non-linear associations with non-susceptibility, and therefore only the co-amoxiclav non-susceptibility models (Models 4 and 9) were shown not to be significantly associated

with the proportion of the population <15 years. Children are frequent recipients of community healthcare and receive disproportionally a greater number of antibiotic prescriptions (Ready et al., 2004). Having a higher proportion of children in the practice has been shown to result in a significant increase in inappropriate antibiotic prescribing (Otters et al, 2004).

A recent systematic review reports higher levels of antibiotic resistance in *E. coli* isolates from urine specimens to ampicillin and ceftazidime in the 0-5 age group, but interestingly lower levels of co-amoxiclav resistance compared with other age groups (Bryce et al., 2016), which may be a factor in why an association was not found for co-amoxiclav and the <15 age group in the statistical models.

Only one model, co-amoxiclav when prescribing ampicillin/amoxicillin (Model 4), had a significant linear association with non-susceptibility for practices with a greater proportion of patients ≥ 65 years. This association was negative, showing a1.4% decrease in the odds of non-susceptibility for every 1% increase in the proportion ≥65 years old. Increasing age has been shown to be a significant risk factor for having bacteria resistant to antibiotics, with ciprofloxacin resistance particularly associated with older age groups (Mulder et al., 2017; Vellinga et al., 2012). This association with age does not explain the finding in Model 4; however it may explain findings from those models measuring ciprofloxacin non-susceptibility (Models 5, 6, 7 and 8) having a significant, albeit a non-linear complex association with practice population ≥ 65 years.

6.5.2.7 Rural location and non-susceptibility

For all five of the statistical models with a significant association between rural location and non-susceptibility, the association was negative (Table 6.7). This suggests that for these models a rural location is associated with fewer non-susceptible *E. coli* in a rural practice population. The magnitude of association with co-amoxiclav non-susceptibility, when prescribing ampicillin / amoxicillin and co-amoxiclav was high for practices in rural locations, with a 38.3% and 41.2% respective decrease in the odds of non-susceptible *E. coli* in the population.

There are a limited number of studies that describe variation in antibiotic resistance in urban and rural locations in developed countries, although a number of studies report on variations in prescribing based on practice location. Authors in the Netherlands suggested greater exposure to resistant bacteria found on cattle farms may be a factor in the small increase in prescribing they found in rural settings (de Jong J. et al., 2014); however in the USA, it was found that urban physicians are more likely to prescribe antibiotics (Mainous, III et al., 1996). A Dutch community prescribing study found no association between rural and urban general practice locations; although the study did not evaluate individual classes of antibiotics (Otters et al, 2004). The strong associations found for reduced odds of non-susceptibility to co-amoxiclav reported in rural locations reported in this chapter are not easily explained. It is possible that there may be interaction between practice location and other potential explanatory practice characteristics, such as deprivation or population case-mix, which may lead to higher levels of prescribing in urban settings (Wang et al, 2009a).

6.5.2.8 Deprivation and non-susceptibility

In this study, seven of the statistical models were found to have a significant linear association with deprivation scores for the general practice locations and non-susceptibility in *E. coli* isolates from urine specimens ; although the magnitude of increase in odds of non-susceptibility for every unit increase in deprivation score was <1% (Table 6.9).

A number of studies have investigated the relationship between prescribing and deprivation. A Welsh study found no association between prescribing and deprivation based on Townsend deprivation scores associated with the practice population, but the authors did report rates of resistance 6% higher in the most deprived quartile (Butler et al, 2007). Another UK study found that practices in the most deprived quintile prescribed 36.5% more antibiotics than those in the least deprived quintile (Covvey et al., 2014b). The authors propose that as employment and income are heavily weighted in the Scottish Index of Multiple Deprivation (SIMD) score used in this study, then these factors may be driving factors for the variation in prescribing.

The dataset for the study described in this chapter included the multiple deprivation index associated with the Local Authority where the practice was located. It is possible that non-susceptibility may be more closely associated with individual indices of deprivation. A study in northern England examined routine antibiotic susceptibility data for *E. coli* isolated from community urine specimens and linked patients postcodes to neighbourhood deprivation scores. The five domains making-up the Indices of Deprivation were separated into quintiles and considered separately in their models. They found that only one of the domains, living

conditions, was significantly associated with resistance against all eight antibiotics included in the models (OR 1.33-3.03) (Nomamiukor et al., 2015).

6.5.2.9 Gender and non-susceptibility

Only one statistical model (Model 4, co-amoxiclav non-susceptibility when prescribing ampicillin / amoxicillin) was found to have a significant, but negative linear association with the proportion of female patients and non-susceptible *E. coli*; with this model suggesting that increases in the proportion of female patients in the practice result in lower numbers of *E. coli* non-susceptible to co-amoxiclav (adjusted OR 0.241 95% CI 0.116 – 0.502, p= <0.001). All the remaining 15 models have significant but complex non-linear associations with gender. The incidence of UTI caused by *E. coli* is higher in females compared with male patients (Foxman, 2010), which may be a factor when assessing this association. It also has been reported that female patients are prescribed significantly more antibiotics than men in their lifetimes (Schroder et al., 2016). The gender of a patient has been found to be a significant factor in determining what type of antibiotic is prescribed, with female patients receiving more amoxicillin prescriptions in a Belgium study (Blommaert et al., 2013). With the findings from these studies, and given the association between increased prescribing and the development of resistance, the findings for the gender variable in Model 4 are not easily explained.

6.5.2.10 Time variable and non-susceptibility

For all the ampicillin / amoxicillin non-susceptibility models and cephalexin non-susceptibility when prescribing cephalosporins model, there was an association with increased non-susceptibility with increases in time during the study period, whereas the opposite was found for all the ciprofloxacin non-susceptibility models (Table

6.11). In Chapter 5 a similar increasing trend in *E. coli* non-susceptibility to third-generation cephalosporins was demonstrated between 2010 and 2013; however in the study reported in Chapter 5, ciprofloxacin also had a rising trend in non-susceptibility in contrast to the these model results. The descriptive work in Chapter 5 was based on AMR surveillance data only to calculate proportions of non-susceptible *E. coli*, and the magnitude for these associations in the statistical models with the 'time' covariate are comparatively small, with all representing a <1% change in non-susceptibility in the number of *E. coli* non-susceptible for each quarter increase in time. A study reported that the proportion of *E. coli* from blood cultures non-susceptible to third-generation cephalosporins and ciprofloxacin declined between 2007 and 2011, and it was suggested that these reductions in non-susceptibility are associated with reduced hospital prescribing. The authors data, however, shows the decline may have ended by 2010-2011 (the period this study commenced) for both these antibiotics, as slight increases in non-susceptibility were reported in this period (Livermore et al., 2013).

Trimethoprim was the only antibiotic of those antibiotics not included in the study described in Chapter 5 that was shown to have a significant rising trend in non-susceptibility over time in the descriptive analysis (Figure 6.9); yet the trimethoprim non-susceptibility statistical model (Model 15) was found to have a complex non-linear association with the 'time' covariate.

6.5.2.11 Number of GPs per 100,000 patients and non-susceptibility

The ten statistical models found to have significant linear associations (albeit with small magnitudes) with the number of GPs per 100,000 patients, suggest that an increase in the number of GPs in the population would result in an increase in the

number of non-susceptible bacteria in that community. These 10 models represented non-susceptibility in just three antibiotics; cephalexin, ciprofloxacin and nitrofurantoin. In contradiction to these findings, it is plausible that greater number of GPs per population would result in fewer consultations per GP, longer appointment times and therefore lower prescribing rates (Wang et al, 2009a); though a study in the USA found that for an increase of one standard deviation in the number of physician offices per capita there was a 25.9% increase in antibiotic prescriptions (Klein et al., 2015). Although this may explain the findings from the statistical models, the private sector model in the USA is different to the UK and therefore may not be comparable. A large English study did not find an association between the numbers of GPs per practice population and prescribing rates (Wang et al, 2009b).

6.5.3 Limitations

Significant statistical associations in these types of modelling studies should be interpreted as suggestive only as they do not necessarily imply cause – effect relationships. This is a retrospective ecological study and therefore is not able to draw inferences about individual risk of antimicrobial resistance. Mutations and selection of resistant strains occur at an individual patient level; however the spread of resistance is at the community level, and therefore population level antibiotic pressure may be more relevant than examining individual usage alone (Bell et al, 2014b;Samore et al., 2006).

DDDs were chosen as the metric for antibiotic prescribing as they are the most commonly applied unit of measurement and are recognised internationally as a

bench-marking measure. Using DDDs also allowed comparison with international studies comparing prescribing with antibiotic resistance. However it is recognised that DDDs do not always accurately reflect prescribing for children or persons with renal impairment (Vernaz et al., 2011).

Multilevel modelling strengthens this study as it allows random effects in the population to be taken into account whilst adjusting for a number of potential predictor or confounding variables. Following a review of the literature only a limited number of studies were found that included practice characteristics and of these comparable interactions between these variables with either not found (Hay et al., 2005b), or were not reported (Wang et al, 2009a). A systematic approach of testing potential interactions was considered for this modelling study; however this would have added over 30 pairwise combinations of variables. Therefore to ensure manageability and aid interpretation it was decided to focus on the main effects of the various variables included in this study.

Given the plausibility of bacteria carrying resistance genes to multiple antibiotics, there will be interdependence between some of the antibiotic combinations when measured against the same antibiotic non-susceptibility results. Therefore with the large amount of testing captured in this study we would expect to encounter a number of type one errors for particular antibiotic combinations. A more stringent significance test was considered; however this was not implemented due to the possibility of increasing the number of false negative associations.

This study was not able to differentiate urine specimens sent from patients in the community residing in long-term care facilities (LTCFs). LTCFs residents have a

higher proportion of UTI and the bacteria causing these infections are more likely to have antibiotic resistance, compared with bacteria isolated from patients living in the community (Rosello et al., 2017).

The antibiotic non-susceptibility data were extracted from routine laboratory reporting and therefore is subject to specimen selection bias as it is likely that urine samples sent for microbiological examination are from patients with treatment failures or those that have complicated and/or severe infections (McNulty et al., 2004). Notwithstanding this, it is encouraging that a study in Ireland in 2012 of urines taken from all adult patients suspected of having a UTI attending 22 practices found similar antibiotic susceptibility proportions. (Vellinga et al, 2012)

6.5.4 Summary

A key objective of this study was to describe the association of antibiotic prescribing in the community with non-susceptibility of *E. coli* to a range of antibiotics commonly used for treating infections in primary care, taking into account other potential explanatory variables, such as GP practice characteristics. The statistical models suggest that small increases in antibiotic prescribing within a general practice increases the number of non-susceptible bacteria isolated in urine samples within the practice population. The magnitude of these associations are not large (between 1 and 6% increase in the odds of non-susceptibility) and therefore reducing antibiotic prescribing may not be seen as worthwhile to only achieve small reductions in AMR. These models, however, are based on one quarters prescribing, with relatively small unit increases in prescribing at a practice level (i.e. equivalent to 10 prescriptions). It

is the cumulative effect of increasing number of non-susceptible bacteria in the practice population over a period of several months that will be instrumental in the emergence and spread of AMR. A systematic review of prescribing and AMR concluded that the residual long-term effect of prescribing is likely to be a key driver for high endemic levels of AMR in the community (Costelloe et al, 2010a).

The descriptive analysis part of the study demonstrated that the large volumes of antibiotics likely to have been used in the treatment of respiratory conditions in winter months, appears to have an immediate short-term effect of increased antibiotic non-susceptibility in bacteria causing unrelated infections. Prudent prescribing in the winter periods, in line with Royal College of General Practitioners guidance (http://www.rcgp.org.uk/clinical-and-research/toolkits/target-antibiotics-toolkit.aspx) is required by individual general practices to maintain a population of susceptible bacteria in their local population, thereby preserving the effectiveness of available antibiotics.

Including potential explanatory variables in the statistical model building enabled the association of these with non-susceptibility to be measured. All 16 models suggested that single-handed GP practices are associated with increased numbers of non-susceptible bacteria in their registered patient population. In future work described in Chapter 7, the interaction between practice variables will be examined, as it is plausible that factors such as the number of patients per GP, location of the practice and deprivation may also be factors when modelling the single-handed practice variable. Although additional study will give insight into interactions between these variables, the results reported in this chapter do suggest that single-handed practices may require additional antibiotic stewardship support and guidance.

Substantial variation in the quantity of antibiotics prescribed by practices in the West Midlands has been demonstrated in this study. Understanding this variation is central to interventions designed at improving antimicrobial stewardship within the community. The outcome measured in the statistical models was non-susceptibility to antibiotics. Several practice characteristics, such as the proportion of children in the practice, gender ratio of the patients and the number of GPs per practice population were found to have significant associations with non-susceptibility in these models. These statistical modelling results therefore may, when combined with information provided in the descriptive analysis and qualitative studies on prescribing practice, such as the GP survey described in Chapter 4, provide an insight into developing targeted interventions in primary care.

7 Overall Discussion

7.1 Summary of findings

7.1.1 Study design

This study was designed to test the hypothesis that surveillance data collected routinely from diagnostic microbiology laboratories in the West Midlands region of England would be able to demonstrate an association between antibiotic prescribing in the community and antibiotic resistance in bacteria causing urinary tract infections.

The work described in this thesis commenced in 2009 with the enrolment of the first laboratories asked to report routine AMR surveillance data. During the study period there have been changes in laboratory methods, with the introduction of new technologies for identification of bacteria and antibiotic susceptibility testing, which were described in Chapters 5 and 6. There has also been a significant move towards harmonising UK and European antibiotic breakpoint standards, which was discussed in Chapter 5.

The overall aim of the study was to determine whether the availability of routine surveillance data can influence antibiotic prescribing habits in the community by demonstrating an association between prescribing and AMR at general practice level. The study was divided into a series of objectives. A key objective was to establish a robust AMR surveillance system in the region (Chapter 3). To help inform the interpretation of these complex AMR surveillance datasets, an understanding of the laboratory methods and protocols used for culture, identification and antibiotic susceptibility testing of bacteria isolated from urine was required (Chapter 2). The interpretation of AMR in bacteria isolated from urine also needs to account for potential sampling bias, by investigating the circumstances in which urine specimens

are sent for microbiological examination by community physicians (Chapter 4). The next stage of the study plan was to determine if routine AMR surveillance data could be analysed to monitor the key drug/bug combinations set out in the UK five year AMR strategy (Department of Health, 2013) (Chapter 5). The final objective was to measure the association between antibiotic prescribing by individual general practices in the West Midlands and non-susceptibility of bacterial pathogens within those practice populations. To achieve this it was necessary to link routine AMR surveillance reports to antibiotic prescribing data for the same general practices (Chapter 6).

7.1.2 Key findings

7.1.2.1 Chapter 2

More than one million urine specimens were processed by laboratories in the West Midlands each year, with approximately half of these being received from primary care settings. Laboratories in the West Midlands used a range of methods for the identification and antibiotic susceptibility testing of bacteria isolated from urine specimens. All but one laboratory identified all or most Gram-negative bacteria isolated from urine specimens to species level, and all but two laboratories used the latest EUCAST or BSAC breakpoint standards to determine antibiotic susceptibility. The introduction of automated susceptibility testing devices in West Midland laboratories may aid the interpretation of surveillance data by increasing the standardisation of methods and the range of antibiotics tested.

7.1.2.2 Chapter 3

Routine AMR surveillance reporting was established in all 15 West Midland laboratories during the study period. The AmWeb application enabled microbiologists and pharmacists to monitor resistance profiles, complete local benchmarking and compile data for infection control reports. The development of the community AMR bulletin provided antibiotic prescribers in the community local AMR reports for their geographic areas to help inform empirical prescribing.

7.1.2.3 Chapter 4

The survey described in this chapter reported that only 50% of GPs indicated that their practice had a policy for taking urine specimens for microbiological investigation. There was variation in the response from GPs regarding the proportion that would send a urine for microbiological examination from the most common presentation (suspected uncomplicated UTI in a young female adult), with 40% indicating they would send a urine sample. There was also variance from national guidance by a proportion of GPs (38%) in the management of catheterised patients. Finally, differences were found in the response from male and female GPs, with a greater proportion of female GPs reporting being influenced by laboratory results, taking specimens and prescribing in some of the clinical scenarios.

7.1.2.4 Chapter 5

The study described in this chapter examined the use of routine AMR surveillance data to monitor antibiotic non-susceptibility for key drug/bug combinations. A linear increase in non-susceptibility to third-generation cephalosporins for *E. coli* and *K. pneumoniae*, and to ciprofloxacin for *E. coli*, in specimens from both hospital and community settings during this study period was reported. The proportions of *E. coli*

and *K. pneumoniae* reported non-susceptible to meropenem and/or imipenem remained low during the study period, with no evidence of linear trend.

Routine antimicrobial resistance surveillance enabled, for the first time in England, the systematic monitoring of resistance in bacteria responsible for urinary tract infections in a defined large population, and thereby provided a representative indication of the burden of resistance in Gram-negative bacteria in hospital and community settings.

7.1.2.5 Chapter 6

Nine of 16 antibiotic prescribing / non-susceptibility combinations had a significant statistical linear correlation with non-susceptibility in *E. coli* isolated from urine specimens taken from patients in the community; demonstrating that small increases in antibiotic prescribing in individual general practices reduces the number of susceptible bacteria in the practice population.

Single-handed general practices were shown to have a significant association with increased numbers of non-susceptible *E. coli* isolates from urine specimens in their practice populations. Increased prescribing of ampicillin / amoxicillin in winter periods was shown to be associated with increased non-susceptibility of *E. coli* isolated from urine specimens.

7.1.3 Strengths and Limitations

Each chapter details the strengths and limitations for the study being reported. In this section the strengths and limitations associated with the key study objectives, drawn from various parts of the study, will be summarised.

7.1.3.1 Delivery and interpretation of routine AMR surveillance data

As described in Chapters 3 and 5, routine AMR surveillance provides a robust, sustainable and cost-effective data for monitoring trends in AMR and detecting emerging public health threats. A further strength of this surveillance is the ability to automate the data collection, which removed the burden of reporting from laboratories and provided the timely delivery of surveillance data.

As discussed in Chapters 3 and 5, interpretation of routine AMR surveillance data are hampered by variation in laboratory methods and bias introduced by specimen sampling policies, particularly for specimens taken in the community (McNulty et al., 2006). Although the results of the laboratory survey reported in Chapter 2 indicate that a wide range of methods and protocols were used by laboratories in the West Midlands, nearly all laboratories had adopted the latest antibiotic breakpoint standards and all took part in an internationally recognised monthly quality control scheme (NEQAS, 2017).

Variation by GPs and practice nurses in referring urine specimens for microbiological examination impedes the generalisability of routine AMR surveillance data (Hayward et al., 2007). It has been reported that results of specimens sent from complicated UTI and treatment failures are the predominant specimens received by in laboratories, as sending urine for examination from the most common presentation

of UTI (uncomplicated UTI in female patients) is not recommended in national guidelines (McNulty et al., 2011). Findings from the survey reported in Chapter 4, and a similar study from Wales (Howard et al., 2001), suggest that GPs do not always follow national guidelines, as a significant proportion of samples from uncomplicated UTI infections (40% and 56% respectively) are sent to laboratories for microbiological examination. Further studies are required to investigate AMR using systematic specimen collection in general practice; however, it is reassuring that a study that collected specimens from all patients suspected of having a UTI (Vellinga et al., 2012) reported similar levels of AMR to that reported in Chapters 5 and Chapter 6 of this thesis.

7.1.3.2 Understanding specimen collection and prescribing protocols

The response rate of the survey of GPs reported in Chapter 4 was low (11.3%); however the demographic profile of responders to the survey was comparable with all West Midland GPs (section 4.5.5). The findings reported from this survey, therefore, provided an insight into why specimens are collected, and in what circumstances antibiotics are prescribed in cases of suspected UTI in the community. This understanding of the variety of clinical conditions leading to urine specimens being examined in laboratories helps interpret routine AMR surveillance as discussed in Chapter 5.

It was not possible to generalise findings from the free-text comments provided by GPs in the survey reported in Chapter 4, as the number of comments was relatively small. The themes that did emerge from this analysis, will require further study using qualitative research methods.

7.1.3.3 Understanding the effect of general practice characteristics and prescribing on antibiotic resistance in organisms isolated from community urine specimens

A strength of the study reported in Chapter 6 was the use of multi-level statistical modelling. This technique allowed for a number of predictor or confounding variables to be assessed for their effect on *E. coli* non-susceptibility, whilst adjusting for variation within the West Midland population. These types of ecological studies do have some limitations and results reported in this chapter should not be used to suggest cause-effect relationships between antibiotic prescribing and non-susceptibility; but rather be used to give an indication, and extent, of associations between various explanatory or confounding variables and antibiotic non-susceptibility

7.2 What this thesis adds

7.2.1 Implications for Public Health

Antimicrobial resistance is one of the most important threats to public health and the problem is accelerating across all parts of the world (WHO, 2015). The use of antibiotics is the key driver of antibiotic resistance (CDC, 2014); therefore reducing inappropriate antibiotic prescribing is central to strategies aimed at tackling this major public health problem.

The results of this thesis have shown that there is a wide variation in antibiotic prescribing between general practices in the West Midlands region (Chapter 6). It

has also shown that the large volume of antibiotics prescribed in the winter periods for respiratory infections is reducing the numbers of susceptible bacterial pathogens in the community causing unrelated infections. The survey of GPs, reported in Chapter 4, provides some insight into the observed variance in prescribing by showing that national guidance / protocols designed to standardise the prescribing of antibiotics and the referral of specimens for microbiological analysis are not consistently followed. Combined with other findings reported in this thesis, such as single-handed general practiced being associated with antibiotic non-susceptibility (Chapter 6), this work will help inform the design of interventions designed to reduce overall prescribing in the community.

Previous intervention strategies have struggled to convince GPs that changes in their prescribing practice can impact AMR in the community (Björkman et al., 2013). This thesis demonstrates that relatively small increases in antibiotic prescribing within a general practice can increase the number of non-susceptible bacteria within the local population. These findings will therefore strengthen the evidence base and support new Public Health campaigns to reduce the consumption of antibiotics.

7.2.2 Implications for clinical practice

The laboratory survey (Chapter 2) found that in 2011 a number of laboratories reported using non-standard techniques (i.e. direct antibiotic susceptibility testing from urine, or the modified Stokes method) which have been shown to be unreliable or unsafe (Gosden et al., 1998). This thesis has demonstrated that it is feasible to capture and analyse routine AMR surveillance data from diagnostic laboratories and

provide web tools for microbiologists and pharmacists to allow benchmarking of results between laboratories. The case studies described in Chapter 3 (section 3.4) show how routine surveillance data are able to change laboratory practice, by improving the quality and safety of individual test results (case studies A and C). The laboratories contacted to inform of 'unusual' susceptibility test results were unaware of increased non-susceptibility results being reported by their laboratories, compared with regional and national averages.

The implementation of routine AMR surveillance in the West Midlands, as described in Chapter 3, has enabled the monitoring of resistance within both hospitals and the community. The reporting tools developed for this study (AmWeb and the Community AMR bulletin), and published studies resulting from this thesis, have helped policy makers develop new prescribing guidance. Anecdotal reports received from GPs following the release of the Community AMR bulletin in 2012 informed that GPs were now prescribing nitrofurantoin for uncomplicated UTI instead of trimethoprim, due to the higher levels of resistance reported in this bulletin. In 2014, based on routine AMR surveillance, PHE changed the national guidelines to recommend nitrofurantoin, in place of trimethoprim, for first-line treatment of uncomplicated UTI (Public Health England, 2017).

As discussed above, this thesis has shown that the actions of individual GPs can affect the development of AMR in their locality. Providing practice level evidence will support community pharmacists and healthcare commissioners in developing appropriate local policies and interventions to help convince community physicians of the benefits of appropriate antibiotic prescribing.

7.2.3 Implications for research

This thesis has demonstrated the value of providing robust antimicrobial susceptibility data to monitor key antibiotic / bacterial combinations as specified in national strategies (Department of Health, 2013). The thesis has also demonstrated the value of linking AMR surveillance reports to other datasets (e.g. general practice demographics and antibiotic prescribing) to measure associations with AMR. Linking AMR reports with prescribing data reported in this thesis has influenced the design of new studies. A PHE pilot study is being planned to link patient-level prescribing data with routine AMR reports, and potentially GP management system data. This will deliver a powerful dataset to enable PHE researchers to reveal the clinical context behind individual antibiotic prescriptions, and help determine patient-level risk factors associated with the development of AMR.

Community prescribers are often a difficult audience to engage with, with the response rate to traditional surveys, even those offering financial incentives, being poor (Hillier et al., 2006). The NHS organisational structure changed in April 2013, with PCTs being replaced by the introduction of Clinical Commissioning Groups (CCGs) (https://www.nhscc.org/ccgs/). The GP survey described in this thesis, found that most GPs used prescribing formularies provided by their PCTs; therefore further research is required to determine if these formularies are still used or if these have been replaced by CCGs. Some of the findings of the GP survey described in Chapter 4, such as different attitudes to prescribing by male and female GPs, and the inclination for some responders to send all urines for microbiological examination, require a qualitative research approach in order to understand the reasoning behind these differences.

7.3 Next steps

Two follow-up studies are being planned. Firstly a statistical study to determine if there are significant interactions between various potential explanatory variables investigated in Chapter 6. As previously discussed the development of the statistical models focused on the main outcomes for the potential explanatory or confounding variables. It is plausible that interactions between these variables may be a factor in their associations with antibiotic non-susceptibility. For example, the findings that single-handed practices are associated with increased numbers of non-susceptible *E. coli* may also be linked to single-handed GPs being associated with other potential explanatory variables such as location (e.g. rural or urban areas) or particular demographics within the registered population (Wilson et al., 1999).

Secondly, routine AMR data from the West Midlands collated for this thesis, were used in a collaborative study with members of the national PHE AMR surveillance unit. This collaborative study was designed to compare AMR in the general community, for adults aged 70 and over, with AMR reported from locations associated with long-term care facilities (LTCFs). The study found four times the rate of AMR in LTCFs compared with the general community (Rosello et al., 2017). It is now intended to analyse and model antibiotic prescribing from these LTCF locations, as higher levels of prescribing in these facilities may be a factor in driving the observed increase in AMR.

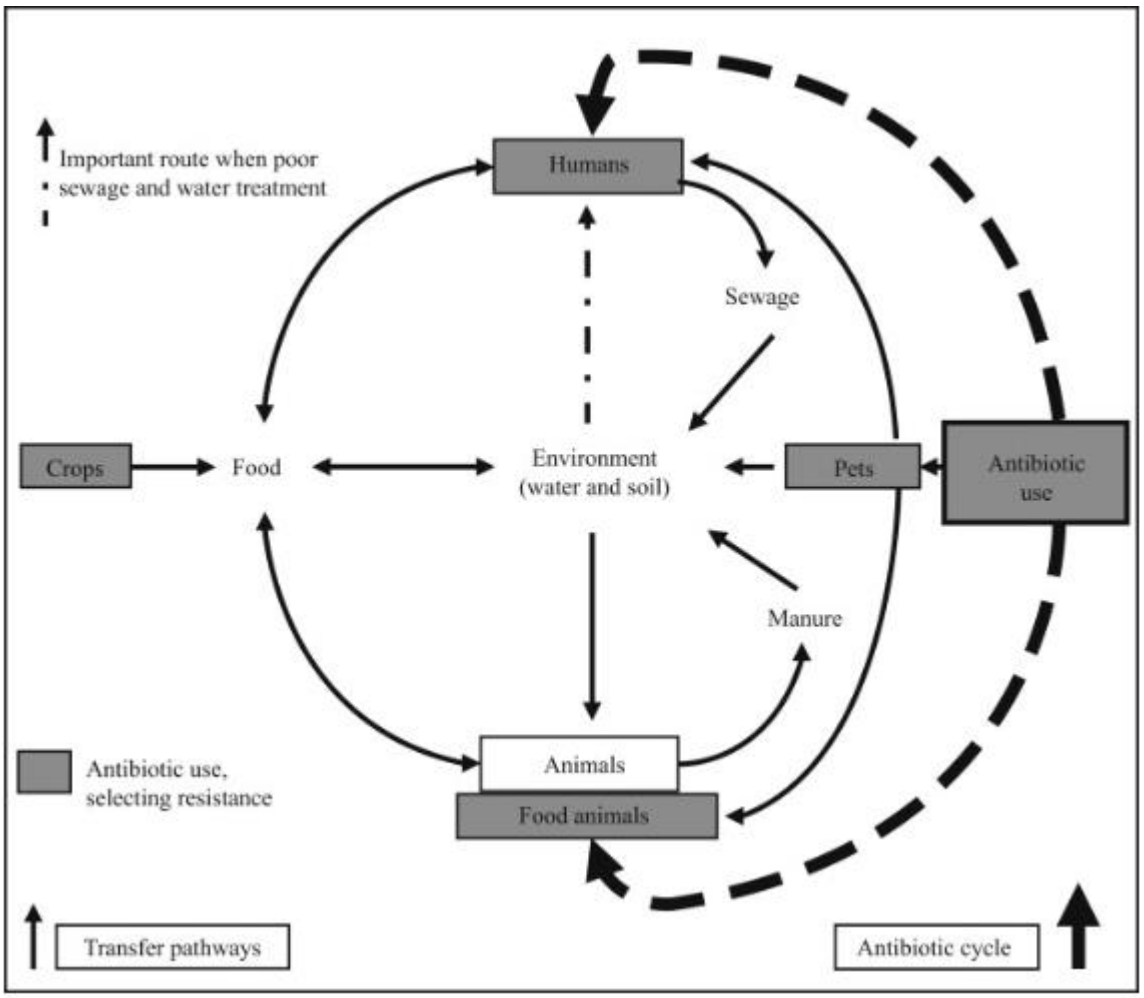
7.4 Closing remarks

One of the aims of antimicrobial stewardship is to preserve the efficacy of antibiotics that are currently available by understanding which infections remain susceptible to therapy by particular drugs, thereby minimising unintended consequences and limiting the spread of AMR (Ashiru-Oredope et al., 2012). This thesis demonstrates that routine AMR surveillance data can both guide effective antibiotic treatment, by informing of the susceptibility of circulating bacteria in the community and hospitals, and be used to inform on the consequence of excessive or inappropriate use.

The studies that make up this thesis have a focus on AMR in bacteria causing community UTIs. Until recently, health professionals and members of the public have been led to believe that AMR is mainly an issue within hospitals (Livermore, 2012); however, frequently newly admitted patients from the community are the source of MDR bacteria in hospitals (Levy, 2002). These patients may be infected or colonised with resistant bacteria as a direct result of inappropriate antibiotic use in the community (Abernethy et al., 2017).

As described in the introduction to Chapter 1, MDR Gram-negative resistance genes are now found widely in the environment and are being distributed in a cyclical manner (Figure 7.1).

Figure 7.1The principle transfer pathways for antibiotic resistance genes (Department of Health, 2012).



The movement of people and rapid changes in agricultural practice are driving the spread of AMR across the world and creating a serious public health crisis (Hawkey, 2015). Multifaceted strategies, based on a one-health approach, are required to reduce risks and mitigate the effects of antibiotic resistance at the interface between humans, animals and the environment (Wellington et al., 2013).

In conclusion, the misuse of antibiotics impacts those providing secondary and primary healthcare, as well as individuals, families and communities. Therefore everyone has a role to play in ensuring this precious resource is preserved for future generations.

Appendix 1: Survey of West Midland laboratories

Screen 1

Introduction

Thank you for entering this short HPA West Midlands survey. The survey should take 15-20 minutes to complete. We greatly appreciate your time in answering these questions. The responses to this survey will be used to better understand antimicrobial surveillance data from urinary infections and help inform analysis and develop meaningful reports.

1. Please select your laboratory from the drop down list*

2. Please select your professional group from the drop down list*

Screen 2

Identification of urinary isolates

3. How are the following organisms identified from urinary isolates. Please tick all relevant boxes for each organism.*

	Microscopy (e.g. gram stain)	Colonial morpholgy	Biochemical tests (e.g. API)	Automated testing device (e.g. Vitek)	Enzyme tests (e.g. oxidase, coagulase)

Non lactose fermenting gram negative bacilli	☐	☐	☐	☐	☐
Lactose fermenting gram negative bacilli	☐	☐	☐	☐	☐
Staphylococcus spp.	☐	☐	☐	☐	☐
Streptococcus spp.	☐	☐	☐	☐	☐
Enterococcus spp.	☐	☐	☐	☐	☐

4. To what level are the following urinary isolates routinely identified. Please select most appropriate option.*

	Family (e.g. Coliform)	Genus (e.g. Klebsiella sp.)	Species (e.g. Klebsiella pneumoniae)
Non lactose fermenting gram negative bacilli	○	○	○
Lactose fermenting gram negative bacilli	○	○	○
Staphylococcus spp.	○	○	○
Streptoccus spp.	○	○	○
Enterococcus spp.	○	○	○

Screen 3

Antimicrobial Susceptbility Tests

5. Please state the method(s) used within the last 12 months to assess antimicrobial susceptibility of bacteria isolated from urinary specimens

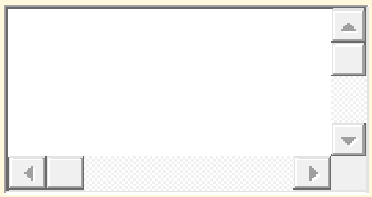
	Please select method(s) used for urinary isolates
Modified Stokes Method	
BSAC Disc Test	
Break Points	
E Test	
Broth/Agar dilution	
Vitek	
Phoenix	
Other	

6. Please can you provide an approximate date that the testing method was introduced?

	Please state approximate date of introduction	Please state approximate date the test was withdrawn (if applicable)
Modified Stokes Method		
BSAC Disc Test		
Break Points		
E Test		
Broth/Agar dilution		

Vitek		
Phoenix		
Other		

7. If more than one method is used to asses antimicrobial susceptibility from urinary isolates then please state the criteria used to determine when a technique is applied?



8. Has the criteria for selecting the testing technique changed over the last 12 months and if so when?

9. If there has been a change in criteria then what was the reason for the change?

Screen 4

Antibiotic Panels

10. Please describe the antibiotic panels used for urinary isolates and whether they are used as first or second line panels (e.g. gram negative first line) *

Panel a)

Panel b)

Panel c)

Panel d)

Panel e)

Panel f)

Panel g)

Panel h)

Panel i)

Panel j)

11. When were the panels introduced (approximate date)? *

Panel a)

Panel b)

Panel c)

Panel d)

Panel e)

Panel f)

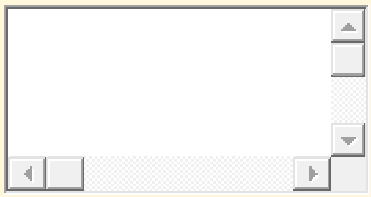
Panel g)

Panel h)

Panel i)

Panel j)

12. Have any of the above panels been modified over the last 12 months and if so which ones and how have they changed?



Screen 5

Antibiotics Tested

13. Please select antibiotics in each panel

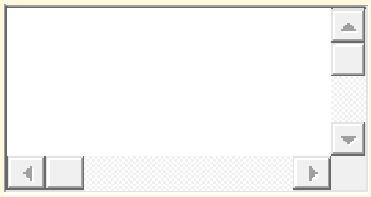
Panel a	Panel b	Panel c

1			
2			
3			
4			
5			
6			
7			
8			

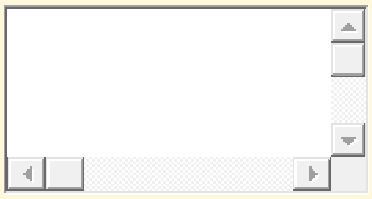
Screen 6

Reporting rules and changes to policies

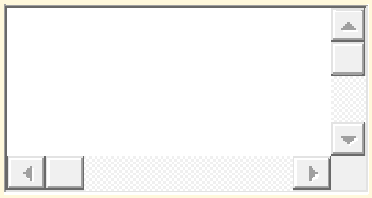
14. Does the laboratory use rule-based reporting (i.e. results reported by rule for particular organisms/antibiotics rather than results of antibiotic susceptibility testing e.g. all H. influenzae isolates reported as resistant to erythromycin by rule)?
    If yes then please describe



15. Have you any plans to change antimicrobial susceptibility testing techniques in the near future?
    If so what changes are planned?



16. Have you any plans to change urine isolates identification methods in the near future?
    If so what changes are planned?



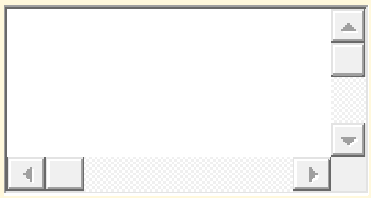
17. Do you plan to change policies involving the criteria used to select organisms (isolated from urine) for identification or the antibiotics tested in the near future?
    If so what changes are planned?

Screen 7

Personal views and observations

18. Have you noticed any particular trends in antimicrobial resistance in the last 12 months?

19. Is there anything else that you would like to add regarding the identification of urinary isolates, antimicrobial testing or antimicrobial resistance surveillance?



Appendix 2: Survey of West Midlands GPs

Welcome to this HPA Survey

The HPA Regional Epidemiology Unit is developing an Antimicrobial Resistance (AMR) surveillance bulletin for GP's in the West Midlands. To help understand potential variation in collection of samples for microbiological investigation and antimicrobial prescribing habits we would be very grateful if you could complete this short survey on the management of UTI.

1. Please provide the name of your practice (primary if you have more than one)*

2. Please enter your national Practice Code or if not known the Practice Postcode

	National Practice Code (e.g. M123456)	OR Practice Post Code
*		

3. Your age?*

 <35 years  35-45 years  46-55 years  >55 years

4. Please can you enter the number of years since qualified.

5. Your gender?*

Male Female

6. Is there a practice policy or protocol for sending urine specimens for microbiological examination?*

Yes No

7. Does your practice use specific prescribing formularies ?*
If YES then please state the source of this guidance (e.g. HPA, PCT, BNF etc)

Yes No

8. If the answer to question 7 is YES then please state the source of the prescribing formularie used in your practice (e.g. PCT, HPA, BNF)

9. Do laboratory antimicrobial susceptibility results for urinary isolates influence your antibiotic prescribing for:

	Always	Frequently	Infrequently	Never
General empirical prescribing:				

In the case of a treatment failure:				
When resistance is reported to initial prescribed agent:				

10. Based on your experience of treating patients presenting with clinically suspected UTI what approximate proportion would you request a urine sample for microbiological examination [%]?* The value must be between 0 and 100, inclusive.

%

11. Has your practice reviewed the management of urinary tract infections within the last 12 months?*

Yes No

For each of the scenario's below please state whether (a) a urine sample would be taken for microbiological examination and (b) an antibiotic agent prescribed (if prescribed empirically)

12. Case 1. A 20 year old lady re-attends surgery and complains that the loin pain and frequent urination symptoms reported to you the previous week had worsened despite finishing a complete course of trimethoprim (no sample was taken previously).

	Yes	No
Would you collect a urine sample for microbiological examination?		

Would you prescribe an antibiotic?	☐	☐

13. Case 3. A 43 year old woman complains of pain passing urine and frequency. She feels well otherwise and has not previously been treated for a UTI.

	Yes	No
Would you collect a urine sample for microbiological examination?	☐	☐
Would you prescribe an antibiotic?	☐	☐

14. Case 4. A 51 year-old man attends your surgery complaining of pain passing urine and perineal tenderness. On examination you find suprapubic tenderness and a temperature of 38.5 C is measured.

	Yes	No
Would you collect a urine sample for microbiological examination?	☐	☐
Would you prescribe an antibiotic?	☐	☐

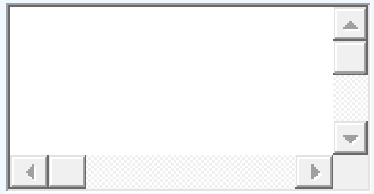
15. Case 5. During a routine antenatal clinic an 18 year old girl who is 20 weeks pregnant produces a cloudy urine sample. She reports no symptoms or discomfort. The urine dipstick tests positive for nitrite but negative for leukocytes and protein.

	Yes	No
Would you collect a urine sample for microbiological examination?	☐	☐
Would you prescribe an antibiotic?	☐	☐

16. Case 6. You visit an 82 year old female in a nursing home. She is catheterised, afebrile and has no symptoms but the staff inform you that the urine is cloudy.

	Yes	No
Would you collect a urine sample for microbiological examination?	☐	☐
Would you prescribe an antibiotic?	☐	☐

17. Please let us know if you have any additional comments regarding this survey.
Please enter any comments in the box below.



Appendix 3: Statistical model results (followed by meta-data description)

Key
Non-linear form

			Qtr2				Qtr3				Qtr4			
Model no.	**Antibiotic non-susceptibility**	**Antibiotic prescribed**	**Adjusted OR (or non-linear form)**	***P* value**	**95% CI(l)**	**95% CI (u)**	**Adjusted OR (or non-linear form)**	***P* value**	**95% CI(l)**	**95% CI (u)**	**Adjusted OR (or non-linear form)**	***P* value**	**95% CI(l)**	**95% CI (u)**
1	ampicillin / amoxicillin	Co-amoxiclav	0.953	<0.001	0.929	0.977	0.968	0.011	0.945	0.993	1.037	0.007	1.010	1.065
2	ampicillin / amoxicillin	ampicillin / amoxicillin	0.994	0.680	0.966	1.023	1.005	0.809	0.966	1.046	1.033	0.130	0.990	1.078
3	ampicillin / amoxicillin	fluoroquinolones	0.950	0.000	0.926	0.974	0.968	0.010	0.944	0.992	1.040	0.004	1.013	1.068
4	co-amoxiclav	ampicillin / amoxicillin	1.082	0.018	1.014	1.156	1.004	0.928	0.928	1.086	1.173	<0.001	1.096	1.255
5	ciprofloxacin	Co-amoxiclav	0.991	0.730	0.944	1.041	0.922	0.002	0.877	0.970	0.992	0.758	0.939	1.047
6	ciprofloxacin	ampicillin / amoxicillin	1.020	0.626	0.942	1.104	0.979	0.662	0.889	1.078	1.018	0.681	0.936	1.107
7	ciprofloxacin	cephalexin	1.001	0.982	0.953	1.050	0.923	0.001	0.878	0.969	0.983	0.536	0.932	1.038
8	ciprofloxacin	fluoroquinolones	1.002	0.931	0.955	1.052	0.915	<0.001	0.871	0.962	0.982	0.497	0.930	1.036
9	co-amoxiclav	Co-amoxiclav	1.081	<0.001	1.037	1.127	1.020	0.392	0.974	1.068	1.179	<0.001	1.128	1.233
10	cephalexin	cephalosporin	1.022	0.467	0.964	1.083	0.989	0.717	0.934	1.048	1.022	0.489	0.960	1.089
11	cephalexin	fluoroquinolones	1.011	0.708	0.955	1.069	0.989	0.691	0.935	1.045	1.057	0.062	0.997	1.121
12	cephalexin	nitrofurantoin	1.019	0.528	0.961	1.080	0.978	0.454	0.924	1.036	1.054	0.079	0.994	1.118
13	nirofurantoin	cephalexin	1.021	0.618	0.940	1.109	0.928	0.079	0.853	1.009	0.966	0.422	0.887	1.051
14	nitrofurantoin	nitrofurantoin	1.041	0.350	0.957	1.131	0.939	0.153	0.862	1.024	0.961	0.415	0.872	1.058
15	trimethoprim	trimethoprim	0.975	0.069	0.949	1.002	0.999	0.962	0.969	1.030	1.005	0.732	0.975	1.036
16	cephalexin	trimethoprim	1.024	0.429	0.966	1.086	0.981	0.529	0.924	1.042	1.029	0.395	0.964	1.098

	Single GP registered in practice				Rural practice location				Deprivation score			
Model no.	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)
1	1.097	0.006	1.027	1.171	0.970	0.181	0.927	1.014	1.000	0.002	1.000	1.000
2	1.083	0.018	1.014	1.156	0.976	0.263	0.934	1.019	1.000	0.001	1.000	1.000
3	1.095	0.008	1.024	1.170	0.984	0.467	0.941	1.028	1.000	0.001	1.000	1.000
4	1.361	<0.001	1.148	1.614	0.617	<0.001	0.530	0.718	1.000	<0.001	1.000	1.000
5	1.458	<0.001	1.267	1.676	1.012	0.816	0.912	1.124	1.005	0.023	1.001	1.010
6	1.448	<0.001	1.258	1.666	0.979	0.701	0.878	1.091	1.005	0.056	1.000	1.010
7	1.370	<0.001	1.180	1.592	0.986	0.792	0.885	1.098	1.005	0.064	1.000	1.009
8	1.371	<0.001	1.182	1.590	0.986	0.800	0.886	1.098	1.006	0.016	1.001	1.011
9	1.398	<0.001	1.171	1.669	0.588	<0.001	0.502	0.689	1.000	<0.001	1.000	1.000
10	1.528	<0.001	1.322	1.767	0.876	0.008	0.794	0.967	1.004	0.073	1.000	1.009
11	1.534	<0.001	1.337	1.759	0.923	0.097	0.839	1.015	1.006	0.005	1.002	1.011
12	1.534	<0.001	1.340	1.756	0.928	0.123	0.844	1.021	1.007	0.004	1.002	1.011
13	1.606	<0.001	1.304	1.979	0.866	0.016	0.771	0.974	1.007	0.027	1.001	1.013
14	1.657	<0.001	1.352	2.031	0.866	0.014	0.773	0.972	1.006	0.047	1.000	1.012
15	1.110	0.009	1.026	1.201	0.991	0.755	0.936	1.049	1.002	0.080	1.000	1.005
16	1.603	<0.001	1.395	1.841	0.909	0.079	0.817	1.011	1.005	0.037	1.000	1.010

	Gender				Proportion ≤14 years old			
Model no.	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)
1	6699.167	0.031	2.283	19700000.000	4.025	<0.001	1.979	8.183
2	5716.261	0.033	2.016	16200000.000	3.202	0.001	1.575	6.511
3	150042.000	0.010	18.072	1250000000.000	3.918	<0.001	1.920	7.993
4	0.241	<0.001	0.116	0.502	3.087	0.243	0.466	20.466
5	14700000.000	0.044	1.574	138000000000000.000	0.000	0.031	0.000	0.187
6	83.872	0.013	2.558	2749.696	11.880	<0.001	3.076	45.884
7	503000000000000.000	0.005	30475.900	830000000000000000000000.000	0.000	0.014	0.000	0.014
8	681000000.000	0.041	2.337	198000000000000000.000	17.048	<0.001	4.426	65.666
9	76.800	0.032	1.462	4035.011	5.209	0.098	0.738	36.750
10	131000000000000000000000.000	<0.001	65100000000.000	266000000000000000000000000000000000.000	23.962	<0.001	6.252	91.842
11	1973.927	<0.001	52.776	73829.140	26.559	<0.001	7.243	97.382
12	774000000.000	0.006	307.532	1950000000000000.000	18.050	<0.001	4.934	66.041
13	206809.600	<0.001	726.828	58800000.000	17.405	0.005	2.352	128.804
14	9624.475	0.001	44.189	2096217.000	13.163	0.010	1.838	94.278
15	0.000	0.007	0.000	0.000	10.779	<0.001	4.818	24.119
16	1612.423	<0.001	39.512	65800.820	31.293	<0.001	7.922	123.606

	Proportion ≥65 years old				GP/100,000 population				Time			
Model no.	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)
1	0.803	0.375	0.495	1.304	1.000	0.843	0.999	1.000	1.005	<0.001	1.002	1.007
2	0.735	0.209	0.455	1.188	1.000	0.438	0.999	1.000	1.005	<0.001	1.002	1.007
3	0.847	0.505	0.521	1.379	1.000	0.842	1.000	1.001	1.005	<0.001	1.002	1.007
4	0.036	<0.001	0.009	0.137	1.000	0.693	0.999	1.002	1.004	<0.001	1.003	1.004
5	6510000000000.000	<0.001	22500000.000	18800000000000000000.000	1.002	0.001	1.001	1.003	0.992	0.006	0.986	0.998
6	6680000000.000	<0.001	525162.800	84900000000000.000	1.002	0.002	1.001	1.003	0.992	0.006	0.986	0.998
7	683000000000000.000	<0.001	1620000000.000	2870000000000000000000.000	1.002	0.003	1.001	1.003	0.993	0.024	0.987	0.999
8	424000000.000	<0.001	27519.870	6550000000000.000	1.002	0.007	1.000	1.003	0.993	0.016	0.987	0.999
9	0.000	0.007	0.000	0.007	1.000	0.839	0.999	1.002	1.004	<0.001	1.003	1.004
10	0.893	0.833	0.311	2.562	1.001	0.044	1.000	1.002	1.009	0.013	1.002	1.017
11	1.135	0.808	0.410	3.138	1.002	0.010	1.000	1.003	1.002	0.002	1.001	1.004
12	1.111	0.842	0.396	3.119	1.001	0.024	1.000	1.003	1.003	0.003	1.001	1.004
13	2.212	0.261	0.555	8.826	1.002	0.026	1.000	1.004	1.006	<0.001	1.003	1.008
14	235000000000.000	<0.001	896843.700	61500000000000000.000	1.002	0.024	1.000	1.004	1.006	0.002	1.002	1.009
15	0.724	0.280	0.402	1.301	1.000	0.164	0.999	1.000	1.001	0.027	1.000	1.001
16	1.208	0.734	0.406	3.595	1.001	0.025	1.000	1.003	1.003	0.049	1.000	1.005

	ddd lag 0				ddd lag -1 Qtr				ddd lag -2 Qtr			
Model no.	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)
1	1.000	0.003	1.000	1.000	NS				NS			
2	1.000	0.001	1.000	1.000	1.000	0.057	1.000	1.000	NS			
3	1.000	0.564	0.999	1.000	NS				NS			
4	1.000	0.003	1.000	1.000	1.000	0.143	1.000	1.000	1.000	0.609	1.000	1.000
5	0.999	0.015	0.999	1.000	1.000	0.436	0.999	1.000	1.000	0.261	1.000	1.001
6	1.000	0.226	1.000	1.000	1.000	0.254	1.000	1.000	1.000	0.506	1.000	1.000
7	1.000	0.555	0.999	1.002	1.000	0.792	0.998	1.001	1.000	0.883	0.998	1.002
8	1.000	0.567	0.999	1.002	0.999	0.329	0.998	1.001	0.999	0.280	0.998	1.001
9	1.000	0.005	1.000	1.000	1.000	<0.001	1.000	1.000	1.000	0.465	0.999	1.000
10	1.001	0.242	0.999	1.002	1.000	0.737	0.998	1.002	1.001	0.556	0.999	1.003
11	1.000	0.008	1.000	1.000	1.000	0.033	1.000	1.000	NS			
12	1.000	0.004	1.000	1.000	0.999	0.310	0.998	1.001	1.000	0.002	1.000	1.000
13	1.000	0.942	0.997	1.002	1.000	0.923	0.997	1.003	0.999	0.558	0.997	1.002
14	0.998	0.036	0.996	1.000	1.003	0.013	1.001	1.005	1.001	0.469	0.999	1.003
15	0.999	0.031	0.999	1.000	1.001	0.008	1.000	1.001	1.001	0.003	1.000	1.001
16	1.000	0.381	0.998	1.001	1.000	0.425	0.999	1.002	1.000	0.713	0.999	1.001

	ddd lag -3 Qtr				ddd lag -4 Qtr			
Model no.	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)	Adjusted OR (or non-linear form)	*P* value	95% CI(l)	95% CI (u)
1	NS				NS			
2	NS				NS			
3	NS				NS			
4	1.000	0.004	1.000	1.000	1.000	0.002	1.000	1.000
5	1.000	0.131	0.999	1.000	1.000	0.567	1.000	1.001
6	1.000	0.470	1.000	1.000	1.000	0.118	1.000	1.000
7	1.000	0.874	0.999	1.002	1.000	0.432	0.999	1.001
8	1.001	0.137	1.000	1.003	1.002	0.034	1.000	1.003
9	1.001	<0.001	1.000	1.001	1.000	0.011	1.000	1.000
10	1.000	0.605	0.999	1.002	1.001	0.303	0.999	1.002
11	NS				NS			
12	NS				NS			
13	1.002	0.013	1.000	1.003	NS			
14	1.000	0.951	0.998	1.002	0.989	<0.001	0.984	0.995
15	1.000	0.231	1.000	1.001	1.001	0.005	1.000	1.001
16	0.999	0.199	0.998	1.000	1.000	<0.001	1.000	1.000

Meta-data used within statistical models

Field	Description
Qtr	Seasonal qtr (see codes table for more details)
Single GP	One registered GP per practice
Gender_ratio	females registered/males registered
pop14	Proportion of GP population aged under 15
pop65+	Proportion of GP population aged 65 and over
GP/100,000 population	GPs per 100,000 practice population
Time	Linear time variable
Deprivation_index	IMD2010 value
Rural location	Whether practice designated as being in a rural location
ddd lag 0	DDDs prescribed per 1000 population this quarter
ddd lag -1 Qtr	DDDs prescribed per 1000 population previous quarter
ddd lag -2 Qtr	DDDs prescribed per 1000 population Q-2
ddd lag -3 Qtr	DDDs prescribed per 1000 population Q-3
ddd lag -4 Qtr	DDDs prescribed per 1000 population Q-4

Season definitions		
Qtr	**Months**	**Season**
1	Mar-May	Spring
2	Jun-Aug	Summer
3	Sep-Nov	Autumn
4	Dec-Feb	Winter

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