THE UNIVERSITY
of EDINBURGH

The Epidemiology of MRSA in Scotland

Cheryl L Gibbons

A thesis submitted for the degree of

Doctor of Philosophy

The Centre for Immunity, Infection and Evolution (CIIE)

The School of Biological Sciences

University of Edinburgh

September 2015

Doctor of Philosophy – The University of Edinburgh – 2016

Declaration

I declare that the work in this thesis is my own (other than stated below) and in all circumstances, I carried out the analysis and interpretation of the data. This work has not been submitted for any degree or professional qualification. All data were acquired from one of the public health agencies in Scotland, as described below.

Cheryl L Gibbons

20th September 2015

Data acquired from and property of -

Chapter 2: Morbidity data from Health Protection Scotland (HPS), and mortality data from the General Registrar Office for Scotland (GROS)

Chapter 3, 4, and 5: Molecular epidemiology data from the Scottish MRSA reference laboratory (SMRSARL)

Chapter 5: Antimicrobial prescribing data from Information Services Division (ISD), part of NHS Scotland

Chapter 6: Hospital-level bacteraemia case data from HPS and hospital-level risk factor data from ISD

Work carried out by other persons –

Dr Thibaud Porphyre wrote the scripts for the antibiogram diversity analyses used in Chapter 4. Dr Margo Chase-Topping carried out preliminary runs of the multi-dimensional scaling analyses in Chapter 3, 4 and 6, and the principle component analysis in Chapter 5. Dr Bram van Bunnik produced connectivity measures for Chapter 6.

Abstract

Staphylococcus aureus (*S. aureus*) is a bacterium that commonly colonises the skin and nares of around one third of otherwise healthy individuals. While colonisation is benign, *S. aureus* can cross skin and mucosal barriers to cause infections that manifest as clinical disease. Clinical outcomes are diverse and range from mild, non-complicated and often self-limiting skin and soft tissue infections (including boils, abscesses and cellulitis) to more severe and life-threatening conditions including pneumonia, toxic shock syndrome and bacteraemia. Medication isn't always needed for mild *S. aureus* infections as often they resolve with time but, for severe or persistent cases, antimicrobial treatment is generally required. Following decades of widespread and intensive usage of topical, enteral and parenteral antimicrobials to treat *S. aureus* infections; AMR has become an established and ubiquitous problem in the treatment of infections caused by this microorganism, especially when in the methicillin resistant form (i.e. MRSA).

The aim of this thesis was to examine aspects of *S. aureus* epidemiology (including MRSA and methicillin-sensitive *S. aureus* (MSSA)) in Scotland using statistical methods and data from several large public health databases. More specifically this involved: descriptions of spatial and temporal trends of morbidity and mortality; comparisons of epidemiological and molecular attributes (including antimicrobial resistance) of (1) MSSA and MRSA, and (2) the dominant clones of MRSA (i.e. EMRSA-15 and EMRSA-16); descriptions of spatial and temporal trends of antimicrobial prescribing in primary and secondary care and any associations between prescribing rates and MRSA antimicrobial resistance; and carrying out a hospital-level risk factor analysis of MRSA, testing hypotheses that hospital size, hospital connectivity (through shared transfer patients) and hospital category have an effect on hospital-level incidences of MRSA in mainland Scottish hospitals.

Results showed that total *S .aureus* bacteraemia and MRSA bacteraemia in Scotland statistically declined over time ($p<0.0001$), but MSSA bacteraemia did not ($p>0.05$). While combined mortality rates (i.e. all MSSA deaths (both primary and secondary cause), or all MRSA deaths (both primary and secondary cause)) mirror these

findings; case-fatality ratios (CFR) show no declines over time for either MRSA or MSSA. Results also show that several epidemiological factors point towards a predominant community source for MSSA isolates (i.e. outside healthcare) and hospital source for MRSA. Evidence for this included: (1) the lack of resistance genes in the MSSA population, (2) MRSA was more associated with long-term care and high-risk patients in the specialties care of the elderly, high dependency units /intensive care units (HDU/ICU), and surgery and conversely MSSA with specialties that commonly served outpatients, and (3) the abundance of non-EMRSA-15/non-EMRSA-16 'other' clones in the MSSA population as compared with the hospital-associated CC22 (EMRSA-15) and CC30 (EMRSA-16) clones. EMRSA-15 was by far the most dominant MRSA clone in Scotland with EMRSA-16 declining significantly and non-EMRSA-15/non-EMRSA-16 clones causing an increasing number of infections (over the time period 2003-2013). EMRSA-16 was resistant to a larger number of antimicrobials than EMRSA-15, typically 9 versus 5, and while resistance varied for EMRSA-16 over the study period, resistance remained stable for EMRSA-15. There was little difference between clinical and screening MRSA isolates. Analyses of antimicrobial prescribing showed that prescribing rates of several drugs increased over time (2003-2013). Prescribing was far higher in primary care settings than in secondary care, although this differed between antimicrobials. Significant positive associations between prescribing and resistance rates were found for gentamicin (pr - $p<0.0001$, se - $p<0.0001$) and trimethoprim (pr - $p<0.01$, se - $p<0.0001$) in both primary (pr) and secondary (se) care, and clindamycin ($p<0.0001$) in primary care only. Finally, in Scotland there is a threshold of connectivity above which the majority of hospitals, regardless of size, are positive for MRSA. Higher levels of MRSA are associated with the large, highly connected teaching hospitals with high ratios of patients to domestic staff.

While there were a number of data limitations, this body of work provides a better understanding of the epidemiology of *S. aureus* including MRSA in Scotland.

Lay summary

Staphylococcus aureus (also known as *S. aureus*) is a bacterium that is found on the skin of approximately one third of the population. Individuals can be colonised by the bacterium without being aware as carriage of the bacterium is symptomless. However, if there is a wound to the skin or tissue, then *S. aureus* can cross the skin barrier and cause an infection. The severity and types of disease that follows can vary from a simple skin infection that clears with or without a short course of antibiotics; to more complex and life-threatening conditions involving multiple organs. Bacteraemia, when bacteria are found in the blood, is a rare but extremely serious outcome of *S. aureus* infection.

Methicillin-resistant *S. aureus,* also known as MRSA, is the antibiotic resistant variant of the bacterium that has been widely reported in the media over the last decade. This variant, although far less common than the methicillin-sensitive bacterium, (known as methicillin-sensitive *S. aureus* or MSSA) tends to cause more serious infections which are more difficult to treat and result in a higher proportion of deaths.

MSSA bacteraemia rates have not changed over time, whereas total *S. aureus* and MRSA bacteraemia rates have steadily declined and importantly, government targets to reduce bacteraemia cases were met for total SAB, but this was driven by a disproportionate reduction in MRSA. Mortality rates mirrored morbidity trends with a decline in total SAB and MRSA but the number of cases who went on to die, a metric known as the case-fatality ratio, has not changed over time for MSSA or MRSA.

Bacteria can be resistant to a number of different antibiotics, which means that, if an individual is infected with an antibiotic-resistant bacterium, then those drugs will not clear the infection. MRSA are resistant to an average of five antibiotics but resistance to as many as 14 is described in Scotland. On the other hand, approximately 20% of MSSA are sensitive to all tested antibiotics, meaning that they can be treated with any drug, and nearly 60% of MSSA are resistant to one antibiotic only (penicillin).

Antibiotic prescribing is considerably higher in primary care settings (such as GPs and community pharmacies) compared to prescribing in secondary care facilities (such as hospitals), although this differs between drugs. Importantly, it was shown that an increase in antibiotic prescribing is associated with an increase in resistance for several antibiotics in Scotland. While prescribing levels of some drugs increased during the period that was investigated, the prescribing levels for the 'drugs of last resort', which are the last line of defence against MRSA, remain low.

Bacteraemias can occur in different hospitals but they are most commonly independently associated with hospitals that are large, receive many transfer patients from other hospitals (a measure known as 'connectivity'), and have a high ratio of patients to domestic staff.

There are several potential implications of this work.

(1) It highlights the need to investigate case-fatality ratios further and to find a way to reduce *S. aureus* related deaths. This could impact funding in this area to provide more resources for the development of bedside rapid testing for timely and appropriate treatment of patients.

(2) It describes levels of antibiotic resistance in MSSA and MRSA, and the association between prescribing and resistance. This has potential policy implications since it could lead to the review of antimicrobial prescribing practices.

(3) It shows that to further reduce bacteraemia due to *S. aureus* and potentially other hospital-associated infections, there needs to be a focus on large, highly connected teaching hospitals with high ratios of patients to domestic staff.

In conclusion, we must continue to monitor MRSA as it is still an important public health problem but the overall population-level burden has declined over time. This is due to the combined efforts and activities to reduce spread and is a great public health success story. MSSA remains a major cause of infection causing outcomes including bacteraemia, but these infections are more easily treated and are less frequently fatal.

Acknowledgements

My PhD was funded by the Scottish Universities Life Sciences Alliances (SULSA), the Medical Research Council (MRC), and Zoetis (previously Pfizer Animal Health). I would especially like to thank Dr Denise Barrault, the Executive Director of SULSA, and MRC for supporting my maternity break, and Dr Michael Pearce of Zoetis for thoroughly reviewing his thesis. I also received a small grant from the Scottish Infection Research Network (SIRN) towards the study described in Chapter 6. The EU-funded project EvoTAR also provided funding that supported work carried out by others in our research group which fed in to this thesis.

I would like to thank my three supervisors; Prof Mark Woolhouse and Dr Margo Chase-Topping of the Institute of Evolutionary Biology and Centre of Immunity, Infection and Evolution (CIIE) at the University of Edinburgh, and Prof Ross Fitzgerald of the Roslin Institute and University of Edinburgh.

I feel particularly indebted to Margo, who is an excellent scientist and an exceptional teacher. She offered advice, friendship, motivated, patiently answered all my questions, trained me to be an epidemiologist, meticulously checked my analyses and chapters, and without her I might have struggled to get to this point. Thank you Margo.

I would also like to thank fellow Epigroup (Woolhouse group) and LBEP (Fitzgerald group) members (past and present) for their fun, friendship, for giving help or suggesting work-related solutions. In particular, I owe a great deal of thanks to Dr Thibaud Porphyre for showing me how to get started with R and giving me advice and encouragement (especially in the early years) when I didn't know how to proceed. I am also very grateful to Dr Bram van Bunnik for his useful discussions, insights, and outstanding proof-reading. I am also very lucky to have a number of talented friends who proof-read parts of this thesis, namely: Dr Annie Cook, Dr Laura Appleby, Dr Joseph White, Rachel Walsh, and Kirsty Headden.

It is important that I mention and thank the public health agencies in Scotland that I have corresponded with over the years and that provided me with data, given their time and shared their expertise: Scottish MRSA reference laboratory (SMRSARL), in particular Dr Giles Edwards, Dr Kirsty Girvan and Dr Elizabeth Dickson; Health Protection Scotland (HPS), especially Dr Oliver Blatchford and Prof Chris Robertson; Information Services Division (ISD), Ishbel Robertson and Victoria Elliot; and the General Registrar Office for Scotland (GROS), Frank Dixon. Giles deserves a very fulsome thank you as the SMRSARL database was his life's work and it was pivotal in this thesis. Additionally, Giles spent a lot of his time fully explaining the database and various aspects of MRSA epidemiology.

I would like to thank my friend Kath Tracey in the Ashworth office for all the laughs, Dr Dan Nussey for his guidance, and Prof Eric Fèvre who took a punt by offering me a research assistant post at the University that eventually led to me starting this PhD.

Thank you to my dear friends and family who have given endless support and encouragement through this experience. My sister Karen doesn't realise how brilliant she is. And finally, to my two favourite boys, Mesoul and Angus, this is for you.

List of Abbreviations

Adj. R^2	Adjusted R^2
AMR	antimicrobial resistance
Antimicrobials	
Ch	chloroimphenicol
Cl	clindamycin
Cp	ciprofloxacin
Cx	cepheroxin
Dp	daptomycin
Er	erythromycin
Fd	fusidic acid
Gn	gentamycin
Km	kanomycin
Lz	linezolid
Mp	mupiricin
Mt	methicillin
Mx	moxifloxacin
No	neomycin
Pn	penicillin
Qp	quinaprist
Rf	rifampicin
St	streptomycin
Su	sulphamethoxazole
Tb	tobramycin
Tc	teicoplanin
Te	tetracyclin
Tr	trimethoprim
Va	vancomycin
AR	antibiogram richness
AOBD	acute occupied bed days
BP	Berger-Parker
BSI	blood stream infection
CA-MRSA	community-associated MRSA
CC	clonal complex
CFR	case- fatality ratio
CIs	confidence intervals
CRA	clinical risk assessment
DDD	Daily Defined Doses
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
ESAC-Net	Antimicrobial consumption interactive database
ED	early discharge
EEA	European Economic Area
EMRSA-15	Epidemic MRSA-15, also known as CC22, ST22 SCCmecIV
EMRSA-16	Epidemic MRSA-16, also known as CC30, ST36 SCCmecII
ES	early switch
EU	European Union
FA-MRSA	farm-associated MRSA
GLM	general linear model
GP	general practitioners
GROS	General Registrar Office for Scotland
HAI	healthcare-associated infections
HA-MRSA	healthcare- (or hospital)-associated (or -acquired) MRSA
HB	NHS Health Boards
Health Boards	

AA	NHS Ayrshire and Arran
BO	NHS Borders
DG	NHS Dumfries and Galloway
FI	NHS Fife
FV	NHS Forth Valley
GC	NHS Greater Glasgow and Clyde
GR	NHS Grampian
LA	NHS Lanarkshire
LO	NHS Lothian
TA	NHS Tayside
HDU	high dependency unit
HMUD	Hospital Medicines Utilisation Database
HPS	Health Protection Scotland
ICU	intensive care units
IE	infective endocarditis
ISD	Information Services Division
LA-MRSA	livestock-associated MRSA
MGE	mobile genetic elements
MLST	multilocus sequence typing
MR	Mortality rate
MRPP	Multi-response Permutation Procedures
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	European Union member states
MSSA	methicillin-sensitive *Staphylococcus aureus*
NHS	National Health Service
NGS	Next generation sequencing
NMS	nonmetric multi-dimensional scaling analysis
OBD	occupied bed days
OR	odds ratio
orfX	open reading frame X
PBP2a	modified penicillin binding protein
PCR	polymerase chain reaction
PFGE	Pulsed Field Gel Electrophoresis
PRISM	Prescribing Information System for Scotland database
pvl	Panton-Valentine Leukocidin toxin gene
ROC	Receiver Operating Characteristic curve analysis
rRNA	ribosomal ribonucleic acid
S. aureus	*Staphylococcus aureus*
SA	*Staphylococcus aureus*
SAB	*Staphylococcus aureus* bacteraemia
SAPG	Scottish Antimicrobial Prescribing Group
SCC	Staphylococcus Cassette Chromosome
SCC*mec*	Staphylococcus Cassette Chromosome carrying the *mec* gene
ScotPHO	Scottish Public Health Observatory
SD	Simpson diversity
SE	Standard error
SEn	Shannon entropy
Sig	Level of statistical significance
SMRSARL	Scottish MRSA reference laboratory
SNPs	single nucleotide polymorphisms
spa	S*taphylococcal* protein A
SSTI	skin and soft tissue infections
ST	sequence type
TSS	toxic shock syndrome
tst	toxic-shock syndrome toxin
UK	United Kingdom
VISA	vancomycin-intermediate *Staphylococcus aureus*

VRSA	vancomycin-resistant *Staphylococcus aureus*
VNTR	variable-number tandem repeats
WGS	Whole genome sequencing
WHO	World Health Organisation
%MRSA	the proportion of total *S. aureus* that was MRSA

Table of contents

Table of figures

Tables of tables

Table of appendix figures

Table of appendix tables

Table of appendix documents

Chapter 1: **Introduction to the thesis**

1.1 Literature review

1.1.1 Clinical background

1.1.1.1 Disease outcomes

Staphylococcus aureus (*S. aureus*) is a bacterium that commonly colonises the skin and nares of around one third of otherwise healthy individuals. More specifically, longitudinal studies have shown that approximately 20% (range 12–30%) of people are persistently colonised, about 30% are intermittent carriers (range 16–70%), and 50% (range 16–69%) are non-carriers [1]. While colonisation itself is benign, *S. aureus* can cross skin and mucosal barriers via an invasive device such as a catheter (primary source), or following a wound (secondary source) [2] to cause an infection that manifests as clinical disease. Clinical outcomes range from mild, non-complicated and often self-limiting skin and soft tissue infections (SSTIs) including boils, abscesses and cellulitis to more severe and life-threatening conditions including pneumonia, toxic shock syndrome (TSS) and bacteraemia (bacteria present in the bloodstream) (Figure 1-1). Bacteraemia can lead to systemic metastatic infections and infective endocarditis (IE), and trigger sepsis, septic shock and multiple organ failure. While SSTIs are considerably more common, there has been a public health focus on reducing the more serious outcomes, in particular bacteraemia, due to high associated burden and case-fatality ratios [3-7]. In the decades before antimicrobials, mortality rates due to *S. aureus* bacteraemia exceeded 80% [8] and *S. aureus* continues to be a leading cause of bacteraemia in high-income countries [9].

1.1.1.2 Methicillin-resistant *S. aureus* (MRSA)

A proportion of the *S. aureus* population is commonly resistant to the antimicrobial agent methicillin therefore giving rise to MRSA. Both methicillin-sensitive *S. aureus* (MSSA) and MRSA cause important healthcare-associated infections (HAIs) but can also cause infections in community-settings. When MRSA is the causative agent of bacteraemia, the risk of treatment failure and mortality is greater and length of hospital stay is extended [10-12]. Additionally, the associated costs of managing

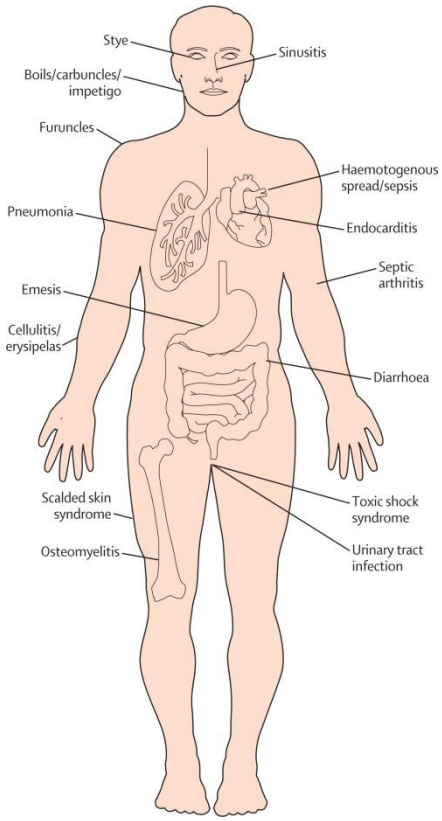
these patients are reportedly 1.4-2.8 times higher for patients with MRSA compared to patients with infections caused by MSSA [13].

1.1.1.3 Treatment

Medication is not always necessary for mild *S. aureus* infections as often they resolve without intervention in time but, for severe or persistent cases, antimicrobial treatment is generally required. Empirical therapy, where treatment begins before a definitive diagnosis is made, is based on clinical experience and often involves the prescribing of broad-spectrum antimicrobials (where antimicrobials act against a wide range of organisms). In contrast, where a definitive diagnosis exists, or if broad-spectrum drugs have not improved a patient's condition, narrow-spectrum antimicrobials (effective against specific families of bacteria), may be prescribed. Where a definitive diagnosis for MSSA bacteraemia has been made, treatment with beta-lactam antimicrobials is the most effective treatment and even superior to vancomycin, which is an important drug in the treatment of MRSA bacteraemia [14].

For MRSA, recent guidelines issued by the Infectious Disease Society of America recommend for example, daptomycin or vancomycin for bacteraemia and endocarditis (plus rifampicin for prosthetic valve infections), and clindamycin, linezolid and vancomycin for pneumonia [15]. Similarly, in 2006, the Joint Working Party of the British Society for Antimicrobial Chemotherapy recommended glycopeptides (i.e. vancomycin, teicoplanin) or linezolid be considered for pneumonia and SSTI if the risk of bacteraemia is high, and a minimum of 14 days treatment with glycopeptides or linezolid for uncomplicated bacteraemia but longer for patients with, or at higher risk of, endocarditis [16]. Following decades of widespread and intensive use of topical, enteral or parenteral antimicrobials to treat *S. aureus* infections; antimicrobial resistance (AMR) has become an established and ubiquitous problem for this microorganism. Therefore, antimicrobial guidelines tend to include a choice of different treatment options to accommodate local resistance profiles.

Figure 1-1: Wide diversity of clinical outcomes caused by *S. aureus* infections.



Source: [1]

1.1.2 *S. aureus* resistance to antimicrobials

1.1.2.1 AMR background

Since their introduction into general clinical usage, antimicrobials have greatly contributed to the improvement of health and to increasing life expectancies globally [17]. However, as long as there have been antimicrobials, there has been resistance and with continued usage, AMR persists. Hence, AMR can be considered as a historic, current and future issue associated with a range of microorganisms. The importance of this public health threat was recently highlighted in the WHO's first global report on surveillance of antimicrobial resistance which advised that every country needed to do more to monitor the problem [18]. Yet, with the rise of AMR and since only two new classes of antimicrobials have been introduced since the 1970s, there has been much discussion on the possibility of a 'post-antibiotic era' in which the successful and powerful drugs of the past will no longer be effective [19].

To address this, there have been calls for improved antimicrobial stewardship to better regulate drug usage, and improved surveillance, monitoring and regulation [20, 21], and even suggestion of an intergovernmental panel on AMR to tackle the issue [22].

1.1.2.2 A history of AMR in *S. aureus*

Chambers and DeLeo [23] described four waves of AMR in *S. aureus* (Figure 1-2). The first began in the 1940s soon after penicillin became routinely established in healthcare with the evolution of penicillin-resistant strains from the lineage known as phage type 80/81 which persists today. Within two decades, approximately 80% of *S. aureus* isolates were penicillin resistant [24].

In the second wave, resistance to the semi-synthetic penicillin-related antibiotic methicillin (also known as meticillin) was first recognised in nosocomial isolates in 1961 [25] following introduction of the β-lactam drug into clinical practice in 1959 [26, 27].

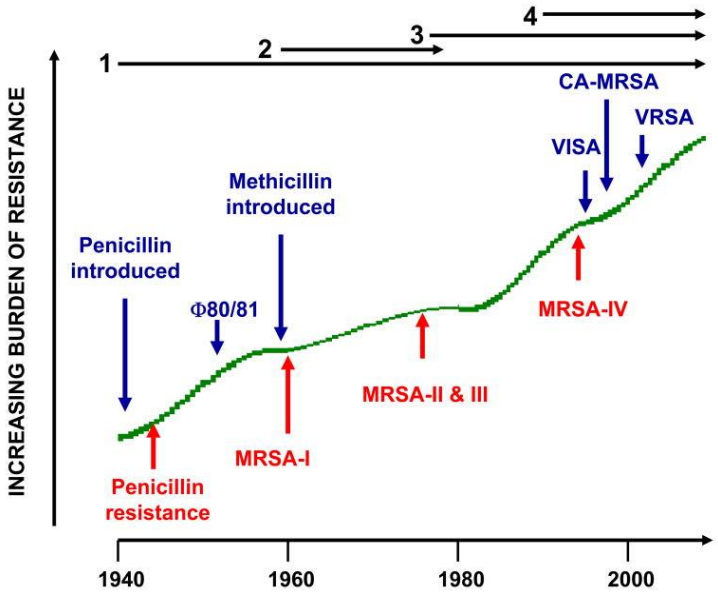
The mid- to late 1970s marked the start of the third wave with the emergence of new resistant strains containing previously unseen variants of the mobile genetic element 'Staphylococcus Cassette Chromosome' (SCC*mec*) (variants SCC*mec*II and III) in nosocomial settings.

In the fourth wave which began in the mid to late 1990s, MRSA strains were no longer restricted to healthcare settings but also recognised as causing infections in the community. Such strains (causing community-associated MRSA (CA-MRSA) infections) tend to contain smaller, more mobile and often previously undiscovered SCC*mec* variants and appear to be causing morbidity and mortality in people without the usual hospital-associated risk factors for the infection. In addition, in 2002 during the fourth wave, vancomycin-intermediate *S. aureus* (VISA) strains and vancomycin-resistant *S. aureus* (VRSA) strains (which are not inhibited at concentrations below 15μl ml^{-1}) were isolated for the first time.

It is possible that we are now in a new, fifth wave with livestock-associated clones (including CC398) causing human infections [28, 29], and with other SCC*mec* types

including SCC*mec*IX, X, XI and divergent *mec* variants (i.e. non-*mecA*) being discovered [30, 31].

Figure 1-2: A timeline of four waves of antibiotic resistance in *S. aureus*.



Φ80/81: phage type 80/81
VISA: vancomycin intermediate *S. aureus*
CA-MRSA: Community-associated MRSA
VRSA: Vancomycin-resistant *S. aureus*
MRSA-I: MRSA with SCC*mec* I; MRSA-II: MRSA with SCC*mec* II; MRSA-III: MRSA with SCC*mec* III; MRSA-IV: MRSA with SCC*mec* IV
Arrows show each of the four waves. Source: [23]

1.1.2.3 How do methicillin-sensitive strains become methicillin-resistant?

S. aureus isolates become methicillin resistant when they acquire, by horizontal gene transfer, one of the SCC, which harbour the *mec* gene-carrying element SCC*mec*. The *mec* gene confers resistance to not only methicillin (and derivative oxacillin) but to all beta-lactams [32, 33] including penicillins, carbapenems and cephalosporins to which resistance can also be acquired independently largely through acquisition of resistance gene-carrying mobile genetic elements (MGEs). Resistance to other antimicrobials, which is largely gained through acquisition of MGEs or through point mutation of chromosomal genes [34], has also been discussed throughout the literature. Rates of such resistance vary between countries, regions and even hospitals with this variation thought to reflect drug usage and selection pressures.

When treated with penicillin and penicillin-related β-lactams; proteins in MSSA strains naturally bind to the structural β-lactam ring found in the antimicrobial compound. Through this interaction, the drug is able to inhibit bacterial cell wall synthesis and clear infection. However, with the acquisition and integration at open reading frame X (*orfX*) of the mobile genetic element SCC*mec* [35]; MSSA strains become methicillin-resistant since the cassette carries *mecA*, the gene that encodes the modified penicillin binding protein, PBP2a (also known as PBP2') [25, 26, 36-38]. Since the binding proteins in methicillin-resistant strains are modified, the bacteria have a low affinity for attachment to all β-lactam rings. The uninhibited binding protein then catalyses the formation of cell wall peptidoglycan [39] allowing bacteria to continue multiplying and infections to persist. Penicillin-resistant strains confer resistance in a different way, producing the plasmid-encoded enzyme penicillinase which hydrolyses and destroys the β-lactam ring [23].

1.1.2.4 Resistance to other antimicrobials

As described in Chapters 3 and 4, resistance to several other antimicrobials is common for MRSA. Chapter 3 also shows that multi-drug resistance in MSSA is rare. The *mecA* gene confers resistance not only to methicillin (and derivative oxacillin) but to all β-lactams [32, 33]. Resistance to all antimicrobial classes has been described in *S. aureus* although resistance to the glycopeptides (vancomycin for instance) is still rare [34]. Resistance to β-lactams, aminoglycosides, erythromycin, clindamycin, tetracycline and chloramphenicol, is gained by acquiring the gene from a MGE; resistance to fluoroquinolone and rifampicin is due to point mutations within chromosomal DNA; and resistance to fusidic acid, trimethoprim, co-trimoxazole and mupirocin can be either by MGE or point mutation [34]. An overview of different antibiotic classes with example of drugs, their data of discovery, and modes of resistance, is shown in Figure 1-3. The modes of resistance are further described in detail elsewhere [40].

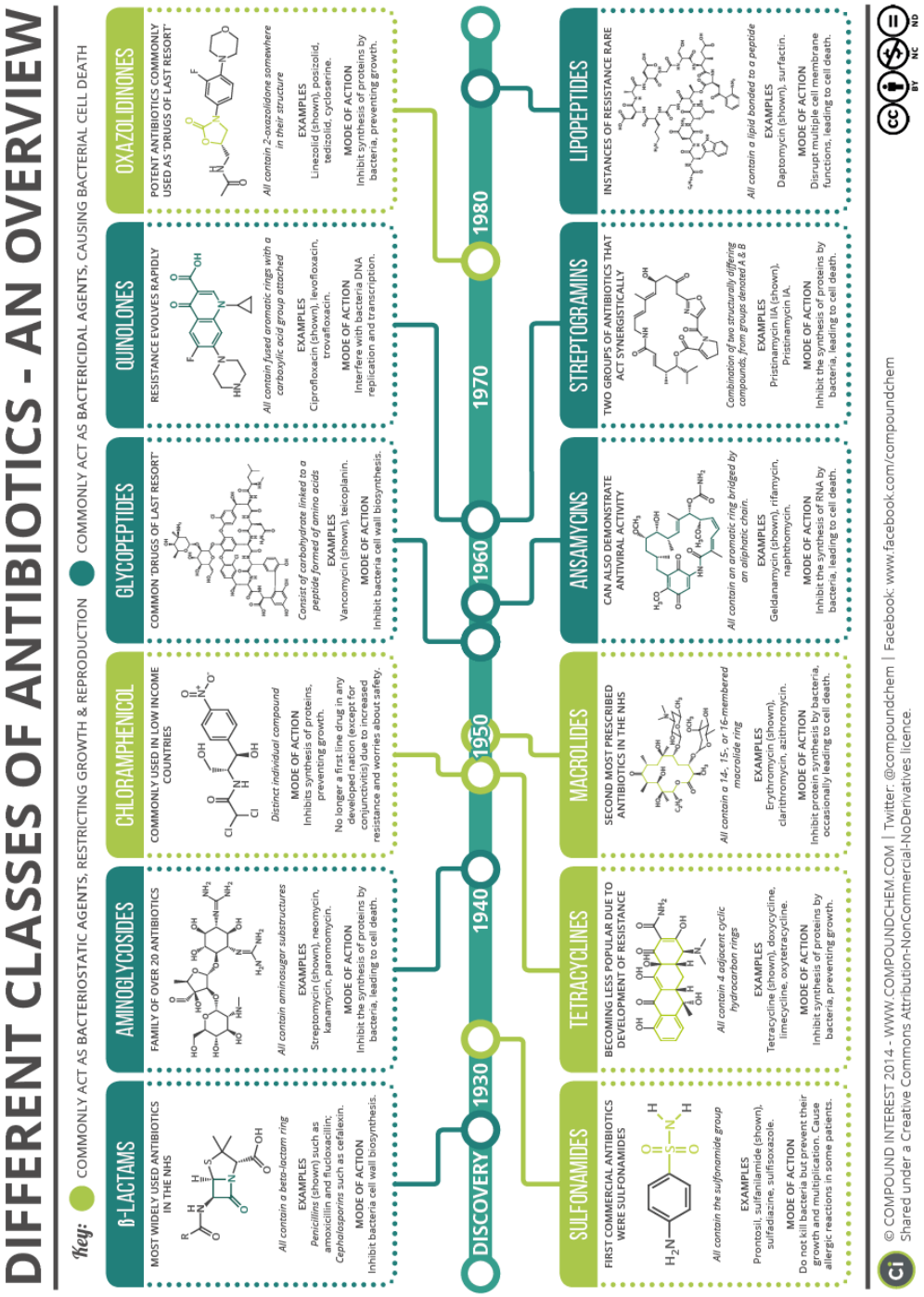
1.1.2.5 Resistance to heavy metals

Heavy metals can be toxic to bacteria and disrupt bacterial cellular processes [41, 42]. However, bacterial species can acquire resistance to heavy metals with determinants against compounds such as zinc, cadmium, mercury, and arsenic carried on plasmids and mercury on the chromosome [43]. Heavy metal resistance is important not only since bacteria can grow in the presence of these materials, but also because the genes for heavy metal resistance can be linked to genes for AMR and thus research suggests that resistance to heavy metals may be a driver of AMR [43]. For example, a cluster of metal resistance determinants exist on the SCC*mec* element, closely linking it to the *mec*A gene and β-lactam resistance [43]. In addition, whereas the growth of cadmium resistant *S. aureus* was inhibited by ampicillin, streptomycin, penicillin and tetracycline; the growth of lead-resistant *S. aureus* was not inhibited in the presence of streptomycin and ampicillin, indicating linked resistance determinants [44].

1.1.2.6 Resistance to antiseptics and disinfectants

Antiseptics (i.e. chemicals applied to skin or living tissue that kill or inhibit the growth of microorganisms, e.g. alcohol gels, surgical hand scrubs) and disinfectants (i.e. chemicals applied to surfaces that kill or inhibit the growth of microorganisms, e.g. bleach, floor cleaner) are vital components of infection control [45]. However, bacteria have adapted to antiseptic and disinfectant exposure by acquiring plasmids and transposons that confer resistance. Furthermore, there is some suggestion in the literature that antiseptic and disinfectant resistance may be linked to reduced antimicrobial susceptibility [45].

Figure 1-3: An overview of different classes of antibiotics.



Source: **[46]**

1.1.2.7 Selective pressures

Mutants containing genes for antimicrobial resistance are selected for when isolates are under selection pressure, and genes only become fixed in a population when they show a strong selective advantage to the bacteria that outweighs any fitness cost [47]. However, previous studies have shown that there is often a fitness cost and reduced growth rate associated with the harbouring of MGEs carrying drug resistance genes, especially if the resistances are not beneficial (such as would be the case in a low selection pressure environment) [48-50]. Furthermore, if resistance does infer a fitness cost, then resistance tends to be eliminated once the selective pressure is removed [50]. This is not a straightforward issue since the effect of resistance on fitness can differ depending on environmental conditions, can be altered by compensatory mutations or epistasis, and some mutations appear to be cost free [49].

While, in general, antimicrobial drug use leads to selection of resistant mutants, resistance can also be gained in the absence of selection pressure through co-resistance where one gene confers resistance to another or multiple antimicrobial classes. For example, erythromycin resistance is conferred by the *msrA* gene (carried on MGE) as well as the *erm* genes (*ermA, ermB, ermC, ermT*, also MGE) which also confer resistance to clindamycin [51]. In addition, clindamycin resistance in *Staphylococcal* species can be either constitutive (meaning that isolates will display the resistant phenotype under routine screening conditions) or inducible (isolates display resistance phenotype only in the presence of erythromycin and clindamycin). Therefore, isolates with the inducible phenotype will appear to be erythromycin resistant and clindamycin sensitive when not tested in the presence of each other [52].

1.1.3 Risk factors

1.1.3.1 Hospital-associated infection (HAI)

S. aureus is a common healthcare- or hospital-associated/acquired infection. In 2009, it was suggested that MRSA caused 171,200 HAIs in Europe each year (44% of all HAIs) [15]. Much of the literature concerning risk factors has focused on MRSA rather than MSSA. Healthcare-associated MRSA (HA-MRSA) tends to affect

individuals in hospitals and residential care homes with specific risk factors including previous hospitalisation or residency at a care facility, recent antimicrobial therapy, indwelling vascular devices or catheters, prior surgical procedures, and age (neonates and the elderly being particularly affected)[27, 53]. Underlying illnesses can also predispose individuals to infection, including the loss of the normal skin barrier, the presence of underlying diseases such as diabetes or AIDS, and defects in neutrophil function [23].

1.1.3.2 Colonisation

Being colonised with MRSA is a known risk factor for infection [54-56] and individuals who are known to have been colonised with MRSA for more than one year are at high risk for subsequent MRSA morbidity and mortality [57]. In a study of Scottish hospital admissions, colonisation was found to be associated with the number of admissions per patient, hospital specialty (department) of admission, age, and source of admission (home, other hospital or care home) [58]. In another Scottish study of anatomical sites for MRSA screening, combining nasal and perineal swabs gave the best 2-site combination that identified 82% of true MRSA carriers [59].

1.1.3.3 Community-associated infection (CA-MRSA)

S. aureus is also a major pathogen outside the hospital environment. CA-MRSA typically affects younger and previously healthy individuals [38] without the typical HA-MRSA risk factors [25, 60]. Some groups within the community are at particular risk including: intravenous drug users (IDUs) [38, 61, 62], men who have sex with men [63], athletes and players of contact sports [64], prisoners and those of lower social-economic status [65], and military personnel [38, 60, 66, 67]. CA-MRSA has also been isolated and noted as causing morbidity in aboriginal communities in Australia [68-75], Canada [76-79], native American Indians in the USA [76, 80], New Zealand [81], Malaysia [30] and elsewhere. A study of CA-MRSA in aboriginal Australians found that such populations were at a higher risk of invasive CA-MRSA and bore a disproportionate burden of invasive infections, a propensity likely to be linked to the high prevalence of skin infections and domestic crowding in this population [68].

1.1.3.4 Livestock- (LA-) or farm- (FA-) associated MRSA

In addition to being a human disease, MSSA and MRSA are also significant veterinary pathogens [82] causing infection in a range of animal hosts [83]. Such infections are important in terms of animal wellbeing, livelihood, economy and also for the potential risk of transmission to humans [82, 84, 85] and within human populations [86, 87]. Particularly at risk from this emerging zoonotic disease known as livestock- or farm-associated MRSA (LA- or FA-MRSA) are those who work closely with livestock and domestic animals including farm workers [28, 87, 88], veterinary staff [89], and their families [87]. MSSA and MRSA belonging to clonal complex *398 (CC398)*, as defined by multilocus sequence typing (MLST), are designated LA-MRSA due to the frequent colonisation of livestock and those with frequent livestock contact. There have been reports of increasing spread to and between individuals with limited or without any direct livestock contact: families of farm workers [90], those living on pig farms but without contact [91], those without any apparent contact [92] and transmission within hospitals [93, 94]. However, livestock contact and the intensity of contact remain the primary risk factors for LA-MRSA carriage, persistent carriage and infection [95].

1.1.3.5 Hospital-level risk factors

A hospital-level risk factor analysis for Scotland is described in Chapter 6 of this thesis. There has been some work on hospital-level risk factors but none incorporating as many variables as in this Chapter and none carried out in Scotland. Hospital size has been found to be associated with hospital-level bacteraemia incidences [96, 97], as has hospital connectedness in England [98] and the Netherlands [96]. By decreasing the number of transfer patients between hospital collectives, the rate of spread of high-risk clones through the hospital network in England was reduced [99]. Similarly, transferring patients within and between hospitals was also found to be a patient-level risk factor of nosocomial infections in a retrospective analysis in France [100], and transfer from another institution, a patient risk-factor for both MSSA and MRSA bacteraemia [101]. Hospital type or category and hence activities and procedures conducted in such facilities were also previously found to be an important hospital-level risk-factor [96, 97].

1.1.4 Surveillance and infection control measures

1.1.4.1 Surveillance

It is a mandatory requirement by law that all NHS diagnostic laboratories in Scotland report every episode of MSSA or MRSA bacteraemia (collectively known as *S. aureus* bacteraemia (SAB)) to Health Protection Scotland (HPS). HPS has monitored trends of MSSA bacteraemia since 2005 and MRSA since 2003. It is also mandatory that all NHS diagnostic laboratories send every blood isolate of SAB (along with accompanying clinical data) to the Scottish MRSA reference laboratory (SMRSARL) for further testing of the isolate e.g. AMR, toxin gene. This SMRSARL contributes information on Scottish SAB to the European Centres for Disease Control (ECDC) antibiotic resistance surveillance system (EARS-Net) [102].

In the Scottish context, a case of SAB is defined as: "A person from whose blood *S. aureus* has been isolated and reported by a diagnostic microbiology laboratory in Scotland in the absence of a positive blood culture with the same organism in the previous two weeks". This therefore excludes positive isolates from a single patient submitted within two weeks of each other which is assumed to be from a single episode. The case definition includes all SAB to avoid the need for potentially subjective assessment of whether an individual case's bacteraemia was associated with healthcare interventions [102].

The Scottish government set targets to reduce all SAB to 70% of the 2005/2006 levels by 2010 [103]. Each year, the Scottish Government and National Health Service (NHS) Scotland agree on a number of national NHS performance targets, which are known as HEAT targets (as explained below). These targets are grouped as one of four priorities: firstly, health improvement for Scottish people; secondly, efficiency and governance improvements; thirdly, access to services; and lastly, treatment appropriate to individuals.

1.1.4.2 Control measures

Several public health policies and initiatives aimed at reducing the burden of MSSA and MRSA were developed and implemented in Scotland. Implementing effective infection control measures to reduce the number of cases and the spread of the

bacteria in nosocomial settings has been a priority in recent years. Several control measures have been implemented.

1.1.4.2.1 MRSA screening

Universal screening (i.e. when all patients are screened) was trialled with the 'Pathfinder' pilot study which was a prospective cohort study of MRSA colonisation in admissions to six acute hospitals in three NHS Health Boards in Scotland [11]. The study found that 7.5% of admissions were colonised and several risk factors were associated with colonisation including: if a patient was re-admitted following previous discharge or admitted from a care home or other hospital, increasing age (i.e. the elderly), and specialty of admission (this was highest in care of the elderly, dermatology, nephrology, and vascular surgery) [11]. There have been further publications focusing on screening in Scottish hospitals including; universal MRSA screening and risk factors for being colonised [58], interim findings from the 'Pathfinder' screening project [104], screening of healthcare workers [105] and patients acceptance of screening [106].

Results from the 'Pathfinder' study and work that followed suggested that clinical risk assessment (CRA) based screening was as effective as universal nasal screening [107]. CRA was then implemented in Scottish hospitals, where three risk based questions are asked and subsequent nasal and perineal swabs taken. MRSA screening of patients in Scotland generally applies to individuals admitted to four acute specialties considered high-risk (medicine for the elderly, dermatology, renal medicine, and vascular surgery) and most elective hospital admissions (excluding paediatrics, obstetrics and psychiatry). This measure has been in place since January 2010 [105] with patient isolation and/or nasal, throat and skin decolonisation of those found to be infected or colonised [108]. Figure 1-4 shows the percentage of admissions to the Aberdeen Royal Infirmary, NHS Grampian, that were screened for MRSA colonisation before and after universal screening was introduced, and the MRSA prevalence at admission (August 2008 to December 2010) [9]. In the same study, screening was associated with a 19% reduction in prevalence of MRSA bacteraemia [9].

1.1.4.2.2 Decolonisation

Nasal decolonisation of patients and healthcare workers with the topical antimicrobial mupirocin has been shown to dramatically decrease the risk of *S. aureus* spread and infection in healthcare settings [109]. In a double-blind, randomised, placebo-controlled clinical trial, intranasal mupirocin ointment eliminated nasal carriage in 58% of healthy workers within two days and 86.7% of individuals by the end of therapy (five days) compared with 9.4% of individuals at the end of treatment with a placebo ($p < 0.001$) [110]. However, while studies investigating the long-term efficacy of decolonisation with mupirocin have found that initial clearance over several weeks is effective; recolonisation, also known as nasal relapse, is common within three months [110, 111].

Other methods to decolonise patients have also been considered such as the long-term use of chlorhexidine baths [112] and pre-admission home-based MRSA decolonisation protocols [113].

1.1.4.2.3 Source isolation

Once carriers or infected individuals have been identified, an important step to reduce onwards transmission is to isolate cases [107]. Case isolation, in a single hospital room for example, can stop colonisation or infection of other hospital patients, staff or the environment. Individuals should then be decolonised to prevent or treat infection [107].

1.1.4.2.4 Antibiotic stewardship

Antibiotic stewardship, the move towards more efficient drug use, has also been high on the agenda as a public health activity that not only stands to positively affect SAB but other conditions and pathogens.

The UK Five Year Antimicrobial Resistance Strategy, 2013 to 2018, further shows commitment to tackling the issue of AMR in the UK. The strategy specifically focuses to; improve the knowledge and understanding of AMR; conserve and steward the effectiveness of existing treatments; and stimulate the development of new antibiotics, diagnostics and novel therapies. An example of how the UK is raising awareness of the issue of AMR has been the participation in the ‘European

Antibiotic Awareness Day' (EAAD), held on the 18th of November each year, since 2008 [114].

The Scottish Antimicrobial Prescribing Group (SAPG) was also established by the Scottish Government in 2008 to develop policy, monitor drug prescribing, provide support and implement change in prescribing behaviours where necessary [115]. Additionally, two strategies known as 'early switch' (ES) and 'early discharge' (ED) are considered important for reducing the number of HAI especially serious MRSA skin infections [116]. They involve patients' antimicrobial therapies being switched from intravenous to oral, and then they are discharged early to finish their treatments and recovery at home. These strategies are also considered cost-effective and to positively impact antimicrobial stewardship [116].

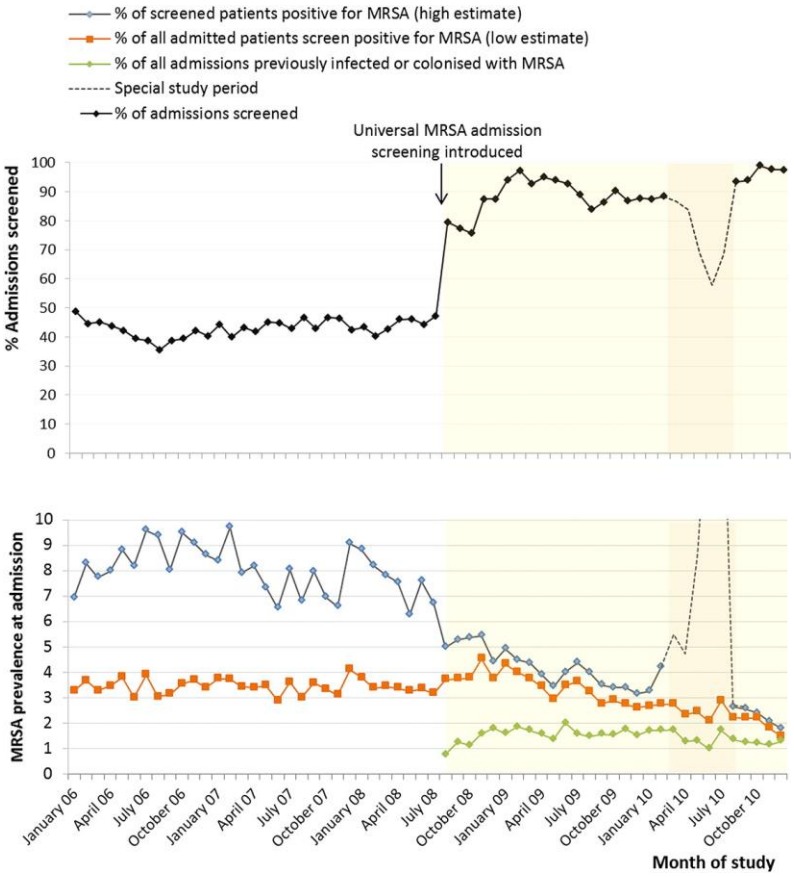
1.1.4.2.5 Hand hygiene, cleaning and audits

Hand hygiene is a vital strategy for reducing transmission of many pathogens including *S. aureus*. Hand hygiene compliance of healthcare workers is especially important in healthcare settings where there is a large at-risk population [117]. In a recent review, Marimuthu *et al* [118] described the effect of improved hand hygiene on nosocomial MRSA control in several studies. It was highlighted that there are a number of different activities that have been included in successful hand hygiene enhancement strategies, leading to increased rates in hand hygiene compliance and reduced rates of infection [118]. Such strategies included: alcohol-based handrubs or gels, staff education, reminders, audits, performance feedback and administrative involvement, culture change programmes, patient empowerment, leadership accountability and marketing/communication [118]. Importantly, hand hygiene campaigns have had previous success in Scotland with a campaign in NHS Grampian, where in combination with other infection control strategies; this resulted in reduced MRSA transmission [119].

Hospital cleaning is also crucial for the control of infectious diseases. Targeted protocols and legislation regarding cleaning and cleaning rotas have been implemented [120], as well as regular hospital audits and spot checks, enhanced hospital cleaning with 'deep cleans' and varied cleaning/decontamination schedules

[121], and novel cleaning technologies [122] have been important strategies. In addition, extra infection control staff can contribute to higher adherence to hand hygiene and more efficient hospital cleaning [108].

Figure 1-4: Adherence to MRSA admission testing and MRSA prevalence during universal surveillance



Study carried out in Aberdeen Royal Infirmary, August 2008 to December 2010
Special study period (February 2010 to August 2010) involved a trial of axillae and groin swabs.
Source [9]

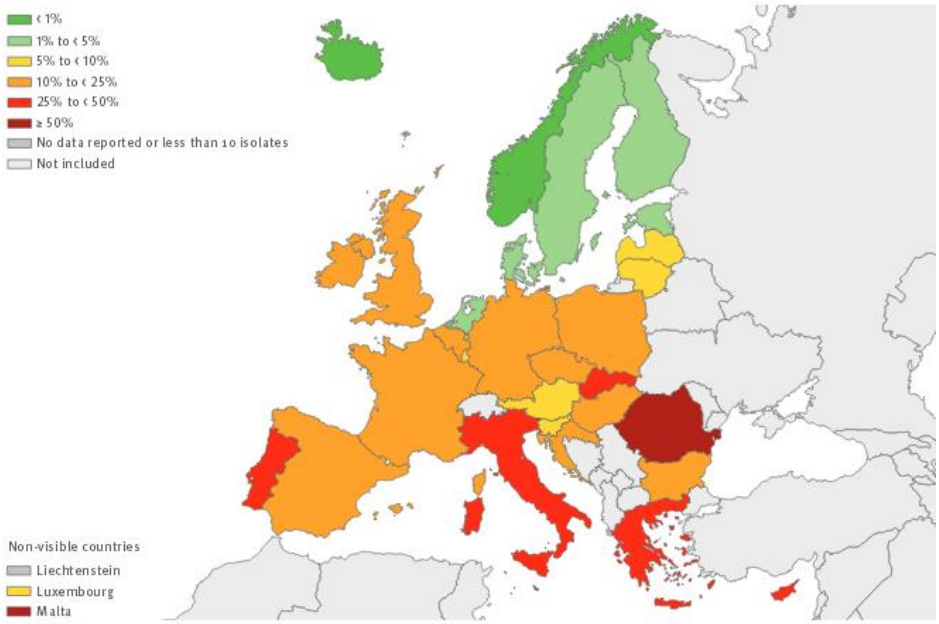
1.1.5 Epidemiological trends

Morbidity and mortality trends in Scotland are described in Chapter 2 of this thesis. SAB morbidity and mortality trends have been previously described for North East Scotland. Firstly, between 2006 and 2010, prevalence density of all SAB in Aberdeen Royal Infirmary, a teaching hospital in NHS Grampian, declined by 41%, from 0.73 to 0.50 cases per 1000 AOBDs (p=0.002), and 30-day all-cause mortality after SAB from 26% to 14% (p=0.013) [9]. Significant declines were observed in

MRSA bacteraemia only [9]. Secondly, in a study of the impact of SAB on mortality within 90 days post admission in an NHS Grampian hospital in 2005-06; MRSA infections were also found to inflate the risk of death compared to individuals with non-*S. aureus* conditions and compared to individuals with MSSA. The risk of death for those with MRSA was 5.6 times higher and with MSSA was 2.7 times higher compared with individuals without SAB [123]. After adjustment for co-morbidity, hospitalisation, age and sex; the risk of death was still 2.9-fold and 1.7-fold higher than non-*S. aureus* conditions for MRSA and MSSA respectively [123].

To put Scottish MRSA in an international context, Figure 1-5 shows the percentage of invasive *S. aureus* isolates that were MRSA (%MRSA) in 2013 by country, for all EU Member States (MS) and EEA counties (Switzerland, Iceland, and Norway). Data are collected and disseminated by the European Centre for Disease Prevention and Control (ECDC). The Figure shows that the United Kingdom (which is reported as one state rather than its four separate components; England, Northern Ireland, Scotland, and Wales) has MRSA levels (%MRSA) between 10-25%, a figure which is in line with several other European countries.

Figure 1-5: The percentage (%) of invasive *S. aureus* isolates that were methicillin-resistant (i.e. MRSA), by country, for EU/EEA countries, 2013.



Source: [124]

1.1.6 Antimicrobial prescribing

1.1.6.1 Daily Defined Doses (DDD)

The international standard which the WHO recommends for comparing antimicrobial usage is the metric DDD which is defined as the assumed average dose per day for a drug used for its main indication in adults [125]. This is calculated to make prescribing internationally comparable between drugs, which may have different routes of administration (e.g. topical, enteral or parenteral) or drug formulations (e.g. capsules, tablets, injection, suspension). This is therefore a proxy for the average number of patients exposed to the antibiotic in question each day.

1.1.6.2 Scottish prescribing

In Scotland, prescribing in primary care (i.e. dispensed from community pharmacies) and secondary care (i.e. in hospitals) is collected and monitored through two systems, the Prescribing Information System for Scotland database (PRISM) and the Hospital Medicines Utilisation Database (HMUD). These prescribing rates are reported as DDD per 1000 population and described further in Chapter 4.

The first Scotland-wide study to describe prescribing rates and the associations with MRSA AMR is presented in Chapter 5 of this thesis. Previous studies of Scottish prescribing have been carried out in NHS Grampian with: (1) a time-series analysis that captured the delay in the effect of prescribing of several antimicrobial classes in a single hospital and its association with the proportion of total *S. aureus* that was MRSA (%MRSA) after varying lag periods [126], and (2) a case-control study in two hospitals which found prescribing rates of macrolides and quinolones to be significantly and positively associated with an increase in %MRSA [127].

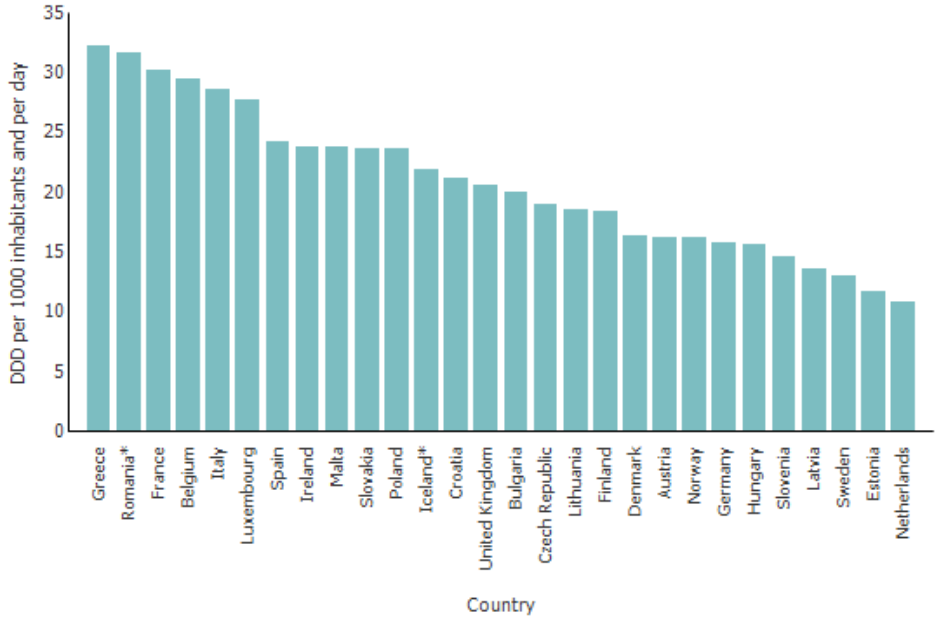
1.1.6.3 European prescribing

To put Scottish prescribing rates in an international context, Figure 1-6A (primary care) and Figure 1-6B (secondary care) show the total antimicrobial consumption (antimicrobials for systemic use) as the total DDD per 1000 population and per day for each MS plus EEA counties, Switzerland, Iceland, and Norway in 2013. National rates of primary and secondary care prescribing are reported by the ECDC for all MS

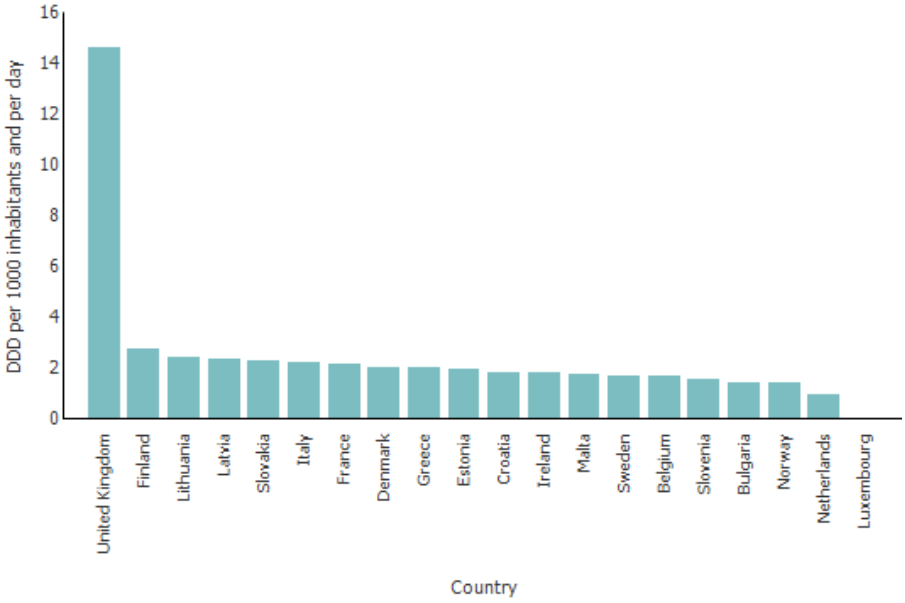
and EEA. The United Kingdom is reported rather than its four separate states, i.e. England, Northern Ireland, Scotland, and Wales. Greece had the highest and the Netherlands the lowest primary care consumption among MS, with the UK being somewhere in between. However, for secondary care consumption, the UK had by far the highest and Luxembourg the lowest. In general, consumption rates in primary care are higher than in secondary care.

Figure 1-6: Antibiotic consumption in Europe in (A) primary care and (B) secondary care for 2013.

(A)



(B)



Source: Antimicrobial consumption interactive database (ESAC-Net)

1.1.7 Molecular epidemiology

1.1.7.1 Clonal complexes

There are at least five distinct lineages of MRSA that have emerged due to independent acquisition of the SCC*mec* cassettes and the *mecA* gene [128] and then subsequent clonal expansion. MRSA is highly clonal and within each clonal group (called a clonal complex (CC)) there is a founder clone and then other strains which have evolved forming lineages through mutation from that founder clone. This differs from MSSA which is thought to be highly diverse [124] and it also differs from the naturally transformable pathogenic species *Streptococcus pneumoniae* and *Neisseria meningitidis* which evolve more frequently by recombination than though mutation [129].

There is also diversity within MRSA lineages due to the acquisition of different SCC*mec* types. The presence of toxin and virulence genes can be used to further classify strains (e.g. isolates can be *pvl* positive indicating that they carry the Panton-Valentine Leukocidin toxin gene (*pvl*)). In addition, the presence of the *pvl* toxin gene as well as genes for other toxins including toxic-shock syndrome toxin (*tst*), is often associated with increased pathogenicity. In contrast to HA-MRSA, but like 2% of all MSSA, CA-MRSA strains are frequently (but not universally) positive for *pvl* which is believed to cause more aggressive infections [38, 39, 130].

1.1.7.2 Major UK clones

While numerous clones of MRSA circulate in the UK, two major epidemic clones have dominated healthcare settings in recent decades [131, 132], namely EMRSA-15 (CC22, ST22 SCC*mec*IV) and EMRSA-16 (CC30, ST36 SCC*mec*II) which are reported to have collectively accounted for greater than 85% of all MRSA infections [10, 126, 131, 133, 134]. As summarised by Wyllie *et al* [132], recent UK studies have noted that these clones followed different epidemic curves with EMRSA-16 first dominating the UK MRSA scene, peaking and then declining to give way to the rise and dominance of EMRSA-15 [10, 126, 131, 133, 134]. A shift in dominance appears to have occurred after 2003 in a large, acute-care teaching hospital in London [34] and after 2006 in Oxfordshire hospitals [132].

From a Scottish perspective, EMRSA-15 and EMRSA-16 comprised 15.4% and 80.0% respectively of all MRSA isolates in Aberdeen Royal Infirmary between 1997 to 2000 [126] but by the later period 2003 – 2007, EMRSA-15 and EMRSA-16 comprised 71.4% and 15.1% in the same hospital. MRSA strain distribution and antimicrobial resistance patterns in NHS Grampian originating from patients attending GP surgeries and hospitals over a 5 year period (from 2003 – 2007) were also described [10]. The study described the proportions of each clone from different clinical specimens (e.g. blood, urine, swab) with EMRSA-15 the most common strain in each category. The authors also found a shift in the relative rates of epidemic strains with an increase in community-associated strains and, in addition, they reported no difference in antibiotic resistance between clinical and screening isolates. A rise in the number of community-associated epidemic strains including CC5-MRSA-IV and CC8-MRSA-IV was also reported in NHS Grampian [10]. EMRSA-15 has been consistently the most dominant clone from 1997 to 2009 [135]. Using Scotland-wide data, characteristics of EMRSA-15 and EMRSA-16 are compared and described in Chapter 4 of this thesis.

1.1.7.3 Main Typing methods

Various phenotypic and genotypic methods for typing, classifying and studying clones and strains of *S. aureus* and MRSA are available.

1.1.7.3.1 Phenotypic methods

Phenotypic antimicrobial testing is routinely conducted on isolates. An isolate's ability to survive in the presence of a wide range of antimicrobials can be tested manually using antimicrobial discs or using automated methods, e.g. by Vitek machine.

Phage typing was widely used for about 30 years, but has now been largely superseded by molecular techniques. It categorises different clones based on their susceptibility to lysis by various bacteriophages and therefore creates a 'lytic spectrum' profile for each isolate.

Biotyping involves three tests (urease production, hydrolysis of Tween 80, and isolate pigmentation) which are carried out to identify phenotypic properties [136].

Isolates can be classified as urease positive (urease becoming pink) or negative (urease becoming yellow); hydrolysis weak, positive, or negative based on the zone cleared in the inoculums; and pigmentation of isolates can be white, yellow or cream. Broadly speaking, EMRSA-15 (CC22) isolates are urease positive and cream/white in pigment and EMRSA-16 (CC30) are urease negative and yellow.

Phenotypic methods are considered less useful for highly sophisticated strain differentiation and classification since they are based on unstable characteristics, can show poor reproducibility [137], and often highlight phenotypic variations occurring in genetically identical bacterial strains [138].

1.1.7.3.2 Genotypic / molecular methods

Pulsed field gel electrophoresis (PFGE) separates *Sma*I-digested genomic DNA fragments by size which creates a banding pattern on an agarose gel that can be visualised and used to compare the relatedness of bacterial isolates. PFGE has a high discriminative power for typing, is suitable for looking at recent evolution of strains [23], is robust, highly reproducible, and is therefore considered to be the 'gold standard' technique for MRSA typing [139, 140]. However, major disadvantages include that it is a laborious and time-consuming method and requires expensive equipment and software [137, 140].

PCR ribotyping also separates digested genomic DNA but targeting the 16S-23S rRNA region of *S. aureus* which is amplified during PCR. This region is polymorphic. Each of the ribosomal RNA operons contains a spacer region of potentially different length, and amplification of these regions may yield a characteristic banding pattern. Comparison of the banding pattern can reveal the relatedness of strains [137, 138]. PCR ribotyping is a PCR-based genotyping technology that is simple, fast, reliable and reproducible, but the discriminatory power is not as high as with other methods (i.e. it can only broadly classify isolates as EMRSA-15 CC22, EMRSA-16 CC30, and 'others').

Spa typing is based on the analysis of variable-number tandem repeats (VNTR) in the polymorphic repeat region of the 3' coding end of the S*taphylococcal* protein A (i.e. *spa*) gene [141] and takes into account the number of point mutations in the

repeat region as well as the number of repeat variations [23]. The repeat region of the *spa* gene is subject to spontaneous mutations, as well as loss and gain of repeats. Since the repeats are assigned an alpha-numerical code, the *spa* type is deduced from the order of specific repeats [139]. *Spa* typing is considered an excellent tool for short-term local epidemiological analyses, as well as for national and international surveillance of *S. aureus*. *Spa* types are written as the letter t- followed by a number assigned to a specific *spa* pattern (e.g. t032). The s*pa* Ridom database (http://www.ridom.de/) holds information on all known and classified s*pa* types. EMRSA-15 CC22 is associated most often with *t032* but also with *t005, t022, t223, t309, t310, t417, t420.* Advantages to this typing method are that it has a high degree of typeability and reproducibility [141], and the use of a single-locus marker is less expensive, time-consuming, and error prone than multilocus techniques, such as multilocus sequence typing (MLST) [141]. PFGE is superior to *spa* typing and probably also MLST in terms of discriminatory power [139].

The MLST sequence-based typing method involves the analysis of ~450-500bp of internal fragments of seven 'housekeeping' genes and the identification of any single nucleotide variations between isolates [26]. Each *S. aureus* strain is assigned a unique sequence type (ST) number and if two isolates are found to have identical sequences at each of the seven loci, they are considered to be a clone and given the same ST number/name. In addition, if two STs differ by single nucleotide polymorphisms (SNPs) at fewer than three loci (i.e. the same at 5 or more loci), they are considered to be different alleles and assigned different STs but still recognised as being closely related and classified as being in the same clonal complex [23]. MLST is highly discriminatory and has made it possible to study the evolutionary history of *S. aureus* and MRSA since it measures variations that accumulate slowly over time. However, MLST is not suitable for routine surveillance of MRSA because of the high cost and the necessity of access to a high-throughput DNA sequencing facility [139]. The MLST database (http://www.mlst.net/) holds information on all sequence types.

1.1.7.3.3 Next generation sequencing (NGS)

Next generation sequencing (NGS) is a term that encompasses the high-throughput technologies for sequence analysis of DNA [142]. NGS involves sequencing DNA fragments and aligning them to a reference genome [143]. Sequencing can be completed in 1-2 days however the aligning stage, bioinformatic and phylogenetic analyses are complex, labour-intensive, and time consuming, and interpretation of results require a level of skill and expertise [143]. Currently, automated algorithms or pipelines are being developed that can identify and provide information on *spa* type, MLST type, AMR, heavy metal resistance, virulence genes, and more [143]. Further considerations surrounding NGS are issues of data storage and ethical considerations [142].

NGS is not currently routine clinical or diagnostic practice in diagnostic or reference laboratories in Scotland although it is well used in research to retrospectively investigate outbreaks and epidemiological surveillance of *S. aureus*, to understand the evolution of the bacteria, to look for associations between phenotype and genotype (i.e. for AMR), and to conduct genome-wide association studies. If they were to become routine, the usefulness of these techniques could be extended to epidemiological surveillance (local, national, global) and outbreak detection (at point of first secondary case) potentially creating a gold standard for typing isolates [144]. Unlike many phenotypic techniques which have a problem with reproducibility and interpretation; NGS provides a comparable, reproducible, portable, scalable, and standardised approach for *S. aureus* typing and detection of virulence and AMR [145]. It would also enable: culture-free sequencing, rapid species identification, antimicrobial susceptibility testing, and testing of virulence determinants and toxins [144].

With the costs of NGS rapidly decreasing and new generation bench top sequencers becoming more accessible [143], NGS techniques could replace traditional microbiological techniques in routine diagnostic and reference laboratories in the very near future.

1.1.8 Scottish health care system

1.1.8.1 Background

The National Health Service (NHS) in Scotland was created following the National Health Service (Scotland) Act 1947 and, since, has provided free public health services to all permanent residents. Health is a matter that is devolved to the Scottish government from the UK parliament. Prescriptions have been free of charge in Scotland since May 2010 although dentist and optician care still carry charges. Private health care is also available in Scotland. In the financial year ending in March 2013, there were: 4,881 general practitioners (GPs); 10,081 hospital doctors and dentists; 46,467 nurses and midwives; and a total of 1,312,946 inpatients in all acute and long stay hospitals in Scotland (Information Services Division).

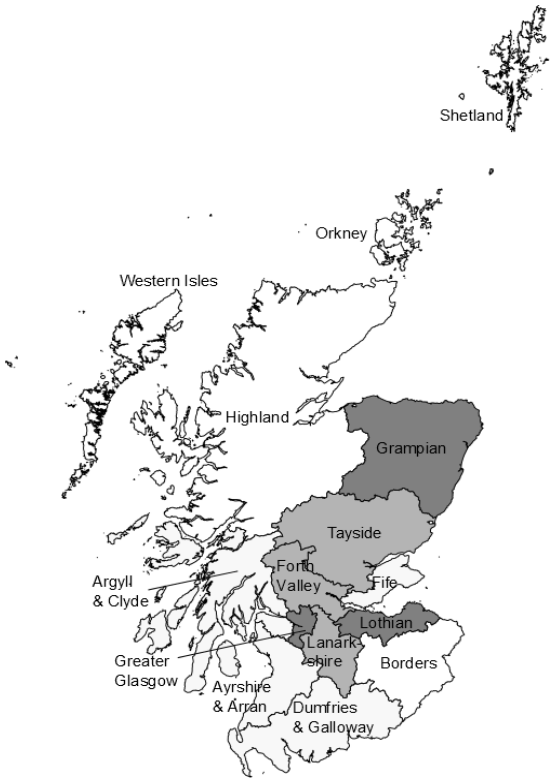
1.1.8.2 Health Boards

Since its reconfiguration in 2007, healthcare in Scotland has been provided by fourteen separate regional Health Boards (HBs). Figure 1-7 shows the (A) old (until 2007) and (B) new NHS Health Board boundaries. Changes involved: NHS Argyll and Clyde being broken up and part-absorbed into what was previously NHS Greater Glasgow and subsequently NHS Greater Glasgow and Clyde, and NHS Highlands. NHS HBs tend to differ in health care policies. The 2013 mid-year population estimates by NHS Health Board and age categories (0-14, 15-64 and 65 & over) are shown in Table 1-1 with NHS Greater Glasgow and Clyde having the largest total population (1,137,930), followed by NHS Lothian (849,700), NHS Lanarkshire (652,580) and NHS Grampian (579,220). The Scottish Public Health Observatory (ScotPHO) states that while percentages belonging to each age group does not vary between NHS HB, in some rural HBs, namely: NHS Borders, NHS Dumfries & Galloway, NHS Western Isles, NHS Highland and NHS Orkney; the percentages of 'working age population' (15-64) is lower, while elderly populations are higher.

In 2013, there were teaching hospitals in four NHS HB only: NHS Grampian (Aberdeen Royal Infirmary), NHS Greater Glasgow and Clyde (Glasgow Royal Infirmary, Southern General, West hospitals), NHS Lothian (Edinburgh Royal Infirmary and Western General) and NHS Tayside (Ninewells).

Figure 1-7: Map of Scottish NHS Health Board boundaries (A) pre and (B) post 2007

(A)



(B)

Table 1-1: 2013 mid-year population estimates by NHS board and age categories (0-14, 15-64 and 65 & over).

NHS Board	0-14	15-64	65 & over	All Ages
Ayrshire & Arran	58,649	237,127	76,434	372,210
Borders	17,754	70,414	25,702	113,870
Dumfries & Galloway	22,470	92,770	35,030	150,270
Fife	60,118	237,776	69,016	366,910
Forth Valley	49,591	197,294	52,795	299,680
Grampian	91,711	390,935	96,574	579,220
Greater Glasgow & Clyde	179,379	774,073	184,478	1,137,930
Highland	50,604	203,235	67,161	321,000
Lanarkshire	111,293	431,385	109,902	652,580
Lothian	135,549	581,448	132,703	849,700
Orkney	3,291	13,719	4,560	21,570
Shetland	4,066	15,031	4,103	23,200
Tayside	63,355	266,667	82,138	412,160
Western Isles	4,175	16,959	6,266	27,400
SCOTLAND Total	852,005	3,528,833	946,862	5,327,700

Source: ScotPHO [146]

1.2 Thesis aims

1.2.1 General aims and motivation

This thesis describes the epidemiology of MSSA and MRSA in Scotland by analysing several large public health databases. The motivation was to explore previously undescribed public health databases and contribute to the body of science concerning the epidemiology of this important infectious disease.

1.2.2 Chapter 2 – MSSA and MRSA morbidity and mortality trends

This chapter examines background spatial and temporal trends in SAB morbidity and temporal trends in *S. aureus* mortality (due to all *S. aureus* disease outcomes) in Scotland. Specific aims were (1) to examine spatial and temporal morbidity trends, (2) to assess whether government targets to reduce all SAB to 70% of the 2005/2006 levels by 2010 were met and maintained, and (3) to compare mortality rates and case fatality ratios where MRSA and MSSA were the primary and secondary causes of death.

1.2.3 Chapter 3 – MRSA declines while MSSA remains a public health concern

This chapter examines microbiological and epidemiological data corresponding to isolates causing SAB from Scotland over an 11 year period. The primary aim was to explore the main microbiological and epidemiological differences between MSSA and MRSA populations in order to explain why MSSA bacteraemia rates have not seen the decline of MRSA rates and why MSSA remains an important public health issue.

1.2.4 Chapter 4 - Antimicrobial resistance of MRSA

The first aim of this chapter was to confirm that EMRSA-15 and EMRSA-16 were the two dominant clones in Scotland and if there was any evidence of a shift in dominance as reported elsewhere in the UK. The second aim was to analyse and explain antimicrobial resistance patterns for the dominant clones in Scotland in terms of resistance to twenty individual antimicrobials, antibiogram length and antibiogram diversity for clinical isolates and non-clinical ‘screening’ isolates. This chapter utilised 8 years of data from the Scottish MRSA reference laboratory.

1.2.5 Chapter 5 - Antimicrobial prescribing and its association with MRSA epidemiology

The work included in this chapter is intended as a pilot study to inform a larger analysis looking at trends and associations between prescribing and resistance for a greater number of antimicrobials and several pathogens. Therefore, the overall purpose is to test methods, and describe pitfalls in using such data and conducting such a study. Specific aims of this study are two-fold. Firstly, to examine spatial and temporal trends in Scottish primary and secondary care prescribing rates. Secondly, to investigate whether or not there are any associations between primary or secondary care prescribing rates and antibiotic resistance in the MRSA population.

1.2.6 Chapter 6 - A hospital-level risk factor analysis of MRSA

The aim of this study is to explore the hypotheses that hospital size, hospital connectivity and hospital category affect the hospital-level incidences of MSSA and MRSA bacteraemia morbidity rates in mainland Scottish hospitals. The objective is twofold; (1) to identify risk factors in hospitals with cases versus those without, and (2) to investigate risk factors associated with increasing number of cases in general hospitals (classification A1, A2, A3 and A4) only. This study has been submitted as a manuscript to the journal BMC Infectious Diseases.

1.2.7 Chapter 7 – Conclusions

This chapter pools overall conclusions from each of the chapters and addresses the aims of each chapter. It also describes limitations and suggests a different approach that could have been taken where appropriate. Any implications or recommendations resulting from this thesis will be discussed.

Chapter 2: **Examining morbidity and mortality trends of MSSA and MRSA in Scotland**

2.1 Background

Staphylococcus aureus (*S. aureus*) is a bacterium that commonly colonises the skin and nares of around one third of otherwise healthy individuals. While colonisation is benign, *S. aureus* can cross skin and mucosal barriers to cause an infection that manifests as clinical disease [2]. Clinical outcomes range from mild, non-complicated and often self-limiting infections to more severe and life-threatening conditions (for more detail information on clinical outcomes, see Chapter 1.1.1.1). Bacteraemia (bacteria present in the bloodstream) is a clinical outcome of *S. aureus* can lead to systemic metastatic infections and infective endocarditis (IE), and it can trigger sepsis, septic shock and multiple organ failure. There has been a public health focus on reducing these more serious outcomes due to high case-fatality ratios [3-7] with specific actions to reduce both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) bacteraemia, collectively known as *S. aureus* bacteraemia (SAB), listed on the Scottish Government and NHS Scotland 'HEAT' targets. Specifically, one target was to reduce all SAB to 70% of the 2005-2006 levels by 2010 [103]. For more detailed information on HEAT targets and disease surveillance, see Chapter 1.1.4. In addition, the UK Five Year Antimicrobial Resistance Strategy, 2013 to 2018, outlines an action plan to undertake to tackle the issue of AMR in the UK [114].

Several public health policies and initiatives aimed at reducing the burden of MSSA and MRSA were developed and implemented in Scotland. These have been instrumental in reducing incidence rates (for more detailed information on Surveillance and Control Measures, see Chapter 1.1.4.2).

Scottish SAB morbidity and mortality trends have been previously described in Aberdeen Royal Infirmary, a teaching hospital in NHS Grampian (for more detailed information on Surveillance and Control Measures, see Chapter 1.1.5).

This data chapter examines Scotland-wide spatial and temporal trends in SAB morbidity, and temporal trends in *S. aureus* mortality (due to all *S. aureus* disease outcomes). Specific aims were to: (1) examine spatial and temporal morbidity trends, (2) assess whether government targets to reduce all SAB to 70% of the 2005-2006 levels by 2010 were met and maintained, and (3) compare mortality rates and case fatality ratios where MRSA and MSSA were the primary and secondary causes of death.

2.2 Materials and methodology

2.2.1 Data

2.2.1.1 Bacteraemia morbidity data

Quarterly MSSA and MRSA bacteraemia case data per NHS Health Board (HB) were obtained from the Health Protection Scotland (HPS) website [153]. The data were dated from April (i.e. quarter 2) 2005 to December (i.e. end of quarter 4) 2013 inclusive for MSSA, and January (i.e. quarter 1) 2003 to December (i.e. end of quarter 4) 2013 for MRSA. Total *S. aureus* (SA), MSSA and MRSA bacteraemia rates per 100,000 AOBDs were calculated using estimates of AOBD from HPS, as per the formula:

$$morbidity\ rate\ =\ (number\ of\ cases\ \ per\ year\ /\ AOBDs\ per\ year) * 100{,}000.$$

Clinical cases of SAB were confirmed where *S. aureus* was isolated from a blood culture and positive isolates from a single patient submitted within two weeks of each other were defined as a single episode [102]. AOBD (acute occupied bed days) is a standardised measure of the number of hospitalised patients and defined as an acute hospital bed occupied for one night [102]. Island HBs (i.e. NHS Orkney, NHS Shetland, and NHS Western Isles) were excluded from all analyses owing to highly variable rates of bacteraemia due to low and fluctuating population sizes. NHS National Waiting Times Centre, also known as the Golden Jubilee National hospital and given its own health board status, was also excluded from all analyses since it does not represent a geographic region like other HBs. The proportion of total SAB cases represented by MRSA was also calculated per quarter and per HB from April 2005 onwards and herein is referred to as '%MRSA'.

2.2.1.2 Mortality data

The number of annual deaths with MSSA (2008-2013) and MRSA (1996-2013) reported as the primary (underlying) and secondary (contributory) cause of death was obtained from the General Register Office for Scotland (GROS). These figures include deaths to bacteraemia and sepsis which were thought to cause over 90% of *S. aureus* deaths (personal communication with Dr Giles Edwards, former infectious diseases consultant in NHS Greater Glasgow and Clyde, and former director of the SMRSARL). No data for MSSA deaths were available pre-2008. Data were not stratified by HB.

2.2.1.2.1 Mortality rates (MR)

MR were calculated and defined as the number of (1) MSSA (2008-2013) and (2) MRSA (2003-2013) deaths per 100,000 AOBDs (stratified by underlying and contributing cause, and combined as total cause of death), as per the formula: $MR = (number\ of\ deaths\ \ per\ year\ /\ AOBDs\ per\ year) * 100{,}000$. Since AOBD data was only available from 2003 onwards, MR prior to this year could not be investigated.

2.2.1.2.2 Breakpoint analysis

Owing to the trend of MRSA mortality data - which increases, levels-off and then declines - a breakpoint analysis was carried out to identify breaks in trends (i.e. where the trend changed direction or rate) and time periods reflecting these breakpoints were established. Two breakpoints were identified; (1) 1999.5 (95% CI 1998.9-2000.2), and (2) 2008.7 (95% CI 2008.1-2009.4). Owing to these breakpoints and since MRSA MR data were only available from 2003 onwards; trends in two time periods were investigated: (T1) 2003-2007 inclusive and (T2) and 2008-2012 inclusive.

2.2.1.2.3 Case fatality ratios (CFR)

CFR were also created and defined as the number of deaths per 100 cases where (1) MSSA (2008-2013) and (2) MRSA ((T1) 2003-2007, and (T2) 2008-2013) were the primary, secondary and combined (both primary and secondary) cause of death, as per the formula:

$CFR = (number\ of\ deaths\ \ per\ year\ /numberof\ \ cases\ per\ year) * 100$

2.2.2 Descriptive and statistical analysis

All statistical analyses were carried out in R (version 3.1.2) [154].

2.2.2.1 Trends in morbidity data

Levels of statistical significance was set at $p<0.05$. Data were stored in Microsoft Excel (2010) spreadsheets. Prior to statistical analysis, skewedness of data (SA, MSSA and MRSA morbidity rates, %MRSA) was assessed and it was concluded that no variable needed to be transformed.

2.2.2.1.1 Temporal trends of morbidity rates

Quarterly trends were investigated for the period 2005 (quarter 2) to 2013 (end of quarter 4) for MSSA, SA and %MRSA, and 2003 (quarter 1) to 2013 (end of quarter 4) for MRSA. Independent variables investigated included: SA bacteraemia morbidity rate (total number of MSSA and MRSA bacteraemia cases per 100,000 AOBD), MSSA bacteraemia morbidity rate (total number of MSSA bacteraemia cases per 100,000 AOBD), and MRSA bacteraemia morbidity rate (total number of MRSA bacteraemia cases per 100,000 AOBD). These were examined using regression analysis (exploring linear, quadratic or cubic where appropriate). Trends in %MRSA over time (2005-2013) were also investigated by regression analyses.

2.2.2.1.2 Spatial trends of morbidity rates

Spatial differences between HBs were investigated. SA, MSSA, MRSA morbidity bacteraemia rates and %MRSA were examined using a one way ANOVA with *post-hoc* Tukey comparisons. %MRSA was additionally tested using a *post-hoc* Bonferroni test for further confirmation. Results show between which set of HB pairs that differences in the mean rate of cases or %MRSA exist (shown in figures). The programme also groups HBs together based on the mean rates or %MRSA, and HBs with statistically different rates or %MRSA will be placed in different groups. Groups are assigned letters to easily identify different groups. Thus, HBs in the same group do not have statistically different means or %MRSA, and hence will be assigned the same letter.

2.2.2.1.3 Trends over space and time

Any interaction between year and HB was initially investigated using the model ($model < - lm(x \sim HB + Year + HB * Year)$) with *x* representing the independent variable which was one of SA, MSSA, or MRSA morbidity bacteraemia rates or %MRSA. However, the standard errors (SE) of the model outputs were very high, suggesting that some of the categories were empty (i.e. there were zero cases for some HB for some quarters). Therefore, the results are not discussed. Instead, temporal trends in each individual HB are shown for each HB.

2.2.2.1.4 Government targets assessment

To assess whether government targets to reduce all SAB to 70% of the 2005/2006 levels by 2010 were met and maintained, the proportion reduction of morbidity cases using 2005 data was calculated and compared.

2.2.2.2 Temporal trends in mortality

2.2.2.2.1 MR versus CFR

MSSA and MRSA MR and CFR were investigated using regression analyses (exploring linear, quadratic or cubic where appropriate) and compared. Prior to statistical analysis, skewedness of data was assessed and it was concluded that none needed to be transformed.

2.3 Results

2.3.1 Trends

2.3.1.1 Temporal trends of morbidity data

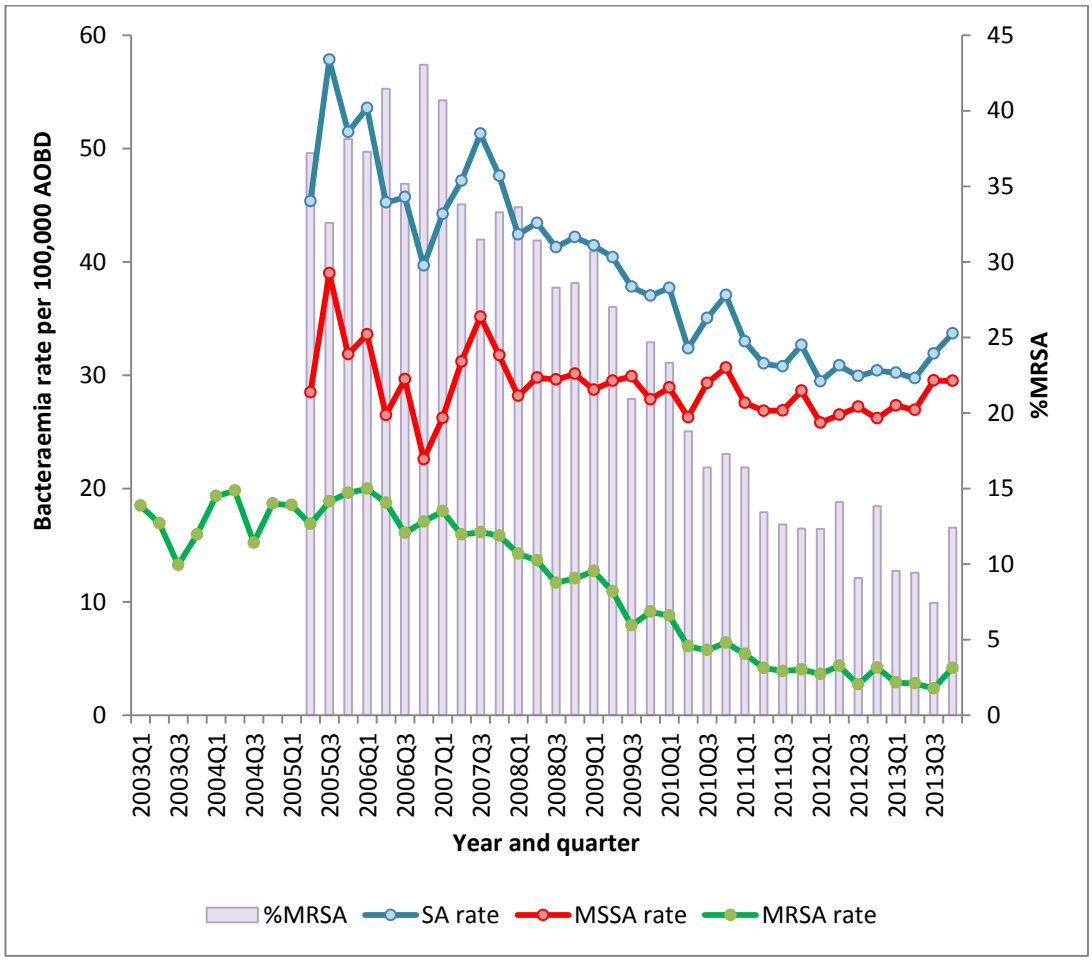
The MSSA bacteraemia rates (per 100,000 AOBD) did not significantly change over time (Table 2-1, $p>0.05$), however MRSA bacteraemia rate did significantly change over time (Table 2-1). The linear model was best fitting ($p<0.0001$, $R^2=0.35$). The total SAB rate significantly declined over time with the linear model being the best fit ($p<0.0001$, $R^2=0.18$). Temporal trends are illustrated in Figure 2-1. In addition, the %MRSA also significantly declined over time (Table 2-1) with the linear model best describing these data ($p<0.0001$, $R^2=0.47$).

Table 2-1: Results from regression analysis: temporal trends of SA, MRSA and MSSA rates, and %MRSA.

Data	Model	Estimate	SE	p-value	Sig.	R^2
SA rate 2005-2013	Year - linear	-2.2	0.23	<0.0001	***	0.18
MSSA rate 2005-2013	Year- linear	-0.16	0.18	0.36		-0.00
MRSA rate 2003-2013	Year- linear	-1.5	0.09	<0.0001	***	0.35
% MRSA 2005-2013	Year- linear	-0.04	0.002	<0.0001	***	0.47

Sig: Significance level = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
SE = standard error
Adj. R^2 = adjusted R^2.

Figure 2-1: Temporal trends in Scottish bacteraemia rates per 100,000 AOBD for SA, MSSA, and MRSA (primary y-axis), and %MRSA (secondary y-axis).



2.3.1.2 Spatial trends of morbidity rates

ANOVAs showed that there were significant differences between HBs in terms of mean SA ($p<0.0001$), MSSA ($p<0.0001$) and MRSA ($p<0.0001$) bacteraemia rates, and %MRSA ($p=0.01$). *Post-hoc* Tukey analyses identified differences between several pairs of HBs (Table 2-2, Figure 2-2). There were 9 different groupings for SA, 5 for MSSA, 7 for MRSA, and 1 for %MRSA.

For %MRSA, while the ANOVA was significant ($p=0.01$) suggesting that at least one group mean was different, the Tukey multiple comparison test failed to identify any significant group differences and thus placed each HB in the same group. This was confirmed by carrying out a Bonferroni test which, although it did identify a few differences between some pairs of HBs, did not find those differences to be significant. This was further scrutinised by transforming the data (natural logarithm and arcsin square root transformation) and repeating both the Tukey and Bonferroni test, however the same result was met.

In General, NHS Fife and NHS Forth Valley had high rates of SA, MSSA and MRSA, while NHS Highland was usually low. NHS Grampian had relatively high rates of SA and MSSA but lower than expected rates of MRSA. For SA bacteraemia rates, NHS Fife had the highest mean with 51.6 cases per 100,000 AOBD and NHS Highland the lowest mean with 25 cases per 100,000 AOBD. For MSSA bacteraemia rates, NHS Fife had the highest mean with 39.2 cases per 100,000 AOBD and NHS Highland the lowest mean with 19.3 cases per 100,000 AOBD. For MRSA bacteraemia, the highest bacteraemia rates were found in NHS Lothian (13.7 cases per 100,000 AOBD) closely followed by NHS Forth Valley (12.6 cases per 100,000 AOBD) and NHS Fife (12.5 cases per 100,000 AOBD), and the lowest in NHS Highland (5.6 cases per 100,000 AOBD). The %MRSA was highest in NHS Borders and NHS Lothian (both 28%) and the lowest in NHS Ayrshire and Arran (18%) (Table 2-2).

Table 2-2: Results of ANOVA and post-hoc Tukey analysis showing the mean, standard deviation (SD), minimum (Min.) and maximum (Max.) for SA rates, MSSA rates, MRSA rates and %MRSA with HB groupings.

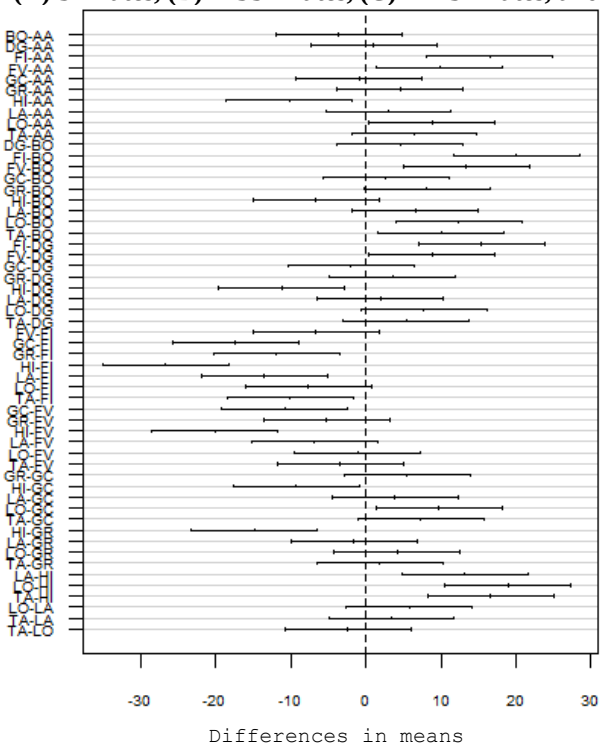
HB	Mean	SD	Min.	Max.	Grouping§
SA rate - ANOVA p<0.0001					
AA	35.2	9.6	18.5	60.5	de
BO	31.6	12.7	7.4	53.9	ef
DG	36.2	13.1	13.4	62	cde
FI	51.6	13.3	27.6	80.1	a
FV	45	11.6	25.7	72.1	ab
GC	34.3	6.1	24.6	49.3	de
GR	39.8	10.5	26.8	69.4	bcde
HI	25	6.9	12.8	45	f
LA	38.2	10.6	19.4	66.3	bcde
LO	44	11.6	26.3	68.3	abc
TA	41.6	10.5	25.9	71.6	bcd
MSSA rate - ANOVA p<0.0001					
AA	28.5	6.9	15	48.6	bc
BO	22.9	11.1	3.7	52.6	cd
DG	28.5	9	13.4	48.9	bc
FI	39.2	10.6	19.6	63.6	a
FV	32.4	8.1	17.8	53.1	b
GC	29.5	5.9	22.2	50.8	b
GR	26.8	4.5	19.8	39.6	bc
HI	19.3	5.7	9	29.2	d
LA	27.9	6.3	8.8	44.8	bc
LO	30.3	5	21.9	41.3	b
TA	29.6	6.2	19.2	52.7	b
MRSA rate - ANOVA p<0.0001					
AA	6.6	4.7	0	16.7	cd
BO	8.7	7.2	0	32.2	abcd
DG	7.7	6.8	0	22.3	bcd
FI	12.5	8.6	1.4	31.4	ab
FV	12.6	7.4	1.9	28.5	ab
GC	10.3	6.2	2	21.3	abcd
GR	7.4	5	0.7	18.2	bcd
HI	5.6	4.5	0	21.8	d
LA	10.2	7.9	1.6	28.3	abcd
LO	13.7	9.4	2	32.9	a
TA	12	8.6	2	29.7	abc
%MRSA - ANOVA p=0.01					
AA	0.18	0.1	0	0.38	a
BO	0.28	0.19	0	0.67	a
DG	0.19	0.13	0	0.41	a
FI	0.23	0.14	0.04	0.5	a
FV	0.27	0.13	0.06	0.55	a
GC	0.24	0.11	0.05	0.43	a
GR	0.21	0.12	0.02	0.43	a
HI	0.22	0.15	0	0.52	a
LA	0.25	0.15	0.05	0.69	a
LO	0.28	0.14	0.07	0.55	a
TA	0.27	0.15	0.08	0.57	a

Health Board (HB) abbreviations: AA = NHS Ayrshire and Arran, BO = NHS Borders, DG = NHS Dumfries and Galloway, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, HI = NHS Highland, LA = NHS Lanarkshire, LO = NHS Lothian, TA = NHS Tayside **.**

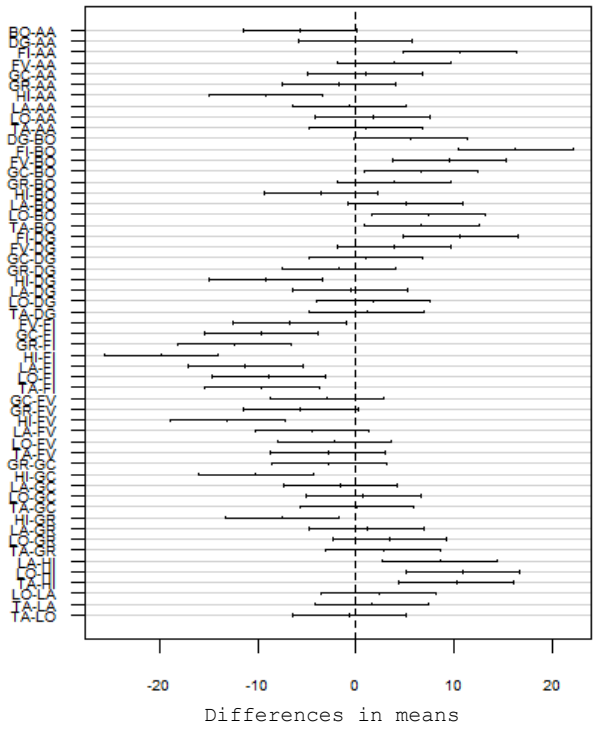
§Groupings: HBs with statistically different rates or %MRSA will be placed in different groups. Groups are assigned letters to easily identify different groups. Thus, HBs in the same group do not have statistically different means or %MRSA, and hence will be assigned the same letter.

Figure 2-2: Results of post-hoc Tukey analysis showing differences in prescribing rates between NHS Health Boards for (A) SA rates, (B) MSSA rates, (C) MRSA rates, and (D) %MRSA.

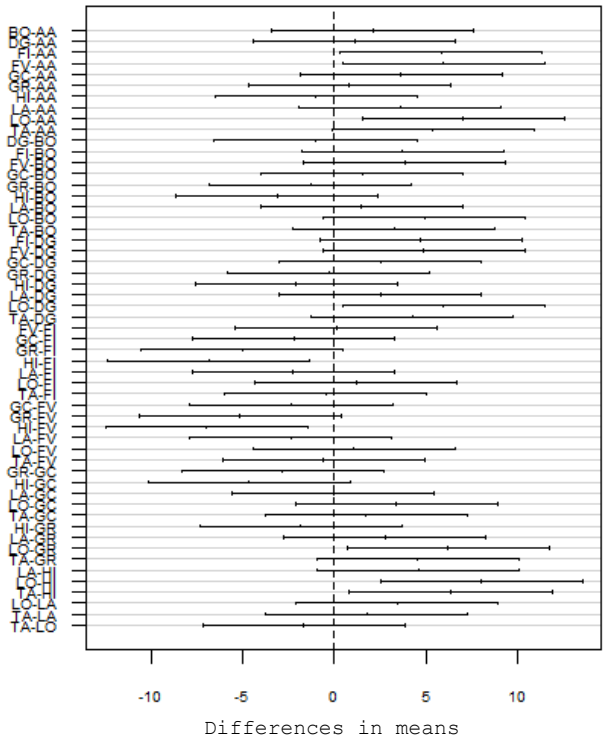
(A) SA rates



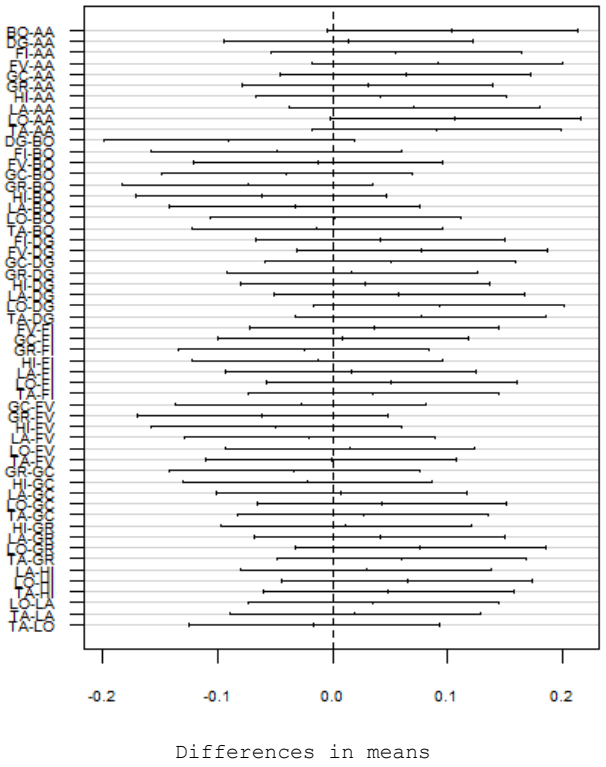
(B) MSSA rates



(C) MRSA rates



(D) %MRSA



Health Board abbreviations: AA = NHS Ayrshire and Arran, BO = NHS Borders, DG = NHS Dumfries and Galloway, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, HI = NHS Highland, LA = NHS Lanarkshire, LO = NHS Lothian, TA = NHS Tayside

2.3.1.3 Trends between NHS Health Boards (HB) over time

All NHS HBs saw a significant decrease in rates of SA (except for NHS Borders which significantly increased, p=0.01), MRSA (except for NHS Borders which did not change), and %MRSA over time (Table 2-3). Rates of MSSA significantly increased over time in NHS Borders (p=0.008), and declined in NHS Dumfries and Galloway (p=0.0007), NHS Greater Glasgow and Clyde (p=0.001), and NHS Lothian (p=0.02). There was no change in MSSA rates in any other HB. Temporal trends for each HB are shown in Figure 2-3.

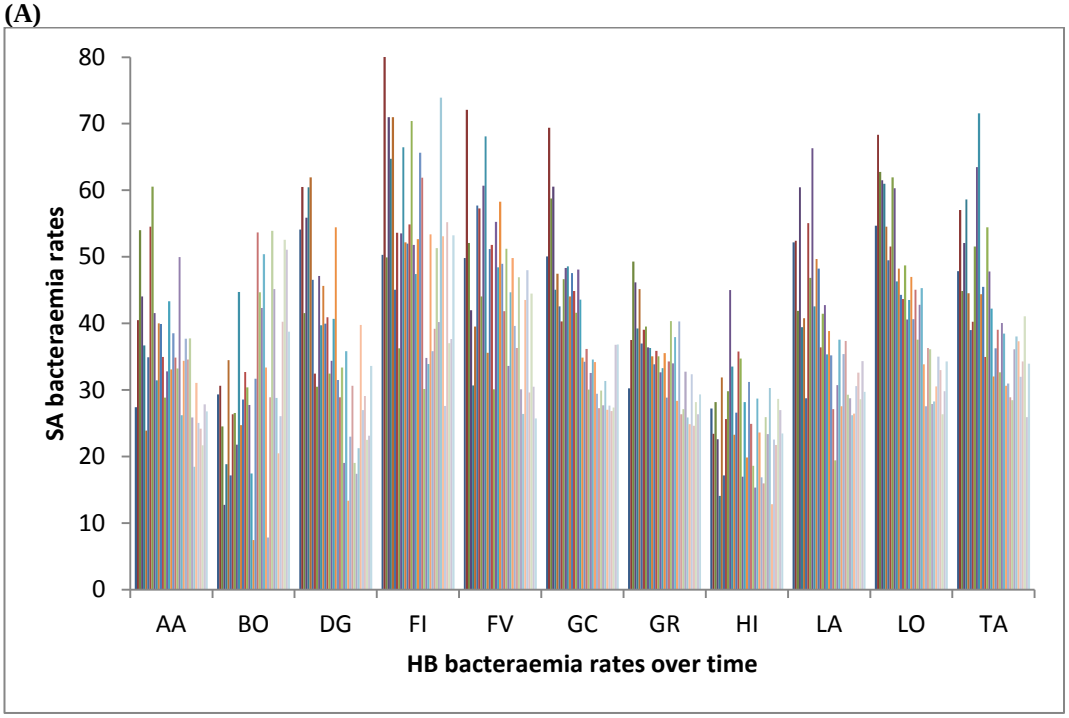
Table 2-3: SA, MSSA and MRSA bacteraemia rates, and %MRSA by HB over time.

	HB	Estimate	SE	p-value	Sig.	R^2
SA	AA	-1.93	0.56	0.002	**	0.24
	BO	2.07	0.78	0.01	*	0.15
	DG	-3.87	0.58	<0.0001	***	0.56
	FI	-2.21	0.82	0.01	*	0.16
	FV	-2.38	0.67	0.001	**	0.26
	GR	-1.69	0.29	<0.0001	***	0.49
	GC	-3.48	0.38	<0.0001	***	0.71
	LA	-2.83	0.53	<0.0001	***	0.45
	LO	-4.14	0.31	<0.0001	***	0.84
	TA	-2.78	0.52	<0.0001	***	0.45
MSSA	AA	-0.83	0.45	0.07	.	0.07
	BO	2.35	0.63	0.0008	***	0.27
	DG	-1.91	0.51	0.0007	***	0.28
	FI	0.47	0.71	0.52		-0.02
	FV	-0.1	0.55	0.85		-0.03
	GR	-0.03	0.31	0.91		-0.03
	GC	-1.2	0.34	0.001	**	0.25
	LA	-0.3	0.43	0.49		-0.02
	LO	-0.74	0.31	0.02	*	0.12
	TA	0.12	0.42	0.78		-0.03
MRSA	AA	-1.1	0.26	0.0001	***	0.34
	BO	-0.29	0.49	0.56		-0.02
	DG	-1.95	0.31	<0.0001	***	0.53
	FI	-2.68	0.36	<0.0001	***	0.62
	FV	-2.28	0.31	<0.0001	***	0.61
	GR	-1.66	0.18	<0.0001	***	0.7
	GC	-2.27	0.14	<0.0001	***	0.89
	LA	-2.54	0.3	<0.0001	***	0.68
	LO	-3.4	0.24	<0.0001	***	0.86
	TA	-2.9	0.3	<0.0001	***	0.74
%MRSA	AA	-2.22	0.6	0.001	***	0.27
	BO	-3.04	1.17	0.01	*	0.15
	DG	-2.97	0.71	0.0002	***	0.33
	FI	-4.23	0.58	<0.0001	***	0.6
	FV	-3.93	0.54	<0.0001	***	0.6
	GR	-3.93	0.5	<0.0001	***	0.64
	GC	-3.76	0.35	<0.0001	***	0.77
	LA	-4.79	0.63	<0.0001	***	0.63
	LO	-4.95	0.44	<0.0001	***	0.79
	TA	-5.07	0.53	<0.0001	***	0.73

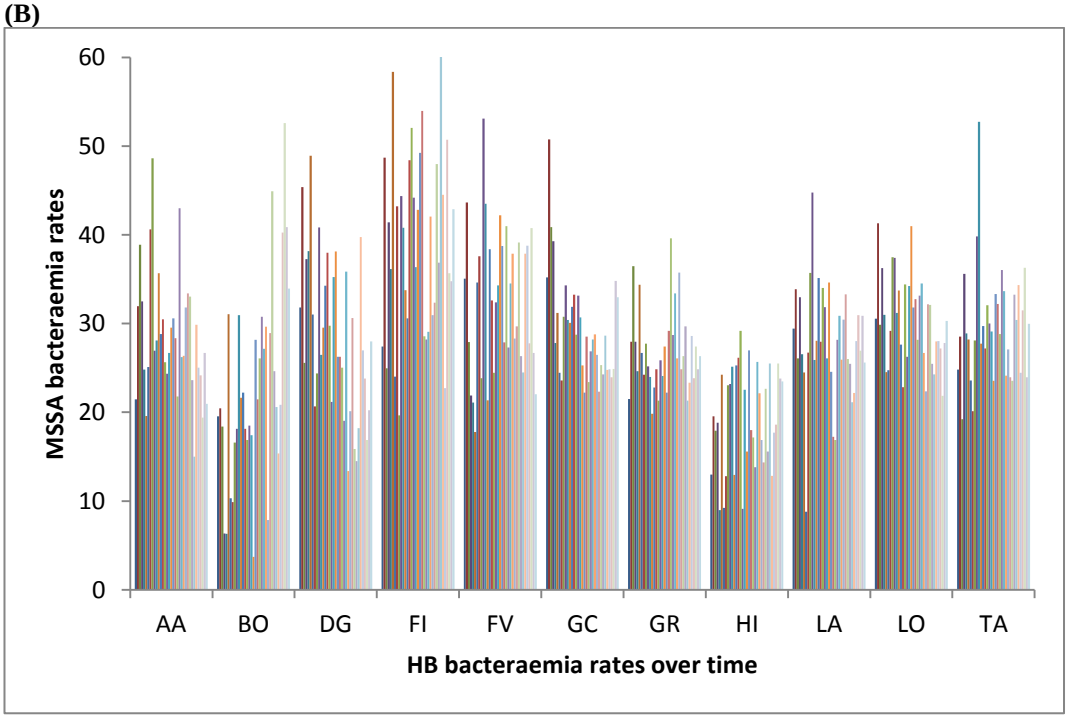
Sig: Significance level = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
SE = standard error, Adj. R = adjusted R^2. Health Board (HB) abbreviations: AA = NHS Ayrshire and Arran, BO = NHS Borders, DG = NHS Dumfries and Galloway, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, HI = NHS Highland, LA = NHS Lanarkshire, LO = NHS Lothian, TA = NHS Tayside

Figure 2-3: Trends between HB over time (year and quarter) for 11 NHS HB in Scotland for (A) SA, (B) MSSA and (C) MRSA bacteraemia, and (D) %MRSA.

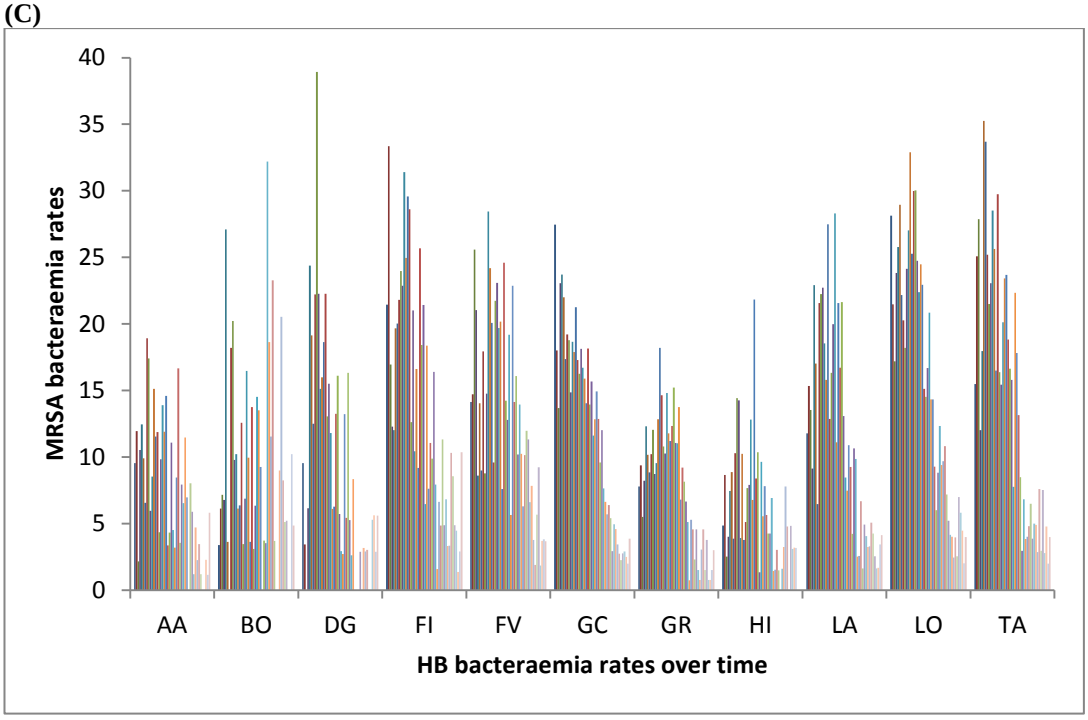
(A)



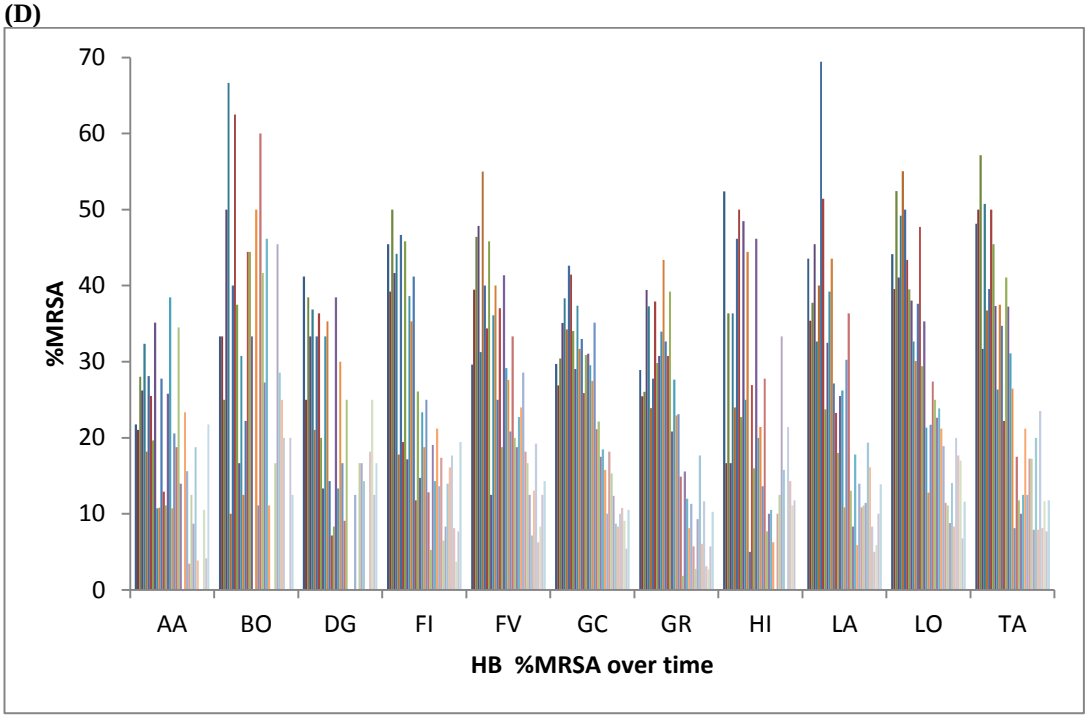
(B)



(C)



(D)



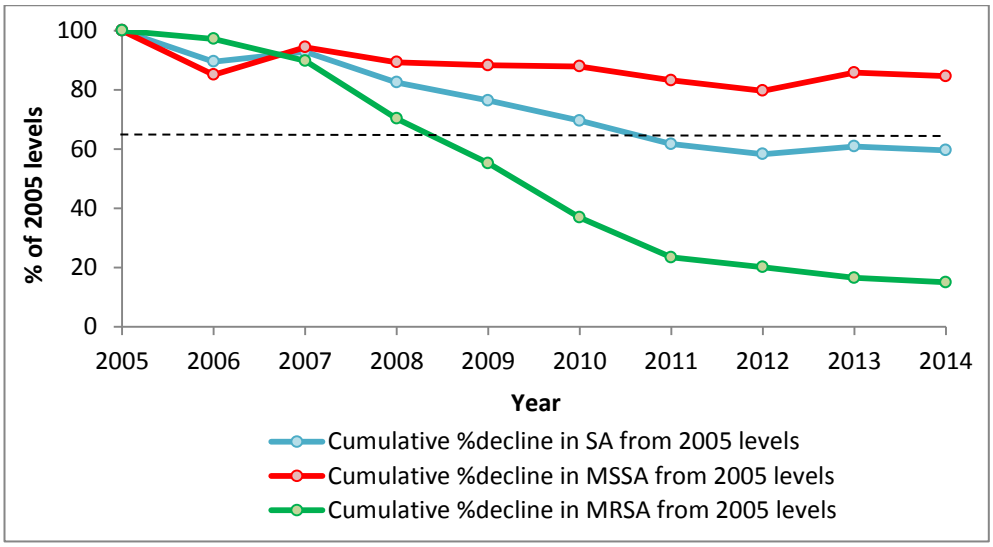
Health Board (HB) abbreviations: AA = NHS Ayrshire and Arran, BO = NHS Borders, DG = NHS Dumfries and Galloway, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, HI = NHS Highland, LA = NHS Lanarkshire, LO = NHS Lothian, TA = NHS Tayside

2.3.1.4 Government target assessment

Government targets aimed to reduce all SAB to 70% of the 2005-2006 levels by 2010. In 2005, there were 2040 SAB cases in Scotland and the SAB rate was 51.1 cases per 100,000 AOBD. In 2010, there were 1835 SAB cases in Scotland, and the SAB rate was 35.5 cases per 100,000 AOBD meaning a 30.5% reduction from 2005 case rate (Figure 2-4). By 2014, SAB rates were 40.4% lower than the 2005 levels. This therefore means that government targets were met by the deadline and maintained in the years that followed (Figure 2-4).

While the overall HEAT target was attained, this was due to disproportionately reductions in MRSA. In 2005, there were 1308 MSSA and 732 MRSA bacteraemia cases in Scotland, and the bacteraemia rate per 100,000 AOBD for MSSA was 32.7 and MRSA was 18.3. In 2010, there were 1486 MSSA and 349 MRSA bacteraemia cases in Scotland, and the rate per 100,000 AOBD for MSSA was 28.8 and MRSA was 6.8. This represents a 12.1% reduction in MSSA rate and a 63.1% reduction in MRSA rate. By 2014, there had been further declines to 15.4% (MSSA) and 85.0% (MRSA) of 2005 levels. This means that if the government targets focused on MSSA and MRSA separately, the targets for MSSA would not have been met by the target year or the years that followed, but that the target for MRSA would have been far exceeded (Figure 2-4).

Figure 2-4: Cumulative reduction in SAB cases from 2005 levels.
Dashed line indicates 30% decline target

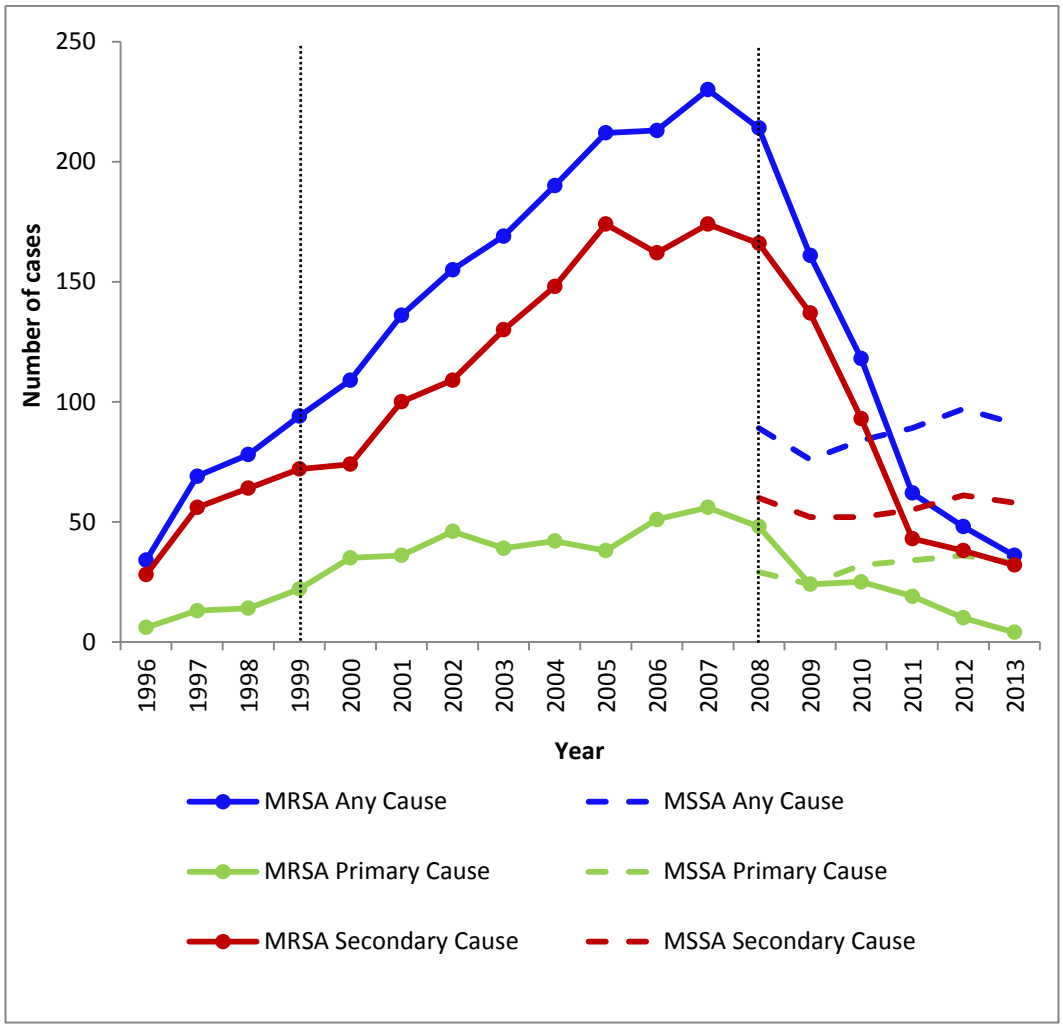


2.3.2 Spatial trends in mortality

2.3.2.1 Number of deaths

The number of deaths, where MSSA and MRSA were the primary (underlying) and secondary (contributing) causes, are shown in Figure 2-5. The total number of deaths due to MRSA (either primary or secondary cause) was 2.4 times higher than those due to MSSA (either cause) in 2008, falling to 2.1 times and 1.4 times in 2009 and 2010 respectively, after which MSSA became a more common cause of death (1.4, 2.0 and 2.5 fold higher than MRSA in 2011, 2012 and 2013 respectively).

Figure 2-5: Total number of MSSA deaths (2008-2013) and MRSA deaths (1996- 2013) in Scotland. Black dashed line indicates breakpoint.



2.3.2.2 Comparison in trends: MR and CFR

MR per 100,000AOBDs were compared where the primary, secondary or combined (i.e. either primary or secondary) cause of death was MSSA or MRSA. There were no changes over the time period 2008-2013 in MR where the primary, secondary or combined cause of death was MSSA ($p>0.05$) (Table 2-4).

MRSA MR increased significantly from 2003 to 2007 ($p=0.008$). However, from 2008 to 2013, MR decreased significantly among patients in which MRSA was the primary cause of death ($p=0.005$), the secondary cause of death ($p=0.003$) or combined primary and secondary cause of death ($p=0.001$) (Table 2-4). These patterns of decline mirror those seen for morbidity rates. Figure 2-6 shows the MR and CFR for (A) MSSA and (B) MRSA.

Case-fatality ratios (CFR) per 100 cases were also compared. There were no significant changes in CFR for either MSSA or MRSA over the time period 2008-2013 (Table 2-4). However, the CFR over the time period 2003-2007, where the combined ($p=0.02$) and secondary causes ($p=0.03$) of death were MRSA, marginally increased.

The CFR where MRSA was the secondary cause of death was consistently higher than other CFR, and was an average of 5.9 times higher (averaged across all study years, range 4.4-7.6) than the CFR where MSSA was the secondary cause, and an average of 4.5 times higher (range 2.2-8) than the CFR where MRSA was the primary cause. The maximum CFR where MSSA was the primary cause of death was 2.7 deaths per 100 cases in 2012, and where MRSA was the primary cause was 8.7 deaths per 100 cases in 2011. The maximum CFR where MSSA was the secondary cause of death was 4.6 deaths per 100 cases in 2012, and where MRSA was the secondary cause was 26.6 deaths per 100 cases in 2010 (Figure 2-6C).

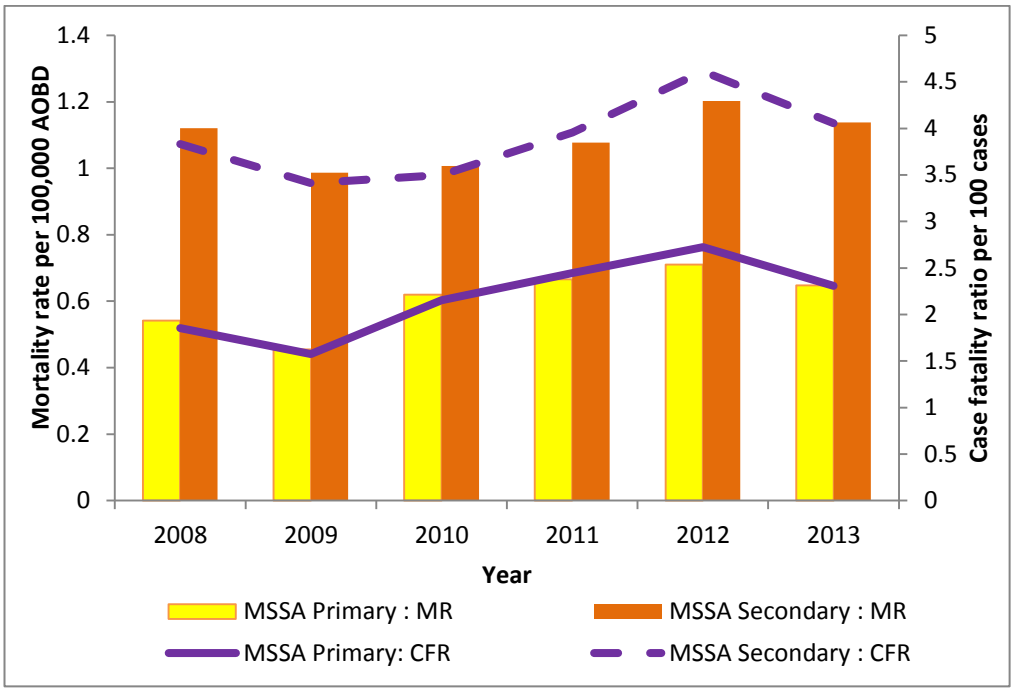
Table 2-4: Model outputs – Mortality rates and case fatality ratio trends over time (2008-2013).

Data			Years	Estimate	SE	p-value	Sig.	R^2
MR	MSSA	All cause	2008 - 2013	0.06	0.03	0.11		0.39
		Primary		0.04	0.02	0.07	.	0.49
		Secondary		0.02	0.02	0.29		0.09
	MRSA	All cause	2003 - 2007	0.28	0.045	0.008	**	0.91
		Primary		0.08	0.03	0.05	.	0.68
		Secondary		0.20	0.07	0.06	.	0.65
	MRSA	All cause	2008 - 2013	-0.68	0.08	0.001	**	0.93
		Primary		-0.14	0.03	0.005	**	0.85
		Secondary		-0.54	0.08	0.003	**	0.89
CFR	MSSA	All cause	2008 - 2013	0.32	0.15	0.1	.	0.43
		Primary		0.17	0.07	0.07	.	0.5
		Secondary		0.15	0.09	0.17		0.26
	MRSA	All cause	2003 - 2007	1.6	0.37	0.02	*	0.82
		Primary		0.48	0.23	0.13		0.45
		Secondary		1.14	0.31	0.03	*	0.76
	MRSA	All cause	2008 - 2013	-1.65	0.6	0.05	.	0.57
		Primary		-0.51	0.52	0.39		-0.01
		Secondary		-1.14	0.64	0.15		0.3

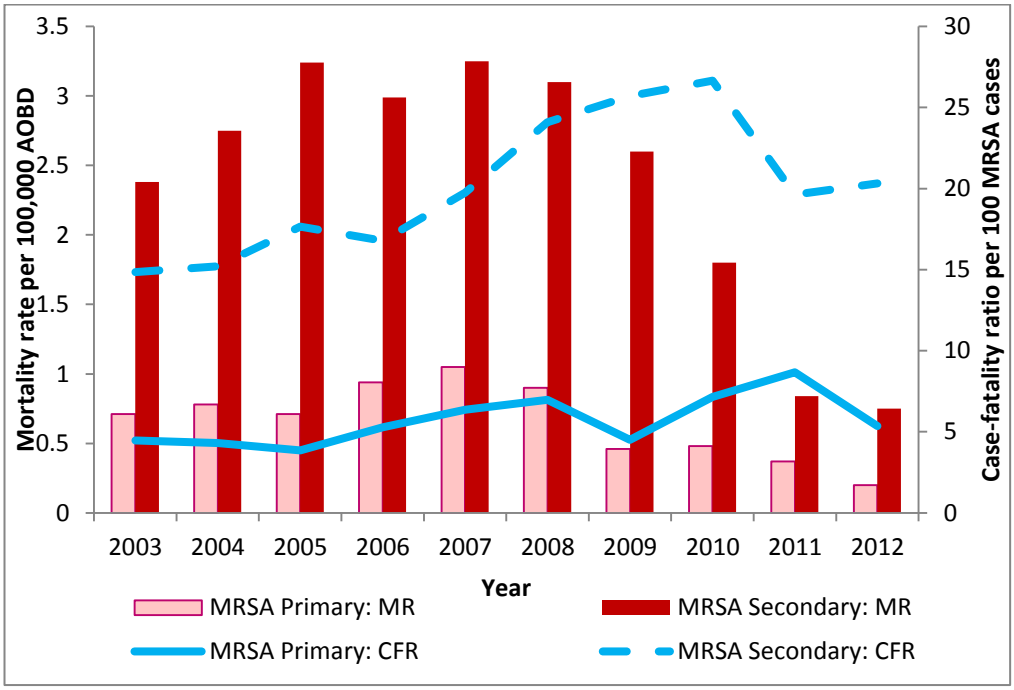
MR: Mortality rate
CFR: case-fatality ratio
Sig: Significance level = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
SE = standard error
Adj. R^2 = adjusted R^2

Figure 2-6: A comparison between mortality statistics: (A) MSSA MR and CFR, (B) MRSA MR and CFR, and (C) MSSA and MRSA CFR.

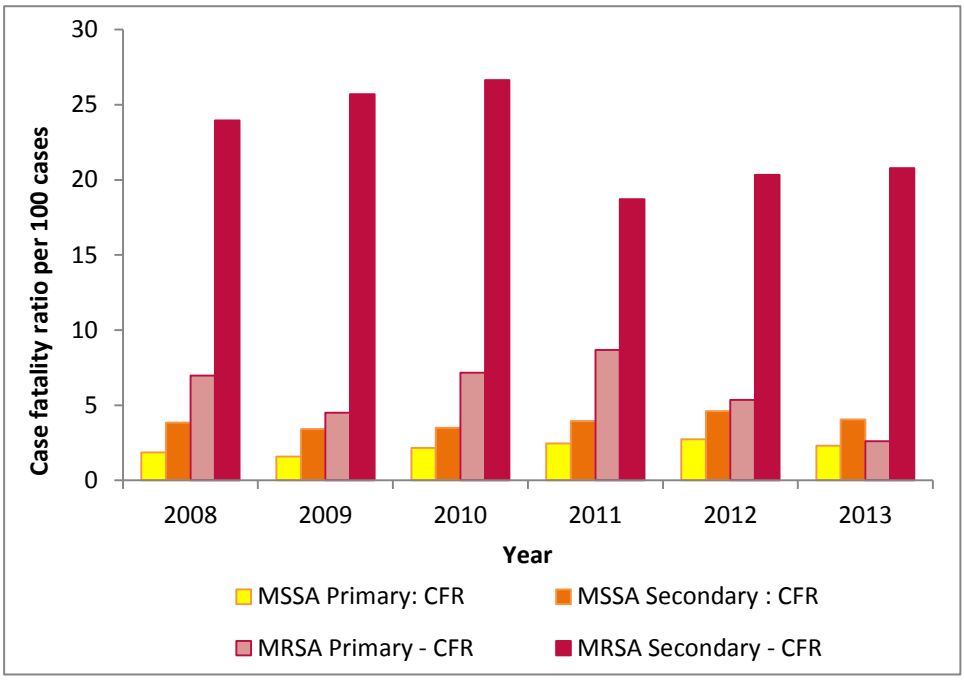
(A)



(B)



(C)



2.4 Discussion

The results in this chapter show that total SAB rates, as well as MRSA bacteraemia rates and the proportion of SAB cases that were MRSA, significantly declined over the study period in all NHS HBs (albeit at different rates). In fact, declines in SAB exceeded targets set by the Scottish Government by the 2010 deadline and rates continued to remain low. This was driven by declines in MRSA and perhaps reflects the continuing efforts to remove MRSA from the hospital environment and reduce the number of cases by a combination of public health strategies. Such declines in morbidity rates were mirrored in mortality rates. This association is expected since with fewer people infected, there are fewer that could succumb to the infection.

Conversely, MSSA rates did not decline over the study period. As a knock-on effect, mortality rates, where the primary or secondary cause of death was an MSSA infection, did not decline either. However, the outcome (i.e. death versus recovery) remained more favourable for individuals with an MSSA rather than an MRSA. This could reflect: fewer treatment options available for MRSA infections, decreased effectiveness of antimicrobials, including vancomycin, which are commonly used to

treat MRSA, and delayed administration of appropriate medication [155]; slower clinical response with an MRSA infection [156]; or the higher pathogenicity and virulence of MRSA compared to MSSA [155]. The incidence of MSSA infection is perhaps difficult to reduce due to the abundance of asymptomatic *S. aureus* carriers who unwittingly pass on the pathogen, or become infected themselves if the bacterium breaches skin or mucosal barriers. One in three individuals is thought to be colonised with *S. aureus*, and carriage is a known risk-factor for infection [54-56].

There was no decline in MSSA or MRSA CFR over time. This may reflect the need to concentrate on developing better patient management practices to avoid death in those with an *S. aureus* infection as well as continued efforts to avoid patients being initially infected. Developing and rolling out rapid real-time diagnostic tools for the detection of bacteria in the blood, correct identification of the microorganism and any antimicrobial resistance may ensure quick and appropriate treatment that could reduce CFR. Current standard protocols for bacteraemia detection involve culturing, which can take several days to isolate and confirm the pathogen.

Where MRSA was the secondary cause of death, the CFR ranged from approximately 24% (2013) to 34% (2010). This CFR represents individuals who already had a primary infection or condition, but due to their co-morbidity (i.e. having MRSA as a secondary infection); the CFR is considerably inflated when compared with the CFR for MRSA or MSSA as a primary cause, or MSSA as a secondary cause.

To test if there were any statistical differences between HBs in terms of rates, analysis of variance (ANOVA) analyses where conducted, and to describe where those statistical differences were, Bonferroni or Tukey analyses were carried out. In the quarterly national surveillance reports by HPS [153], funnel plots are used to illustrate the rate as well as identify any HB outliers (with rates above or below what would be expected). This is a useful, descriptive way of depicting this information although it does not give any indication of any statistical differences between HB rates. The funnel plots were not included here in order to avoid repetition with national surveillance reports. In addition, the models used in this chapter differed

from those used in national surveillance (national surveillance used case data with a Poisson distribution, offset by AOBD) to determine if rate had significantly changed over time. The outcomes are the same as have been described here.

There is a potential limitation of the data in that morbidity data represent cases of bacteraemia while mortality data represent all types of MSSA or MRSA deaths. This would perhaps mean that they are not comparable. However, the patterns of MR and CFR presented in this chapter are largely consistent with that found in the literature. In a multi-national study, the 30-day CFR of SAB was 20.3% [3]; in a 7 year nationwide US study of bacteraemia, the overall SAB crude mortality rate was 25.4% (ICU wards 34.4%, non-ICU 18.9%) [4]; in a US study of over-65 year olds, SAB mortality rates were 49.4% [5]; in a population-based surveillance in the Calgary Health Region, Canada, the overall case-fatality rate due to MRSA and MSSA was found to be 39% and 24% respectively [6]; and in a Japanese study the 7-day and 30-day mortality rates of MRSA bacteraemia were 12.0% and 25.3% respectively [7]. In addition, while it may also be a limitation that mortality was only investigated for 5 years for MSSA, it seems probable that at least for mortality rates, trends would have paralleled morbidity trends. In addition, there are often caveats associated with the use of death certificate data for measuring mortality since the cause of death can often be recorded inaccurately (e.g. incorrect ICD-10 codes, primary cause given but not secondary cause, deaths within 30-days of an infection not related to the infection). In spite of this, death certificates are the best available complete source of death records in Scotland and therefore their use in this study is still relevant. It is particularly useful since cause of death is split into primary, secondary and often tertiary causes so it can be surmised if the deceased had been living with long-term morbidity, was co-infected at the time of death, or what a person died *with* but not necessarily *of* [157].

2.5 Conclusion

MRSA morbidity and mortality rates continue to decline over time. However, there have been no statistical declines in MSSA, although the outcome (death versus

recovery) is more favourable for individuals with the methicillin-sensitive pathogen. In addition, there have been no declines in CFR in recent years and this is perhaps an area for further policy development. On the patient level, MRSA can be considered more severe than MSSA owing to the higher risk of death associated with infections due to this pathogen. However, at the population level, MSSA causes significantly more cases and thus remains an important public health issue.

Chapter 3: **MSSA remains a public health issue while MRSA declines**

3.1 Introduction

Worldwide, a proportion of the *S. aureus* population is typically resistant to the semi-synthetic penicillin-related antimicrobial agent methicillin, therefore giving rise to methicillin-resistant *S. aureus* (MRSA). Resistance to methicillin was first recognised in nosocomial isolates in 1961 [25] following introduction of the β-lactam drug into clinical practice in 1959 [26], for more information on the history of antimicrobial resistance in *S. aureus*, see Chapter 1.1.2.2). Since, MRSA has been an important healthcare-associated infection (HAI) globally and it is often described as a 'superbug' due to its persistence in nosocomial settings, ability to cause outbreaks and resistance to an extensive number of antimicrobials. However, in recent years it has been widely reported by public health bodies, the media and in the published medical literature [2, 9, 134, 158-160] that the proportion of *S. aureus* isolates that is MRSA, as well as UK MRSA bacteraemia rates, have significantly declined over time (see Chapter 1.1.5 for information on Epidemiological Trends and Chapter 1.1.7 for Molecular Epidemiology). Recent literature shows that this decline is most likely due to several public health interventions that have been implemented such as antimicrobial stewardship, universal patient screening to identify carriers and decolonisation to eliminate reservoirs, and case isolation, heightened hand-hygiene and hospital cleaning to stop transmission [118], and others (for more information on Surveillance and Control Measures, see Chapter 1.1.4).

Despite such efforts, the declines of MRSA are not echoed in methicillin-sensitive *S. aureus* (MSSA) trends and MSSA morbidity and mortality rates remain unchanged [131]. The reasons and mechanisms underlying this inconsistency are not well understood.

Bacteraemias caused by MRSA tend to have more serious complications and are considered more difficult to treat than those caused by MSSA. This could reflect the

reduced number of options available for treatment of MRSA infections due to antimicrobial resistance (AMR) to multiple drugs and drug classes including vancomycin which is commonly used to treat MRSA bacteraemias [155, 161], delayed administration of appropriate medication [155], slower clinical response with an MRSA infection [156], or the higher pathogenicity and virulence of MRSA compared to MSSA [155]. Consequently, patients with MRSA tend to have a higher risk of death which was 5.6 times higher for hospital patients with MRSA and 2.7 times higher with MSSA compared with individuals without *S. aureus* bacteraemia (SAB) in a study from 2005-06 in Ninewells hospital, NHS Tayside [123]. After adjustment for co-morbidity, hospitalisation, age and sex; the risk of death (also described as the death hazard in this publication) was still 2.9-fold and 1.7-fold higher than non-*S. aureus* conditions for MRSA and MSSA respectively. Furthermore, MRSA is also associated with longer lengths of hospital stay and higher financial costs in managing individual patients [101, 162, 163] with the associated costs of managing these patients are reportedly 1.4-2.8 times higher for patients with MRSA compared to patients with infections caused by MSSA [13]. However, MSSA is still an important infection which carries high individual and population burden which, unlike MRSA, has not diminished over time.

In this study, microbiological and epidemiological data corresponding to isolates causing SAB in Scotland over an 11 year period were examined. The primary aim was to explore microbiological and epidemiological differences between the MSSA and MRSA populations in order to provide insight as to why MSSA bacteraemia rates have not seen the decline of MRSA rates and why MSSA remains an important public health issue.

3.2 Materials and methods

3.2.1 Data collection

3.2.1.1 EARS-Net

For the purpose of this study, the Scottish component of the EARS-Net (European Antimicrobial Resistance Surveillance Network (EARS-Net)) database was used. EARS-Net is a European-wide network of national surveillance systems, collecting country-level data on antimicrobial resistance for public health purposes. The

network is coordinated and funded by the European Centre for Disease Prevention and Control (ECDC) having been transferred from the Dutch National Institute for Public Health and the Environment (RIVM) in January 2010 (previously known as the European Antimicrobial Resistance Surveillance Network (EARSS)).

3.2.1.2 Scottish MRSA reference laboratory (SMRSARL)

The SMRSARL began contributing to EARS-Net from 2002 (quarter 2). This contribution includes the first blood isolate from an individual patient episode, i.e. bacteraemia (both MSSA and MRSA), from across Scotland [102]. Blood isolates from inpatients and outpatients in hospitals and general practitioner (GP) surgeries were sent on from one of the twenty-two diagnostic laboratories in Scotland (that initially receive and process the specimens) to the reference laboratory for further identification and antimicrobial screening. The isolation of *S. aureus* from blood cultures is known as a bloodstream infection (BSI) or SAB [164].

3.2.1.3 Data for this study

For this analysis, data for the years 2003-2013 were extracted from the SMRSARL database (n=20,316). The years 2002 and 2014 were excluded as they were not full years of data collection. In addition, isolates that were considered duplicates of a single patient-visit (matched on patient name-code, age, sex, date collected and location) and that matched on isolate characteristics (antibiogram, PFGE and PCR-ribotype) were also removed. The community health index (CHI), which is a patient identifier, was not available as it was removed from the database prior to release of the data to protect confidentiality. There was a total of 14,260 MSSA isolates and 5758 MRSA bacteraemia isolates (n=20,018) included in this study.

The proportion of total SAB that was MRSA was also calculated for each year of the study and herein is referred to as, '%MRSA'.

3.2.2 Isolate classification and antimicrobial testing

3.2.2.1 Confirmation of species

Isolates received from diagnostic laboratories by the SMRSARL were confirmed as *S. aureus* using a latex slide agglutination test (Staphytect Plus©) following

incubation for 24 hours on blood agar and, if tested positive for *S. aureus,* were prepared for antimicrobial sensitivity testing using Vitek® sensitivity cards. If the isolates tested negative for *S. aureus,* they were subject to further tests to check if they were false negatives of the previous test and indeed *S. aureus,* or a different species. These tests included: a rapid agglutination test (PASTOREX© Staph-plus kit, to identify *S. aureus*), a tube coagulase test (to further identify *S. aureus*), PCR testing for the *nuc* gene (for identification of *S. aureus*), PCR testing for the *mec* gene (for identification of MRSA), and the Vitek®2 gram positive ID card (for species-level organism identification).

3.2.2.2 Typing

Isolates confirmed as *S. aureus* were typed using standard procedures at the SMRSARL by using a combination of pulsed-field gel electrophoresis (PFGE), PCR ribotyping, biotyping (profiling based on a combination of isolate pigmentation, urease and tween tests), sequence typing (ST) (seldom carried out) and, more recently, *spa* typing. The SMRSARL types MSSA and MRSA isolates as one of three clones / clonal groups. (Group 1) Clonal complex 22 (CC22), which includes the major hospital-associated clone ST22 SCC*mec*IV. If an isolate is ST22 SCC*mec*IV and MRSA, it is commonly referred to as EMRSA-15. (Group 2) Clonal complex 30 (CC30), which includes the major hospital-associated clone ST36 SCC*mec*II. If an isolate is ST36SCC*mec*II and MRSA, it is commonly referred to as EMRSA-16. (Group 3) Non-CC22 / non-CC30 isolates referred to as 'others' which includes many other ST and *spa* types. The full diversity of the 'others' clonal group is not known as ST was rarely carried out on isolates in the database and *spa* typing has only become common practice more recently (i.e. since 2007).

3.2.2.3 Antimicrobial testing

Antimicrobial testing was carried out using an automated method and following standard operating procedures for *in vitro* testing of bacterial susceptibility to antimicrobial agents with Vitek® sensitivity cards. Susceptibility to the following antimicrobials was tested consistently over the study period; ceferoxin/cefoxitin (Cx), chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Km), linezolid (Lz),

methicillin/oxacillin (Mt), mupirocin (high level) (Mp), neomycin (No), penicillin (Pn), rifampicin (Rf), streptomycin (St), sulphamethoxazole (Su), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), and trimethoprim (Tr). In addition, susceptibility to daptomycin (Dp), moxifloxacin (Mx), quinupristin (Qp), and vancomycin (Va) was tested for but not consistently over the whole study period and therefore these antimicrobials were not included in the main analyses.

3.2.2.4 Toxin testing

Testing for the presence of Panton-Valentine Leukocidin toxin gene (*pvl*) and toxic shock syndrome toxin gene (*tst*) was carried out using polymerase chain reaction (PCR) as is standard procedure at SMRSARL.

3.2.3 Descriptive and statistical analysis

Unless otherwise stated, All statistical analyses were carried out in R (version 3.1.2) [154].

3.2.3.1 Background epidemiology

Temporal trends of MSSA and MRSA were examined for the years 2003-2013. The change over time in the number of MSSA and MRSA counts (offset by population size of Scotland) was investigated using a generalized linear model (using a Poisson distribution and a log link-function). The %MRSA was examined using regression analysis with linear, quadratic and cubic models tested where appropriate.

3.2.3.2 Univariate analysis - Isolate characteristics

3.2.3.2.1 Antimicrobial resistance

The percentage of the MSSA and MRSA isolates resistant to twenty antimicrobials was calculated and displayed using radar charts. Any statistical difference in the percentages between the two groups was calculated using either a Pearson's chi square test or Fisher's exact test, depending on the sample size. The change in the percentage resistant to each antimicrobial over time was investigated for both MSSA and MRSA populations using linear regression analyses.

3.2.3.2.2 Antibiogram length

An antibiogram is the combination or string of antimicrobials that an isolate is resistant to, e.g. if an isolate is resistant to penicillin, methicillin and erythromycin, the antibiogram is PnMtEr and the antibiogram length is 3. The antibiogram length is the total number of antimicrobials that an isolate showed phenotypic resistance to. 'Intermediate' resistance phenotypes (which are neither sensitive nor fully resistant to an antimicrobial) were considered not resistant in this study. The distribution of antibiogram lengths of MSSA and MRSA were examined and student's t-tests were used to assess if there were significant differences in mean antibiogram lengths. A student's t-test was used as the central limit theorem states that for large sample sizes (n>30, as a rule of thumb) the sample mean will be approximately normally distributed.

3.2.3.2.3 Clone

The association of MSSA or MRSA and clone type / clonal group, (whether isolates were CC22, CC30, or the heterogeneous group of non-CC22 / non-CC30 'others') was tested using a Pearson's chi-squared test. The percentage of different clone types (by MSSA and MRSA) resistant to twenty antimicrobials was calculated and displayed in radar charts and any significant difference between these percentages calculated using Pearson's chi-squared test or Fisher's exact test (depending on sample size, i.e. if one of the entries in the 2x2 table is ≤5, then a Fisher's exact test is used, and if >5 then a chi-squared test is used).

3.2.3.2.4 Toxins

Toxin typing was inconsistently carried out pre-2010 with <5% of all isolates screened in 2003, 2004, and 2005, <10% in 2006 and 2007, and <12% in 2008 and 2009. In 2010, the percentage of all isolates screened increased to >90%, and in 2011, 2012 and 2013 more than 98% of all isolates were screened for the *pvl* and *tst* toxin genes. Therefore, information on presence of the *pvl* toxin genes was only available for 42.6% (n=6071) of MSSA isolates and 17.1% (n=983) of MRSA, and information on the presence of *tst* toxin genes was available for 41.1% (n=5858) of MSSA isolates and 16.4% (n=942) of MRSA isolates. Where information was available, the difference in the percentage of MSSA and MRSA isolates positive for

either toxin gene was tested using a Pearson's chi-squared test. Since the level of testing for the toxin genes varied over time, a sensitivity analysis, using Pearson's chi-squared test, was carried out to assess that there was no difference in the rate of presence of toxin genes over time. Data from the years 2010 to 2013 inclusive was compared to data from all years, for both MSSA and MRSA, for both toxins. The analyses showed that there was no difference in the rate of toxin presence when comparing years 2010 to 2013 to all years, for both toxins, for both MSSA and MRSA ($p<0.0001$). Therefore, the results describe toxin presence for all years.

3.2.3.3 Univariate analysis - Isolate origin or niche

3.2.3.3.1 Patient origin

Within the database, the origin of the isolates was defined as inpatients, outpatients, patients attending GP surgeries or 'other'. The numbers of outpatient and inpatient comprised 2.5% and 93.8% of MSSA and 1.7% and 96.7% of MRSA isolates respectively. As such, for the purpose of analysis of patient origin, corresponding to the origin designated as GP or other (or where information was missing), 631 isolates were removed. The difference between the proportion of MSSA and MRSA isolates that were originally isolated from inpatients and outpatients was tested using a Pearson's chi-squared test or Fisher's exact test (depending on the sample size).

3.2.3.3.2 Hospital specialty

Only isolates from inpatients were considered for this analysis (MSSA = 13,371 isolates, MRSA = 5568 isolates) as there were insufficient numbers of isolates originating from outpatients, GP and unspecified other. Isolates with missing (MSSA = 3091 isolates, MRSA = 1306) and unspecified 'other' specialty (MSSA = 1563 isolates, MRSA = 486 isolates) were also excluded. To test if MRSA was distributed equally across all specialties (i.e. if patients in certain specialties were more at risk); a 2x12 table was analysed using chi-squared analysis.

3.2.3.3.3 Patient age and sex

Statistical differences in the mean age of patients between MSSA and MRSA were determined by Student's t-test. Pearson's chi-squared tests or Fisher's exact tests (depending on sample sizes) were used to assess the significance of any difference between MSSA and MRSA for the proportion of isolates from males. A logistic

regression model ($(organism\ (MSSA\ or\ MRSA) \sim age\ +\ sex\ +\ age * sex$) was also used to investigate if a patient developing MRSA or MSSA bacteraemia was related to their age or sex.

3.2.3.3.4 Device

Information on whether an intravenous line, central line, catheter or any other device was fitted the time the patient developed bacteraemia was only available for 24.0% (n=3423) of MSSA isolates and 23.3% (n=1343) of MRSA isolates. The association between having a device and a MSSA or MRSA bacteraemia was tested within this subset using a Pearson's chi-squared test.

3.2.3.4 Multivariate analysis - nonmetric multi-dimensional scaling (NMS)

3.2.3.4.1 NMS background

To further investigate differences between MSSA and MRSA, a NMS analysis was carried out. NMS is a data reduction and nonparametric ordination technique that is appropriate for data that are non-normal and does not have the assumption of linear relationships among variables that is common to other data reduction techniques. The ordination is based on information in a primary matrix which is reduced to typically two or three axes (dimensions). Analyses aim to visualise the level of dissimilarities between isolates in an *N*-dimensional space or scatterplot using a ranked distance matrix that was calculated with a Euclidian distance measure. The dimensionality of the plot was determined by plotting a measure of fit (known as stress) to the number of dimensions with the optimal dimensionality being the number of dimensions with the lowest stress. This optimisation strategy aims to create a configuration which represents the smallest departure from monotonicity in the relationship between distance in the original space and that in the reduced ordination space. Five hundred iterations were used for each NMS run, using random starting coordinates. Several NMS runs were performed to ensure that the solution was stable and represented a configuration with the best possible fit. The NMS was performed in PC-ORD version 6 (MJM software Design, Gleneden Beach, OR).

3.2.3.4.2 Data for NMS

Initially, information on AMR, antibiogram length, clone/clonal group, toxins, patient origin, biotyping (urease, tween), hospital specialties, patient age and sex, and

device was included in the primary matrix. However, missing data meant that toxin, device and biotyping data were excluded from the NMS analysis. In addition only inpatient data was included as other patients origins comprised less than 5% of the isolates (GP= 0.15%, outpatients=2.3%, unspecified other=0.05% of all isolates with known patient origin) in the database. The initial NMS was run with 14 variables (age, antibiogram length, sex, clonal type (CC22, CC30, 'other'), and hospital specialties (accident and emergency, renal, medicine, paediatrics, surgery, care of elderly, high dependency unit/intensive care unit, orthopaedics)) and 13,658 rows. Unfortunately this file was too large for the programme to run so the database had to be reduced to approximately 5,000 rows. This was done using a stratified random approach. The data was stratified by year and clone and data were selected randomly in relation to the proportions in the full database. Sex was removed in subsequent NMS runs as it did not contribute in this multivariate analysis. In the final NMS analysis, for each isolate the continuous variables age and antibiogram length were included in the primary matrix, and information on clone and specialties was included with the binomial variables (present or absent to each): CC22, CC30, accident and emergency, renal, medical, surgery, paediatrics, care of elderly, high dependency unit/intensive care unit and orthopaedics. The NMS analysis was carried out on a random sample of 5000 inpatient isolates only.

3.2.3.4.3 NMS results

To identify variables that were associated with either group, a second matrix was superimposed on the ordination to create a joint plot (radiating lines). The direction of the lines indicates either a positive or negative relationship with an axis and the variables of the secondary matrix, and the length shows the strength of the correlation on that axis (only with quantitative variables). The ordination was rotated in order to maximise the difference between MRSA and MSSA along Axis 1. Information in the second matrix included: AMR, antibiogram length, isolate clone/clonal group (classed as CC22, CC30, and non-CC22/non-CC30'others'), presence of *pvl* or *tst* toxin genes, patient gender and age, specialty that patient was in when blood specimen taken, whether a patient had a device fitted, and year. Furthermore, Multi-response Permutation Procedures (MRPP) analysis was

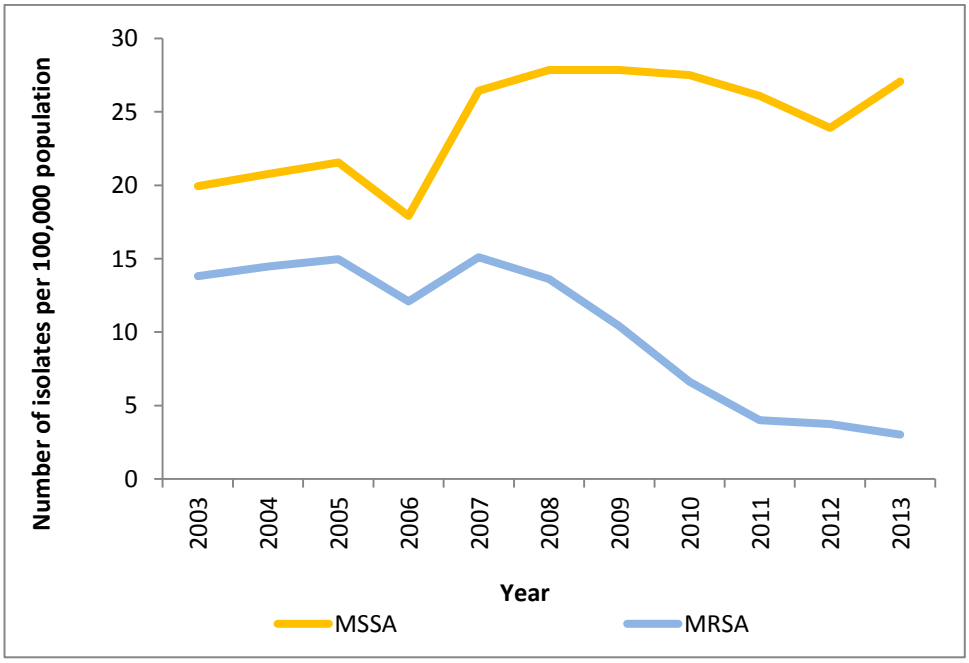
performed to test the hypothesis of no difference between the 2 groups (i.e. MSSA and MRSA) and therefore the association between variables with either group.

3.3 Results

3.3.1 MSSA and MRSA trends

For all years examined in this study, MSSA bacteraemia was more common than MRSA. In 2003, the number of MSSA isolates was 1.5 times higher than MRSA but by 2013 this had increased to 9.8 times, and the proportion of total SAB that was caused by MRSA had also statistically declined ($p<0.0001$).This increase in magnitude over time was in part due to the overall significant increase in the number of MSSA bacteraemia isolates ($p<0.0001$), and also due to the significant decrease in MRSA bacteraemia by an average of 13% per year over the whole study period ($p<0.0001$). These trends are shown in Figure 3-1. Overall, the percentage of total SAB isolates that was methicillin resistant was 28.8%. This varied by year with the percentage approximately 40% for 2003-2006 inclusive, falling to below 30% in 2009, to below 20% in 2010, and to below 10% by 2013.

Figure 3-1: Trends in the number of Scottish MSSA and MRSA bacteraemia isolates (for EARS-NET) by year.



3.3.2 Univariate analysis of isolate characteristics - MSSA and MRSA differ by AMR, clone type and toxins

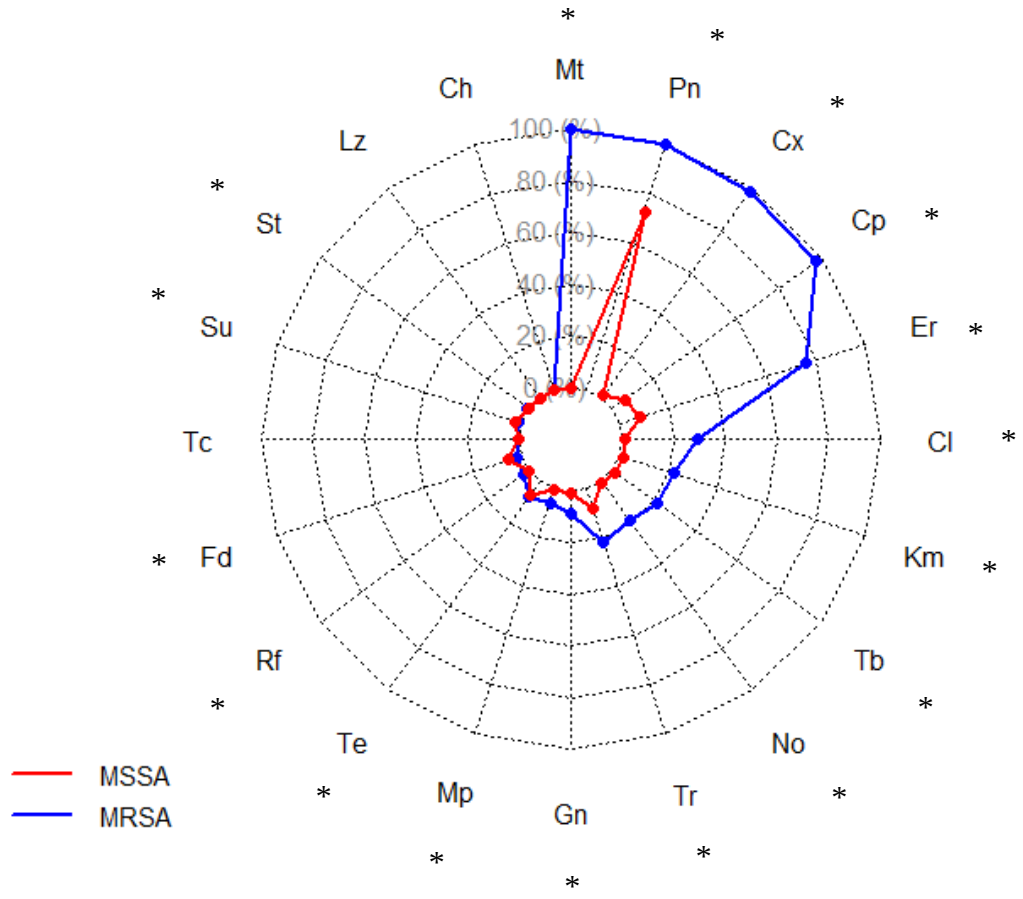
3.3.2.1 AMR

The percentages of both MSSA and MRSA isolates that were resistant to 20 frequently tested antimicrobials are shown in Figure 3-2. The radar chart shows, as suggested above, that multi-drug resistance in the MSSA population was low. While more than 70% of isolates were resistant to penicillin, only 5% to 9% of the population was resistant to ciprofloxacin, erythromycin, fusidic acid, tetracycline and trimethoprim, and less than 2% was resistant to each of the other antimicrobials. Moreover, 21.0% of the MSSA population was fully sensitive to all twenty antimicrobials tested.

In contrast, MRSA was multi-drug resistant. Averaged over the whole study period, resistance to methicillin (by definition) and penicillin was 100%, ceferoxin/cefoxitin and ciprofloxacin was greater than 98%, erythromycin was 76%, clindamycin, kanamycin, neomycin, tobramycin and trimethoprim between 19% and 30%, gentamycin, mupirocin and tetracycline between 5% and 9%, fusidic acid and rifampicin between 2% and 3%, and resistance below 2% for all other antimicrobials (n=5) (Figure 3-2).

The percentage resistant to each antimicrobial was significantly different between the MSSA and MRSA populations ($p<0.0001$ for each antimicrobial other than streptomycin where $p=0.03$) except for chloramphenicol, linezolid and teicoplanin where there was no difference in the proportion resistant between the two groups (i.e. MSSA and MRSA) ($p<0.05$) (Figure 3-2). Vancomycin resistance (not shown) was only tested from 2008 onwards but 100% of both MSSA and MRSA isolates were sensitive to this third-line antimicrobial.

Figure 3-2: Radar chart showing percentage of the MSSA (n=14,260) and MRSA (n=5758) populations that are resistant to 20 antimicrobials.



Asterisks indicate significant differences between the populations ($p<0.05$). Antimicrobial abbreviations: ceferoxin/cefoxitin (Cx), chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Km), linezolid (Lz), methicillin/oxacillin (Mt), mupirocin (high level) (Mp), neomycin (No), penicillin (Pn), rifampicin (Rf), streptomycin (St), sulphamethoxazole (Su), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), trimethoprim (Tr).

3.3.2.2 Antibiogram length

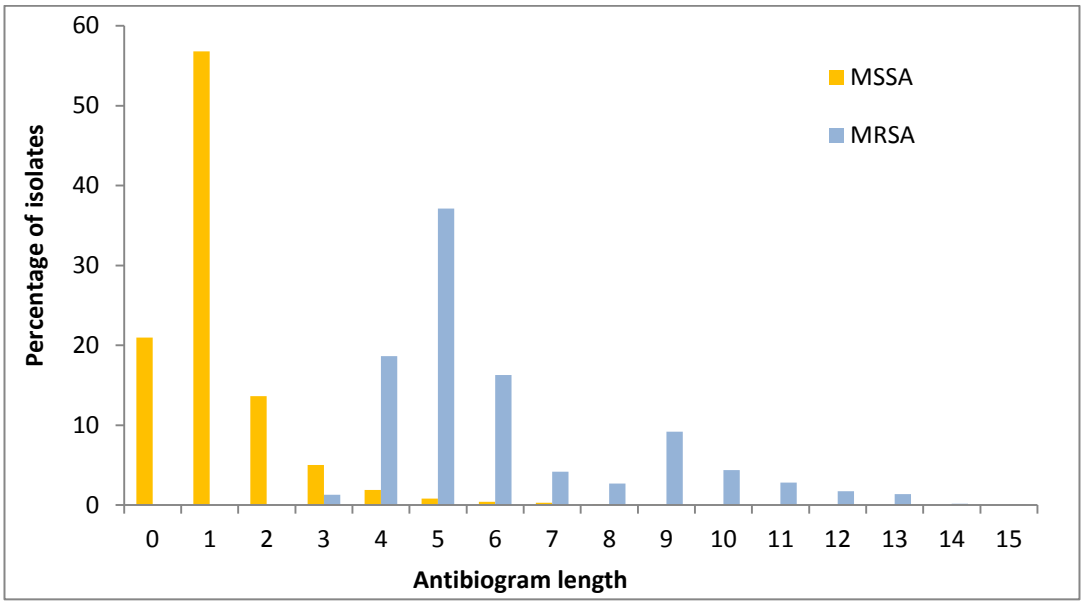
MSSA and MRSA differed statistically in terms of the number of antimicrobials that the two groups were phenotypically resistant to ($p<0.0001$). The MSSA population was resistant to a median of 1 and MRSA resistant to a median of 5 antimicrobials (Table 3-1). The distribution of antibiogram lengths of MSSA is skewed to the left while that of MRSA is almost bimodal (Figure 3-3).

Table 3-1: Summary of antibiogram lengths for MSSA and MRSA.

Organism	Min	1st Qu	Median	Mean	3rd Qu	Max
MSSA	0	0	1	0.83	1	9
MRSA	2	5	5	6.1	7	15

Qu = quartile; Min = minimum; Max = maximum

Figure 3-3: Distribution of frequencies of antibiogram lengths of MSSA and MRSA.



3.3.2.3 Clone / clonal 'group'

There was a statistical difference between MSSA and MRSA in terms of clone type composition: 5.9% of MSSA and 74.2% of MRSA belonged to CC22 ($p<0.0001$), and 20.0% and 22.2% of MSSA and MRSA respectively belonged to CC30 ($p<0.001$). While Figure 3-4 shows very little difference between MSSA and MRSA for CC30, the large sample size resulted in the significant result. Only 3.5% of MRSA isolates belonged to any other clone, whereas 74.1% of MSSA isolates belonged to a non-CC22 / non-CC30 'other' clone ($p<0.0001$) (Figure 3-4).

The median antibiogram lengths for MSSA CC22, CC30 and 'others' were 2, 1 and 1 respectively and there where statistical differences in the distributions of these lengths ($p<0.0001$) (Table 3-2). MSSA 'others' was more diverse in terms of numbers of antibiograms with 270 different antibiograms compared to 62 for CC22

and 80 for CC30. The most common antibiogram for each clone was 'Pn' followed by 'fully sensitive'. There were also statistical differences between the median antibiogram lengths of MRSA CC22, CC30 and 'others' ($p<0.0001$) with the CC30 clone having the longest antibiogram (Table 3-2). The percentage of total MSSA and total MRSA belonging to each clone/clonal group (i.e. C22, CC30 or 'others') is shown in Figure 3-4, a summary of antibiogram lengths for MSSA and MRSA by clone shown in Table 3-2, the distribution of frequencies of antibiogram lengths of MSSA and MRSA by clone in Figure 3-5, and the percentages resistant to each antimicrobial by clone for MSSA and MRSA populations are shown in Figures 3-6A and 3-6B.

Differences between clone types of MRSA are described in greater detail in Chapter 4 of this thesis.

Figure 3-4: Percentage of total MSSA and total MRSA belonging to each designated clonal group.

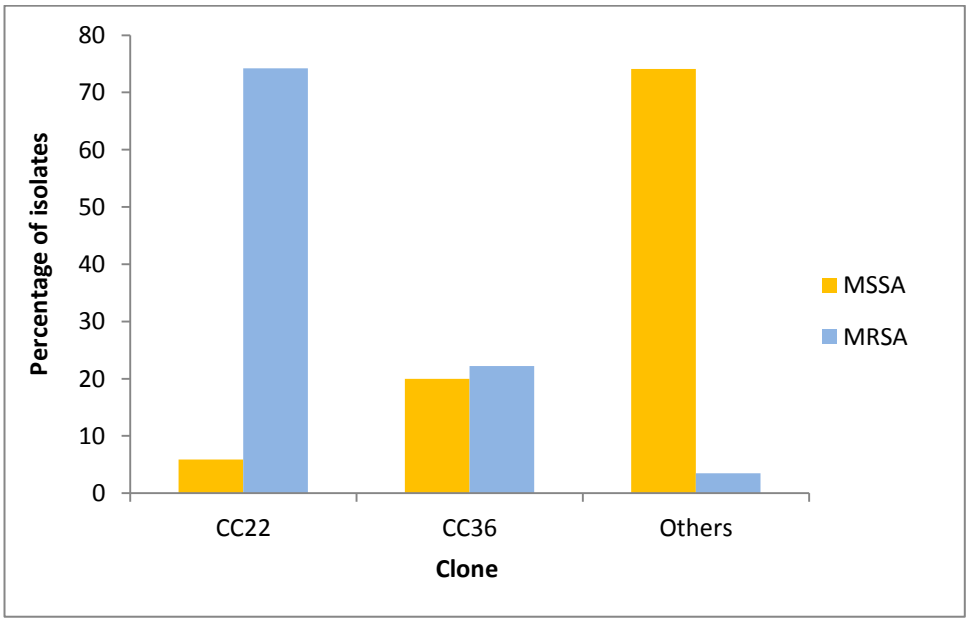


Table 3-2: Summary of antibiogram lengths for each clone by organism (MSSA or MRSA).

Organism	Clone	Min	1st Qu	Median	Mean	3rd Qu	Max
MSSA	CC22	0	1	2	2	3	7
	CC30	0	1	1	1.0	1	8
	'Others'	0	1	1	1.1	1	9
MRSA	CC22	2	4	5	5.2	6	13
	CC30	2	8	9	9.2	10	15
	'Others'	2	4	6	7.0	10	15

Qu = quartile; Min = minimum; Max = maximum

Figure 3-5: Distribution of frequencies of antibiogram lengths of MSSA and MRSA by clone.

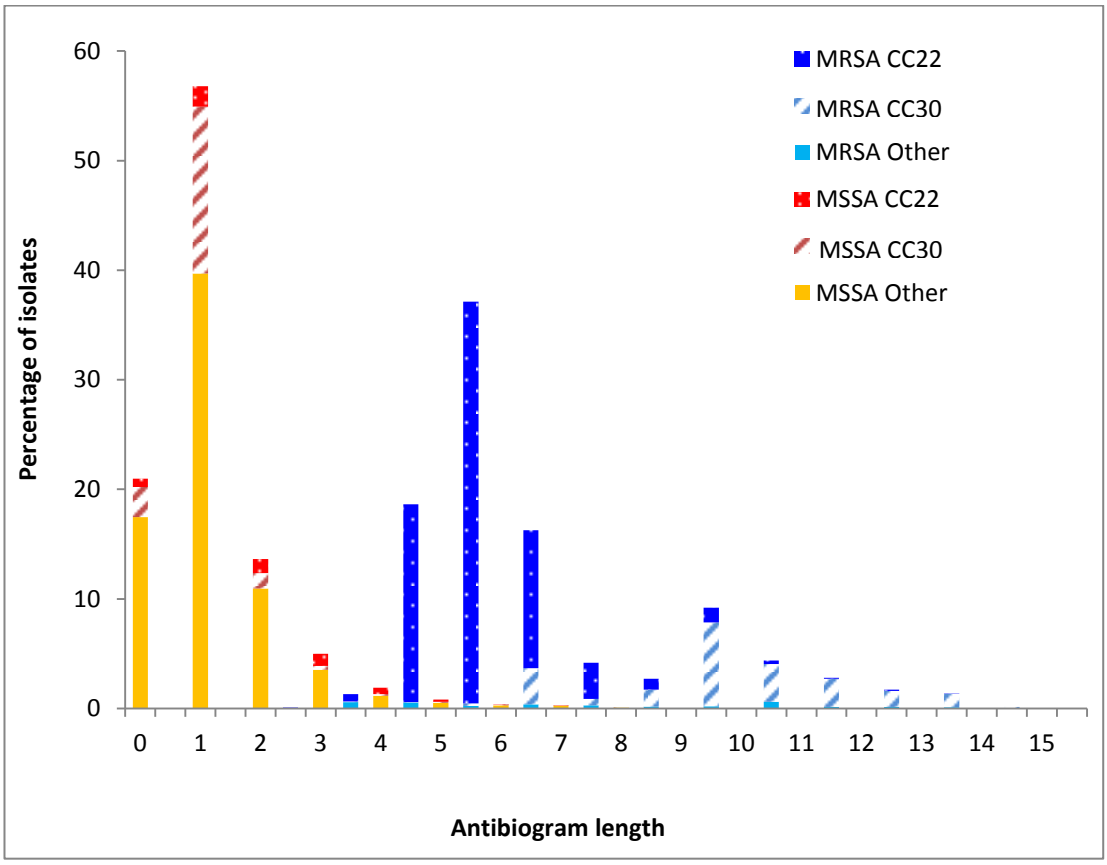
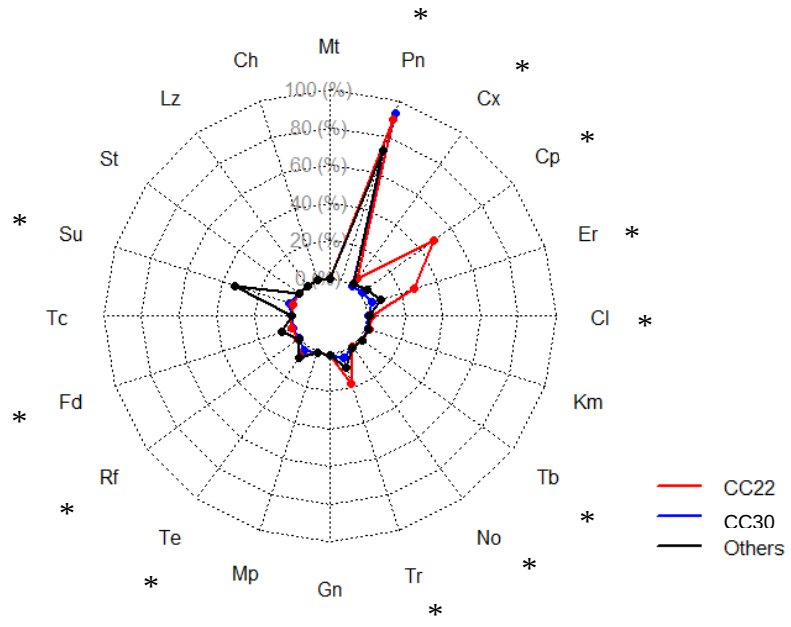
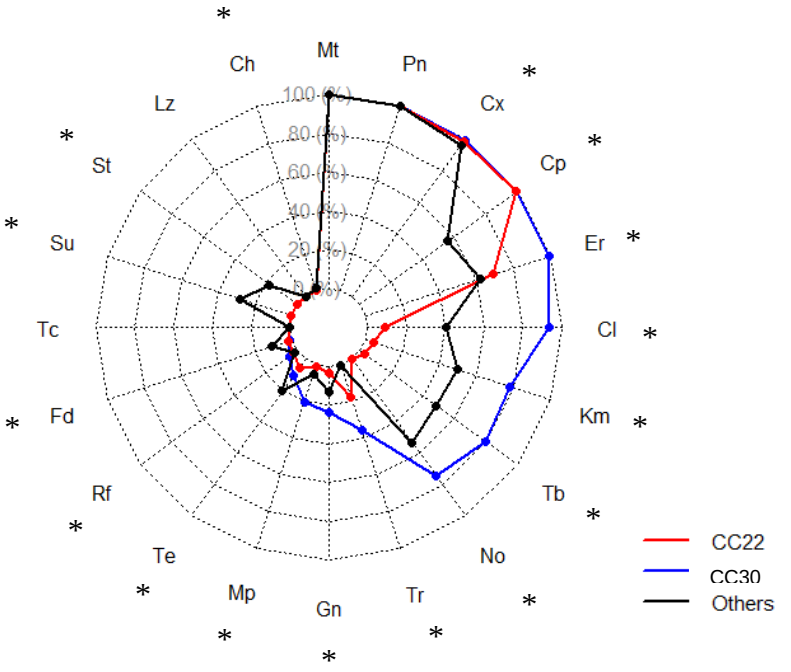


Figure 3-6: Radar chart showing percentage resistant to 20 antimicrobials in different clones of the (A) MSSA and (B) MRSA populations. Asterisks represents statistical difference (p<0.05).

(A) Mt, Km, Gn, Mp, Tc, St, Lz, Ch – no statistical differnce (p>0.05). All other antimicrobials significant (Tb, No p<0.02; Rf, Su, Cl p<0.01, all others p<0.0001). CC22 n = 839, CC30 n = 2858, Others n = 10563



(B) Mt, Pn, Lz, Tc - no statistical difference (p>0.05); all other antimicrobials significant (all p<0.0001 except Ch and Cx where p<0.01). CC22 n = 4275, CC30 n = 1279, Others n = 204.



3.3.2.4 Toxins

The presence of toxin genes in MSSA and MRSA was compared (Table 3-3). Of all MSSA and MRSA isolates, 42.6% and 17.1% respectively were tested for the *pvl* toxin gene and of those tested, 2.0% and 2.2% were positive. There was no significant difference between MSSA and MRSA in terms of the proportion with toxin genes ($p>0.05$). Similarly, 41.1% and 16.4% of MSSA and MRSA were tested for the *tst* toxin gene and of those tested, 16.0% and 7.4% were positive. There was a significant difference between MSSA and MRSA in terms of the proportion with toxin genes ($p<0.0001$).

When investigating *pvl* gene presence between clonal group (between organisms, MSSA and MRSA), there was a statistical difference between the percentages of *pvl* positivity in the CC22 clone between MSSA and MRSA populations as well as for 'others' ($p<0.0001$), but no difference between MSSA and MRSA for CC30 ($p>0.05$). MRSA 'others' had the highest proportion positive of *pvl* (14.8%).

When investigating *tst* gene presence between clonal group (between organisms, MSSA and MRSA), there was a statistical difference between MSSA and MRSA for CC22 ($p=0.01$) as well as CC30 ($p<0.0001$), but no difference for 'others' ($p>0.05$). Both MSSA and MRSA CC30 had high levels of *tst*.

Table 3-3: Presence of toxin genes.

Organism	Toxin	Positive	Negative	Unknown	Proportion tested	Proportion positive	significance level
MSSA	*pvl*	122	5949	8189	42.6	2.0	p>0.05
MRSA	*pvl*	22	961	4775	17.1	2.2	
MSSA	*tst*	938	4920	8402	41.1	16.0	p<0.0001
MRSA	*tst*	70	872	4816	16.4	7.4	
MSSA CC22	*pvl*	12	308	519	38.1	3.8	p<0.0001
MRSA CC22	*pvl*	2	778	3495	18.2	0.3	
MSSA CC30	*pvl*	34	1061	1763	38.3	3.1	p>0.05
MRSA CC30	*pvl*	2	79	1198	6.3	2.5	
MSSA others	*pvl*	76	4579	5905	44.1	1.6	p<0.0001
MRSA others	*pvl*	18	104	82	59.8	14.8	
MSSA CC22	*tst*	9	294	536	36.1	3.0	p<0.0001
MRSA CC22	*tst*	1	758	3516	17.8	0.1	
MSSA CC30	*tst*	757	315	1786	37.5	70.6	p=0.01
MRSA CC30	*tst*	67	12	1200	6.2	84.8	
MSSA others	*tst*	171	4311	6078	42.4	3.8	p>0.05
MRSA others	*tst*	2	102	100	51	1.9	

3.3.3 Isolate origin or niche - MSSA and MRSA differ by niche

3.3.3.1 Patient origin

The overall majority of isolates were isolated from inpatients (97.4%) as compared to other patient origins (outpatients (2.3%), GP surgeries (0.15%), and unspecified others (0.05%)). As the numbers for other patient origins were low, comparisons were made statistically between inpatients and outpatients only. Inpatients were 2.4 times and outpatients 3.7 times more likely to have a MSSA bacteraemia than an

MRSA bacteraemia. MSSA bacteraemia was 38.0 times and MRSA bacteraemia was 58.0 times higher in inpatients than outpatients (Table 3-3). There was a significant difference between the proportion MRSA that was isolated from inpatients versus outpatients ($p<0.001$) with 42.4% of inpatient bacteraemias and 27.3% of outpatient bacteraemias caused by MRSA (Table 3-4).

Table 3-4: Patient origins.

Patient origin	MRSA cases Number (%)	MSSA cases Number (%)	%MRSA in each patient origin$
GP	5 (0.09)	24 (0.17)	20.83
Inpatient	5568 (98.2)	13371 (97.2)	41.64
Outpatient	96 (1.69)	352 (2.56)	27.27
Other	1 (0.02)	9 (0.07)	11.11
Total	5670 (100)	13756 (100)	41.22
Missing	88	504	17.46

$ %MRSA in each patient origin = MRSA cases/MSSA cases

3.3.3.2 Hospital specialty

Information on the different hospital specialties that inpatients were present in when their SAB occurred or when blood specimen taken, was also compared for the MSSA and MRSA populations. Within all specialties, there were more MSSA bacteraemia than MRSA. The highest within-specialty percentages of infections that were MSSA were found in paediatrics (94.8%), obstetrics and gynaecology (89.9%), and accident and emergency (85.0%). The highest within-specialty percentage of bacteraemia that was MRSA was found in Care of the Elderly (i.e. of all bacteraemias in CoE, 43.6% was MRSA), followed by high dependency unit / intensive care unit (40.2%), and surgery (36.9%) (Table 3.5). Across all specialties (and excluding isolates where the specialty was unknown or listed as ‘other’), MSSA was most common in medicine (26.5%), followed by accident and emergency (13.2%), and renal (11.3%) and MRSA most common in medicine (28%), followed by high dependency unit /

intensive care unit (13.5%), renal (12.7%), and surgery (11.2%). Figure 3-7A shows the percentage of total MSSA isolates or MRSA isolates found in each hospital specialty, and Figure 3-7B shows the within-specialty proportion of isolates that were MSSA and MRSA.

There was a difference between MSSA and MRSA in the percentages of each specialty ($p<0.0001$). MRSA was associated with specialty and there were differences with respect to MRSA across specialties (i.e. risk factor >1, Table 3-6). MRSA was overrepresented in care of the elderly ($p<0.0001$), high dependency units /intensive care units ($p<0.0001$), renal ($p=0.03$), surgery ($p<0.0001$). MSSA was overrepresented in accident and emergency ($p<0.0001$), infectious diseases ($p<0.001$), obstetrics and gynaecology ($p=0.0001$), orthopaedics ($p<0.0001$), paediatrics ($p<0.0001$), paediatrics ICU ($p<0.0001$), and in these specialties, the odds of MRSA was <1 (Table 3-6).

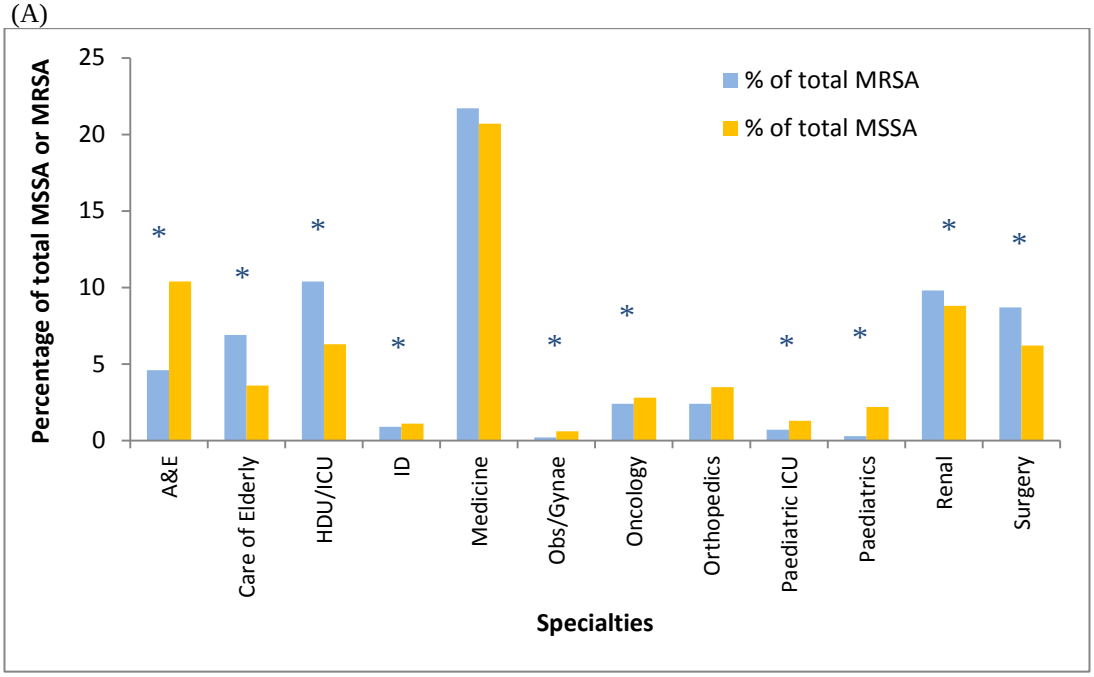
Table 3-5: Proportion of MSSA and MRSA isolates found in each hospital specialty.

Specialty	Within specialty %MRSA$	% of total MSSA	% of total MRSA
A&E	15	10.4	4.6
Care of Elderly	43.6	3.6	6.9
HDU/ICU	40.2	6.3	10.4
ID	26	1.1	0.9
Medicine	29.7	20.7	21.7
Obs/Gynae	10.1	0.6	0.2
Oncology	25.6	2.8	2.4
Orthopaedics	21.5	3.5	2.4
Paediatric ICU	17.4	1.3	0.7
Paediatrics	5.2	2.2	0.3
Renal	30.9	8.8	9.8
Surgery	36.3	6.2	8.7
Other	23.7	11	8.4
Missing	29.7	21.7	22.7

$ %MRSA in each specialty = MRSA cases/MSSA cases. Specialties: A&E = Accident and Emergency, HDU/ICU = high dependency unit / intensive care unit, ID = infectious diseases, Obs/Gynae = obstetrics and gynaecology

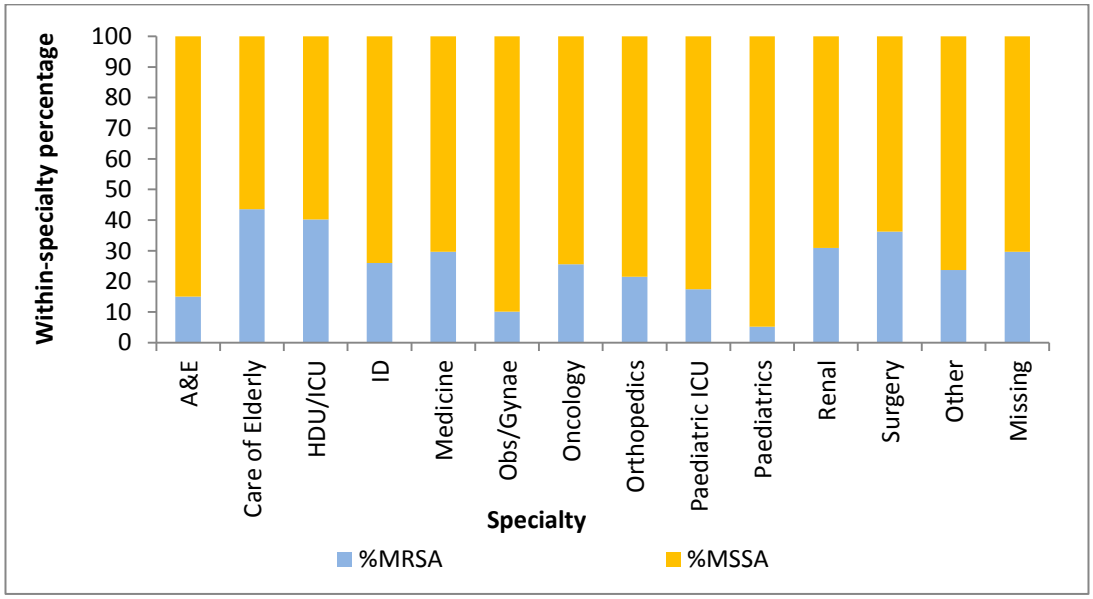
Figure 3-7: The hospital specialties that an individual was in when they developed their bacteraemia or had the blood specimen taken. (A) The percentage of total MSSA isolates or MRSA isolates found in each hospital specialty, (B) the within-specialty proportion.

(A)



Asterisks highlights where a statistical difference between the proportions MSSA and MRSA exist (excluding 'missing' and 'others').
Specialty: A&E = Accident and Emergency, HDU/ICU = high dependency unit / intensive care unit, ID = infectious diseases, Obs/Gynae = obstetrics and gynaecology
Note: This graph and asterisk represent differences between MSSA and MRSA, e.g., is there a difference in the proportion of MRSA in A&E compared with the proportion of MSSA in A&E?

(B)



Specialties: A&E = Accident and Emergency, HDU/ICU = high dependency unit / intensive care unit, ID = infectious diseases, Obs/Gynae = obstetrics and gynaecology

Table 3-6: Specialties where patients are more at risk of MRSA.

Specialty	Organism	n	Chi-square	p-value	OR (95% CI)
Accident and emergency	MRSA	222	179.9	<0.001	0.38 (0.32-0.44)
	MSSA	1288			
Care of elderly	MRSA	393	91.04	<0.001	1.93 (1.68-2.22)
	MSSA	511			
HDU / ICU	MRSA	599	92.81	<0.001	1.72 (1.54-1.92)
	MSSA	890			
Infectious diseases	MRSA	53	6.692	0.01108	0.67 (0.47-0.91)
	MSSA	149			
Medicine	MRSA	1241	0.6192	0.8053	0.99 (0.91-1.07)
	MSSA	2944			
Obstetrics and gynaecology	MRSA	9	15.09	0.00014	0.28 (0.12-0.56)
	MSSA	77			
Oncology	MRSA	133	3.914	0.0495	0.82 (0.66-1.00)
	MSSA	386			
Orthopaedics	MRSA	136	20.38	<0.001	0.64 (0.52-0.78)
	MSSA	496			
Paediatrics ICU	MRSA	36	17.12	<0.001	0.48 (0.32-0.69)
	MSSA	179			
Paediatrics	MRSA	16	94.3	<0.001	0.12 (0.07-0.20)
	MSSA	303			
Renal	MRSA	526	4.557	0.03302	1.13 (1.01-1.26)
	MSSA	1131			
Surgery	MRSA	499	34.45	<0.001	1.42 (1.26-1.60)
	MSSA	874			

OR = Odds Ratio, HDU/ICU = high dependency unit / intensive care unit
Note: This table describes the risk of having MRSA in each specialty, e.g. what are the odds of having MRSA in A&E compared with the odds of having MSSA in A&E?

3.3.3.3 Patient age and sex

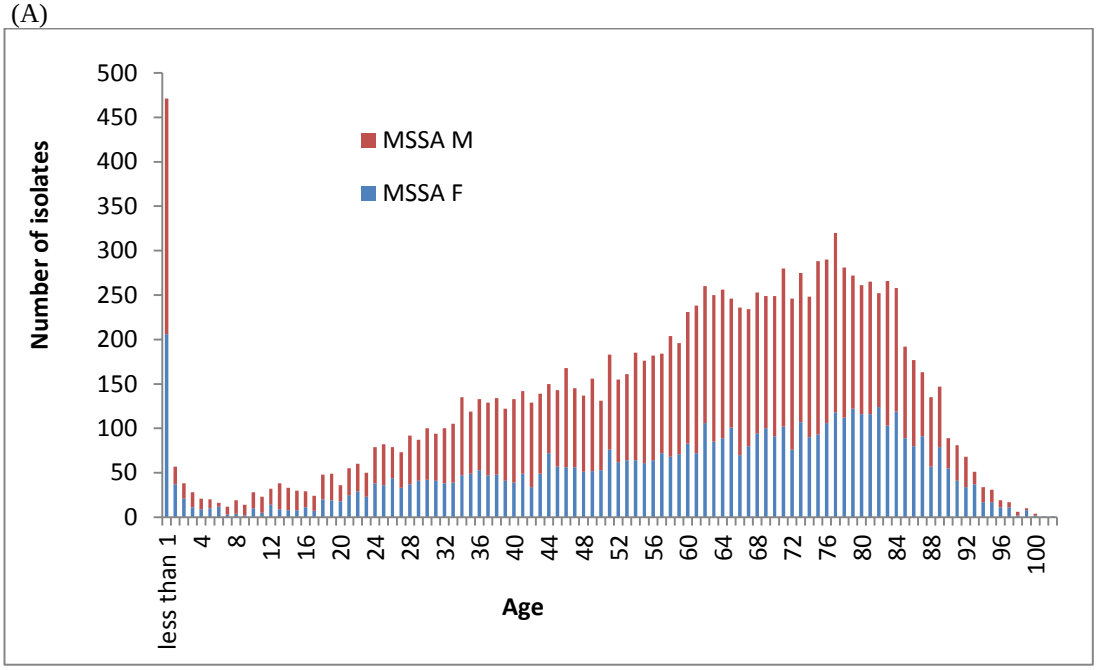
The mean age for MSSA was statistically lower than that for MRSA (58.1 years versus 67.8 years, p<0.0001) (Figure 3-8A, B, C). In addition, 3.4% of all MSSA bacteraemias occurred in individuals aged less than 1 year, while for all MRSA infections that figure was 1.0%. There was a statistically significant difference (albeit small) in the proportion of isolates from males versus females with 60.4% of MSSA isolates and 66.0% of MRSA isolates from males (p<0.0001) (Figure 3-8A, B, C).

In investigating the relationship between the organism (either MSSA or MRSA) with patient age and sex; patient age did not differ with sex and vice versa (i.e. no

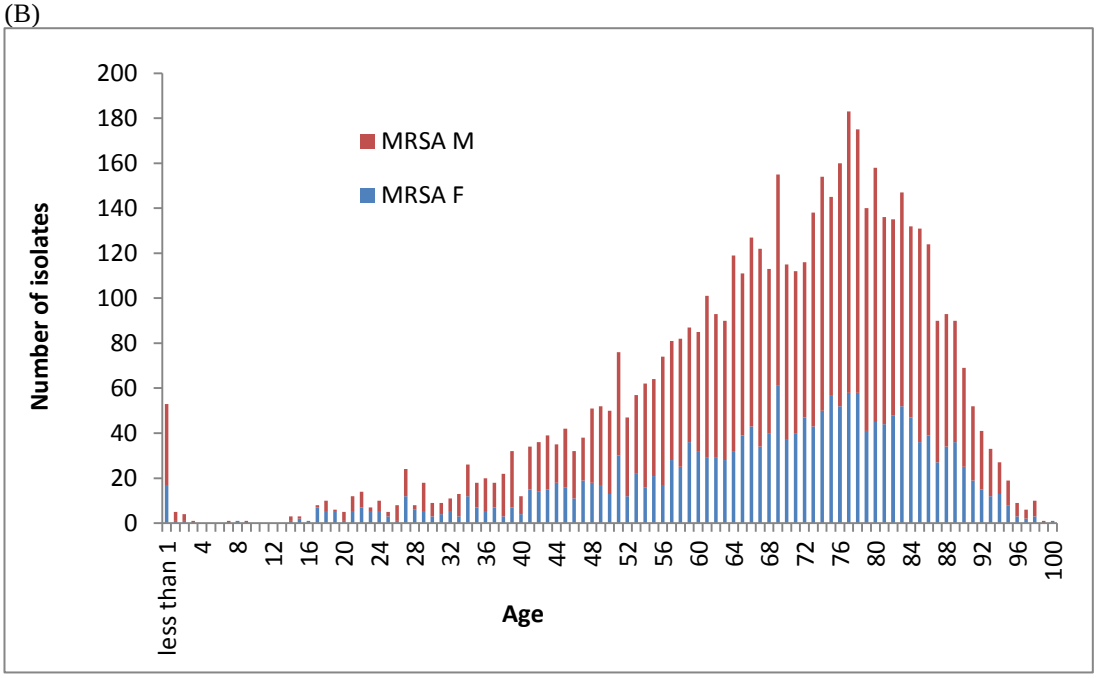
interaction was found to be significant with the model $((organism\ (MSSA\ or\ MRSA) \sim age\ +\ sex\ +\ age * sex)$, (p>0.05)). The only variable that was univariately significant was age (i.e. as age advances, an individual is at an increased risk of MRSA, p<0.0001).

Figure 3-8: Distribution of age and sex for (A) MSSA, (B) MRSA and (C) both MSSA and MRSA for comparable age groups.

(A)

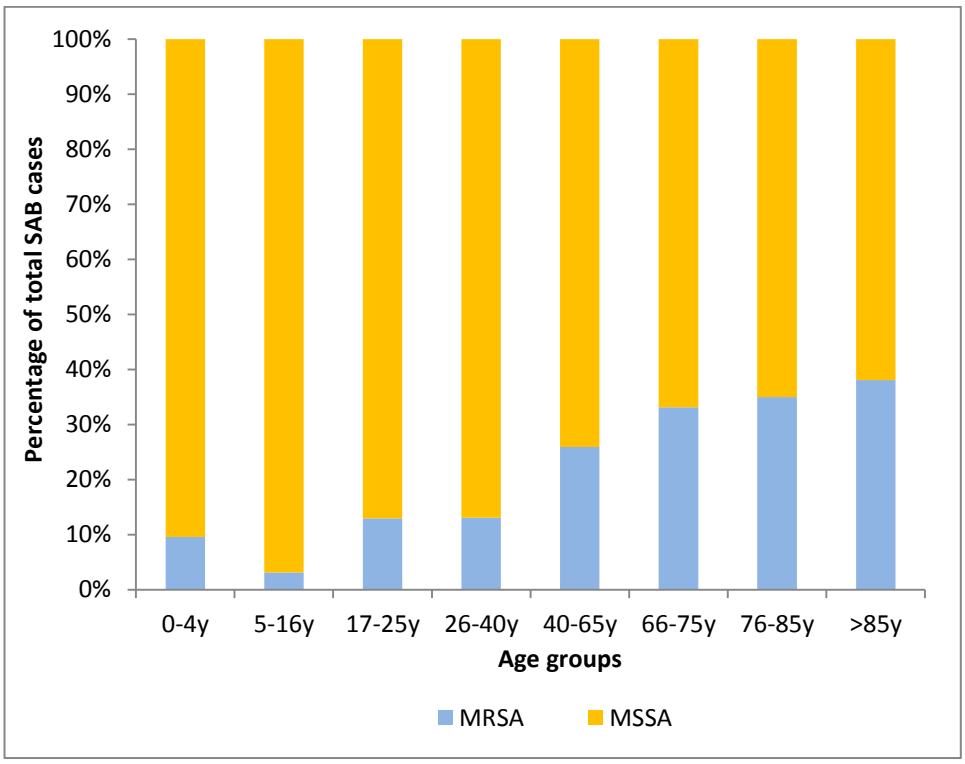


(B)



M= males and F=Females

(C)



3.3.3.4 Device

While much of the information on whether an inpatient was fitted with an intravenous line or catheter at the time of bacteraemia was missing (records exist for 24.0% of MSSA isolates, and 23.3% of MRSA isolates only); the data show that 30.0% and 37.4% of MSSA and MRSA isolates respectively were associated with a patient who had a device. This difference was statistically significant between the two groups ($p < 0.0001$).

3.3.4 Multivariate analysis

The result of the NMS analysis (Table 3-7, Figure 3-9A) was a three-dimensional solution that explained a total of 79.86% of the variation in the data. Additional dimensions provided no significant improvement in fit. The three axes comprised 38.5%, 20.4% and 20.7% of the variation in the data respectively. The first axis, which was rotated to maximise the difference between MSSA and MRSA, described 38.5% of variation in the data. Axes 2 and 3 separated the different specialties (Figure 9B).

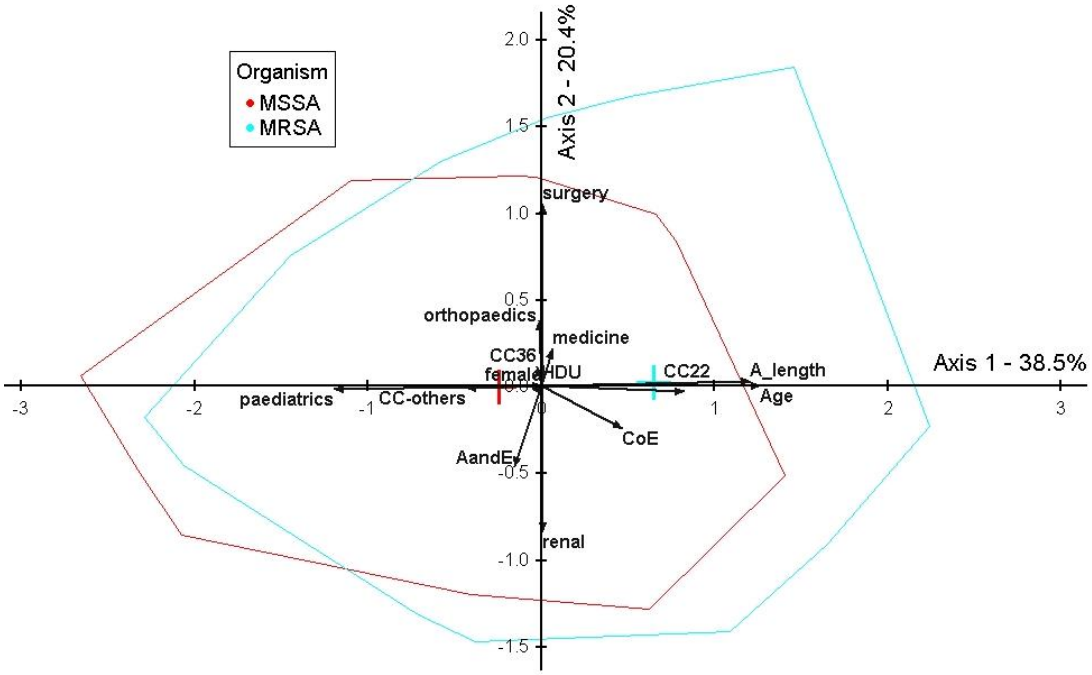
Overall there was considerable overlap between MSSA and MRSA with representatives in all specialties and in all three clones/clonal groups identified. The best separation of MSSA and MRSA was along NMS Axis 1 with MRSA to the right and MSSA to the left. As highlighted by the radiating lines on the joint plot (Figure 3-9), MRSA was associated with older patients, longer antibiogram lengths, CC22 clone and the specialty care of the elderly, and by contrast MSSA was associated with younger patients, shorter antibiogram lengths, non-CC22 and non-CC30 'others' and hospital specialties paediatrics, and accident and emergency.

MRPP was performed to test the null hypothesis of no difference between MSSA and MRSA. Results show (Table 3-7) that the null hypothesis of no difference between the groups can be rejected and the two groups occupy different regions of space as shown by the strong chance-correlated within-group agreement (A) and significance level ($p<0.0001$).

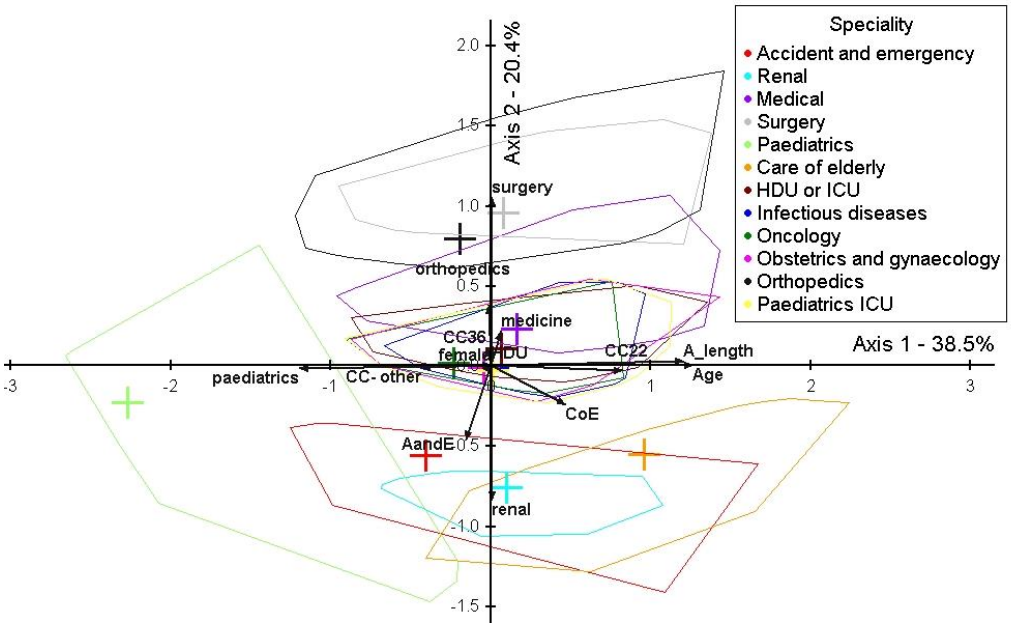
Overall, the NMS and MRPP results show that MSSA and MRSA differ by a combination of patient age, antibiogram length, isolate clone, and hospital specialty. However, as aforementioned while MSSA and MRSA differ, there is some overlap. This means that some MRSA isolates are similar to MSSA isolates (given this combination and the data-reduction of these variables) and some MSSA isolates have attributes more alike that of MRSA.

Figure 3-9: Results of NMS – 2D ordination graphs (Axes 1 and 2) with joint plot with variables associated with (A) MSSA and MRSA, and (B) hospital specialties.

(A)



(B)



NMS differentiated two groups identified as MSSA (red polygon) and MRSA (blue polygon). '+' indicated the centre point of a polygon. The polygons overlap meaning that the two groups share some characteristics. The black radiating lines indicate variables that are significantly associated with either group; for quantitative variables (i.e. age and antibiogram length), the longer the branch, and the stronger the association.
Key: A_length = antibiogram length, specialties: CoE = Care of Elderly, A and E = accident and emergency

Table 3-7: Diagnostic results of NMS.

Diagnostic		NMS results
Final stress[a]		17.86
Monte Carlo test[b]		0.0196
Number of iterations		68
Orthogonality , Axis pair 1 vs 2	r	0.010
	% = 100(1- r^2)	100
Orthogonality , Axis pair 1 vs 3	r	-0.147
	% = 100(1- r^2)	97.8
Orthogonality , Axis pair 2 vs 3	r	-0.040
	% = 100(1- r^2)	99.8
% of variation explained	Axis 1	38.5
	Axis 2	20.4
	Axis 3	20.7
	Total	79.6
MRPP		
(H_0: no difference between groups, MSSA v MRSA)A[c]		0.03782210
	p[d]	0.00000

[a] <5, excellent, no prospect of misinterpretation; 5-10 good , no real risk of drawing false inferences; 10-20 fair, provides a useable picture; >20 poor, dangerous to interpret; 35-40 random placement of samples (*10*)

[b] better-than-random solution

[c] chance-corrected within group agreement: a=1 all identical within group

[d] solution significantly stronger than expected by chance

3.4 Discussion

3.4.1 MRSA bacteraemia decline

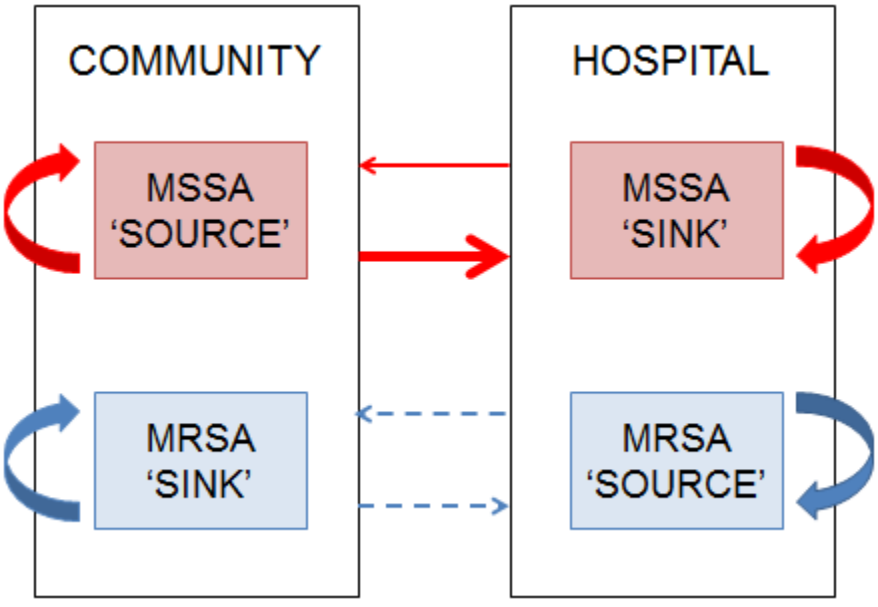
The number of MRSA bacteraemia cases and the proportion of total SAB caused by MRSA have declined steadily in recent years in Scotland. These trends are attributed to a combination of strategies including government legislation on targets, surveillance and reporting, and interventions such as enhanced and intensive cleaning and disinfection, decolonisation programmes, heightened hygiene awareness with alcohol gels and hand washing, physical barriers and other novel approaches [11, 148-152]. Ongoing action to remove MRSA from the hospital environment and particularly in high-risk specialties for MRSA, stands to not only reduce bacteria spread, risk of transmission between patients and therefore lower infection rates in the hospital, but it will also result in fewer discharged patients being colonised or infected. This could have a knock-on effect over time; gradually reducing the number of MRSA isolates spreading in the community and thus reducing the chance of colonised or infected individuals subsequently entering hospital.

3.4.2 Sink-Source Model

It would perhaps be predicted that such actions to eradicate MRSA as a public health problem would also have led to the reduction in MSSA cases but a decline was not observed and MSSA bacteraemia rates remain high. This could be explained by an ecological ‘sink-source’ model where a ‘sink’ population is only maintained by immigration from the ‘source’ (Figure 3-10) [165, 166]. Therefore the source which, in this context is the population of circulating MSSA in the community outside the hospital environment, is a self-sustaining reservoir from which isolates continually bombard or flow to the sink (i.e. hospital environment) with each colonised or infected patient, hospital visitor or member of hospital staff. Given that approximately one third of healthy individuals are asymptomatically colonised with *S. aureus*, this is highly plausible. Isolates could then potentially cause opportunistic community-acquired hospital-onset (CA-HO) infections in colonised hosts, could be spread to other susceptible hosts in the hospital to cause hospital-acquired colonisations or infections, or could be transferred to a surface of hospital furniture for example, where they would remain until spread to humans or removed by

cleaning or cell death. Without constant influx from the source, there is a high probability that the MSSA hospital population (i.e. the sink) would become extinct through stochastic events or, in ecological terms, habitat destruction [165]. For bacterial populations in hospitals, this destruction or removal could involve extensive cleaning or disinfection, or be the result of antimicrobial selection pressures or patient decolonisation. Owing to high selection pressures in hospitals, it is presumed that, given correct dosages, MSSA would be immediately destroyed when challenged with antimicrobials since the vast majority of isolates are susceptible to most antimicrobials in common usage. However, it is speculated that the MSSA population in hospitals can be maintained as it is constantly re-seeded with new wild-type isolates from the community (i.e. source). Hospitals also remain a reservoir for at-risk individuals who are typically physically unwell, who may be immunocompromised or who may have had their skin barriers breached through operation or device. With this combination of a near-constant bacterial population and at-risk individuals; MSSA bacteraemias continue to occur.

Figure 3-10: The ecological sink-source model as applied to MSSA and MRSA circulating isolates in community and hospital environments.



The large red arrow between MSSA source and sink represents the constant and substantial flow of isolates, single red arrow between MSSA sink and source represents a lesser flow of isolates. The broken blue arrows between MRSA sink and source represents diminishing flow of isolates over time. The curly red and blue arrows represent circulation of MSSA and MRSA in the community and hospital setting. Over time, this would also diminish for MRSA.

3.4.3 AMR in MSSA and MRSA

In contrast to MSSA, antibiogram data showed that MRSA were commonly resistant to several antimicrobial classes. MRSA are likely better adapted to the hospital environment than MSSA and could therefore withstand hospital selection pressures. Harbouring many large and non-beneficial AMR genes in a low selection pressure environment has often been associated with reduced bacterial fitness, lessening the ability to spread [49]. On the other hand, in the hospital environment and under high selection pressures, resistance genes can be advantageous for MRSA and a population can be maintained since there is no shortage of potential hosts to colonise or infect.

The MRSA population-level resistance to several antimicrobials (including erythromycin, clindamycin, kanamycin, tobramycin, neomycin, trimethoprim and tetracycline) fluctuated by about 10% over the study period. Fluctuations such as this can be caused by the frequent loss and gain or 'shuffling' of resistance genes carried on mobile genetic element (MGEs) which can be the result of multi-drug, intermittent and or changing antimicrobial usage with MGEs only becoming fixed in a population if they exhibit a selective advantage [34, 47]. It is assumed that once the selection pressure is switched off, then resistance will be lost as it is no longer advantageous to retain it and both mathematical and statistical modelling has shown that selection pressures can rapidly change the population-level of resistance over short time periods (within days or months) [127, 167-169]. However, co-selection of resistances due to genetic linkages of the resistance genes and compensatory mutations to counter the costs of carrying resistance genes, mean that resistance to an antimicrobial not currently in use may also be observed and may potentially take a long time to be lost [49]. This picture is further complicated since the cost of AMR can be a result of epistasis, environmental conditionals that affect fitness in different ways, or some mutations can be cost free [49]. Of note, this analysis showed that resistance to the antimicrobial mupirocin, which is of great interest since it is used to decolonise individuals harbouring MRSA, did not statistically increase over time. However, even when there are statistical differences and fluctuations in resistance levels, it is sometimes unclear what the biological implications are.

3.4.4 The community as a predominant source of MSSA and hospitals as a predominant source of MRSA

While (especially) CC22 and CC30 MRSA may be well adapted to the hospital environment due to extended AMR, these clones may be less well adapted to the community environment than MSSA where the driving force of isolate reduction is the biological cost to the bacteria [49]. In the community where there are much lower selection pressures, it is assumed that MRSA isolates are burdened by large unnecessary resistance genes, e.g. are biologically less fit, and therefore spread less readily than MSSA. MRSA isolates would therefore be less likely to re-seed hospitals from the community 'source' (as is probable with MSSA) since it is hypothesised that MRSA cannot spread well in the community and moreover, with diminishing hospital colonisations and infections over time, fewer discharged infected or colonised patients re-enter the community. Therefore, it is unlikely that the community is the true 'source' and hospitals 'sinks' for MRSA isolates, but instead hospitals should be considered the 'source' and community the 'sink' for MRSA (Figure 3-10). However, with diminishing or in the absence of a constant flow of isolates from source-to-sink, the sink population cannot be sustained. With MRSA more at home in hospitals, it is therefore more likely to cause hospital-acquired (i.e. infected in the hospital) than community–acquired infections (colonised or infected in the community), although community-acquired infections and infections that were hospital-acquired with a subsequent community onset, are also possible.

Several epidemiological aspects point towards a predominant 'community' source for MSSA isolates and hospital source for MRSA. Firstly, in contrast to MRSA, the lack of resistance genes in the MSSA population suggests that MSSA is more adapted to a low selection pressure environment with penicillin resistance presumably exhibiting a current or historic selective advantage. Secondly, while both MSSA and MRSA occur in all hospital specialties, the data suggest that MSSA is more associated with accident and emergency, obstetrics and gynaecology, oncology, orthopaedics, and paediatrics than MRSA which are specialties that commonly serve outpatients and therefore (particularly accident and emergency) see a regular patient turnover. Many of the infections in these specialties could be community-associated

but with a hospital onset (CA-HO). This is often defined as a patient who is colonised or infected while in the community and whose infection develops and is diagnosed within 48 hours of admission. In contrast, MRSA is more associated with long-term care and high-risk patients in the specialties care of the elderly, high dependency units /intensive care units (HDU/ICU), and surgery. It is speculated that the majority of these infections would be hospital-associated, i.e. those that develop more than 48 hours post-admission. The specialties that were found to be more associated with MRSA in this thesis and also previously described as high risk for hospital-acquired infections including MRSA elsewhere [11, 170], are precisely the areas that have been subject to increased patient screening, intense cleaning and hygiene protocols in recent years. Therefore, this 'niche' for MRSA has been challenged and possibly destructed. Thirdly, the abundance of non-CC22 / non-CC30 'other' clones in the MSSA population as compared with the hospital-associated CC22 and CC30 clones again suggests that the community is a more common source for MSSA and the hospital a more common reservoir of MRSA. The NMS analysis described these differences well, but as was observed there is much overlap between the MSSA and MRSA populations and so the categorisation as highlighted by the NMS cannot be used as a tool to diagnose a bacteraemia patient. Therefore, microbiological analyses remain vital.

3.4.5 Limitations

There were several limitations in this study. While every effort was made to remove duplicated isolates from the same patient-visit, there may be further duplicates that were not excluded. For example, duplicates of patients who had a blood specimen taken on subsequent days during the same bacteraemia event, patients who had a blood specimen taken on the same day during the same bacteraemia event at two different locations (following hospital transfer), the same patient-event but with some laboratory or typing error in describing the isolate diagnostics or patient information, or some other reason. The unique patient identity number, community health index or 'CHI', is considered sensitive information and it was therefore removed before accessing these data which made accurately matching patients more challenging. In addition, there was missing information on specialty for 27.7% of MSSA isolates and

29.3% of MRSA isolates and furthermore, it cannot be confirmed that the specialty that the patient was in when the isolate was collected represented the specialty the patient was in when colonised or infected. This may be due to 'boarding' (within-hospital movements) which is very common. In addition, not all hospital specialties are represented in the data which might indicate no cases or perhaps patients are moved when bacteraemia is suspected.

3.4.6 To further this study

To take this analysis further, it would be very useful to sequence the isolates discussed here using next generation sequencing (NGS) which would identify other genes of interest, e.g. other virulence determinants or heavy metal resistance determinants, as well as confirm *spa type,* AMR and toxin types. Bioinformatic and phylogenetic analyses could identify clusters and trace isolates as they move through and evolve in the hospital and community environments. If these data were combined, through large data linkage projects, to link data on patient demographic data, depravity scores, other disease statuses, number of hospital admissions, residency in care home, history of antimicrobial prescribing, history of surgery, disease outcomes/death for example; then a wide range of research question could be addressed.

Similarly, to better understand the hospital and community dynamics of MRSA and MSSA populations, a screening study of individuals entering hospitals or a 'capture-recapture' phylogenetic study to trace strains through the hospital and community environments and therefore test the source-sink hypothesis, may be interesting. The hypothesis could even be tested mathematically if, for example, the assumption that one third of all individuals entering the hospital are colonised is used and if certain information is available, e.g. the proportion of hospital arrivals that use alcohol gels to decolonise their hands, hospital cleaning rates, patient screening and decolonisation rates (which will vary between specialty), in-hospital patient-movements (i.e. boarding), R_0 for MSSA and MRSA, and potentially other factors. Furthermore, laboratory or modelling analyses may enable a more thorough understanding of the difference in fitness between MRSA and MSSA.

3.5 Conclusion

For several decades, MRSA has been considered an important public health problem but the overall population-level burden has steadily decreased in recent years. On the other hand, MSSA continues to be a public health problem as it has always been and causes significantly more cases than MRSA. However, the difference is that MSSA more commonly causes shorter illnesses, lower individual-level burden, lower mortality rates and lower costs to the healthcare system than MRSA due to more extensive choices of successful drug treatments and perhaps other microbiological aspects of the bacterial populations that could not be shown here given the data availability (e.g. possible differences in virulence and pathogenicity).

The results of this study suggest that there are epidemiological differences between MRSA and MSSA including antimicrobial resistance patterns and to some degree 'preferred' niche. These differences may give some understanding as to why MRSA has declined but MSSA has persisted as a public health problem. Here, it is suggested that since MSSA is so abundant in the community (it is estimated that approximately one third of individuals are colonised with *S. aureus*) it will continuously bombard or re-infect the hospital environment via colonised or infected patients, members of staff or visitors. This is related to assumption that MRSA is more preventable than MSSA [171]. People shed MRSA and MSSA into the general environment [172] and the bacteria can survive on hospital items and furniture, survive in the environment including air, be passed by hands and spread to other people. Cleaning, antibacterial washes and antimicrobial use (if susceptible) will remove both MSSA and MRSA from people and the environment in hospitals, but MSSA will be replaced and re-introduced more quickly as it circulates in the community at higher levels.

Screening and decolonisation of high-risk patients in high-risk specialties for MRSA may have had an important impact on MRSA declines. To reduce the number of MSSA bacteraemia cases in hospitals, it may also be important to begin screening and decolonising high-risk patients in high-risk specialties for MSSA. However, as with tackling MRSA, a multifactorial approach will likely be the way to reduce MSSA bacteraemia [127]. In addition, the importance of using alcohol gels to clean

hands on entry to hospitals for every visitor, staff member and patient should be more widely promoted.

Chapter 4: **Antimicrobial resistance of MRSA in Scotland**

4.1 Background

Antimicrobial resistance (AMR) is an historic, current and future issue associated with a range of microorganisms in a diversity of settings including hospitals, the community, livestock, the food chain and the environment [173]. The importance of this public health threat was recently highlighted in the WHO's first global report on surveillance of antimicrobial resistance which advised that every country needed to do more to monitor the problem [18]. Since this publication, there has been much discussion on the possibility of a 'post-antibiotic era' and steps that could be taken to potentially mitigate this situation including an intergovernmental panel on AMR [22].

Bacteria become resistant to antimicrobials as a result of point mutations in chromosomal genes, or through the acquisition of resistance gene-carrying mobile genetic elements [34]. Antimicrobial resistant mutants are selected for and survive in the presence of these drugs with resistance genes becoming fixed in populations when they show a strong selective advantage to the bacteria that outweighs any fitness cost [47]. Bacteria can be concurrently resistant to multiple antimicrobials and the proportion of a bacterial population that is resistant to any given substance is thought to reflect drug usage and selection pressures. For more background information on antimicrobial resistance in *S. aureus*, including selective pressures, molecular mechanisms of resistance, how MSSA becomes MRSA, see Chapter 1.1.2.

Continued surveillance at the local, national and international levels can reveal the extent of AMR, and continued analysis of trends will indicate which antimicrobials are relevant and useful for the treatment or prevention of disease in particular situations or geographic regions. In the long run, this may highlight the need for better antibiotic stewardship, which in turn may help to reduce resistance rates and perhaps preserve the efficiency or longevity of these drugs. Trends may also help to

evaluate the effectiveness of the UK Five Year Antimicrobial Resistance Strategy, which specifically focuses to; improve the knowledge and understanding of AMR; conserve and steward the effectiveness of existing treatments; and stimulate the development of new antibiotics, diagnostics and novel therapies [114].

Staphylococcus aureus (S. aureus) is a universal coloniser of the skin that can cross skin and mucosal barriers to manifest as clinical disease. Following decades of widespread and intensive usage of topical, oral and intra-venous antimicrobials to treat *S. aureus* infections, AMR has become an established and ubiquitous problem in the treatment of infections caused by this microorganism. Resistance rates vary between countries, regions and even hospitals and this variation is thought to reflect drug usage and selection pressures. For more information on AMR in *S. aureus*, see Chapter 1.1.2.1, and for the history of AMR in *S. aureus*, see Chapter 1.1.2.2.

Numerous clones of methicillin-resistant *S. aureus* (MRSA) circulate in the UK but two major epidemic clones have dominated in UK hospitals in recent decades namely EMRSA-15 (CC22, ST22 SCC*mec*IV) and EMRSA-16 (CC30, ST36 SCC*mec*II) [131, 132]. Collectively, infections caused by these clones are reported to account for more than 85% of all MRSA infections [10, 126, 131, 133, 134]. As summarised by Wyllie *et al* [132], recent UK studies identified that these clones followed different epidemic curves. EMRSA-16 initially dominated the UK MRSA scene, peaked and then declined giving way to the rise and dominance of EMRSA-15 [10, 126, 131, 133, 134]. For more information on the major UK clones and the Scottish perspective, see Chapter 1.1.7.2.

The reasons behind this dominance shift and clone displacement are not established but it has been suggested that it could be linked to: increased fitness of EMRSA-15 isolates which would enhance survival, spread and natural selection; interventions against MRSA that are particularly effective against EMRSA-16; microbial competition; and genetic differences [135, 174]. *In vitro* studies have shown that EMRSA-15 isolates are fitter than EMRSA-16 isolates, evidenced by the ability of EMRSA-15 to grow independently in rich broth, competitiveness for nutrients in culture, and the ability to survive stress and desiccation [34, 174].

The objective of this study was to analyse temporal data of antimicrobial resistance for dominant clones in Scotland from 2003 to 2012 by examining resistance to 20 individual antimicrobials, in particular, the proportion of clinical isolates with antimicrobial resistance and the diversity of resistance. An additional objective was to provide a comparison of clinical and non-clinical 'screening' isolates (as defined below). This was to determine if screening isolates are an appropriate proxy measure of clinical isolates that could identify the circulating clones and characteristics of those clones in the Scottish MRSA population.

4.2 Materials and methods

4.2.1 Data collection

From 2003 to 2012, the Scottish MRSA reference laboratory (SMRSARL) collected MRSA isolates in the baseline survey 'snapshot'. The programme was a volunteer referral system that requested diagnostic laboratories across Scotland to send all MRSA isolates received during a single specified week, four times per year, to SMRSARL. In this survey, MRSA isolated in hospitals and GP surgeries from all body sites, whether colonisation or infection, sent to any diagnostic laboratory for identification, were referred to the reference laboratory for further testing.

Data from the snapshot database were divided into two time periods for the purpose of analysis: Time period A: 2003-2006 inclusive and Time period B: 2009-2012 inclusive. Data collection was interrupted in 2007 and 2008, so data from this time frame were not included in this study. Isolates were designated as either clinical (wound swabs, blood, genital, respiratory or urine samples from infected individuals) or screening (nasal, throat, axilla and groin swabs (as previously described [10])). MRSA isolates from screening samples were analysed separately from clinical isolates. The two groups were compared to establish whether they differed in terms of antimicrobial resistance, and other epidemiological parameters discussed below, to determine if screening isolates were a useful representation of clinical isolates. While the main focus of this study was the similarities and differences between EMRSA-15 and EMRSA-16, all other non-EMRSA-15 and non-EMRSA-16 clone types (herein collectively referred to as "others") are discussed but no formal statistical analyses

were done on this group. Isolates were assigned to one of these three clonal 'groups' as per typing methods at the SMRSARL (see below).

Table 4-1 shows the number of isolates and the percentage of total isolates represented by clones EMRSA-15, EMRSA-16 and non-EMRSA-15 and non-EMRSA-16 'others' for each year and time period both clinical and screening isolates. There were totals of 1221 and 1055 clinical isolates in time periods A and B, respectively, and 467 and 1018 screening isolates in time periods A and B, respectively. Data collected included patient age, sex, NHS health board of origin, and specimen type (blood, genital, respiratory, wound, urine, and other). Isolates with specimen information missing were removed from the analysis (time period A: total = 99, of which, EMRSA-15 n=76, EMRSA-16 n= 21, others n= 2; time period B: total = 99, of which, EMRSA-15 n=134, EMRSA-16 n= 15, others n= 17).

Table 4-1: Number of isolates and percentages of total isolates represented by clones by year for clinical and screening isolates.

	2003	**2004**	**2005**	**2006**	**2009**	**2010**	**2011**	**2012**	**total**	**time A**	**time B**
Clinical isolates											
EMRSA-15	266	220	205	288	250	281	191	157	1858	979	879
EMRSA-16	62	44	51	57	32	22	19	11	298	214	84
'Others'	4	10	3	11	15	17	24	36	120	28	92
Total	332	274	259	356	297	320	234	204	2276	1221	1055
Screening isolates											
EMRSA-15	60	73	105	121	152	308	225	150	1194	359	835
EMRSA-16	22	22	17	24	24	40	19	7	175	85	90
'Others'	2	4	9	8	7	22	32	32	116	23	93
Total	84	99	131	153	183	370	276	189	1485	467	1018

4.2.2 Isolate classification and antimicrobial testing

Briefly, isolates received from diagnostic laboratories by the SMRSARL were confirmed as *S. aureus* using a latex slide agglutination test (Staphytect Plus©), a

rapid agglutination test (PASTOREX© Staph-plus kit), a tube coagulase test, and a PCR testing for the *nuc* gene. PCR testing for the *mec* gene was carried out for identification of MRSA, and the Vitek®2 gram positive ID card for species-level organism identification. Details are fully described in Chapter 3.2.2.

Isolates confirmed as *S. aureus* were typed as EMRSA-15, EMRSA-16 or 'others' following standard procedures at the SMRSARL using a combination of pulsed-field gel electrophoresis (PFGE), PCR ribotyping, biotyping (profiling based on a combination of isolate pigmentation, urease and tween tests), sequence typing (ST) (seldom carried out) and more recently, *spa* typing (seldom carried out in time period A).

Antimicrobial testing was carried out using an automated method and following standard operating procedures for *in vitro* testing of bacterial susceptibility to antimicrobial agents using Vitek® sensitivity cards. Susceptibility to the following antimicrobials was tested consistently over the study period; ceferoxin/cefoxitin (Cx), chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Km), linezolid (Lz), methicillin/oxacillin (Mt), mupirocin (high level) (Mp), neomycin (No), penicillin (Pn), rifampicin (Rf), streptomycin (St), sulphamethoxazole (Su), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), and trimethoprim (Tr). Resistance susceptibility to daptomycin (Dp), moxifloxacin (Mx), quinupristin (Qp), togamycin (Tg) and vancomycin (Va) was also tested for but not consistently over the whole study period. Therefore, these four antimicrobials were not included in the main analyses.

Testing for the presence of Panton-Valentine Leukocidin toxin gene (*pvl*) and toxic shock syndrome toxin gene (*tst*) was carried out using polymerase chain reaction (PCR) as is standard procedure at SMRSARL.

4.2.3 Data management and statistical analysis

Unless otherwise stated all analyses refer to clinical isolates.

4.2.3.1 Background epidemiology

The percentage of total isolates that were EMRSA-15, EMRSA-16 or 'others', by year and time period, were described. This confirmed EMRSA-15 as the dominant clone in the Scottish MRSA population. Differences in the percentage of isolates that were EMRSA-15 and EMRSA-16 across time periods were examined using a General Linear Model (GLM). Descriptive analyses of age, sex, clinical specimen (i.e. blood, genital, respiratory, swab or urine) and presence of toxin genes *pvl* and *tst* were also conducted. To investigate if a patient's age (log transformed) varied by patient sex, clone, and/or time period, the following linear regression model was used: $(\log(age) \sim sex * clone * time\_period)$. Associations between clinical specimen type and clone were examined using stratified $2x2$ tables. As a result of insufficient samples for time period A, the proportions of isolates that tested positive for the *pvl* or *tst* toxin genes were analysed for time period B only, and *spa* typing was also only available for time period B. Only descriptive analyses were conducted for 'others' as, especially in time period A, they were too few in number, and too heterogeneous, encompassing several MLST / *spa*-types.

Unless otherwise stated, all analyses were carried out in R (version 3.1.2) [154]. Statistical significance was set at $p<0.05$.

4.2.3.2 Antimicrobial Resistance

4.2.3.2.1 Univariate analysis of single antimicrobials

The percentage of clinical EMRSA-15, EMRSA-16 and 'others' resistant to 20 antimicrobials was calculated for both time periods A and B. This information was displayed on radar charts. Pearson's chi square tests or Fisher's exact tests were used to assess the significance of changes in percentage over time for bacterial populations and time. The choice of Pearson's chi square tests or Fisher's exact tests was dependent on sample sizes; i.e. where ≤5 counts appeared in one box of the 2x2 table, then the Fisher's exact test was used, where all counts in the 2x2 table where >5, then the Pearson's chi square test was used. Difference between clinical and screening isolates were plotted on radar charts and analysed by Pearson's chi-square tests or Fisher's exact tests.

4.2.3.2.2 Multivariate analysis using Nonmetric Multidimensional Scaling

To further investigate differences between EMRSA-15 and EMRSA-16 isolates, a data reduction and nonparametric ordination technique called nonmetric multi-dimensional scaling (NMS) analysis was carried out. NMS is appropriate for data that are non-normal and does not have the assumption of linear relationships between variables that is common to other data reduction techniques. The analysis aims to visualise the level of dissimilarities between isolates in an *N*-dimensional space or scatterplot using a ranked distance matrix that was calculated with a Euclidian distance measure. The dimensionality of the plot was determined by plotting an inverse measure of fit (known as stress) to the number of dimensions with the optimal dimensionality being the number of dimensions with the lowest stress. This optimisation strategy aims to create a configuration which represents the smallest departure from monotonicity in the relationship between distance in the original space and that in the reduced ordination space. Five hundred iterations were used for each NMS run, using random starting coordinates. Several NMS runs were performed to ensure that the solution was stable and represented a configuration with the best possible fit.

The NMS was performed using two matrices created from the data; matrix 1 and matrix 2. Information on antimicrobial resistance (binary (resistant versus not, 1/0)) for antimicrobials with variation greater than 5% (i.e. more than 5% of isolates resistant or more than 5% of isolates not resistant): including Cl, Cp, Er, Gn, Km, No, Tb, Te, Tr and removing Mt, Pn, Cx, Gn, Mp, Rf, Fd, Tc, Su, St, Lz, Ch) was included in matrix 1 and used to perform the NMS.

To identify variables that were associated with either group of interest (i.e. EMRSA-15, EMRSA-16), matrix 2 was superimposed on the ordination to create a joint plot (radiating lines). The direction of the lines indicates either a positive or negative relationship with an Axis and the variables of matrix 2, and (if the variable is continuous) the length shows the strength of the correlation on that axis. The ordination was rotated in order to ensure maximum separation of the groups of interest (EMRSA-15, EMRSA-16) along Axis 1. Variables in matrix 2 included information on antimicrobial resistance, antibiogram length (see below), isolate

pigmentation, urease test result, clinical specimen type, patient gender and age, NHS Health Board, year and time period. Multi-response Permutation Procedures (MRPP) analysis was performed to test the hypothesis of no difference between the two groups, EMRSA-15 and EMRSA-16, and the association of variables with either group. The NMS was performed in PC-ORD version 6.03 (MJM software Design, Gleneden Beach, OR). Scatterplots and 80% confidence ellipses were drawn to visualise the results.

4.2.3.3 Antibiogram lengths

An antibiogram is the combination or string of antimicrobials to which an isolate is resistant, e.g. if an isolate is resistant to penicillin, methicillin and erythromycin, the antibiogram is PnMtEr and the antibiogram length is 3. Antibiogram length is the total number of antimicrobials to which an isolate shows phenotypic resistance. Student's t-tests were used to assess if there were significant differences in mean antibiogram lengths. A Student's t-test was used, as the central limit theorem states that for large sample sizes (n>30, as a rule of thumb) the sample mean will be approximately normally distributed. Antibiogram length was also included in the aforementioned NMS analysis as an overlay from matrix 2.

4.2.3.4 Antibiogram diversity

Clinical and screening isolates were analysed separately. Four ecological diversity indices related to Rényi's measures of generalised entropy were used to compare antibiogram diversity of EMRSA-15 and EMRSA-16 for both time periods A and B. This method, with specific reference to antimicrobial resistance, was described by Mather *et al* [175].

The exponential of Rényi's entropy (D_α) estimates the effective number of species (or in this case, antibiograms) where α represents different weights of and adjusts for the number of antibiograms (richness) and relative abundances of antibiograms. Briefly, the diversity indices compared were: antibiogram richness (AR), a count of antibiograms (which ignores the relative abundance or weight of each antibiogram, e.g. rare and common antibiograms are considered equal), D_0; Shannon entropy (SEn), the probability of any two isolates drawn at random from an infinitely large

population being the same antibiogram, log(D_1); Simpson diversity (SD), the relative abundance of each antibiogram, 1/D_2; and Berger-Parker (BP), the proportion of the most common antibiogram in the sample, 1/D_∞. Owing to differences in sample sizes between EMRSA-15 isolates, EMRSA-16 isolates and time periods, resampling of 99 isolates (this was the sample size of the smallest clinical subset, EMRSA-16 time period B) and 85 isolates (the sample size of the smallest screening subset, EMRSA-16 time period A) was carried out 1000 times (bootstrapped) within the other respective subsets. This sampling 'with replacement' method provided a mean and confidence intervals (CIs) for the diversity measures of EMRSA-15 in time periods A and B and of EMRSA-16 in time period A for clinical isolates, and same for diversity measures of EMRSA-15 in time periods A and B, and of EMRSA-16 in time period B for screening isolates. This allowed diversity measures of clones to be compared. This method was described by Mather *et al* [175]. This information is given in a table and in a diversity figure in which each index is represented along the x-axis and the effective number of antibiograms is on the y-axis. 'Others' were not included in this analysis since the sample sizes in time period A for clinical (n=28) and screening (n=23) isolates were the lowest of all clone-time period groups. Sampling with replacement using these low sample sizes would have resulted in wide, confidence intervals for the 'other' clones and uncertain conclusions.

4.3 Results

4.3.1 Background epidemiology of clinical isolates

Over the study period, there were two major epidemic clones of MRSA in Scotland; EMRSA-15 (CC22, ST 22 SCC*mec*IV) and EMRSA-16 (CC30, ST36 SCC*mec*II). The number of EMRSA-15 isolates received over the course of the study was 4.6 times higher than EMRSA-16 in time period A and 10.5 times higher in time period B (Table 4-2 and Figure 4-1). Together, EMRSA-15 and EMRSA-16 comprised 97.7% of all clinical MRSA isolates in Scotland in time period A and 91.3% in time period B. Non-EMRSA-15 / non-EMRSA-16 *spa* types corresponding to sequence types including but not limited to ST1 (t127), ST5 (t002), ST8 (t008), ST80 (t044), ST88(t186), and ST239 (t037) made up the remainder. Appendix Table 1 lists all s*pa* types associated with EMRSA-15, EMRSA-16 and 'others' for time period B.

The percentage of all isolates that were EMRSA-15 did not significantly change over time (p>0.05), while the percentage of isolates that were EMRSA-16 significantly decreased over time (p=0.002). The percentage of 'others' increased over time (p=0.01) from 1.2% in the first year of the study to 17.6% in the final year. While t002 was the most common *spa* type associated with 'others' (time period B, n=14), it was not possible to conclude whether this clone could emerge as the next 'major' clone as there was insufficient information from time period A. Figure 4-1 shows general trends and percentage of isolates that were EMRSA-15, EMRSA-16, and 'others' by year.

The mean patient ages for EMRSA-15 (71.2 years) for both time periods were the highest, followed by EMRSA-16 (66.8 years) and then 'others' (49.7 years) (Table 4-3). There were significant differences in the mean age of patients between EMRSA-15 and EMRSA-16 (p=0.01), EMRSA-15 and 'others' (p<0.001), and EMRSA-16 and 'others' (p<0.01) for time period A, and between EMRSA-15 and EMRSA-16 (p=0.02), EMRSA-15 and 'others' (p<0.0001), and EMRSA-16 and 'others' (p<0.0001) for time period B. No clone differed in mean age between time periods (i.e. no difference between EMRSA-15 time period A versus B, p>0.05). The proportion of EMRSA-15 isolates from males was statistically significantly lower than that of EMRSA-16 for time period B (49.5% and 64.3% respectively, p=0.01) (Table 4-3). There were no other significant differences in the proportion of isolates from males between clones and time periods. In investigating the relationship between patient's (log) age and sex, clone, and/or time period$\log(age) \sim sex * clone * time_period$), no interaction was found to be significant (p>0.05), indicating that there is no difference in the age with respect to sex, clone and time period.

Table 4-4 lists the number of EMRSA-15, EMRSA-16 and 'others' from each clinical specimen for time periods A and B. Of all clinical EMRSA-15, EMRSA-16 and 'others', the majority of specimens (68%, n=1552/2276) were from wound /skin swabs (other than for screening). Overall, urine and respiratory specimens comprised approximately 12% each and genital and blood comprised 4% of total isolates. After wound /skin swabs, the next most common known specimen type for EMRSA-15

time period A (13.9%) and B (14.2%) respectively was urine, for EMRSA-16 time period A (19.2%) and B (15.5%) was respiratory, and for 'others' time period A (10.7%) was respiratory and B (7.6%) was genital. There were no or marginal statistically significant differences in the proportions of each isolates from each clinical specimen for EMRSA-15 between time period A and B ($p=0.05$) and EMRSA-16 between time period A and B ($p>0.05$). There were statistically significant differences in the proportions of a clone from each clinical specimen between EMRSA-15 and EMRSA-16 for time period A ($p<0.01$) and time period B ($p<0.0001$). Blood specimens, which indicate the disease outcome with the highest morbidity and mortality, i.e. bacteraemia or bloodstream infections (BSI), comprised less than 5.2% of infections for each clone and time period but were highest for EMRSA-16 time period A (5.1%).

Associations between specimen type and clone were examined using stratified 2x2 tables (Table 4-4B). For all clinical specimen types except wound /skin swabs there was no difference across time frames ($p>0.05$) and therefore a common odds ratio was generated. Respiratory samples were 1.74 times more likely to be EMRSA-16 than EMRSA-15 ($p<0.0001$). Urine and genital samples were more likely to be EMRSA-15 than EMRSA-16. Blood samples were equally likely to be EMRSA-15 or EMRSA16 ($p=1.0$). There were differences across the time periods with respect to wound /skin swabs specimens. In time period A there was no difference between EMRSA-15 and EMRSA-16, however, in time period B wound /skin swabs specimens were almost 2x more likely to be EMRSA-16 than EMRSA-15 (odds ratio 1.95 (CI: 1.11-3.61)).

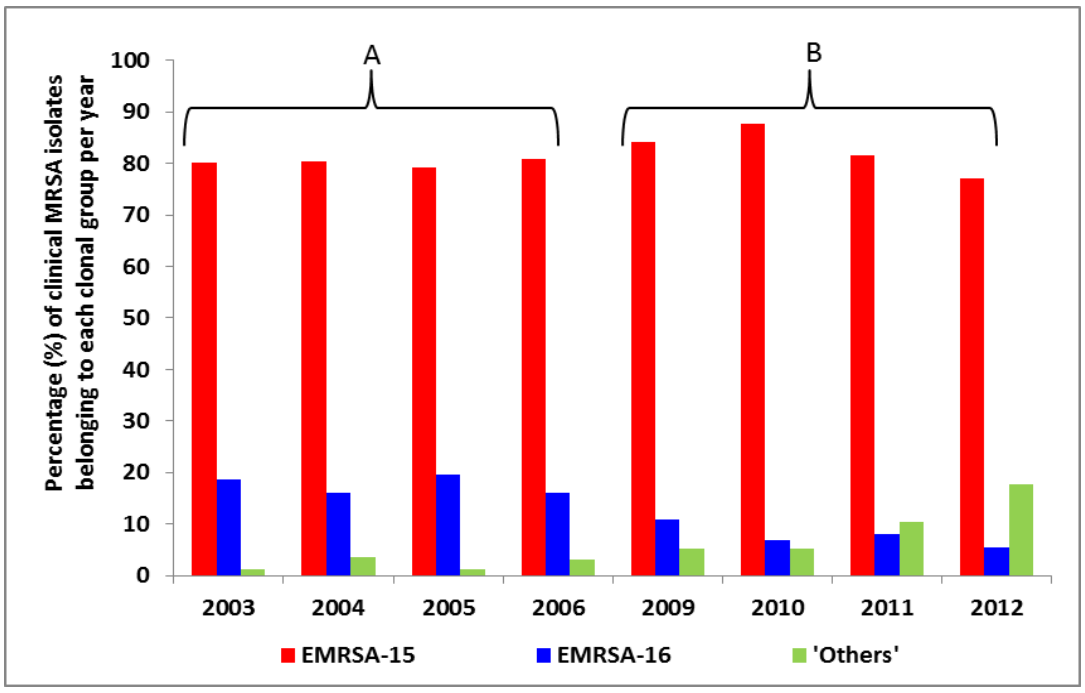
In time period A, only 1.6% and 0.2% of total clinical isolates were tested for the presence of the *pvl* and *tst* genes, however in time period B 98.7% of isolates were tested for both toxins. Therefore, only isolates from time period B are examined in terms of toxin genes. For EMRSA-15, EMRSA-16 and 'others', the percentages of isolates that were tested for *pvl* presence were 98.6%, 97.6%, and 100%, and the percentage with the *pvl* gene present was 1.2%, 9.8%, and 19.6%. For EMRSA-15, EMRSA-16 and 'others', the percentages of isolates tested for *tst* presence were 98.6%, 97.6%, and 100%, and the percentages with the *tst* gene present were 0.5%,

94.4%, and 4.3%. There were statistical differences in the proportions with *pvl* toxin and *tst* toxin with EMRSA-16 having higher proportions of both *pvl* and *tst* gene than EMRSA-15 (both p<0.0001), and higher odds of a *pvl* positive (odds ratio =0.1) or *tst* positive (odds ratio =0.0003) isolate being EMRSA-16 than EMRSA-15.

Table 4-2: Percentages of clinical isolates represented by each clone type, by year and time period.

	2003	2004	2005	2006	2009	2010	2011	2012	total	time A	time B
Percentage EMRSA-15	80.5	80.5	77.6	80.6	84.1	87.4	82.1	76.5	81.4	79.9	83.0
Percentage EMRSA-16	18.4	15.8	21.3	16.4	11.1	6.7	9.3	5	13.1	17.8	8.1
Percentage 'Others'	1.1	3.7	1.1	3.1	4.8	5.9	8.6	18.5	5.5	2.3	8.9

Figure 4-1: The percentage of clinical MRSA isolates that represented EMRSA-15, 16, and 'others' by year and time period.



Time periods A and B are highlighted with parenthesis.

Table 4-3: Age and sex of individuals that clinical isolates were taken from.

		Age		Sex	
Clone and time period	***N***	**Mean**	**Missing**	**Proportion Male**	**Missing**
EMRSA-15					
Time A	979	70.8	95	49.6	8
Time B	879	71.2	0	49.5	1
EMRSA-16					
Time A	214	67.5	25	56.1	2
Time B	84	66.2	0	64.3	0
'Others'					
Time A	28	51.8	1	64.3	0
Time B	92	49.3	0	48.4	1

'Missing' indicates where there are missing data for individuals. The denominator used to calculate 'Proportion Male' was number of males + number of females with missing excluded.

Table 4-4: Different clinical specimens from which MRSA was recovered by clone and time period.

Clinical specimen	**Clone and time period**						**Row Total**
	EMRSA-15		**EMRSA-16**		**'Others'**		
	A	B	A	B	A	B	
Blood							
n	40	33	11	1	1	2	88
%	4.1	3.8	5.1	1.2	3.6	2.2	3.9
Genital							
n	30	49	3	1	1	7	91
%	3.1	5.6	1.4	1.2	3.6	7.6	4
Respiratory							
n	117	84	41	13	3	3	261
%	12	9.6	19.2	15.5	10.7	3.3	11.5
Swab (wound or SSTI)							
n	656	588	142	67	22	77	1552
%	67	66.9	66.4	79.8	78.6	83.7	68.2
Urine							
n	136	125	17	2	1	3	284
%	13.9	14.2	7.9	2.4	3.6	3.3	12.5

SSTI = Skin and soft tissue infections

4.3.2 AMR

4.3.2.1 Levels and patterns of AMR – Univariate analysis

The percentage of EMRSA-15 (Figure 4-2A), EMRSA-16 (Figure 4-2B) and 'others' (Figure 4-2C) resistant to 20 consistently tested antimicrobials were compared for two time periods. All EMRSA-15 isolates were resistant to methicillin (by definition, all are MRSA) and penicillin (Figure 4-2A). Resistance to cefoxitin and ciprofloxacin significantly decreased in time period B from 99.3% to 96.8% ($p<0.001$) and 99.8% to 98.4% ($p<0.01$), respectively. Resistance significantly increased for clindamycin from 5.1% to 9.0% ($p<0.01$), kanamycin from 1.5% to 7.7% ($p<0.0001$), trimethoprim from 14.9% to 20.4% ($p<0.01$), gentamicin from 1.5% to 5.4% ($p<0.0001$), mupirocin from 0.3% to 1.8% ($p<0.01$) and tetracycline from 4.9% to 9.2% ($p<0.001$). Of the remaining antimicrobials, with the exception of erythromycin, resistance to all was less than 1.5% in time period A and less than 2.5% in time period B. There were no significant differences between the time periods for any of these antimicrobials.

All EMRSA-16 isolates were resistant to methicillin and penicillin (Figure 4-2B). In time period A, >90% of isolates were resistant to cefoxitin (100%), ciprofloxacin (100%), erythromycin (99.1%), and clindamycin (90.6%), and resistance to ciprofloxacin and erythromycin statistically significantly declined by 10.7% and 8.5% respectively in the second time period ($p<0.0001$ and $p<0.001$). In time period A, between 80-90% of isolates were resistant to kanamycin (86.4%), tobramycin (85.1%) and neomycin (83.6%) but this fell to 63.1%, 71.2% and 57.5% respectively in the second time period ($p<0.0001$, $p=0.01$, and $p<0.0001$ respectively). The greatest increase in resistance was observed in the second time period for tetracycline which increased from 3.3% to 22.6% ($p<0.0001$). Resistance to trimethoprim (A=38.8%, B=42.9%), gentamicin (A=22.4%, B=29.8%) and mupirocin (A=16.4%, B=27.4%) was moderate in both time periods but did not significantly change. Resistance to all other antimicrobials was between 0% and 2.4% in time period A, and 0% and 4.8% in time period B and there were no significant differences between the time periods for any of these other antimicrobials.

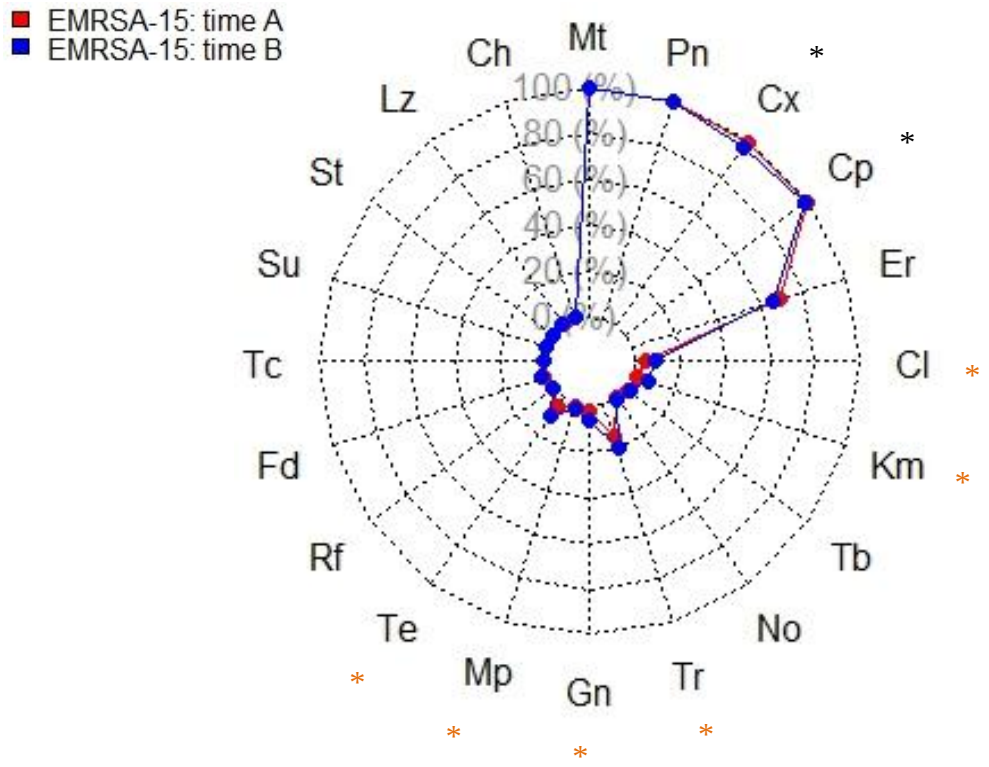
Out of those 20 consistently tested antimicrobials, resistance to two antimicrobials declined and resistance to six antimicrobials increased among EMRSA-15, and resistance to five antimicrobials declined and to one antimicrobial increased among EMRSA-16. Some additional antimicrobials were also tested for (i.e. moxifloxacin, quinupristin, vancomycin, togamycin, and daptomycin) albeit not consistently across the study period. Resistance to moxifloxacin was only tested in time period A and for EMRSA-15 (n=711) and EMRSA-16 (n=152) isolates, 2.4% and 2.0% of isolates respectively were resistant. No isolates were resistant to quinupristin (EMRSA-15, time period A, n=711; EMRSA-16, time period A, n=152), vancomycin (EMRSA-15, time period B, n=879; EMRSA-16, time period B, n=84), or togamycin (EMRSA-15, time period B, n=879; EMRSA-16, time period B, n=84). Resistance to daptomycin was only tested in 2011 and 2012 for EMRSA-15 (n=166) and EMRSA-16 (n=11) isolates, no isolates were resistant.

For non-EMRSA-15 / non-EMRSA-16 'others', it is difficult to determine a pattern in resistance since the group contained several different clones (Figure 4-2C). Aside from resistance to mupirocin and chloroimphenicol, which slightly increased (but not significantly), resistance to all other antimicrobials declined or did not change between time periods A and B. Resistance to clindamycin fell by 23.8% ($p<0.01$), kanamycin by 29.2% ($p=0.01$), tobramycin by 30.1% ($p<0.01$), neomycin by 40.6% ($p<0.001$), trimethoprim by 31.2% ($p<0.01$), gentamicin by 22.7% ($p=0.01$), tetracycline by 22.7% ($p=0.01$), sulphamethoxazole by 40.7% ($p<0.0001$) and streptomycin by 34.9% ($p<0.0001$).

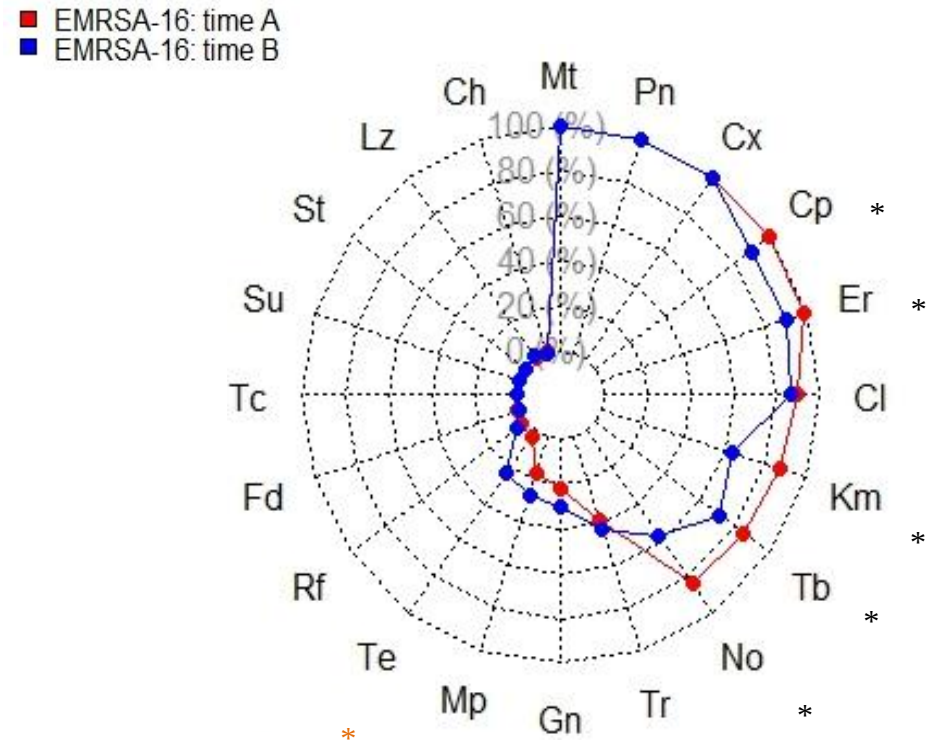
Figure 4-2: Radar chart of antimicrobial resistance in (A) EMRSA-15, (B) EMRSA-16 and (C) 'others' for time periods A and time period B.

Percentages shown represent the percentage of each clone population with each time period that was resistant to each antimicrobial for time periods A and B.
Orange / black asterisks indicate significant increase / decrease in resistance (significant if $p \leq 0.05$).
The following abbreviations are used to indicate each antimicrobial; cefoxitin (Cx), chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Km), linezolid (Lz), methicillin (Mt), mupirocin (Mp), neomycin (No), penicillin (Pn), rifampicin (Rf), streptomycin (St), sulphamethoxazole (Su), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), and trimethoprim (Tr).

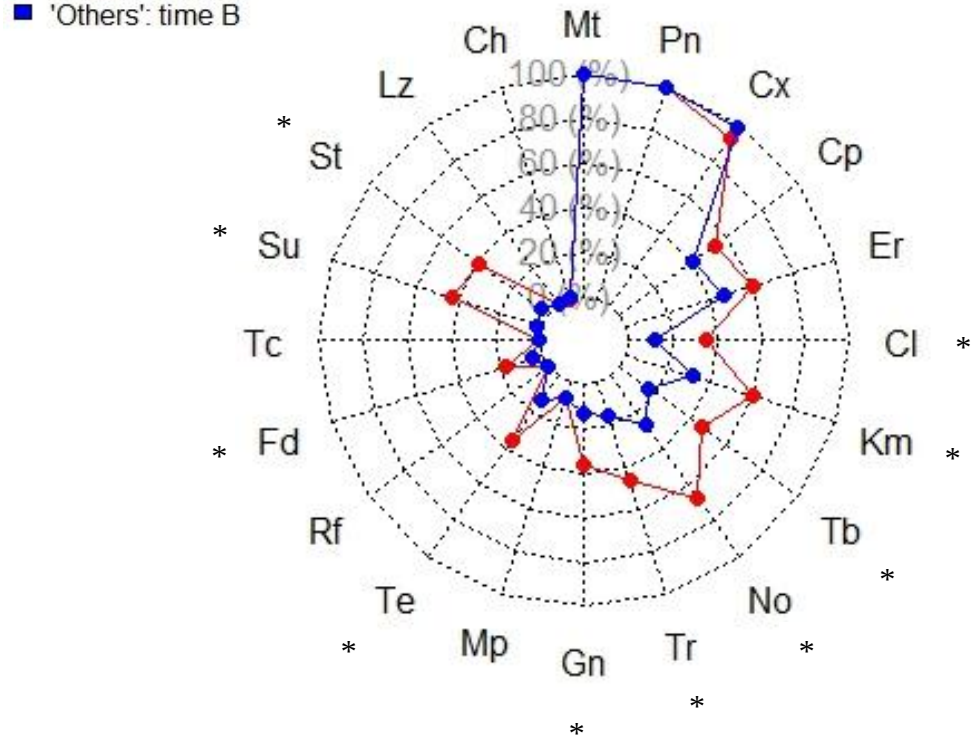
A)



B)


EMRSA-16: time A
EMRSA-16: time B
Mt
Pn
Cx
Cp
Er
Cl
Km
Tb
No
Tr
Gn
Mp
Te
Rf
Fd
Tc
Su
St
Lz
Ch
100 (%)
80 (%)
60 (%)
40 (%)
20 (%)
0 (%)

C)


'Others': time A
'Others': time B
Mt
Pn
Cx
Cp
Er
Cl
Km
Tb
No
Tr
Gn
Mp
Te
Rf
Fd
Tc
Su
St
Lz
Ch
100 (%)
80 (%)
60 (%)
40 (%)
20 (%)
0 (%)

4.3.2.2 Multivariate MDS analysis

The result of the NMS analysis (Table 4-5, Figure 4-3A, B, C), which only included EMRSA-15 and EMRSA-16 isolates, was a two-dimensional solution that explained a total of 84.2% of the variation in the data. Additional dimensions provided only a slight (non-significant, $p>0.05$) improvement in fit. The first axis, shown on the joint plot and which was rotated to maximise the difference between EMRSA-15 and EMRSA-16, described 74.5% and Axis 2 only 9.7% of the variation in the data (Figure 3A).

EMRSA-15 isolates were associated with a positive urease test, cream or white isolate pigmentation, shorter antibiogram lengths, urine and genital specimens, and sensitivity to clindamycin, erythromycin, kanamycin, neomycin and tobramycin on Axis 1. EMRSA-16 isolates were associated with a negative urease test, yellow isolate pigmentation, longer antibiogram lengths, respiratory specimens, the presence of *tst* toxin genes and resistance to kanamycin, neomycin, tobramycin, clindamycin, and erythromycin on Axis 1(Figure 3A). EMRSA-15 and EMRSA-16 also differed by resistance to trimethoprim and gentamycin on Axis 2 (Figure 3A). Isolate pigment and the urease test, which are used as diagnostic tools to broadly distinguish between EMRSA-15 and EMRSA-16 isolates, were shown to differentiate between the two groups effectively. Results from the NMS support and validate the results shown in Figure 2.

In the analysis, a positive Tween test result, the presence of *pvl* toxin genes, patient age and sex, wound/skin swabs and blood specimens, and resistance to ciprofloxacin and tetracycline were common to both groups (i.e. EMRSA-15 and EMRSA-16). Although both groups (EMRSA-15 and EMRSA-16) shared some properties (as seen by overlapping confidence ellipses), the centroid points (as shown by a cross) for each group are distinct (Figure 3B). Furthermore, MRPP was performed to test the null hypothesis of no difference between EMRSA-15 and EMRSA-16 ($p<0.0001$). Results (Table 4-5) show that the null hypothesis can be rejected since the two groups occupy different regions of space as shown by the strong chance-correlated within-group agreement (A) and significance level (p).

Figure 3C shows EMRSA-15 and EMRSA-16 for both time periods. The close proximity of the centroids for EMRSA-15 for both time periods indicates that there is little change between the time periods, and in contrast the centroids for EMRSA-16 for both time periods are more distant indicating change between the time periods. Furthermore, the confidence ellipse for EMRSA-16 time period B encompasses all other clone-time periods, again highlighting a diverse antibiogram and small sample size.

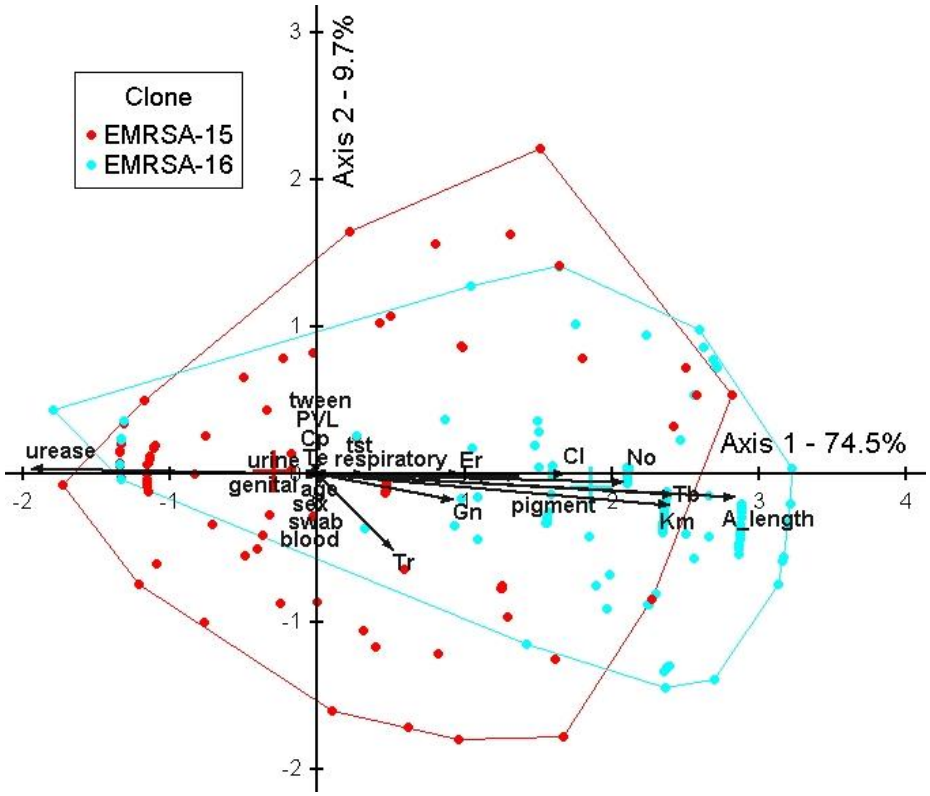
Table 4-5: Results of NMS.

Diagnostic	NMS results
Final stress[a]	6.91543
Monte Carlo test[b]	0.000196
Number of iterations	132
Orthogonality , Axis pair 1 vs 2	
r	0.0000
% =100(1- r^2)	100
MRPP (H_0: no difference between EMRSA-15 and EMRSA-16)	
A[c]	0.19
p[d]	<0.0001
% of variation explained	
Axis 1	74.5
Axis 2	9.7
Total	84.2

[a] <5, excellent, no prospect of misinterpretation; 5-10 good , no real risk of drawing false inferences; 10-20 fair, provides a useable picture; >20 poor, dangerous to interpret; 35-40 random placement of samples (*10*)
[b] better-than-random solution
[c] chance-corrected within group agreement: a=1 all identical within group
[d] solution significantly stronger than expected by chance

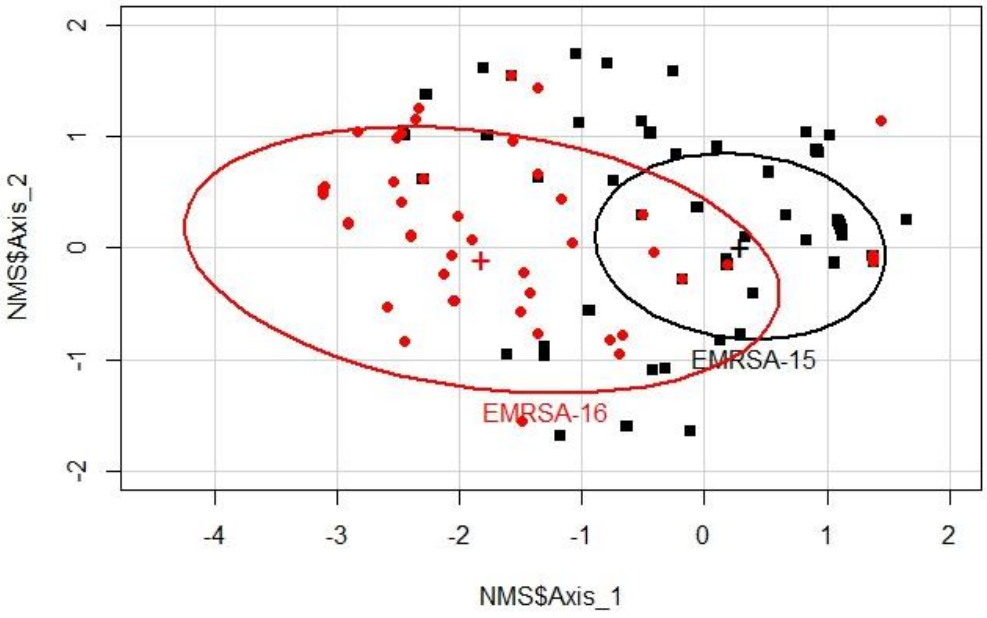
Figure 4-3: Results of NMS – 2-dimentional ordination graphs.

(A) EMRSA-15 versus EMRSA-16 with joint plot

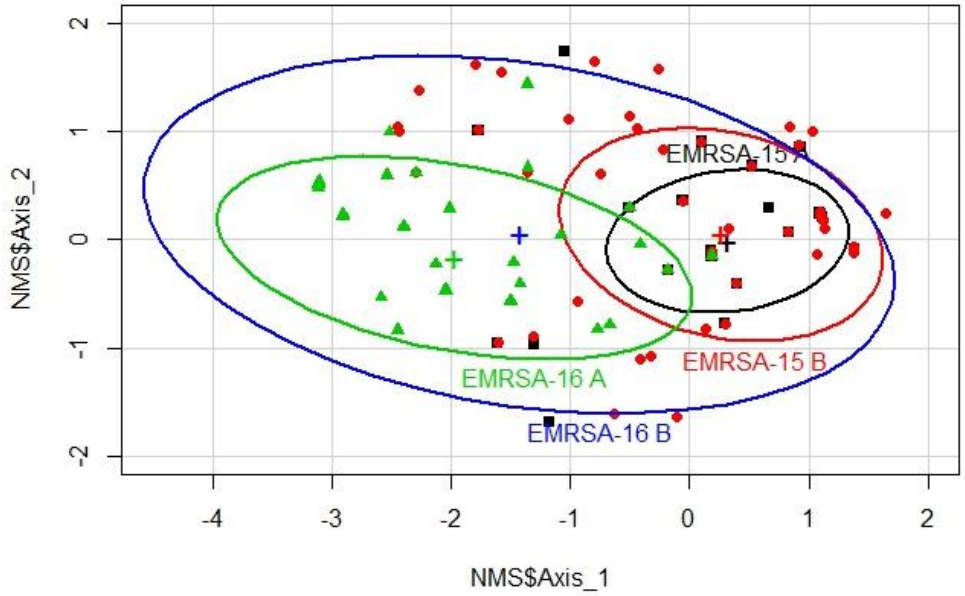


Key: A_length = antibiogram length, Antimicrobial abbreviations; ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), gentamicin (Gn), kanamycin (Km), neomycin (No), tetracycline (Te), tobramycin (Tb), trimethoprim (Tr), toxin genes: *pvl* and *tst*, specimen types: blood, genital, respiratory, urine, wound/skin swab.

(B) EMRSA-15 versus EMRSA-16 with confidence ellipses



(C) EMRSA-15 (time period A and B) versus EMRSA-16 (time period A and B)



4.3.2.3 Antibiogram length

Mean antibiogram length of EMRSA-15 was 5.0 in time period A and 5.2 in time period B (Table 4-6). Among clinical EMRSA-15 isolates, the antibiogram length was 5 or shorter among in 79.8% isolates from time period A and 73.2% from time period B. By contrast, 0.5% and 9.5% (time period A and time period B respectively) of EMRSA-16 isolates had an antibiogram length of 5 or below. The distribution of antibiogram lengths was strikingly different between the EMRSA-15 and EMRSA-16 populations (Figure 4-4A, Table 4-6). These results are consistent with those shown in Figure 4-2. Figures 4A and B show that the shortest and longest antibiogram lengths for each clone and for both time periods were not common (i.e. the maximum length of 10 occurs only once).

The mean antibiogram lengths of EMRSA-15 and EMRSA-16 were significantly different (student's t-test, time period A: $p< 0.0001$, time period B: $p< 0.0001$). The mean antibiogram lengths of EMRSA-15 in time period A and B were significantly different (increasing from a mean antibiogram length of 5.0 to 5.2, $p<0.0001$). The mean antibiogram lengths of EMRSA-16 in time period A and B decreased from a mean antibiogram length of 9.3 to 8.7 but this was not significantly different ($p>0.05$). A summary of the distribution of antibiogram lengths is given in Table 4-6.

The NMS analyses shown above (Figure 4-3) further highlight that antibiogram length is a key factor in discriminating between EMRSA-15 and EMRSA-16 isolates. However, it cannot be used as a diagnostic tool since both clones can have wide, overlapping distributions of antibiogram length (Table 4-6).

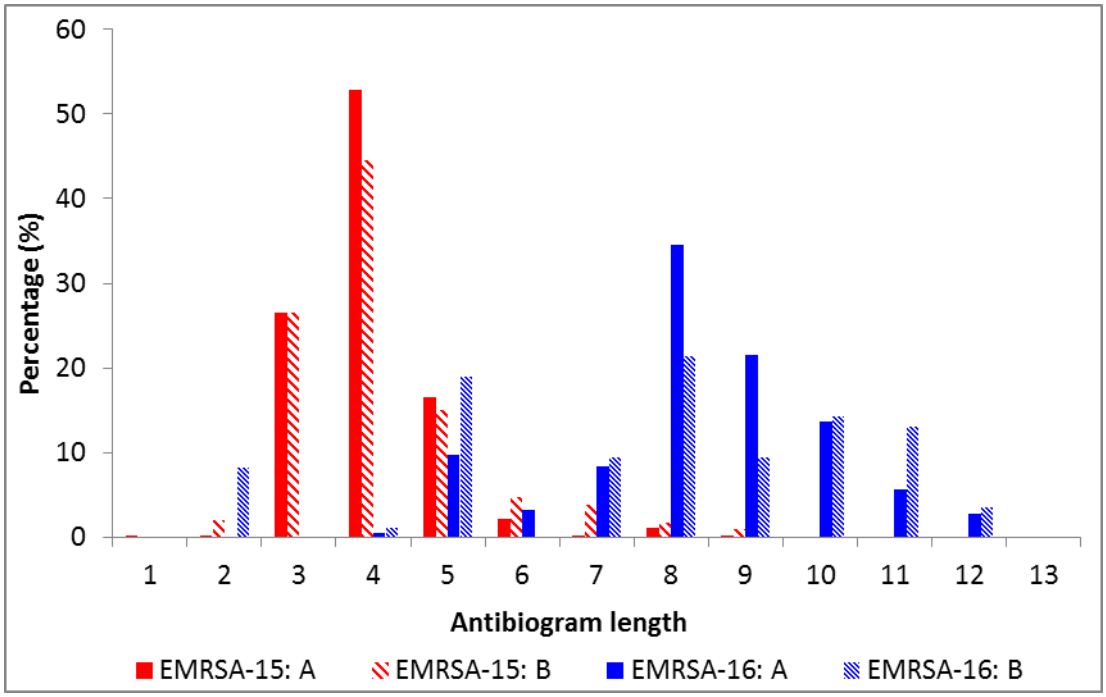
Table 4-6: Summary of antibiogram lengths of clinical isolates.

Clone and time period	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
EMRSA-15: A	2	4	5.0	5.0	5	10
EMRSA-15: B	3	4	5.0	5.2	6	12
EMRSA-16: A	5	9	9.0	9.3	10	13
EMRSA-16: B	3	6	9.0	8.7	11	13
‘Others’: A	3	5	7.0	8.4	13	14
‘Others’: B	3	3	4.0	5.2	7	11

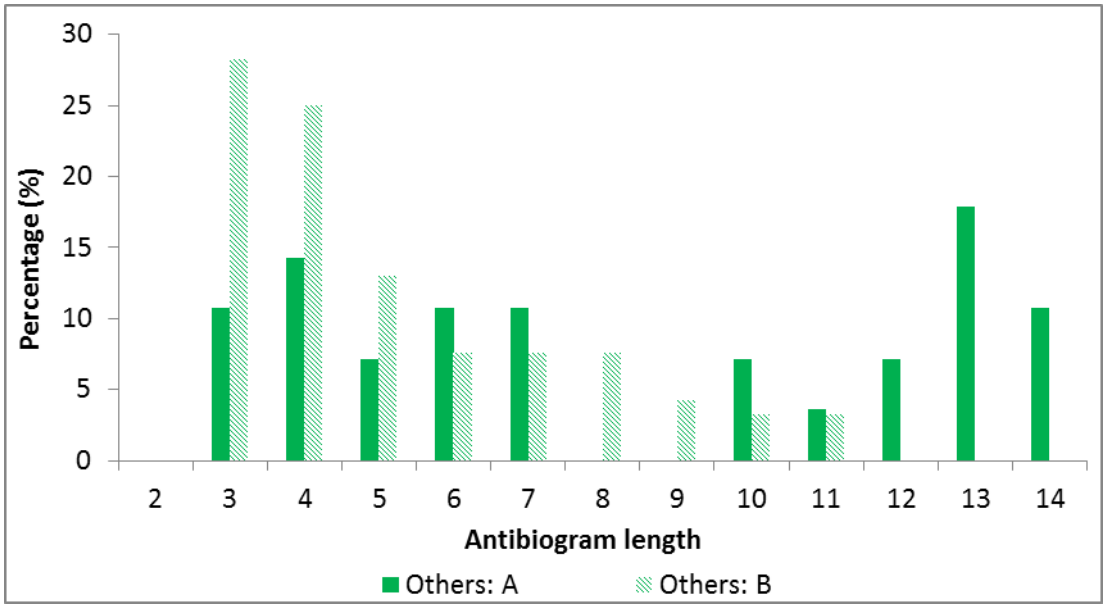
Figure 4-4: The frequency distributions of antibiogram length in time periods A and B.

Distribution of frequencies for antibiogram lengths of (A) EMRSA-15 isolates (red) and EMRSA-16 isolates (blue) and (B) 'others' (green) for time period A (solid bar) and time period B (hatched bar).

A)



B)



4.3.2.4 Antibiogram diversity

Antibiogram diversity of EMRSA-15 and EMRSA-16 was compared within the two time periods by calculating four diversity indices; antibiogram richness (AR), Shannon entropy (SEn), Simpson diversity (SD) and Berger-Parker (BP). Due to differences in sample sizes, data were adjusted using EMRSA-16 time period B as the reference clone – time period since its sample size was the smallest, and re-sampling in each of the other clone and time periods made indices comparable. This created measures of the expected diversity (rather than the observed).

Each of the four diversity indices suggested that antibiogram diversity was higher among EMRSA-16 than EMRSA-15 within both time periods (Figure 4-5). In both clones, diversity was higher in time period B than time period A but not significantly so ($p>0.05$). Table 4-7 shows the mean measures of expected antibiogram diversity, adjusted for by sample size. Since the proportion of EMRSA-15 isolates resistant to individual antimicrobials and the mean antibiogram length of EMRSA-15 isolates were lower than for EMRSA-16 isolates (Figures 4-2, 4-3 and 4-4), it was expected that the antibiogram diversity of the EMRSA-15 population would be lower since fewer antimicrobial combinations were possible.

The most common antibiogram (represented by the diversity index Berger-Parker) of EMRSA-15 isolates in both time periods was 'PnMtCxErCp' which represented 49.0% and 38.0% in time periods A and B respectively. The dominance of this single antibiogram reflects the relative stability of antimicrobial resistance patterns for EMRSA-15 (the proportions resistant didn't vary greatly over time as seen in Figure 4-2 and 4-3, and the clone has similar mean antibiogram lengths for two time periods, Figure 4-4). This antibiogram of EMRSA-15 isolates was found once in the EMRSA-16 population in 2003. The most common antibiogram of EMRSA-16 in time period A was 'PnMtCxErClCpKmNoTb' which represented 33.0% of all EMRSA-16 isolates and 'PnMtCxErClCp' in time period B which represented 29.0% of all EMRSA-16 isolates (Table 4-7). This captures the reduction in the level of resistance to kanamycin, neomycin and tobramycin as seen in Figure 4-2. The lower Berger-Parker estimates for EMRSA-16 isolates indicate that the EMRSA-16 population was dominated by a higher number of antibiograms than the EMRSA-15

population for both corresponding time periods. There were no EMRSA-15 isolates with the most common EMRSA-16 antibiogram in time period A, but the most common antibiogram of EMRSA-16 appeared every year in the EMRSA-15 population in time period B. There were no isolates in either time period or either clone that were resistant to all 20 antimicrobials and only few (≤5) had antibiograms of the observed maximum length (Table 4-8, 'others' also shown). In time period A, three antibiograms were common to EMRSA-15 and EMRSA-16, and in time period B, four antibiograms were common to these two clones (Table 4-9).

Table 4-7: Mean measures of expected antibiogram diversity adjusted for by sample size.

Clone - time period	Sample size	AR (2.5, 97.5% CI)	SEn (2.5, 97.5% CI)	SD (2.5, 97.5% CI)	BP (2.5, 97.5% CI)	Most common antibiogram
EMRSA 15: A	979	11 (7-15)	1.50 (1.24-1.77)	0.32 (0.26-0.40)	0.49 (0.38-0.58)	PnMtCxErCp
EMRSA 15: B	879	19 (14-24)	2.03 (1.71-2.32)	0.26 (0.17-0.28)	0.38 (0.30-0.49)	PnMtCxErCp
EMRSA 16: A	214	20 (16-25)	2.32 (2.10-2.57)	0.12 (0.13-0.21)	0.33 (0.30-0.49)	PnMtCxErClCpKm NoTb
EMRSA 16: B	84	29*	2.84*	0.09*	0.29*	PnMtCxErClCp

*= reference clone-year and therefore no confidence intervals are given.
Key to diversity indices: Antibiogram richness (AR), Shannon entropy (SEn), Simpson diversity (SD) and Berger-Parker (BP).

Figure 4-5: Expected diversity of antibiograms.

Diversity of the effective number of antibiograms calculated by four different diversity indices. These indices occurs along the x-axis. The solid curves (red, brown, blue, and black) represent diversity of EMRSA-15 and EMRSA-16 for both time periods. Shading around curves represents confidence intervals (no CIs for EMRSA-16 time period B as this was the reference clone-year). Key to diversity indices: Antibiogram richness (AR), Shannon entropy (SEn), Simpson diversity (SD) and Berger-Parker (BP).

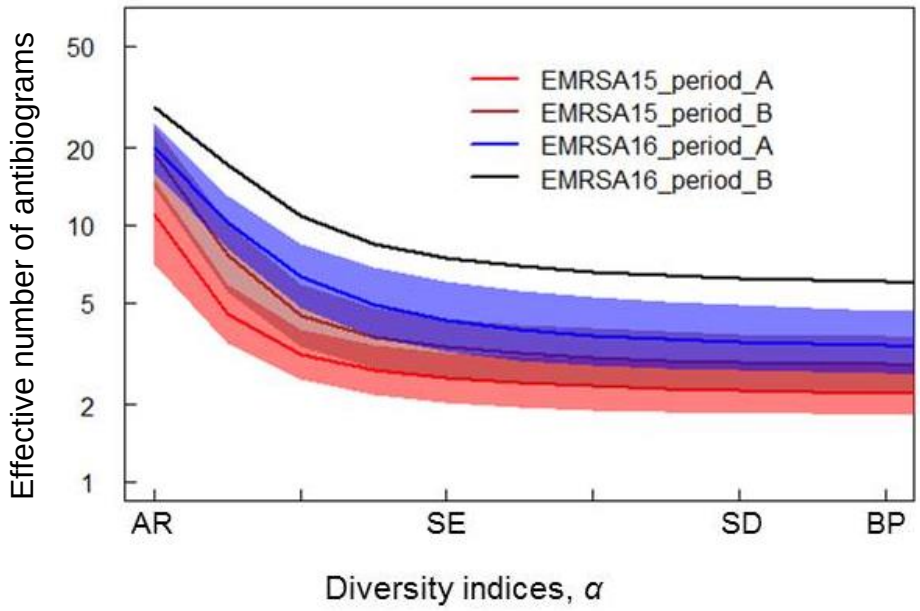


Table 4-8: Antibiograms of maximum observed length for each clone and time period.

Clone and time period	Maximum observed antibiogram length	Antibiogram	Number of observations
EMRSA-15: A	10	'PnMtCxErGnFdCpTrKmTb'	2
EMRSA-15: B	12	'PnMtCxErClGnCpTrTeMpKmTb'	1
		'PnMtCxErClGnFdCpTrTeMpKm'	1
EMRSA-16: A	13	'PnMtCxErClGnCpTrTeMpKmNoTb'	6
		'PnMtCxErClGnCpTrRfMpKmNoTb'	1
EMRSA-16: B	13	'PnMtCxErClGnCpTrMpKmNoTbLz'	1
		'PnMtCxErClGnCpTrRfMpKmNoTb'	1
		'PnMtCxErClGnCpTrTeMpKmNoTb'	1
Others: A	14	'PnMtCxErGnCpTrSuTeKmStNoTb'	3
Others: B	11	'PnMtCxErClGnCpSuKmStTb'	1
		'PnMtCxErClGnCpTrMpKmTb'	1
		'PnMtCxErClGnCpTrTeMpKm'	1

Table 4-9: Shared antibiograms between clones.

Antibiograms of both EMRSA-15 and EMRSA-16 populations are listed with the number of observations (n) and the percentage of each clone (%).

		EMRSA-15		EMRSA-16	
Time period	Antibiogram	n	%	n	%
A	'PnMtCxErClCp'	41	4.7	20	9.3
	'PnMtCxErClCpTr'	7	0.8	2	0.9
	'PnMtCxErCp'	477	54.3	1	0.5
B	'PnMtCx'	8	0.9	7	8.3
	'PnMtCxErClCp'	44	5.0	16	19.0
	'PnMtCxErClGnCpTrKm'	1	0.1	1	1.2
	'PnMtCxErClGnCpTrTeMpKmTb'	1	0.1	1	7.1

4.3.3 Clinical isolates versus screening isolates

Overall, clinical and screening isolates were very similar. The screening isolates followed a similar pattern to the clinical isolates with EMRSA-15 being the dominant clone. Overall, EMRSA-15, EMRSA-16, and 'others' made up 80.4%, 11.8% and 7.8% of all isolates, respectively. In time period A, EMRSA-15, EMRSA-16, and 'others' made up 76.9%, 18.2% and 4.9% of all isolates, respectively, and 82.0%, 8.8% and 9.2% of all isolates (respectively) in time period B (Table 4-10 and Figure 4-6). There was a significant difference in the percentages between clinical and screening isolates in time period A ($p=0.01$) but not time period B ($p>0.05$).

There were no statistical differences in the age of patients from whom isolates were obtained between clinical and screening isolates within each clone and time period ($p>0.05$ in each case). There were no statistical differences in the sex of patients between clinical and screening isolates within each clone and time period ($p>0.05$ in each case) except in time period A ($p<0.01$) when the proportion of isolates from males which were EMRSA-16 was higher in screening samples (73.5%) compared with clinical samples (56.1%).

For EMRSA-15, EMRSA-16 and 'others' in time period B, the percentage of screening isolates with the *pvl* gene present was 0.36%, 2.2%, and 12.9%, respectively, and the percentage of screening isolates with the *tst* gene was 0.12%, 87.8% and 5.4%, respectively. There was no difference between clinical and screening isolates for either toxin ($p>0.05$).

There was a statistical difference between the antimicrobial resistances of screening and clinical isolates for: trimethoprim (EMRSA-15 time period A, $p<0.0001$ resistance in screening isolates higher, EMRSA-16 time period A, $p<0.02$, resistance in clinical isolates higher), clindamycin (EMRSA-15 time period B, $p<0.002$, resistance in clinical isolates higher), kanamycin (EMRSA-15 time period B, $p<0.002$, resistance in clinical isolates higher), mupirocin (EMRSA-16 time period A, $p<0.0001$, resistance in screening isolates higher), and ciprofloxacin (EMRSA-16 time period B, $p<0.01$, resistance in screening isolates higher)(see Figure 4-7 A, B and C)). There were no differences in the proportion resistant between screening or clinical isolates for any other antimicrobials, for any clone or time period ($p>0.05$).

There were statistically significant differences in the mean antibiogram lengths (Table 4-6 and 4-11) between screening (mean length, time period A= 5.15; time period B=4.98) and clinical (mean length, time period A= 4.99; time period B=5.20) EMRSA-15 isolates (time period A, $p = 0.01$, and time period B, $p<0.001$). For EMRSA-16 isolates, there was no statistically significant difference between the mean antibiogram lengths of screening (mean length, time period A= 9.17; time period B=8.45) and clinical (mean length, time period A= 9.30; time period B=8.70) isolates (time period A, $p>0.05$, and time period B, $p>0.05$).

As was seen with clinical isolates, the antibiogram diversity of EMRSA-16 among screening isolates was higher than that among EMRSA-15 isolates but the difference was not significantly different (Figure 4-8, Table 4-12). There was no difference between EMRSA-15 isolates between time periods. Owing to the re-sampling methods (i.e. bootstrapping) and restrictions of small sample sizes, CIs around EMRSA-16 were narrow. EMRSA-16 in time period B was the reference for this analysis so no CIs were generated. The most common antibiograms for clinical

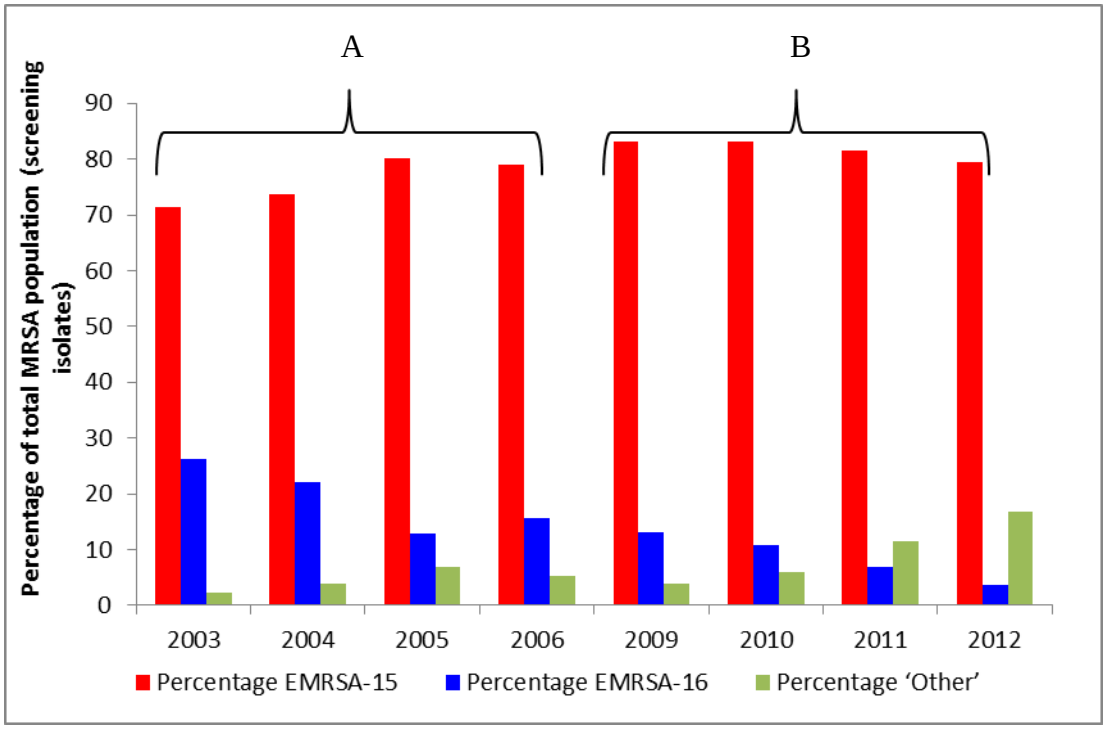
isolates were the same as those for screening isolates and, again, there were no isolates that were resistant to all antimicrobials.

Differences between clinical and screening isolates are summarised in Table 4-13.

Table 4-10: Number of isolates and percentages represented by each clone type by year (screening isolates only).

	2003	2004	2005	2006	2009	2010	2011	2012	total	time A	time B
Total isolates	84	99	131	153	183	370	276	189	1485	467	1018
Percentage EMRSA-15	71.4	73.7	80.2	79.1	83.1	83.2	81.5	79.4	80.4	76.9	82.0
Percentage EMRSA-16	26.2	22.2	13	15.7	13.1	10.8	6.9	3.7	11.8	18.2	8.8
Percentage 'Others'	2.4	4	6.9	5.2	3.8	5.9	11.6	16.9	7.8	4.9	9.1

Figure 4-6: The percentage of isolates that were EMRSA-15, 16, and 'others' by year (screening isolates only).

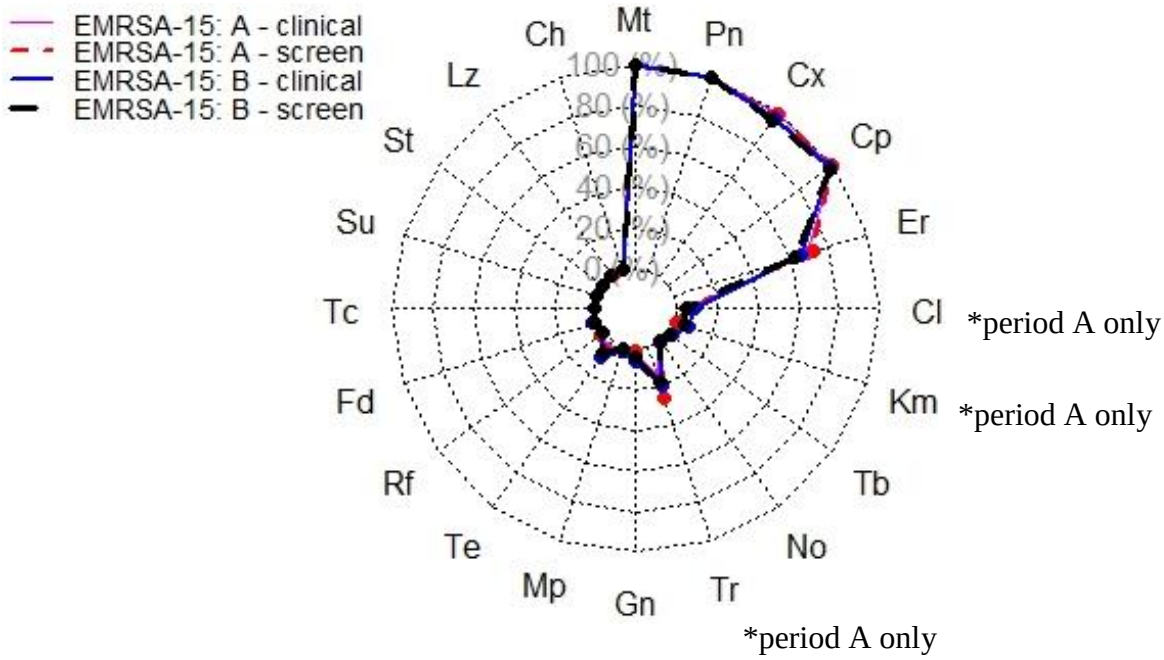


Time periods A and B are highlighted with parenthesis.

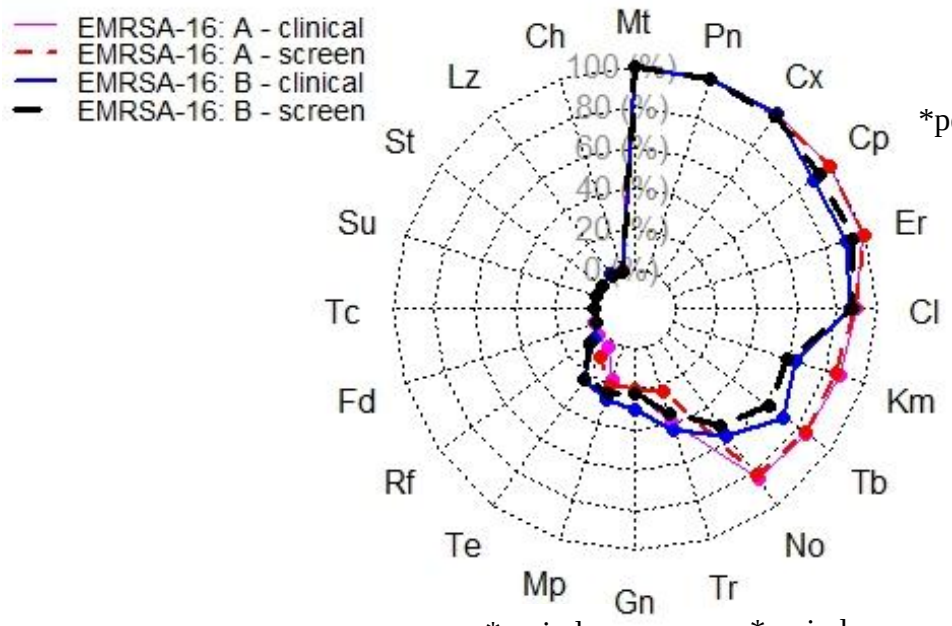
Figure 4-7: Radar chart of antimicrobial resistance of clinical and screening isolates for time period A and B: (A) EMRSA-15 (B) EMRSA-16, and (C) 'others'.

Asterisks denote statistical differences in the proportion resistant between clinical and screening isolates. Radar charts show the percentage of a) EMRSA-15, b) EMRSA-16, and c) 'others' that were resistant to each antimicrobial for time period A and B. The following abbreviations are used to indicate each antimicrobial; cefoxitin (Cx), chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), kanamycin (Km), linezolid (Lz), methicillin (Mt), mupirocin (Mp), neomycin (No), penicillin (Pn), rifampicin (Rf), streptomycin (St), sulphamethoxazole (Su), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), and trimethoprim (Tr).

A)



B)

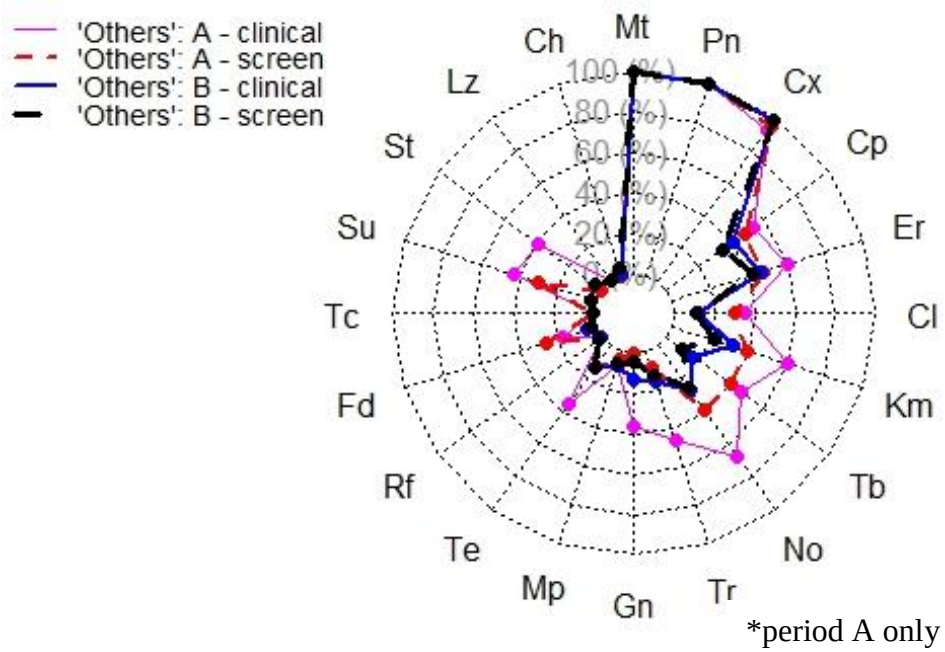


*period B only

*period A only

*period A only

C)



*period A only

*period A and B

Table 4-11: Summary of antibiogram lengths of screening isolates only.

Clone and time period	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
EMRSA-15: A	3	5	5	5.15	6	9
EMRSA-15: B	2	4	5	5.0	5	11
EMRSA-16: A	5	8	9	9.2	10	13
EMRSA-16: B	3	6	9	8.5	10.6	13
'Others': A	3	4	5	6.2	10	10
'Others': B	3	4	4	4.8	6	11

Table 4-12: Mean measures (with 2.5, 97.5% CI) of antibiogram diversity adjusted by sample size.

Clone -time period	Sample size	AR (2.5, 97.5% CI)	SEn (2.5, 97.5% CI)	SD (2.5, 97.5% CI)	BP (2.5, 97.5% CI)	Most common antibiogram
EMRSA-15: A	359	12 (9-16)	1.7 (1.5-1.9)	0.25 (0.21-0.32)	0.40 (0.32-0.49)	PnMtCxErCp
EMRSA-15: B	835	16 (12-20)	1.9 (1.6-2.1)	0.25 (0.19-0.32)	0.39 (0.32-0.49)	PnMtCxErCp
EMRSA-16: A	85	19*	2.07*	0.23*	0.43*	PnMtCxErClCp KmNoTb
EMRSA-16: B	90	32 (30-33)	2.9 (2.8-2.95)	0.10 (0.09-0.10)	0.21 (0.19-0.22)	PnMtCxErClCp

*= reference clone and therefore no CIs

Figure 4-8: Expected diversity of antibiograms of screening isolates.

Figure illustrates diversity in the effective number of antibiograms as calculated by four different indices. These indices occur along the x-axis. The solid curves (red, brown, blue, and black) represent diversity of EMRSA-15 and EMRSA-16 for both time periods. Shading around curves represents confidence intervals (no CIs for 'EMRSA-16 time period B as this was the reference clone-year). Key to diversity indices: Antibiogram richness (AR), Shannon entropy (SEn), Simpson diversity (SD) and Berger-Parker (BP).

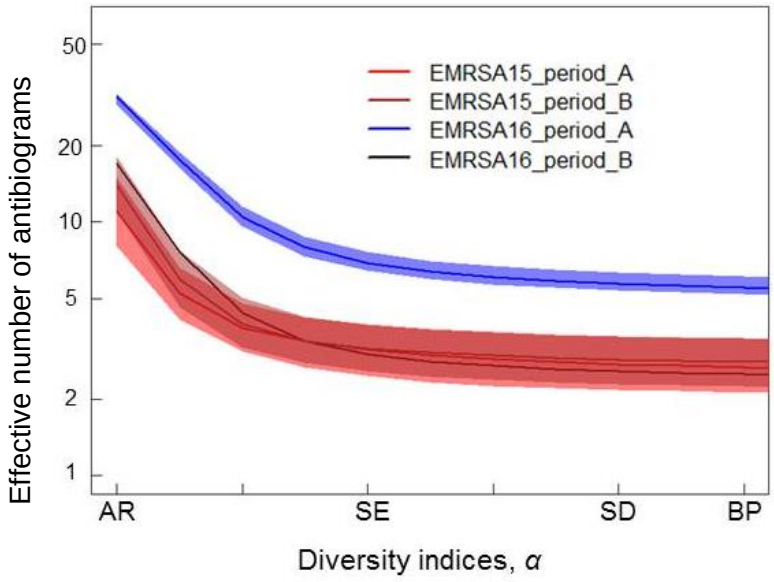


Table 4-13: Differences between clinical and screening isolates summarised.

	Clinical	**Screening**	**Significant difference?**
Dominant clone	EMRSA-15	EMRSA-15	N/A
Clone			
% EMRSA-15	A: 79.9, B: 83.0	A: 76.9, B: 82.0	A: **p=0.01**, B: p>0.05
% EMRSA-16	A: 17.8, B:8.1	A: 18.2, B: 8.8	
% 'Others'	A: 2.3, B: 8.9	A: 4.9, B: 9.1	
Mean age			
EMRSA-15	A: 70.8, B: 71.2	A: 71.3, B: 72.2	A: p>0.05, B: p>0.05
EMRSA-16	A: 67.5, B: 66.2	A: 64.3, B: 70.8	A: p>0.05, B: p>0.05
'Others'	A: 51.8, B: 49.3	A: 56.2, B: 64.0	A: p>0.05, B: p>0.05
% Male			
EMRSA-15	A:49.6, B: 49.5	A: 50.6, B: 50.4	A: p>0.05, B: p>0.05
EMRSA-16	A: 56.1, B: 64.3	A: 73.5, B: 52.2	A: **p<0.01**, B: p>0.05
'Others'	A: 64.3, B: 48.3	A:52.2, B: 52.7	A: p>0.05, B: p>0.05
pvl toxin - % positive			
EMRSA-15	B: 1.2	B: 0.36	B: p>0.05
EMRSA-16	B: 9.8	B: 2.2	B: p>0.05
'Others'	B: 19.6	B: 12.9	B: p>0.05
tst toxin - % positive			
EMRSA-15	B: 0.5	B: 0.12	B: p>0.05
EMRSA-16	B: 94.4	B: 87.8	B: p>0.05
'Others'	B: 4.3	B: 5.4	B: p>0.05
Antibiogram mean length			
EMRSA-15	A:4.99, B: 5.20	A: 5.15, B: 4.98	A: **p<0.01**, B: **p<0.001**
EMRSA-16	A: 9.30, B: 8.70	A: 9.17, B:8.45	A: p>0.05, B: p>0.05
Antimicrobial resistance*			
EMRSA-15			
Cl	B: 9.0	B: 5.0	B**: p<0.002**
Km	B: 7.7	B:4.8	B: **p<0.002**
Tr	A: 14.9	A: 26.0	A: **p<0.0001**
EMRSA-16			
Cp	B: 89.3	B: 93.3	B: **p<0.01**
Mp	A: 16.4	A: 20.0	A: **p<0.0001**
Tr	A: 38.8	A:23.6	A: **p<0.02**
Antibiogram diversity	No difference for either clone or time period for any of the four diversity measures		
Most common antibiogram			
EMRSA-15	A: PnMtCxErCp, B: PnMtCxErCp	A: PnMtCxErCp, B: PnMtCxErCp	N/A
EMRSA-16	A: PnMtCxErClCp KmNoTb B: PnMtCxErClCp	A: PnMtCxErClCp KmNoTb B: PnMtCxErClCp	N/A

* Only antimicrobials with statistically significant differences between EMRSA-15 and EMRSA-16 are shown

4.4 Discussion

4.4.1 Clone dominance and differences

EMRSA-15 and EMRSA-16 have been the two most dominant MRSA clones over the past decade. EMRSA-15 remains by far the most common with the number of EMRSA-16 isolates declining. There is no indication yet of an emerging sequence type from the 'others' group that could potentially be the next major clone. However, in a 16-year surveillance study of MRSA isolates in NHS Grampian, increases in the number of community strains, in particular CC5, was recently reported [119].

Results in this chapter show that EMRSA-15 and EMRSA-16 populations differ in terms of the level and pattern of antimicrobial resistance, their antibiogram lengths and (to a lesser extent) antibiogram diversity. As is likely true for all bacteria, but particularly at times of dominance and latterly decline; MRSA has both benefitted from and been hindered by antimicrobial resistance.

There have been some changes in the MRSA antibiogram over time. While this largely has to do with EMRSA-16 isolates losing antibiotic resistance determinants (predominantly losing resistance to kanamycin, tobramycin and neomycin), the data imply that this also has to do with clones changing in frequency.

4.4.2 Selecting for AMR

Mutants containing genes for antimicrobial resistance are selected when isolates are under selection pressure, and genes only become fixed in a population when they give a strong selective advantage to the bacteria which outweighs any fitness cost [47]. Previous studies have shown that there is often a fitness cost and reduced growth rate associated with resistance, and the harbouring of mobile genetic elements (MGEs) with drug resistance genes, and especially if the resistances are not beneficial such as would be the case in a low selection pressure environment [48-50]. Furthermore, if resistance does carry a fitness cost, resistance tends to be eliminated once the selective pressure is removed [50]. This is not a straightforward issue since the effect of resistance on fitness can differ depending on environmental conditions, and can be altered by compensatory mutations or epistasis, and some mutations may be cost free [49]. In simple terms, it might be argued that EMRSA-15 isolates are not

burdened by large unnecessary resistance genes and are therefore biologically fitter, and spread more readily between people, patients, visitors, hospital staff and the hospital environment (including furniture, floors and air), as well as in the non-hospital environment.

In contrast, EMRSA-16 isolates tend to harbour resistance genes to a larger number of antimicrobials. It could be suggested that so many large genes hinder the clone, reducing its ability to readily spread from person-to-person at a rate faster than it is removed in order to sustain the population (although there are likely several other factors influencing spread of the clone). Isolates can be removed from the environment by cleaning as well as by natural bacterial death, and in humans and animals by the immune system, antimicrobials, antibacterials or by radiation. If bacteria are not replaced by new isolates of the same clone (e.g. from colonised or infected hospital staff, visitors, patients or through air conditioning systems) or dispersed through the hospital population and environment faster than the rate of removal, then the number of isolates of that clone will diminish over time (as observed). Of course, it is possible that low levels of bacteria will continue to remain since cleaning is not always 100% effective, antimicrobials not always taken correctly, or due to antimicrobial resistance.

Since differences in levels and patterns of resistance to a single antimicrobial most likely reflect current (at the time of sample isolation) or historical antimicrobial selective pressures, it is reasonable to infer that EMRSA-16 isolates exist(ed) in a niche of high antimicrobial usage. Furthermore, it has previously been proposed that EMRSA-16 may be particularly well adapted to the hospital environment [135]. Specifically, the niche occupied may be intensive care units or care of the elderly specialties in major hospitals for example, which in recent years have been subject to targeted interventions and hygiene policies and practices [105]. This targeting of particular specialties could have caused the observed impact on the EMRSA-16 population.

Unlike the EMRSA-15 population, the level and pattern of resistance to several antimicrobials changed in the EMRSA-16 population by time period B. This ‘gene

shuffling', in which the resistance to ciprofloxacin, erythromycin and aminoglycosides fell, and resistance to tetracycline increased, could be a result of adaptation to survive in changing environments or selection pressures. MGEs are key in the adaptation to new niches [47], and gene shuffling indicates frequent transfer and loss of MGEs carrying resistance genes. Looking specifically at the proportion of EMRSA-15 clinical isolates resistant to different antimicrobials per individual year of time period B, there was evidence for increase in resistance to trimethoprim, tetracycline, and to a lesser extent gentamicin, with resistance to kanamycin and clindamycin fluctuating but increasing overall. It has been suggested (personal communication with Giles Edwards, former director of the Scottish MRSA Reference Laboratory and consultant microbiologist) that a subset of EMRSA-15 isolates have adapted to fill the vacating niche of EMRSA-16 isolates and have hence taken on traits which would have been common to the EMRSA-16 clone in order to survive there. However, there are several questions surrounding this hypothesis, such as why EMRSA-16 would not survive but EMRSA-15 could. This may be due to the rate at which EMRSA-15 spreads and is replaced; that it is being removed as EMRSA-16 would be (e.g. by cleaning and antimicrobials) but it is replaced faster than it is removed.

4.4.3 Co-resistance

Although antimicrobial drug use selects for resistant mutants, resistance can also be acquired in the absence of selection pressure by co-resistance where one gene confers resistance to multiple antimicrobial classes. For example, erythromycin resistance is conferred by the *msrA* gene (carried on MGE) as well as the *erm* genes (*ermA, ermB, ermC, ermT*, also MGE) which also confer resistance to clindamycin [51]. In addition, clindamycin resistance in *Staphylococcus* species can be either constitutive (meaning that isolates will display the resistant phenotype under routine screening conditions) or inducible (isolates display resistance phenotype only in the presence of erythromycin and clindamycin). Therefore, isolates with the inducible phenotype will appear to be erythromycin resistant and clindamycin sensitive when not tested in the presence of each other [52]. The presented results have only included isolates with constitutive resistance only. If inducible resistance had also

been included, for time period A, the proportion of EMRSA-15 isolates resistant to clindamycin would have increased from 5.4% to 21.8%, and for EMRSA-16 isolates from 90.2% to 94.7%. There was no inducible resistance recorded for time period B for either clone. Inducible resistance becomes particularly important when deciding on the most appropriate treatment regimens for patients.

Co-resistance is also conferred for aminoglycosides kanamycin, tobramycin and neomycin due to acquisition of any one of the genes *aadD, aacA-aphD, ant4* [176, 177]. These genes are carried on large MGEs together with heavy metal resistance genes and penicillinase [33] and it has been suggested that having this large plasmid may be negatively associated with the survival capacity of the bacteria. This may have contributed to the reduction of the proportion resistant to all three antimicrobials in the second time period for EMRSA-16 isolates. Gentamicin is also an aminoglycoside that confers resistance through acquisition of the *aacA-aphD* gene only.

In addition, bacterial species can acquire resistance to heavy metals. This is important not only since bacteria can grow in the presence of these materials, but also because the genes for heavy metal resistance can be linked to genes for AMR. Furthermore, research suggests that resistance to antimicrobial metals may be a driver of AMR [43]. For example, a cluster of metal resistance determinants can exist on the SCC*mec* element, closely linking it to the *mec*A gene and β-lactam resistance [43]. Resistance to antiseptics (i.e. chemical applied to skin or living tissue that kills or inhibits the growth of microorganisms, e.g. alcohol gels, surgical hand scrubs etc) is also possible and there is some suggestion that this can lead to reduced antimicrobial susceptibility by intrinsic or acquired mechanisms of resistance [45].

4.4.4 Mupirocin

The use of the topical antimicrobial mupirocin has greatly increased in recent years since it emerged that nasal decolonisation of patients and healthcare workers dramatically decreases the risk of *S.aureus* nosocomial spread and infection [109]. Hence, any increase in resistance would be of great clinical interest. Resistance statistically increased for EMRSA-15 with high-level mupirocin resistance gained

through acquisition of the *mupA* and *mupB* genes which are carried on MGEs [51]. Of further interest, *mupA* can also confer resistance to gentamicin, tetracycline, and trimethoprim as well as mupirocin [178].

4.4.5 Clinical versus screening isolates

Table 4-13 highlights that there were very few differences in the pattern of resistance between clinical and screening isolates, which is in agreement with previous research [10, 56]. This suggests that it is the colonising strains that go on to be clinically important and therefore, this furthers the argument for decolonising hospital admissions to prevent re-seeding of hospitals and hospital outbreaks, and especially hospitalised patients pre-surgery to prevent infection. However, even if patients are screened and found not to be colonised, they may still go on to develop a clinical episode since the bacteria may be acquired in hospital (i.e. hospital-acquired infection) or due to a false negative result.

It also suggests that screening isolates can be analysed in order to identify trends in clinical isolates such as the emergence of new *spa* types or increasing resistances.

4.4.6 Non-EMRSA-15 / non-EMRSA-16 'others'

The 'others' are a group of non-EMRSA-15 / non-EMRSA-16 isolates of several different sequence types (ST) and *spa* types and therefore this group was not expected to behave in a predictable manner. The levels of resistance varied and antibiogram lengths changed for both clinical and screening isolates between the two time periods, which could suggest a shift in clone dominance within the group. Unfortunately, this could not be investigated as sequence and *spa* typing was rarely carried out in time period A.

4.4.7 Limitations

There were further limitations of the data and assumptions made to carry out these analyses.

Firstly, it is difficult to say whether all 'screening' isolates were removed from the database of 'clinical' isolates. The definition of 'screening' isolates (as used by [10]) was: any nasal, throat, axilla or groin swabs from which bacteria were grown. The

major assumption here was that specimen types listed as ‘skin swabs’ and swabs from various body sites were indeed wound and skin and soft tissue infections, i.e. true infections rather than screens. While removing screening isolates results in slight changes in the percentages of isolates that are EMRSA-15, EMRSA-16 and ‘others’, and despite the fact that there were some statistically significant differences in the percentages resistant to different antimicrobials, this limitation made very little difference to the overall results.

Secondly, antibiogram length was described in this study given resistance to 20 consistently tested antimicrobials. Resistance to untested antimicrobials could theoretically have been possible which could have resulted in longer maximum length antibiograms. This said, the twenty consistently tested antimicrobials were considered to be most relevant for MRSA (personal communication with Giles Edwards) and it is unlikely that the overall picture of resistance would have been altered by including further antimicrobials in the analyses.

Thirdly, the snapshot database is a volunteer referral system and while cooperation and adherence was very good, some isolates for some years may have been missed.

Lastly, this chapter suggests that there is a link between survival and dominance of EMRSA-15 isolates and, conversely, the decline of EMRSA-16 isolates with the level and pattern of antimicrobial resistance, antibiogram length and diversity. However, there may be several other influences on survival that could not be explained by these data, for example, the fact that EMRSA-15 and EMRSA-16 isolates also differ genetically and have different *SCCmec* types [134]. Experimental investigation may be helpful to further understand the impact of these differences.

4.4.8 How this builds on previous publications

Previously, MRSA strain distribution and antimicrobial resistance patterns in NHS Grampian (northeastern Scotland) originating from patients attending GP surgeries and hospitals over a 5 year period (from 2003 – 2007) were described [10]. The study described the proportions of each strain type from different clinical specimens (e.g. blood, urine, swab) with EMRSA-15 the most common strain in each category. The authors also found a shift in the relative rates of epidemic strains with an

increase in community-associated strains, and in addition they reported no difference in antibiotic resistance between clinical and screening isolates.

The findings of this chapter agree with those of NHS Grampian but this chapter covers a longer, more recent, time period, is Scotland-wide and gives a more detailed comparison of strain types, describing AMR, antibiogram length and diversity.

4.5 Conclusion

EMRSA-15 isolates were dominant throughout this study. Historically, clones have dominated, declined and then been replaced by another clone, and although this looks unlikely to happen to EMRSA-15 isolates in the short-term, it appears to have already happened to EMRSA-16. However, there is no clear single sequence type or clonal complex emerging from the 'others' group at present.

The diversity of antibiograms was found to be wide which could reflect frequent loss and transfer of resistance genes between isolates [33]. However, it could also be suggested that EMRSA-15 and EMRSA-16 populations do not share a spatial niche as distinct resistance patterns were observed. The levels and patterns of resistance are likely reflective of selective pressures within these spatial (and also temporal) niches which may vary due to the clinical usage, the volume consumed, and the method of administering in addition to the method of gaining resistance.

There are several antimicrobials to which the resistance in the population remains very low and therefore they are still available for treatment of MRSA infections caused by EMRSA-15. The main pattern of resistance and most common antibiogram in the EMRSA-15 population remained stable throughout this study but NHS Scotland must remain vigilant. This is especially important if EMRSA-15 isolates do move into the vacating niche of EMRSA-16 isolates and become more multi-drug resistant. Furthermore, NHS Scotland must continue to monitor the multi-resistances of EMRSA-16 isolates since treating these now apparently rare infections could become more challenging.

Chapter 5: **Antimicrobial prescribing and its relationship with antimicrobial resistance in MRSA**

5.1 Background

Since their introduction into general clinical usage, antimicrobials have greatly contributed to the improvement of health and to increasing life expectancies globally [17]. However, with the rise of antimicrobial resistance (AMR) and since only two new classes of drugs have been introduced since the 1970s [20], there is great concern that we could be entering a post-antibiotic era where successful and powerful drugs of the past will no longer be effective. To address this, there have been calls for improved antimicrobial stewardship to better regulate drug usage, as well as improved surveillance, monitoring and regulation [20-22] (for more background information on antimicrobial stewardship, see Chapter 1.1.4.2.4).

A better understanding of prescribing rates may provide an appreciation of the effects of population-level antimicrobial usage on antimicrobial resistance and the epidemiology of infectious diseases. For methicillin-resistant *Staphylococcus aureus* (MRSA), there have been several studies that have investigated this effect. In a time-series analysis, the delay (after varying lag periods) in the effect of prescribing levels of several antimicrobial classes in a hospital in NHS Grampian and its association with the proportion of total *S. aureus* that was MRSA (%MRSA) was captured [126]. Also in NHS Grampian was a case-control study of two hospitals which found prescribing rates of macrolides and quinolones to be significantly and positively associated with an increase in %MRSA [127]. However, in a study of MRSA isolates in a single London hospital, the correlation between prescribing and antimicrobial resistance was reported to be strongest with ciprofloxacin and cephalosporin [34]. There was also an association between antimicrobial use in the previous 12 months and the %MRSA isolates from non-clinical colonised individuals in England [179]; a strong association between antimicrobial usage within the previous 6 months and MRSA infection in a community-acquired MRSA outbreak in rural Alaska [180]; and, likewise, a strong association of antimicrobial usage within the previous 12

months and nasal colonisation in Taiwanese children [181]. In contrast there was no association found between prescribing and skin infections in two studies [182, 183] and in a meta-analysis that included those two and the Alaskan study, the odds ratio was close to 1 [184].

The work in this chapter was intended as a pilot study to inform a larger analysis looking at trends and associations between prescribing and resistance levels for a greater number of antimicrobials and several pathogens. Therefore, the overall purpose was to test methods, and describe pitfalls of using such data and carrying out such a study. This is discussed at the end of the chapter. The specific aims of this chapter were two-fold. Firstly, to examine spatial and temporal trends in Scottish primary and secondary care prescribing rates. This included the use of the multivariate ordination technique, principal component analysis (PCA). Secondly, to investigate whether there were associations between primary or secondary care prescribing rates and antibiotic resistance in the MRSA population.

5.2 Methods

5.2.1 Data

5.2.1.1 Scottish prescribing data

5.2.1.1.1 Measurement of prescribing

The international standard which the WHO recommends for comparing antimicrobial usage is the metric daily defined doses (DDD), defined as the assumed average maintenance dose per day for a drug used for its main indication in adults [185]. This is therefore a proxy measure for the average number of patients exposed to the drug in question each day. Prescribing rates per 1000 population (by quarter and HB) were calculated using population data as the denominator and number of DDD as the numerator.

5.2.1.1.2 Prescribing databases

Two datasets containing information on antimicrobial prescribing were received from Information Services Division (ISD), NHS Scotland.

The first dataset was extracted from the Prescribing Information System for Scotland (PRISM) database and contained information on antimicrobial prescribing in primary care, i.e. dispensed from community pharmacies, measured in DDD with variables including: antimicrobial name and group, NHS Health Board (HB), drug formulation, year (financial year, starting 1st April 2004/05 - 2011/12 inclusive) and quarter (1=March, April, May; 2=June, July, August; 3=September, October, November; 4=December, January, February). HB boundaries were reconfigured in 2007: NHS Argyll and Clyde was broken up and part-absorbed into what was previously NHS Greater Glasgow and subsequently NHS Greater Glasgow and Clyde, and NHS Highlands. The PRISM database contains information from pre- and post-reconfiguration but the database only uses the new HB names and boundaries. Pharmacies in regions where the boundaries changed were re-assigned to reflect the new HBs by ISD prior to releasing the data.

The second dataset was taken from the Hospital Medicines Utilisation Database (HMUD) and contained information on antimicrobial prescribing in secondary care measured by DDD and again with information on: antimicrobial name and group, HB, drug formulation, year (financial year 2007/08 – 2011/12 inclusive) and quarter. Herein, when years are discussed, the year represents the beginning of a financial year. i.e. 2007 represents financial year 2007/08. HMUD also reflects new HB boundaries.

5.2.1.1.3 NHS Health Boards

Data collection was incomplete for some HBs. Therefore only seven HBs with consistently the most complete reporting to HMUD could be included in this study. The HBs included were: NHS Ayrshire and Arran, NHS Fife, NHS Forth Valley, NHS Greater Glasgow and Clyde, NHS Grampian, NHS Lanarkshire and NHS Lothian. This was applied to both the primary and secondary prescribing datasets to allow for direct comparison. Approximately 81% of annual prescribing in primary care and between 56-72% of annual prescribing in secondary care was in these seven HBs, and the same HBs contain approximately 80% of the total Scottish population. Hence these HBs were considered a good representation of prescribing in Scotland.

5.2.1.1.4 Antimicrobials

Fourteen antimicrobials were initially selected for this study. These were: chloramphenicol (Ch), ciprofloxacin (Cp), clindamycin (Cl), erythromycin (Er), fusidic acid (Fd), gentamicin (Gn), linezolid (Lz), neomycin (No), rifampicin (Rf), streptomycin (St), teicoplanin (Tc), tetracycline (Te), tobramycin (Tb), and trimethoprim (Tr). These were selected since they represent classes of antimicrobials that are relevant to *S. aureus* infections (personal communication with Dr Giles Edwards, former infectious diseases consultant in NHS Greater Glasgow and Clyde, and former director of the Scottish MRSA Reference Laboratory). Initial investigation of general patterns showed that six antimicrobials, ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline and trimethoprim, had the highest prescribing rates in primary and secondary care when rates from both primary and secondary care were combined and ranked. These six antimicrobials became the focus of further analyses. Combined they accounted for between 16-22% of total annual primary prescribing (between 97-99% of the 14 antibiotics initially selected) and between 13-19% of total annual secondary prescribing (between 93-95% of the 14 antimicrobials initially selected).

5.2.1.2 Scottish population data

Mid-year population estimates at 1) HB-level and 2) the national-level were obtained from the General Register Office for Scotland website for the years 2004 – 2011 inclusive.

5.2.1.3 Scottish resistance data

Data on the proportion of MRSA isolates resistant to the six different antimicrobials (ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and trimethoprim) were extracted from the Scottish MRSA reference laboratory (SMRSARL) database. Specifically, this included information on MRSA isolates that had caused bacteraemia, by quarter and HB for the years 2003-2012. This information was collected by the SMRSARL for contributing to the EARS network (European Antimicrobial Resistance Surveillance Network). The proportion resistant to an antimicrobial was calculated as the number of isolates (for a given quarter, year and HB) that were resistant as the numerator, and the total number of MRSA isolates

(for the corresponding quarter, year and HB) as the denominator. Herein, 'the proportion of MRSA isolates that were resistant' shall be referred to as the 'proportion resistant'.

5.2.2 Descriptive and statistical analysis

All statistical analyses were done using R (version 3.1.2) [154].

5.2.2.1 General Scottish trends

General patterns of 14 antimicrobials prescribed in either primary or secondary care were first described. Then descriptive analyses of six antimicrobials (ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and trimethoprim) were conducted.

Temporal trends of prescribing rates in primary care for financial years 2004/05 - 2011/12 and secondary care for financial years 2007/08 – 2011/12, measured by the number of DDDs per 1000 population, were examined using regression analysis (exploring linear, quadratic or cubic where appropriate). Spatial trends in primary and secondary care prescribing rates, i.e. number of DDDs per 1000 population, between HBs were examined using a one-way analysis of variance (ANOVA). The reference HB for each model was the one with the highest estimate of prescribing rate. To identify where the statistical differences between HBs lay, *a post-hoc* multiple comparisons testing using Tukey's test was carried out. One of the outputs from this analysis is that HBs are grouped together based on mean prescribing rates, and HBs with statistically different rates are placed in different groups. Groups are assigned letters to easily identify different groups. Thus, HBs in the same group do not have statistically different means, and hence will be assigned the same letter. In addition, any interaction between year and HB was also investigated using the model; $prescribing\_rate \sim HB + Year + HB * Year$.

Level of statistical significance for all models was set at $p \leq 0.05$. Skewed variables were first log-transformed to normalise the data and where necessary the log of x+1 was taken to avoid problems taking the log of 0: primary care data ($log10(DDD)$) for ciprofloxacin, clindamycin, erythromycin, tetracycline and trimethoprim, and ($log10(DDD + 1)$) for gentamycin; secondary care data ($log10(DDD)$) for

ciprofloxacin, erythromycin, gentamycin and trimethoprim, and ($log10(DDD + 1)$) for clindamycin.

5.2.2.2 Principal Component Analysis (PCA)

Principal component analysis (PCA), the multivariate ordination technique that reduces the dimensionality of a dataset while largely maintaining the variation [186], was used to examine relationships between prescribing of selected antimicrobials over space and time. A PCA reduces the dimensionality of a dataset by identifying directions (axis) along which data have the greatest distance or variation. This distance is represented by loading scores. PCA therefore creates new variables called principle components that can be plotted in order to visualise similarities and differences between groups, in this case prescribing rates between and within primary and secondary care.

The first PCA was based on the combined primary and secondary care prescribing rate (DDD per 1000 population) of ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and trimethoprim (transforming variables were appropriate and aforementioned). Following preliminary runs of the analysis, two outliers (1= secondary data from the 4th quarter, 2011 from NHS Greater Glasgow and Clyde; 2= secondary data from the 4th quarter, 2011 from NHS Lothian) were removed since data for both were further than two standard deviations from the mean, suggesting that data from this quarter were not complete.

To find variables associated with different groups in a PCA, a second matrix with additional data is superimposed on the ordination to create a joint plot (radiating lines). The direction of the lines indicates either a positive or negative relationship with an axis and the variables of the second matrix, and the length shows the strength of the correlation on that axis. The ordination was rotated in order to show the strongest correlations along axis 1. Variables in the second matrix included information on NHS Health Board, year, quarter, season (quarters 2/3 = spring/summer and quarters 3/4= autumn/winter), and the proportion of antimicrobial resistance (to ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and trimethoprim) in the MRSA population. Multi-response permutation procedures

(MRPP) analysis was performed to test the hypothesis of no difference between groups.

Two additional PCAs were run on primary care prescribing data only and secondary care prescribing data only. Joint plots were also plotted to visualise within-group differences over years and between HBs. MRPP carried out to test for no difference over time, and between HBs. All PCA were performed in PC-ORD version 6.03 (MJM software Design, Gleneden Beach, OR).

5.2.2.3 Associations between prescribing and MRSA antimicrobial resistance

Associations between the prescribing rate in both primary and secondary care and the proportion of the MRSA population resistant to the six different antimicrobials (ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and trimethoprim) were investigated using linear regression analyses. This would capture any association between prescribing and resistance in the same quarter (*t0*). To capture any possible delay or lag in the effect that prescribing had on resistance trends, resistance data were also staggered so that they represented a delay of one quarter (*t1*), two quarters (*t2*), and so on until six quarters (*t6*) (with one quarter being three months). In these univariate analyses, the proportion resistant to each antimicrobial in each time period (*t1*, *t2* etc.) were the dependent variables, and prescribing rate (in either primary or secondary care) the independent or explanatory variable. Resistance data for each antimicrobial were log transformed ($\log_{10}(X+1)$) to normalise the data.

In a multivariate analysis, the proportion of isolates resistant to each antimicrobial at *t0* was the dependent variables, and prescribing rates (both primary and secondary care), HB and year the independent variables. Correlation analyses were conducted to check that no two variables were strongly correlated (correlation coefficient >0.8). A backward elimination approach was applied and statistical significance set at $p \leq 0.05$. The akaike information criterion (AIC) and quantile-quantile-plots were used to compare nested models, and p-values and adjusted R^2 values to compare non-nested models. The robustness of final models was tested by applying a forward stepwise approach and examining the diagnostic statistics, and the stability of the

final models was also checked by removing variables one at a time and assessing the effect of the remaining variables. The final models were deemed to be both robust and stable. They were then run using the staggered resistance data that represented lag period *t1-t6* as the independent variable.

5.3 Results

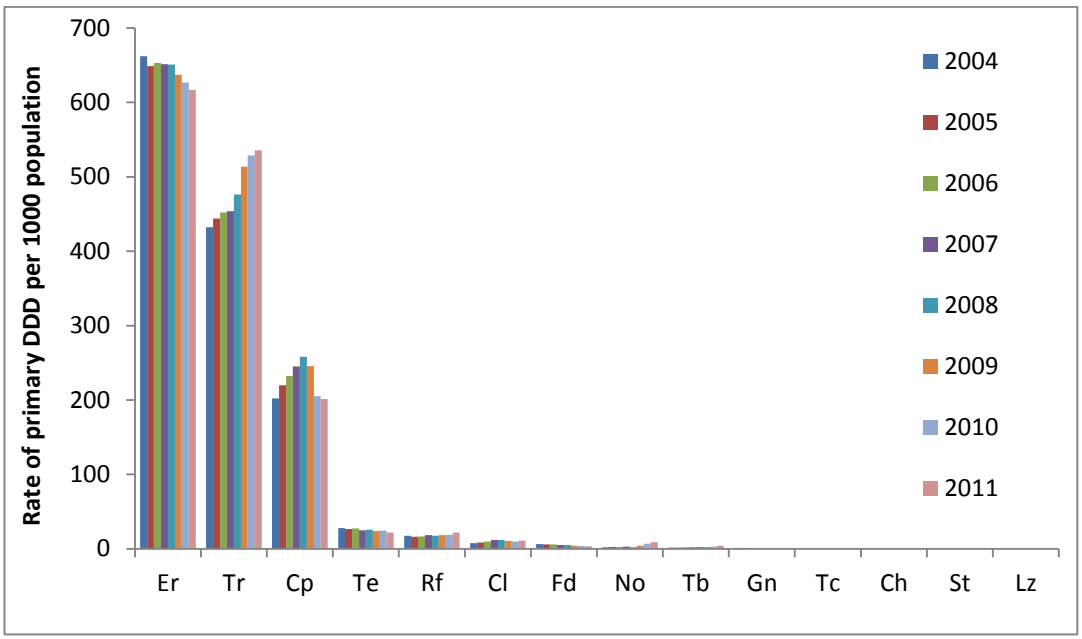
5.3.1 General patterns

Among the 14 antimicrobials initially investigated, the three most commonly prescribed from primary healthcare sources over the entire study period were erythromycin, trimethoprim and ciprofloxacin (Figure 5-1A). Among the 14 antimicrobials initially investigated that were prescribed from secondary healthcare sources, ciprofloxacin and trimethoprim were also high (Figure 5-1B). In secondary care, moderate rates of prescribing (between 10-50 DDDs per 1000 population) were also observed for erythromycin, gentamycin and clindamycin, and low rates of prescribing (below 10 DDDs per 1000 population) for all other selected antibiotics (Figure 5-1B).

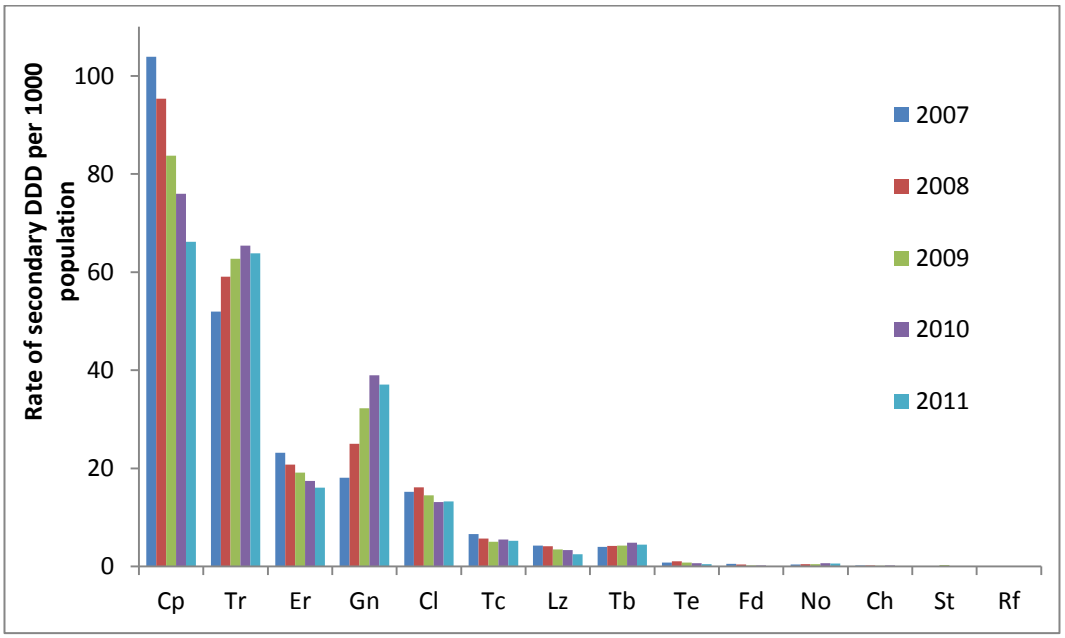
Despite being the two most commonly prescribed antibiotics in secondary care in this study, the rate of prescribing of ciprofloxacin and trimethoprim for the years 2007 – 2011 was between 2.4 - 3 and 8.1 - 8.7 times lower than that seen in primary prescribing for both respective antibiotics (Table 5-1). Prescribing rates were between 6.2-6.8 times higher in primary care for the 14 antimicrobials combined, and between 6.5- 7.0 times higher in primary care for the 6 selected antimicrobials combined. The difference between prescribing in primary and secondary prescribing was largest for erythromycin (28 - 38 times higher in primary care) and tetracycline (24-44 times higher in primary care). Prescribing was consistently higher in secondary care for clindamycin and gentamycin (Table 5-1). The range reflects variation in prescribing rates over years.

Figure 5-1: Antibiotic prescribing rate per 1000 population, by year, in (1A) primary and (1B) secondary care. Raw data (i.e. no transformations) with HB and quarter collapsed.

A) Primary care prescribing



B) Secondary care prescribing



Antimicrobial abbreviations: Ch, chloramphenicol; Cp, ciprofloxacin; Cl, clindamycin; Er, erythromycin; Fd, fusidic acid; Gn, gentamicin; Lz, linezolid; No, neomycin; Rf, rifampicin; St, streptomycin; Tc, teicoplanin; Te, tetracycline; Tb, tobramycin; Tr, trimethoprim.

Table 5-1: Difference in prescribing rates per 1000 population between primary and secondary care

Year	clindamycin	ciprofloxacin	erythromycin	gentamicin	tetracycline	trimethoprim
2007	0.8	2.4	28.1	0.06	29.9	8.7
2008	0.7	2.7	31.4	0.05	24.4	8.1
2009	0.7	2.9	33.3	0.03	29.1	8.2
2010	0.8	2.7	35.9	0.02	37.6	8.1
2011	0.8	3	38.3	0.02	44	8.4

i.e. Primary prescribing of erythromycin was 28.1 times higher than in secondary care in 2007.

5.3.1.1 PCA results – differences between primary and secondary prescribing

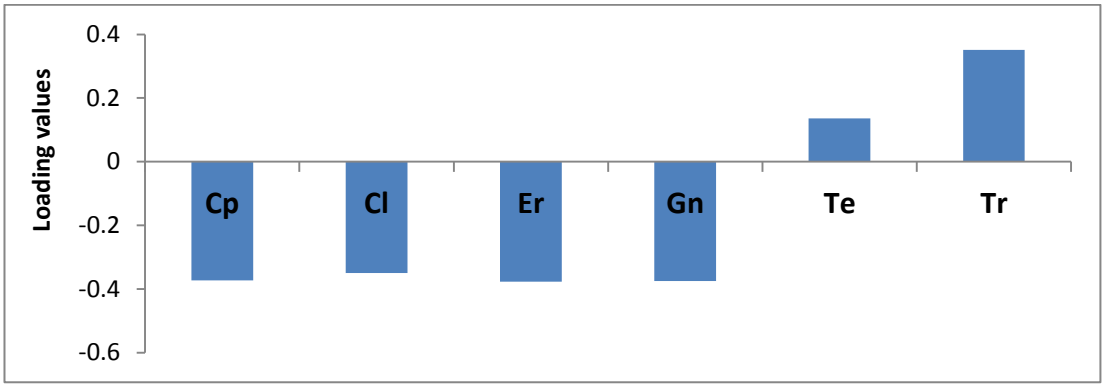
A PCA was conducted on combined primary and secondary care prescribing data showed differences between the two groups (MRPP = chance-corrected within-group agreement, A = 0.84, $p<0.0001$). There was only one significant axis ($p=0.001$) that had an Eigenvector value over 1 and that explained 78.5% of total variation in the data (Table 5-2). Loading scores, which indicate where the main differences in the data lie, were highest for ciprofloxacin, erythromycin, and gentamycin, closely followed by clindamycin and trimethoprim (Figure 5-2). Axis 1, representing 78.5% of total variation, distinguished primary care which was characterised by high levels of ciprofloxacin, erythromycin, tetracycline and trimethoprim prescribing and low levels of gentamycin prescribing, from prescribing in secondary care which had low levels of ciprofloxacin, erythromycin, tetracycline and trimethoprim, and high levels of gentamycin prescribing. Clindamycin was prescribed in both primary and secondary care as represented by the second (non-significant) axis which explained 15.9% of variation in the data (Figure 5-3).

Table 5-2: PCA results for combined primary and secondary care data.

PCA / Axis	Eigenvector values from real data	Eigenvalue from randomisations				% of Variance	Cum.% of Var.
		Minimum	Average	Maximum	p-value		
Primary and secondary data combined							
1	4.65	1.09	1.21	1.36	0.001	77.58	77.58
2	0.94	1.03	1.11	1.21	1	15.69	93.27
3	0.2	0.95	1.03	1.11	1	3.33	96.6
4	0.14	0.88	0.96	1.03	1	2.28	98.88
5	0.05	0.78	0.89	0.99	1	0.82	99.7
6	0.02	0.66	0.81	0.92	1	0.3	100

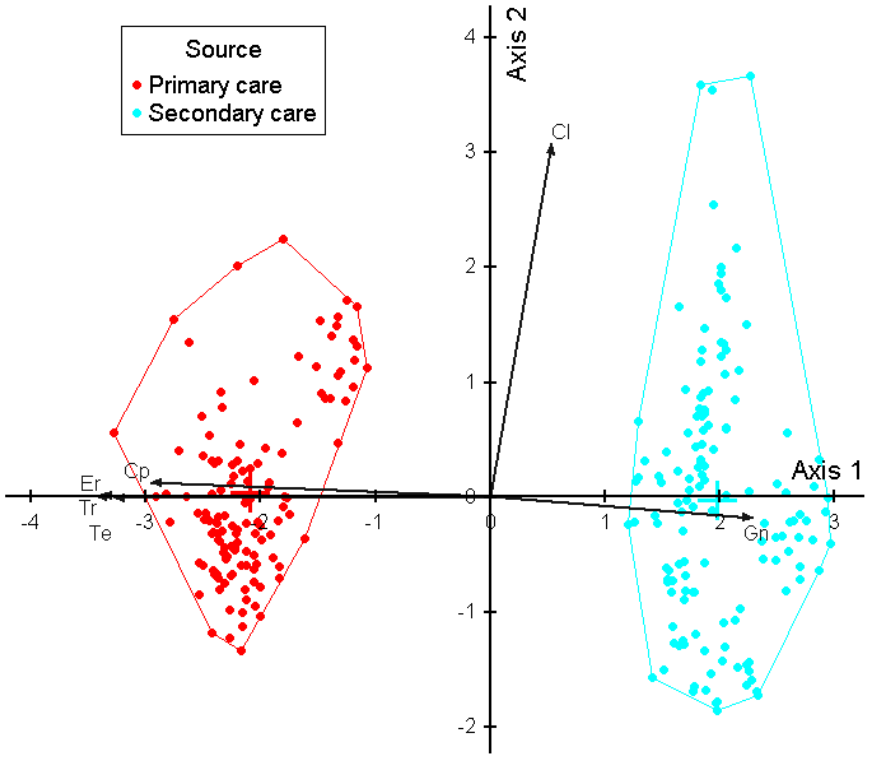
Cum.% of Var. : Cumulative percentage of variance
% of Variance : Percentage of variance

Figure 5-2: PCA loading values for combined primary and secondary care prescribing data.



Antibiotic abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

Figure 5-3: PCA – Joint plot showing differences in prescribing between primary and secondary care.



Radiating lines were superimposed on the ordination to create a joint plot which identifies variables associated with either group. The direction of a line indicates either a positive or negative relationship with an axis and the variables of the secondary matrix, and the length shows the strength of the correlation on that axis. The ordination was rotated in order to show the strongest correlations along axis 1. Antibiotic abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim. Polygons were drawn around the outermost data points to distinguish each group (primary care and secondary care).

5.3.2 Trends

5.3.2.1 Temporal trends in prescribing

5.3.2.1.1 Regression analyses

In primary care, the prescribing rates of ciprofloxacin ($p<0.001$), gentamicin ($p=0.01$), and tetracycline ($p<0.001$) statistically declined over the study period (Table 5-3A). Despite being statistically significant, the adjusted R^2 values were consistently low suggesting the model explained very little of variation in the data. The cubic regression model for ciprofloxacin ($p>0.05$) described the data better than the linear and quadratic models. The linear model was the best fitting for gentamicin, and there were no statistical differences between any of the three regression models

for tetracycline ($p>0.05$). Primary care prescribing of clindamycin ($p=0.0002$) and trimethoprim ($p<0.0001$) increased over the study period, with the linear regression providing the best fitting models for clindamycin, and either the linear or quadratic models being best fitting for trimethoprim with no difference between the two ($p>0.05$). There was no change in the primary care prescribing rates of erythromycin. Figure 5-4A shows primary prescribing data over time with regression fitted.

In secondary care, the prescribing rates of ciprofloxacin ($p<0.0001$), clindamycin ($p=0.02$), erythromycin ($p=0.001$), and tetracycline ($p<0.01$) statistically declined over the study period (Table 5-3B). As with the primary care data, adjusted R^2 values were consistently low. There were no statistical differences between the three regression models for ciprofloxacin, clindamycin and tetracycline (each $p>0.05$), but either the linear or quadratic regressions best described erythromycin prescribing. The prescribing rates for gentamicin (<0.0001) and trimethoprim ($p<0.01$) statistically increased over time with there being no difference between the three regressions for gentamicin ($p>0.05$) and with the linear regression providing the best fit for trimethoprim. Figure 5-4B shows primary prescribing data over time with regression fitted.

Table 5-3: Model outputs from regression analyses of temporal changes in antimicrobial prescribing in (A) primary and (B) secondary care.

All years and health boards combined. In the model column, for the best model for each antimicrobial is in bold and italicised; where there was no difference between models, more than one is in bold.

A) Primary care prescribing

Model	Estimate	SE	p-value	Sig.	Adj. R^2	Trend
Ciprofloxacin'						
Y	-0.01	0.003	0.06	.	0.01	
Y*Y	-0.001	0.0004	0.002	**	0.04	↓
Y*Y*Y	-0.0002	0.00004	0.00008	***	0.07	↓
Clindamycin'						
Y	0.02	0.005	0.0002	***	0.06	↑
Y*Y	0.002	0.001	0.01	**	0.03	↑
Y*Y*Y	0.0001	0.0001	0.04	*	0.02	↑
Erythromycin'						
Y	-0.002	0.002	0.25		0.001	
Y*Y	-0.0002	0.0002	0.23		0.002	
Y*Y*Y	-0.00003	0.00002	0.22		0.002	
Gentamicin''						
Y	-0.004	0.001	0.01	**	0.03	↓
Y*Y	-0.0004	0.0002	0.02	*	0.02	↓
Y*Y*Y	-0.00004	0.00002	0.04	*	0.02	↓
Tetracycline'						
Y	-0.01	0.004	0.0004	***	0.05	↓
Y*Y	-0.002	0.0005	0.0004	***	0.05	↓
Y*Y*Y	-0.0002	0.0001	0.0006	***	0.05	↓
Trimethoprim'						
Y	4.1	0.3	<0.0001	***	0.40	↑
Y*Y	0.4	0.04	<0.0001	***	0.40	↑
Y*Y*Y	0.005	0.0005	<0.0001	***	0.38	↑

' data log transformed
" data log(x+1) transformed
Sig: Significant = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
Trend indicates direction of change if regression is significant (↑ = increase, ↓ = decrease)
SE = standard error
Adj. R^2 = adjusted R^2
Model: linear regression (Y) *Primary_DDD_rate ~ Year*
quadratic regression (Y*Y) *Primary_DDD_rate ~ Year*Year*
cubic regression (Y*Y*Y) *Primary_DDD_rate ~ Year*Year*Year*

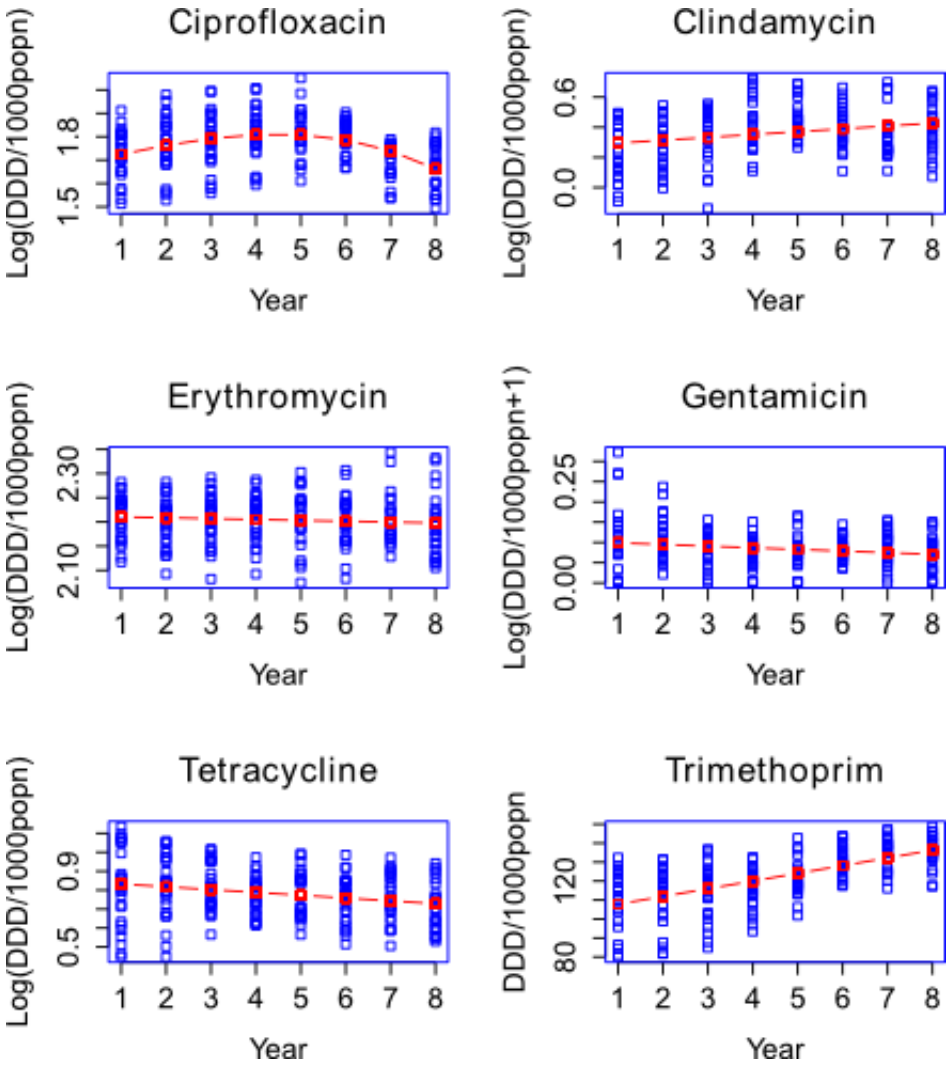
B) Secondary care prescribing

Model	Estimate	SE	p-value	Sig.	Adj. R^2	Trend
Ciprofloxacin'						
Y	-0.05	0.01	<0.0001	***	0.26	↓
Y*Y	-0.01	0.001	<0.0001	***	0.24	↓
Y*Y*Y	-0.001	0.0002	<0.0001	***	0.22	↓
Clindamycin'						
Y	-0.02	0.01	0.02	*	0.03	↓
Y*Y	-0.003	0.001	0.02	*	0.03	↓
Y*Y*Y	-0.001	0.0003	0.03	*	0.03	↓
Erythromycin'						
Y	-0.05	0.01	0.0009	***	0.07	↓
Y*Y	-0.01	0.002	0.0006	***	0.07	↓
Y*Y*Y	-0.001	0.0004	0.001	**	0.07	↓
Gentamicin'						
Y	0.09	0.01	<0.0001	***	0.2	↑
Y*Y	0.01	0.002	<0.0001	***	0.18	↑
Y*Y*Y	0.002	0.0005	<0.0001	***	0.15	↑
Tetracycline						
Y	-0.02	0.01	0.01	**	0.04	↓
Y*Y	-0.004	0.001	0.01	**	0.05	↓
Y*Y*Y	-0.001	0.0003	0.005	**	0.05	↓
Trimethoprim'						
Y	0.02	0.01	0.01	**	0.04	↑
Y*Y	0.004	0.002	0.02	*	0.03	↑
Y*Y*Y	0.001	0.0003	0.03	*	0.03	↑

' data log transformed
" data log(x+1) transformed
Sig: Significant = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
Trend indicates direction of change if regression is significant (↑ = increase, ↓ = decrease)
SE = standard error
Adj. R = adjusted R^2
Model: linear regression (Y) *lm(Primary_DDD_rate ~ Year)*
quadratic regression (Y*Y) *lm(Primary_DDD_rate ~ Year*Year)*
cubic regression (Y*Y*Y) *lm(Primary_DDD_rate ~ Year*Year*Year)*

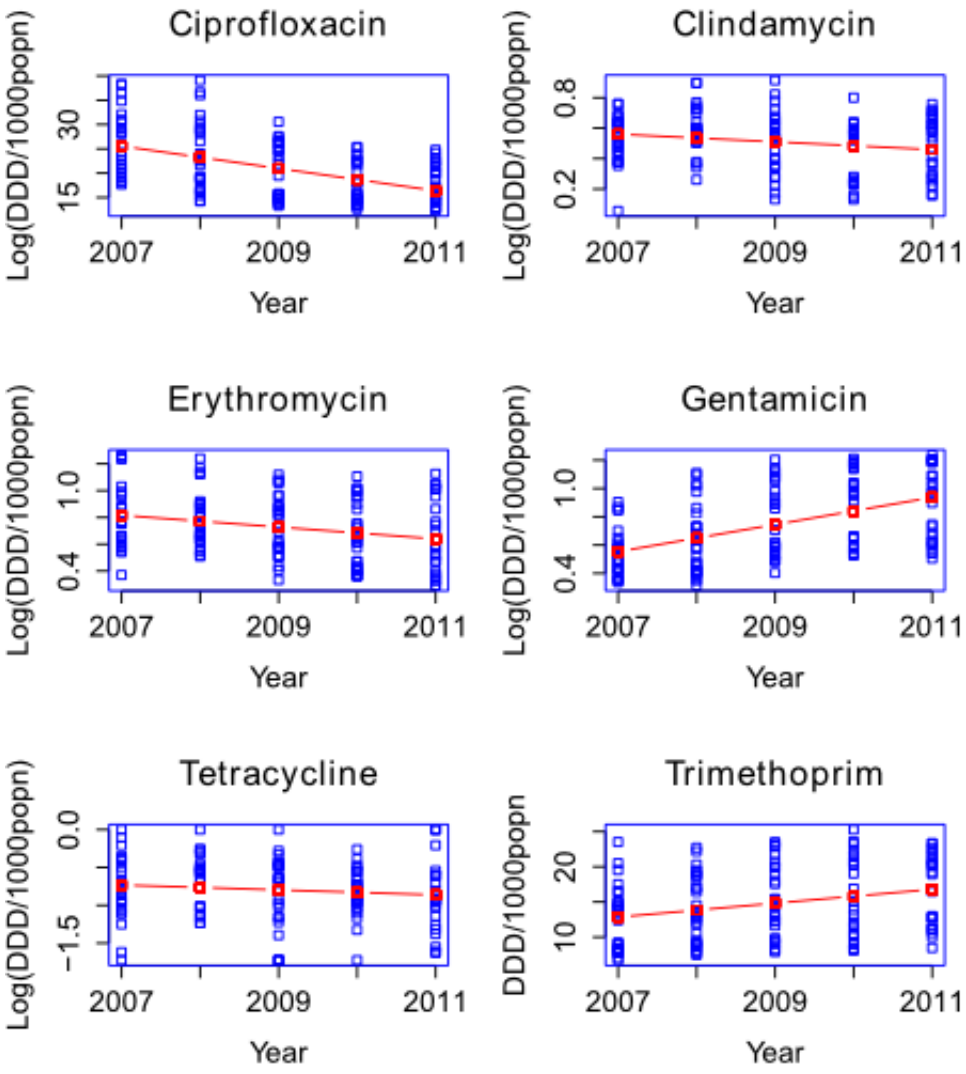
Figure 5-4: Plots of regression models fitted to data showing prescribing rates over time in (A) primary and (B) secondary care.

(A)



x-axis year: 1 = 2004/05, 2 = 2005/06, 3 = 2006/07, 4 = 2007/08, 5 = 2008/09, 6 = 2009/10, 7 = 2010/11, 8 = 2011/2012
y-axis = DDD (daily defined doses) per 1000 population

(B)



y-axis = DDD (daily defined doses) per 1000 population

5.3.2.2 PCA results– Differences over time

A PCA of primary care prescribing data showed differences in prescribing over time (MRPP, $p<0.0001$). There were two significant axes ($p=0.001$) with eigenvector values over 1.0 which explained 53% of total variation in the data (Table 5-4). While the eigenvector value for a third axis was close to 1.0, it was not significant and therefore not described. The loading scores (Figure 5-5A), which indicate where the main differences in the data lie, were highest in Axis 1 for tetracycline followed by ciprofloxacin, and Axis 2 for ciprofloxacin and erythromycin. The joint plot (Figure 5-6Ai) appears to show a shift from left to right along Axis 1 which explains 29.0% of the total variation and represents a reduction in tetracycline prescribing and an increase in trimethoprim and clindamycin prescribing over time. This is consistent with the above regression analyses. Axis 2, which explains 24.0% of the variation in the data, is associated with gentamycin and ciprofloxacin prescribing. The ellipse plot also indicates a shift over time (Figure 5-6Aii).

A further PCA was carried out on secondary care prescribing data and also showed there to be differences in prescribing over time (MRPP, $p<0.0001$). There were two significant axes ($p=0.001$) with eigenvector values over 1 which explained 65.5% of total variation in the data (Table 5-4). The loading scores (Figure 5-5B) were highest in Axis 1 for ciprofloxacin, clindamycin and gentamicin, and Axis 2 for trimethoprim, erythromycin and again gentamicin. The joint plot (Figure 5-6Bi) appears to show a shift from left to right along Axis 1 (34.0% of the total variation) which distinguished between high levels of ciprofloxacin and clindamycin prescribing which declined over time, and low levels of gentamycin prescribing which increased over time. Axis 2 (31.5% of the variation) separated erythromycin which declined and trimethoprim that increased over time. Again, this is consistent with the above regression analyses. The ellipse plot also indicates a shift over time (Figure 5-6Bii).

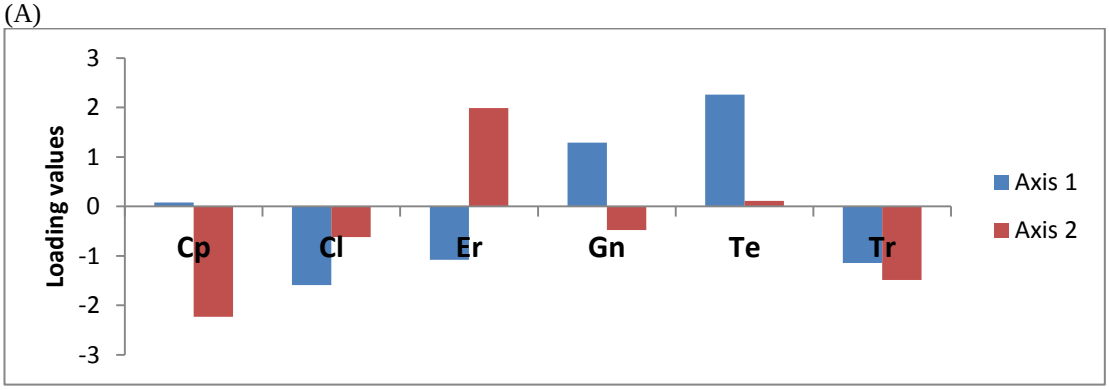
Table 5-4: PCA results for (A) primary and (B) secondary care data.

Axis	Eigenvector values from real data	Eigenvalue from randomisations				% of Variance	Cum.% of Var.
		Minimum	Average	Maximum	p-value		
(A) Primary							
1	2.07	1.11	1.29	1.61	0.001	28.64	28.64
2	1.83	0.99	1.15	1.32	0.001	23.71	52.35
3	0.98	0.91	1.04	1.15	0.962	16.05	68.4
4	0.64	0.79	0.94	1.05	1	13.45	81.85
5	0.33	0.7	0.84	0.95	1	10.88	92.72
6	0.15	0.58	0.73	0.9	1	7.28	100
(B) Secondary							
1	2.07	1.11	1.29	1.61	0.001	34.53	34.53
2	1.83	0.99	1.15	1.32	0.001	30.57	65.09
3	0.98	0.91	1.04	1.15	0.962	16.28	81.38
4	0.64	0.79	0.94	1.05	1	10.68	92.05
5	0.33	0.7	0.84	0.95	1	5.46	97.51
6	0.15	0.58	0.73	0.9	1	2.49	100

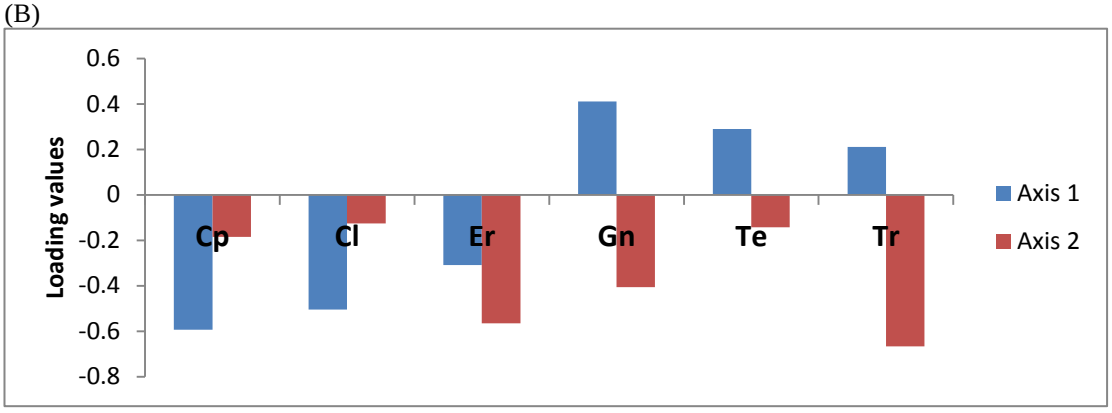
Cum.% of Var. : Cumulative percentage of variance
% of Variance : Percentage of variance

Figure 5-5: PCA loading values for (A) primary and (B) secondary care.

(A)



(B)



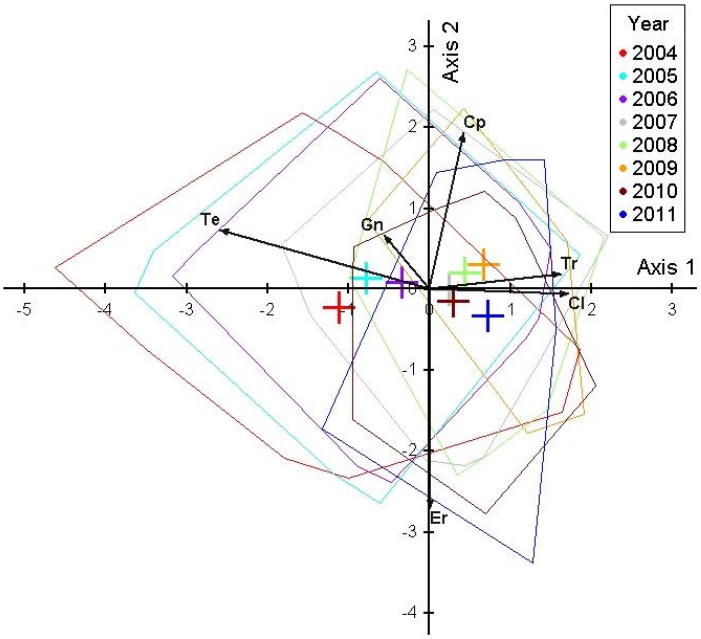
Antibiotic abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

Figure 5-6: PCA - Differences over time in (A) primary and (B) secondary care prescribing.

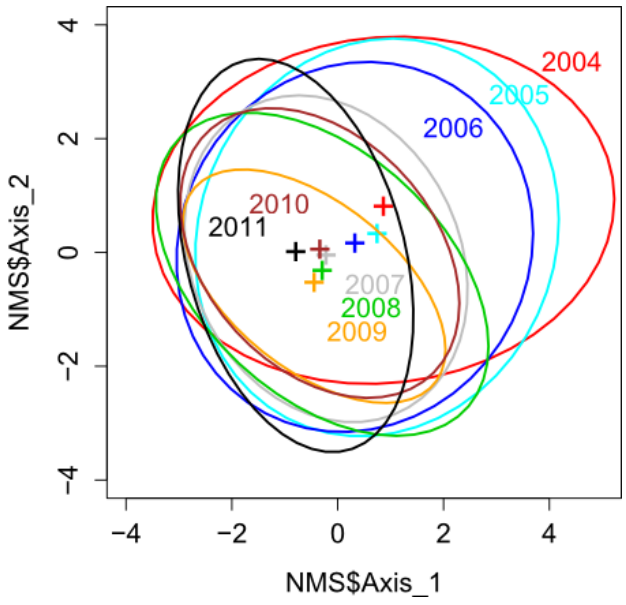
i – The joint plots show radiating lines that were superimposed on the ordination to identify variables associated with the grouping variable (i.e. year). The direction of a line indicates either a positive or negative relationship with an axis and the variables of the secondary matrix, and the length shows the strength of the correlation on that axis. Crosses indicate the centroid of the polygon which joins the outer data points for each group. The ordination was rotated in order to show the strongest correlations along axis 1.

ii – The confidence ellipses show with 95% confidence where data in ordination space lie. Crosses indicate central points of ellipses. Data points removed to make it easier to see the patterns

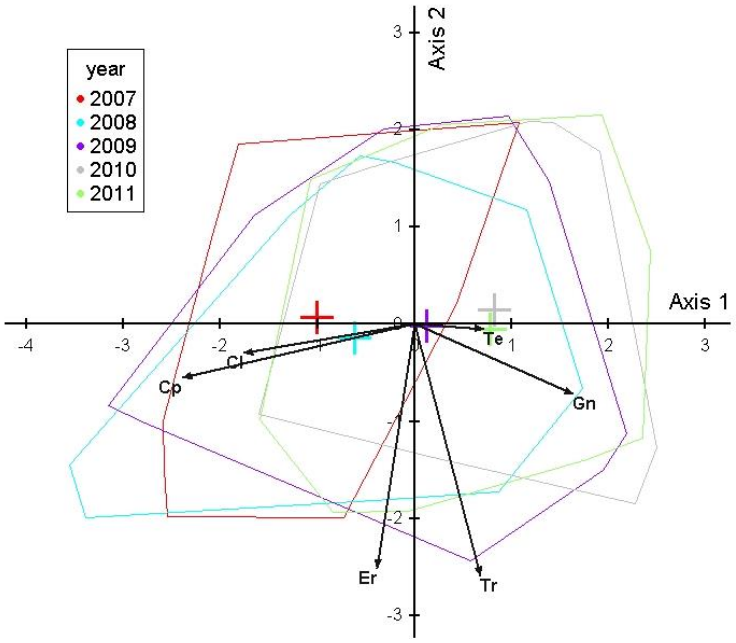
(Ai)



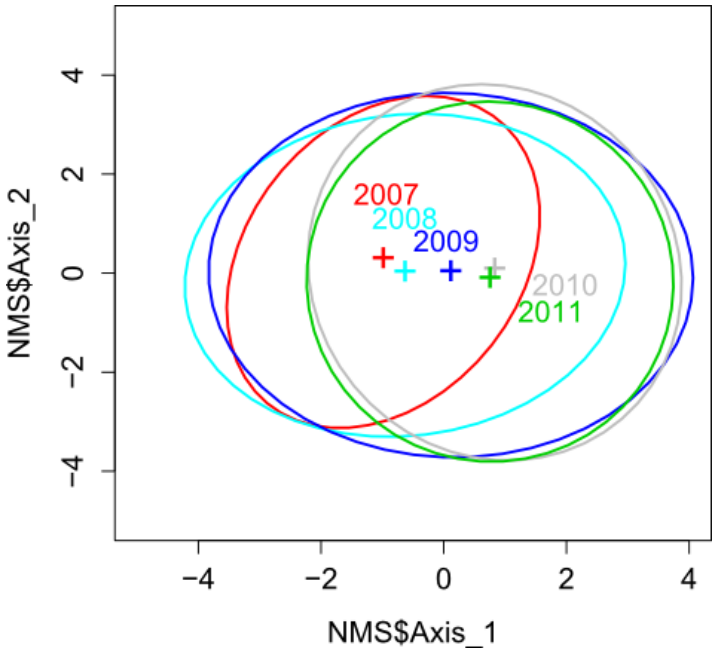
(Aii)



(Bi)



(Bii)



Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

5.3.2.3 Trends in prescribing between NHS Health Boards

5.3.2.3.1 ANOVA and *post-hoc* Tukey analyses

ANOVA analyses showed that for each of the six antimicrobials, i.e. clindamycin, ciprofloxacin, erythromycin, gentamycin, tetracycline, and trimethoprim, there were differences between HBs ($p<0.0001$) in prescribing rates in both primary and secondary care (Tables 5-5A and 5-5B).

Post-hoc Tukey analyses showed pairwise differences in prescribing rates between HBs (Tables 5-5A and 5-5B). Each letter represents different groups of HB where the prescribing rates were similar. No one HB was consistently high across all antimicrobials examined. For each antimicrobial, there are several different groupings with tetracycline prescribing in primary care having the fewest (3 groups) meaning lower heterogeneity in prescribing rates. In secondary care, ciprofloxacin had the fewest number of groupings (three groups), while both gentamicin and trimethoprim had the highest number (6 groups). Figure 5-7A and 5-7B shows the between-HB differences in prescribing means for both primary and secondary care respectively. These interval plots also show for which pairs of HBs the prescribing rates are significantly different since the confidence intervals do not overlap.

Table 5-5: Results of ANOVA and post-hoc Tukey analysis in (A) primary and (B) secondary care showing the mean, standard deviation (SD), minimum and maximum prescribing rates (DDD per 1000 population) and HB groupings.

(A)

HB	Mean	Minimum	SD	Maximum	Grouping§
Ciprofloxacin' ANOVA - p<0.0001					
AA	1.75	1.55	0.08	1.86	c
FI	1.81	1.75	0.04	1.88	b
FV	1.92	1.74	0.07	2.05	a
GC	1.75	1.64	0.04	1.82	c
GR	1.78	1.49	0.14	1.92	bc
LA	1.75	1.65	0.05	1.84	c
LO	1.62	1.51	0.06	1.72	d
Clindamycin' ANOVA p<0.0001					
AA	0.23	-0.06	0.11	0.47	cd
FI	0.32	0.07	0.12	0.5	bc
FV	0.51	0.31	0.09	0.73	a
GC	0.37	0.16	0.09	0.5	b
GR	0.32	0.07	0.11	0.56	bc
LA	0.22	-0.14	0.18	0.57	d
LO	0.58	0.35	0.1	0.71	a
Erythromycin' ANOVA p<0.0001					
AA	2.24	2.13	0.06	2.34	a
FI	2.2	2.11	0.04	2.27	b
FV	2.2	2.11	0.03	2.24	b
GC	2.21	2.16	0.03	2.27	ab
GR	2.15	2.07	0.05	2.25	c
LA	2.18	2.12	0.04	2.24	b
LO	2.24	2.15	0.04	2.3	a
Gentamycin'' ANOVA p<0.0001					
AA	0.04	0.0004	0.04	0.16	c
FI	0.12	0.04	0.07	0.32	a
FV	0.06	0.003	0.03	0.15	bc
GC	0.12	0.08	0.02	0.17	a
GR	0.08	0.003	0.05	0.18	b
LA	0.08	0.03	0.02	0.12	b
LO	0.08	0.03	0.02	0.12	b
Tetracycline' ANOVA p<0.0001					
AA	0.66	0.56	0.06	0.76	c
FI	0.73	0.54	0.09	0.9	c
FV	0.67	0.45	0.13	0.89	c
GC	0.84	0.75	0.03	0.91	b
GR	0.96	0.84	0.06	1.08	a
LA	0.88	0.7	0.13	1.14	b
LO	0.69	0.51	0.11	0.87	c
Trimethoprim ANOVA p<0.0001					
AA	130.36	120.71	7.62	148.76	ab
FI	131.42	112.41	11.28	147.4	a
FV	118.35	106.07	8	140.54	cd
GC	121.7	105.26	10.28	139.61	bc
GR	128.28	117.3	6.74	143.89	abc
LA	109.61	79.94	23.34	145.96	d
LO	111.88	95.81	9.27	127.21	d

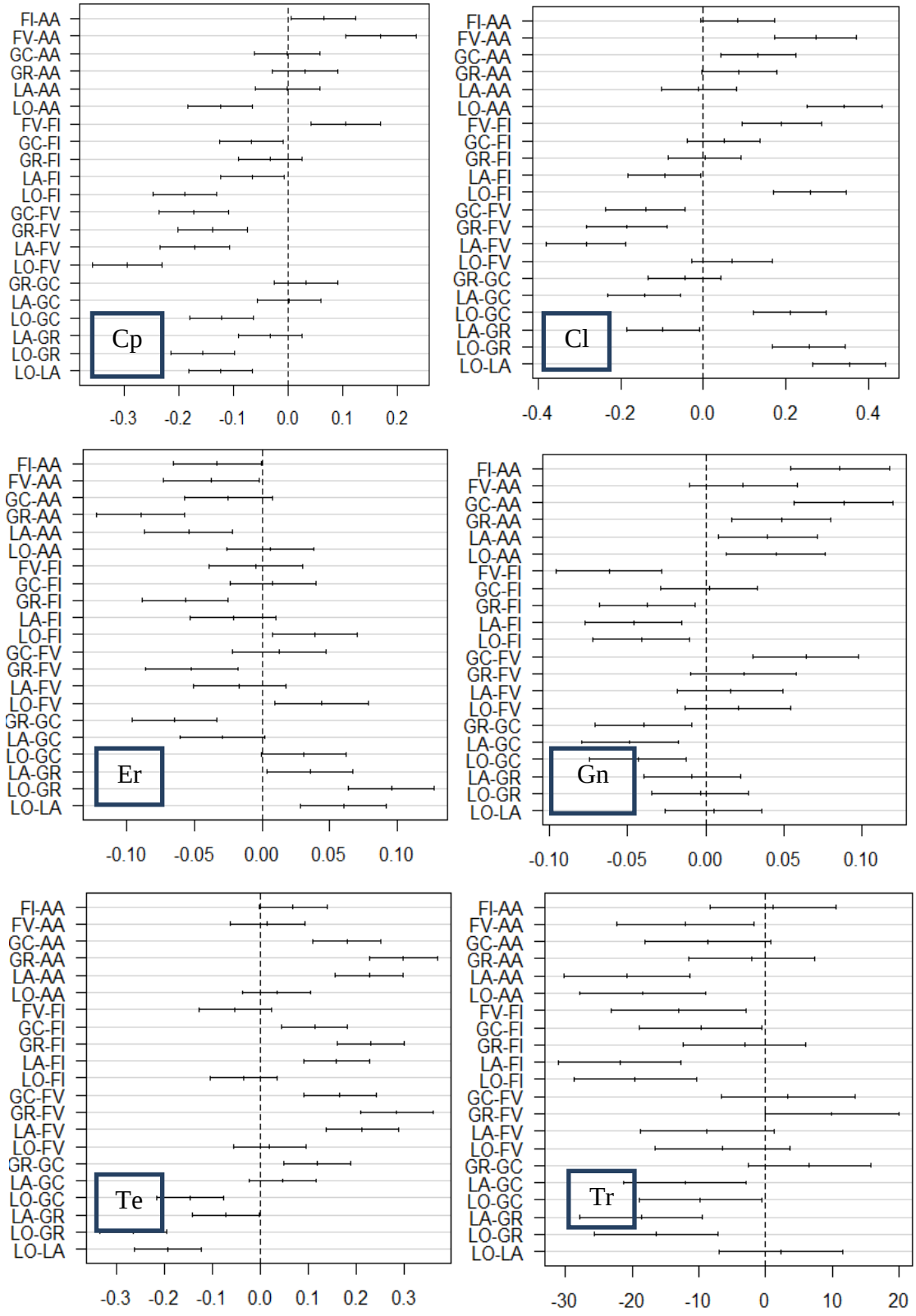
(B)

HB	Mean	Minimum	SD	Maximum	Grouping§
Ciprofloxacin' ANOVA - p<0.0001					
AA	29.37	22.27	6.37	39.09	a
FI	16.36	12.64	2.96	22.66	c
FV	16.97	13.04	2.23	20.78	c
GC	21.55	12.51	7.6	34.85	b
GR	23.26	15.68	2.89	27.61	b
LA	15.58	12.23	2.51	21.69	c
LO	25	18.16	4.17	30.9	a
Clindamycin' ANOVA p<0.0001					
AA	0.68	0.44	0.15	0.91	a
FI	0.34	0.06	0.14	0.69	d
FV	0.43	0.22	0.2	0.77	cd
GC	0.37	0.13	0.19	0.72	d
GR	0.6	0.4	0.08	0.75	ab
LA	0.54	0.37	0.08	0.64	bc
LO	0.62	0.54	0.05	0.71	ab
Erythromycin' ANOVA p<0.0001					
AA	1.12	0.99	0.09	1.27	a
FI	0.54	0.29	0.14	0.77	d
FV	0.49	0.29	0.12	0.74	c
GC	0.91	0.81	0.06	1.02	b
GR	0.86	0.69	0.1	1.03	b
LA	0.69	0.51	0.08	0.83	c
LO	0.51	0.34	0.09	0.64	d
Gentamycin' ANOVA p<0.0001					
AA	0.54	0.36	0.13	0.74	de
FI	0.51	0.36	0.09	0.67	e
FV	0.69	0.31	0.32	1.09	cd
GC	0.81	0.58	0.16	1	bc
GR	1.07	0.85	0.14	1.21	a
LA	0.97	0.5	0.26	1.24	ab
LO	0.59	0.49	0.06	0.73	de
Tetracycline' ANOVA p<0.0001					
AA	-1.09	-1.73	0.57	0	c
FI	-0.68	-1.23	0.37	0	a
FV	-0.8	-1.63	0.42	0	abc
GC	-0.52	-1.14	0.27	-0.11	a
GR	-0.73	-1.31	0.27	-0.26	ab
LA	-0.73	-1.61	0.33	-0.21	ab
LO	-1.05	-1.39	0.18	-0.76	bc
Trimethoprim ANOVA p<0.0001					
AA	15.1	12.38	2.25	20.6	c
FI	11.21	6.71	1.84	13.17	d
FV	9	7.47	1.48	12.99	e
GC	21.97	17.53	1.9	25.27	a
GR	17.81	14.36	2.18	21.8	b
LA	18.96	13.71	3.22	23.3	b
LO	9.34	7.02	1.25	11.14	de

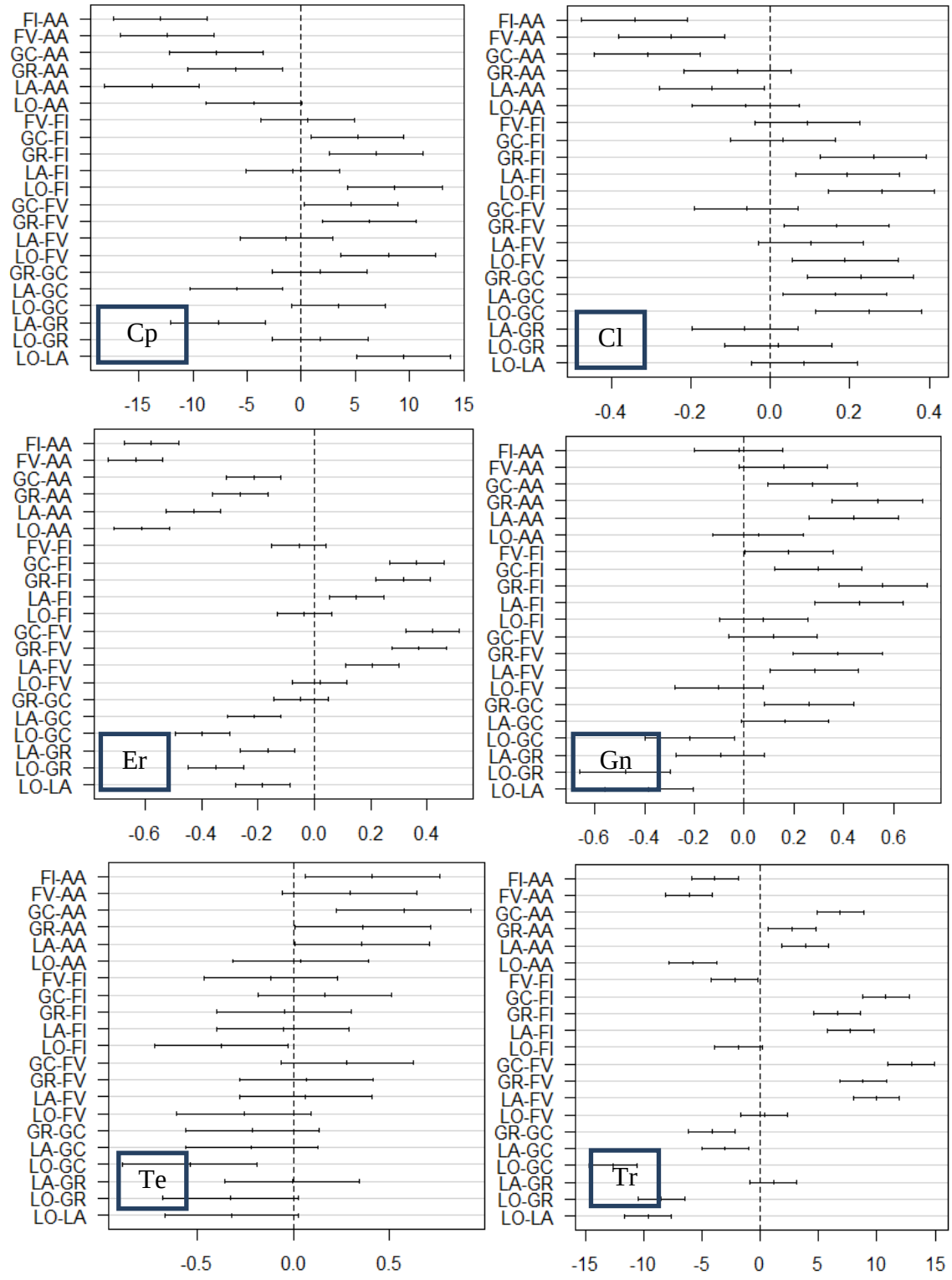
' data log transformed; " data log(x+1) transformed; HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian; **§Groupings:** HBs with statistically different rates will be placed in different groups. Groups are assigned letters to easily identify different groups. Thus, HBs in the same group do not have statistically different means or %MRSA, and hence will be assigned the same letter.

Figure 5-7: Results of post-hoc Tukey analysis showing differences in prescribing rates between HBs for each antibiotic in (A) primary and (B) secondary care with 95%CI.

(A)



(B)



x-axis = Differences in mean levels of Daily Defined Doses (DDD) per 1000 population. y-axis: Pairwise HB comparisons; HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian. Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

5.3.2.4 PCA results– differences between HBs

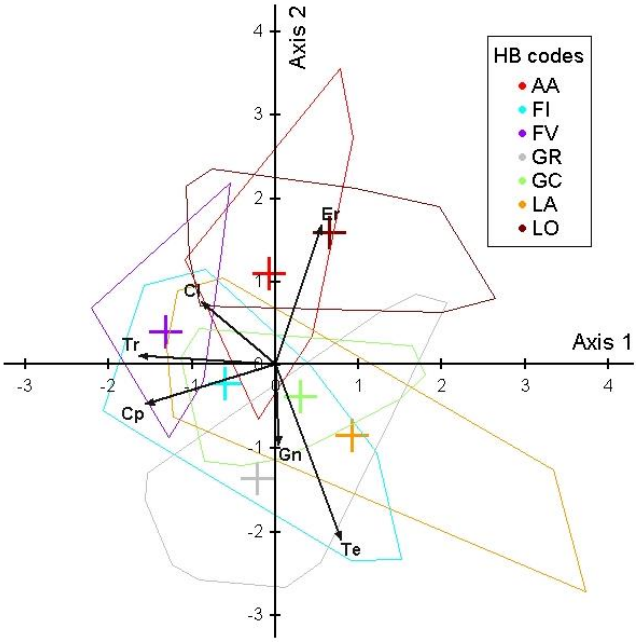
In the two aforementioned PCAs of primary care and secondary care prescribing rates, differences between HBs was also investigated. In both primary and secondary care, there were differences in prescribing rates between HBs (MRPP, $p<0.0001$). The results from the PCA for primary care, as visualised in the joint plot (Figure 5-8Ai), largely agree with those from the ANOVA and Tukey analyses, for example prescribing of ciprofloxacin was highest in NHS Forth Valley, NHS Fife, and NHS Grampian, clindamycin highest in NHS Lothian and NHS Forth Valley, and gentamicin highest in NHS Fife, NHS Greater Glasgow and Clyde and NHS Grampian. Results from the PCA for secondary care, as visualised in the joint plot (Figure 5-8Bi), were also largely similar to the ANOVA and Tukey analyses: NHS Ayrshire and Arran had the highest rates of prescribing of ciprofloxacin, clindamycin and erythromycin, NHS Greater Glasgow and Clyde had high rates of prescribing of erythromycin, gentamicin and trimethoprim, and both NHS Fife and NHS Forth Valley had comparably low rates of prescribing of all antimicrobials other than tetracycline. Figures 5-8Aii and 5-8Bii show the confidence ellipses for HBs on the ordination which highlights much overlap in prescribing rates between each HB.

Figure 5-8: PCA – between HBs in (A) primary care and (B) secondary care prescribing.

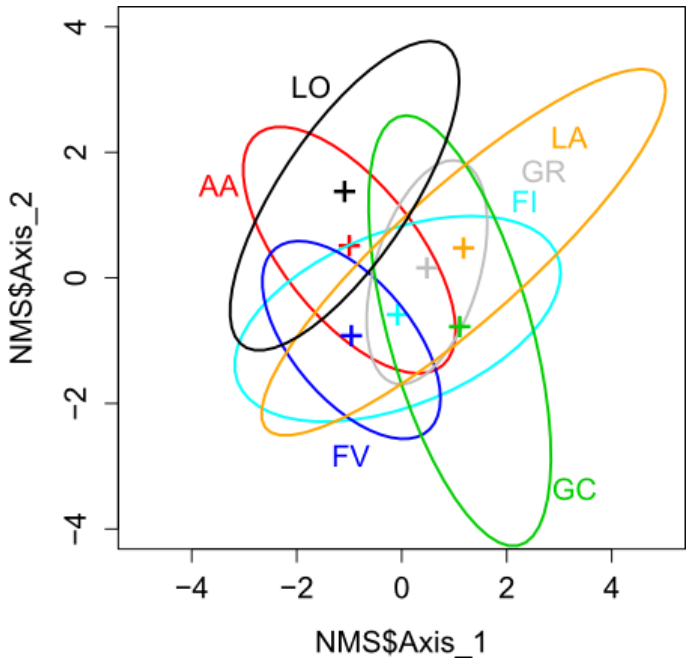
i –Joint plots show radiating lines that were superimposed on the ordination to identify variables associated with the grouping variable (i.e. year). Line direction indicates either a positive or negative relationship with an axis and the variables of the secondary matrix, and the length shows the strength of correlation on that axis. Crosses indicate the centroid of the polygon. The ordination was rotated to show the strongest correlations along axis 1.

ii –Confidence ellipses, with 95% confidence limits, show the location of the data points in ordination space lie. Crosses indicate central points of ellipses.

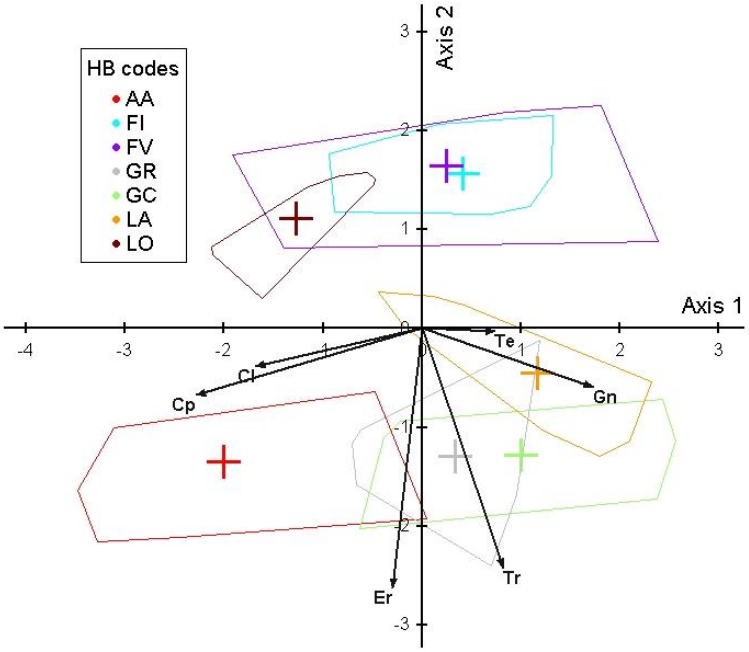
(Ai)



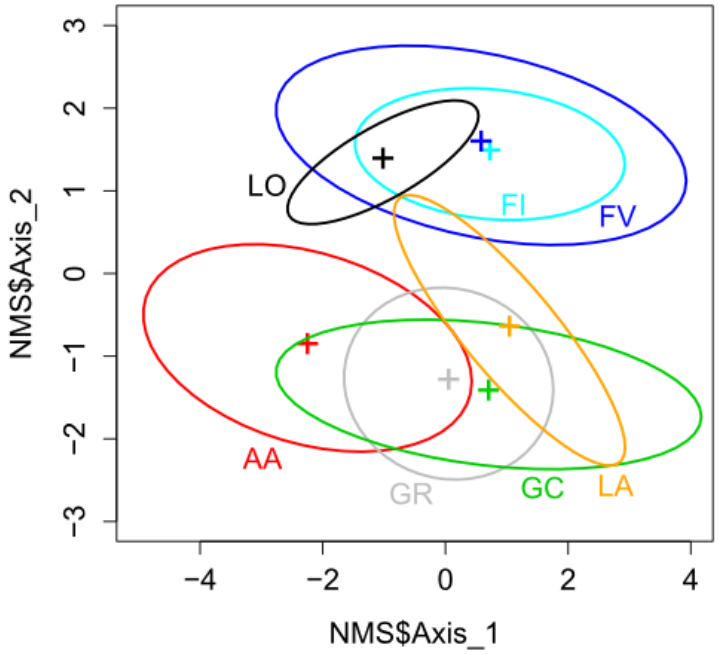
(Aii)



(Bi)



(Bii)



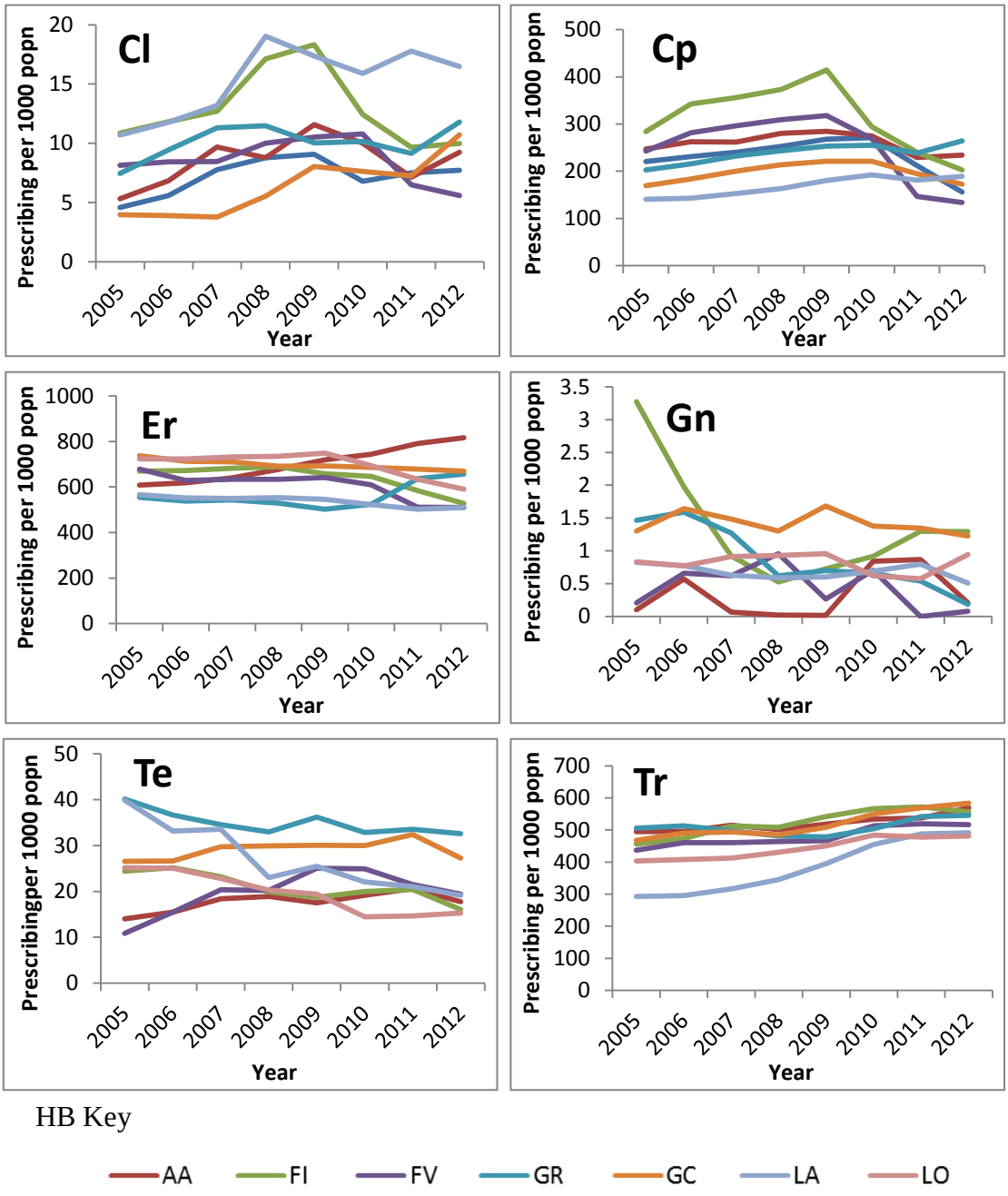
HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian. Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

5.3.2.5 Trends in prescribing by NHS Health Boards over time

Prescribing rates were further investigated using the model ($mod <- lm(prescribing\ rate \sim HB + Year + HB * Year)$) to determine if there were differences in prescribing rates in each NHS HB over time. Model outputs are given in Appendix Table 2A (primary care) and Appendix Table 2B (secondary care). There were significant interactions between HB and year in both primary and secondary care indicating differences in prescribing in each HB over time for each antimicrobial ($p<0.0001$) except tetracycline in secondary care ($p>0.05$). An additive model ($mod <- lm(prescribing\ rate \sim HB + Year)$) for tetracycline in secondary care was not significant ($p>0.05$). Thus, prescribing rates over time differed between HBs. Differences in prescribing rates between HBs for primary care are plotted in Figure 5-9A and for secondary care in Figure 5-9B.

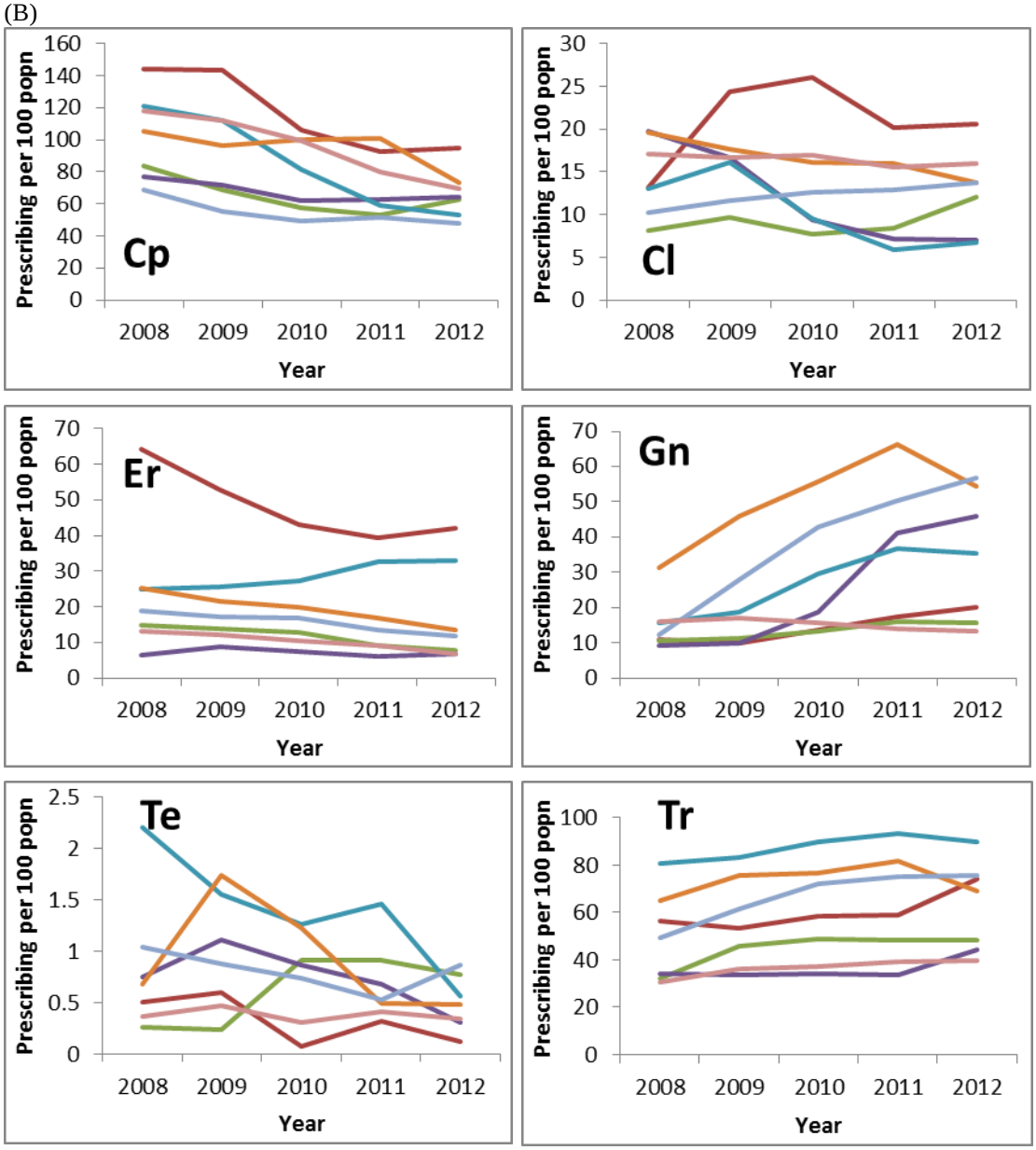
Figure 5-9: Prescribing rates of antibiotics in (A) primary and (B) secondary care, by NHS health boards.

(A)



HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian. Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

(B)



HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian. Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.

5.3.3 The association between prescribing rates and resistance

5.3.3.1 Univariate analyses

Associations between prescribing rates and antimicrobial resistance were examined using univariate analysis with prescribing rates as the independent variables and the proportion resistant at *t0* and each time lag (*t1–t6*) as the dependent variables. Model outputs for primary care are shown in Appendix Tables 3A and for secondary care in Appendix Tables 3B. Prescribing rates were positively associated with the concurrent proportion resistant (*t0*), and also at each time lag (*t1 – t6*) for clindamycin and gentamicin in primary care, and for gentamicin and trimethoprim in secondary care. Primary care prescribing rates of trimethoprim were positively associated with trimethoprim resistance in *t0* and *t1*. No other associations were found ($p>0.05$).

5.3.3.2 Multivariate analyses

In a multivariate analysis, the proportions of isolates resistant to each antimicrobial at *t0* were the dependent variables, and prescribing rates (both primary and secondary care), HB and year were the independent variables. When a significant model was identified, it was then run using staggered resistance data representing lag period *t1-t6* as the independent variable. Significant associations between prescribing and resistance were only found for clindamycin, gentamycin and tetracycline.

The best fitting model to describe clindamycin resistance was an additive model including the variables primary care prescribing, year and NHS HB (Table 5-6). This model was positive and significant ($p<0.0001$) at *t0* and each time lag (*t1-t6*). This model was significant albeit with low adjusted R^2 values. The best fitting model to describe gentamicin resistance included primary care prescribing and secondary care prescribing with an interaction term (Table 5-6). This model was positive and significant ($p<0.0001$) at *t0* and each time lag (*t1-t6*) but with low adjusted R^2 values. The best fitting model to describe trimethoprim resistance included secondary care prescribing only (Table 5-6) and this was positive and significant ($p<0.0001$) at *t0* and each time lag (*t1-t6*).

In the two aforementioned PCAs; antibiotic resistance data was included as an overlay in matrix 2. Prescribing and resistance data represented concurrent years (i.e.

t0). The joint plots (primary care by years - Figure 5-10Ai, primary care by HB – Figure 5-10Aii; secondary care by years - Figure 5-10Bi, secondary care by HB – Figure 5-10Bii) show weak associations, particularly for secondary care in which resistance and prescribing of erythromycin, gentamycin, tetracycline and trimethoprim were on radiating lines in similar directions that occupied proximal ordination spaces (Figure 5-10Bi). This is also the case for gentamycin and trimethoprim in primary prescribing (Figure 5-10Ai).

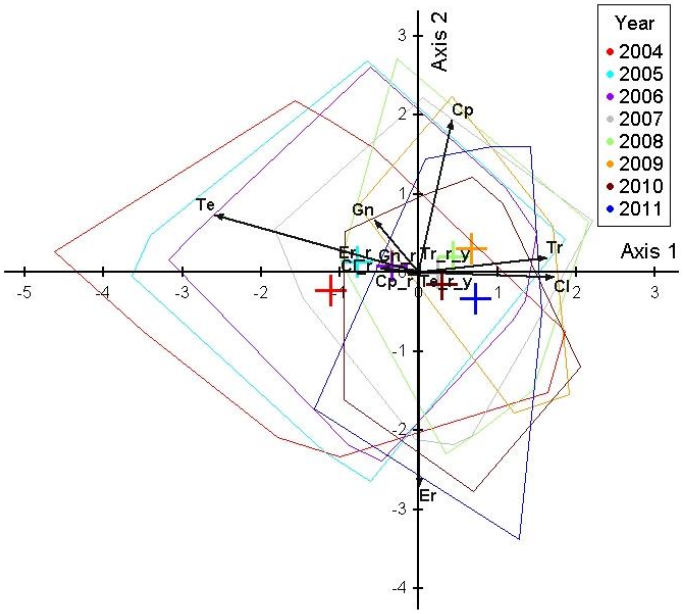
Table 5-6: Results of multivariate analysis for clindamycin, gentamycin and trimethoprim – association between proportion resistant and prescribing rates, by year and HBs.

Variables and Interactions		Estimate	SE	Var p-value	Sig.	Adj. R^2	Model p-value
Clindamycin resistance ~ primary prescribing rate (Pr) + Year (Y) + HB							
t0	Pr	0.23	0.07	0.002	**	0.18	<0.0001
	Y	-0.01	0.01	0.005	**		
	HB	0.03	0.01	<0.0001	***		
t1	Pr	0.27	0.08	0.0005	***	0.19	<0.0001
	Y	-0.01	0.01	0.01	*		
	HB	0.03	0.01	<0.0001	***		
t2	Pr	0.26	0.07	0.0006	***	0.23	<0.0001
	Y	-0.02	0.01	0.004	**		
	HB	0.03	0.01	<0.0001	***		
t3	Pr	0.24	0.07	0.002	**	0.23	<0.0001
	Y	-0.01	0.01	0.01	*		
	HB	0.03	0.01	<0.0001	***		
t4	Pr	0.22	0.08	0.004	**	0.23	<0.0001
	Y	-0.01	0.01	0.04	*		
	HB	0.03	0.01	<0.0001	***		
t5	Pr	0.16	0.08	0.03	*	0.23	<0.0001
	Y	-0.01	0.01	0.13			
	HB	0.04	0.01	<0.0001	***		
t6	Pr	0.13	0.08	0.1	.	0.22	<0.0001
	Y	-0.01	0.01	0.37			
	HB	0.04	0.01	<0.0001	***		
Gentamicin resistance ~ primary prescribing rate (Pr) * secondary prescribing rate (Sec)							
t0	Pr	0.71	0.19	0.0002	***	0.29	<0.0001
	Sec	0.14	0.03	<0.0001	***		
t1	Pr	0.46	0.19	0.02	*	0.25	<0.0001
	Sec	0.15	0.03	<0.0001	***		
t2	Pr	0.46	0.18	0.01	*	0.21	<0.0001
	Sec	0.12	0.03	<0.0001	***		
t3	Pr	0.44	0.19	0.02	*	0.18	<0.0001
	Sec	0.12	0.03	<0.0001	***		
t4	Pr	0.39	0.19	0.04	*	0.15	<0.0001
	Sec	0.11	0.03	0.0002	***		
t5	Pr	0.46	0.19	0.02	*	0.17	<0.0001
	Sec	0.11	0.03	0.0001	***		
t6	Pr	0.56	0.19	0.004	**	0.18	<0.0001
Trimethoprim resistance ~ secondary prescribing rate (Sec)							
t0	Sec	0.02	0.0038	<0.0001	***	0.15	<0.0001
t1	Sec	0.02	0.0036	<0.0001	***	0.14	<0.0001
t2	Sec	0.01	0.0038	0.0006	***	0.08	0.0006
t3	Sec	0.01	0.0037	0.0009	***	0.08	0.0009
t4	Sec	0.01	0.0037	0.0003	***	0.09	0.0003
t5	Sec	0.01	0.0037	<0.0001	***	0.11	<0.0001
t6	Sec	0.02	0.0036	<0.0001	***	0.13	<0.0001

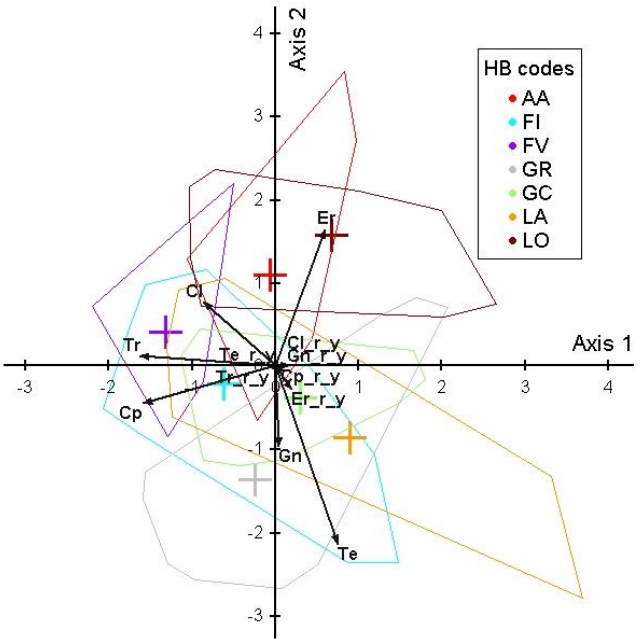
Sig.: Significant = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '
SE: standard error; Adj. R^2 : adjusted R^2; Var. p-value: variable

Figure 5-10: PCA – the previously shown joint plots for (A) primary care prescribing (i) over years and (ii) NHS Health Boards and for (B) secondary care prescribing (i) over years and (ii) NHS Health Boards with additional information on AMR superimposed.

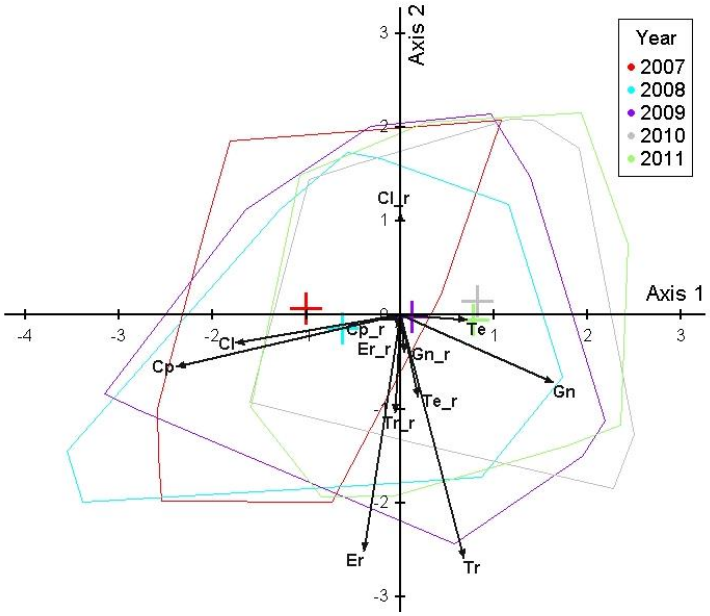
(Ai)



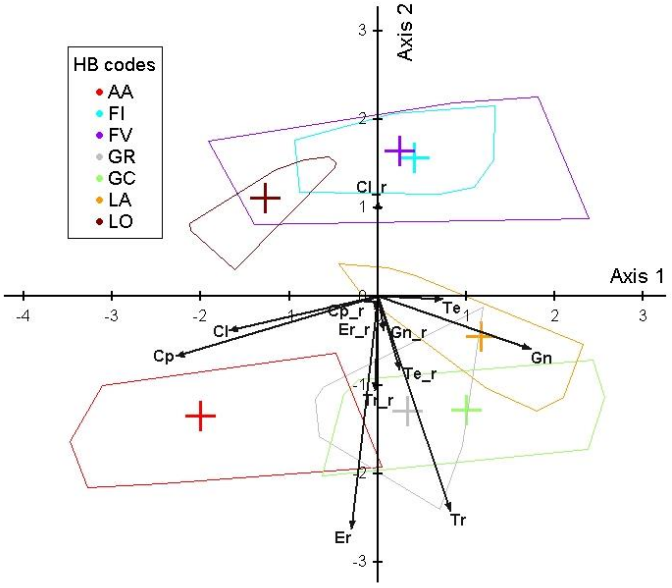
(Aii)



(Bi)



(Bii)



HB abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian.
Antimicrobial abbreviations: Cl, clindamycin; Cp, ciprofloxacin; Er, erythromycin; Gn, gentamycin; Te, tetracycline; Tr, trimethoprim.
Resistance in MRSA population to each antimicrobial: r_CpMRSA, Cp; r_ClMRSA, Cl; r_ErMRSA, Er; r_GnMRSA, Gn; r_TeMRSA, Te; r_TrMRSA, Tr.

5.4 Discussion

5.4.1 The volume of prescribing in primary and secondary care

Despite concerns of over-prescribing of antimicrobials and the threat of the post-antibiotic era, prescribing rates of several antimicrobials increased over this study period. Primary care prescribing was far higher than that in secondary care, although this differed between antimicrobials. This agrees with other studies reported in the literature: in the UK in 2005, approximately 80% of all antibiotics were prescribed by GPs, most of which were for respiratory infections [187]. Similarly, for the period 2009-2010 in the Netherlands, 80% of prescribing occurred in primary care [188].It is difficult to reduce antimicrobial consumption [127], particularly when the burden of bacterial disease remains high, and where patients expect to be treated with drugs and therefore there is pressure on medical professionals to prescribe [189].

5.4.2 Spatial differences

This study also found that, for all antimicrobials studied, the rate of prescribing differed between Health Boards. This could suggest spatial variation in prescribing policies and implementation of those policies. It may also reflect the different hospital types or specialties within those HBs with prescribing pressures differing between locations to meet the needs of infections or conditions particular to those hospitals or specialties. It may also reflect a variation in demographics, e.g. age, sex, ethnicities, or other social characteristics of an HB, e.g. low social economic status, or high rates of drug abuse.

5.4.3 AMR and prescribing

It is presumed that in high antimicrobial selection pressure environments, i.e. where there is high levels of antimicrobial prescribing and consumption, it would be a selective advantage for bacteria to be resistant to those drugs, and hence it would also be expected that with intensifying usage resistance would also increase [48-50]. In this study, significant positive associations between prescribing and resistance were found for gentamicin and trimethoprim in both primary and secondary care, and clindamycin in primary care only. For clindamycin and gentamycin, resistance was

seen concurrently with prescribing but was also detectable up to 18 months later (6 quarters). In a meta-analysis of the effect of prescribing on resistance in different bacterial populations, there was strong evidence of associations which were strongest in the month directly after prescribing, but detectable up to 12 months after prescribing [184]. The association between prescribing of several antimicrobial classes and an observed change in the proportion of *S. aureus* that was MRSA in an Aberdeen hospital was seen for each drug class within 7 months, (only 1 month delay described for macrolides, penicillins with extended spectrum, and third-generation cephalosporins) [126].

Although trimethoprim resistance was strongly linked to hospital use, clindamycin resistance was a response to primary healthcare prescribing and gentamicin resistance to both primary and secondary care. Other antimicrobials resistances were not clearly linked to either primary or secondary care prescribing. This may imply that clindamycin resistance is being imported into hospitals on MRSAs, MSSAs or some other route. In Chapter 4 of this thesis, it was noted that levels of clindamycin resistance remained high over the study period (2003-06, 2008-2012) in the healthcare-associated and nursing home-associated EMRSA-16 clone but levels of resistance increased from low to moderate levels in non-EMRSA-15 and non-EMRSA-16 'other' clones. Additionally, the proportion of 'others' resistant to trimethoprim also increased over the study period of Chapter 4. Since the antibiograms of EMRSA-15s and EMRSA-16s remained relatively stable (Chapter 4), and the 'others' group seemed to be expanding, it is possible that the associations between prescribing and resistance in this Chapter may apply to the 'others' clonal group only. Owing to small sample sizes, this could not be accurately tested.

The multivariate models showed significant positive associations between resistance and prescribing in both primary and secondary care. This is an important finding as it might be assumed that since MRSA bacteraemia is primarily a hospital acquired infection, only stewardship in secondary care settings is important, but this study suggests otherwise, e.g. that stewardship in all settings is important. This is something that could be investigated further. However, model outputs showed low adjusted R^2 values meaning that while these were the best fitting models to describe

the data given the available variables, there may have been other variables which influenced the level of clindamycin, gentamycin and trimethoprim resistance. These variables are discussed below.

The lack of association between resistance and prescribing for other antimicrobials could be due to fixed resistances in bacterial population which might have resulted from historic prescribing, or maintained due to compensatory mutations diminishing or cancelling any fitness cost to the bacteria [49]. It is thought not to be beneficial to harbour resistance genes against drugs which are not in current use since resistance can carry a fitness cost. Thus, resistance tends to be eliminated once the selective pressure is removed [50]. Alternatively, it may be that resistance to one antimicrobial is genetically linked to another resulting in resistance to an antimicrobial that is not currently in use. Examples of such co-resistance include the acquisition of any of the *erm* genes (*ermA, ermB, ermC, ermT,* carried on mobile genetic elements (MGEs)) which confer resistance to both erythromycin and clindamycin [51], and acquisition of any one of the genes *aadD, aacA-aphD, ant4* (again MGEs) which confer resistance to aminoglycosides kanamycin, tobramycin and neomycin [176, 177].

5.4.4 Limitations

There were several limitations to this study. These limitations would be important issues to consider in a subsequent, larger study involving additional antimicrobials and pathogens. Incomplete data for both primary and secondary prescribing meant that this study was restricted to using seven HBs with the most complete data. Furthermore, only hospitals which used compatible pharmacy administration systems, e.g. Ascribe RX® Pharmacy System, were capable of electronically submitting data to HMUD which may have introduced bias and led to important trends in other HBs being missed. However, the HBs selected represent approximately 80% of the Scottish population and so the most significant trends were likely identified.

Only 14 antimicrobials were initially selected to look at general patterns, and 6 antimicrobials for further analysis. This may have led to missed trends and means that this study cannot give estimates of total antibiotic usage. However, the selected

antibiotics represent those in common clinical usage and that are important for MRSA treatment and resistance. A disadvantage of both primary and secondary prescribing data is that they represent only a proxy for antibiotic usage. Primary prescribing data represent all prescriptions that are dispensed by community pharmacies, but not necessarily used or taken by the patient, and secondary prescribing data represent drugs dispensed from hospital pharmacies to different hospital departments and, again, not necessarily administered to patients. While these prescribing datasets represent only a proxy measure for antibiotic usage; this was the best data available for researching population-level prescribing trends in Scotland at the time of the analysis.

No prescribing information was available for topical agents or items without associated DDDs. This included mupirocin, the use of which is thought to have greatly increased in the past few years to treat MRSA skin infections and nasal colonisations since it emerged that nasal decolonisation of patients and healthcare workers dramatically decreases the risk of *S. aureus* nosocomial spread and infection [109]. Perhaps an additional constraint of this study is that results cannot be applied at the individual-level since this was a population-based study that cannot adjust for individual level doses or characteristics [189].

5.5 Conclusion

This study serves as a pilot study for a much larger analysis examining trends and associations between prescribing and resistance for a greater number of antimicrobials and pathogens. As a stand-alone project, sound methods were developed for investigating spatial and temporal trends, and associations between prescribing and resistance for MRSA. There are several potential public health messages arising from this study.

Firstly, it was found that antibiotic usage of several antimicrobials increased and the rate of this increase should be monitored to prevent extreme over-use and drugs potentially becoming obsolete.

Secondly, the rate of prescribing of different antimicrobials differed between HBs and over years which could be due to several factors but likely mirrors differing HB-specific prescribing guidelines but also represents a lack of consistency in treatment. Thirdly, resistance was found to be associated with prescribing rates for three antimicrobials over this study period, although there are also likely to be other factors contributing to resistance (for example historic prescribing).

Secondly, the rate of antimicrobial prescribing differed between HBs and over years. This could be due to several factors but likely mirrors differing HB-specific prescribing guidelines and inconsistencies in treatment.

Chapter 6: **A hospital-level risk factor analysis of *Staphylococcus aureus* bacteraemia in Scotland**

6.1 Background

Healthcare-associated infections (HAIs) have been an unwanted aspect of healthcare systems for several decades. *Staphylococcus aureus* (*S. aureus*) is a common HAI which results in a spectrum of clinical conditions ranging from mild and often self-limiting skin and soft tissue infections to more serious illnesses including bacteraemia, pneumonia and toxic shock syndrome. Despite representing only a proportion of all symptomatic *S. aureus* infections, *S. aureus* bacteraemia (SAB) contributes globally to a considerable and disproportionate disease burden owing to their high associated mortality rates from life-threatening complications such as infective endocarditis and metastatic infections [158]. Therefore, SAB is considered a serious disease outcome of *S. aureus* infection and of high clinical importance. Furthermore, when methicillin-resistant *S. aureus* (MRSA) is the causative agent of bacteraemia, the risk of treatment failure and mortality is greater, and associated costs of managing these patients are higher than patients with bacteraemia caused by methicillin-sensitive *S. aureus* (MSSA) bacteraemia [10-12].

To design successful and targeted intervention programmes, risk factor analyses are often carried out to identify behaviours and characteristics associated with transmission, morbidity and mortality. Many studies on risk factors for *S. aureus* and MRSA infections including SAB have been performed at the patient-level, identifying characteristics such as previous hospitalisation, recent antimicrobial therapy, indwelling vascular devices, prior invasive or surgical procedures, often within risk groups such as the elderly and those with underlying health conditions [53, 60]. For more information on risk factors, see Chapter 1.1.3.

However, these patient-level characteristics do not fully explain observed heterogeneity in the number of bacteraemia cases between hospitals. Therefore, hospital-level risk factors must also be considered, for example, hospital size,

hospital connectedness where hospitals are connected through shared patients, and hospital type (for examples of these risk factors in the literature, see Chapter 1.1.3.5).

Controlling the spread of MRSA depends on knowledge of where the bulk of disease burden lies and understanding why infections occur where they do. Therefore, the aim of this study was to determine whether hospital connectivity, type and size significantly influence hospital-level MRSA morbidity rates in mainland Scottish hospitals. The approach was twofold: firstly to identify risk factors for the presence of MRSA bacteraemia in all Scottish mainland hospitals, and, secondly, to identify risk factors for high rates of MRSA bacteraemia for General hospitals only.

6.2 Methods

6.2.1 Hospital selection

Initially all 264 facilities listed in the financial year 2007/08 Information Services Division (ISD) Scotland cost book reports (R020 and R020LS) were considered [190]. For analysis, a hospital was defined as a secondary healthcare facility with at least one inpatient per year and with at least one hospital specialty (previously known as departments). These facilities were scrutinised and those with a facility index other than 'H' (for hospital) were excluded. The excluded facility indices were: residential homes (index R), nursing homes (index V), GP surgeries and health centres (index B), clinics (index C), prisons (index P) and others. Within facility index H, dental hospitals, clinics (hospital- and non-hospital clinics), closed locations, day hospitals, the NHS National Waiting Times Centre (Golden Jubilee national hospital), Clydebank, Glasgow, and the State hospital, Carstairs, South Lanarkshire, and a further 3 hospitals were excluded since their function and costs were considered not comparable to other hospitals included in the study. In addition, island Health Boards; NHS Orkney, NHS Shetland and NHS Western Isles were also excluded since they are considered to contain atypical hospitals. Therefore the study included hospitals from the Scottish mainland only. This resulted in 198 National Health Service (NHS) hospitals being included in the study for the financial year 2007/08, and 66 facilities being excluded. All hospitals in Scotland and the number of MRSA bacteraemia cases are shown in Figure 6-1.

6.2.2 Data collection

6.2.2.1 Case data

MSSA and MRSA bacteraemia case data by hospital for the financial years 2006/07 – 2010/11 (5 years) were received from Health Protection Scotland (HPS). The financial year 2007/08 (6 April 2007 – 5 April 2008) was chosen as the study year because at the time it was the only year for which data were available for all risk factors (hospital size, type and connectivity). While the study period was only one year, size (measured in occupied bed days (OBDs)) did not vary considerably from year to year (See Appendix Figure 1A), and size (measured in metres squared) and hospital type did not change over the five year period. While this study only used data from a single year, hospitals that had MRSA bacteraemia cases, i.e. were MRSA 'positive', tended to remain positive over the five-year period; and hospitals that did not have any MRSA bacteraemia cases, i.e. were MRSA negative, tended to remain negative over the five-year period (Appendix Table 4). Almost 90% of hospitals had the same status (either MRSA bacteraemia positive or negative) over the five years (financial years 2006/07 to 2010/11 inclusive) for which data were available. There were three hospitals that were only positive for the year of this study. All models were run with and without these hospitals with no change in the results.

6.2.2.2 MSSA agreement with MRSA: justification for MRSA data only

This study focuses on MRSA bacteraemia only. However, the justification for not including MSSA bacteraemia was scrutinised by testing the agreement between MSSA and MRSA data. The association between the presence of MRSA and MSSA for all hospitals was examined for the financial year 2007/08 (chi-square test) and across all years for which data were available (2006/07 – 2010/11, n=5 years) (Table 6-1). The analysis across financial years was performed using a stratified 2x2 analysis which calculates the Breslow Day statistic. This tests the null hypothesis that there is no difference between MSSA and MRSA where $p \leq 0.05$, i.e. just as likely to have one or the other, and the relationship between MSSA and MRSA is the same across all years (homogeneity of odds).

6.2.2.3 Risk factors

Data regarding potential risk factors were obtained from ISD for each hospital for the financial year 2007/08 (a full list of variables in Appendix Table 5). As the aim was to test specific hypotheses regarding the effect of hospital size, type and connectivity; the majority of the data that were obtained represented different measures of those characteristics. Other measures including average occupancy rate, total number of whole time equivalent (medical and dentistry, nursing and midwifery, domestic, and support services) staff, patient-staff ratios (number of patients to medical and dentistry, nursing and midwifery, domestic, and support services staff), and cleaning by hospital size were considered (Appendix Table 5).

6.2.2.3.1 Hospital size

Measures of size included occupied bed days (OBD), surface area (m^2), average staffed beds, total inpatients discharged, total staff members and others (Appendix Table 5).

6.2.2.3.2 Hospital type

Within the ISD database, Scottish hospitals are grouped into the following categories: Category A (General (mainly acute) including Teaching (A1), large General (A2), General (A3), and Sick children's (A4)); Category B (Long stay), Category C (Mental); Category D (psychiatry of learning difficulties); Category E (Maternity); and Category J (Community). Information on the presence and absence of 46 specialties was also acquired (Appendix Table 5).

6.2.2.3.3 Connectivity

To quantify movements of patients between hospitals, patient admission data were obtained from ISD. Patient admission data covered all admissions to healthcare facilities in Scotland for the calendar year 2007 (1 January – 31 December 2007). From this dataset movements were extracted for patients between hospitals either as direct transfers, i.e. from one hospital directly to another hospital, or as indirect transfers, i.e. when a patient was discharged from one hospital into the community and subsequently, within the period covered by the data, admitted to another hospital. From these data, a movement matrix was derived for all connected hospitals in

Scotland [191] and then various summary measures of hospital connectivity generated (Table 6-2).

6.2.3 Descriptive and statistical analyses

All statistical analyses were carried out in R (version 3.1.2) [154].

6.2.3.1 Descriptive analyses

Descriptive analyses were conducted to summarise hospital characteristics and the number of cases per hospital for both MSSA and MRSA. Descriptive analyses of potential hospital-level risk factors were also done. Several variables were $\log_{10}(x+1)$ transformed to correct for non-normal distribution of the data and others were categorised. A full list of all 39 explanatory variables is shown in Appendix Table 5. Hospital connectivity variables were examined as both continuous and categorical variables. Cut-off levels of connectivity variables were determined using receiver operating characteristic (ROC) curve analysis [14], above which hospitals were considered positive for MRSA bacteraemia. The cut-off chosen was one that maximized the sum of the sensitivity and specificity. Confidence intervals (CIs, 25th and 75th percentiles) for the cut-off values were generated from 10,000 bootstrap simulations. The presence of a hospital specialty (n=46) and the association with MRSA cases was investigated using Chi-square or Fisher's exact tests where appropriate. Specialties that were statistically significant in this univariate screening were included in a nonmetric multi-dimensional scaling (NMS) analysis. Data regarding hospital specialties was summarised using NMS to reduce the number of variables. The NMS was performed in PC-ORD version 6.08 (MJM software Design, Gleneden Beach, OR) using the 36 specialties that were sufficiently represented across all hospitals, i.e. present in at least 5% of the hospitals, so 10 specialties were excluded from this analysis. Multi-response Permutation Procedures (MRPP) analysis [15] was used *a posteriori* to test the hypothesis of no difference between hospital status (presence/absence of MRSA bacteraemia) and hospital type (Category A, B, C, D, E, J). The association between the presence of a specialty within a hospital and the number of MRSA bacteraemia cases was examined for each of the 46 hospital specialties using Chi-square or Fisher's exact where appropriate. Odds

ratios (OR) are given for specialties with statistically significant Chi-square tests (Table 6-3).

6.2.3.2 Regression analyses

For both the univariate and multivariate analyses, two models were considered. Model 1 was a logistic regression model with binomial distribution fitted to presence-absence data for all hospitals (n=198) to identify risk factors for having cases versus not. Model 2 was a generalised linear model with a Poisson distribution fitted to count data, offset by the logarithm of OBD, to identify risk factors associated with higher rates of MRSA bacteraemia in General (Category A) hospitals only (n=38).

Before undertaking the multivariate analysis, each variable was examined individually in a univariate analysis. Correlational analyses were performed to look for correlations between explanatory variables and matrices were constructed to screen for confounders. Correlation was considered strong if the coefficient was >0.8, moderate if >0.5 and <0.8, and weak if below 0.5. Correlation coefficients of size and connectivity variables are shown in screening matrices in Appendix Figure 2A (size) and 2B (connectivity). There were strong correlations between several different potential measures of size (Appendix Figure 2A) and connectivity (Appendix Figure 2B). Variables that were statistically significant in a univariate analysis ($p<0.05$) and not highly correlated to other variables were taken forward to the multivariate statistical analysis. A $p<0.05$ screening level was chosen as there were many variables that were significant in the univariate analysis. This is not surprising given that these variables were chosen *a prioir* as possible risk factors.

A backward elimination approach was applied in the multivariate analysis. Statistical significance was set at $p\leq0.05$. Likelihood-ratio tests and the akaike information criterion (AIC) were used to select the most parsimonious model. The robustness of the final model was tested by applying a forward stepwise approach and examining the diagnostic statistics. The stability of the final model was also checked by removing the variables in the model and assessing the effect of the remaining variables. The final models were deemed to be both robust and stable.

6.3 Results

6.3.1 General epidemiology

6.3.1.1 Hospitals Summary

There were a total of 1,018,597 inpatients discharged from 198 hospitals (range 1 to 75159 inpatients, median = 338 inpatients per hospital) in Scotland over the financial year 2007/08, representing a total of 7,506,822 occupied bed days (OBD) (range 990 to 322500 OBDs, median =12170OBDs per hospital). The size of hospitals (measured in m^2) ranged from 100 to 15200 m^2 (median = 3621), the number of specialties per hospital ranged from 1 to 29 (median = 2), the proportion of specialties per hospital that were acute ranged from 0 – 1 (median = 0.5) and the average occupancy rate per hospital ranged from 28.6 - 97.3% (median = 78.2%). Thirty-eight hospitals in our study were classed as General hospitals (mainly acute), 52 as long-stay hospitals, 34 as mental hospitals, 13 as psychiatry of learning disabilities hospitals, 3 as maternity hospitals, and 58 as community hospitals.

6.3.1.2 Cases

From the 198 hospitals included in this study, the majority (n=152, 77%) reported no MRSA bacteraemia cases (Figure 6-1). The remaining 46 hospitals reported 662 MRSA bacteraemia cases in total (range 0 to 72 MRSA cases at the hospital-level). From these 662 cases, 47 cases died from all-cause mortality within 30 days, giving a 30-day case-fatality ratio of 7.1%. MRSA bacteraemia occurred in 0.06% of all inpatients in Scotland.

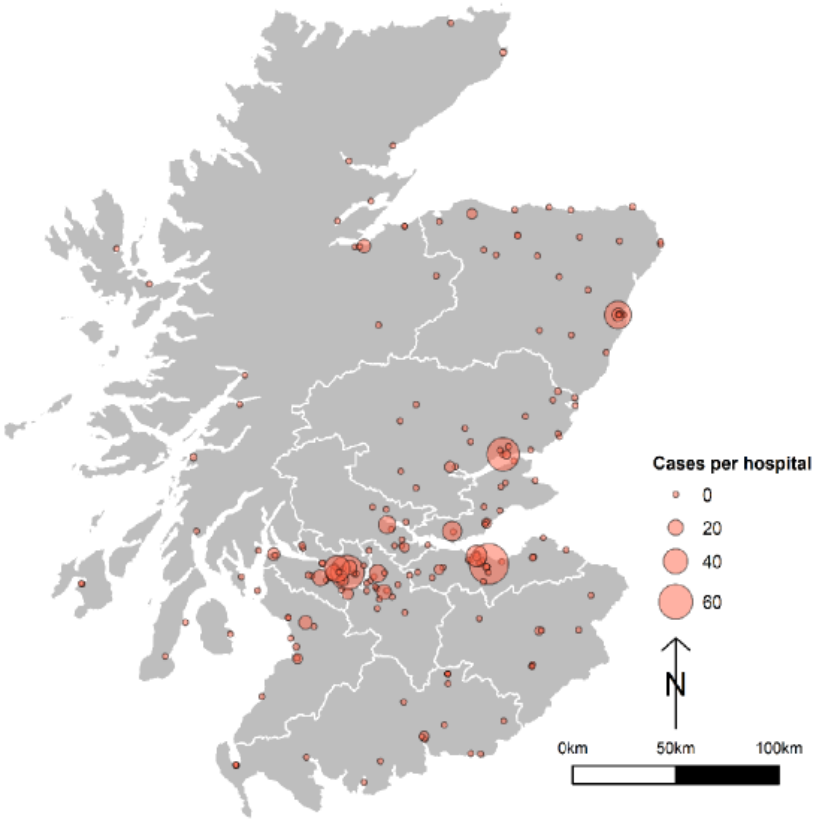
During the study year, MRSA was the causative agent of 36.6% of all *Staphylococcus aureus* bacteraemia (SAB) infections although this varied between NHS Health Boards. The highest percentage of total SAB due to MRSA in NHS Lanarkshire (43.7%, n=126), and the lowest percentages in NHS Borders (24.0%, n=25), and NHS Dumfries and Galloway (23.7%, n=38). MSSA and MRSA bacteraemia co-occurred in the same 39 hospitals.

Of the 198 hospitals included in this study, the majority (n=149, 75%) reported no MSSA bacteraemia cases. The remaining 49 hospitals reported 1147 MSSA bacteraemia cases in total (range 0 to 108). Of these 1147 cases, 51 died from all-

cause mortality within 30 days, giving a 30-day case-fatality ratio of 4.4%. MSSA bacteraemia occurred in 0.11% of all inpatients in Scotland.

Figure 6-1: Map of mainland Scotland with NHS Health Boards.

Circles show the 198 hospitals included in this study for financial year 2007/08. Each circle represents one hospital. Number of cases per hospital is continuous (ranging from 0-72) and the size of circle represents the number of cases with increasing number of cases illustrated by increasingly larger circles. The legend highlights the size of a circle that represents 0, 20, 40 and 60 cases.



6.3.1.3 MSSA agreement with MRSA - Justification for MRSA data only

There was a significant association between MSSA and MRSA for financial year 2007/08 (chi square test: 124.5, $p<0.001$) and this was consistent for all financial years for which data was available (Breslow Day Statistic, 2.969, $p=0.5642$). Hospital's that were positive for MRSA were 101.3 (95%CI = 59.44-179.8) times more likely to be positive for MSSA. The data are shown in Table 6-1. Owing to the substantial and significant agreement in the data, MRSA herein is the focus of the study.

Table 6-1: Number of hospitals that were positive or negative for MRSA and MSSA.

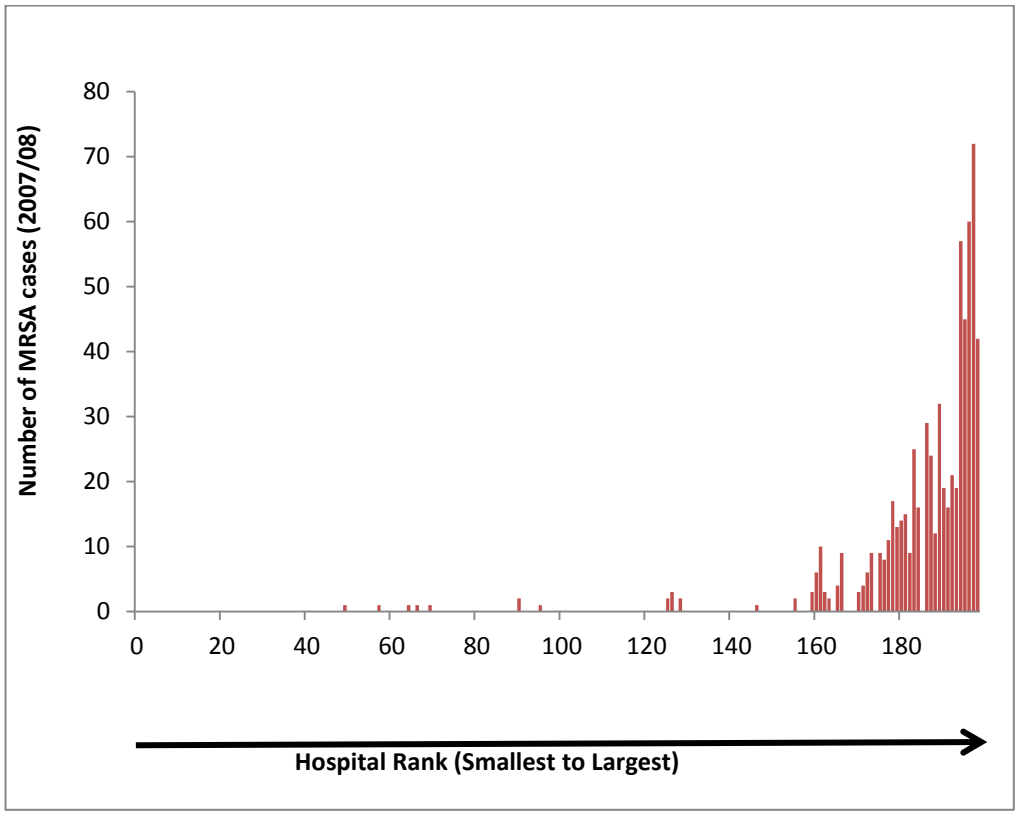
	MSSA									
Year	2006/2007		2007/2008		2008/2009		2009/2010		2010/2011	
MRSA	N	P	N	P	N	P	N	P	N	P
P	8	34	6	40	3	37	2	37	4	33
N	146	10	143	9	148	9	140	13	129	14

P, positive for MSSA/MRSA; N, negative for MSSA/MRSA.
For the financial year 2006/07 to 2010/11. Total 198 hospitals, 5 financial years.

6.3.2 Univariate analysis of risk factors

Model outputs from the univariate regression analyses are shown in Appendix Table 6 (Model 1) and Appendix Table 7 (Model 2). Several measures of hospital size (size (m^2), average number of staffed beds, OBDs, total patients discharged) were found to be statistically significant predictors for the presence of MRSA bacteraemia cases (model 1, $p<0.0001$) and number of cases in General hospitals (model 2, $p<0.0001$). Figure 6-2 shows the distribution of MRSA bacteraemia cases with respect to hospital size. Hospitals were ranked by size, as measured by occupied bed days. Several proxy measures of size were highly and positively correlated with correlation coefficients above 0.8 (Appendix Figure 2A).

Figure 6-2: Number of MRSA bacteraemia cases for the 198 hospitals included in this study. Hospitals were arranged by increasing size (represented by number of occupied bed days (OBD)).



Hospital connectivity, both as a continuous or as a threshold variable, was statistically significant with both models (Appendix Tables 6 and 7). Table 2 shows the best cut-off levels for the connectivity measures identified using ROC curve analysis. For all cut-offs found, hospitals above the threshold were at significantly increased risk for the presence of MRSA bacteraemia (Table 6-2). Several measures of connectivity were also highly and positively correlated with correlation coefficients above 0.8 (Appendix Figure 2B).

Table 6-2: Summary of hospital connectivity measures.

Name	Definition	Cut-off	Percentiles[a]	p-value[b]
Patients in	Total number of patients moving to this hospital from other hospitals adjusted by number of staffed beds	3.61	3.26-4.09	<0.001
In-degree	Number of hospitals that transferred patients to this hospital [11]	11	6-25	<0.001
Out-degree	Number of hospitals that receive patients from this hospital [11]	8	8-12	<0.001
Closeness centrality	Normalised measure of centrality of a node in a network based on the mean length of all shortest paths from that node to every other reachable node in the network [23].	0.3898	0.3898-0.3915	<0.001

[a]from 10,000 bootstrap simulations
[b]Fisher's exact test

Hospital type was an important factor. In regression analyses with model 1; being a General hospital ($p<0.0001$), having increased number of specialties ($p<0.0001$), and having a higher proportion of specialties that were acute ($p<0.0001$) were risk factors for the presence of MRSA bacteraemia. In the regression analyses with model 2; being a Teaching hospital ($p<0.0001$), having increased number of specialties ($p<0.0001$), and having a higher proportion of specialties that were acute ($p<0.0001$) were risk factors for increasing MRSA bacteraemia rates.

Table 6-3 lists all specialties included in the NMS. It also lists results of univariate screening using Chi-square or Fisher's exact where appropriate and providing OR for specialties that with statistically significant Chi-square or Fisher's exact tests. The univariate screening of specialties was carried out prior to the NMS analysis and to aid selection of variables for the NMS analysis. Several specialties had an increased risk of MRSA (OR>1, and significant p-values ($p\leq0.05$) were positively associated with MRSA). Hospitals with intensive care units (ICUs) were identified as having the highest significant odds of MRSA bacteraemia compared to hospitals without ICUs (OR = 196 (95%CI = 28.0-8096)). Hospitals with the specialties Geriatric Psychiatry, Learning Disabilities and General Practice were at a lower risk of MRSA (OR<1, i.e. protective against MRSA).

Table 6-3: List of 46 hospital specialties and association with hospital-level MRSA bacteraemia
Results of univariate screening using Chi-square or Fisher's exact where appropriate and OR (for specialties that with statistically significant Chi-square tests and no cells where n=0). Specialties in bold print (n=36) were used in the NMS (Figure 6-3).

Specialty	No. hospitals	MRSA bacteraemia	x^2	p	OR (95%CI)
Accident and Emergency	**11**	**10**	**24.0**	**<0.001**	**41.9 (5.52-1832)**
Acute other	46	1	1.26	0.412	-
Adolescent psychiatry	46	0	0.398	1.00	-
Burns	**11**	**9**	**17.98**	**<0.001**	**18.24 (3.50-177)**
Cardiac Surgery	46	5	13.25	0.001	-
Cardiology	**16**	**15**	**39.9**	**<0.001**	**73.1 (10.2-3095)**
Child psychiatry	46	1	2.81	0.232	-
Clinical genetics	46	3	7.66	0.012	-
Communicable Diseases	6	6	16.2	0.0001	-
Coronary Care Unit	**19**	**19**	**59.4**	**<0.001**	**-**
Dermatology	**12**	**11**	**27.05**	**<0.001**	**47.46 (6.36-2055)**
Ear Nose Throat	**19**	**17**	**42.46**	**<0.001**	**44.0 (9.38-401)**
Gastroenterology	**13**	**11**	**23.65**	**<0.001**	**23.6 (4.73-223)**
General Medicine	**38**	**32**	**101.9**	**<0.001**	**171.4 (35.7-1533)**
General Practice	**69**	**5**	**15.2**	**<0.001**	**0.168 (0.049-0.461)**
General psychiatry	**41**	**10**	**0.0389**	**1.00**	**-**
Geriatric Assessment	**48**	**36**	**95.21**	**<0.001**	**42 (15.49-117)**
Geriatric long stay	**77**	**14**	**1.802**	**0.227**	**-**
Geriatric Psychiatry	**70**	**8**	**8.46**	**0.0045**	**0.306 (0.116-0.728)**
General Surgery	**38**	**32**	**92.87**	**<0.001**	**84.57 (24.1-359)**
Gynaecology	**21**	**20**	**57.34**	**<0.001**	**116 (16.6-4844)**
High Dependency Unit	**26**	**24**	**67.7**	**<0.001**	**81.8 (17.7-734)**
Haematology	**26**	**25**	**76.6**	**<0.001**	**180 (25.7-7425)**
Intensive Care Unit	**27**	**26**	**80.7**	**<0.001**	**196 (28.0-8096)**
Learning Disabilities	**18**	**1**	**3.48**	**0.079**	**0.177 (0.004-1.197)**
Medical other	**18**	**11**	**15.93**	**0.003**	**6.51 (2.103-21.08)**
Medical Paediatrics	**17**	**14**	**29.59**	**<0.001**	**21.7 (5.50-122)**
Nephrology	**11**	**11**	**31.7**	**<0.001**	**-**
Neurology	**11**	**10**	**24.0**	**<0.001**	**41.9 (5.52-832)**
Neurosurgery	46	4	7.46	0.011	14.4 (1.35-713)
Obstetrics GP	**12**	**3**	**0.129**	**1.00**	**-**
Obstetrics Specialist	**21**	**17**	**35.97**	**<0.001**	**21.7 (6.3-92.9)**
Ophthalmology	**20**	**18**	**45.84**	**<0.001**	**48.2 (10.3-438)**
Oncology*	**11**	**10**	**24.01**	**<0.001**	**41.9 (5.52-1832)**
Oral surgery	**17**	**15**	**35.9**	**<0.001**	**36.3 (7.65-334)**
Orthopaedics	**32**	**29**	**83.7**	**<0.001**	**84.7 (21.9-459)**
Paediatrics	**17**	**14**	**30.15**	**<0.001**	**22.4 (5.66-12.6)**
Rehabilitation Medicine	**16**	**11**	**20.2**	**<0.001**	**9.24 (2.71-35.6)**
Respiratory medicine	**15**	**14**	**36.56**	**<0.001**	**66.06 (9.18-2811)**
Rheumatology	**11**	**9**	**17.98**	**<0.001**	**18.2 (3.50-177)**
Special Care Baby Unit	**46**	**12**	**23.7**	**<0.001**	**17.5 (4.32-100)**
Spinal paralysis	46	1	2.81	0.232	-
Surgical paediatrics	46	3	3.56	0.083	-
Vascular surgery	**9**	**9**	**25.3**	**<0.001**	**-**
Thoracic	46	5	13.3	0.001	-
Urology	**20**	**20**	**63.2**	**<0.001**	**-**
Young Chronic Sick	**9**	**6**	**8.326**	**0.006**	**7.45 (1.493-47.4)**

'*': includes medical and clinical oncology; '-': OR could not be calculated as 1 cell contained a zero, OR = Odds Ratio, x^2=Chi square

6.3.3 Multivariate analysis of risk factors

6.3.3.1 NMS results

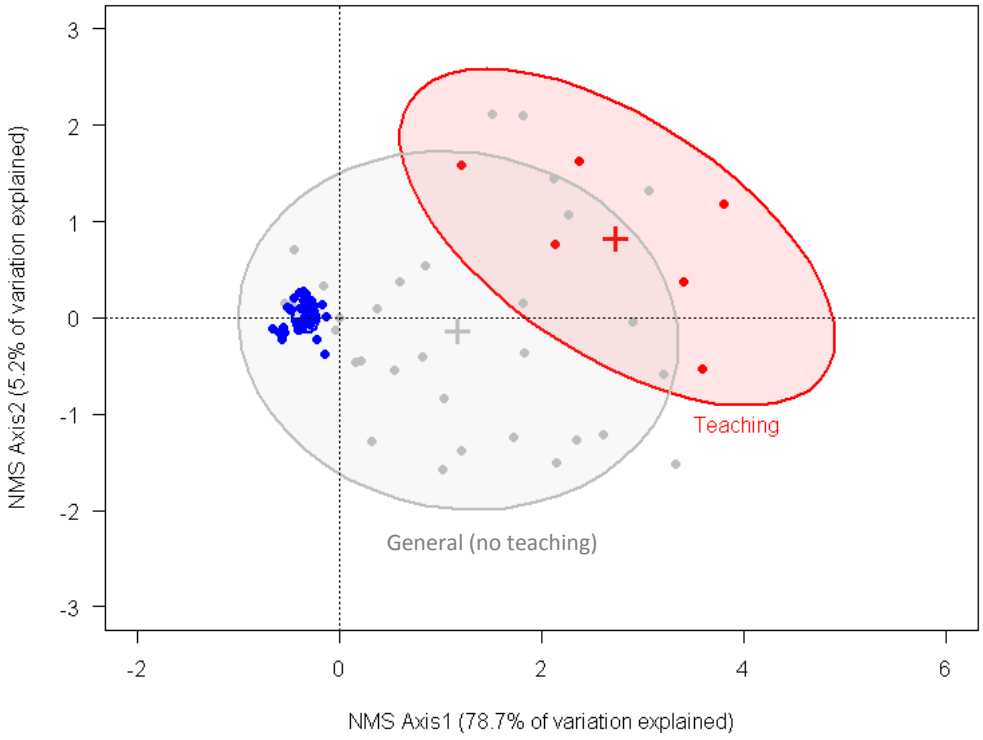
The NMS aimed to describe differences between hospitals with MRSA bacteraemia versus those that did not, based on presence of specialties for the 198 hospitals included in this study. The result of the NMS analysis was a two-dimensional solution that explained a total of 83.9% of the variation in the data. Additional dimensions provided no significant improvement in fit. The first axis, which was rotated to maximise the difference between hospitals with and without MRSA, explained 78.7% of the variation.

MRPP was performed to test the null hypothesis of no difference between hospitals with MRSA versus those without. The null hypothesis of no difference between the groups can be rejected and the two groups occupy different regions of space as shown by the strong chance-correlated within-group agreement (A) and significance level ($p<0.0001$).

Progression along NMS Axis 1 was associated with an increasing number of MRSA bacteraemia cases, increasing hospital size (OBD) and an increasing number of specialties (Figure 6-3).

Three significantly (MRPP, $p<0.001$) different hospital groups were identified, distributed along NMS Axis 1 (Figure 6-3), namely: Teaching, General (no teaching), and "Other". The "Other" group corresponded to the ISD hospitals designated categories B, C, D, E, J. Hospitals designated "Other" were at lower risk of MRSA than General Hospitals with no Teaching (Categories A2, A3, A4) which in turn had a lower risk than Teaching hospitals (Category A1). There was some overlap between the three groups meaning that there were some hospitals with MRSA that were similar to hospitals without MRSA, and vice versa.

Figure 6-3: Nonmetric multidimensional scaling (NMS) of the specialties (n=36) for the 198 hospitals included in this study.
Two significant axes explain a total of 83.9% of the variation in the data with NMS axis 1 explaining 78.7%, representing an increasing number of MRSA cases, increasing hospital size (OBD), and an increasing number of total specialties (from left to right along NMS Axis 1). 80% confidence ellipses drawn to designate significant groups based on hospital type as defined by Information Services Division (ISD): Teaching (Category A1; n=6, red points), General, no teaching (Category A2, A3, A4; n=32, grey points) and Other (Categories B, C, D, E, J; n=160, blue points).

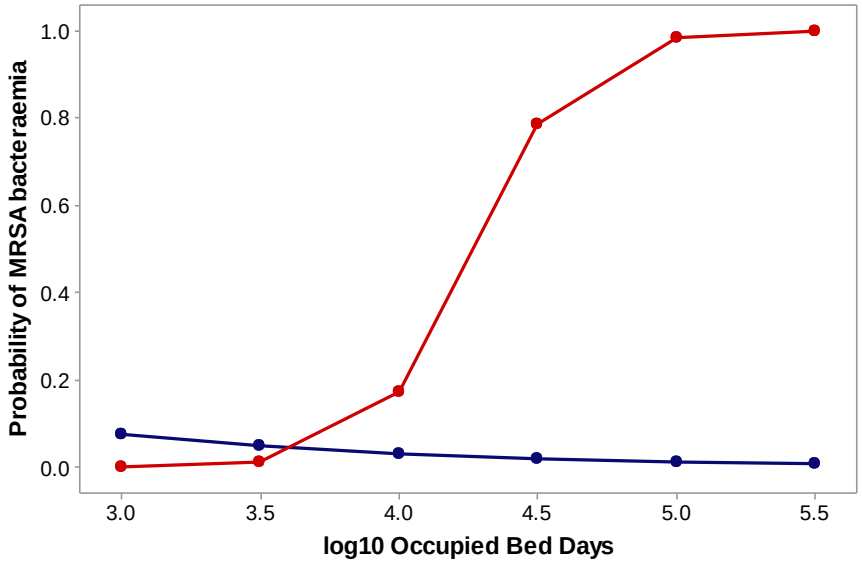


6.3.3.2 Model 1 - Risk factors for presence of MRSA bacteraemia cases (n=198 hospitals).

Results (Figure 6-5) showed that the probability of a hospital having at least one MRSA bacteraemia case increased with hospital size (log_{10}OBD) but only for hospitals that exceeded the threshold for the connectivity variable outdegree (interaction: log_{10}OBD x outdegree threshold, p=0.0009)). Hospitals above the threshold were statistically significantly (i.e. $p \leq 0.05$) at greater risk (OR (95%CI): 309 (13-7213)) for having at least one MRSA bacteraemia case than hospitals below the threshold (OR (95%CI): 0.40 (0.042-3.92)), regardless of their size (Figure 6-5).

Figure 6-4: Probability of a hospital having at least one case of MRSA bacteraemia.

For hospitals above (red line, n=56) and below (blue line, n=142) the Outdegree threshold (Table 6-1).



6.3.3.3 Model 2 - Risk factors for higher rates of MRSA bacteraemia (n=38 hospitals).

After adjusting for size (model included an offset for OBD); results showed (Table 6-4) that the total number of patients transferred (proxy measure of connectivity), being a teaching hospital and the ratio of patients-to-domestic staff were all significant predictors of the level of MRSA bacteraemia in General hospitals. However, the significant effects of the total number of patients transferred varied depending on whether the General hospital was Teaching or non-Teaching (interaction, p=0.005). In General, non-Teaching hospitals, the rate of MRSA bacteraemia increased with the total number of patients transferred (p<0.001). Whereas, for General, teaching hospitals the number of patient transfers, which is already high, had no significant effect on the levels of MRSA bacteraemia (p=0.360) (Table 6-4).

Table 6-4: Risk factors for higher rates of MRSA bacteraemia in General Scottish mainland hospitals (n=38).

Variable	Estimate (SE)	p-value	Risk Ratio (95% CI)[a]
Total patients transferred (log_{10})	1.73 (0.332)	0.0682	1.69 (0.962-2.97)
Teaching hospital	10.21 (2.18)	<0.001	27134 (377-1954565)
Ratio of patients to domestic staff	0.253 (0.060)	<0.001	1.29 (1.14-1.45)
Total patients transferred (log_{10}) In a teaching hospital	2.411 (0.554)	<0.001	11.15 (3.76-33.04)

[a]Risk ratio and 95% confidence intervals estimated using the modified Poisson Regression approach [24].

6.4 Discussion

In this study, data on MRSA bacteraemia were obtained for all Scottish mainland hospitals, defined as a secondary healthcare facility with at least one inpatient per year and with at least one hospital specialty. Only 23% of the hospitals examined in this study reported at least one case of MRSA bacteraemia. This proportion was small however; the hospital definition included long stay, mental and community hospitals which were typically small facilities, providing very few specialties. Generally, hospitals that reported at least one case of MRSA bacteraemia over the study period were large hospitals. However, even large hospitals rarely had large numbers of reported MRSA bacteraemia cases unless they were also well connected to the wider hospital network. For all connectivity measures generated in this study, there seemed to be a cut-off below which a hospital had no or lower than expected MRSA bacteraemia cases from a model that included size alone. Although further testing would be required, such a measure is highly useful in identifying hospitals at high risk for MRSA bacteraemia (and potentially other HAIs) as it could help focus surveillance efforts at a national level. Hospital connectivity was also a significant risk factor in the level of MRSA bacteraemia observed in General hospitals thus providing further evidence that size alone cannot fully predict a hospital's MRSA bacteraemia status.

6.4.1 Well-connected hospitals and transfer patients

Since well-connected hospitals receive large volumes of transfer patients from several other facilities; a hospital's level of connectivity is important in disease transmission. Previous studies have shown that transfer patients have a comparatively higher risk of nosocomial infection than non-transferred patients owing to different demographics, health status at time of transfer (often acutely unwell and critical) and other patient-level risk characteristics [100]. Compared with non-transfer patients, transfer patients have also spent an increased length of time in the healthcare network and therefore have had increased chance of pathogen exposure, carriage or infection, and opportunities to seed and spread infections in their new hospitals.

6.4.2 Hospital type

When examining hospital type; Teaching hospitals were highlighted as having a large number of MRSA bacteraemia cases. Teaching hospitals are large, well connected and have a high number of specialties and teasing apart the effect of connectivity, size and hospital type is not easy. As all Teaching hospitals were well connected, there was no additional effect of further increasing connectivity. By comparison, increasing connectivity in non-teaching hospitals, led to significant increases in MRSA bacteraemia. Well-connected hospitals (which also tend to be large and Teaching hospital) are also more likely to generate cases in both the in-coming transfer population as well as the rest of the hospital population (who also have patient-level risk factors for bacteraemia). With a higher proportion of specialties that are acute, well-connected hospitals tend to have the capacity to carry out specialist, complex or high-risk procedures on often acutely physically unwell individuals, who may be infectious, immune-compromised and high-risk for bacteraemia. For the same reasons, these hospitals will also receive the most acutely unwell emergency cases direct from the community increasing the at-risk hospital population and introducing new potential sources of infection.

6.4.3 Limitations

6.4.3.1 Community versus hospital acquired bacteraemia

Unfortunately, a restriction of the data meant that acute hospital admissions with bacteraemia and cases that developed bacteraemia during their stay in hospital (i.e. hospital-associated versus community-associated cases) could not be distinguished. In a 3-year study of admissions to a large general hospital in NHS Lanarkshire, 26.6% of all SAB cases and 18.8% of all MRSA bacteraemia cases were acute admissions, and the majority (81%) of acute admissions with MRSA bacteraemia were admitted from nursing homes and had been transferred from another hospital or had been an inpatient within the previous 6 months [192]. Similarly, in a five year study of bacteraemia trends in a teaching hospital in NHS Grampian, 44% of MSSA and 62% of MRSA bacteraemia were considered hospital-associated (defined as occurring >48 hours after admission or ≤14 days from discharge) [9]. While this may not reflect the picture across all Scottish hospitals, bias could be introduced into this dataset since acute cases occurring in the community (including nursing homes) may be more likely to attend hospitals with accident and emergency departments. This would therefore increase the counts of bacteraemia cases for those hospitals.

6.4.3.2 Direct and indirect hospital transfers

Measures of connectivity in this study were estimated from referral data for both direct and indirect hospital-to-hospital transfers of patients in both directions regardless of bacteraemia status. Indirect transfers were those where the patient returns to the community or non-hospital-facility (i.e. nursing home) for a given period before being readmitted. In the case of the Scottish transfer network for the financial year 2007/08, approximately 15% of readmissions occurred within 7 days of discharge, 40% of cases were readmitted within 30 days, and 70% within 90 days. Pathogen carriage and infection can be very long lasting months or even years [193]. As a result, indirect transfers could lead to transmission on subsequent admissions (and therefore the source of the colonisation or infection not being the same the hospital where the bacteraemia was counted as occurring). Robotham *et al* [194] also suggested that readmissions to hospitals from the community were a critical consideration for control of HAIs to be successful.

6.4.3.3 Important caveats

This study has some further limitations that must be considered. The definition of a hospital was intended to capture facilities with overnight patients receiving medical care. Nursing homes, for example, were excluded since not all patients would receive medical care, the demographics and risk factors of patients would likely differ from true hospitals, and nursing homes operate in a different way to hospitals. Despite this, the incidence of colonisation and all-type infection, i.e. outcomes other than bacteraemia, and level of transmission within nursing homes and to hospitals is expected to be high. Nursing homes are thought to harbour a vast number of *Staphylococcus aureus* colonisation and infections and therefore are thought to be an important reservoir for hospital infections [195-198].

It is also expected that since bacteraemia is a notifiable condition, the number of cases would not be affected by substantial underreporting. It is acknowledged that by only studying the disease outcome bacteraemia may have influenced our results since severe outcomes tend to be associated with riskier procedures, certain hospital specialties and certain hospital categories. It is also accepted that MSSA and MRSA colonisation and other disease outcomes of *S. aureus* infection, such as wound infections, are likely occur in a significant proportion of hospitals throughout Scotland.

It is an important finding that high patient-to-staff ratios were also identified as a risk factor for higher levels of MRSA bacteraemia. Patient-to-staff ratio of domestic workers was used as a proxy measure for the ratio of patients to cleaning staff. Hospital cleaning remains a vital strategy for effective infection control which was highlighted by this small but significant risk from our model. This result, however, supports the previously reported effect of heightened hygiene awareness and enhanced cleaning on reducing the number of new infections and microbial contamination of the hospital [148, 151, 172, 199].

6.4.3.4 Unavailable information

There were a number of potential risk-factors that data were not available for analysis. There was no access to universal data on within-hospital movements known

as ‘boarding’. Boarding, where patients are moved to other wards to accommodate influxes of new patients or to more appropriate wards or specialties which may be of greater benefit to the patient [200], is thought to increase the risk of infection transmission. In addition, referrals (including both direct and indirect transfers) from outside Scotland were not considered. As suggested by Ciccolini *et al* [201], a regional approach, for example a UK-wide study, may be more appropriate than a Scotland only study as some patients are transferred across these borders. Unfortunately, the full repertoire of variables was not available for all hospitals elsewhere in the UK and the interconnectedness of hospitals in Scotland with elsewhere in the UK was not known. Data on the composition or demography of patients, such as mean age, sex, or proportion belonging to certain religions, ethnicities and social groups at different hospitals was also lacking. Such demographic mixes could differ between hospitals and since groups may have different patient-population-based risk-factors for bacteraemia; hospital demography could have contributed to hospital-level differences. Data on antibiotic usage are currently unavailable at the hospital-level. The number of daily defined dose (DDDs) per antibiotic would very likely be an important risk factor for MRSA although it would perhaps correlate with hospital size or the proportion of specialties that are acute. There was also no measure of universal hospital-level infection control or policy data to include in the study.

6.5 Conclusion

This is the first Scotland-wide study utilizing hospital-level characteristics to examine differences in MRSA bacteraemia morbidity among hospitals. This study showed that 23% of Scottish mainland hospitals reported cases of MRSA bacteraemia. Effort to reduce MRSA transmission and the number of bacteraemia cases should therefore be focused on large, well-connected hospitals. Further research should be conducted with respect to reducing the ratio of patients to domestic staff as a means of reducing the levels of MRSA bacteraemia infections, especially in teaching hospitals where the levels are very high.

Health Protection Scotland (HPS) and Scottish Government prioritise the implementation of effective infection control measures to reduce the spread of pathogens in nosocomial settings. MRSA bacteraemia may be considered as a marker for the ability to contain transmission of important pathogens in healthcare settings. Programmes that prevent MRSA bacteraemia transmission will likely be relevant to other epidemiologically important healthcare pathogens that spread by patient-to-patient transmission.

Chapter 7: **Thesis conclusion**

7.1 Main conclusions from chapters

7.1.1 Chapter 2 – MSSA and MRSA morbidity and mortality trends

MSSA bacteraemia rates did not significantly decline over time while total *S. aureus* and MRSA bacteraemia rates did. Government targets to reduce bacteraemia cases by 30% of the 2005/2006 levels were met for total SAB and targets were exceeded by the 2010 deadline with rates remaining low thereafter. However, this was due to a disproportionate reduction in MRSA SAB which reached 30% of the 2005/2006 levels by 2010. MSSA did not decline to 30% of the 2005/2006 levels. In addition, there were no changes in mortality rates where the primary, secondary or combined cause of death was MSSA, however where MRSA was the primary, secondary and combined cause of death, mortality rates significantly declined over time. There were no significant declines in case-fatality ratio for either MSSA or MRSA.

7.1.2 Chapter 3 – MRSA declines while MSSA remains a public health concern

In this chapter, 11 years of microbiological and epidemiological data for isolates causing MSSA and MRSA bacteraemia in Scotland were examined. The main differences between MSSA and MRSA were: (1) antimicrobial resistance, with MRSA being multi-drug resistant and having longer antibiograms, (2) clone, with MRSA more associated with CC22, which includes the hospital-associated sequence type ST22, and MSSA with non-CC22 and non-CC30 'other' clones, (3) patient age, with MRSA associated with older patients, and (4) hospital specialties with MRSA associated with care of elderly, high dependency units /intensive care units, renal and surgery, and MSSA with accident and emergency, infectious diseases, obstetrics and gynaecology, orthopaedics, paediatrics and paediatrics ICU.

These data suggest that the natural niche of MRSA is in the hospital and for MSSA it is in the community, i.e. outside the healthcare environment. The 'sink-source' model was used to illustrate this. While MRSA has declined, which is likely a result of targeted interventions such as screening, decolonisation and cleaning, MSSA has not and it remains a public health concern. It is suggested that since one third of all

patients, visitors and staff are commonly colonised, MSSA constantly enters the hospital from the community at a rate faster than it can be removed.

7.1.3 Chapter 4 - Antimicrobial resistance of MRSA

Using 8 years of data, antimicrobial resistance was described for MRSA. Specifically, resistance to 20 individual antimicrobials, antibiogram length and antibiogram diversity for clinical isolates and non-clinical 'screening' isolates were compared for EMRSA-15 (CC22, ST22 SCC*mec*IV) and EMRSA-16 (CC30, ST36SCC*mec*II) isolates. EMRSA-15 was by far the most dominant MRSA clone in Scotland. EMRSA-16 declined significantly, and non-EMRSA-15/non-EMRSA-16 'other' clones are causing an increasing number of infections. EMRSA-16 was resistant to a larger number of antimicrobials than EMRSA-15, typically 9 compared with 5, and although resistance varied for EMRSA-16 over the study period, resistance remained stable for EMRSA-15. There was little difference between clinical and screening MRSA isolates.

7.1.4 Chapter 5 - Antimicrobial prescribing and its association with MRSA epidemiology

This chapter describes as a pilot study for a much larger analysis looking at trends and associations between prescribing and resistance for a greater number of antimicrobials and pathogens. As a stand-alone project, a sound methodology was developed for investigating spatial and temporal trends in Scottish primary and secondary care prescribing rates and associations between prescribing and resistance in MRSA. Analyses of antimicrobial prescribing showed that rates of several drugs increased from 2003 to 2013. Primary care prescribing was in general far higher than that in secondary care, although this differed between antimicrobials. Significant positive associations between prescribing and resistance rates were found for gentamicin and trimethoprim in both primary and secondary care, and clindamycin in primary care only.

7.1.5 Chapter 6 - A hospital-level risk factor analysis of MRSA

The aim of this study was to explore the hypotheses that hospital size, hospital connectivity and hospital category have an effect on the hospital-level incidences of

MRSA in mainland Scottish hospitals. In Scotland, size was not the only significant risk factor identified for the presence and rate of MRSA bacteraemia. The probability of a hospital having at least one case of MRSA bacteraemia increased with hospital size only if the hospital exceeded a certain level of connectivity. Higher levels of MRSA bacteraemia were associated with the large, highly connected teaching hospitals with high ratios of patients to domestic staff. A hospital's level of connectedness within a network may be a better measure of a hospital's risk of MRSA bacteraemia than size.

7.2 Key policy implications

There are several potential implications of this work.

This thesis highlights the need to investigate case-fatality ratios further and to find a way to reduce deaths of cases. This could impact policy since it could become the evidence behind a government target, e.g. to reduce CFR to 70% of that in 2015 etc. It may also impact funding in this area to provide more resources for the development of bedside rapid testing for timely and appropriate treatment of patients. Further down the line, it could become routine policy to test patients with suspected bacteraemia using a bedside rapid test.

If the sink-source model of MSSA and MRSA is verified and found to be accurate, then there should be more effort to reduce MSSA traffic into hospitals. This could impact policy and lead to MSSA screening and decolonisation for high-risk patients entering high-risk specialties for MSSA.

This thesis highlights the extent of multi-drug resistance of MRSA although this is largely stable for the most common clone, EMRSA-15. The 'snapshot' programme provides invaluable insight into resistance of MRSA and it is important that this programme is maintained. Surveillance of MRSA through the 'snapshot' and EARS databases should remain a priority as potential policy implications can arise from monitoring trends. In the future, this may highlight which 'other' clones will become clinically important and to which antimicrobials resistance is developing.

This thesis describes levels of AMR in MSSA and MRSA. This has potential policy implications since it could lead to the review of antimicrobial prescribing practices.

This thesis has shown that prescribing is associated with resistance at the Health Board level. It should be emphasised, and could become a key educational and training point, that resistance can be tackled locally in GP surgeries and hospitals by prudent use of antimicrobials and antibiotic stewardship.

To further reduce MRSA and potentially other hospital-associated infections; large, highly connected teaching hospitals with high ratios of patients to domestic staff could be identified and targeted for more extensive health protection activities such as screening all patients, extra cleaning, and heightened surveillance. Since connectivity is a measure of how connected hospitals are in terms of shared patients, screening and decolonising patients prior to transfer might be another policy recommendation to reduce MRSA in the healthcare environment.

This thesis used several large public health databases. These are valuable sources of public health information and the continued practice of collecting, compiling, analysis and distributing data should be maintained. The experience of this PhD was that there are often missing data, or no available data on some factor that would have been interesting to investigate. Therefore, it is suggested that there is a policy for when new major databases are being planned; a consultation with potential users of the database takes place. In addition, owing to legislation surrounding release of data (particularly surrounding confidentiality of patient information), the time between requesting data and acquiring it can often be lengthy and the process often unclear. During this PhD, successful collaborations were formed between the holders of data and me. For future collaborations, there needs to be more explicit guidelines on standard practice and protocol for requesting and clearing data for quick release from, for example, the Scottish Government, ISD or HPS.

7.3 Questions unanswered by this thesis

There are several questions that resulted from this body of work, or that arose during this PhD which could not be answered due to time constraints, data or other resource limitations. Below are listed some of these questions.

1. How to reduce MSSA bacteraemia?

Screening and decolonisation of high-risk patients in high-risk specialties for MRSA has had an important impact on MRSA declines. To reduce the number of MSSA bacteraemia cases in hospitals, it may also be important to begin screening and decolonising high-risk patients in high-risk specialties for MSSA. However, as with tackling MRSA, a multifactorial approach will likely be the way to reduce MSSA bacteraemia. For example, decolonisation of patients prior to transfer may reduce hospital-level infections in the patients' next hospital. In addition, the importance of using alcohol gels to clean hands, and potentially further activities, on entry to hospitals for every visitor, staff member and patient should be more strongly advocated.

2. How to reduce case-fatality ratios for SAB?

There needs to be a better understanding of factors that contribute to a case going on to succumb to the infection. One factor may be that the length of response time between patients becoming ill, being diagnosed and receiving appropriate treatment is too long. A potential strategy would be to lessen the response time which will likely require the development of real-time or bedside diagnostics.

3. Which resistances carry the highest fitness costs?

Answering this question would likely require laboratory experiments to determine if some resistance genes affect the spread and growth of bacteria more than others, or if a fitness cost is instead due to a certain combination of resistance genes, or if it's a case of numbers, e.g. that there is a threshold number of genes above which fitness is compromised.

4. What caused EMRSA-16 to decline?

It is speculated that EMRSA-16 declined due to a specific focus on cleaning, screening and decolonisation of people and the hospital environment in high-risk specialties that was perhaps the natural niche for EMRSA-16. Furthermore, it is speculated that the multi-drug resistances of EMRSA-16 carried too high a fitness cost and negatively affected the bacteria's ability to spread. Laboratory experiments may help to answer this question.

5. Is there a subset of EMRSA-15 that will fill the EMRSA-16 vacated niche?

If it is indeed true that EMRSA-16 had a 'niche' in the hospital and that it was eliminated from it, will EMRSA-15 or a different clone expand to fill that niche? If so, will it take on characteristics, such as certain resistances, to enable them to survive in that niche? This seems unlikely since if EMRSA-16 could not survive there, another clone would also meet the same challenges.

6. How frequently do MSSA and MRSA enter a hospital and spread?

This would require screening patients, visitors, staff and the hospital environment and perhaps a capture-recapture design. It could help to confirm the sink-source model.

7. How can the sink-source model be confirmed?

A screening study of individuals entering hospitals or a 'capture-recapture' phylogenetic study to trace strains through the hospital and community environments could test the source-sink hypothesis. It could also perhaps be tested mathematically.

8. How important are nursing homes for MRSA and MSSA in Scotland?

Nursing homes are thought to be a source of MSSA and MRSA infection but it is not known how they fit in to the sink-source model and it is not known how much bacteraemia is attributable to Scottish nursing homes. Screening studies may show this. In addition, the level of connectivity or the connectivity network of nursing homes to hospitals could also be an important risk factor for hospital-level infections.

9. How do hospital level risk factors apply to other diseases?

It is expected that size, connectivity and hospital type will be important hospital-level risk factors for other hospital-associate infections but it would be interesting to apply data of other diseases to confirm this.

10. How have private cleaning contracts affected MRSA, SAB and other HAI?

Private cleaning contracts in some hospitals became more common in the last decade. It would be an important finding if this was found to be a hospital-level or patient-level risk factor for infection.

7.4 Research recommendations for the future

Below are listed some research recommendations for the future.

Large data linkage projects to link data on isolates from SMRSARL, or cases, or deaths due to MRSA, with other data would enable the study of a number of wider research questions. The data requested would be specific to the research questions, e.g. patient demographic data, depravity scores, other disease statuses, number of hospital admissions, residency in care home, history of antimicrobial prescribing, history of surgery, disease outcomes/death etc. Data, if available, can be acquired through ISD with the linkage process taking up to one year. Furthermore, the isolates could be whole genome sequenced (WGS) with bioinformatic and phylogenetic analyses conducted which could identify clinically-relevant clusters and a wide range of genetic determinants including AMR and virulence genes, help to understand strain evolution, as well as elicit any associations between isolate and variables from the linked datasets.

A field study in hospitals and the community to investigate MRSA and MSSA transmission by collecting information and taking swabs from staff / patients / visitors / environment at several time points. This study would provide information on the proportion of admissions and discharges that were colonised or infected, help to understand the role of staff / patients / visitors / environment in transmission, and provide molecular epidemiology information such as levels of AMR and diversity of strains and how this related to patient outcome (infection type, death etc.) or

specialty. It would also help to test the 'sink-source' model and the rate of strain removal. Isolates could also be whole genome sequenced and phylogenetic studies conducted to 'follow' isolates at hospital admission, through the hospital setting and back into the community (as per the sink source model) and provide insight into (AMR) gene acquisition and loss.

Develop mathematical models that test different strategies to tackle AMR. For example, (a) models that can provide estimates of a threshold of drug prescribing, above which, AMR develops, or (b) models suggesting how best to rotate antimicrobials to prevent resistance developing, or (c) developing early warning systems.

Develop tools to reduce CFR, for example, bedside diagnostics to ascertain the pathogen and any AMR for more timely and appropriate treatment of patients.

7.5 Data limitations

In every chapter, there were limitations surrounding data. The large, public health databases used in this thesis were often collected for purposes other than the analyses described here.

The largest database used was that from the SMRSARL. In its entirety, the full database contained 240 columns of information for more than 120,000 isolates spanning 17 years. It is an unparalleled source of *S. aureus* information with many of the original isolates still existing in storage at the laboratory. However, the full database did not represent a single baseline study, instead including information of all isolates processed by the Reference Laboratory which included special studies, sub-projects, research, as well as testing for the EARS, snapshot and pathfinders studies described in this thesis and routine testing. Therefore, for Chapters 3, 4 and 5 of this thesis, the EARS and snapshot sub-databases were extracted from this full database and analysed. However, these extracts had many missing and irrelevant data (for purpose of this thesis anyway) and as a result, only a small number of variables could be used for analyses in this thesis. It would have been useful and interesting to have had information on whether an isolate was from a hospital-associated or

community-associate case, information on antiseptic resistance and heavy metal resistance, a greater number of demographic variables, and others that could potentially be addressed through a data linkage study.

The primary and secondary care prescribing databases used in Chapter 5were also very large and incomplete with many data missing for certain time periods or regions. As a result, only 7 NHS Health Boards and 6 antimicrobials were examined. In addition, no information of prescribing was available for topical agents or items without associated DDDs. This included mupirocin, which is used in the decolonisation of colonised individuals.

The data used in Chapter 6, the hospital-level risk factor analysis, also had a number of issues including highly correlated variables and the fact that it was difficult to tease apart many variables as they were often closely related. Furthermore, there were a number of potential risk-factors that data were not available for and which could have potentially widened the scope of this chapter too.

7.6 Methodological critique

The descriptive and analytical methods used in this thesis are standard statistical methods that are appropriate for the types of data used and questions addressed. Analyses were discussed with a statistician either prior to being carried out or during the results stage.

Methods included: Pearson's Chi square and Fisher's exact tests to test for independence and/or association, t-tests to test for differences in means in continuous variables, ANOVA with Bonferroni or Tukey corrections to test for differences in means between categories, PCA and MDS as techniques to reduce the number of variables and visualise data, diversity plots, and modelling to investigate trends in space and time.

A risk factor analyses was carried out in Chapter 6 again using standard methods beginning with normalising data, checking for correlations, screening variables, and then building and validating the model using a backwards and forwards stepwise process.

To test if there were any statistical differences between HBs in terms of rates, analysis of variance (ANOVA) analyses where conducted, and to describe where those statistical differences were, Bonferroni or Tukey analyses were carried out. In the quarterly national surveillance reports by HPS [153], the funnel plots are used to illustrate the rate as well as identify any HB outliers (with rates above or below what would be expected). This is a useful way of depicting this information although it does not give any indication of any statistical differences between HB rates. The funnel plots were not included here in order to avoid repetition with national surveillance reports. In addition, the models used in this chapter differed from those used in national surveillance (national surveillance used case data with a Poisson distribution, offset by AOBD) to determine if rate had significantly changed over time. The outcomes are the same as have been described here.

7.7 Ethical approval, data sources and access

No ethical approval was required for the overall PhD project since it did not involve any direct contact with humans or animals. However, approval was required for accessing some of the individual datasets which held personal data and data on disease episodes affecting humans and animals, as described below.

In order to receive the SMRSARL database, my supervisor needed to formally apply for access through the then director of the reference laboratory and the 'gate holders of the data', the Caldicott Guardian at NHS Greater Glasgow and Clyde. Release of the data was approved so long as personal identifiers were removed and codes replaced names. The application and approval stages took approximately 12 months. The approval email is shown in Appendix Document 1.

Similarly, in order to receive the data used in Chapter 6, the annual counts of bacteraemia cases per hospital, my supervisor needed to formally apply for access through a consultant of public health medicine at HPS and the Caldicott Guardian at ISD. Release of the data was approved with the application and approval stages taking approximately 18 months. The approval email is shown in Appendix Document 2.

The data used in this PhD thesis was acquired from several additional sources.

The number of bacteraemia cases by quarter and by HB, as was used in Chapter 2, is published on the HPS website and freely available, and hence no approval was required to access this dataset. The number of MRSA deaths is published and freely available from the GROS website. However, a special request was made for the number of MSSA deaths by year as this is not published. This request was made by emailing GROS and having follow-up discussions with the information analyst. Data were supplied free of charge. The number of AOBDs, and the hospital-level risk variables were accessed freely through the ISD website and so again, no approval was required to access these data.

The prescribing datasets that were used in Chapter 5 were received following an email request to ISD. No formal approval was required. However, since the preparation of the databases was a lengthy process, we needed to pay for this service. It was approximately 12 months from applying for the data until receiving it.

7.8 Novelty: what this thesis adds to the literature

Each of the data chapters represents a publishable piece of work. Chapter 2 describes and compares MR and CFR of MRSA and MSSA in Scotland which, to my knowledge, has not been done before. Chapter 3 and Chapter 4 describe trends in national–level rates of resistance to 20 antimicrobials for both MRSA and MSSA, and discuss several other microbiological factors of interest, which have not been published elsewhere. In addition, the 'sink-source' model has been adapted to describe the flow of MRSA and MSSA through the community and healthcare settings which is an original use of this model. Chapter 5 investigates national-level prescribing data and how it is associated to resistance which has not yet been published for Scotland. Chapter 6 describes a hospital-level risk factor analyses which has not been conducted elsewhere as comprehensively as was shown in this thesis for *S. aureus*.

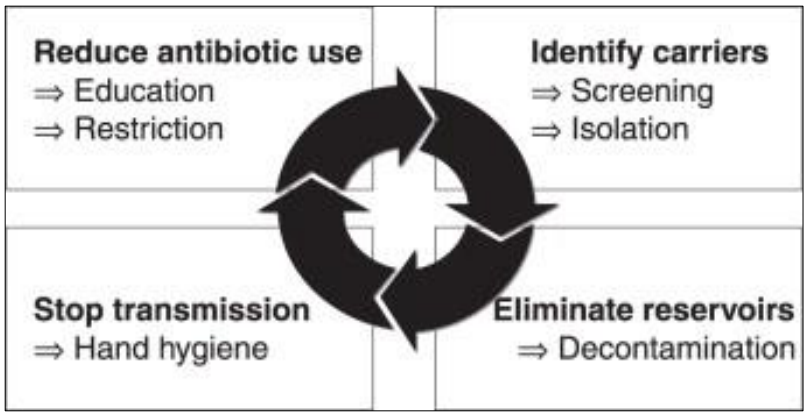
7.9 Concluding remarks

MRSA is still a public health problem but the overall population-level burden has declined over time. This is a great public health success story as serious cases have declined and MRSA is no longer so great an issue as it once was. It was once sensationalised in the media as the next major public health disaster. This did not happen but MRSA should not be forgotten and public health bodies should remain diligent and cognisant of this continued risk. Public health measures must be adhered to and remain in place in order to avoid a resurgence.

In addition to MRSA, *Clostridium difficile*, another important hospital infection, has also declined and therefore there have been multiple benefits from public health activities. Despite this decline, there has been an increase in carbapenemase producing Enterobacteriaceae (CPE) which is an extremely antimicrobial-resistant family of bacteria. While Enterobacteriaceae (which includes *Salmonella, Escherichia coli, Klebsiella sp, Proteus sp* and *Shigella sp*) are part of the normal bacterial gut flora, they can cause urinary tract infections, intra-abdominal infections and bloodstream infections. Carbapenems are a class of very broad spectrum intravenous drugs which are reserved for serious infections or when other therapeutic options have failed (i.e. drug of last resort).

To control these HAIs, it may be useful to consider the principles that were applied to control MRSA, namely; (1) identify cases, (2) stop transmission, (3) eliminate reservoirs, and in the case of antimicrobial resistance (4) antibiotic stewardship (Figure 7-1). However, as with MSSA, there may need to be a greater focus on some of these principles. For example, supposing the sink-source paradigm is true and MSSA constantly bombards and enters the hospital environment at a rate faster than it can be removed; then perhaps there needs to be a greater focus on identifying carriers by expanding screening to include MSSA too and eliminate these reservoirs through decolonisation. Furthermore, stopping transmission of MSSA could be achieved if stricter infection control measures were in place at hospital entrances. These suggestions, however, may be impractical and perhaps expensive.

Figure 7-1: Standard approaches for the control of MRSA in hospitals and potentially other pathogens.



Source: [121]

While it has been a recognised issue in the medical and scientific world for decades, AMR is now a hot topic in the media and is gaining government priority and funding to tackle the issue worldwide. This is much needed and extremely important if new drugs are to be developed and diseases are to be controlled. Public health messages are also crucial to ensure engagement of the general public.

As with elsewhere, disease surveillance systems in Scotland are not flawless. Cases in the community may be under-ascertained (i.e. not attend a GP surgery or hospital), or cases attending healthcare may be under-reported (i.e. diagnosed incorrectly, reported incorrectly or not at all) [157]. In the case of MSSA and MRSA, since bacteraemia is a serious condition leading to death if untreated, it is expected that under-ascertainment of bacteraemia in Scotland is low. Reporting of *S. aureus* bacteraemia cases is mandatory in Scotland and correct reporting of all deaths is also required by law so it is expected that under-reporting of morbidity and mortality events is also very low. There is, however, often uncertainty surrounding the cause of death in instances where a patient is suffering from co-morbidities (i.e. sequelae that an individual died *with* (e.g. secondary and tertiary causes) but not necessarily *of* (i.e. the primary cause of death)).

Throughout this thesis, I was involved in a number of important collaborations with individuals and groups at the SMRSARL, HPS, and within the university. These led to data and information sharing, and the analysis of datasets that perhaps otherwise would have remained unexplored. This thesis has shown that collaboration is key in this field, particularly as studies expand to become multi-disciplinary, and also that collaboration between public health bodies and researchers can produce fruitful results.

Chapter 8: **Appendices**

8.1 Appendices from Chapter 4

Appendix Table 1: Spa types associated with clinical EMRSA-15, EMRSA-16 and others in time period B only.

EMRSA-15				EMRSA16		'Others'	
Spa type	**obs.**	**Spa type**	**obs.**	**Spa type**	**obs.**	**Spa type**	**obs.**
t032	236	t2237	1	t018	32	t002	14
t022	42	t2377	1	t871	22	t008	10
t6057	29	t2892	1	t012	8	t127	7
t020	16	t307	1	t019	6	t186	5
t7894	14	t3211	1	t253	6	t398	5
t025	9	t3212	1	t122	1	t437	2
t379	7	t3490	1	t275	1	t612	2
t1214	6	t3861	1	t486	1	t7344	2
t628	5	t5302	1	t7356	1	t010	1
t717	5	t651	1	Missing	6	t015	1
t554	4	t8133	1			t044	1
t849	4	t819	1			t068	1
t005	3	t8374	1			t088	1
t1287	3	t8492	1			t10948	1
t1370	3	t8695	1			t1222	1
t1977	3	t965	1			t128	1
t2004	3	t9760	1			t1467	1
t2113	3	t9826	1			t172	1
t5892	3	t9923	1			t2143	1
t5951	3	Missing	432			t216	1
t1415	2					t2191	1
t557	2					t304	1
t578	2					t324	1
t756	2					t344	1
t7900	2					t345	1
t7943	2					t4364	1
t10040	1					t442	1
t1021	1					t458	1
t1036	1					t657	1
t1041	1					t690	1
t10466	1					t701	1
t11099	1					t786	1
t1378	1					t843	1
t1468	1					Missing	20
t1516	1						
t1790	1						
t1924	1						
t1963	1						
t2062	1						
t223	1						
t2236	1						

obs= observations, number of times the spa type appears in the database.

8.2 Appendices from Chapter 5

Appendix Table 2: Model output showing interaction between HB and time for (A) primary and (B) secondary prescribing rates.

A) Primary care prescribing

Model	Coefficient	Std. Error	p-value	Significance level
Ciprofloxacin', p<0.0001, Adj. R^2 = 0.65				
Year	0.02	0.005	<0.0001	***
LO - REF				
AA	0.28	0.04	<0.0001	***
FI	0.3	0.04	<0.0001	***
FV	0.41	0.04	<0.0001	***
GC	0.15	0.04	<0.0001	***
GR	0.43	0.04	<0.0001	***
LA	0.2	0.04	<0.0001	***
LO*Y - REF				
AA*Y	-0.03	0.01	<0.0001	***
FI	-0.03	0.01	0.0003	***
FV	-0.03	0.01	0.003	**
GC	-0.01	0.01	0.31	
GR	-0.06	0.01	<0.0001	***
LA	-0.02	0.01	0.02	*
Clindamycin', p<0.0001, Adj. R^2 = 0.69				
Year	0.07	0.01	<0.0001	***
LA - REF				
AA	0.19	0.05	0.0004	***
FI	0.29	0.05	<0.0001	***
FV	0.55	0.05	<0.0001	***
GC	0.37	0.05	<0.0001	***
GR	0.47	0.05	<0.0001	***
LO	0.52	0.05	<0.0001	***
LA*Y - REF				
AA	-0.04	0.01	0.0002	***
FI	-0.04	0.01	<0.0001	***
FV	-0.06	0.01	<0.0001	***
GC	-0.05	0.01	<0.0001	***
GR	-0.08	0.01	<0.0001	***
LO	-0.04	0.01	0.0005	***
Erythromycin', p<0.0001, Adj.R^2 = 0.60				
Year	0.02	0	<0.0001	***
AA - REF				
FI	0.12	0.02	<0.0001	***
FV	0.09	0.02	<0.0001	***
GC	0.09	0.02	<0.0001	***
GR	-0.04	0.02	0.03	*
LA	0.07	0.02	0.0004	***
LO	0.15	0.02	<0.0001	***
AA*Y - REF				
FI*Y	-0.03	0.004	<0.0001	***
FV*Y	-0.03	0.004	<0.0001	***
GC*Y	-0.03	0.004	<0.0001	***
GR*Y	-0.01	0.004	0.004	**
LA*Y	-0.03	0.004	<0.0001	***
LO*Y	-0.03	0.004	<0.0001	***
Gentamycin'', p<0.0001, Adj.R^2 = 0.47				

Year	0.005	0.003	0.09	.
AA - REF				
FI	0.17	0.02	<0.0001	***
FV	0.03	0.02	0.16	
GC	0.12	0.02	<0.0001	***
GR	0.15	0.02	<0.0001	***
LA	0.07	0.02	0.0005	***
LO	0.07	0.02	0.0005	***
AA*Y - REF				
FI*Y	-0.02	0.004	<0.0001	***
FV*Y	0	0.005	0.92	
GC*Y	-0.01	0.004	0.11	
GR*Y	-0.02	0.004	<0.0001	***
LA*Y	-0.01	0.004	0.07	.
LO*Y	-0.01	0.004	0.13	
Tetracycline', p<0.0001, Adj.R^2 = 0.84				
Year	0.04	0.01	<0.0001	***
FV - REF				
AA	0.12	0.03	0.0003	***
FI	0.35	0.03	<0.0001	***
GC	0.31	0.03	<0.0001	***
GR	0.52	0.03	<0.0001	***
LA	0.6	0.03	<0.0001	***
LO	0.38	0.03	<0.0001	***
FV*HB - REF				
AA*Y	-0.04	0.01	<0.0001	***
FI*Y	-0.07	0.01	<0.0001	***
GC*Y	-0.04	0.01	<0.0001	***
GR*Y	-0.06	0.01	<0.0001	***
LA*Y	-0.09	0.01	<0.0001	***
LO*Y	-0.09	0.01	<0.0001	***
Trimethoprim, p<0.0001, Adj. R^2 = 0.85				
Year	9.6	0.43	<0.0001	***
LA - REF				
AA	53.09	3.14	<0.0001	***
FI	46.36	3.1	<0.0001	***
FV	40.85	3.29	<0.0001	***
GC	37.58	3.1	<0.0001	***
GR	55.97	3.1	<0.0001	***
LO	30	3.1	<0.0001	***
LA*Y - REF				
AA*Y	-7.21	0.62	<0.0001	***
FI*Y	-5.46	0.61	<0.0001	***
FV*Y	-6.49	0.75	<0.0001	***
GC*Y	-5.66	0.61	<0.0001	***
GR*Y	-8.29	0.61	<0.0001	***
LO*Y	-6.16	0.61	<0.0001	***

’ data log transformed

” data log(x+1) transformed

Health Board abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian

Significance level: Significant = ≤ 0.001 = ‘***’; ≤0.01 = ‘**’; ≤0.05 = ‘*’; Not significant = <0.1 & >0.05 = ‘.’; 1 = ‘ ’

Std. Error = standard error

Y = Year

Health Board * Y = Interaction, within-Health Board trend over time (Year)

- = represents reference Health Board

B) Secondary care prescribing

Model	Coefficient	Std. Error	p-value	Significance level
Ciprofloxacin, p<0.0001, Adj. R^2 = 0.86				
Year	-0.62	0.41	0.14	
GR – REF	-	-	-	-
AA	6311.15	1149.85	<0.0001	***
FI	1640.59	1142.59	0.15	
FV	489.99	1142.59	0.67	
GC	8364.08	1142.59	<0.0001	***
LA	1330.2	1142.59	0.25	
LO	4159.52	1173.87	0.0006	***
GR*Y – REF	-	-	-	-
AA*Y	-3.14	0.57	<0.0001	***
FI*Y	-0.82	0.57	0.15	
FV*Y	-0.25	0.57	0.66	
GC*Y	-4.16	0.57	<0.0001	***
LA*Y	-0.67	0.57	0.24	
LO*Y	-2.07	0.58	0.0006	***
Clindamycin', p<0.0001, Adj. R^2 = 0.67				
Year	0.03	0.02	0.06	.
LA – REF	-	-	-	-
AA	2.24	47.66	0.96	
FI	10.6	47.34	0.82	
FV	311.98	47.34	<0.0001	***
GC	266.08	47.34	<0.0001	***
GR	103.67	48.71	0.04	*
LO	69.73	48.71	0.15	
LA*Y – REF	-	-	-	-
AA*Y	-0.001	0.02	0.97	
FI*Y	-0.01	0.02	0.82	
FV*Y	-0.16	0.02	<0.0001	***
GC*Y	-0.13	0.02	<0.0001	***
GR*Y	-0.05	0.02	0.04	*
LO*Y	-0.03	0.02	0.16	
Erythromycin', p<0.0001, Adj. R^2=0.93				
Year	0.03	0.01	0.003	**
GC – REF	-	-	-	-
AA	162.32	29.25	<0.0001	***
FI	222.22	29.05	<0.0001	***
FV	133.41	29.05	<0.0001	***
GR	189.39	29.89	<0.0001	***
LA	158.29	29.05	<0.0001	***
LO	180.4	29.89	<0.0001	***
GC*Y – REF	-	-	-	-
AA*Y	-0.08	0.01	<0.0001	***
FI*Y	-0.11	0.01	<0.0001	***
FV*Y	-0.07	0.01	<0.0001	***
GR*Y	-0.09	0.01	<0.0001	***
LA*Y	-0.08	0.01	<0.0001	***
LO*Y	-0.09	0.01	<0.0001	***
Gentamycin', p<0.0001, Adj. R^2 = 0.90				
Year	0.2	0.01	<0.0001	***
FV – REF	-	-	-	-
AA	245.54	38.07	<0.0001	***
FI	303.98	37.82	<0.0001	***
GC	203.7	37.82	<0.0001	***
GR	233.4	38.91	<0.0001	***

LA	81.59	37.82	0.03	*
LO	425.29	38.91	<0.0001	***
FV*Y – REF	-	-	-	-
AA*Y	-0.12	0.02	<0.0001	***
FI*Y	-0.15	0.02	<0.0001	***
GC*Y	-0.1	0.02	<0.0001	***
GR*Y	-0.12	0.02	<0.0001	***
LA*Y	-0.04	0.02	0.03	*
LO*Y	-0.21	0.02	<0.0001	***
Tetracycline', Interaction NS, additive model NS				
Year	-0.01	0.06	0.83	
FV – REF	-	-	-	-
AA	-144.2	161.6	0.37	
FI	-79.8	160.5	0.62	
GC	233.6	160.5	0.15	
GR	149.3	165.2	0.37	
LA	166.4	160.5	0.3	
LO	-5.38	165.2	0.97	
FV*Y – REF	-	-	-	-
AA*Y	0.07	0.08	0.38	
FI*Y	0.04	0.08	0.62	
GC*Y	-0.12	0.08	0.15	
GR*Y	-0.07	0.08	0.37	
LA*Y	-0.08	0.08	0.3	
LO*Y	0.003	0.08	0.98	
Trimethoprim, p<0.0001, Adj.R^2 = 0.92				
Year	1.9	0.24	<0.0001	***
LA – REF	-	-	-	-
AA	1719.68	672.93	0.01	*
FI	2059.62	668.44	0.003	**
FV	2793.96	668.44	<0.0001	***
GC	2413.4	668.44	0.0004	***
GR	1794.43	687.8	0.01	*
LO	2315.86	687.8	0.001	**
LA*Y – REF	-	-	-	-
AA*Y	-0.86	0.34	0.01	*
FI*Y	-1.03	0.33	0.002	**
FV*Y	-1.4	0.33	<0.0001	***
GC*Y	-1.2	0.33	0.0005	***
GR*Y	-0.89	0.34	0.01	*
LO*Y	-1.16	0.34	0.001	***

' data log transformed

Health Board abbreviations: AA = NHS Ayrshire and Arran, FI = NHS Fife, FV = NHS Forth Valley, GR = NHS Grampian, GC = NHS Greater Glasgow and Clyde, LA = NHS Lanarkshire, LO = NHS Lothian

Significance level: Significant = ≤ 0.001 = '***'; ≤0.01 = '**'; ≤0.05 = '*'; Not significant = <0.1 & >0.05 = '.'; 1 = ' '

Std. Error = standard error

Y = Year

NS = Non-significant

Health Board * Y = Interaction, within-Health Board trend over time (Year)

- = represents reference Health Board

Appendix Table 3: Univariate analyses: association between proportion resistant and (A) primary and (B) secondary care prescribing rate.

(A)

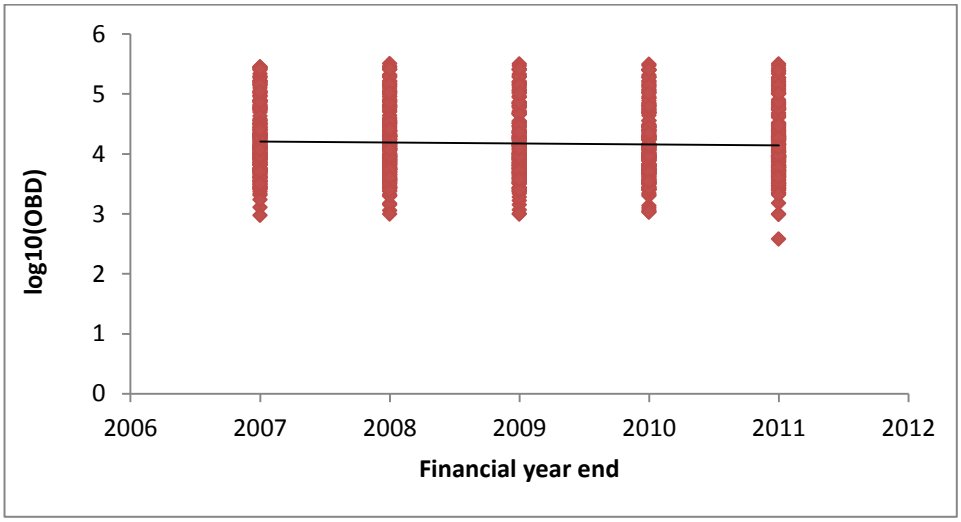
Data	Estimate	Std. Error	P-value	Significance	Adj. R2
Ciprofloxacin					
R_MRSA t0	-0.03	0.12	0.82		-0.005
R_MRSA t1	0.03	0.13	0.82		-0.005
R_MRSA t2	0.04	0.13	0.79		-0.005
R_MRSA t3	-0.05	0.13	0.74		-0.005
R_MRSA t4	-0.03	0.14	0.82		-0.005
R_MRSA t5	-0.06	0.14	0.68		-0.005
R_MRSA t6	-0.21	0.14	0.13		0.008
Clindamycin					
R_MRSA t0	0.29	0.07	0.0001	***	0.06
R_MRSA t1	0.34	0.07	<0.0001	***	0.09
R_MRSA t2	0.34	0.07	<0.0001	***	0.09
R_MRSA t3	0.34	0.08	<0.0001	***	0.09
R_MRSA t4	0.33	0.08	<0.0001	***	0.09
R_MRSA t5	0.3	0.08	0.0002	***	0.07
R_MRSA t6	0.28	0.08	0.0006	***	0.06
Erythromycin					
R_MRSA t0	-0.18	0.33	0.59		0.6
R_MRSA t1	-0.09	0.34	0.78		-0.005
R_MRSA t2	-0.09	0.34	0.79		-0.005
R_MRSA t3	-0.4	0.34	0.25		0.002
R_MRSA t4	0.25	0.34	0.47		-0.003
R_MRSA t5	-0.13	0.35	0.71		-0.005
R_MRSA t6	-0.27	0.35	0.44		-0.002
Gentamicin					
R_MRSA t0	0.53	0.12	<0.0001	***	0.08
R_MRSA t1	0.56	0.13	<0.0001	***	0.08
R_MRSA t2	0.62	0.13	<0.0001	***	0.1
R_MRSA t3	0.59	0.14	<0.0001	***	0.08
R_MRSA t4	0.58	0.14	<0.0001	***	0.08
R_MRSA t5	0.6	0.15	<0.0001	***	0.08
R_MRSA t6	0.59	0.16	0.0003	***	0.07
Tetracycline					
R_MRSA t0	0.06	0.06	0.31		0.0002
R_MRSA t1	0.09	0.06	0.12		0.007
R_MRSA t2	0.06	0.05	0.27		0.001
R_MRSA t3	0.08	0.05	0.13		0.007
R_MRSA t4	0.08	0.05	0.12		0.008
R_MRSA t5	0.1	0.06	0.06	.	0.01
R_MRSA t6	0.1	0.06	0.09	.	0.01
Trimethoprim					
R_MRSA t0	0.003	0.001	0.01	**	0.03
R_MRSA t1	0.002	0.001	0.02	*	0.02
R_MRSA t2	0.002	0.001	0.1	.	0.009
R_MRSA t3	0.001	0.001	0.31		0.0002
R_MRSA t4	0.001	0.001	0.29		0.0008
R_MRSA t5	0.001	0.001	0.28		0.0009
R_MRSA t6	0.001	0.001	0.34		-0.0004

(B)

Data	Estimate	Std. Error	P-value	Significance	Adj. R2
Ciprofloxacin					
R_MRSA t0	0.001	0.002	0.53		-0.005
R_MRSA t1	0.0005	0.002	0.77		-0.007
R_MRSA t2	0.001	0.002	0.75		-0.007
R_MRSA t3	0.001	0.002	0.63		-0.007
R_MRSA t4	0.001	0.002	0.52		-0.005
R_MRSA t5	0.001	0.002	0.78		-0.009
R_MRSA t6	0.001	0.001	0.25		-0.003
Clindamycin					
R_MRSA t0	-0.004	0.09	0.96		-0.007
R_MRSA t1	-0.12	0.09	0.18		0.006
R_MRSA t2	-0.16	0.09	0.06	.	0.02
R_MRSA t3	-0.14	0.09	0.11		0.01
R_MRSA t4	-0.14	0.09	0.13		0.01
R_MRSA t5	-0.17	0.09	0.06	.	0.02
R_MRSA t6	-0.15	0.09	0.09	.	0.02
Erythromycin					
R_MRSA t0	0.02	0.08	0.78		-0.007
R_MRSA t1	0.07	0.09	0.44		-0.003
R_MRSA t2	0.07	0.09	0.41		-0.003
R_MRSA t3	0.1	0.09	0.25		0.003
R_MRSA t4	0.01	0.09	0.88		-0.009
R_MRSA t5	0.01	0.09	0.88		-0.009
R_MRSA t6	0.1	0.09	0.27		0.002
Gentamycin					
R_MRSA t0	0.14	0.03	<0.0001	***	0.18
R_MRSA t1	0.15	0.03	<0.0001	***	0.19
R_MRSA t2	0.13	0.03	<0.0001	***	0.17
R_MRSA t3	0.13	0.03	<0.0001	***	0.18
R_MRSA t4	0.13	0.03	<0.0001	***	0.16
R_MRSA t5	0.12	0.03	<0.0001	***	0.15
R_MRSA t6	0.12	0.03	0.0003	***	0.12
Tetracycline					
R_MRSA t0	0.01	0.03	0.61		-0.005
R_MRSA t1	-0.0004	0.03	0.99		-0.008
R_MRSA t2	0.01	0.02	0.64		-0.006
R_MRSA t3	-0.003	0.02	0.89		-0.009
R_MRSA t4	-0.01	0.02	0.56		-0.006
R_MRSA t5	-0.03	0.02	0.22		0.005
R_MRSA t6	-0.01	0.03	0.57		-0.007
Trimethoprim					
R_MRSA t0	0.02	0.004	<0.0001	***	0.18
R_MRSA t1	0.02	0.004	<0.0001	***	0.17
R_MRSA t2	0.01	0.004	<0.0001	***	0.11
R_MRSA t3	0.01	0.004	0.0002	***	0.1
R_MRSA t4	0.02	0.004	0.0001	***	0.12
R_MRSA t5	0.01	0.004	0.0003	***	0.12
R_MRSA t6	0.02	0.004	0.0004	***	0.12

8.3 Appendices from Chapter 6

Appendix Figure 1: One year of data - Size (log10(OBD)) does not change over 5 year time period.



Appendix Table 4: Hospital MRSA 'status'.

The status of a hospital, either MRSA positive (i.e. had cases) versus MRSA negative (i.e. did not have cases), is shown for five financial years, 2006/07 to 2010/11 inclusive. A white box indicates that a hospital is negative and a black box indicates that a hospital was positive for that year. Hospitals that were positive for the study year (2007/2008) tended to be positive in other years. For hospitals with an '*' in the Status for Analysis column, the analyses were run with and without them to check that there was no change in the model.

Hospital Code	2006/2007	2007/2008	2008/2009	2009/2010	2010/2011	Status for Analysis
A101H						Negative
A105H						Negative
A110H						Negative
A201H						Negative
A207H						Negative
A211H						Negative
B103H						Negative
B105H						Negative
B114H						Negative
B128H						Negative
B129H						Negative
B130H						Negative
C101H						Negative
C106H						Negative
C108H						Negative
C110H						Negative
C113H						Negative
C114H						Negative
C122H						Negative
C204H						Negative
C310H						Negative
C403H						Negative

C406H					[redacted]	Negative
F705H						Negative
F708H						Negative
F709H						Negative
F711H						Negative
F712H						Negative
F716H						Negative
F810H						Negative
G111H						Negative
G206H						Negative
G302H						Negative
G303H						Negative
G408H					[redacted]	Negative
G505H						Negative
G515H					[redacted]	Negative
G606H						Negative
G610H					[redacted]	Negative
H104H						Negative
H106H						Negative
H108H						Negative
H201H						Negative
H208H						Negative
H210H						Negative
H211H						Negative
H212H						Negative
H213H				[redacted]	[redacted]	Negative
H217H						Negative
H219H						Negative
H223H						Negative
L103H						Negative
L104H						Negative
L105H						Negative
L203H						Negative
L204H						Negative
L206H						Negative
L207H						Negative
L209H						Negative
L211H						Negative
L213H					[redacted]	Negative
L216H						Negative
L218H						Negative
L304H						Negative
L305H						Negative
L307H						Negative
N103H						Negative
N121H						Negative
N151H						Negative
N161H						Negative
N181H						Negative
N198H					[redacted]	Negative
N331H						Negative
N332H						Negative
N335H						Negative
N336H						Negative
N337H						Negative
N351H				[redacted]	[redacted]	Negative
N352H						Negative
N353H						Negative
N431H						Negative
N432H						Negative
N434H						Negative
N451H						Negative
N492H						Negative
N494H					[redacted]	Negative
N496H					[redacted]	Negative
N499H					[redacted]	Negative
S102H						Negative
S103H						Negative

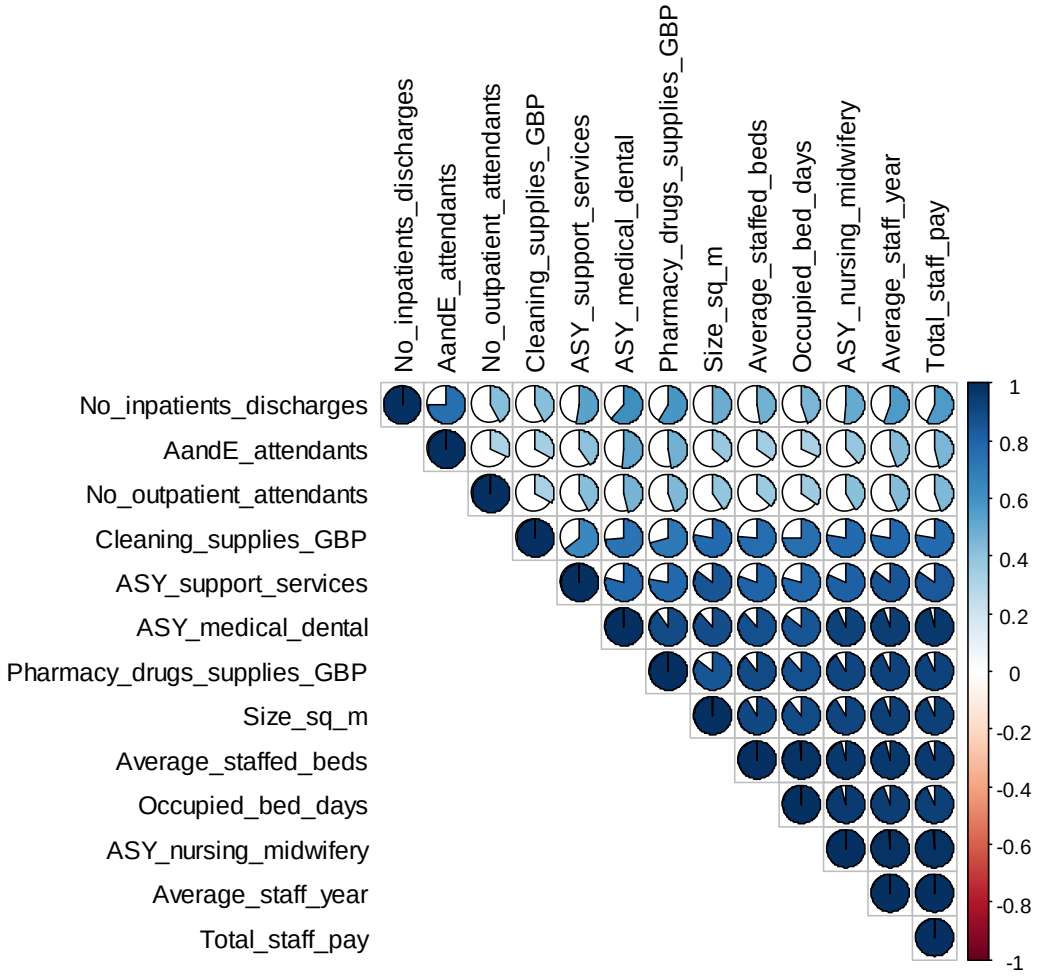
S107H						Negative
S108H						Negative
S109H						Negative
S210H						Negative
S214H						Negative
S217H						Negative
S225H						Negative
S305H						Negative
S306H						Negative
S310H						Negative
S312H						Negative
S313H						Negative
S315H						Negative
T105H						Negative
T114H						Negative
T115H						Negative
T205H						Negative
T207H						Negative
T208H					[redacted]	Negative
T209H						Negative
T215H						Negative
T304H						Negative
T305H						Negative
T309H						Negative
T310H						Negative
T311H						Negative
T316H						Negative
T317H						Negative
V105H						Negative
V106H						Negative
V107H						Negative
V113H						Negative
V202H						Negative
V204H				[redacted]		Negative
V211H						Negative
V214H						Negative
Y101H						Negative
Y103H						Negative
Y106H						Negative
Y107H						Negative
Y108H						Negative
Y109H						Negative
Y110H						Negative
Y114H						Negative
Y120H						Negative
Y123H						Negative
Y124H						Negative
Y126H						Negative
Y129H				[redacted]		Negative
Y130H						Negative
Y134H						Negative
Y136H						Negative
Y139H						Negative
A111H	[redacted]					Positive
A210H						Positive
B120H						Positive
C206H						Positive
C313H						Positive
C418H						Positive
F704H						Positive
F805H						Positive
G107H						Positive
G207H						Positive
G306H						Positive
G307H						Positive
G405H						Positive
G513H						Positive
G516H						Positive

H202H						Positive
L106H						Positive
L302H						Positive
L308H						Positive
N101H						Positive
N102H						Positive
N411H						Positive
S113H						Positive
S114H						Positive
S116H						Positive
S209H						Positive
S308H						Positive
S314H						Positive
T101H						Positive
T107H						Positive
T202H						Positive
V102H						Positive
V201H						Positive
Y104H						Positive
A103H						Negative
A208H						Positive
A215H						Negative
B118H						Negative
C121H						Positive
C407H						Positive*
F701H						Negative
F703H						Negative
G109H						Positive
H101H						Negative
H103H						Positive
H214H						Negative
H215H						Negative*
N183H						Positive*
N333H						Positive*
N334H						Positive
N433H						Positive*
S201H						Positive
T312H						Positive
T313H						Negative
Y144H						Positive

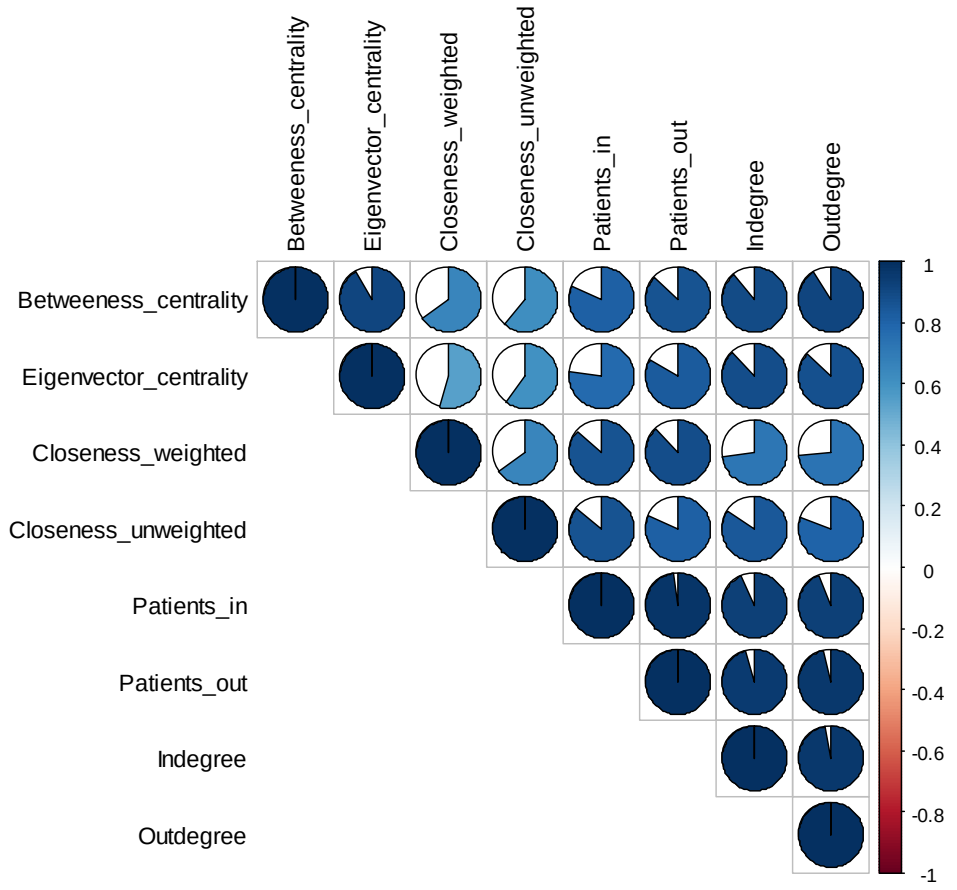
Appendix Figure 2: Correlation analyses for potential variables of (A) size and (B) connectivity.

The colour key (right-hand side of figure) shows the correlation coefficient in relation to colour; i.e. dark blue represents two variables with a strong positive correlation and dark red represents two variables with a strong negative correlation coefficient. The pie charts represent the correlation coefficient for a pair of variables (i.e. if the whole pie is dark blue, then correlation coefficient = 1, if half of the pie is blue, then the correlation coefficient = 0.5). Variables are ordered by increasing coefficient.

(A)



(B)


Betweeness_centrality
Eigenvector_centrality
Closeness_weighted
Closeness_unweighted
Patients_in
Patients_out
Indegree
Outdegree
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Appendix Table 5: List of all the variables considered in the risk factor analysis (Model 1: Logistic Regression and Model 2: Poisson regression).

Variable name	Variable type	Definition
Size (m^2)	Q	Hospital size measured in square metres
Average number of staffed beds	Q	Average number of available staffed beds for inpatients or day cases.
Occupied bed days (OBD)	Q	Sum of the number of occupied beds for each day of the period. A bed occupied by an inpatient at the bed count is counted as one OBD. If it is simultaneously reserved for a second patient, it is counted as two OBD.
Total patients discharged	Q	Total number of patients discharged.
Total average staff per year	Q	Number of staff members (WTE).
Average occupancy rate	Q	The average proportion of beds that were occupied by patients.
Average length of stay	Q	The average patient length of stay in acute specialties per hospital in days (This was only carried out for model 2 since model 1 dataset included hospitals with no acute specialties and hence length of stay measured in weeks).
ASY medical and dental	Q	Total average medical and dental staff per year (WTE).
ASY nursing and midwifery	Q	Total average nursing and midwifery staff per year (WTE).
ASY domestic staff	Q	Total average domestic staff per year (WTE (includes cleaners, cleaning supervisors and cleaning managers)).
ASY support services staff	Q	Total average support services staff per year (WTE (includes general services, hotel services, maintenance and estates, sterile services))
PSR – medical and dental	Q	Patient to medical and dentistry staff ratios; total number of inpatients per hospital per year, divided by total number of staff members registered to and paid from that hospital per year (WTE).
PSR – nurses and midwives	Q	Patient to nurses and midwives staff ratios; the total number of inpatients per hospital per year, divided by the total number of staff members registered to and paid from that hospital per year (WTE).
PSR – domestic staff	Q	Patient to domestic staff ratios total number of inpatients per hospital per year, divided by total number of staff members registered to and paid from that hospital per year (WTE)
Patients in	Q/C	Connectivity measure. Total number of patients moved to hospital A from other hospitals.
Patients out	Q/C	Connectivity measure. Total number of patients moved from hospital A to other hospitals.
Patients total	Q/C	Connectivity measure. Total number of patients moved to and from hospital A to other hospitals.
Indegree	Q/C	Connectivity measure. Total number of hospitals that transferred patients to hospital A.
Outdegree	Q/C	Connectivity measure. Total number of hospitals that received patients sent from hospital A.
Closeness Centrality	Q/C	Connectivity measure. The mean distance (in terms of the number of steps from hospital A to hospital B for each hospital).
Teaching hospital	C	If hospital was a teaching hospital (A1) versus any other type.
Category A	C	If hospital was a category A hospital (General, mainly acute).
Category B	C	If hospital was a category B hospital (long stay).

Category C	C	If hospital was a category C hospital (mental).
Category D	C	If hospital was a category D hospital (psychiatry of learning difficulties).
Category E	C	If hospital was a category E hospital (maternity).
Category J	C	If hospital was a category J hospital (community).
Hospital group	C	Three hospital groups that emerged from the NMS analysis (Figure 3). 1, Teaching hospital; 2, General hospital, no teaching; 3, Other (Categories B, C, D, E and J).
Sum of specialties	Q/C	Total number of hospital specialties. See Supplementary Table 2 for complete list of specialties.
Proportion of acute specialties	Q	Proportion of all specialties (Supplementary Table 2) that were acute
Long Stay Specialties	C	Presence of only long stay specialties (Adolescent Psychiatry, Child Psychiatry, General Psychiatry, Geriatric Psychiatry, Geriatric Long Stay, Learning Disabilities, Young Chronic Sick).
Intensive Care Unit	C	Intensive care unit.
Haematology	C	If the hospital had a haematology specialty.
Accident and Emergency (A&E)	C	If the hospital had an Accident and Emergency.
A&E Attendance	Q	Total number of patients attending Accident and Emergency.
Cleaning supplies	Q	Total cost of cleaning supplies (GBP).
Number of cleaning cost units	Q	Total cleaning number of cost units.
Standardised cleaning supplies	Q	The cleaning supplies (GBP) divided by hospital size (in square meters).
Standardised cleaning cost	Q	The cleaning number of cost units divided by hospital size (in square meters).

Q=quantitative / continuous; C=categorical; WTE, Whole Time Equivalent; ASY, Average staff per year; PSR, Patient Staff Ratio; GBP, Great British Pound, NMS: non-parametric multi-dimensional scaling;

Appendix Table 6: Output of univariate analyses, binomial model with presence-absence of MRSA bacteraemia model.

Variable	Variable type	AIC	Estimate	SE	p-value
Teaching hospital	Hospital classification	197.35	17.93	979.61	ns
Category A	Hospital classification	127.16	4.09	0.53	<0.0001
Category B	Hospital classification	214.44	-0.46	0.41	ns
Category C	Hospital classification	196.08	-17.6	1118.62	ns
Category D	Hospital classification	213.48	-1.33	1.06	ns
Category E	Hospital classification	214.15	-14.37	840.27	ns
Category J	Hospital classification	198.55	-2.04	0.62	<0.01
NMS presence of specialties	Specialties / activity type	107.3	4.42	1.12	<0.0001
Sum of specialties	Specialties / activity type	113.58	0.44	0.09	<0.0001
Proportion specialties acute	Specialties / activity type	165.66	0.04	0.01	<0.0001
Only long-stay specialties	Specialties / activity type	175.86	-3.68	1.02	<0.0001
Only acute specialties	Specialties / activity type	210.54	0.82	0.36	<0.05
Both specialty types	Specialties / activity type	204.73	1.15	0.35	<0.001
Log(Patients in)	Connectivity	59.72	5.78	1.08	<0.0001
Log(Patients out)	Connectivity	65.06	4.62	0.89	<0.0001
Log(Indegree)	Connectivity	90.97	5.85	0.88	<0.0001
Log(Outdegree)	Connectivity	78.44	6.38	0.99	<0.0001
Log(Closeness unweighted)	Connectivity	96.09	32.41	5.2	<0.0001
Closeness weighted	Connectivity	104.17	3086.36	630.78	<0.0001
Log(Eigenvector centrality)	Connectivity	99.36	862.42	132.71	<0.0001
Log(Betweeness centrality)	Connectivity	84.65	2.41	0.34	<0.0001
Closeness_threshold	Connectivity	127.37	3.89	0.49	<0.0001
Outdegree_threshold	Connectivity	105.47	4.54	0.59	<0.0001
Log(size in square metres)	Size	120.73	3.59	0.53	<0.0001
Log(Average staffed beds)	Size	118.23	4.31	0.62	<0.0001
Log(Occupied bed days)	Size	120.27	3.94	0.57	<0.0001
Log(total patients discharged)	Size	99.4	2.82	0.42	<0.0001
Log(total average staff per year)	Size/ Staff	103.93	4.07	0.59	<0.0001
Log(ASY medical and dental)	Size/ Staff	111.27	2.85	0.4	<0.0001
Log(ASY nursing and midwifery)	Size / Staff	110.05	4.14	0.6	<0.0001
Log(ASY domestic staff)	Size / Staff	128.01	1.2	0.2	<0.0001
Log(ASY support services staff)	Size / Staff	121.63	0.03	0.01	<0.0001
Log(Cleaning supplies GBP)	Size / Cleaning	125.68	3.16	0.48	<0.0001
Log(Number of cleaning cost units)	Size / Cleaning	121.26	3.61	0.54	<0.0001
Log(Pharmacy drugs supplies GBP)	Size / Prescribing	125.79	2.18	0.32	<0.0001
PSR – medical and dental	Staff / Patient staff ratios	210.71	-0.001	0.001	ns
PSR – nurses and midwives	Staff / Patient staff ratios	160.37	0.12	0.02	<0.0001
PSR – domestic staff	Staff / Patient staff ratios	153.4	0.001	0.0004	<0.001
PSR – support services staff	Staff / Patient staff ratios	208.72	0.001	0.001	ns
Cleaning 1	Cleaning	212.71	-2.99	3.01	ns
Cleaning 2	Cleaning	212.66	0.03	0.02	ns
Cleaning staff	Cleaning	214.1	-0.01	0.05	ns
Average occupancy rate	Other	207.23	0.04	0.01	<0.01

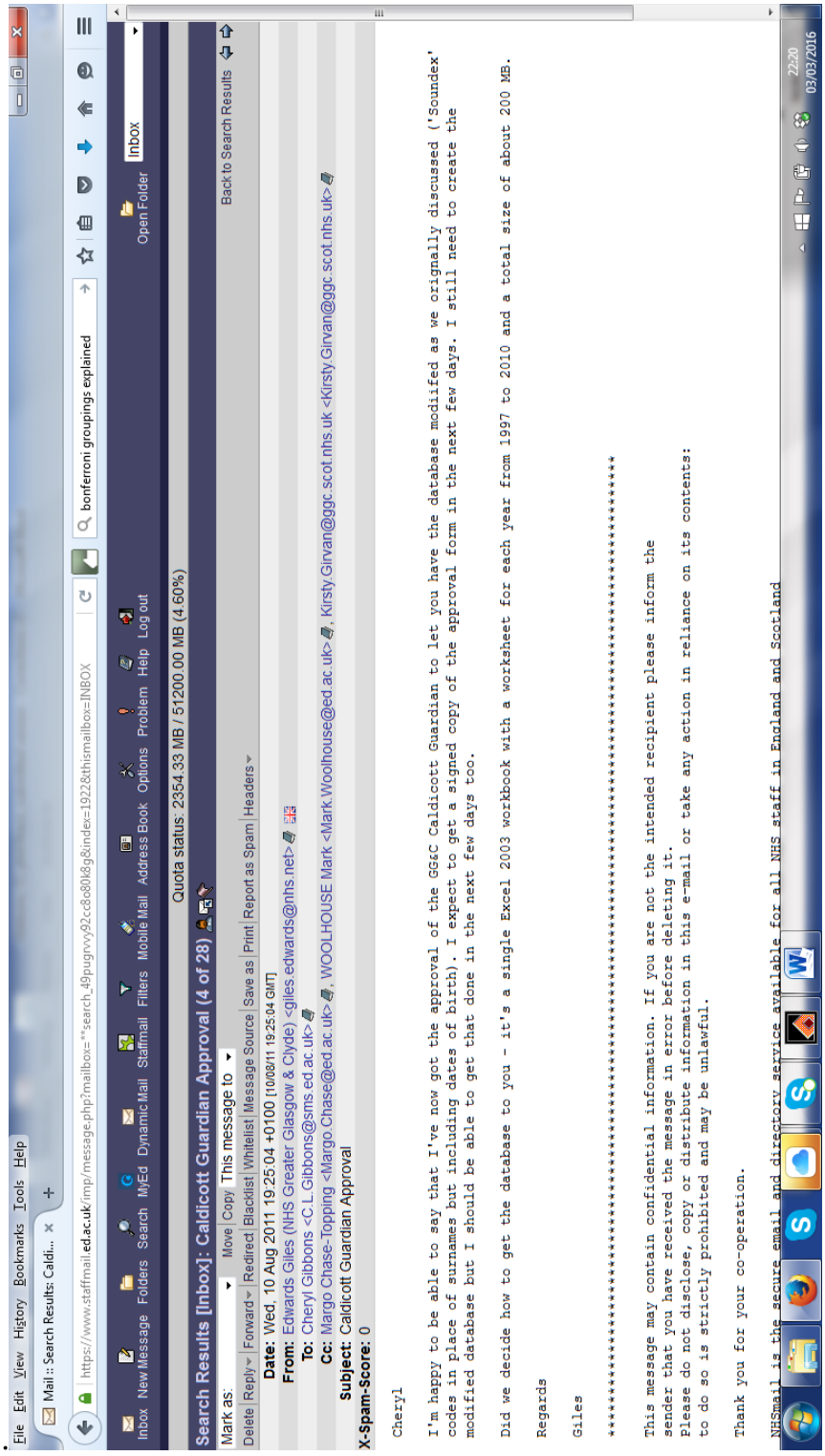
SE: Standard Error; AIC: Akaike Information Criterion
NMS: non-parametric multi-dimensional scaling; ASY: Annual staff per year; GBP: Great British pounds; PSR: Patient-staff-ratio

Appendix Table 7: Output of univariate analyses, Poisson model with MRSA bacteraemia case data.

Variable	Variable type	AIC	Estimate	SE	p-value
Teaching hospital	Hospital classification	210.79	0.63	0.08	<0.0001
NMS presence of specialties	Specialties / activity type	238.68	0.26	0.05	<0.0001
Sum of specialties	Specialties / activity type	250.41	0.03	0.01	<0.0001
Proportion specialties acute	Specialties / activity type	233.76	0.04	0.01	<0.0001
Only acute specialties	Specialties / activity type	234.26	0.5	0.09	<0.0001
Both specialty types	Specialties / activity type	234.26	-0.5	0.09	<0.0001
Log(Patients in)	Connectivity	231.33	0.92	0.15	<0.0001
Log(Patients out)	Connectivity	221.26	1.23	0.19	<0.0001
Log(Indegree)	Connectivity	228.81	2.38	0.39	<0.0001
Log(Outdegree)	Connectivity	232.41	2.35	0.4	<0.0001
Closeness unweighted	Connectivity	224.97	8.94	1.39	<0.0001
Log(Closeness weighted)	Connectivity	235.14	2001.88	357.66	<0.0001
Log(Eigenvector centrality)	Connectivity	241.5	184.84	35.86	<0.0001
Log(Betweeness centrality)	Connectivity	233.14	0.65	0.11	<0.0001
Log(size in square metres)	Size	232.34	0.98	0.17	<0.0001
Log(Average staffed beds)	Size	239	1.04	0.2	<0.0001
Log(Occupied bed days)	Size	236.03	1.06	0.19	<0.0001
Log(total patients discharged)	Size	236.37	0.92	0.17	<0.0001
Log(total average staff per year)	Size/ Staff	228.77	1.12	0.18	<0.0001
Log(ASY medical and dental)	Size/ Staff	223.68	1.06	0.16	<0.0001
Log(ASY nursing and midwifery)	Size / Staff	230.73	1.1	0.18	<0.0001
Log(ASY domestic staff)	Size / Staff	247.7	-0.07	0.03	<0.05
Log(ASY support services staff)	Size / Staff	256.1	0.25	0.07	<0.0001
Log(Cleaning supplies GBP)	Size / Cleaning	254.61	0.3	0.07	<0.0001
Log(Number of cleaning cost units)	Size / Cleaning	232.15	0.98	0.17	<0.0001
Log(Pharmacy drugs supplies GBP)	Size / Prescribing	241.48	0.6	0.11	<0.0001
PSR – medical and dental	Staff / Patient staff ratios	257.96	-0.005	0.001	<0.001
PSR – nurses and midwives	Staff / Patient staff ratios	270.27	0.004	0.008	ns
PSR – domestic staff	Staff / Patient staff ratios	231.93	2.37e-05	4.88e-06	<0.0001
PSR – support services staff	Staff / Patient staff ratios	232.38	0.002	0.0005	<0.0001
Cleaning 1	Cleaning	267.2	8.13	4.97	ns
Cleaning 2	Cleaning	270.34	0.001	0.002	ns
Cleaning staff	Cleaning	269.9	-0.02	0.02	ns
Average occupancy rate	Other	258.06	0.02	0.01	<0.001
Average length of stay	Other	269.8	-0.02	0.025	ns

SE: Standard Error; AIC: Akaike Information Criterion
NMS: non-parametric multi-dimensional scaling; ASY: Annual staff per year; GBP: Great British pounds; PSR: Patient-staff-ratio

Appendix Document 1: Approval granting access to the SMRSARL database from the NHS Greater Glasgow and Clyde Caldicott Guardian



Search Results [Inbox]: Caldicott Guardian Approval (4 of 28)

Quota status: 2364.33 MB / 51200.00 MB (4.60%)

Date: Wed, 10 Aug 2011 19:25:04 +0100 [10/08/11 19:25:04 GMT]
From: Edwards Giles (NHS Greater Glasgow & Clyde) <giles.edwards@nhs.net>
To: Cheryl Gibbons <C.L.Gibbons@sms.ed.ac.uk>
Cc: Margo Chase-Topping <Margo.Chase@ed.ac.uk>, WOOLHOUSE Mark <Mark.Woolhouse@ed.ac.uk>, Kirsty.Girvan@ggc.scot.nhs.uk <Kirsty.Girvan@ggc.scot.nhs.uk>
Subject: Caldicott Guardian Approval
X-Spam-Score: 0

Cheryl

I'm happy to be able to say that I've now got the approval of the GG&C Caldicott Guardian to let you have the database modified as we orignally discussed ('Soundex' codes in place of surnames but including dates of birth). I expect to get a signed copy of the approval form in the next few days. I still need to create the modified database but I should be able to get that done in the next few days too.

Did we decide how to get the database to you - it's a single Excel 2003 workbook with a worksheet for each year from 1997 to 2010 and a total size of about 200 MB.

Regards

Giles

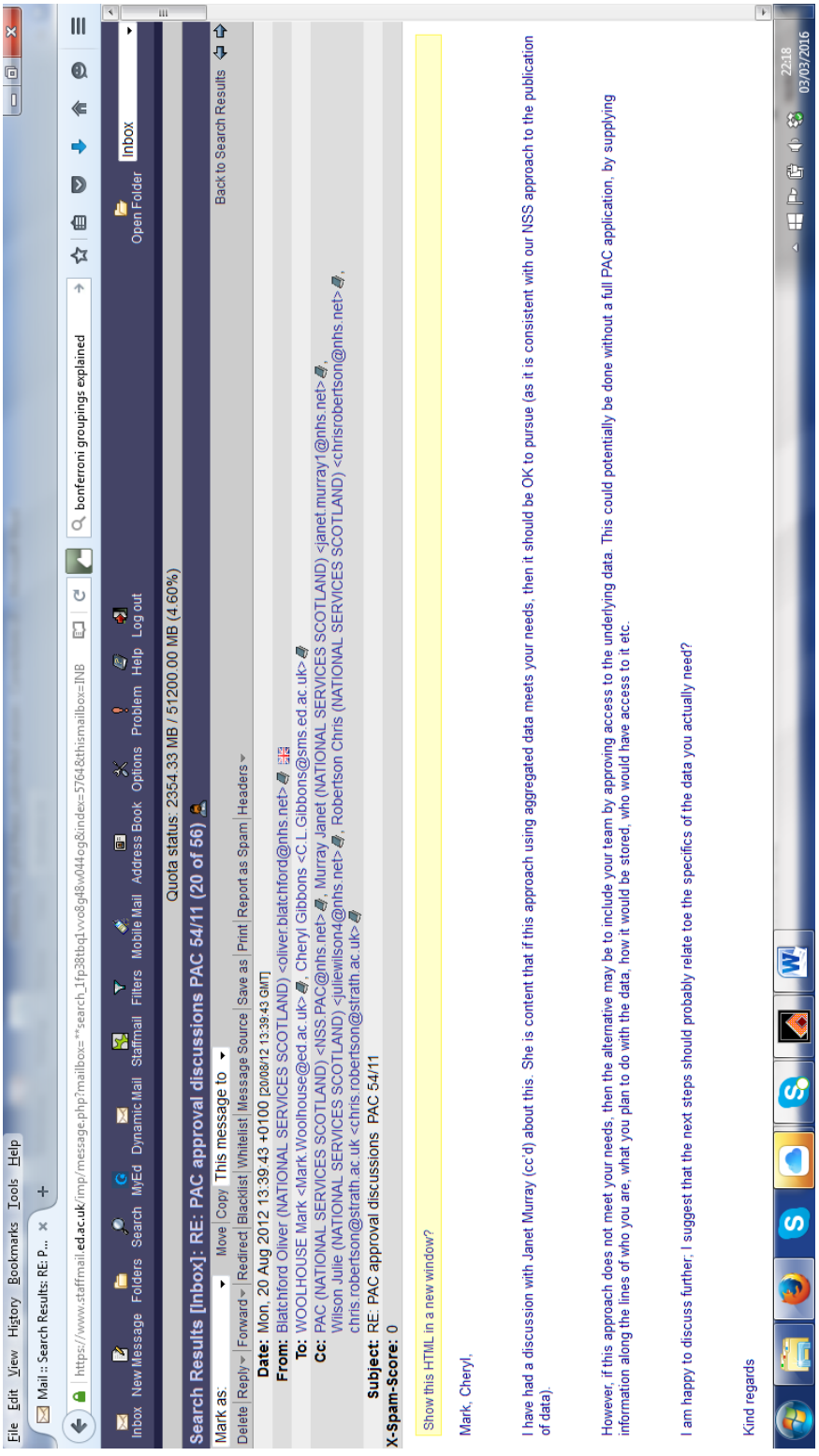
****

This message may contain confidential information. If you are not the intended recipient please inform the sender that you have received the message in error before deleting it.
Please do not disclose, copy or distribute information in this e-mail or take any action in reliance on its contents: to do so is strictly prohibited and may be unlawful.

Thank you for your co-operation.

NHSmail is the secure email and directory service available for all NHS staff in England and Scotland

Appendix Document 2: Approval for the release of hospital-level bacteraemia data from the Caldicott Guardian at ISD.



Search Results [Inbox]: RE: PAC approval discussions PAC 54/11 (20 of 56)

Date: Mon, 20 Aug 2012 13:39:43 +0100 (20/08/12 13:39:43 GMT)
From: Blatchford Oliver (NATIONAL SERVICES SCOTLAND) <oliver.blatchford@nhs.net>
To: WOOLHOUSE Mark <Mark.Woolhouse@ed.ac.uk>, Cheryl Gibbons <C.L.Gibbons@sms.ed.ac.uk>
Cc: PAC (NATIONAL SERVICES SCOTLAND) <NSS.PAC@nhs.net>, Murray Janet (NATIONAL SERVICES SCOTLAND) <janet.murray1@nhs.net>, Wilson Julie (NATIONAL SERVICES SCOTLAND) <juliewilson4@nhs.net>, Robertson Chris (NATIONAL SERVICES SCOTLAND) <chrisrobertson@nhs.net>, chris.robertson@strath.ac.uk <chris.robertson@strath.ac.uk>
Subject: RE: PAC approval discussions PAC 54/11
X-Spam-Score: 0

Show this HTML in a new window?

Mark, Cheryl,

I have had a discussion with Janet Murray (cc'd) about this. She is content that if this approach using aggregated data meets your needs, then it should be OK to pursue (as it is consistent with our NSS approach to the publication of data).

However, if this approach does not meet your needs, then the alternative may be to include your team by approving access to the underlying data. This could potentially be done without a full PAC application, by supplying information along the lines of who you are, what you plan to do with the data, how it would be stored, who would have access to it etc.

I am happy to discuss further, I suggest that the next steps should probably relate toe the specifics of the data you actually need?

Kind regards

Chapter 9: **References**

1. Wertheim, H.F.L., et al., *The role of nasal carriage in Staphylococcus aureus infections.* The Lancet Infectious Diseases, 2005. **5**(12): p. 751-762.
2. Wilson, J., et al., *Trends in sources of meticillin-resistant Staphylococcus aureus (MRSA) bacteraemia: data from the national mandatory surveillance of MRSA bacteraemia in England, 2006-2009.* Journal of Hospital Infection, 2011. **79**(3): p. 211-7.
3. Tom, S., et al., *Case fatality ratio and mortality rate trends of community-onset Staphylococcus aureus bacteraemia.* Clinical Microbiology and Infection, 2014. **24**(10): p. 1469-0691.
4. Wisplinghoff, H., et al., *Nosocomial Bloodstream Infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Surveillance Study.* Clinical Infectious Diseases, 2004. **39**(3): p. 309-317.
5. Kaye, K.S., et al., *Effect of nosocomial bloodstream infections on mortality, length of stay, and hospital costs in older adults.* Journal of the American Geriatrics Society, 2014. **62**(2): p. 306-11.
6. Laupland, K.B., Ross, T., and Gregson, D.B., *Staphylococcus aureus bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000-2006.* Journal of Infectious Diseases, 2008. **198**(3): p. 336-43.
7. Kaku, N., et al., *Influence of antimicrobial regimen on decreased in-hospital mortality of patients with MRSA bacteremia.* Journal of Infection and Chemotherapy, 2014. **20**(6): p. 350-5.
8. Skinner, D. and Keefer, C.S., *Significance of bacteremia caused by staphylococcus aureus: A study of one hundred and twenty-two cases and a review of the literature concerned with experimental infection in animals.* Archives of Internal Medicine, 1941. **68**(5): p. 851-875.
9. Lawes, T., et al., *Trends in Staphylococcus aureus bacteraemia and impacts of infection control practices including universal MRSA admission screening in a hospital in Scotland, 2006-2010: retrospective cohort study and time-series intervention analysis.* British Medical Journal, 2012. **2**(3): p. 2011-000797.
10. Hunt, A.C., et al., *Methicillin-Resistant Staphylococcus aureus in Northeastern Scotland in 2003 to 2007: Evolving Strain Distribution and Resistance Patterns.* Journal of Clinical Microbiology., 2011. **49**(5): p. 1975-1978.
11. Reilly, J.S., et al., *Universal screening for meticillin-resistant Staphylococcus aureus: interim results from the NHS Scotland pathfinder project.* Journal of Hospital Infection, 2010. **74**(1): p. 35-41.
12. Wyllie, D.H., Peto, T.E.A., and Crook, D., *MRSA bacteraemia in patients on arrival in hospital: a cohort study in Oxfordshire 1997-2003.* British Medical Journal, 2005. **331**(7523): p. 992.

13. Goetghebeur, M., et al., *Methicillin-resistant Staphylococcus aureus: A public health issue with economic consequences.* Canadian Journal of Infectious Diseases and Medical Microbiology, 2007. **18**(1): p. 27-34.
14. McDanel, J.S., et al., *Comparative effectiveness of beta-lactams versus vancomycin for treatment of methicillin-susceptible Staphylococcus aureus bloodstream infections among 122 hospitals.* Clinical Infectious Diseases, 2015. **61**(3): p. 361-7.
15. Gould, I.M., et al., *New insights into meticillin-resistant Staphylococcus aureus (MRSA) pathogenesis, treatment and resistance.* International Journal of Antimicrobial Agents, 2012. **39**(2): p. 96-104.
16. Gemmell, C.G., et al., *Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK.* Journal of Antimicrobial Chemotherapy, 2006. **57**(4): p. 589-608.
17. The Lancet Infectious Diseases, *Antibiotic resistance: long-term solutions require action now.* The Lancet Infectious Diseases, 2013. **13**(12): p. 995.
18. World Health Organisation, *Antimicrobial resistance: global report on surveillance 2014*, 2014, WHO: Geneva.
19. Reardon, S., *WHO warns against 'post-antibiotic' era*, in *Nature news*, 2014.
20. Howard, S.J., et al., *Antibiotic resistance: global response needed.* The Lancet Infectious Diseases, 2013. **13**(12): p. 1001-3.
21. Earnshaw, S., et al., *Global collaboration to encourage prudent antibiotic use.* The Lancet Infectious Diseases, 2013. **13**(12): p. 1003-4.
22. Woolhouse, M. and Farrar, J., *Policy: An intergovernmental panel on antimicrobial resistance.* Nature, 2014. **509**(7502): p. 555-7.
23. Chambers, H.R. and DeLeo F.R., *Waves of resistance: Staphylococcus aureus in the antibiotic era.* Nature Reviews Microbiology, 2009. **7**(9): p. 629-641.
24. Appelbaum, P.C., *Microbiology of Antibiotic Resistance in Staphylococcus aureus.* Clinical Infectious Diseases, 2007. **45**(Supplement 3): p. S165-S170.
25. Zetola, N., et al., *Community-acquired meticillin-resistant Staphylococcus aureus: an emerging threat.* The Lancet Infectious Diseases, 2005. **5**(5): p. 275-86.
26. Enright, M.C., et al., *The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA).* Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(11): p. 7687-92.
27. Elston, J.W.T. and Barlow G.D., *Community-associated MRSA in the United Kingdom.* Journal of Infection, 2009. **59**(3): p. 149-155.
28. Wulf, M. and Voss A., *MRSA in livestock animals—an epidemic waiting to happen?* Clinical Microbiology and Infection, 2008. **14**(6): p. 519-521.
29. van Cleef, B.A., et al., *Prevalence of Livestock-Associated MRSA in Communities with High Pig-Densities in The Netherlands.* PLoS ONE, 2010. **5**(2): p. e9385.
30. Ghasemzadeh-Moghaddam, H., et al., *Methicillin-susceptible Staphylococcus aureus from clinical and community sources are genetically diverse.* International Journal of Medical Microbiology, 2011. **301**(4): p. 347-353.
31. Shore, A.C., et al., *Detection of Staphylococcal Cassette Chromosome mec Type XI Carrying Highly Divergent mecA, mecI, mecR1, blaZ, and ccr Genes in Human Clinical Isolates of Clonal Complex 130 Methicillin-Resistant*

Staphylococcus aureus. Antimicrobial Agents and Chemotherapy, 2011. **55**(8): p. 3765-3773.
32. Shore, A.C. and Coleman, D.C., *Staphylococcal cassette chromosome mec: Recent advances and new insights.* International Journal of Medical Microbiology, 2013. **303**(6–7): p. 350-359.
33. Lindsay, J.A., *Hospital-associated MRSA and antibiotic resistance—What have we learned from genomics?* International Journal of Medical Microbiology, 2013. **303**(6–7): p. 318-323.
34. Knight, G.M., et al., *Shift in dominant hospital-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) clones over time.* Journal of Antimicrobial Chemotherapy, 2012. **67**(10): p. 2514-22.
35. Church, D.L., et al., *Comparison of automated repetitive-sequence-based polymerase chain reaction and spa typing versus pulsed-field gel electrophoresis for molecular typing of methicillin-resistant Staphylococcus aureus.* Diagnostic Microbiology and Infectious Disease, 2011. **69**(1): p. 30-7.
36. Foster, T.J., *The Staphylococcus aureus "superbug".* Journal of Clinical Investigation, 2004. **114**(12): p. 1693-6.
37. Horgan, M., et al., *A study of the prevalence of methicillin-resistant Staphylococcus aureus in pigs and in personnel involved in the pig industry in Ireland.* The Veterinary Journal, 2010, 190(2):255-9.
38. Otter, J.A. and French G.L., *Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Europe.* The Lancet Infectious Diseases, 2010. **10**(4): p. 227-239.
39. Diederen, B.M.W. and Kluytmans J.A.J.W., *The emergence of infections with community-associated methicillin resistant Staphylococcus aureus.* Journal of Infection, 2006. **52**(3): p. 157-168.
40. Dancer, S.J., *The effect of antibiotics on methicillin-resistant Staphylococcus aureus.* Journal of Antimicrobial Chemotherapy, 2008. **61**(2): p. 246-53.
41. Hassen, A., et al., *Resistance of environmental bacteria to heavy metals.* Bioresource Technology, 1998. **64**(1): p. 7-15.
42. Silver, S., Misra, T.K., and Laddaga, R.A., *DNA sequence analysis of bacterial toxic heavy metal resistances.* Biological Trace Element Research, 1989. **21**: p. 145-63.
43. Nair, R., et al., *Resistance to zinc and cadmium in Staphylococcus aureus of human and animal origin.* Infection Control and Hospital Epidemiology, 2014. **35**(3): p. 677834.
44. Chudobova, D., et al., *Effect of ampicillin, streptomycin, penicillin and tetracycline on metal resistant and non-resistant Staphylococcus aureus.* International Journal of Environmental Research and Public Health, 2014. **11**(3): p. 3233-55.
45. Sheldon, A.T., *Antiseptic "Resistance": Real or Perceived Threat?* Clinical Infectious Diseases, 2005. **40**(11): p. 1650-1656.
46. Brunning, A. *Compond Interest. Webpage: http://www.compoundchem.com/2014/09/08/antibiotics/. UK*. 2015 [Accessed August 2015].

47. Lindsay, J.A., *Staphylococcus aureus genomics and the impact of horizontal gene transfer.* International Journal of Medical Microbiology, 2014. **304**(2): p. 103-109.
48. Nielsen, K.L., et al., *Fitness cost: a bacteriological explanation for the demise of the first international methicillin-resistant Staphylococcus aureus epidemic.* Journal of Antimicrobial Chemotherapy, 2012. **67**(6): p. 1325-32.
49. Andersson, D.I. and Hughes, D., *Antibiotic resistance and its cost: is it possible to reverse resistance?* Nature Reviews Microbiology, 2010. **8**(4): p. 260-271.
50. Bjorkman, J. and Andersson, D.I., *The cost of antibiotic resistance from a bacterial perspective.* Drug Resistance Updates, 2000. **3**(4): p. 237-245.
51. Gordon, N.C., et al., *Prediction of Staphylococcus aureus Antimicrobial Resistance by Whole-Genome Sequencing.* Journal of Clinical Microbiology, 2014. **52**(4): p. 1182-91.
52. Prabhu, K., Rao, S., and Rao, V., *Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples.* Journal of Laboratory Physicians, 2011. **3**(1): p. 25-27.
53. Cookson, B., et al., *Meticillin-resistant Staphylococcus aureus (MRSA): screening and decolonisation.* International Journal of Antimicrobial Agents, 2010. **37**(3): p. 195-201.
54. Harinstein, L., Schafer, J., and D'Amico, F., *Risk factors associated with the conversion of meticillin-resistant Staphylococcus aureus colonisation to healthcare-associated infection.* Journal of Hospital Infection, 2011. **79**(3): p. 194-197.
55. Corbella, X., et al., *Staphylococcus aureus nasal carriage as a marker for subsequent staphylococcal infections in intensive care unit patients.* European Journal of Clinical Microbiology and Infectious Diseases, 1997. **16**(5): p. 351-7.
56. von Eiff, C., et al., *Nasal Carriage as a Source of Staphylococcus aureus Bacteremia.* New England Journal of Medicine, 2001. **344**(1): p. 11-16.
57. Datta, R. and S.S. Huang, *Risk of Infection and Death due to Methicillin-Resistant Staphylococcus aureus in Long-Term Carriers.* Clinical Infectious Diseases, 2008. **47**(2): p. 176-181.
58. Reilly, J.S., et al., *Universal screening for meticillin-resistant Staphylococcus aureus in acute care: risk factors and outcome from a multicentre study.* Journal of Hospital Infection, 2012. **80**(1): p. 31-5.
59. Matheson, A., et al., *Nasal swab screening for methicillin-resistant Staphylococcus aureus--how well does it perform? A cross-sectional study.* Infection Control and Hospital Epidemiology, 2012. **33**(8): p. 803-8.
60. Kock, R., et al., *Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe.* Eurosurveillance, 2010. **15**(41): p. 19688.
61. Huang, H., et al., *Injecting drug use and community-associated methicillin-resistant Staphylococcus aureus infection.* Diagnostic Microbiology and Infectious Disease, 2008. **60**(4): p. 347-350.
62. Qi, W., et al., *Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus in Zurich, Switzerland (2003): Prevalence of Type IV SCCmec and a New SCCmec Element Associated with Isolates from*

Intravenous Drug Users. Journal of Clinical Microbiology., 2005. **43**(10): p. 5164-5170.
63. Diep, B.A., et al., *Emergence of Multidrug-Resistant, Community-Associated, Methicillin-Resistant Staphylococcus aureus Clone USA300 in Men Who Have Sex with Men.* Annals of Internal Medicine, 2008. **148**(4): p. 249-257.
64. Kazakova, S.V., et al., *A Clone of Methicillin-Resistant Staphylococcus aureus among Professional Football Players.* New England Journal of Medicine, 2005. **352**(5): p. 468-475.
65. Hota, B., et al., *Community-Associated Methicillin-Resistant Staphylococcus aureus Skin and Soft Tissue Infections at a Public Hospital: Do Public Housing and Incarceration Amplify Transmission?* Archives of Internal Medicine, 2007. **167**(10): p. 1026-1033.
66. Millar, B.C., Prendergast B.D., and Moore J. E., *Community-associated MRSA (CA-MRSA): an emerging pathogen in infective endocarditis.* Journal of Antimicrobial Chemotherapy, 2008. **61**(1): p. 1-7.
67. Millar, B.C., et al., *Proposed definitions of community-associated meticillin-resistant Staphylococcus aureus (CA-MRSA).* Journal of Hospital Infection, 2007. **67**(2): p. 109-113.
68. Wehrhahn, M., et al., *Clinical and laboratory features of invasive community-onset methicillin-resistant Staphylococcus aureus infection: a prospective case–control study.* European Journal of Clinical Microbiology and Infectious Diseases, 2010. **29**(8): p. 1025-1033.
69. Tong, S.Y.C., et al., *Trimethoprim-sulfamethoxazole compared with benzathine penicillin for treatment of impetigo in Aboriginal children: A pilot randomised controlled trial.* Journal of Paediatrics and Child Health, 2010. **46**(3): p. 131-133.
70. Tong, S.Y.C., et al., *Community-Associated Strains of Methicillin-Resistant Staphylococcus aureus and Methicillin-Susceptible S. aureus in Indigenous Northern Australia: Epidemiology and Outcomes.* Journal of Infectious Diseases, 2009. **199**(10): p. 1461-1470.
71. Tong, S.Y.C., et al., *Global Implications of the Emergence of Community-Associated Methicillin-Resistant Staphylococcus aureus in Indigenous Populations.* Clinical Infectious Diseases, 2008. **46**(12): p. 1871-1878.
72. Gubbay, J.B., et al., *Clinical features, epidemiology, antimicrobial resistance, and exotoxin genes (including that of Panton-Valentine leukocidin) of gentamicin-susceptible methicillin-resistant Staphylococcus aureus (GS-MRSA) isolated at a paediatric teaching hospital in New South Wales, Australia.* Pathology, 2008 **40**(1): p. 64-71.
73. Maguire, G.P., et al., *Emerging epidemic of community-acquired methicillin-resistant Staphylococcus aureus infection in the Northern Territory.* Medical Journal of Australia., 1996. **164**(12): p. 721-3.
74. McDonald, M., et al., *Use of a single-nucleotide polymorphism genotyping system to demonstrate the unique epidemiology of methicillin-resistant Staphylococcus aureus in remote aboriginal communities* Journal of Clinical Microbiology., 2006 **44**(10): p. 3720-7.
75. Stevens, C.L., et al., *Community-acquired methicillin-resistant Staphylococcus aureus in Central Australia.* Communicable Diseases Intelligence, 2006. **30** (4): p. 462-6.

76. Byrd, K.K., et al., *Methicillin-Resistant Staphylococcus aureus-Associated Hospitalizations among the American Indian and Alaska Native Population.* Clinical Infectious Diseases, 2009. **49**(7): p. 1009-1015.
77. Embil, J., et al., *Methicillin-resistant Staphylococcus aureus in tertiary care institutions on the Canadian prairies 1990-1992.* Infection Control and Hospital Epidemiology., 1994. **15**(10): p. 646-51.
78. Ofner-Agostini, M., et al., *Methicillin-Resistant Staphylococcus aureus in Canadian Aboriginal People.* Infection Control and Hospital Epidemiology, 2006. **27**(2): p. 204-207.
79. Simor, A.E., et al., *Mupirocin-resistant, methicillin-resistant Staphylococcus aureus strains in Canadian hospitals.* Antimicrobial Agents and Chemotherapy, 2007. **51**(11): p. 3880-6.
80. Groom, A.V., et al., *Community-Acquired Methicillin-Resistant Staphylococcus aureus in a Rural American Indian Community.* JAMA: The Journal of the American Medical Association, 2001. **286**(10): p. 1201-1205.
81. Rings, T., Findlay R., and Lang S., *Ethnicity and methicillin-resistant S. aureus in South Auckland.* New Zealand Medical Journal., 1998 **Apr 24;111(1064):151.**
82. Ben Zakour, N.L., Guinane C.M., and Fitzgerald J.R., *Pathogenomics of the staphylococci: insights into niche adaptation and the emergence of new virulent strains.* FEMS Microbiology Letters, 2008. **289**(1): p. 1-12.
83. Cuny, C., et al., *Emergence of methicillin-resistant Staphylococcus aureus (MRSA) in different animal species.* International Journal of Medical Microbiology, 2010. **300**(2-3): p. 109-117.
84. van Loo, I., et al., *Emergence of Methicillin-Resistant Staphylococcus aureus of Animal Origin in Humans.* Emerging Infectious Diseases, 2007. **13**(12).
85. Rybak, M.J. and LaPlante K.L., *Community-associated methicillin-resistant Staphylococcus aureus: a review.* Pharmacotherapy, 2005. **25**(1): p. 74-85.
86. Hartmeyer, G.N., et al., *Pig-associated methicillin-resistant Staphylococcus aureus: Family transmission and severe pneumonia in a newborn.* Scandinavian Journal of Infectious Diseases, 2010. **42**: p. 318-320.
87. Huijsdens, X.W., et al., *Community-acquired MRSA and pig-farming.* Annals of Clinical Microbiology and Antimicrobials., 2006. **5**(26).
88. Voss, A., et al., *Methicillin-resistant Staphylococcus aureus in pig farming.* Emerging Infectious Diseases., 2005 **11**(12): p. 1965-6.
89. Wulf, M., et al., *Methicillin-resistant Staphylococcus aureus in Veterinary Doctors and Students, the Netherlands.* Emerging Infectious Diseases, 2006. **12**(12).
90. Garcia-Graells, C., et al., *Dynamic of Livestock-Associated Methicillin-Resistant Staphylococcus aureus CC398 in Pig Farm Households: A Pilot Study.* PLoS ONE, 2013. **8**(5).
91. Van Den Broek, I.V., et al., *Methicillin-resistant Staphylococcus aureus in people living and working in pig farms.* Epidemiology and Infection., 2009. **137**(5): p. 700-8.
92. Ward, M.J., et al., *Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic Resistance Dynamics of Staphylococcus aureus Clonal Complex 398.* Applied and Environmental Microbiology., 2014. **80**(23): p. 7275-82.

93. Wulf, M.W., et al., *First outbreak of methicillin-resistant Staphylococcus aureus ST398 in a Dutch hospital, June 2007.* Eurosurveillence, 2008. **13**(9).
94. Cuny, C., et al., *Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs.* PLoS ONE, 2009. **4**(8): p. 0006800.
95. Graveland, H., et al., *Persistence of Livestock Associated MRSA CC398 in Humans Is Dependent on Intensity of Animal Contact.* PLoS ONE, 2011. **6**(2): p. e16830.
96. Donker, T., Wallinga, J., and Grundmann, H., *Patient Referral Patterns and the Spread of Hospital-Acquired Infections through National Health Care Networks.* PLoS Computational Biology, 2010. **6**(3): p. e1000715.
97. Wakefield, D.S., et al., *Variation in Methicillin-Resistant Staphylococcus aureus Occurrence by Geographic Location and Hospital Characteristics.* Infection Control, 1987. **8**(4): p. 151-157.
98. Donker, T., et al., *Hospital networks and the dispersal of hospital-acquired pathogens by patient transfer.* PLoS ONE, 2012. **7**(4): p. 25.
99. Donker, T., Wallinga, J., and Grundmann, H., *Dispersal of antibiotic-resistant high-risk clones by hospital networks: changing the patient direction can make all the difference.* Journal of Hospital Infection, 2013. **24**(13): p. 021.
100. Eveillard, M., et al., *Association Between Hospital-Acquired Infections and Patients' Transfers.* Infection Control and Hospital Epidemiology, 2001. **22**(11): p. 693-696.
101. de Kraker, M.E.A., et al., *Clinical Impact of Antimicrobial Resistance in European Hospitals: Excess Mortality and Length of Hospital Stay Related to Methicillin-Resistant Staphylococcus aureus Bloodstream Infections.* Antimicrobial Agents and Chemotherapy, 2011. **55**(4): p. 1598-1605.
102. Health Protection Scotland (HPS), *Protocol for the Scottish Mandatory Surveillance Programme for Staphylococcus aureus Bacteraemia*, 5th Edition, December 2012: Scotland, UK.
103. NHS Scotland, *HEAT Targets Due for Delivery in 2009/10: Summary of Performance: NHS Scotland Performance and Business Management*, 2011: Edinburgh.
104. Reilly, J.S., et al., *Universal screening for meticillin-resistant Staphylococcus aureus: interim results from the NHS Scotland pathfinder project.* Journal Hospital Infection, 2010. **74**(1): p. 35-41.
105. Hawkins, G., et al., *Should healthcare workers be screened routinely for meticillin-resistant Staphylococcus aureus? A review of the evidence.* Journal of Hospital Infection, 2011. **77**(4): p. 285-289.
106. Currie, K., et al., *Methicillin-resistant Staphylococcus aureus screening as a patient safety initiative: using patients' experiences to improve the quality of screening practices.* Journal of Clinical Nursing, 2014. **23**(1-2): p. 221-31.
107. Coia, J.E., Leanord, A.T., and Reilly, J., *Screening for meticillin resistant Staphylococcus aureus (MRSA): who, when, and how?* British Medical Journal, 2014. **348**.
108. Coia, J.E., et al., *Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities.* Journal of Hospital Infection, 2006. **63**(1): p. 3.

109. Hetem, D.J. and Bonten, M.J., *Clinical relevance of mupirocin resistance in Staphylococcus aureus.* Journal of Hospital Infection, 2013. **85**(4): p. 249-56.
110. Fernandez, C., et al., *A double-blind, randomized, placebo-controlled clinical trial to evaluate the safety and efficacy of mupirocin calcium ointment for eliminating nasal carriage of Staphylococcus aureus among hospital personnel.* Journal of Antimicrobial Chemotherapy, 1995. **35**(3): p. 399-408.
111. Doebbeling, B.N., et al., *Long-term efficacy of intranasal mupirocin ointment. A prospective cohort study of Staphylococcus aureus carriage.* Archives of Internal Medicine, 1994. **154**(13): p. 1505-8.
112. Sangal, V., et al., *Impacts of a long-term programme of active surveillance and chlorhexidine baths on the clinical and molecular epidemiology of meticillin-resistant Staphylococcus aureus (MRSA) in an Intensive Care Unit in Scotland.* International Journal of Antimicrobial Agents, 2012. **40**(4): p. 323-31.
113. Currie, K., et al., *Cross-sectional survey of meticillin-resistant Staphylococcus aureus home-based decolonization practices in Scotland.* Journal of Hospital Infection, 2012. **80**(2): p. 140-3.
114. Department of Health and Department of Food & Rural affairs, *UK Five Year Antimicrobial Resistance Strategy, 2013 to 2018*, 2013: UK.
115. Davey, P., et al., *Time for action-Improving the design and reporting of behaviour change interventions for antimicrobial stewardship in hospitals: Early findings from a systematic review.* International Journal of Antimicrobial Agents, 2015. **45**(3): p. 203-12.
116. Eckmann, C., et al., *Antibiotic treatment patterns across Europe in patients with complicated skin and soft-tissue infections due to meticillin-resistant Staphylococcus aureus: a plea for implementation of early switch and early discharge criteria.* International Journal of Antimicrobial Agents, 2014. **44**(1): p. 56-64.
117. World Health Organization, *WHO Guidelines on Hand Hygiene in Health Care: a Summary*, 2009: Geneva.
118. Marimuthu, K., Pittet, D., and Harbarth, S., *The effect of improved hand hygiene on nosocomial MRSA control.* Antimicrobial Resistance and Infection Control, 2014. **3**(34): p. 2047-2994.
119. Lawes, T., et al., *Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis.* British Medical Journal Open, 2015. **5**(3): p. 2014-006596.
120. Borg, M.A., et al., *Prevention of meticillin-resistant Staphylococcus aureus bloodstream infections in European hospitals: moving beyond policies.* Journal of Hospital Infection, 2014. **5**(14): p. 00153-4.
121. Harbarth, S., *Control of endemic methicillin-resistant Staphylococcus aureus--recent advances and future challenges.* Clinical Infectious Diseases, 2006. **12**(12): p. 1154-62.
122. Dancer, S.J., *Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination.* Clinical Microbiology Reviews, 2014. **27**(4): p. 665-90.
123. Wolkewitz, M., et al., *Mortality associated with in-hospital bacteraemia caused by Staphylococcus aureus: a multistate analysis with follow-up*

beyond hospital discharge. Journal of Antimicrobial Chemotherapy, 2011. **66**(2): p. 381-6.
124. European Centre for Disease Prevention and Control, *Antimicrobial resistance surveillance in Europe 2013. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net),* 2014, ECDC: Stockholm.
125. WHO Collaborating Centre for Drug Statistics Methodology, *Guidelines for ATC classification and DDD assignment 2013 (http://www.whocc.no/filearchive/publications/1_2013guidelines.pdf),* 2012, World Health Organisation: Oslo.
126. Monnet, D.L., et al., *Antimicrobial drug use and methicillin-resistant Staphylococcus aureus, Aberdeen, 1996–2000.* Emerging Infectious Diseases, 2004. **10**(8).
127. Mahamat, A., et al., *Impact of infection control interventions and antibiotic use on hospital MRSA: a multivariate interrupted time-series analysis.* International Journal of Antimicrobial Agents, 2007. **30**(2): p. 169-176.
128. Aires de Sousa, M., et al., *Comparison of genetic backgrounds of methicillin-resistant and -susceptible Staphylococcus aureus isolates from Portuguese hospitals and the community.* Journal of Clinical Microbiology, 2005. **43**(10): p. 5150-7.
129. Feil, E.J., et al., *How Clonal Is Staphylococcus aureus?* Journal of Bacteriology, 2003. **185**(11): p. 3307-3316.
130. Vandenesch. F., et al., *Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence.* Emerging Infectious Diseases., 2003 **9**(8): p. 978-84.
131. Wyllie, D.H., et al., *Decline of meticillin-resistant Staphylococcus aureus in Oxfordshire hospitals is strain-specific and preceded infection-control intensification.* British Medical Journal Open, 2011. **1**(1).
132. Wyllie, D., Paul, J., and Crook, D., *Waves of trouble: MRSA strain dynamics and assessment of the impact of infection control.* Journal of Antimicrobial Chemotherapy, 2011. **66**(12): p. 2685-8.
133. Johnson, A.P., et al., *Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS).* Journal of Antimicrobial Chemotherapy, 2001. **48**(1): p. 143-144.
134. Ellington, M.J., et al., *Decline of EMRSA-16 amongst methicillin-resistant Staphylococcus aureus causing bacteraemias in the UK between 2001 and 2007.* Journal of Antimicrobial Chemotherapy, 2010. **65**(3): p. 446-8.
135. Rao, G.G., Kearns, A.M., and Edwards, G.F., *Decline and fall of epidemic meticillin-resistant Staphylococcus aureus-16.* Journal of Hospital Infection, 2011. **79**(3): p. 269-70.
136. Coia, J.E., et al., *Characterisation of methicillin-resistant Staphylococcus aureus by biotyping, immunoblotting and restriction enzyme fragmentation patterns.* Journal of Medical Microbiology, 1990. **31**(2): p. 125-132.
137. Oliveira, A.M. and Ramos M.C., *PCR-based ribotyping of Staphylococcus aureus.* Brazilian Journal of Medical and Biological Research, 2002. **35**: p. 175-180.

138. Kostman, J.R., et al., *A Universal Approach To Bacterial Molecular Epidemiology By Polymerase Chain Reaction Ribotyping.* Journal of Infectious Diseases, 1995. **171**(1): p. 204-208.
139. Harmsen, D., et al., *Typing of Methicillin-Resistant Staphylococcus aureus in a University Hospital Setting by Using Novel Software for spa Repeat Determination and Database Management.* Journal of Clinical Microbiology, 2003. **41**(12): p. 5442-5448.
140. Senna, J.P.M., et al., *Comparison of Pulsed-Field Gel Electrophoresis and PCR Analysis of Polymorphisms on the mec Hypervariable Region for Typing Methicillin-Resistant Staphylococcus aureus.* Journal of Clinical Microbiology, 2002. **40**(6): p. 2254-2256.
141. Koreen, L., et al., *spa Typing Method for Discriminating among Staphylococcus aureus Isolates: Implications for Use of a Single Marker To Detect Genetic Micro- and Macrovariation.* Journal of Clinical Microbiology, 2004. **42**(2): p. 792-799.
142. Anderson, A.W. and I. Schrijver, *Next Generation DNA Sequencing and the Future of Genomic Medicine.* Genes, 2010. **1**(1): p. 38-69.
143. Lindsay, J.A., *Evolution of Staphylococcus aureus and MRSA during outbreaks.* Infection, Genetics and Evolution, 2014. **21**: p. 548-53.
144. Price, J., et al., *The usefulness of whole genome sequencing in the management of Staphylococcus aureus infections.* Clinical Microbiology and Infection, 2013. **19**(9): p. 784-789.
145. Leopold, S.R., et al., *Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes.* Journal of Clinical Microbiology, 2014. **52**(7): p. 2365-70.
146. The Scottish Public Health Observatory (ScotPHO). *Population: estimates by NHS Board (Website: http://www.scotpho.org.uk/population-dynamics/population-estimates-and-projections/data/nhs-board-population-estimates)*. 2015 [Accessed August 2015].
147. Seaton, R.A., et al., *Economic evaluation of treatment for MRSA complicated skin and soft tissue infections in Glasgow hospitals.* European Journal Clinical Microbiology and Infectious Diseases, 2014. **33**(3): p. 305-11.
148. Dancer, S.J., et al., *Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study.* BMC Medicine, 2009. **7**(28): p. 1741-7015.
149. Dancer, S.J., *Control of transmission of infection in hospitals requires more than clean hands.* Infection Control and Hospital Epidemiology, 2010. **31**(9): p. 958-60.
150. Dancer, S.J., *Put your ties back on: scruffy doctors damage our reputation and indicate a decline in hygiene.* British Medical Journal, 2013. **13**(346).
151. Petti, S., Polimeni, A., and Dancer, S.J., *Effect of disposable barriers, disinfection, and cleaning on controlling methicillin-resistant Staphylococcus aureus environmental contamination.* American Journal of Infection Control, 2013. **41**(9): p. 836-40.
152. Mitchell, B.G., et al., *Controlling methicillin-resistant Staphylococcus aureus (MRSA) in a hospital and the role of hydrogen peroxide decontamination: an*

interrupted time series analysis. British Medical Journal Open, 2014. **4**(4): p. 2013-004522.
153. Health Protection Scotland (HPS). *The Staphylococcus aureus Bacteraemias Quarterly Report of Cumulative Data from all NHS Boards in Scotland, http://www.hps.scot.nhs.uk/pubs/Publication_Detail.aspx*. 2015.
154. R Core Team, *R: A Language and Environment for Statistical Computing*, 2015, R Foundation for Statistical Computing: Vienna, Austria.
155. Cosgrove, S.E., et al., *Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis.* Clinical Infectious Diseases, 2003. **36**(1): p. 53-9.
156. Lodise, T.P. and McKinnon, P.S., *Clinical and economic impact of methicillin resistance in patients with Staphylococcus aureus bacteremia.* Diagnostic Microbiology and Infectious Disease, 2005. **52**(2): p. 113-22.
157. Gibbons, C.L., et al., *Measuring underreporting and under-ascertainment in infectious disease datasets: a comparison of methods.* BMC Public Health, 2014. **14**(147): p. 1471-2458.
158. Johnson, A.P., *Methicillin-resistant Staphylococcus aureus: the European landscape.* Journal of Antimicrobial Chemotherapy, 2011. **66**(suppl 4): p. iv43-iv48.
159. Agha, M., *Epidemiology and Pathogenesis of C. difficile and MRSA in the Light of Current NHS Control Policies: A Policy review.* Annals of Medicine and Surgery, 2012. **1**: p. 39-43.
160. Thompson, D.S., Workman, R., and Strutt, M., *Decline in the rates of meticillin-resistant Staphylococcus aureus acquisition and bacteraemia in a general intensive care unit between 1996 and 2008.* Journal of Hospital Infection, 2009. **71**(4): p. 314-9.
161. Corey, G.R., *Staphylococcus aureus Bloodstream Infections: Definitions and Treatment.* Clinical Infectious Diseases, 2009. **48**(Supplement 4): p. S254-S259.
162. Filice, G.A., et al., *Excess costs and utilization associated with methicillin resistance for patients with Staphylococcus aureus infection.* Infection Control and Hospital Epidemiology, 2010. **31**(4): p. 365-73.
163. Abramson, M.A. and Sexton, D.J., *Nosocomial methicillin-resistant and methicillin-susceptible Staphylococcus aureus primary bacteremia: at what costs?* Infection Control and Hospital Epidemiology, 1999. **20**(6): p. 408-11.
164. Wyllie, D.H., Crook, D.W. and Peto, T.E.A., *Mortality after Staphylococcus aureus bacteraemia in two hospitals in Oxfordshire, 1997-2003: cohort study.* British Medical Journal, 2006. **333**(7562): p. 281.
165. Sokurenko, E.V., Gomulkiewicz, R., and Dykhuizen, D.E., *Source-sink dynamics of virulence evolution.* Nature Reviews Microbiology, 2006. **4**(7): p. 548-555.
166. Pulliam, H.R., *Sources, Sinks, and Population Regulation.* The American Naturalist, 1988. **132**(5): p. 652-661.
167. Lipsitch, M., Bergstrom, C.T., and Levin, B.R., *The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions.* Proceedings of the National Academy of Sciences, 2000. **97**(4): p. 1938-43.

168. Bonten, M.J., Austin, D.J., and Lipsitch, M., *Understanding the spread of antibiotic resistant pathogens in hospitals: mathematical models as tools for control.* Clinical Infectious Diseases, 2001. **33**(10): p. 1739-46.
169. Aldeyab, M.A., et al., *Modelling the impact of antibiotic use and infection control practices on the incidence of hospital-acquired methicillin-resistant Staphylococcus aureus: a time-series analysis.* Journal of Antimicrobial Chemotherapy, 2008. **62**(3): p. 593-600.
170. Dancer, S.J., et al., *MRSA acquisition in an intensive care unit.* American Journal of Infection Control, 2006. **34**(1): p. 10-7.
171. Lawes, T., Edwards, B., and Gould, I.M., *Are all Staphylococcus aureus equal?*: Lancet Infectious Diseases, 2015 Jul;15(7):762.
172. Dancer, S.J., *Importance of the environment in meticillin-resistant Staphylococcus aureus acquisition: the case for hospital cleaning.* Lancet Infectious Diseases, 2008. **8**(2): p. 101-13.
173. Woolhouse, M.E.J. and Ward, M.J., *Sources of Antimicrobial Resistance.* Science, 2013. **341**(6153): p. 1460-1461.
174. Knight, G.M., Budd, E.L., and Lindsay, J.A., *Large mobile genetic elements carrying resistance genes that do not confer a fitness burden in healthcare-associated meticillin-resistant Staphylococcus aureus.* Microbiology, 2013. **159**(Pt 8): p. 1661-72.
175. Mather, A.E., et al., *An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations.* Proceedings of the Royal Society B: Biological Sciences, 2011.
176. Holden, M.T., et al., *A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant Staphylococcus aureus pandemic.* Genome Research, 2013. **23**(4): p. 653-64.
177. Malachowa, N. and DeLeo, F., *Mobile genetic elements of Staphylococcus aureus.* Cellular and Molecular Life Sciences, 2010. **67**(18): p. 3057-3071.
178. Patel, J.B., Gorwitz, R.J., and Jernigan, J.A., *Mupirocin Resistance.* Clinical Infectious Diseases, 2009. **49**(6): p. 935-941.
179. Costelloe, C., et al., *Effect of antibiotic prescribing in primary care on meticillin-resistant Staphylococcus aureus carriage in community-resident adults: a controlled observational study.* International Journal of Antimicrobial Agents, 2012. **39**(2): p. 135-41.
180. Baggett, H.C., et al., *Community-onset methicillin-resistant Staphylococcus aureus associated with antibiotic use and the cytotoxin Panton-Valentine leukocidin during a furunculosis outbreak in rural Alaska.* Journal of Infectious Diseases, 2004. **189**(9): p. 1565-73.
181. Lo, W.-T., et al., *Nasal carriage of a single clone of community-acquired methicillin-resistant Staphylococcus aureus among kindergarten attendees in northern Taiwan.* BMC Infectious Diseases, 2007. **7**(1): p. 51.
182. Campbell, K.M., et al., *Risk factors for community-associated methicillin-resistant Staphylococcus aureus infections in an outbreak of disease among military trainees in San Diego, California, in 2002.* Journal of Clinical Microbiology, 2004. **42**(9): p. 4050-3.
183. Paganini, H., et al., *[Community-acquired methicillin-resistant Staphylococcus aureus infections in children: multicenter trial].* Archivos argentinos de pediatría, 2008. **106**(5): p. 397-403.

184. Costelloe, C., et al., *Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis.* British Medical Journal, 2010. **340**.
185. WHO Collaborating Centre for Drug Statistics Methodology, *Guidelines for ATC classification and DDD assignment 2013 (http://www.whocc.no/filearchive/publications/1_2013guidelines.pdf),*, 2012, World Health Organisation: Oslo.
186. Ringner, M., *What is principal component analysis?* Nature Biotechnology, 2008. **26**(3): p. 303-304.
187. Standing Medical Advisory Commitee, *The path of least resistance*, 1998, Department of Health: London, UK.
188. Kuyvenhoven, M.M., van Balen, F.A., and Verheij, T.J., *Outpatient antibiotic prescriptions from 1992 to 2001 in the Netherlands.* Journal of Antimicrobial Chemotherapy, 2003. **52**(4): p. 675-8.
189. Hay, A.D., et al., *The relationship between primary care antibiotic prescribing and bacterial resistance in adults in the community: a controlled observational study using individual patient data.* Journal of Antimicrobial Chemotherapy, 2005. **56**(1): p. 146-153.
190. Information Services Scotland (ISD). *NHS Scotland financial cost books, http://www.isdscotland.org/Health-Topics/Finance/Costs/ (Accessed 2013)*.
191. Ciccolini, M., et al., *Efficient surveillance for healthcare-associated infections spreading between hospitals.* Proceedings of the National Academy of Sciences, 2014. **111**(6): p. 2271-2276.
192. Neish, B., Gillespie, T., and Liddell, K.G., *Improving surveillance of MRSA bacteraemia: Scottish data prompt query of significance of MRSA isolated from blood in acute admissions*: British Medicial Journal. 2005 Nov 12;331(7525):1143.
193. Huang, S.S., et al., *Quantifying interhospital patient sharing as a mechanism for infectious disease spread.* Infection Control and Hospital Epidemiology, 2010. **31**(11): p. 1160-9.
194. Robotham, J.V., et al., *Meticillin-resistant Staphylococcus aureus (MRSA) in hospitals and the community: model predictions based on the UK situation.* Journal of Hospital Infection, 2007. **2**: p. 93-9.
195. Hudson, L.O., et al., *Diversity of methicillin-resistant Staphylococcus aureus strains isolated from residents of 26 nursing homes in Orange County, California.* Journal of Clinical Microbiology, 2013. **51**(11): p. 3788-95.
196. McKinnell, J.A., et al., *A systematic literature review and meta-analysis of factors associated with methicillin-resistant Staphylococcus aureus colonization at time of hospital or intensive care unit admission.* Infection Control and Hospital Epidemiology, 2013. **34**(10): p. 1077-86.
197. Jans, B., et al., *Epidemiology of multidrug-resistant microorganisms among nursing home residents in Belgium.* PLoS ONE, 2013. **8**(5).
198. Lee, B.Y., et al., *The importance of nursing homes in the spread of methicillin-resistant Staphylococcus aureus (MRSA) among hospitals.* Medical Care, 2013. **51**(3): p. 205-15.
199. Dancer, S.J., White, L., and Robertson, C., *Monitoring environmental cleanliness on two surgical wards.* International Journal of Environmental Health Research, 2008. **18**(5): p. 357-64.

200. McMurdo, M.E.T. and Witham M.D., *Unnecessary ward moves.* Age and Ageing, 2013.
201. Ciccolini, M., et al., *Infection prevention in a connected world: The case for a regional approach.* International Journal of Medical Microbiology, 2013. **303**(6–7): p. 380-387.