

Submitted for the degree of Doctor of Philosophy

**Modelling the relationship between
antibiotic consumption and resistance in
heterogeneous populations**

Tamsin Callie Mortimer Dewé

Department of Infectious Disease Epidemiology
Imperial College London

Supervised by:

Dr. Nicholas Croucher

Prof. Christophe Fraser

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Statement of Originality

I declare that the work presented here is my own, except where otherwise referenced.

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Abstract

Antibiotic resistance occurs when bacteria can withstand treatment with antibiotics designed to kill them or inhibit their growth, and has been identified as a major threat to global health. Mathematical and statistical models can help us understand the epidemiology and evolution of resistance, but there are important features that are not well captured by existing models. In particular, the coexistence of antibiotic-resistant and sensitive bacterial strains at intermediate frequencies is persistently observed across a wide range of bacterial and host populations, resistance mechanisms, and rates of antibiotic treatment.

In this research, an agent-based model of multi-strain bacterial populations was developed, with a novel competition mechanism that enables coexistence of resistant and sensitive bacteria across a wide range of parameters. Results from this model also demonstrated the influence of bacterial population structure, in addition to antibiotic consumption, in shaping resistance in heterogeneous populations. The model was then used to examine the influence of horizontal gene transfer on resistance dynamics, and determine the conditions for sustaining coexistence when resistance is mobile. Likelihood-free inference was used to estimate model parameters, which enabled simulations that mirrored the heterogeneity of an observed bacterial population.

As a counterpart to this model of resistance carriage in commensal organisms, a statistical model of resistance in clinical isolates was compared to antibiotic consumption data from veterinary and medical settings across Europe. Results from this analyses supported the importance of antibiotic consumption as a driver of resistance in organisms causing disease; however, they also showed that this relationship is affected by other factors. Taken together, the results of this research underline the importance of a One Health approach, and good antibiotic stewardship, in controlling antibiotic resistance. However, they also imply that more specific interventions are not necessarily transferable to different contexts.

Abbreviations

ABC	Approximate Bayesian computation
ABM	Agent-based model
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
AIC	Akaike information criterion
BIC	Bayesian information criterion
BOLFI	Bayesian optimisation for likelihood-free inference
BSI	Bloodstream infection
CBP	Clinical breakpoint
CC	Clonal complex
CI	Confidence interval
DDD	Defined daily dose
ECOFF	Epidemiological cut-off value
ESBL	Extended-spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GIT	Gastrointestinal tract
GLMM	Generalised linear mixed model
HGT	Horizontal gene transfer
HP-CIA	Highest-priority critically important antibiotic [1]
HPD	Highest posterior density
IBM	Individual-based model
ICE	Integrative and conjugative element
IPC	Infection prevention and control
LA-MRSA	Livestock-associated methicillin-resistant <i>Staphylococcus aureus</i>
LFI	Likelihood-free inference
LMIC	Low and middle-income country
MCMC	Markov chain Monte Carlo
MDR	Multi-drug resistant
MGE	Mobile genetic element
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NFDS	Negative frequency-dependent selection
NP	Natural product
OR	Odds ratio
PCU	Population-corrected unit
PNSP	Penicillin non-susceptible <i>Streptococcus pneumoniae</i>
SCC	Staphylococcal cassette chromosome
SMC	Sequential Monte Carlo
SMC-ABC	Sequential Monte Carlo approximate Bayesian computation
SS	Summary statistic
TMPS	Trimethoprim/sulfamethoxazole
UI	Uncertainty interval
UTI	Urinary tract infection
VRE	Vancomycin-resistant enterococci
VSE	Vancomycin-sensitive enterococci
WGS	Whole-genome sequencing

Table of Antibiotic Classes

Antibiotic class	Example(s)	Molecular Target
Aminoglycosides	Gentamicin Kanamycin Spectinomycin Streptomycin	Protein synthesis: 30S ribosomal subunit
Amphenicols	Chloramphenicol	Protein synthesis: 50S ribosomal subunit
Azoles	Metronidazole	DNA replication and repair
Beta-lactams: penicillins	Amoxicillin Ampicillin Methicillin Piperacillin	Cell wall turnover: penicillin-binding proteins
Beta-lactams: cephalosporins	Ceftazidime Ceftiofur Ceftazidime	Cell wall turnover: penicillin-binding proteins
Beta-lactams: carbapenems	Meropenem	Cell wall turnover: penicillin-binding proteins
Glycopeptides	Vancomycin	Cell wall turnover: peptide precursor units
Lincosamides	Clindamycin	Protein synthesis: 50S ribosomal subunit
Macrolides	Clarithromycin Erythromycin	Protein synthesis: 50S ribosomal subunit
Mupirocin	Mupirocin	Protein synthesis: isoleucyl-tRNA synthetase
Oxazolidinones	Linezolid	Protein synthesis: 50S ribosomal subunit
Phosphonates	Fosfomycin	Cell wall turnover: peptidoglycan precursors
Pleuromutilins	Tiamulin	Protein synthesis: 50S ribosomal subunit
Polymyxins	Colistin	Cell wall turnover: cell membrane disruption
Pyrimidines	Trimethoprim	Folate synthesis: inhibition of dihydrofolate reductase
Quinolones	Ciprofloxacin Nalidixic acid	DNA replication: DNA gyrase and topoisomerase IV
Streptogramins	Virginiamycin	Protein synthesis: 50S ribosomal subunit
Sulfonamides	Sulfamethoxazole	Folate synthesis: inhibition of dihydropteroate synthetase
Tetracyclines	Doxycycline Tetracycline	Protein synthesis: 30S ribosomal subunit
Tetracycline derivatives: glycylcyclines	Tigecycline	Protein synthesis: 30S ribosomal subunit

Table 1: **Major antibiotic classes, including their mechanisms of action, and examples of specific antibiotics.** Adapted from multiple sources [2–5].

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Chapter 1

Introduction

Antimicrobial resistance (AMR) is the ability of microorganisms to resist treatment with antimicrobials, which include antibiotics, antivirals, antiparasitics, and antifungals. Resistance means that micro-organisms no longer respond to treatment with these substances [6]. AMR has been identified as a leading threat to global human and animal health by the World Health Organisation (WHO) [7, 8], the World Organisation for Animal Health (WOAH) [9], the Food and Agriculture Organisation of the United Nations (FAO) [10], and the United Nations Environment Programme (UNEP) [11] - together, the Quadripartite - and the World Bank [12]. It is also on the UK's National Risk Register of the most serious risks facing the UK [13].

Antibiotic resistance occurs when bacteria develop the ability to resist treatment with antibiotics designed to kill them or inhibit their growth. As it is the exclusive focus of this thesis, I will use the term 'AMR' interchangeably with 'antibiotic resistance' throughout.

In this Introduction, I will explain the history of antibiotics, how they work, and why they are important. I will also outline the development and mechanisms of antibiotic resistance, and explain why it is a global health threat. Key features of bacterial ecology and the epidemiology and evolutionary drivers of resistance will be detailed.

I will then explain how mathematical and statistical models have helped further our understanding of AMR, and the limitations of these approaches, before outlining the work presented in this thesis.

1.1 Antibiotics

1.1.1 Natural origins of antibiotics

Antibiotics and other bioactive compounds (natural products, or NPs) are naturally produced by more than 25,000 distinct microbial species, particularly soil-dwelling bacteria and fungi [5, 14–16]. These NPs are chemically diverse, with a wide range of biological activities [17]. Antibiotics enable direct suppression of neighbouring microbes, but also have other functions at sub-inhibitory concentrations, for example as signalling molecules or mediators of interactions with host organisms [5, 15, 18].

1.1.2 History of antibiotic discovery

1.1.2.1 Ancient use

It was Pasteur who first proposed that microbes could kill other microbes [19], but antibiotic treatments have been used since ancient times. Substances containing NP-producing microorganisms, such as soil and mouldy bread, were recognised as anti-infective therapies in Ancient Egypt [5]. The antibiotic tetracycline, an NP of the actinomycetes genus *Streptomyces* [20], has been detected in human bones from late-Roman Sudanese Nubia [21, 22], although it is not known whether tetracycline exposure was deliberate or a by-product of these people’s dietary regime.

1.1.2.2 Modern discovery

The first antibiotic to be used in hospitals was pyocyanase, derived from the bacterium *Pseudomonas aeruginosa* by Emmerich and Löw in the late 19th century [5, 23, 24]. However, it was clinically unreliable, and systematic searches of synthetic compounds with antibacterial activity were pioneered by Ehrlich, resulting in the discovery of salvarsan [25] and in 1935, Prontosil [26]. Prontosil belonged to the sulfonamide class and was the first antibiotic drug on the commercial market [24].

The sulfonamides were “the first truly effective, broad spectrum antimicrobials in clinical use” [5], and are still manufactured. They were introduced into veterinary medicine in the 1930s [27] and are one of the main antibiotic classes used today [28]. However, in

human medicine, they have been superseded by penicillin. Antibiotic activity arising from *Penicillium* mould was first observed by Fleming in 1928 [29], although penicillin itself was only purified in 1940 by Florey and Chain [30]. After further work enabling semi-synthetic production of penicillin in large quantities [31–34], it entered the market in 1945, becoming the first NP to be widely used clinically [24], and was established in veterinary medicine by 1950 [27].

1.1.2.3 The golden age

Penicillin’s discovery, and subsequent commercial manufacture, triggered a ‘golden age’ of antibiotic discovery that lasted until the 1960s. Antibiotic classes introduced to clinical practice during this era include aminoglycosides, amphenicols, cephalosporins, glycopeptides, macrolides, polymyxins, quinolones, and tetracyclines [5] (see Table 1 in the preface). The important beta-lactam methicillin, then known as ‘celbenin’, was released onto the UK market in 1959 [35].

The golden age was initiated by Waksman, who was the first to use the term ‘antibiotic’, and whose team discovered streptomycin, the first antibiotic effective against Gram-negative bacteria in 1943 [36]. It was Waksman who pioneered systematic surveys of NPs for antibiotic activity [5, 15] and who first identified the actinomycetes as prolific producers of antibiotics [37–39]. Fifty-five percent of the antibiotics discovered between 1945 and 1978 arose from the *Streptomyces* [40]. Even today, 64% of known NP antibiotic classes arise from the actinomycetes [5].

1.1.2.4 Decline

Antibiotic discovery rapidly slowed in the 1970s, with few new antibiotic classes being identified since then (although key discoveries include the carbapenems and oxazolidinones) [5, 24]. Systematic surveys of NPs generated from (mostly actinomycete) fermentation assays repeatedly reidentified compounds that were already known [5, 17]. Subsequently, pharmaceutical companies divested from NP discovery and diverted resources to high-throughput screening programmes for synthetic antibiotics; these were also unsuccessful [41, 42]. The majority of new antibiotics identified since then, including those in current clinical trials, are therefore derivatives of existing NPs or synthetic classes [5, 24, 43].

The O'Neill Review was a landmark AMR report, commissioned by the UK government, which sought to analyse the burden of AMR and propose interventions [44]. Boosting early drug discovery was recommended as one of the solutions to containing AMR [45], and there is certainly potential for under-explored environments and organisms to yield new NPs [5]. However, disincentives for pharmaceutical investment include barriers to new product authorisations, cheap market prices, and other economic factors [16, 46]. There are also biological limitations: there are a limited range of prokaryotic cellular processes that can be targeted effectively, as outlined below, and the speed with which bacteria can adapt makes it likely that the evolution of resistance will continue to outpace pharmaceutical development.

1.1.3 Mechanisms of antibiotic action

Bacteria are prokaryotes (single-celled microorganisms that lack a nucleus or other membrane-bound organelles), whereas host organisms such as animals or plants are eukaryotes (their cells have a nucleus). There is sufficient difference between the cellular machinery of prokaryotic and eukaryotic cells for antibiotics to act selectively on bacteria rather than the host organism.

Antibiotics work by targeting cellular processes that are essential to the survival of bacteria (bactericidal antibiotics), or that enable replication (bacteriostatic antibiotics). Bactericidal and bacteriostatic antibiotics antagonise each other [47, 48]. This has been shown to be at least partly due to bacteriostatic antibiotics reducing cellular respiration rates, which blocks bactericidal activity [48]. Bactericidal antibiotics trigger a common cellular pathway [49]. Beta-lactams, aminoglycosides (excluding spectinomycin) and quinolones have all been shown to stimulate production of free hydroxyl radicals, which cause lethal damage, whereas bacteriostatic antibiotics do not [49].

The three cellular processes considered 'proven targets' for antibiotics are bacterial cell wall turnover, protein synthesis (including RNA activity), and DNA replication and repair [2, 24]. A summary of the major antibiotic classes and their mechanisms of action is provided in Table 1 in the preface.

1.1.3.1 Cell wall turnover

Bacteria have a peptidoglycan cell wall that provides structure and integrity, protecting the cell from osmotic lysis [50, 51]. The cell wall is absent from eukaryotic cells, and is regularly recycled [52], making it an obvious target for antibiotic activity. The cell wall is a peptidoglycan layer that is assembled by enzymatic linkage of peptide strands. Antibiotics targeting cell wall turnover either target the enzymes facilitating this linkage (e.g. beta-lactam antibiotics), or tie up the peptide substrate, making it unavailable (e.g. glycopeptides) [2].

1.1.3.2 Protein synthesis

Protein synthesis is conducted by ribosomes. While both prokaryotic and eukaryotic cells utilise ribosomal machinery, there are sufficient differences between the two for the bacterial ribosome to be an effective target for antibiotic activity [2]. There are multiple different molecular steps in the machinery of protein synthesis; thus, drugs that interfere with these processes are amongst the broadest classes of antibiotics [49]. They can be further categorised depending on the ribosomal component that they affect: inhibitors of the 50S subunit (e.g. macrolides) or inhibitors of the 30S subunit (e.g. tetracyclines). Most antibiotics inhibiting ribosomal function are bacteriostatic, apart from the aminoglycosides (with the exception of spectinomycin), and most commonly block initiation or elongation of peptide chains [49].

1.1.3.3 DNA replication and repair

Type II topoisomerase enzymes untangle coils of DNA arising from DNA replication, by transiently cleaving and re-ligating DNA strands [2]. The synthetic class of quinolone antibiotics bind to type II topoisomerases such as DNA gyrase, and prevent re-ligation of the broken DNA strands. The accumulation of broken double-stranded DNA within the cell eventually triggers cell death [2].

1.1.4 Use of antibiotics

Antibiotics are widely used in both human and animal health, for the treatment of bacterial diseases as well as prophylactically. Antibiotics are also used as agricultural growth promoters in some parts of the world [53].

1.1.4.1 Therapeutic use

In both medical and veterinary clinical practice, antibiotic administration is ideally informed by positive culture to identify a bacterial pathogen, and antibiotic susceptibility testing (AST) to assess resistance. However, the turnaround times and expense of diagnostic tests means that antibiotic therapy is often commenced without the benefit of AST [54].

Other factors governing prescribing choices include the site of infection, the immunocompetence of the patient, and any side effects. For example, effective treatment of bacterial meningitis is dependent on the ability of antibiotics to penetrate the blood-brain barrier and reach sufficiently high concentrations within the cerebrospinal fluid [55]. As another example, tetracyclines are usually contraindicated in young children and animals, due to permanent effects on developing bones and teeth [56].

1.1.4.2 Prophylactic use

Antibiotics are also used prophylactically (i.e., in the absence of clinical signs) in individual humans and animals, including prior to surgery or immunosuppressive therapy. They may also be used prophylactically in groups of animals, for example during periods with higher risk of infectious disease (e.g. weaning or transport) [57]. The European Union banned prophylactic use of antibiotics in groups of animals in 2022; in individual animals, they are only to be used under exceptional circumstances [58].

In the farming context, there is also metaphylaxis, which is midway between group prophylaxis and therapeutic use. Metaphylaxis refers to treatment of a group of animals where some of them are showing signs of clinical disease [57, 59]. Since 2022, metaphylactic use of antibiotics in farmed animals in the EU has only been permitted when the risk of infection within the group is high, and alternatives are not available [58].

1.1.4.3 Antibiotics as growth promoters

The growth-promoting effects of antibiotics in animals and humans is well-documented [60–62]. In some countries, antibiotics are used routinely as growth promoters in animals [53]. Use of antibiotics in this way has been banned in multiple countries, including in Sweden since 1986 [63], the European Union (EU) and the UK since 2006 [64], and in the USA since 2017 [65].

1.1.5 Impact of antibiotics

Antibiotics are one of the most significant discoveries in the history of modern medicine [4, 5, 46, 66, 67]. They have been extensively used to treat bacterial infections since the mid-20th century, with drastic effect. In the USA, crude mortality rates for infectious diseases (largely bacterial) declined by 8.2% per year: from 797 deaths per 100,000 people in 1937 to 75 deaths per 100,000 in 1952 [68]. This reduction coincided with the introduction of sulfonamides, penicillin, and streptomycin onto the open market. Mortality rates attributable to pneumonia caused by *Streptococcus pneumoniae*, a human commensal and opportunist pathogen, declined from 30.5% to 12.3% when treated with sulfonamides, and to 5.1% when treated with more effective antibiotics such as penicillin [69].

Lack of access to antibiotics still costs lives [66]: over a million children die each year of untreated pneumonia and sepsis [70], which are commonly caused by bacteria; pneumonia, diarrhoea and tuberculosis (caused by *Mycobacterium tuberculosis*) were amongst the top 20 causes of death globally in 2010 [71]. The impact of antibiotics on animal health is less well quantified, however, they are an essential tool in the treatment of disease in animals.

1.2 Antibiotic resistance (AMR)

1.2.1 Natural origins of AMR

The ability of bacteria to resist the activity of antibiotic NPs, like the NPs themselves, is also ancient. Metagenomic analysis of actinomycete DNA extracted from 30,000 year old permafrost sediments detected well-characterised genes encoding resistance to beta-lactam (*bla*), tetracycline (*tetM*), and glycopeptide (*vanX*) antibiotics [72]. This finding

is supported by earlier phylogenetic analyses showing that resistance genes, including *tet* and *van*, evolved well before the antibiotic era [73, 74]. Contemporary soil-dwelling *Streptomyces* show an enormous diversity of resistance: in one study, 480 different strains were identified, all multi-drug resistant (MDR), with almost 200 unique resistance profiles [75]. This diversity is perhaps unsurprising, given the vast range of NPs produced in natural environments.

Production of, and resistance to, NPs is not confined to soil: a recent paper showed that a methicillin resistance gene (*mecC*) evolved in *Staphylococcus aureus* bacteria on European hedgehogs in the 19th century [76], probably in response to NPs produced by the dermatophyte *Trichophyton erinacei*.

1.2.2 History of AMR discovery

Although AMR is not a new phenomenon, its emergence in pathogenic (or potentially pathogenic) bacteria has accelerated with the invention and widespread adoption of antibiotics. Clinical resistance to most antibiotics used in human medicine has been detected within a few years of their release onto the commercial market [5].

Non-responsiveness to salvarsan treatment was first reported by Silberstein in 1929, who also noted an increasing proportion of treatment failures in the 1920s compared to the 1910s [77]. In Fleming’s landmark paper on the discovery of penicillin, he described its inhibitory effects on growth of staphylococcal and streptococcal bacteria, amongst others [29]. However, he also observed that growth of other bacteria, including *Escherichia coli* and *Haemophilus influenzae*, appeared unaffected [29]. This finding was later confirmed by Abraham & Chain [78]. Contemporaneously with Abraham & Chain’s work, resistance of *S. pneumoniae* and other organisms to sulfonamides was being reported [79–81].

Methicillin was developed in response to penicillin resistance in staphylococci [82]. However, resistance to this new antibiotic was detected by Patricia Jevons within a year of its release onto the UK market [83]. The UK’s *Staphylococcus* Reference Laboratory screened 5,440 *S. aureus* isolates, largely originating from clinical samples in 1960 [83]. Of these, 3 were resistant to methicillin, and all originated from the same hospital, indicating in-hospital transmission. Later analysis using Bayesian phylogenetic reconstruction

showed that the *SCCmec* element carrying the *mecA* gene, which confers methicillin resistance, emerged in UK *S. aureus* in the 1940s - well before the release of this drug onto the open market [35]. This implies that selection for this resistance was driven by clinical use of other beta-lactam antibiotics, such as penicillins.

Resistance to vancomycin, a glycopeptide, was detected in *Enterococcus faecium* isolated from hospital patients in 1986 [84, 85]. Unusually, this was some decades since its discovery and commercial release; however, due to concerns about toxicity, and the availability of alternative treatment options, it was little-used in clinical practice before the 1980s [86].

1.2.3 Mechanisms of AMR

Antibiotic resistance works via three main routes: minimising the concentration of antibiotic within the bacterial cell; modifying the antibiotic's target molecule(s), so that antibiotic binding is reduced or prevented; and modifying the antibiotic itself, so that it becomes inactive. These mechanisms have been reviewed at length by Wright, Davies & Davies, and Blair, amongst others [4, 46, 87]; what follows is an overview of some key examples.

1.2.3.1 Minimising antibiotic concentration within the cell

The concentration of antibiotic within the bacterial cell can be minimised in two ways: by reducing the entry of antibiotics into the cell in the first place; or by accelerating their exit.

Reducing entry The bacterial cell wall acts as a barrier to penetration by molecules including antibiotics. Some bacteria have a thick outer peptidoglycan layer around their cell membrane, which provides additional structural integrity. These bacteria are known as Gram positives, due to their reaction to the cellular stain of the same name, and include *Enterococcus*, *Staphylococcus*, and *Streptococcus* genera. Gram-negative bacteria such as *E. coli* and *Salmonella* species have a thinner peptidoglycan layer and a second, outer, cell membrane. This outer membrane acts as a fine molecular sieve, meaning that larger antibiotics, such as vancomycin, can't access the cell wall [87, 88].

There are other ways for molecules to enter Gram-negative cells. Specific proteins called porins are embedded within the outer membrane, acting as channels from the outside to the inside of the cell [87]. In the Enterobacterales, a taxonomic order that includes *Escherichia*, *Klebsiella*, and *Salmonella* species, these channels are non-specific. They restrict entry based on molecular qualities such as size, and can be down-regulated or replaced with more selective channels [87]. This is a well-recognised type of intrinsic resistance [88, 89], but can also be an acquired mechanism, for example in carbapenem resistance [90].

Accelerating exit Once antibiotics have entered the bacterial cell, they can be removed through efflux pumps. Some pumps are highly specific, whereas others have a broad spectrum of activity against multiple antibiotic classes [87]. These MDR efflux pumps have been found in all bacteria [87]; the best characterised are the resistance nodulation division family in Gram-negative species, which when overexpressed, export a wide range of antibiotics [91]. Efflux pumps are another intrinsic mechanism of resistance in Gram-negative bacteria; examples include tetracycline resistance in *P. aeruginosa* [92], and macrolide resistance in *E. coli* [93]. Increased expression of efflux pumps can also occur due to mutations in regulatory pathways [87], including in MDR *E. coli* [94]. All bacteria carry genes encoding these pumps on their chromosomes; however, some genes have been mobilised onto MGEs [87].

1.2.3.2 Modifying antibiotic target(s)

Resistance can also occur through modification of the target molecule(s) within bacterial cells, which make them insensitive to antibiotic action. These modifications can arise from changes to the genome encoding the target itself, or by post-translational modification [24, 87].

Modifying the genome Examples of antibiotics modifying the genome include mutation in the chromosomal *gyrA* gene, which confers structural changes to the gyrase target of fluoroquinolone antibiotics [87, 95, 96]. In *S. pneumoniae*, modification of existing *pbp* genes through interspecies recombination generates novel penicillin-binding proteins that

are still functional but have reduced affinity for penicillin [97].

Modifying after translation Examples of post-translational modification include the addition of chemical groups interfering with antibiotic binding. The *erm*-mediated methylation of 16S ribosomal RNA means that macrolides, lincosamides, and streptogramins cannot bind to their target [87]. The majority of resistance to tetracyclines in Gram-positive pathogens is induced by *tetM*, which encodes ribosomal protection proteins that overlap the tetracycline binding site [98].

1.2.3.3 Modifying the antibiotic itself

The third main route by which antibiotic resistance develops is through modification of the antibiotic itself. Enzymatic hydrolysis of antibiotic compounds is a major contributor, and affects antibiotic classes including aminoglycosides, amphenicols, and macrolides [87]. Beta-lactam antibiotics are inactivated by beta-lactamase enzymes [78], which form the primary resistance mechanism to these antibiotics in Gram-negative bacteria [29, 99]. Beta-lactamases have varying degrees of activity, and include the extended-spectrum beta-lactamases (ESBLs), which degrade cephalosporins as well as penicillins [99, 100].

Alternatively, antibiotic molecules can be altered by the addition of chemical groups, which prevents the drug from binding to its protein target. Due to their size and chemical structure, aminoglycoside antibiotics are particularly susceptible to modification of this kind (e.g. through phosphorylation or acetylation), which results in high levels of resistance [24, 87].

1.2.4 Measuring AMR

Antibiotic resistance can be measured phenotypically, by assessing bacterial growth in the presence of antibiotics, or genotypically, by identifying known resistance genes through whole genome sequencing (WGS) and other molecular methods. The predominant phenotypic techniques for antimicrobial susceptibility testing (AST) used in Europe are broth microdilution and disk diffusion [101, 102]. Results can be interpreted using clinical breakpoints (CBPs) or epidemiological cut-off values (ECOFFs) to distinguish resistant organisms.

1.2.4.1 Broth microdilution

Broth microdilution involves inoculation of bacteria into serial, two-fold dilutions of a specific antibiotic [103, 104]. Bacterial growth within each well is assessed optically. Resistance is measured by the minimum inhibitory concentration (MIC): the lowest concentration of antibiotic that prevents bacterial growth [104]. This is considered accurate within one two-fold dilution [103].

1.2.4.2 Disk diffusion

Disk diffusion tests involve inoculation of bacteria onto agar plates, onto which are placed paper disks containing standardised concentrations of antibiotics [103]. A zone of inhibited bacterial growth occurs around each disk, the size of which is dependent on the rate at which the antibiotic diffuses through the agar, and the susceptibility of the isolate [103]. Resistance is measured by the diameter of the zone of inhibited bacterial growth, to the nearest millimetre.

1.2.4.3 Clinical breakpoints (CBPs)

Bacterial isolates are categorised as susceptible or resistant according to CBPs established by expert sources. The MIC value or inhibitory zone diameter generated from broth microdilution or disk diffusion, respectively, is compared against published breakpoint tables for the two different methods. These breakpoints are determined for individual combinations of bacteria and antibiotics and are reviewed and updated each year, based on microbiological and pharmacological knowledge and clinical considerations [105].

In human medicine, CBPs published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) are used to inform antibiotic prescribing choices for a given patient and infection. In Europe, separate CBPs are published to indicate the thresholds for ‘susceptible, standard dosing regimen’ (‘S’) and ‘resistant’ (‘R’) [106]. Isolates with MIC values less than or equal to (for broth microdilution), or with inhibitory zone diameters greater than or equal to (for disk diffusion), the published ‘S’ CBP are considered susceptible [107]. For broth microdilution and disk diffusion techniques, isolates with MIC values greater than, or in-

inhibitory zone diameters less than, respectively, the ‘R’ CBP are considered resistant [107]. Isolates with AST results in between these two values are categorised as ‘I’ for ‘susceptible, increased exposure’ [107], indicating that therapeutic success is likely if exposure of the organism to the antibiotic is increased [106].

In veterinary medicine, veterinary CBPs are produced by CLSI and the Antibiogram Committee of the French Society of Microbiology (CA-SFM); where these are not established, human CBPs can be used [108]. Unlike in human medicine, no single scheme has published methods and CBPs for all relevant combinations of bacteria and antibiotics [109]. This is an area under active development by the Veterinary Committee on AST (VetCAST).

1.2.4.4 Epidemiological cut-off values (ECOFFs)

In both people and animals, CBPs are used to assess AMR in the clinical context (i.e. to inform treatment options). For surveillance purposes, a different method of interpreting AST results can be used in addition to CBPs. ECOFFs are the threshold for denoting ‘wild-type’ or fully susceptible isolates [110] and are determined from distributions of MIC results for a given combination of bacterial species and antibiotic [111]. This method allows for the identification of reduced susceptibility below the point of treatment failure (often corresponding with the combined ‘I’ and ‘R’ categories assessed using CBPs) [102] and is a more sensitive way to detect emerging resistance within bacterial populations. ECOFFs are used to report AMR in animals and food in the UK [108, 112], and in animals, food, and humans across Europe [102].

1.2.5 Burden of AMR

The burden of AMR is affected by both prevalence and clinical impact.

1.2.5.1 Prevalence in human health

Current situation Established surveillance programmes for AMR in humans focus on clinical isolates from patients [113–115]. The Global Antimicrobial Resistance and Use Surveillance System (GLASS) has been collecting data from human healthcare systems internationally since 2016, and has now enrolled 114 countries, territories, or areas [114].

In those countries reporting clinical AST results from human patients, high levels (above 50%) of resistance were observed in bacteria causing bloodstream infections (BSIs) [114]. However, there are considerable limitations to comparing AST results internationally, due to substantial differences in healthcare systems across the world, particularly in terms of diagnosis, testing, and reporting coverage [114]. In countries where routine AMR surveillance is less well-developed, AST data can be sparse and/or tends to be biased towards complex infections, including treatment failures, which results in resistance being over-estimated [114]. For example, the median rate of third-generation cephalosporin-resistant *E. coli* causing BSIs in 76 reporting countries is 41.8%; when restricted to 19 countries with high testing coverage, the median is 10.6% [114].

In the UK, AMR in *E. coli* BSIs is reported voluntarily, although coverage was 84% in 2021 [116]. The percentage of bloodstream isolates reported resistant to amoxicillin/clavulanic-acid (a beta-lactam antibiotic and a beta-lactamase inhibitor, respectively, administered concurrently) in 2021 was 41.2%; to the highest-priority critically important antibiotic [1] (HP-CIA) third-generation cephalosporins, it was 13.5% [115]. The percentage of bloodstream isolates reported resistant to another HP-CIA, ciprofloxacin, was 17.2% in 2021 [115].

In the EU, monitoring of AMR in the major foodborne pathogens *Campylobacter* and *Salmonella* spp. has been harmonised since 2014 [102]. Resistance in non-typhoidal *Salmonella* isolated from human cases in 2019-2020 was high for ampicillin (29.8%), sulfonamides (30.1%), and tetracyclines (31.2%) [102]. Of the HP-CIAs, resistance to ciprofloxacin was 14.1%, and 0.8% to each of the third-generation cephalosporins cefotaxime and ceftazidime [102]. Resistance to tetracycline and to ciprofloxacin in *C. jejuni* (43.7% and 61.2%, respectively) and *C. coli* (74.0% and 65.8%) from human patients was very or extremely high [102].

Recent trends AMR in bacterial isolates from people increased worldwide over the preceding three decades [6, 117–119], although this trend may be slowing in some regions [114, 120]. Between 2017 and 2020, resistance levels for many of the reported pathogen-antibiotic combinations remained broadly stable [114]. However, resistance to the HP-CIA meropenem and third-generation cephalosporins in *E. coli* BSIs increased by 80.0% and 18.8%, respectively [114]. BSIs caused by *Salmonella* spp. resistant to

ciprofloxacin, a fluoroquinolone HP-CIA, increased by 64.2% over the same period, and those caused by MRSA increased by 10.7% [114].

In the EU (excluding the UK), most combinations of bacterial species isolated from invasive infections and antibiotics tested for resistance showed either statistically significant decreasing trends, or no significant trend, in the population-weighted percentage of AMR between 2017 and 2021 [121]. Important exceptions to this include the significant increasing trends in: carbapenem-resistant *E. coli* (0.1% to 0.2%) and *K. pneumoniae* (8.1% to 11.7%); vancomycin-resistant *E. faecium* (13.4% to 17.2%); and *Acinetobacter* spp. with combined resistance to carbapenems, fluoroquinolones, and aminoglycosides (32.1% to 36.8%) [121].

Reporting and interpretation of AMR in people has been impacted by the COVID-19 pandemic, due to changes in social contact, hospital admissions, and infection prevention and control (IPC) [121, 122]. In the UK, the percentages of several key BSI pathogens resistant to one or more key antibiotics increased between 2017 and 2019, but dropped dramatically in 2020 [115]. These pathogens include *E. coli* (+12.2% between 2017 and 2019, -16.2% in 2020), *K. pneumoniae* (+29.2% between 2017-2019, -14.1% in 2020), *Pseudomonas* spp. (+5.7% between 2017-2019, -14.9% in 2020), and *S. pneumoniae* (+31.1% between 2017-2019, -36.0% in 2020) [115]. Of these, the number of resistant BSIs caused by both *E. coli* and *S. pneumoniae* remain lower than in 2017 (-11.8% and -23.2%, respectively) [115]. Exceptions to this trend include resistant BSIs caused by *Enterococcus* spp. (+43.8% since 2017) and *S. aureus* (-16.6% since 2017) [115].

1.2.5.2 Clinical impact on human health

The difficulties of estimating the clinical impact of AMR have been reviewed at length [123–126]. Adverse outcomes of resistant infections include longer hospital stays and/or increased cost of treatment, or death [124, 127, 128]. However, measuring these accurately is vulnerable to confounding and methodological limitations [123–125], and careful study design is needed [124, 125, 129].

Length of hospital stay Resistant infections can cause longer hospital stays, due to delays in appropriate treatment and/or treatment failure [128]. Acquisition of BSIs caused

by MRSA resulted in an additional 12.8 days (95% CI: 6.2-26.1) in hospital, for patients admitted in Australia hospitals between 2005 and 2010 [130]. However, the increased length of stay was not significantly different to that caused by acquisition of an MSSA BSI, which was 11.0 days (95% CI: 6.4 to 14.9) [130]. Similar findings have been reported from Europe. Data from 2010-2011 showed acquisition of BSIs caused by MRSA and MSSA resulted in longer hospital stays of 13.3 days (95% CI: 13.2-13.4) and 11.5 days (95% CI: 11.5-11.6), respectively [131]. While the CI of these estimates did not overlap, computation of the excess length of stay for MRSA BSIs when compared directly to MSSA BSIs did not show a significant increase (2.5 days, 95% CI: -3.2-8.3) [131]. By contrast, BSIs caused by Enterobacterales resistant to third-generation cephalosporins significantly increased the excess length of hospital stay (4.9 days, 95% CI: 1.1-8.7) when compared to susceptible infections [131].

Mortality Measuring deaths caused by resistant infections is more straightforward than measuring other clinical outcomes and forms the basis for the majority of burden assessments [120, 126, 132]. Drug-resistant infections have been shown to have a higher mortality rate than infections caused by their susceptible counterparts. Meta-analyses show increased mortality rates for bacteraemias in people caused by: MRSA compared to MSSA (odds ratio, OR: 1.93 [95% CI: 1.54-2.42]) [133]; vancomycin-resistant enterococci (VRE) compared to vancomycin-sensitive enterococci (VSE) (OR: 2.52 [95% CI: 1.9-3.4]) [134]; and ESBL-producing Enterobacterales (OR: 2.35 [95% CI: 1.90-2.91]) [135].

The number of deaths caused by resistant infections has previously been estimated at 35,000 in the USA [136] and at least 25,000 in the EU [119, 137], each year. However, the first comprehensive assessment of the global burden of AMR was only published in 2022. The landmark report by Global Research on Antimicrobial Resistance (GRAM) used predictive statistical modelling to estimate that 1.27 million deaths (95% uncertainty intervals [UI]: 0.91-1.71 million) globally were directly attributable to antibiotic resistance in 2019 [120]. This estimate was based on data obtained from systematic literature reviews, surveillance systems, and other sources, and compared deaths from drug-resistant infections to an alternative scenario, where the infections were caused by fully-susceptible bacteria. The number of deaths attributable to AMR exceeds the previous, less-rigorous

estimate of 700,000 deaths per year put forward in the O'Neill Review [44].

The GRAM report showed that the death rate attributable to AMR to be highest in western sub-Saharan Africa, at 27.3 (95% UI: 20.9-35.3) deaths per 100,000 people, and other lower-income regions, largely driven by the prevalence of infection [120]. Of the six bacterial species with the most deaths attributable to AMR in this study, five are zoonotic: *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *Acinetobacter baumannii*, and *P. aeruginosa*. These, along with the human commensal and pathogen *S. pneumoniae*, together accounted for 929,000 (95% UI: 660,000-1,270,000) AMR-attributable deaths in 2019 [120].

1.2.5.3 Economic costs in human health

Productivity losses due to AMR morbidity and mortality, along with increased healthcare costs associated with treating resistant infections, have been estimated at €1.5 billion annually in the EU [137]. However, the economic cost of AMR is difficult to quantify, in part due to variation in the underlying costs of care [132, 138, 139]. Estimates of cost are also limited by the methodological assumptions of the study [139, 140].

1.2.5.4 Prevalence in animal health

Internationally-collated AMR surveillance data from animals are not yet available, although this is an area under active development [141–143]. Several countries have developed, or are developing, surveillance systems for AMR in bacteria isolated from diagnostic samples from animals [108, 143–146]. However, these systems are not harmonised or standardised [109, 143, 144]. Similar to AMR surveillance in human patients, this type of surveillance is subject to biases resulting from passive surveillance and/or preferential diagnostic testing (which over-represents resistant infections) [143, 146].

The most comprehensive clinical AMR surveillance programme in animals is France's Resapath scheme, which comprises an extensive network of 71 laboratories, including private veterinary laboratories, and has been running since 1982 [147]. It reports the results of over 60,000 ASTs performed on bacteria isolated from animals for diagnostic purposes, each year [148]. In 2021, 18.1% and 17.7% of AST results reported involved bacteria isolated from cattle and poultry, respectively [148].

Resistance to antibiotics frequently used in veterinary medicine was extremely high

in *E. coli* isolated from the gastrointestinal tract of young cattle in 2021: 84% were resistant to amoxicillin, 83% were resistant to streptomycin, and 77% were resistant to tetracycline [149]. This is important because diarrhoea in calves is common, and antibiotics are often used in treatment. By contrast, *E. coli* isolated from mastitis cases in adult cattle (which arise from faecal contamination) were much more susceptible [148]: the percentage of these isolates resistant to the same antibiotics was 44%, 26%, and 24%, respectively [149]. In broiler chickens, AMR in *E. coli* was much lower, with the highest levels of non-susceptibility reported as 37% (to amoxicillin) and 28% (to tetracycline) [149].

1.2.5.5 Clinical impact in animal health

The veterinary impact of AMR on animal health and welfare is far less studied than on human health, and estimates of morbidity and mortality are lacking.

1.2.5.6 Economic costs in animal health and production

The economic costs of AMR in the animal health sector, while conjectured [150], remain largely unquantified [151]. However, productivity losses due to untreatable disease, and/or financial losses due to increased cost of treatment and/or diagnostics, could be anticipated [150, 152]. The Global Burden of Animal Diseases (GBADs) programme, funded by WOAH, is the first to undertake a systematic quantification of the burden of AMR in animals [153], but results are not yet available.

1.2.5.7 Prevalence in bacteria carried by healthy animals

The background prevalence of AMR carried by the microflora of the human population, outside hospital settings, is not routinely monitored. AMR in healthy animals at slaughter has, however, been monitored in a harmonised way across Europe since 2014 [154]. This programme focuses on major food-producing animal species that were historically higher users of antibiotics: pigs, poultry (broiler chickens and fattening turkeys), and veal calves [155]. Sampling is representative of healthy animals entering the food chain, and their meat at point of sale [155]. In this programme, AST and WGS are performed on major zoonotic and commensal organisms (*E. coli*, non-typhoidal *Salmonella*, and *Campylobacter* spp.), for resistance to a panel of antibiotics important to human health [155, 156].

In Europe The most recent data, spanning 2020-2021, shows the prevalence of resistance to classes of antibiotics that are commonly used in veterinary medicine, such as beta-lactams, tetracyclines, and sulfonamides, was moderate (>10% to 20%) to extremely high (>70%) in *E. coli* and *Salmonella* spp. isolated from animals in most European countries [157]. Resistance to HP-CIA third-generation cephalosporins was seldom detected in these bacteria. These patterns of resistance were similar to those detected in non-typhoidal *Salmonella* isolated from human patients [157].

Interpretation of AMR trends has been disrupted by the UK's exit from the EU, with 2019-2020 being the last two-year period in which UK-wide AMR data was reported within this programme [102, 157]. In 2020, resistance to ciprofloxacin in *Salmonella* isolated from broilers and turkeys across Europe was very high (52.9% and 53.7% of total isolates, respectively). Over 2019-2020, resistance to ciprofloxacin was very high to extremely high in *C. jejuni* and *C. coli* isolated from: broilers (72.8% and 61.9%, respectively); turkeys (77.9% and 80.4%, respectively); pigs (52.4% in *C. coli* only); and calves (56.8% and 80.6%, respectively) [102]. Resistance to fluoroquinolones in human clinical isolates of *Salmonella* spp. (14.1%) and *Campylobacter* spp. (61.2% and 65.8% for *C. jejuni* and *C. coli*, respectively) tended to be lower in 2020 [102].

Key indicators measured under this programme are based on AMR in *E. coli* and are weighted by the size of livestock populations in different countries [157]. The proportion of *E. coli* isolated from animals at slaughter and found to be susceptible to the entire panel of antibiotics tested tended to increase between 2014-2020, with 14 of 29 countries showing a statistically significant increasing trend over this time, and only four countries demonstrating a decreasing trend [102]. There is much variation in the percentage of fully-susceptible *E. coli* reported by different countries: in 2021, this ranged between 6.5% and 85.9% [108, 157].

In the UK In the UK, which continues to align with the EU programme, substantial improvements have been achieved in the key indicators since 2014 [158]. These include an increase in the percentage of fully-susceptible *E. coli* isolated from caecal samples from 18% of isolates in 2014/2015 to 39% in 2020/2021, and a decrease in MDR *E. coli* from 57% of isolates to 31% over the same period [158, 159]. This coincides with a 55% reduction

in sales of antibiotics for food-producing animals [158]. Some hotspots of AMR persist: resistance to the fluoroquinolone ciprofloxacin in *C. coli* was detected in 59% of isolates from broilers, and 37% of isolates from turkeys, in 2020; both increased since 2014 [108]

Globally An analysis of results from 901 research studies (point prevalence surveys of AMR in *E. coli*, *Salmonella* and *Campylobacter* spp., plus *S. aureus* isolated from healthy animals) from low- and middle-income countries (LMICs) was published in 2019 [160]. This showed that the proportion of antibiotics tested to which resistance was above 50% increased in chickens (0.15 to 0.41) and pigs (0.13 to 0.34), but plateaued at a lower level in cattle (between 0.12 and 0.23), between 2000 and 2018 [160]. However, the study does not distinguish trends in resistance to individual antibiotics, or antibiotic classes, meaning that this headline result is vulnerable to bias in study design. Additionally, given the reliance on research studies rather than national or regional surveillance programmes, the results can not be considered representative of the underlying population.

1.3 Bacterial ecology

The evolutionary drivers of AMR cannot be explained without understanding the complicated ecological landscape within bacterial populations. Here, I will lay out some important features of bacterial species, and define ecological and genomic concepts that underpin later sections of this thesis.

1.3.1 Relationships with other organisms

Bacteria occupy a vast diversity of habitats and can interact with other organisms in different ways. Some are environmental, existing predominantly outside eukaryotic hosts, such as *P. aeruginosa*, which colonises soil, water, and artificial environments [161, 162]. Others are commensals, which are part of the normal microbial flora of people and animals, and reside in the gastrointestinal tract and other bodily cavities, or on the skin, without causing disease. Examples of commensals include *S. pneumoniae* in the human upper respiratory tract [163, 164], or *Enterococcus* spp. in the gastrointestinal tract of people and animals [165]. Commensal organisms are important in the development of AMR,

as they can be exposed to antibiotic treatment administered to their host for unrelated infections [166].

‘Pathogen’ is used to describe bacteria that cause disease. Both environmental and commensal bacteria can act as opportunistic pathogens, i.e. will infect eukaryotes and cause disease under specific circumstances. For example, *P. aeruginosa* is an important cause of hospital-acquired infections and an opportunistic pathogen of people with cystic fibrosis [161, 162, 167]. *S. pneumoniae* can cause invasive disease, such as meningitis or sepsis, particularly in children who are immunosuppressed [164]. *Mannheimia haemolytica* is a commensal of the bovine upper respiratory tract, but is implicated in bovine respiratory disease complex when other factors, such as viral infection and environmental stressors, are present [168]. Obligate pathogens are a further group of bacteria that are dependent on a host to complete their life cycle, such as the causative agents of tuberculosis (*M. tuberculosis*) and gonorrhoea (*Neisseria gonorrhoeae*) in people [169, 170].

Bacteria can be further divided into generalists, which are able to exist in a range of different environments (including within different host species), for example *Clostridium difficile* [171–173]; and specialists, which are adapted to a specific environment or host, for example *S. pneumoniae* [164]. The extent of this adaptation may differ between species within a genus, or between lineages within a species [174]. For example, the genus *Campylobacter* includes multi-host generalist and host-restricted specialist species [175]. *C. jejuni*, which is a commensal of birds and other animals, but an important foodborne pathogen of people, is differentiated into multiple distinct lineages associated with different animal host species [176, 177]. *S. aureus*, a commensal of the skin and upper respiratory tract and opportunistic pathogen of both people and a range of animal species, is also made up of multiple distinct host-associated lineages [178].

1.3.2 Bacterial strains

A clone is a particular subpopulation of bacterial cells, all descended from a common ancestor [179]. A bacterial strain in a natural or ecological context is a set of isolates of the same species with similar genomes and/or phenotypic characteristics [180, 181]. Defining strains can be a problem within bacterial population genetics, particularly in populations where loci are frequently exchanged by recombination [174, 182]. Nevertheless, the exis-

tence of groups of very closely-related isolates is a feature of most bacterial populations. Bacteria with multi-strain population structures include *Campylobacter* spp. and *S. aureus*, as described above, *S. pneumoniae*, and *E. coli* [183]. Other species, such as *Yersinia pestis* [184], are monomorphic: composed of a single strain.

1.3.3 Competition

The more similarities there are between strains (or species) within a given environment, the more likely they are to compete with each other for a finite resource [185]. Competition can be direct or indirect, and the specific mechanisms deployed by bacteria to compete with other strains are diverse [186–188].

Direct competition includes both ‘exploitative’ and ‘interference’ mechanisms [187, 188]. Exploitative competition strategies use up external resources, making them less available to competitors. Better adaptation to a specific niche can result in higher rates of growth. Interference strategies involve interacting with competing organisms and impeding their growth. This can occur through killing (e.g. via bacteriocins, such as colicins secreted by *E. coli* [189]) or disruption of normal cellular processes (e.g. via metabolites or enzymes, as occurs in *Staphylococcus epidermidis*’ inhibition of *S. aureus* in the nasal passages [190, 191]). Actively harming competing cells reduces their lifespan, or prevents them from colonising the same niche in the first place [192], as has been shown to occur between competing strains of *S. aureus* [193].

Indirect competition can occur within hosts, for example when one strain/species triggers an immune response that disproportionately affects competitors, as has been shown in *H. influenzae* and *S. pneumoniae* co-colonisation of the nasopharynx [194].

1.3.4 Effect of competition on population structure

Competition shapes the diversity and stability of complex microbial systems. Multi-strain structuring of bacterial populations can occur through neutral genetic drift or through selection for ‘ecotypes’ adapted to different ecological niches [174, 195, 196]. However, both drift and strong competition between species ultimately reduce diversity [197]. Thus, processes of balancing selection are required to maintain stable multi-strain population structures.

Negative frequency-dependent selection (NFDS) is a type of balancing selection where genotypes and/or phenotypes are most beneficial when they are rare [198]. Competition can facilitate NFDS in many ways, including direct competition for resources, or indirect competition (e.g. immune-mediated). A type of NFDS, non-transitive competition (essentially rock-paper-scissors) between bacterial species or strains promotes stability [199], but only when the environment is structured into different niches [200, 201].

1.4 Evolution of AMR

Antibiotic resistance can be intrinsic, i.e. attributable to inherent structural or functional characteristics of specific bacteria [87]. Several examples of this type of resistance are mentioned in section 1.2.3. Antibiotic resistance genes can also arise by spontaneous mutation or can be acquired vertically through inheritance, or from other bacteria through horizontal gene transfer (HGT) [202].

1.4.0.1 Mutation

Chromosomal mutations conferring antibiotic resistance can occur spontaneously or in response to selective pressures. Frequent detection of chromosomally-encoded resistance to ciprofloxacin in soil-dwelling *Streptomyces*, arising from different point mutations [75], reflects the natural sequence variation of this genomic region [203]. On the other hand, multiple examples exist of increased frequencies of bacterial mutations in the presence of antibiotics [204–207].

1.4.0.2 Horizontal gene transfer (HGT)

HGT occurs via transformation, which is the uptake of naked DNA by bacteria, or by the transmission of mobile genetic elements (MGEs) between bacterial cells.

Transformation Transformation requires competence machinery, which actively pulls fragments of DNA into the bacterial cell [208, 209]. Bacteria that possess this machinery, allowing them to actively take up and incorporate foreign DNA into their own genome, are called ‘naturally competent’. Many bacterial species, including important examples

such as *S. pneumoniae* and *H. influenzae*, are naturally competent [208], and through this have been able to acquire AMR genes from other bacterial species [210–212].

Mobile genetic elements (MGEs) MGEs are segments of DNA that facilitate their own transfer between genomes, and include prophage or ‘phage’ (the latent form of bacteriophage that is integrated in the chromosome), plasmids, and integrative and conjugative elements (ICEs). The existence of MGEs were first discovered by several Japanese research groups, including those led by Rintaro Nakaya and Tsutomu Watanabe, when they showed that MDR in *Shigella dysenteriae* was transferable to other species of Enterobacterales through cell-to-cell contact (detailed in [213]). The same phenomenon was reported in *Salmonella enterica* serovar Typhimurium by Naomi Datta [214].

Integration of phage into the genome is called transduction, and usually occurs within (rather than between) bacterial species. Despite the ubiquity and diversity of phage, there are very few examples of them transmitting resistance [215].

By contrast, ICEs and plasmids are frequently implicated in the transfer of resistance [216–221]. Different AMR genes can be arranged together into MDR cassettes, which form modular cargo within ICEs and plasmids. Notable examples of gene acquisition in this way include: the Tn916 family of ICEs [222], conferring MDR to *S. aureus*, *S. pneumoniae*, and *C. difficile*, amongst others [223]; and conjugative IncA/C plasmids conferring MDR to *Salmonella enterica* serovars [224].

1.4.1 Antibiotic consumption as an evolutionary driver

AMR is a natural phenomenon, but spread of resistant genotypes is driven by the selection pressure of antibiotic use. When a person or animal is treated with antibiotics, susceptible bacteria are killed off or have their growth impeded, while resistant bacteria continue to multiply. The association between antibiotic consumption and resistance is demonstrable for multiple pathogens, on varying scales from individual to country level.

1.4.1.1 Associations between antibiotic consumption and resistance

In individuals Monitoring the faecal flora of individual human patients before and after treatment with clarithromycin showed elevated levels of clarithromycin-resistant entero-

cocci even years after treatment [225]. There is an association between a history of individual use of antibiotics of any type and nasopharyngeal carriage of penicillin non-susceptible *S. pneumoniae* (PNSP) [226]. Administration of the third-generation cephalosporin ceftiofur to beef cattle resulted in transient increases in ceftiofur-resistant *E. coli* in faeces that were significantly different from the unmedicated control group ($p = 0.01$) [227]. A systematic review of the effects of oral antibiotics in individual pigs showed an increase in AMR *E. coli* after treatment in 32/36 studies [228]. Effects were highest for aminoglycoside, quinolone and tetracycline administration. A meta-analysis of 12 human studies showed that urinary tract *E. coli* (pooled OR: 2.5 [95% CI:2.1-2.9]) or respiratory tract isolates of various species (pooled OR: 2.4 [95% CI: 1.4-3.9]) were significantly more likely to be resistant if the person had received antibiotics from their primary care provider within the preceding two months [229].

In hospitals The pandemic MRSA clone EMRSA-15 is highly transmissible within hospitals, but emerged from a methicillin-sensitive, community-associated population [230], demonstrating the advantage of acquiring resistance in a high-antimicrobial setting.

Within-hospital studies repeatedly show a positive correlation between antibiotic therapy and antibiotic resistance in *S. aureus*, *E. coli*, and other Gram-negative bacteria [231–233]. However, it seems that resistance in a specific pathogen can be less closely associated with antibiotic treatment for that pathogen than it is with treatment for some other reason [234, 235]. Tedijanto and colleagues called this background antibiotic consumption, and estimated that for most combinations of bacterial species and antibiotic, less than 20% of bacterial exposures to antibiotics are due to treatment of disease caused by that species [235].

In the community A systematic review of the associations between antibiotic consumption and resistance acquired in the community showed that 67% of 243 studies found a positive correlation [236]. Arason *et al.* also linked PNSP carriage to antibiotic consumption in the wider community [226], which has persisted despite the introduction of vaccination [97].

On farms On intensive farms where antibiotics are often administered *en masse*, there are multiple reports linking usage of a given antibiotic to an increased prevalence of resistance to that antibiotic, particularly in gastrointestinal organisms. Administering fluoroquinolones to poultry flocks substantially and significantly increased the prevalence of ciprofloxacin-resistant *C. coli* and *C. jejuni* [237]. Vancomycin-resistant enterococci were detected at higher frequencies on poultry and pig farms that used avoparcin as a growth promoter, but not on antibiotic-free farms [238–241]. ESBL-producing *E. coli* were significantly more likely to be detected on pig farms with a prior history of third/fourth-generation cephalosporin consumption [242]. In another UK study, the presence of AMR genes in Enterobacterales was significantly associated with antibiotic use on farms, and MDR was more frequent in isolates from pigs than from sheep and cattle, which also reflects differences in antibiotic use [243].

At national level On an international scale, several significant associations between countries’ antibiotic consumption and the prevalence of resistance have been reported. These include: *S. pneumoniae* [244–246], *Streptococcus pyogenes* [244], *E. coli* [246], *Neisseria gonorrhoeae* [247], and *Helicobacter pylori* [248] in people, and *E. coli* in livestock [249, 250]. A national-level metagenomic analysis of AMR genes in human sewage found increased abundance of genes conferring resistance to a given antibiotic class was significantly associated with increased use of that class, but not with total antibiotic use [251].

It is notable that in Europe, where people, livestock and livestock products move freely between countries, there are strong country-to-country differences in the prevalence of resistance in people and livestock [245, 246, 248, 249], as well as large disparities in antibiotic consumption [28, 252]. Geographic gradients in PNSP prevalence have been shown, with resistance tending to be lower in northern European countries and higher in southern European countries [97, 121, 245, 253]. This corresponds to a similar south-north gradient in antibiotic consumption [246]. In a univariate analysis of European country-level data, a strong positive correlation (correlation coefficient r : 0.49) was shown between antimicrobial usage and the average prevalence of *E. coli* resistant to third-generation cephalosporins and fluoroquinolones [254].

1.4.1.2 Reducing consumption (usually) reduces resistance

There is good evidence to show that interventions to decrease antibiotic consumption are usually associated with reduced resistance [236, 255–257]. However, the magnitude of these changes are not necessarily aligned. For example, a meta-analysis of 81 different interventions in livestock showed that most resulted in a 10-15% reduction in resistant isolates in intervention groups compared to control groups [256]. These results are remarkably consistent, given the wide range of interventions in this study, affecting multiple livestock and bacterial species, antibiotic classes, and sample types. Interventions that involved the complete cessation of a particular antibiotic were associated with larger, but still incomplete, reductions in resistance. For example, bans on the glycopeptide growth promoter avoparcin were associated with a pooled reduction in resistant enterococci of 22% (95% CI: 12-32%) [256]. This is a substantial and significant drop, but not of the same magnitude as the reduction in consumption. More than a decade after European Union-wide bans on avoparcin and virginiamycin use in livestock, low levels of glycopeptide- and streptogramin-resistant enterococci were still detectable in various countries and animal species [250].

1.4.1.3 Changes in antibiotic consumption are reflected in resistance over varying timescales

The time taken for AMR prevalence to reflect changes in antibiotic consumption varies widely, and seems to follow two broad patterns: short-term (days to months) and long-term (years). For example, immediate increases in fluoroquinolone-resistant *Campylobacter* in poultry [237], and cephalosporin- and tetracycline-resistant *E. coli* in cattle [227] were observed after antibiotic administration in experimental studies. An intervention that reduced fluoroquinolone prescribing in human primary care by around 40% resulted in a rapid reduction in urinary tract infections (UTIs) caused by non-susceptible *E. coli* of around 25% (although note that again, the magnitude of the reduction in non-susceptibility does not correspond to the reduction in consumption) [255]. A reversion to baseline antibiotic consumption in all three studies was followed by corresponding and significant changes in resistance, within weeks. These short-term dynamics are also reflected in

seasonal patterns of higher antibiotic consumption and increased *S. pneumoniae* resistance during winter [258, 259].

There are also multiple examples of AMR prevalence changing more slowly - over several years - in response to shifts in antibiotic consumption. Long-term increases in macrolide, aminopenicillin and cephalosporin use have been temporally associated with increased resistance in invasive *S. pneumoniae* [260–262]. Fosfomycin use has been similarly associated with resistance in *E. coli* and *K. pneumoniae* UTIs in people [263], and decreasing trimethoprim resistance in these organisms was linked to decreasing community consumption [263]. The avoparcin ban in livestock feed was followed by marked reductions in vancomycin-resistant enterococci (VRE) prevalence (from 75% to 6% of enterococcal isolates) in Danish broiler chickens over three years [264]. Substantial declines in tetracycline use in France over five years were associated with a delayed and smaller decline in *E. coli* resistance [265]. And in a 14-year period in France in which total veterinary antibiotic use declined by almost half [266], resistance to most antibiotics in *E. coli* causing disease in animals also declined [267].

1.4.1.4 Coexistence of resistant and susceptible bacteria despite high antibiotic use

Despite antibiotics inhibiting their growth and transmission, susceptible bacteria continue to circulate in bacterial populations, even when antibiotic consumption is high [53, 268]. This persistent coexistence of resistant and sensitive strains is a major challenge to our understanding of AMR evolution, having been observed across many bacterial species, including *S. pneumoniae* [216, 217], *S. aureus* [269], *Salmonella enterica* [270], *E. coli* [271, 272], and *C. difficile* [273].

The ubiquity of coexistence and the acceleration in resistance during the antibiotic era, when there was a clear selective benefit to carrying resistance genes, suggest that these benefits are counterbalanced by a cost. This is biologically plausible: phenotypic changes caused by acquisition of a resistance genes, such as producing an enzyme to degrade antibiotics, or altering the cell wall, must result in a suboptimal physiology that diverts resources away from other functions. It could therefore be expected that the cell becomes less efficient at reproducing. As bacteria compete for hosts, resistance genes

compete for bacteria, and high-cost acquisitions can be selected against. In one example, experimentally-induced vancomycin resistance resulted in a decreased growth rate of MRSA *in vitro* [274]. Subsequent loss of *mecA*, which confers methicillin resistance, enabled these strains to outcompete *mecA*-bearing vancomycin-resistant strains.

The impact of resistance fitness costs may also be detected epidemiologically. High prevalence of VRE in Norwegian broilers, attributable to co-selection for reduced susceptibility to narasin (see section 1.4.1.5), eventually declined [275, 276] despite narasin still being used in feed. Simm and colleagues hypothesise that this was due to vancomycin-susceptible enterococci with reduced narasin susceptibility eventually out-competing their vancomycin-resistant counterparts [276]. Similarly, increased susceptibility of *S. aureus* in US hospital patients has been attributed to increasing prevalence of penicillin-susceptible *S. aureus* strains out-competing MRSA strains [277].

However, this inference from population genetics is not always easy to detect experimentally. The fitness cost of resistance can be highly variable [278] and/or influenced by the environment [279]. Some studies indicate a fitness benefit associated with specific resistance genes [278, 280–282].

1.4.1.5 The role of co-selection

Increasing or stable resistance to an antibiotic, despite declining consumption, can be facilitated by co-selection for resistance to another drug. Harkins and colleagues used WGS of historical MRSA isolates and Bayesian phylogenetic reconstruction to infer when this lineage emerged, and when the staphylococcal cassette chromosome *mec* element (SCC*mec*), carrying the *mecA* gene conferring methicillin resistance, was acquired [35]. They showed that the SCC*mec* element was most likely acquired by an *S. aureus* lineage in early 1941, 14 years before methicillin was used therapeutically [35]. The most obvious reason for the emergence of MRSA is the widespread use of penicillin at that time, as *mecA* confers resistance to penicillin and other beta-lactams.

Co-selection has certainly been implicated in the preservation of AMR at population level. After the growth promoter avoparcin was banned, the prevalence of VRE decreased substantially in Danish poultry (from 73% to 21%), but remained constant at around 20% in pigs [264]. The persistence of VRE in pigs was associated with a multi-resistant

clone bearing the *vanA* gene for vancomycin resistance along with *ermB*, which conveys macrolide resistance [283]. VRE prevalence in pigs only decreased once consumption of the macrolide tylosin reduced significantly [264]. Additionally, while VRE declined rapidly in Danish broilers [264] and in German poultry meat samples (from 100% to 26% over the same period) [284], there was no corresponding decline in VRE in Norwegian broilers [285]. This stable prevalence of VRE was subsequently attributed to co-selection for vancomycin resistance and reduced susceptibility to the coccidiostat narasin, which was introduced in Norway as a replacement for avoparcin [276].

Co-selection can also involve heavy metals, which are used in livestock feed [286] and linger in the environment. Resistance to heavy metals has been linked to AMR loci [286, 287]. Co-selection to heavy metals could be involved in the persistence of glycopeptide- and fluoroquinolone-resistant isolates on farms where those antibiotics had never been used [239, 285, 288].

1.4.2 Other factors affecting AMR distribution

1.4.2.1 Strain dynamics

Additional mechanisms contributing to the distribution of resistance include clonal expansion of successful lineages, as has been reported for the MDR *S. Typhimurium* clone DT104 [289], fluoroquinolone-resistant *Salmonella* Typhi [290, 291], and different resistant clones of *S. pneumoniae* [292].

While the emergence of a resistant clone is often linked to antimicrobial use [230], other factors - such as virulence, fitness, or lack of immunity - can contribute to its success [118, 270, 293, 294]. For example, dissemination of susceptible *Salmonella* clones occurred despite varying background levels of antibiotic consumption [295, 296], and a pandemic clone of MRSA was able to displace established MRSA strains [230]. Successive waves of regional emergence and spread of an imported MDR clone of PNSP were observed during a period of declining antibiotic use in Iceland [297, 298] and Sweden [299, 300], indicating that in this case, other aspects of the strain's phenotype had a greater effect on the population dynamics than declining antibiotic consumption.

1.4.2.2 Biosecurity and hygiene

Poor biosecurity and/or hygiene can allow introductions of resistant bacteria into hospitals, communities and farms, from other hosts [301–306] or via fomites [303, 307]. Introductions from the environment, which can act as a reservoir for resistant genes, also occur [308–310]. Resistant bacteria are commonly detected in environmental samples from pig and poultry farms [237, 241, 285, 302, 307, 311–313], or nearby in air and soil [314], and have been detected extensively in wastewater samples, including from hospitals [251, 315].

Introductions from external sources could explain the presence of resistant isolates [316, 317] and genes [318] on organic farms, where antibiotic use is severely restricted; or in agriculture in countries where specific antibiotics are not used in animals [319, 320].

1.4.3 The role of HGT

AMR evolution and transmission is facilitated by HGT. Co-selection for vancomycin and tylosin resistance in Danish pigs, as mentioned above, occurred because the *vanA* and *ermB* genes occur on the same MGE [283]. The *tcrB* gene for copper resistance, also implicated in co-selection for VRE, is carried on a conjugative plasmid [286]. This type of genetic linkage is common [321]; it has also been implicated in, for example, pandemic spread of the MDR *S. Typhimurium* clone DT104 [322, 323].

Originally highlighted by genotyping techniques such as multilocus sequence typing (MLST) [324], high-throughput genome sequencing has provided a high-resolution view of how HGT drives the spread of resistance through the underlying bacterial population structure. The evolutionary dynamics of resistance, as mediated by HGT, are further complicated by the relative costs and benefits of MGEs. Fitness costs of specific plasmids have been shown to be dependent on the traits they encode [325], as well as being strain-specific [326–328].

MGEs should enable rapid adaptation to antibiotic exposure. However, while acquisition of resistance can occur multiple times within a bacterial species [216, 230, 269, 273, 329, 330], the distribution of resistance is largely clonal in many important pathogens [269, 270, 273, 294]. Coexistence suggests that resistance-bearing MGEs are not moving freely between strains. We also know that slower-moving ICEs are more clonally-associated, and

more associated with resistance genes, than rapidly-moving phage [216, 217, 220, 331, 332]. Plasmid associations with different Gram-negative strains are also not random [325]. Strain-specific fitness costs, as well as bacterial defence mechanisms [217, 221, 333–337], could both be contributing to this pattern.

1.4.4 AMR transmission between different host species

So far, I have been addressing resistance within animal or human populations. Of course, resistant bacteria or resistance-encoding genes can be transmitted between different host species. Resistance can be transmitted from animals to people through food [338–340], the environment [341, 342], or direct contact [342, 343]. Transmission of AMR from people to animals has also been reported, including of MRSA (to poultry) [344] and tetracycline-resistant *Streptococcus agalactiae* (to cattle) [345].

Cross-species AMR transfer events can be transient, or resistance can fail to establish in the new host population. Tang *et al.* showed that interventions to reduce livestock antibiotic use resulted in an overall reduction in human AMR [256], indicating that usage in livestock could be an important driver of AMR in people. However, only three of the papers included in Tang’s meta-analysis examined AMR in the wider community: the majority of studies investigated AMR carriage in farmers and farm workers, who are in close contact with livestock, and therefore more likely to acquire livestock-associated strains [275, 343, 346]. Such carriage is often transient, infrequently followed by expansion in the wider community, and is not representative of carriage by the general population. For example, livestock-associated MRSA CC398 has successfully infected pig farmers, their families, and members of the community in areas of high farm densities [343, 347]. However, transmission of this clone between people in hospital settings was initially much lower than other MRSA strains [348–350], indicating that it was insufficiently adapted for ongoing human-to-human transmission. Ongoing evolution within the CC398 lineage indicates adaptation to the hospital environment, including increased virulence [351] and the acquisition of resistance to mupirocin, the mainstay of MRSA decolonisation [352].

Thus, successful expansion in the new host depends on transmission opportunities, selection pressures, and other factors within the new host population, as well as the necessary genetic machinery. HGT facilitates the spread of resistance genes into different host pop-

ulations, via the direct introduction into bacteria already adapted to the recipient host species [353], or by helping resistant bacteria from the donor species adapt to the new host [354, 355]. WGS shows how cross-species transmission of AMR varies between bacterial species that are: multi-host-adapted (e.g. *C. difficile*); divided into generalist and specialist lineages (e.g. *E. coli*, *S. aureus*, *Salmonella*, *Campylobacter*); or host-restricted, albeit closely related to bacteria with a wider host range (e.g. *S. pneumoniae*).

Multi-host-adapted bacteria Knetsch and colleagues demonstrated frequent cross-continental and human-to-animal or animal-to-human spread of *C. difficile* RT078 [173]. They also showed a diversity of AMR genes and an accessory genome that was indistinguishable between human and animal strains.

Bacteria with generalist and specialist lineages By contrast, WGS of cephalosporin-resistant *E. coli* isolates from farmers, chicken meat, pigs, poultry, and human patients in the Netherlands showed genetically distinct lineages in different host species [346]. This finding overturned previous work that used low-resolution MLST to conclude that livestock were a source of ESBL-producing *E. coli* expansion in people [356–358]. Two UK studies sequenced collections of *E. coli* isolated from various sources. Ludden and others utilised ESBL-positive and ESBL-negative *E. coli* from livestock farms, retail meat, and human BSIs [359]; whereas Day and her colleagues used veterinary diagnostic samples, farm slurry, retail meat, human BSIs, human faeces, and sewage [360]. Both studies showed that the isolates of human and livestock origin were genetically distinct.

However, de Been and colleagues showed that resistance to third-generation cephalosporins was disseminated between animals and humans via plasmids, specifically IncI1 plasmids bearing ESBL genes (9/13 found in both human and livestock isolates), and IncK plasmids bearing AmpC genes (9/10 found in both human and livestock isolates) [346]. By contrast, while Ludden and colleagues found frequent HGT of MGEs between *E. coli* lineages, they showed that these MGEs were largely distinct between livestock and human isolates, with only 5% of human isolates bearing AMR-associated MGEs potentially shared with livestock isolates [359]. The disparity in these results could be explained by the small sample size in de Been’s study, but would also be explained by this work including isolates from

faecal samples from farmers, who are not representative of the wider population.

Analysis of the core genome of major *S. aureus* lineages from animals and humans shows that it does not usually determine host adaptation [361–363], although sometimes this can be attributable to a single nucleotide mutation [364]. MGEs carrying host-adaptation genes such as the human-specific immune response evasion cluster *chp*, *sak* and *scn* [365], or ruminant-specific von Willebrand binding protein (*vwb*) [364], have been associated with several species jumps by *S. aureus* [344, 366–368]. Price and colleagues were the first to show that livestock-associated MRSA (LA-MRSA) clonal complex 398 (CC398) likely originated from a human MSSA strain [344]. Evolutionary analysis of *S. aureus* CC398, including LA-MRSA, in the UK demonstrated distinct clades in animals and people, but with frequent cross-species transmission, the majority of which events originated in livestock [369]. AMR differences between CC398 lineages have also been described [178, 344, 369], with Price *et al.* showing that the human-to-livestock species jump was associated with the acquisition of *tetM* and *mecA* genes, encoding resistance to tetracyclines and methicillin, respectively, and the loss of human immune evasion genes carried by phage [344]. Subsequent work demonstrated that the *tetM* is borne on a Tn916 transposon, and *mecA* on SCC*mec*, both of which arose from separate, single acquisitions, and have subsequently been stably inherited [178]. By contrast, the human immune evasion cluster is carried on ϕ Sa3 prophage, which, while much more frequent in human-associated lineages than livestock-associated lineages, is more dynamic [178].

Mather and colleagues showed that the pandemic strain of MDR *S. Typhimurium* DT104 was largely maintained separately within animal and human populations, with limited transmission events in either direction [370]. Resistance genes were also largely maintained separately, with greater diversity of AMR genes and phenotypic resistance profiles in isolates originating from humans [370, 371]. There was also evidence for HGT of AMR genes between lineages [370].

Host-specific bacteria *S. pneumoniae* is a commensal of the human upper respiratory tract and an opportunistic pathogen. MGEs, particularly ICEs, are an important route of acquisition of AMR genes [216, 217], as is homologous recombination [97, 294]. AMR genes have been transferred between closely related streptococci through transformation [212,

372, 373]. An important source of AMR genes for *S. pneumoniae* is *S. mitis* [212, 373–375], which is also restricted to the human host. However, sparse sampling and WGS of non-pneumococcal streptococci makes it difficult to identify the source of many AMR genes acquired through interspecies transformations, for example those borne on Tn916-type ICEs [374]. By contrast, genomic characterisation of the porcine and human pathogen *S. suis* showed a much greater prevalence and diversity of AMR-gene-bearing MGEs than in other streptococcal species (including *S. pneumoniae*) [376]. This may be due to its exposure to a wider variety of genetic donors, but these findings also imply it could act as a reservoir of MGEs and AMR genes that are transmissible between host species.

1.5 Modelling infectious diseases

Mathematical and computational models are tools to understand the complex interactions between infectious agents, their hosts, and their environment [377]. Mathematical models were first applied to infectious disease transmission by Bernoulli in 1760 [378], but it was not until the 20th century that some of the key concepts underpinning the field were developed [379, 380]. These, along with important theoretical developments later in that century, are summarised by Anderson & May in their seminal text [381]. The field has expanded rapidly since the turn of the millennium, with substantial increases in the amount of published literature [382], and the use of models to inform public and animal health policy [377].

A detailed exposition of different modelling techniques, and their application to infectious disease epidemiology, is beyond the scope of this thesis. However, there are two methodological approaches with direct relevance to this work that I will first outline here: compartmental models, because they have dominated prior efforts to model AMR [383–387]; and individual-based models (IBMs), which are an emerging area in infectious disease epidemiology more generally [387], and which I have used in this current work.

1.5.1 Compartmental models

Compartmental models describe the host population in aggregate, categorising it into different subgroups or compartments according to infection state [377, 388, 389]. Movement

of hosts between compartments represents the natural history of the infection.

1.5.1.1 Structure

One of the simplest forms is a two-compartment model representing susceptible **S** and infectious **I** hosts. This SI model can be represented graphically, as shown in Fig. 1.1. This structure is appropriate for lifelong infections, for example, herpesvirus, and excludes a pre-infectious period.

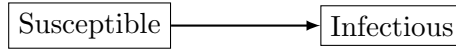


Figure 1.1: **Structure of an SI compartmental model**

For infections that can be cleared by the host, an SIS model is used (Fig. 1.2), which allows movement from the infectious compartment back to the susceptible compartment. This model is appropriate for recurrent infections without an appreciable period of immunity after the infectious stage.

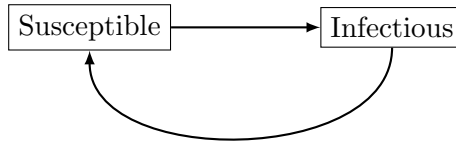


Figure 1.2: **Structure of an SIS compartmental model**

The structural flexibility of these models is detailed in comprehensive texts by Anderson & May and Keeling & Rohani [388, 390]. Compartmental models can be expanded by the use of additional compartments according to the natural history of infection. For immunising infections, an immune or recovered compartment **R**, representing hosts that are no longer infectious but are invulnerable to further infection, is added to create an SIR model (Fig. 1.3). A pre-infectious or exposed compartment **E**, to represent hosts that have been exposed but are not yet infectious, could also be considered. Depending on the infectious process and immunological response, reversion to susceptibility may (SEIRS, e.g. for influenza) or may not (SEIR, e.g. for measles) be appropriate. Additional compartments can be added to incorporate interventions, for example treatment or vaccination.

Figure 1.3: **Structure of an SIR compartmental model**

1.5.1.2 Dynamics

The movement of hosts between compartments is usually modelled in continuous time, governed by a series of differential equations, which specify the rate of movement between different compartments [388, 391]. These rates are incorporated into the model diagrams accordingly.

For example, the SIS model described above (Fig. 1.2) would be annotated to form Fig. 1.4, and represented by two differential equations:

$$\frac{dS}{dt} = \gamma I - \beta SI \quad (1.1)$$

$$\frac{dI}{dt} = \beta SI - \gamma I \quad (1.2)$$

Where $S + I = 1$. S and I represent the proportion of the host population in each compartment, β is the transmission parameter (the product of contact rates between hosts and the probability of transmission when contact occurs between a susceptible and an infectious host), and γ is the recovery rate.

The SIR model shown in Fig. 1.3 is annotated as shown in Fig. 1.5, and represented mathematically as:

$$\frac{dS}{dt} = -\beta SI \quad (1.3)$$

$$\frac{dI}{dt} = \beta SI - \gamma I \quad (1.4)$$

$$\frac{dR}{dt} = \gamma I \quad (1.5)$$

Where $S + I + R = 1$, R represents the proportion of the host population in the recovered compartment, and the other parameters are as before.

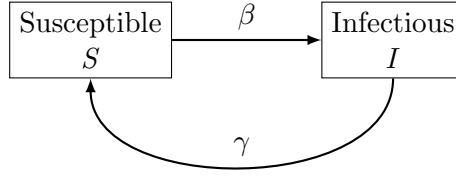


Figure 1.4: **Diagram of an SIS model, showing rates of movement between compartments**

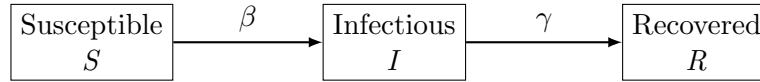


Figure 1.5: **Diagram of an SIR model, showing rates of movement between compartments.**

These models incorporate several assumptions:

1. Frequency-dependent transmission: transmission between hosts is independent of the total population size N . More explicitly, $S = X/N$ and $I = Y/N$, where X and Y are the number of hosts that are susceptible and infectious, respectively.
2. Homogenous mixing: hosts interact with equal probability with other hosts.
3. Closed population: demography is excluded, in that there are no births, deaths, or migration incorporated in the model.

These assumptions are appropriate in large populations in which disease transmission occurs quickly enough that demographic processes are not influential [388].

Other important parameters in these models are the infectious period $1/\gamma$ and the basic reproductive ratio $R_0 = \beta/\gamma$, which is the number of infections arising from the index infection in an entirely susceptible population [388].

1.5.1.3 Stochasticity

The models described above are deterministic, that is, the movement of hosts between compartments is determined exactly, with no random variation. This means that under the same starting conditions, the outcome is always the same [392]. This is appropriate in large populations, where random effects tend to cancel each other out due to the law of large numbers [393]. However, stochasticity is important when the incidence of infection is

small, for example in small populations, or during the emergence of an infection [392, 393]. Stochasticity is most commonly incorporated into continuous-time compartmental models by taking an events-driven approach, for example using Gillespie’s direct algorithm [394].

1.5.2 Individual-based models (IBMs)

Compartmental models consider the number or proportion of hosts experiencing different infection states; however, they do not distinguish hosts individually. By contrast, individual- or agent-based models (ABMs) record the infection status of each host at each point in time [392]. In these models, population-level phenomena emerge from interactions between individual hosts and other agents [395]. Such models are suited to complex biological systems, and can capture both heterogeneity and stochasticity, but are computationally intensive [392]. They are most useful when individuals’ properties and/or behaviour depend on their history, with sufficient complexity that it would require a very large number of compartments to replicate in a compartmental model. IBMs have been increasingly applied to infectious disease problems in recent years. A systematic review documented a three-fold increase in papers published annually in this field between 2006 and 2016 (from 38 to 115) [396].

1.5.3 Fitting models to epidemiological data

Models can be parameterised using observed information (e.g. duration of infectious period), however, transmission characteristics usually need to be inferred [388]. In compartmental models, this is usually via a maximum likelihood technique: the likelihood that the observed data arose from the model is calculated, and the set of parameters that maximise this likelihood is identified [388]. For stochastic models, the likelihood can be calculated sequentially, for example under Markov chain Monte Carlo approaches [392]. However, calculating likelihoods becomes computationally expensive or intractable with increasing model complexity, particularly under stochastic conditions or when data are incomplete, necessitating likelihood-free inference (LFI) techniques [392].

1.6 Modelling AMR

Mathematical models are increasingly applied to AMR problems [387]. However, coexistence of resistant and sensitive bacterial strains remains particularly challenging for modellers to simulate. In this section, I will outline previous developments in this area, and explain why coexistence remains a persistent barrier to effective models of AMR.

Modelling AMR in commensal organisms such as *S. pneumoniae* requires some technical adjustments to the language used above. Strictly, ‘infection’ refers to a pathogenic process, whereas ‘carriage’ or ‘colonisation’ are more appropriate terms for modelling commensals when not acting as opportunistic pathogens. However, many models of AMR in commensals, including the one developed for this current work, ignore invasive disease, due to its comparative infrequency [383]. For brevity, I use ‘infection’ interchangeably with these more accurate terms in these instances.

1.6.1 Compartmental models of AMR

Computational models of AMR in the existing literature are dominated by compartmental approaches [387, 397]. Seventy-six percent of 201 AMR modelling papers identified in a recent systematic review were compartmental [387]. This review included papers modelling antiviral, antifungal, and antiparasitic resistance, as well as antibiotic resistance, which was the focus of approximately 65% of the papers. For bacterial commensals, where re-infection occurs frequently, an SIS or SIRS model structure is used.

1.6.1.1 Modelling fitness cost

A fitness cost, counteracting the obvious benefits to bacterial strains of carrying resistance in the presence of antibiotic consumption, is an obvious explanation for coexistence. Previous work using compartmental models has imposed the cost of resistance on the rate of bacterial transmission or the duration of carriage [383, 385].

In the simplest models, resistance persists in a population only once antibiotic use is sufficiently high to overcome the imposed cost [398] (Fig. 1.6). Above a second consumption threshold, the advantages of resistance are so great that it fixes in the population; it is only between these two thresholds that coexistence is stable. The magnitude of the fitness

cost affects the value of these thresholds [385, 398], and the difference between them tends to be small. This is inconsistent with reality, where coexistence persists despite many-fold increases in antibiotic consumption [244, 245, 249], across many species and different resistance mechanisms (see section 1.4.1.4). Another limitation is the difficulty estimating fitness costs from *in vitro* studies [278, 279, 282, 399].

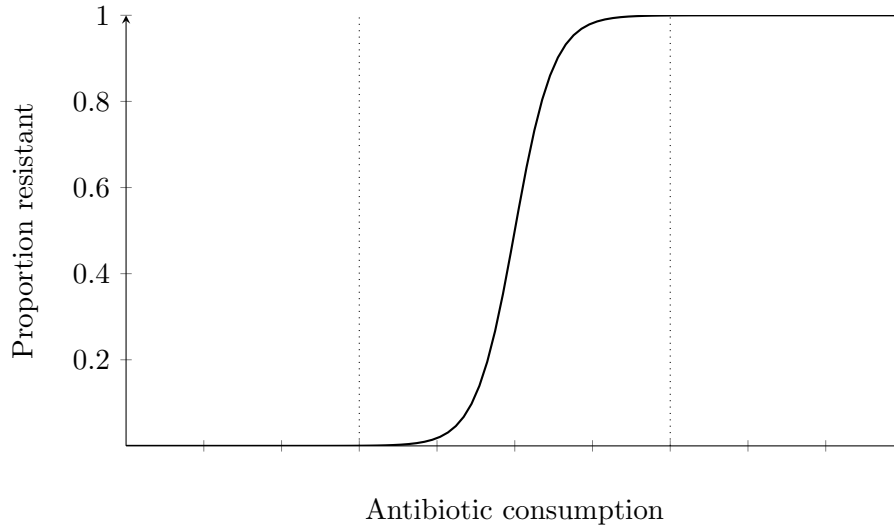


Figure 1.6: **Modelling the relationship between antibiotic consumption and resistance.** In the simplest models, resistance exists at intermediate frequencies over a limited range of antibiotic consumption (dotted lines).

1.6.1.2 Structural neutrality

Austin and colleagues subsequently showed a much more gradual relationship between antibiotic consumption and AMR, but only by giving sensitive strains a structural advantage in their model [400]. Hosts infected with resistant bacteria could be super-infected with sensitive bacteria, but not vice versa. However, this kind of bias does not necessarily make sense biologically, and clouds our understanding of the processes that contribute to maintaining coexistence. Models attempting to understand the evolution of coexistence should be structurally neutral and not allow “coexistence for free” [384].

1.6.1.3 Heterogeneity

The relatively narrow parameter window within which coexistence can be modelled [383, 385, 398] can be expanded somewhat by introducing heterogeneity, for example, in du-

ration of carriage [385], host population structure [383, 386, 401], or rates of antibiotic consumption [386, 401]. Despite such improvements, existing models still indicate a delicate balance between resistance cost and antibiotic consumption within a single system.

1.6.1.4 Modelling competition

Colijn and colleagues modelled resistant and sensitive bacteria as separate strains, and showed that coexistence was promoted when the strains competed more strongly with themselves (within-strain competition) than with each other (between-strain competition) [383]. Without this, the principle of competitive exclusion applies: two strains competing for the same ecological niche cannot coexist [402].

Colijn *et al.* expressed this as a representation of strain-specific versus strain-non-specific immunity (where ‘strain’ was determined by resistance status), and postulated that an association between resistance and some pneumococcal serotypes, against which immunity can be specific [403], could result in within-strain competition outweighing between-strain competition [383]. Colijn *et al.* also showed that the extent of effective competition between resistant and sensitive strains affects the relationship between antibiotic consumption and resistance [404]. Subsequent work has adopted a similar structure, with bacterial strains - and therefore competition - being determined by resistance status [385, 386].

1.6.2 Other modelling approaches

The application of IBMs to AMR problems remains limited: another systematic review showed that only 12% of 273 models of AMR (in this case, including antibiotic, antiviral, antiparasitic and antifungal resistance) published between 2006 and 2016 used an IBM approach [387]. Publications applying IBMs to antibiotic resistance specifically were dominated by within-hospital studies, largely of MRSA [405]. The majority of studies explicitly investigating coexistence utilise compartmental models [383, 385, 386, 398, 400], although there are exceptions [401, 406].

1.7 Aims of this work

The over-arching aim of this work was to use mathematical and statistical modelling to better understand the relationships between antibiotic consumption and resistance in heterogeneous populations. In this thesis, I present a novel multi-strain model of AMR that achieves coexistence across a wide range of antibiotic consumption rates and fitness costs (Chapter 2), and assess its sensitivity to parameter variations and heterogeneity. I then extend this model to explore the effect of HGT on coexistence, and the impact of bacterial transmission between host populations (Chapter 3). In Chapter 4, I develop an algorithm for fitting this model to epidemiological data using likelihood-free inference (LFI), and show how it can replicate heterogeneities in real-world data. Finally, in Chapter 5, I use a statistical model to assess the associations between antibiotic consumption and resistance in different host populations.

Chapter 2

Results: Agent-based model (ABM)

2.1 Introduction

In Chapter 1, I described characteristics of AMR that are observed in natural populations, but are not well-captured in existing models: the ubiquity of coexistence and the persistence of AMR in antibiotic-free environments.

Coexistence remains a relatively rare outcome of existing models of AMR. In reality, however, coexistence is a remarkably common phenomenon, spanning multiple host and bacterial species, environments, and antimicrobial classes, as outlined in the Introduction (section 1.4.1.4). Coexistence also spans different genetic mechanisms of resistance. Within *S. pneumoniae* for instance, co-trimoxazole and beta lactam resistance arises from modification of the protein targets of the antibiotic [407, 408], tetracycline resistance is caused by the TetM protein inhibiting antibiotic binding [409], chloramphenicol and aminoglycoside resistance is the consequence of antibiotic modification [410, 411]. Macrolide resistance can also be caused by antibiotic modification, as well as antibiotic efflux [412]. Co-existence of sensitive and resistant phenotypes are observed across all these mechanisms.

The evidence also shows that antibiotic consumption varies widely between different antibiotic classes and host populations, as well as undergoing temporal variation between seasons and/or demonstrating longer-term trends [28, 53, 108, 244, 245, 252, 258, 268,

413]. It is highly unlikely that such a sensitive balance between consumption and cost, as indicated by existing models, is repeatedly attained in such diverse and overlapping systems. There is therefore a need for a model of AMR that achieves robust coexistence despite variation in antibiotic consumption and fitness cost.

The key to unlocking coexistence could be bacterial competition. In the Introduction, I outlined the importance of competition in maintaining diversity and stability in bacterial populations. I also indicated the ongoing influence of competition on the distribution of AMR observed in different ecosystems, for example, in the expansion or decline of resistant or susceptible clones [276, 277, 297–300]. Previous modelling work on AMR shows that competition between resistant and sensitive strains affects coexistence [383, 404], but only for a limited range of parameters. I argue that instead of using resistance status to define bacterial strains, as has been done previously [383, 385, 386, 404, 406], strains should be modelled according to other genetic features [414]. Competition between bacteria should be determined by how biologically similar they are, and therefore how likely they are to compete for resources. This has been demonstrated *in vitro* [415] and in population genetics, where acquisition and loss of resistance genes occurs independently of strain identity [277].

Distinguishing bacterial strains is not always straightforward. Often, variable phenotypes such as serotype will have a distribution that mirrors the underlying collection of strains, and the two can be treated as approximately synonymous [416]. In *S. pneumoniae*, for example, strains could be modelled as serotypes (which determine the primary interaction with the host immune system [403]), or by sequence clusters (which indicate genetic relatedness and imply phenotypic similarity [198]). If resistance is associated with strains that compete more strongly with themselves than with each other, strain-based competition should facilitate coexistence by allowing for frequency-dependent selection on AMR. Strain-based competition rather than resistant/sensitive competition should also allow for persistence of costly resistance genes when antibiotic selection pressure is reduced or absent (see 1.4.1), provided that strains compete more strongly with themselves than with other strains (i.e. competitive exclusion is avoided [197]).

Additionally, competition between bacteria colonising hosts should be modelled as occurring continuously, rather than solely at the moment of co-colonisation, as has been

done in previous models. Ongoing competition occurs via both exploitative and interfering mechanisms, as outlined in the Introduction (section 1.3.3). The strain-based competition structure proposed here also allows the cost of resistance to be imposed on competition fitness, i.e., the ability of an isolate to out-compete another within a host, as well as on transmission rate or duration of carriage, as has previously been modelled [383, 385]. Inclusion of both of these elements is supported by contemporaneous work by Davies *et al.* [406], who demonstrated that explicitly modelling within-host competition between two co-colonising strains (resistant and sensitive) produced widespread coexistence.

In the Introduction, I described how AMR prevalence can change in the short- and long-term in response to changing antibiotic consumption. These disparate temporal scales are difficult to reconcile within a single dynamic process, and do not fit into existing AMR models. Given the wide variety of aetiologies and epidemiologies described, different rates of AMR change in response to consumption change are to be expected. However, there is no obvious pattern across bacterial species, antibiotics, and host populations [227–229, 237, 255, 264, 265, 417–419].

The short- and long-term timeframes observed under different circumstances could represent two different manifestations of a key bacterial dynamic: competition. When there is a sudden increase in antibiotic consumption, there is a reduction in absolute numbers of susceptible bacteria (due to antibiotic activity) and an increase in resistant bacteria (which become relatively more fit). Increased antibiotic consumption increases the benefits of resistant cells relative to a fixed cost. If the increased consumption is transient, this temporary advantage is soon removed, and the bacterial population reverts to (or approaches) its previous equilibrium.

By contrast, longer-term increases in antibiotic consumption promote more lasting restructuring of the bacterial population. Any compensatory mutations that ameliorate the fitness costs associated with resistance [420–422] will aid this process. These long-term changes to the population structure would explain why interventions to reduce antibiotic consumption after periods of prolonged use can take several years to manifest in a reduction in AMR [102, 108], as I outlined in 1.4.1. Similar timeframes have been observed for large-scale population changes in *S. pneumoniae* after the introduction of vaccination [423, 424].

Simulating complex dynamics between different combinations of hosts, bacterial strains,

resistance genes, and MGEs (see Chapter 3) would rapidly become unmanageable using a compartmental model. I developed an agent-based model (ABM) to simulate continuous, strain-based competition, and assess its effect on resistance frequency and the occurrence of coexistence. This model is highly flexible while preserving an essential criterion of coexistence modelling: structural neutrality [384].

2.2 Methods

An agent-based model was constructed in C++. The model does not distinguish between non-pathogenic carriage and invasive disease and therefore the terms ‘infection’ and ‘colonisation’ are used interchangeably. All code is stored in the repository <https://github.com/tamsindewe/Thesis.git>.

2.2.1 Model components

The model includes three types of agent: hosts, bacteria, and genes.

Hosts are characterised by rates of entry into, and departure from, the population of interest μ , and by the population’s background rate of antibiotic consumption α . They are assumed to mix randomly within a single population. Hosts are also characterised by their infection status: they can either be uninfected, or colonised by one or more bacterial populations.

Bacteria exist within hosts and are characterised by their strain, which represents a distinct genotype. Each bacterial strain i is assigned individual properties: transmission rate (β_i); natural rate of clearance by the host immune response (γ_i); and competition fitness (f_i). Bacteria are not modelled as individual cells, but rather, a single agent which represents a population of cells of that type within a single host. Infection of a host by a ‘bacterium’ therefore represents successful colonisation by that clone.

Bacterial properties can be modified by the presence of additional genes. These genes are modelled as agents that represent a gene’s presence within a given bacterial population colonising a host. Genes can confer antibiotic resistance and/or an associated fitness cost on the bacterial agent. This fitness cost c was modelled as a reduction in: bacterial transmission rate (c_β); duration of carriage (by increasing the rate of immune clearance,

c_γ); or competition fitness (c_f). A change in state of a bacterial agent of strain i from susceptible (S) to resistant (R) therefore resulted in new parameters:

$$\beta_{i,R} = \frac{\beta_{i,S}}{1 + c_\beta} \quad (2.1)$$

$$\gamma_{i,R} = \gamma_{i,S}(1 + c_\gamma) \quad (2.2)$$

$$f_{i,R} = f_{i,S}(1 - c_f) \quad (2.3)$$

2.2.2 Model processes and concepts

Bacterial agents infect new hosts by cloning themselves, along with their properties and any acquired genes. These clones attempt transmission independently of the colonisation or treatment status of the new host. No structural restrictions are placed on host superinfection, which can include superinfection with bacteria of the same strain. The infectivity of each bacterial agent within a co-colonised host is decreased proportionate to the number of co-colonising populations, such that the overall infectivity of the host is not increased by superinfection with multiple bacterial strains. Antibiotic consumption occurs randomly at a given rate, and is independent of colonisation status. Antibiotic consumption by an individual host clears any resident susceptible bacteria and blocks new infections by sensitive strains within that time step.

Bacterial agents compete directly with others. Competition is a continuous process, where bacteria already co-colonising the same host try to eliminate each other. Pair-wise interactions between all bacteria resident in a host are conducted in random order. Competition involves two probabilities for each pair-wise interaction: the probability that a competitive event occurs (i.e. one bacterial agent is eliminated, rather than both allowed to co-exist); and the probability of a given agent winning this encounter.

The probability of competition occurring p_c between two bacterial agents depends on whether the bacteria belong to the same (within-strain competition, $p_c = p_w$) or different (between-strain competition, $p_c = p_b$) strains. The probability of a given bacterial agent remaining in a co-colonised host in a given time step (p_a) is:

$$p_a = \prod_{k=1}^{n-1} \left[1 - p_c \left(\frac{f_k}{f_r + f_k} \right) \right] \quad (2.4)$$

where n is again the number of isolates infecting the host, and f_r and f_k are the competition fitnesses of the target isolate and its co-colonising isolates, respectively.

2.2.3 Scheduling

The model is stochastic and runs in discrete time. For every process occurring at rate r , the probability p of it happening within a given time step is:

$$p = 1 - e^{-rt} = 1 - e^{-rl/365} \quad (2.5)$$

where t is time in years and l is the length of the chosen time step, in days. Occurrence of events within the time step is stochastic: the event is actualised if a random number drawn from a uniform distribution $U(0, 1)$ is less than the calculated value for p .

Within each time step, a series of processes occur, either internal to individual hosts or external. Initial external processes are conducted first. The model then loops through every individual host to complete within-host processes, before performing additional external processes. These processes are summarised below, and in Fig. 2.1.

1. External processes:

- (a) Seeding of any bacteria introduced at this time step (see 2.2.4);
- (b) Identification of hosts treated with antibiotics;
- (c) Identification of hosts leaving the population (e.g. through death or ageing-out);

2. Internal processes:

- (a) Identification and implementation of bacterial clearance:
 - i. Clearance by host immune system;
 - ii. Clearance by antibiotics;
 - iii. Clearance by competition;
- (b) Identification of new infections.

3. External processes:

- (a) Implementation of new infections;
- (b) Implementation of hosts leaving the population;
- (c) Implementation of new hosts entering the population.

2.2.4 Model initialisation

Hosts were initialised at the beginning of the model's run time, and infected at random at the desired time step with bacterial agents of distinct strains, according to the starting prevalence of each strain. Resistance genes, if present, were seeded into bacterial agents according to the desired distribution. In strain-associated resistance, resistance was seeded into some strains, but not others, and was fixed within the strains in which it was present. When resistance was not strain-associated, it was seeded into all strains, but at a low frequency in each (Fig. 2.2).

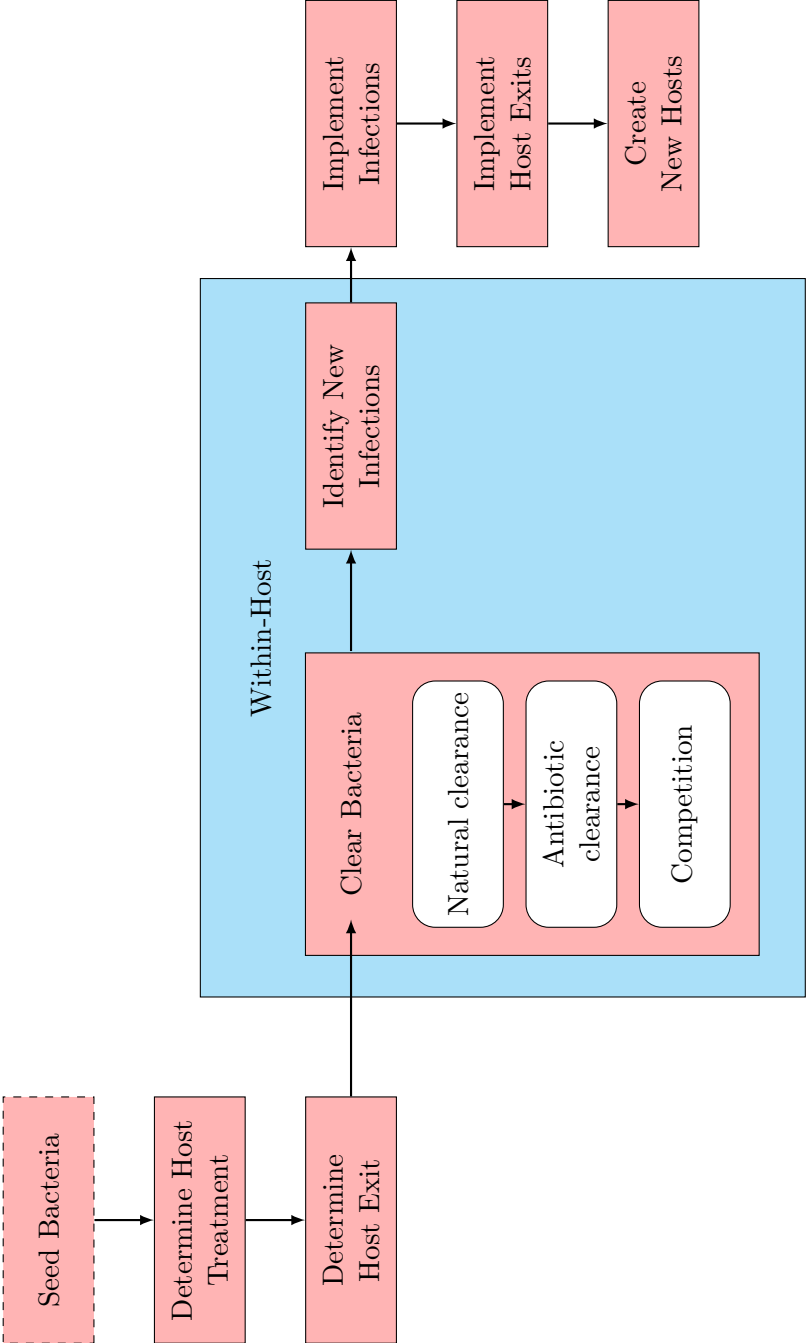


Figure 2.1: **Model workflow.** Processes performed during a single time step.

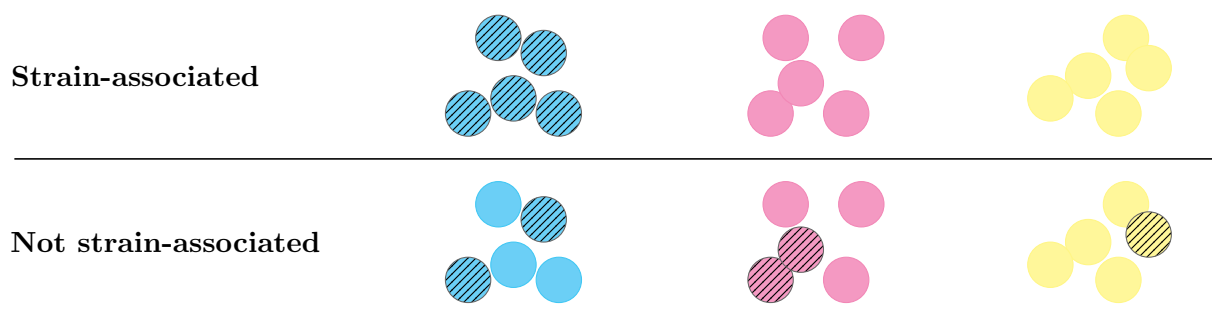


Figure 2.2: **Distribution of resistance in strains at model initialisation.** Bacterial agents (circles) belonging to different strains (colours) are seeded with resistance (shading) at model initialisation. Top: resistance is seeded within the blue strain only, and is fixed within that strain. Bottom: resistance is seeded at low frequencies in all strains.

2.2.5 Parameterisation

Preliminary simulations were performed to determine burn-in period (5 years), length of time step (1 week), and number of hosts (100,000). Model run-time was 100 years. As the antibiotic era is less than 100 years old, and resistance dynamics seem to have stabilised during periods when prescribing has been constant, this should be sufficient time for a biologically plausible representation of the system to reach equilibrium.

The model was broadly parameterised for *S. pneumoniae*, an important human respiratory commensal and opportunistic pathogen, and the target species for previous models of coexistence [383, 385, 386, 401, 404]. The natural history and population genetics of *S. pneumoniae* are also well-characterised [425–427]. While not zoonotic, this organism shares key features with other commensals, including zoonoses: it is highly recombinogenic, and has a multi-strain population structure. A broad range of parameter values were explored. Initial, non-strain-specific parameters are shown in Table 2.1. The parameters were subsequently restricted to investigate specific behaviours of interest using different scenarios.

2.2.5.1 Homogeneous strains

Strain properties The model was seeded with a given number of identifiable strains, initially with identical properties (Table 2.2). Both two- and multi-strain systems were explored.

	Parameter	Value	Reference
μ	Rate of host exit and replacement	$\frac{1}{5}$	<i>S. pneumoniae</i> is carried at much higher prevalences in infants and young children under the age of 5 [426, 428]
p_w	Probability of within-strain competition	0.0-1.0	-
p_b	Probability of between-strain competition	0.0-1.0	-
α	Rate of antibiotic consumption (courses/host/year)	0.0-5.0	Assuming 1 course is equivalent to 7 DDD, this exceeds the range of total antibiotic consumption reported at national level [114, 429]

Table 2.1: **Model parameters.**

	Parameter	Value	Reference
n	Number of strains	2-20	Expansion of pre-existing models [383–386, 398, 400, 406]
R_0	Basic reproduction number	2.0-5.0	Approximate range in Europe [430], allowing for loss due to competition
D	Duration of carriage	1-4 months	Typical range of carriage duration reported for pneumococcal serotypes [427]
γ	Rate of natural clearance	$\frac{12}{D}$	-
β	Rate of bacterial transmission	$R_0\gamma$	-
f	Competition fitness	1.0	-
r	Proportion of hosts infected with each strain at initialisation	0.1	-

Table 2.2: **Strain-specific parameters.**

Distribution	Number of strains with resistance	Prevalence of resistance within each strain
Strain-associated (single strain)	1	1
Strain-associated (multiple strains)	$\frac{n}{2}$	1
Non-strain-associated	n	0.1

Table 2.3: **Distribution of resistance at initialisation.**

	Parameter	Value	Reference
c_f	Cost on competition fitness	0.0-1.0	Possible range
c_β	Cost on transmission parameter	0.0-1.0	Wider range than previously shown to maintain coexistence [383]
c_γ	Cost on duration of carriage	0.0-1.0	Wider range than previously shown to maintain coexistence [385, 401]

Table 2.4: **Resistance parameters.** Different types and magnitude of fitness costs.

Resistance properties Simulations were parameterised with a single resistance gene. Resistance was distributed in a strain-associated or non-strain-associated manner (see section 2.2.4), as summarised in Table 2.3, and could impose a cost (Table 2.4).

2.2.5.2 Heterogeneous strains

The impact of bacterial heterogeneity on coexistence was examined by introducing variation in either the competition fitness of individual strains, or the rates of transmission and natural clearance. Some general parameters were adjusted based on the results of the previous section (Table 2.5), otherwise as before (Table 2.2).

Effect of competition fitness All strains had identical rates of transmission and natural clearance, such that:

$$\gamma_{i=1}, \gamma_{i=2}, \dots, \gamma_{i=n} = \bar{\gamma} \quad (2.6)$$

$$\beta_{i=1}, \beta_{i=2}, \dots, \beta_{i=n} = \bar{R}_0 \bar{\gamma} \quad (2.7)$$

Inherent competition fitness f_i of each individual strain i was adjusted to range between 1 (the default) and 0.6. Strains were matched so that each pair of strains had identical properties, and are described for 10 strains in Table 2.6.

	Parameter	Value	Reference
n	Number of strains	10, 20	Results of previous section
p_w	Probability of within-strain competition	1.0	Results of previous section
p_b	Probability of between-strain competition	0.0-0.5	Results of previous section
$\bar{R}_0$	Mean basic reproduction number	3.0	Results of previous section
$\bar{\gamma}$	Mean rate of natural clearance	6.05	Inverse of median carriage duration of the 10 most prevalent serotypes in the Maela dataset [427]
α	Rate of antibiotic consumption	0.0-2.5	Exceeding range of total antibiotic consumption reported at national level [114, 429]

Table 2.5: **Model parameters: heterogeneous strains.** All other parameters as per Table 2.2.

Parameter	A, F	B, G	C, H	D, I	E, J
γ_i	6.054	6.054	6.054	6.054	6.054
R_{0_i}	3.000	3.000	3.000	3.000	3.000
f_i	1	0.9	0.8	0.7	0.6

Table 2.6: **Strain-specific parameters for 10 matched strains with heterogeneous competition fitness.**

Resistance was initialised as previously described (Tables 2.3 and 2.4).

Effect of epidemiological parameters Heterogeneity of epidemiological parameters was introduced into n strains, by generating an evenly-spread range of values for γ_i , such that:

$$\frac{1}{n} \sum_{i=1}^n \gamma_i = \bar{\gamma} \quad (2.8)$$

Because continuous competition disproportionately increases the rate of clearance of longer-duration strains, this heterogeneity in γ_i necessitated the introduction of simultaneous heterogeneity in R_{0_i} , to maintain strain diversity. A range of values for R_{0_i} was generated for each strain, such that:

$$\frac{1}{n} \sum_{i=1}^n R_{0_i} = \bar{R}_0 \quad (2.9)$$

Sets of strains were created for simulations where $n \in \{10, 20\}$ and each individual strain had unique properties (Tables 2.7 and 2.8, respectively). Resistance was initialised

Parameter	A	B	C	D	E	F	G	H	I	J
γ_i	4.036	4.485	4.933	5.381	5.830	6.278	6.727	7.175	7.624	8.072
R_{0_i}	3.250	3.194	3.139	3.083	3.028	2.972	2.917	2.861	2.806	2.750
f_i	1	1	1	1	1	1	1	1	1	1

Table 2.7: **Strain-specific parameters for 10 unique strains with heterogeneous carriage duration ($1/\gamma$) and R_0 .**

Parameter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
γ_i	4.036	4.249	4.461	4.673	4.886	5.098	5.311	5.523	5.735	5.948	6.160	6.373	6.585	6.798	7.010	7.222	7.435	7.647	7.860	8.072
R_{0_i}	3.250	3.224	3.197	3.171	3.145	3.118	3.092	3.066	3.039	3.013	2.987	2.961	2.934	2.908	2.882	2.855	2.829	2.803	2.776	2.750
f_i	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 2.8: **Strain-specific parameters for 20 unique strains with heterogeneous carriage duration ($1/\gamma$) and R_0 .**

as previously described (Tables 2.3 and 2.4).

Effect of initial resistance prevalence To assess the effect of initial conditions on the frequency of resistance, further simulations were performed on strain-associated resistance within a 10-strain system. For these simulations, resistant strains (strains A-E) were paired with individual sensitive strains (F-J), so that they differed only in their resistance status. Pairs of matched strains were formed, with each pair sharing identical epidemiological properties (Table 2.9). Different starting prevalences of resistant and sensitive strains were tested (Table 2.10).

Effect of the proportion of resistant strains The effect on resistance frequency and coexistence of the proportion of strains with which resistance is associated, was explored further. The same strain-specific parameters γ_i and R_{0_i} outlined in Table 2.9 were retained, and each strain was seeded into 10% of hosts. Resistance was strain-associated and distributed into: a single strain (variously, A, C or E; overall resistance prevalence ≈ 0.10); five strains (A-E; overall resistance prevalence ≈ 0.5); or all except one strain

Parameter	A, F	B, G	C, H	D, I	E, J
γ_i	4.036	5.045	6.054	7.063	8.072
R_{0_i}	3.250	3.125	3.000	2.875	2.750
f_i	1	1	1	1	1

Table 2.9: **Strain-specific parameters for 10 matched strains with heterogeneous carriage duration ($1/\gamma$) and R_0 .**

Scenario	Individual strain prevalence (A-E)	Individual strain prevalence (F-J)	Overall resistance prevalence
1	0.02	0.18	≈ 0.1
2	0.10	0.10	≈ 0.5
3	0.18	0.02	≈ 0.9

Table 2.10: **Effect of initial resistance prevalence: distribution of strains (and resistance) at initialisation.** Strains A-E are fully resistant; strains F-J are fully susceptible. Individual strain prevalence shows the proportion of hosts seeded with that strain.

(excluding A, C, or E; overall resistance prevalence ≈ 0.90).

2.2.6 Model behaviour

Multiple-node Windows machines on the Department of Infectious Disease Epidemiology computing cluster were used to perform large-scale scans of the parameter space and explore model behaviour. All data analysis and visualisation was performed in R [431]. Particular attention was paid to the conditions necessary to maintain strain diversity, which is a key characteristic of many bacterial populations, and to coexistence of resistant and sensitive strains.

2.3 Results

A note on terminology: ‘co-colonisation’ is defined as colonisation of an individual host with two or more bacterial agents at the same time. I use ‘strain diversity’ and ‘coexistence’ to refer to the stable persistence of unique bacterial strains, and resistant and sensitive bacteria, respectively, at the population level.

2.3.1 Homogeneous strains

2.3.1.1 System characteristics

Two-strain system Within- and between-strain competition and R_0 all interact to determine the prevalence of infection (Fig. 2.3). Prevalence increases when R_0 increases or the probability of competition decreases. In a two-strain system, prevalence of infection is more sensitive to the probability of within-strain competition p_w than to the probability of between-strain competition p_b .

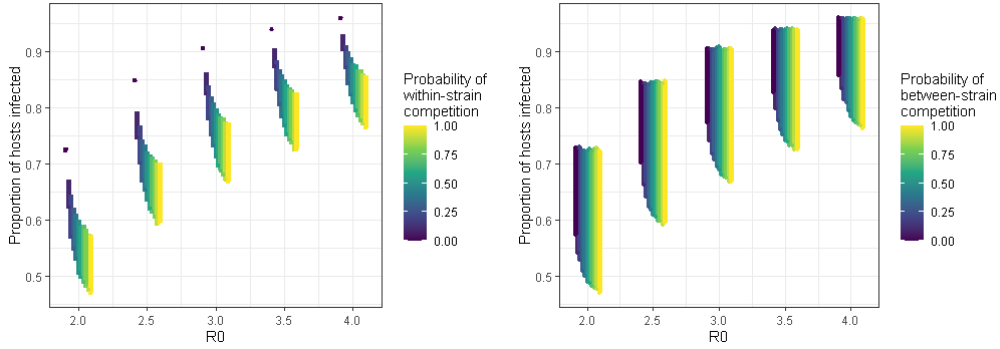


Figure 2.3: **Sensitivity of infection prevalence to competition and R_0 in a two-strain system.** **Left.** Varying between-strain competition probabilities between 0 and 1, for given within-strain competition probabilities (shown by colour). **Right.** Varying within-strain competition probabilities between 0 and 1, for given between-strain competition probabilities (shown by colour). $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

Multi-strain system The impact of within- and between-strain competition on several key system characteristics are shown in Figs. 2.4 and 2.5, and compared to results from two-strain simulations. Strain diversity is the proportion of strains at initialisation remaining at equilibrium; multi-agent infection is the proportion of infected hosts carrying more than one bacterial agent; and multi-strain infection is colonisation with more than one identifiable bacterial strain. In a two-strain system, the proportion of hosts infected with multiple unique strains is insensitive to variations in within-strain competition, provided that $p_w > p_b$ (see Appendix, Fig. 6.1).

These relationships change most dramatically when strain numbers are increased from two to 10, especially at lower probabilities of between-strain competition. Under these conditions, bacterial agents are less likely to encounter (and compete with) agents belonging to the same strain than they are in a two-strain system; more bacteria are therefore retained within the system.

2.3.1.2 Strain dynamics

Two-strain system Initial simulations performed using two unique strains, with identical parameterisation, showed the impact of competition parameters on strain dynamics. In this model, the probabilities of within- and between-strain competition affect strain diversity. When within-strain competition is large and between-strain competition is small, homogenous strains are stably maintained within the system (Fig. 2.6). When within-

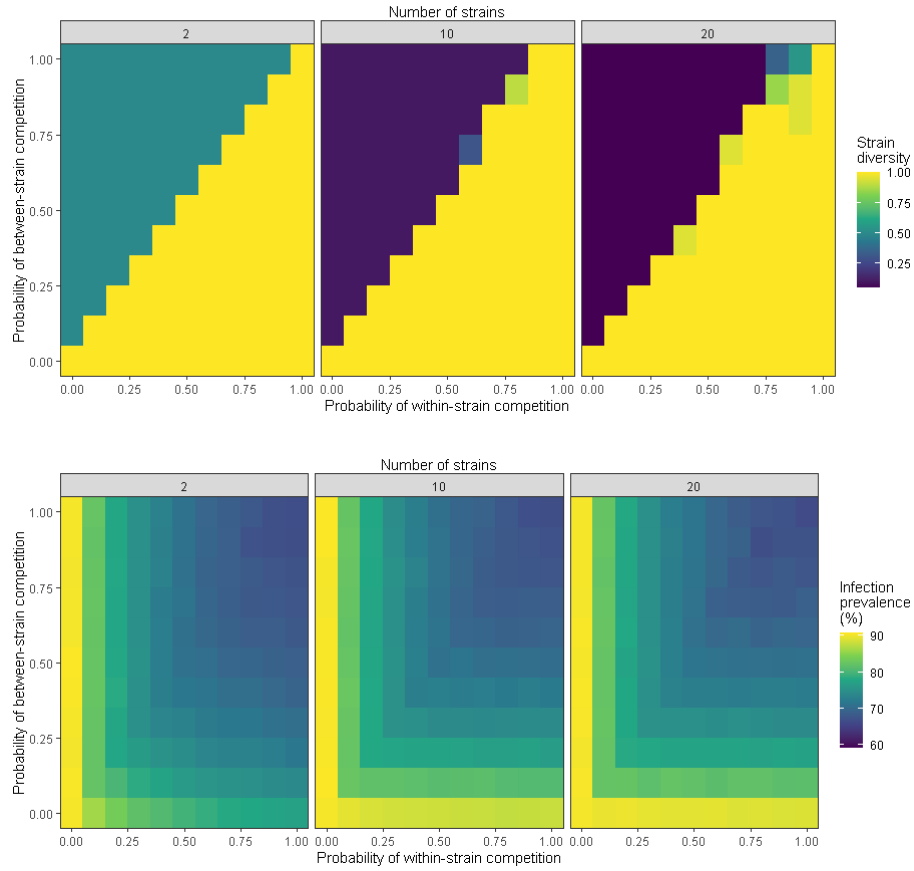


Figure 2.4: **Effect of competition probabilities on system characteristics.** Strain diversity (top), infection prevalence (bottom). $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

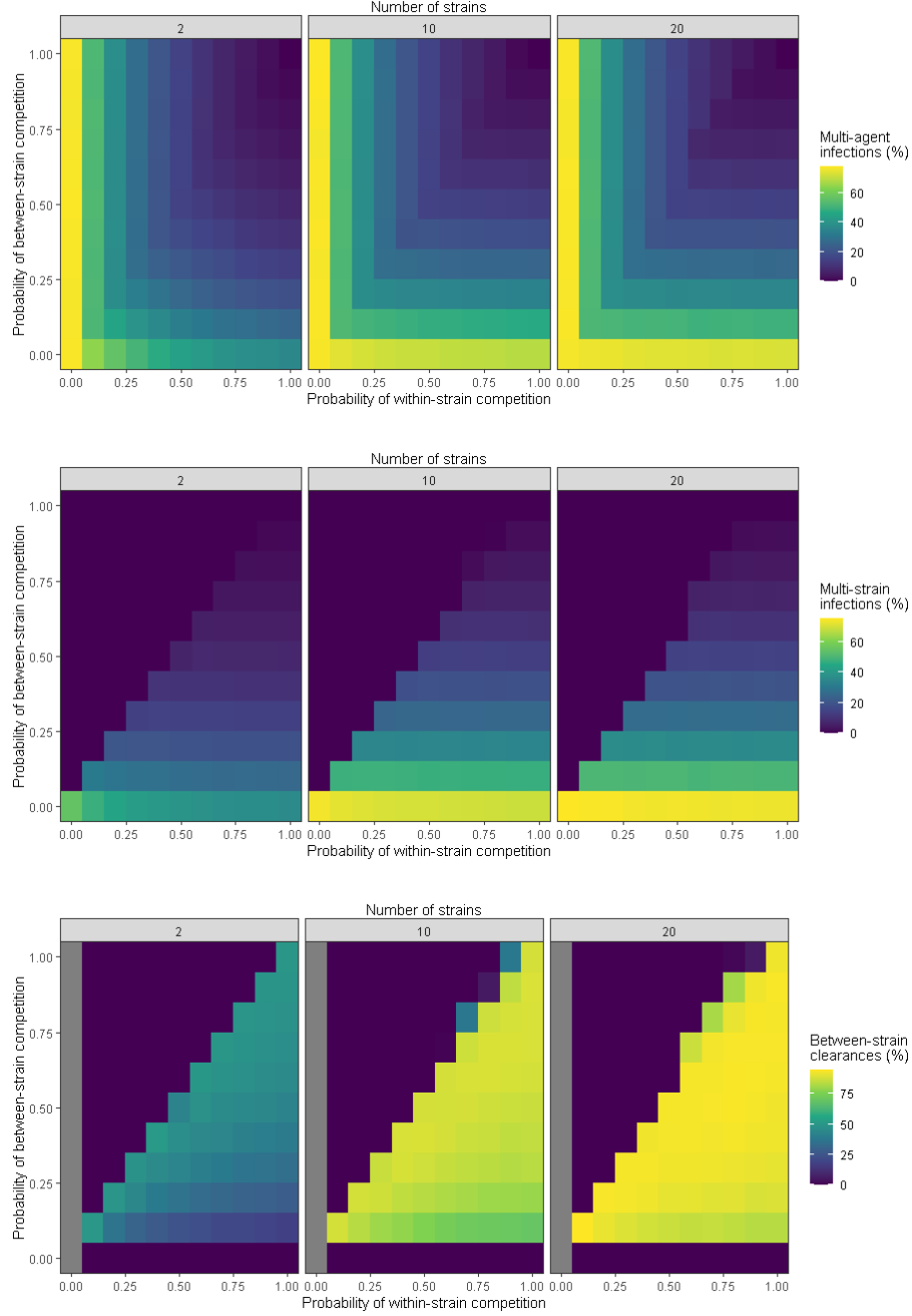


Figure 2.5: **Effect of competition probabilities on system characteristics.** Colonisation with multiple bacterial agents (top), colonisation with multiple distinct strains (middle), and proportion of clearances attributable to between-strain competition (bottom) in systems with different numbers of strains. $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

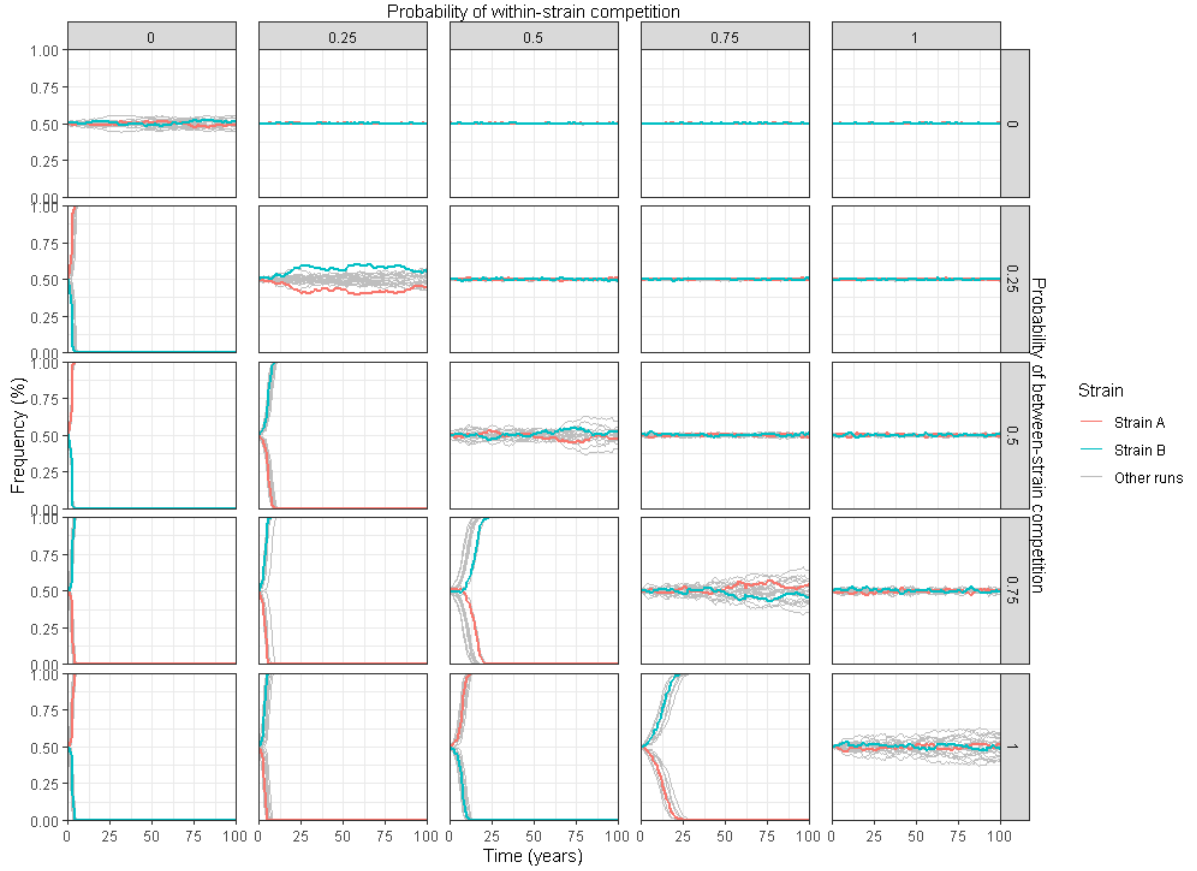


Figure 2.6: **Dynamics of a homogenous two-strain bacterial system in a host population of 100,000.** Frequencies of individual strains in a single simulation are shown in colour. Frequencies of both strains in additional simulations are shown in grey. Results of 10 simulations. $n = 2$, $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

strain competition is equal to between-strain competition, the dynamics are less stable, but both strains coexist over the run time of the model (here, 100 years), in a host population of 100,000. When within-strain competition is considerably less than between-strain competition, one of the strains rapidly goes to fixation over the course of the simulation. The time taken for fixation to occur decreases with decreasing within-strain competition. It also decreases with smaller population size (see Appendix, 6.2).

It is also clear from this plot that for a given probability of within-strain competition, increasing between-strain competition destabilises the frequencies of individual strains; conversely, for a given probability of between-strain competition, increasing within-strain competition stabilises the frequencies.

These patterns are captured in Fig. 2.7, which shows how competition affects the dis-

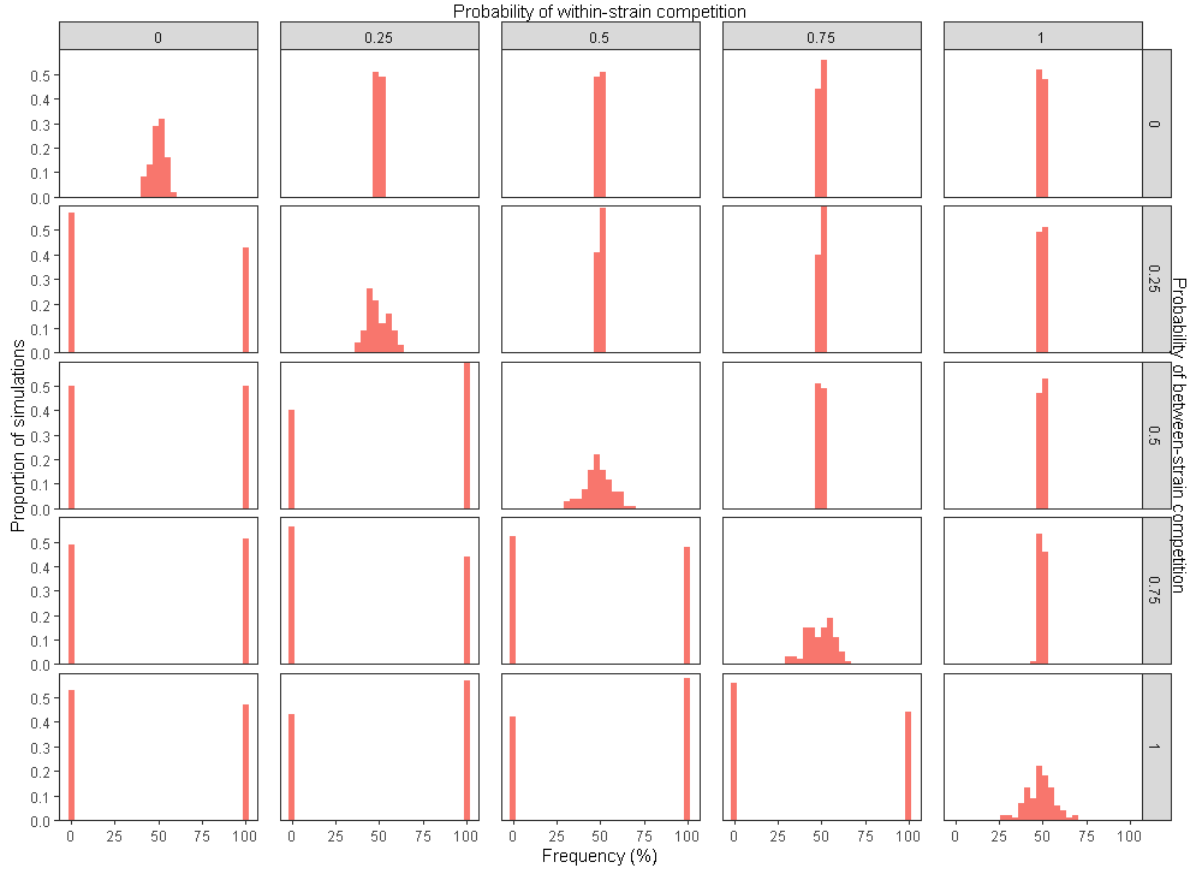


Figure 2.7: **Distribution of the frequency of one strain.** In a host population of 100,000, after 100 years of simulation time. Results of 100 simulations. $n = 2$, $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

tribution of the frequency of an individual strain after 100 years of simulation time. When within-strain competition exceeds between-strain competition, the distribution of individual strain frequencies are centred around 50%. When between-strain competition exceeds within-strain competition, individual strain prevalences follow a bimodal distribution (either 0% or 100%).

Multi-strain system Dynamics are similar within a multi-strain system: one strain goes to fixation when between-strain competition exceeds within-strain competition. The remaining strains are eliminated. Equilibrium frequencies of individual strains differ according to the number of strains in the system: under conditions where 10 strains are stably maintained, frequencies of individual strains centre around 10% (see Appendix, Fig. 6.3); whereas 20 stably-maintained strains centre on frequencies of 5% each.

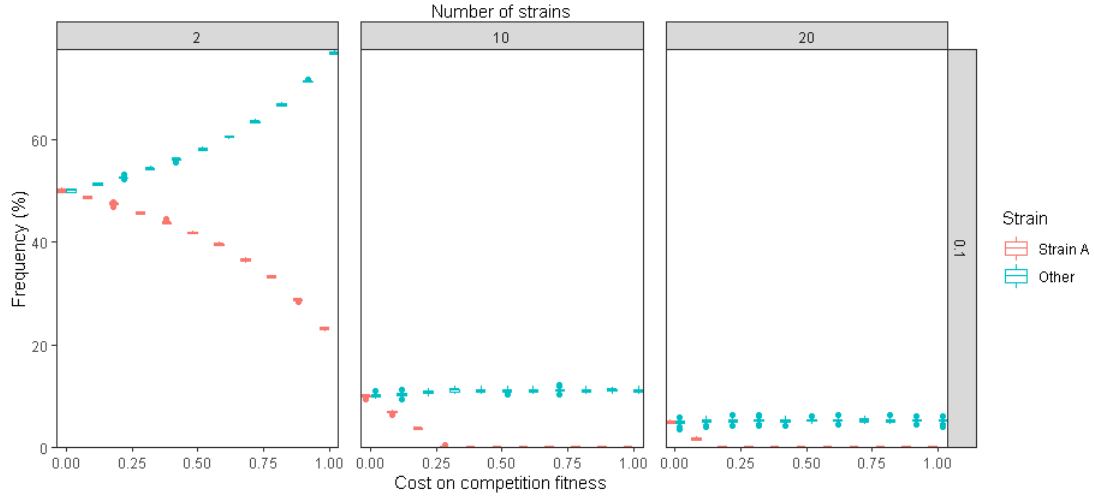


Figure 2.8: **Strain-based competition enables less fit strains to persist in multi-strain populations.** Number of strains in the system shown along top. Strain diversity is maintained in two-strain systems even when cost on competition fitness is maximal; multi-strain systems are more sensitive. Results of 10 simulations. $p_w = 1, p_b = 0.1, \gamma = 6.05, R_0 = 3, \alpha = 0$.

2.3.1.3 Effect of cost without antibiotics

A cost was introduced on competition fitness under conditions where strain dynamics are stable and diversity is maintained (probability of within-strain competition is high; probability of between-strain competition is low). In the absence of antibiotic consumption, strain diversity was maintained across the range of possible costs on competition fitness within a two-strain system. Within multi-strain systems, strain diversity was not maintained across the full range of costs (Fig. 2.8). Sensitivity of strain diversity to a cost on competition fitness increased with increasing strain complexity. This is because as strain numbers increase, the proportion of clearances attributable to between-strain competition increases (see Fig. 2.5, bottom panel).

2.3.1.4 Coexistence

The results above show that within-strain competition needs to exceed that of between-strain competition for strain diversity to be achieved. Strain diversity increases as within-strain competition increases. Retaining strain diversity is essential to effective modelling of resistance in complex systems; results in this section therefore focus on simulations

where within-strain competition is maximal ($p_w = 1$).

Two-strain system I have assumed that one course of antibiotics lasts 7 days, and therefore that 5 antibiotic courses per year in this model is equivalent to 96 DDD/1000/day. The range of antibiotic consumption shown here, as defined by the model parameterisation, is more than double that reported globally [114, 429]. Fig. 2.9 demonstrates coexistence across a very large range of values for antibiotic consumption and cost on competition fitness, when resistance is strain-associated and between-strain competition is low ($p_b \in \{0, 0.1\}$, left panel, top and middle). Unsurprisingly, coexistence is unaffected by cost on competition fitness when resistance is strain-associated and competition between strains is absent ($p_b = 0$).

By contrast, when resistance is not strain-associated (right panel), coexistence is achieved in a much narrower parameter window. The frequency of coexistence under these conditions is also much less sensitive to the probability of between-strain competition.

The effect of strain-associated resistance on coexistence is smaller at higher probabilities of between-strain competition ($p_b = 0.5$, Fig. 2.9, bottom panel). Here, competitive exclusion of strains is more likely. When resistance is strain-associated, the resistant strain is much more likely to be eliminated (when there is a strong net cost of resistance) or reach fixation (when there is a strong net benefit). This is reflected in the smaller proportion of hosts co-colonised with resistant and sensitive bacteria as between-strain competition increases (Fig. 2.10, bottom).

Multi-strain system Results are broadly similar when $n = 10$ (Fig. 2.11) and $n = 20$: coexistence is achieved over a large parameter space when resistance is strain-associated and competition probabilities maintain strain diversity (Fig. 2.12).

These results also show when conditions are favourable to coexistence, coexistence becomes less frequent as strain numbers increase (i.e. the bacterial population becomes more complex) (Fig. 2.13, top panel). The impact of within-strain competition decreases as strain numbers increase. Fig. 2.13, bottom panel, shows the proportion of successful competition events (clearance of a bacterial agent from a host due to competition) attributable to within-strain competition for simulations with different numbers of strains.

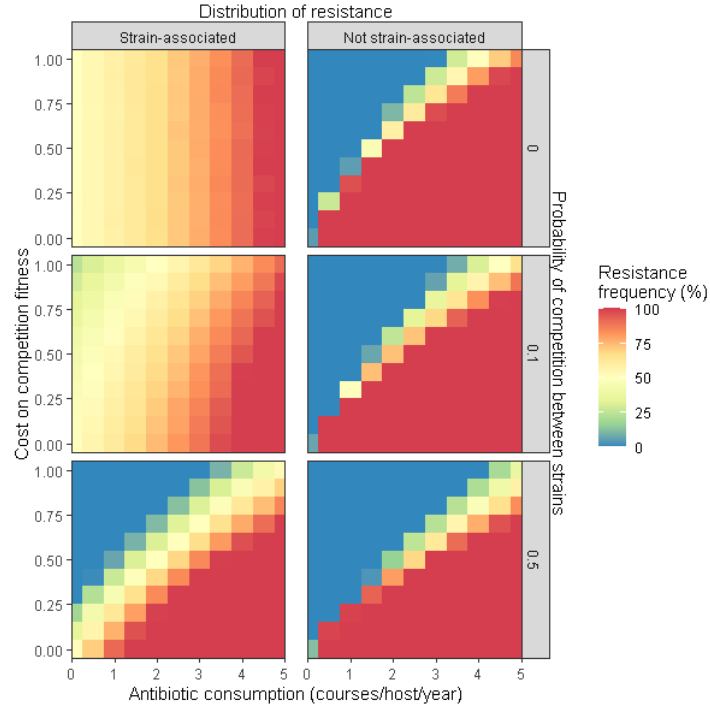


Figure 2.9: **Coexistence of resistant and sensitive bacteria in a two-strain system.** Frequency of resistance after 100 years of simulation time. $n = 2$, $\gamma = 6.05$, $R_0 = 3$, $p_w = 1$.

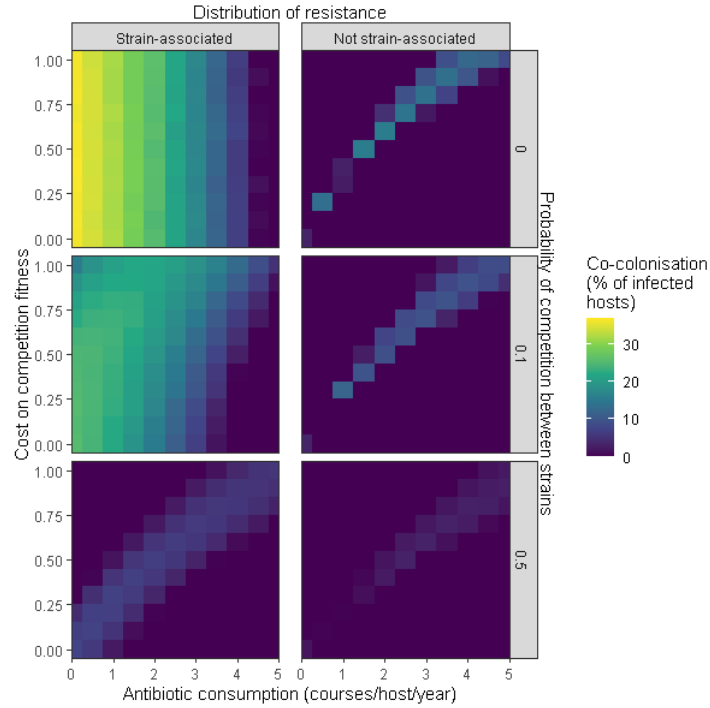


Figure 2.10: **Co-colonisation of hosts with resistant and sensitive bacteria in a two-strain system.** Percentage of infected hosts carrying resistant and sensitive bacteria after 100 years of simulation time. $n = 2$, $\gamma = 6.05$, $R_0 = 3$, $p_w = 1$.

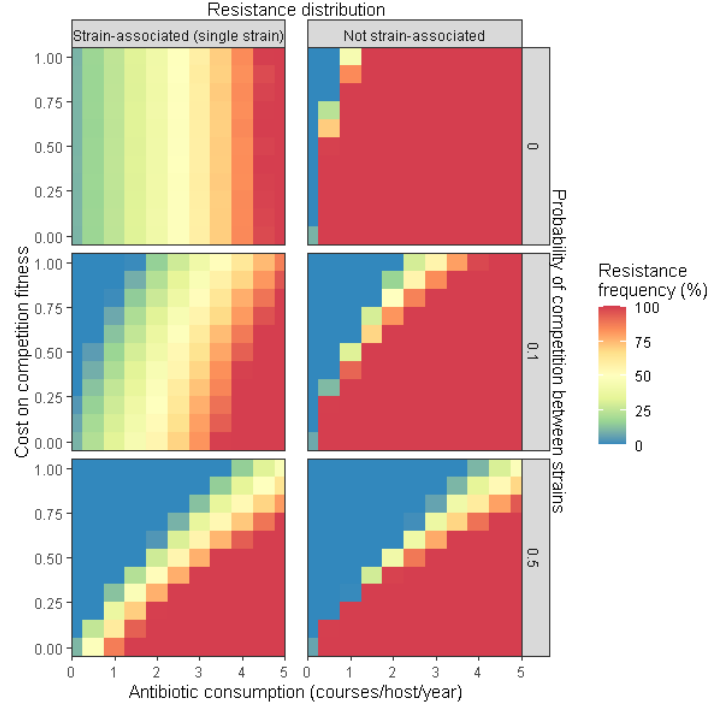


Figure 2.11: **Coexistence of resistant and sensitive bacteria in a 10-strain system.** Frequency of resistance after 100 years of simulation time. $n = 10$, $\gamma = 6.05$, $R_0 = 3$, $p_w = 1$.

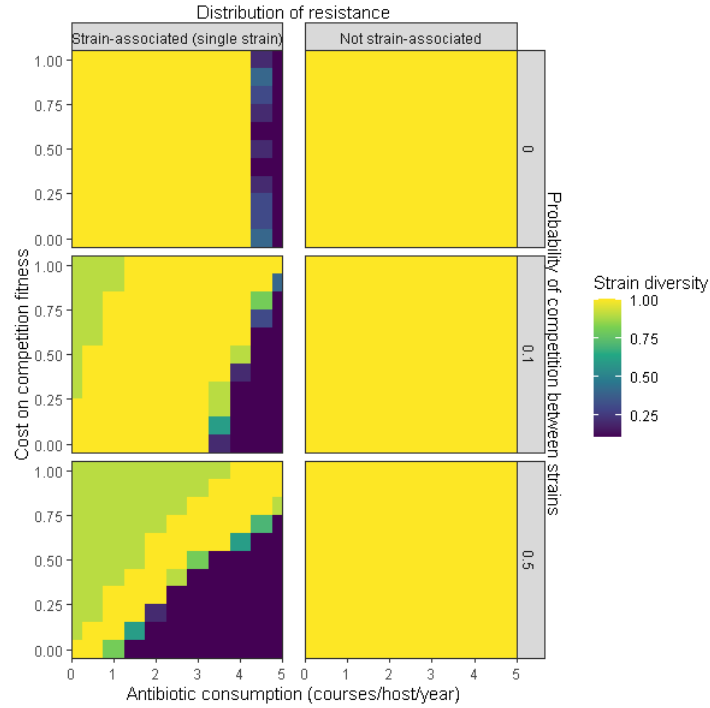


Figure 2.12: **Diversity of bacterial strains in a 10-strain system.** Proportion of strains remaining after 100 years of simulation time. $n = 10$, $\gamma = 6.05$, $R_0 = 3$, $p_w = 1$.

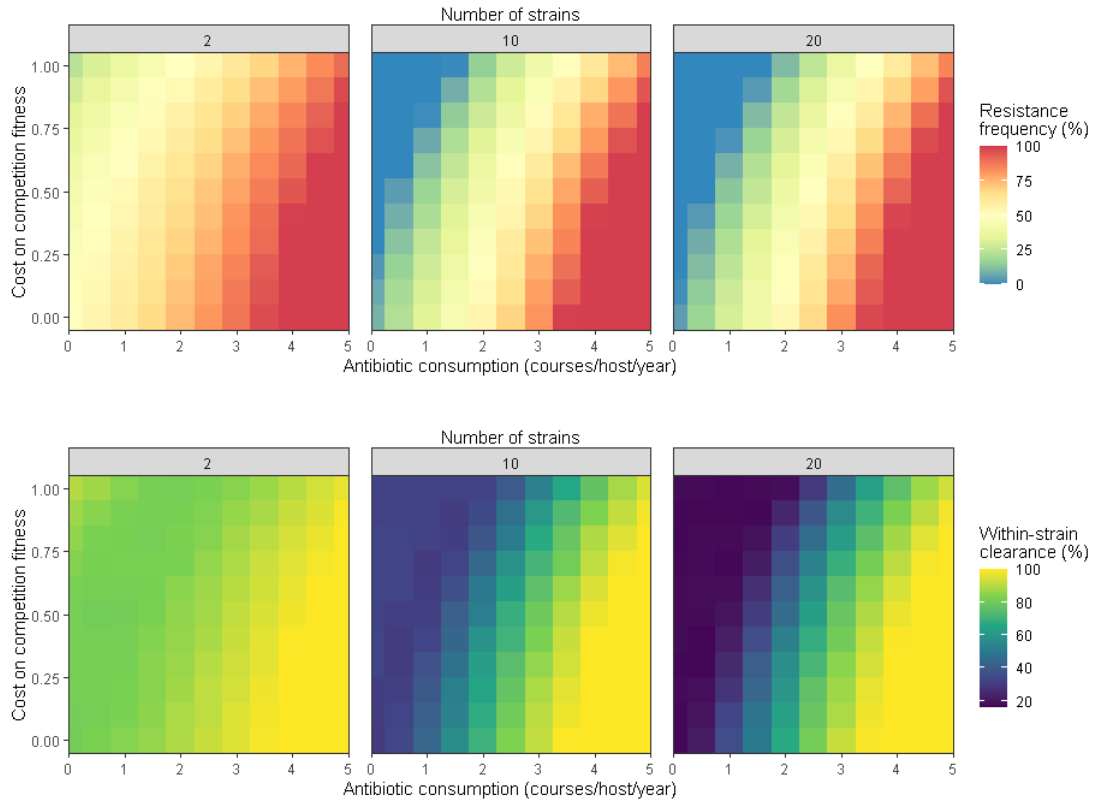


Figure 2.13: **Effect of number of strains on coexistence.** Frequency of resistance after 100 years of simulation time (top) and proportion of competitive clearances attributable to within-strain competition (bottom). Resistance is associated with a single strain. $\gamma = 6.05$, $R_0 = 3$, $p_w = 1$, $p_b = 0.1$.

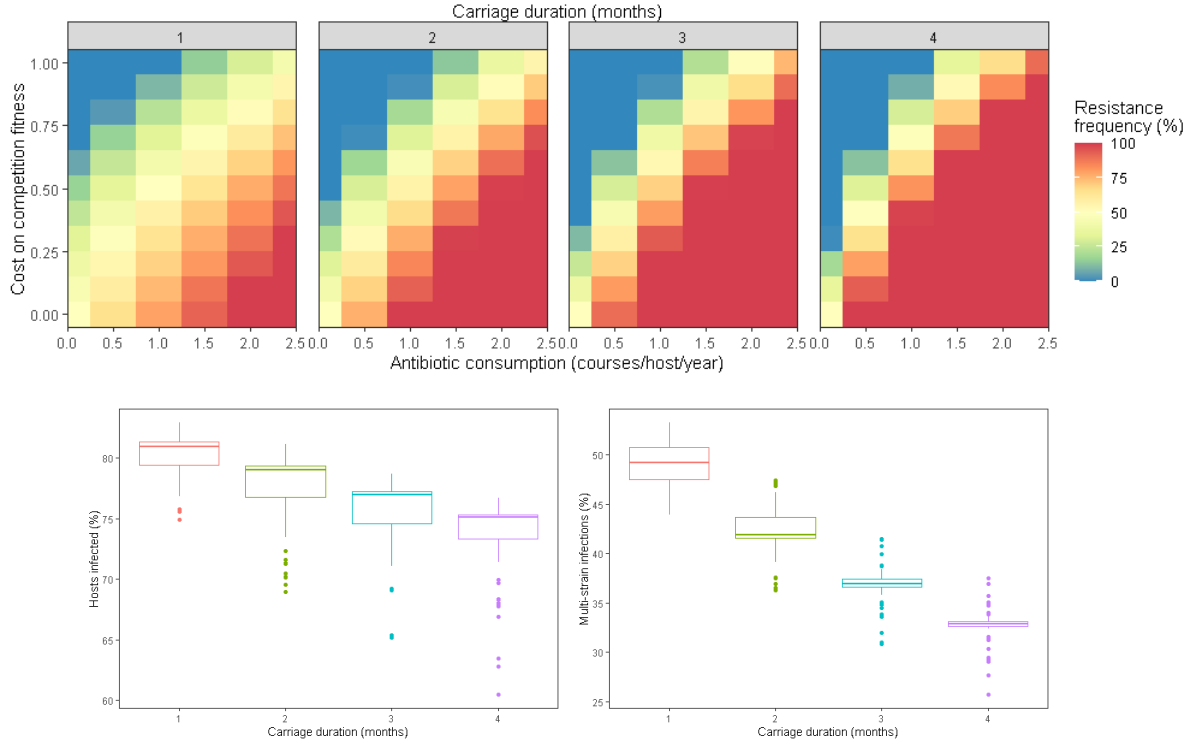


Figure 2.14: **Effect of carriage duration on coexistence.** Frequency of resistance after 100 years of simulation time (top); percentage of hosts infected (bottom left) and percentage of infections involving multiple strains (bottom right). $n = 10$, resistance associated with 50% of strains. $\gamma = 6.05$, $p_w = 1$, $p_b = 0.1$. Bottom row: $c_f \in \{0, 0.1, \dots\}$ and $\alpha \in \{0, 0.5, \dots, 2.5\}$.

Effect of epidemiological parameters Additional simulations were performed where resistance was associated with 50% of strains, over a more realistic range of rates of antibiotic consumption. Overall, increasing the number of strains with which resistance is associated from 1 to $n/2$ reduced the range of antibiotic consumption rates at which coexistence was observed. This result is explored further in section 2.3.2.3.

These simulations showed that coexistence becomes more frequent as carriage duration decreases (Fig. 2.14, top) or R_0 increases (Fig. 2.15, top). With decreasing carriage duration or increasing R_0 , infection prevalence increases, as does the percentage of hosts carrying multiple strains. The percentage of infected hosts co-colonised with resistant and sensitive strains and the contribution of within-strain competition also increase.

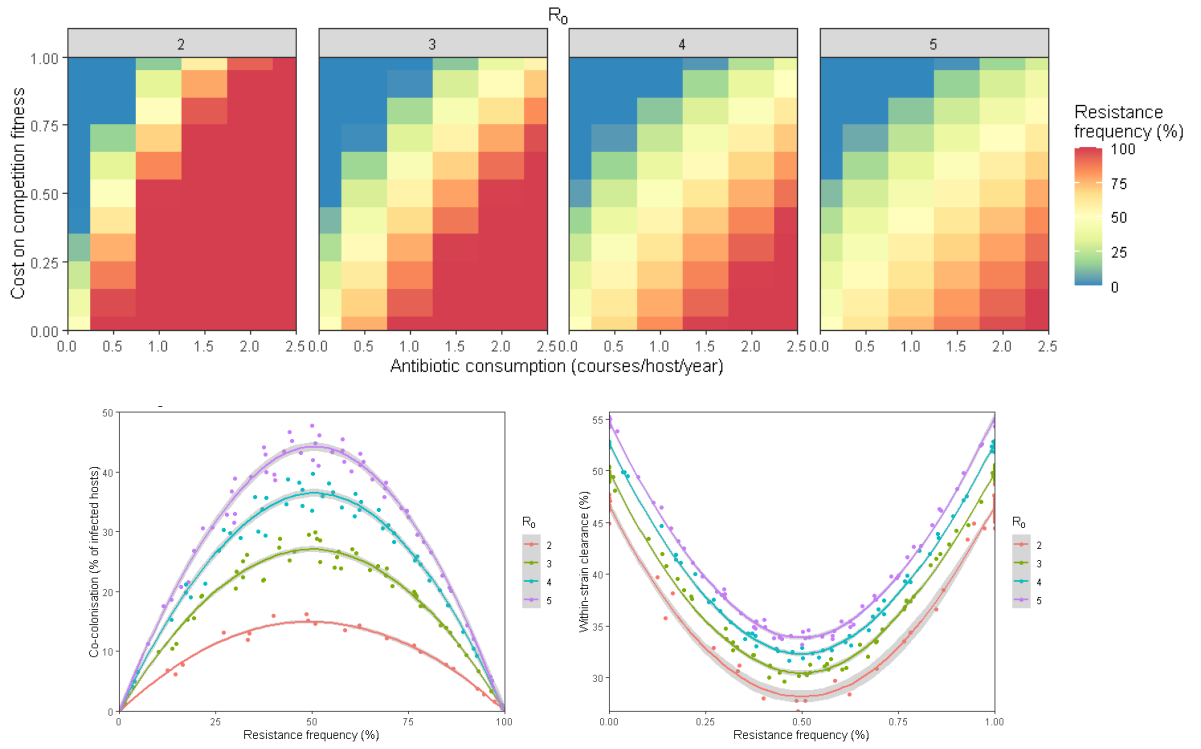


Figure 2.15: **Effect of R_0 on coexistence.** Top, frequency of resistance after 100 years of simulation time. Bottom, co-colonisation of hosts with resistant and sensitive strains (left) and percentage of successful competition events attributable to within-strain competition (right), at different values of R_0 . $n = 10$, resistance associated with 50% of strains. $\gamma = 6.05$, $p_w = 1$, $p_b = 0.1$. Bottom row: $c_f \in \{0, 0.1, \dots, 1\}$ and $\alpha \in \{0, 0.5, \dots, 2.5\}$.

2.3.2 Heterogeneous strains

2.3.2.1 Strain dynamics

Effect of between-strain competition on strains Heterogeneity in γ_i and R_{0i} changes the equilibrium prevalence of each strain i , in the absence of antibiotics or cost (Fig. 2.16). When between-strain competition does not occur ($p_b = 0$, top), strain prevalence varies according to R_{0i} . When between-strain competition is low ($p_b = 0.1$, bottom), clearance by competition disproportionately affects longer-duration strains.

Effect of antibiotics on strains Antibiotic consumption also disproportionately affects longer-duration strains when there is heterogeneity in γ_i and R_{0i} . Fig. 2.17 demonstrates the change in frequency of individual strains with increasing antibiotic consumption within a multi-strain system: strains A-D decrease in frequency; strain E is largely unchanged; and strains F-J increase in frequency. Absolute numbers of bacterial agents in the system reduce for strains A-G; remain constant for strain H; and increase for strains I and J. This indicates that the shortest-duration strains directly benefit from reduced competitive interactions with longer-duration strains.

2.3.2.2 Coexistence

The introduction of heterogeneity into strains had little impact on co-existence overall. Coexistence was promoted by resistance being associated with a limited number of strains (Fig. 2.18), high probability of within-strain competition, and low probability of between-strain competition, as before. However, strain diversity was less stable than when strains were homogeneous (Fig. 2.19). For the parameter sets tested here, coexistence was a slightly more frequent outcome of simulations involving heterogeneous competition fitness rather than epidemiological parameters (Fig. 2.18), although strain diversity was reduced (Fig. 2.19).

Impact of resistance distribution As indicated in the results of sections 2.3.1.4 and Fig. 2.18, the initial distribution of resistance has a major effect on coexistence. Fig. 2.20 illustrates the population dose-response curves for different distributions of resistance at

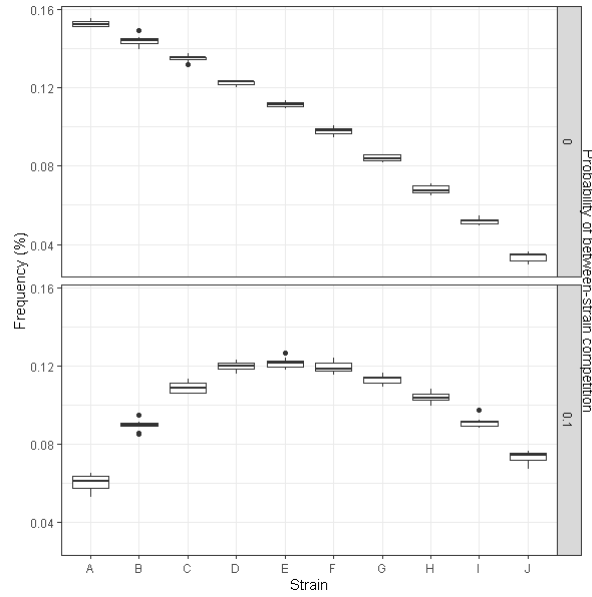


Figure 2.16: **Effect of between-strain competition on strain prevalence.** Strains in descending order of γ_i and R_{0i} , as described in section 2.2.5.2. Results of 10 simulations for each parameter combination. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$. No antibiotics, resistance or cost.

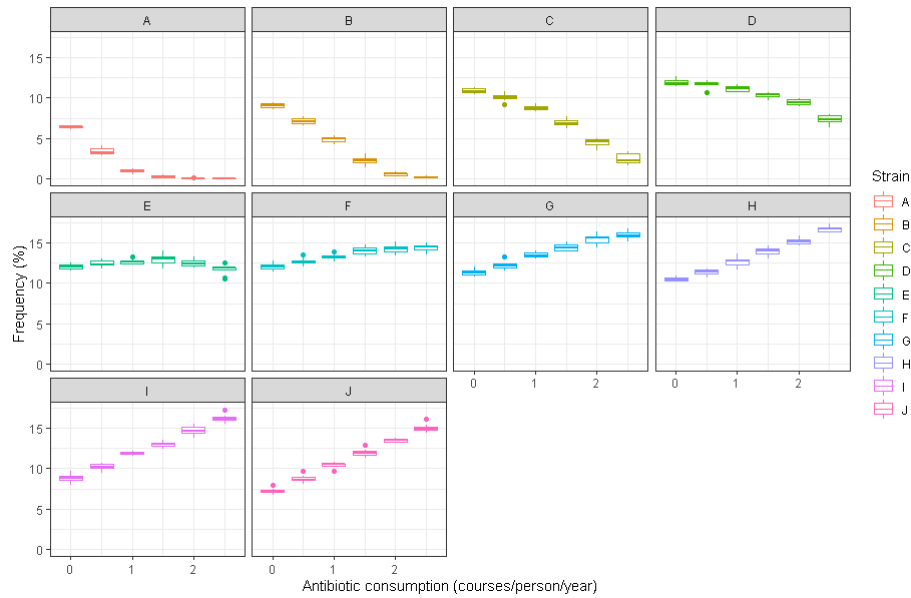


Figure 2.17: **Effect of antibiotic consumption on strain prevalence.** Strains in descending order of γ_i and R_{0i} , as described in section 2.2.5.2. Results of 10 simulations for each parameter combination. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$. No resistance or cost.

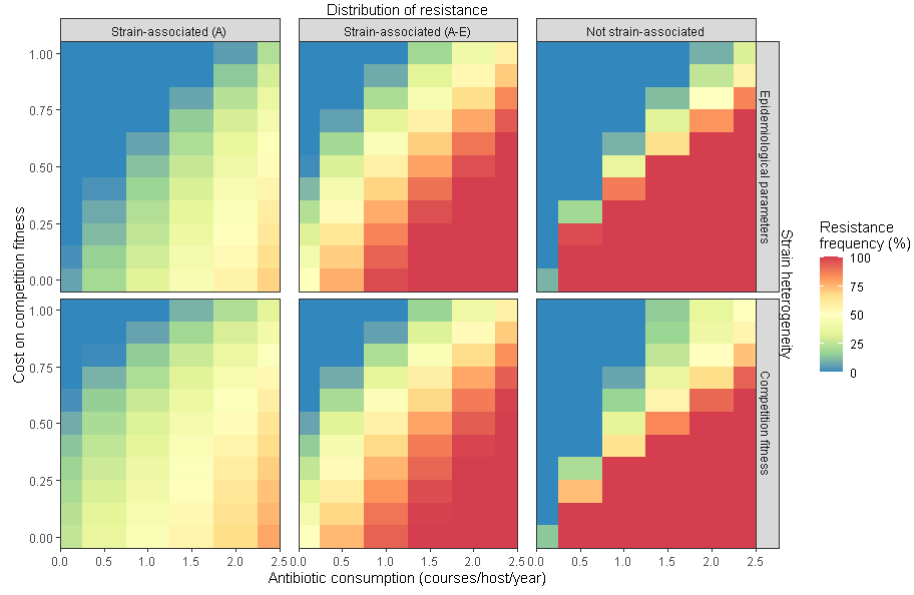


Figure 2.18: **Effect of strain heterogeneity on coexistence.** Strains with heterogeneity in epidemiological parameters (top), or competition fitness (bottom), as described in section 2.2.5.2. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$.

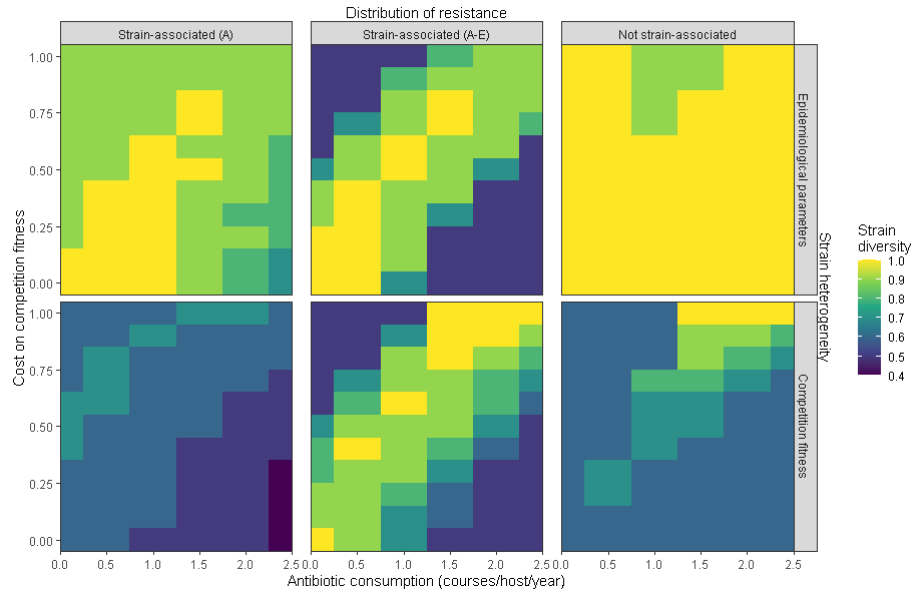


Figure 2.19: **Effect of strain heterogeneity on strain diversity.** Strains with heterogeneity in epidemiological parameters (top), or competition fitness (bottom), as described in section 2.2.5.2. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$.

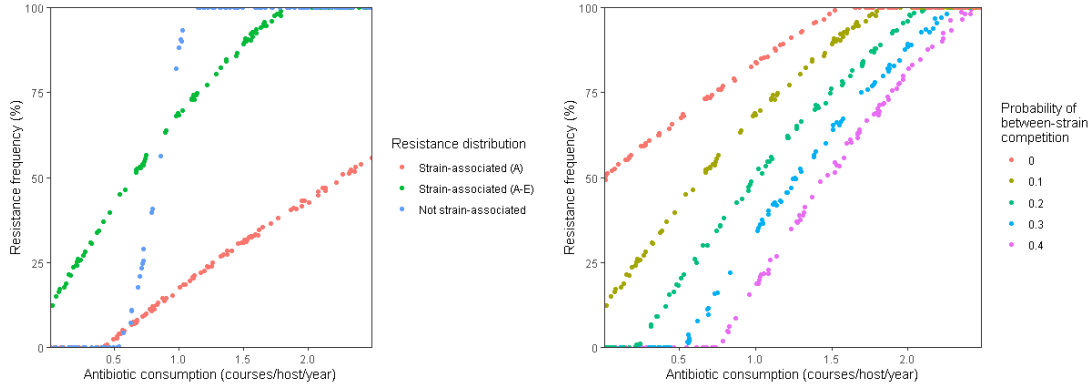


Figure 2.20: **Effect of resistance distribution at initialisation (left) and between-strain competition (right) on the relationship between antibiotic consumption and resistance frequency.** Heterogeneity in strain epidemiological parameters; strains in matched pairs. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $c_f = 0.4$. Left, $p_b = 0.1$. Right, resistance strain-associated (50% of strains).

initialisation. (The dose being rate of antibiotic consumption by the population; response being the overall frequency of resistance.) Other parameters are held constant. The gradient of the curve increases as the number of strains associated with resistance increases; the steepest curve is demonstrated when resistance is not associated with specific strains.

2.3.2.3 Strain-associated resistance

Additional simulations were performed to quantify the impact of different parameters and starting conditions of strain-associated resistance on the relationship between antibiotic consumption and resistance frequency.

Between-strain competition Increasing the probability of between-strain competition increases the gradient of the dose-response curve (Fig. 2.20, right). As p_b approaches p_w , the relative contribution of p_w is reduced, thereby reducing balancing selection on multiple resistant and sensitive strains.

Effect of initial resistance prevalence The equilibrium frequency of resistance is determined by the type and magnitude of fitness cost, and antibiotic consumption (Fig. 2.21). Simulations in which the initial overall frequencies of resistance were varied (but distribution of resistance within strains remained constant), converged on the same overall resistance frequency at equilibrium. This result was highly reproducible between model

simulations.

Effect of the proportion of resistant strains The equilibrium frequency of resistance is also affected by the proportion of strains with which resistance is associated, as alluded to in section 2.3.1.4. Increasing the proportion of resistant strains in the system increases the equilibrium frequency of resistance (Fig. 2.22).

2.3.2.4 Other types of cost

Using this model, coexistence is less common when costs are imposed on transmission fitness or on duration of carriage (Fig. 2.23). This suggests resistance drops out of the population more quickly when costs are on transmission or carriage. These two cost types both impact all resistance-bearing bacterial agents, whereas cost on competition fitness only affects agents that are co-colonising a host within a given time step.

2.4 Discussion

The results presented here demonstrate that modelling continuous within-host competition between bacterial strains, some of which are associated with resistance, achieves coexistence across a wide range of antibiotic consumption rates. This range exceeds the range of rates of antibiotic consumption by people reported globally [114, 429]. To my knowledge, this is the first model addressing resistant/sensitive coexistence where the probability of competition occurring between bacteria depends on strain identity alone.

While previous models have designated strains according to their resistance status [383, 384, 386, 400, 432], this is not necessarily biologically sensible [414]. Other biological features, such as host affinity or nutritional requirements, would be expected to impact competition between bacteria irrespective of their resistance status. Thus, competition should occur independently of resistance status. My results show that strain-based competition achieves persistent coexistence of resistant and sensitive bacteria across this wide range, while maintaining strain diversity. This outcome requires resistance to be associated with some strains but not others. When resistance status is unlinked from strain designation, the relationship between antibiotic consumption and resistance prevalence is much more

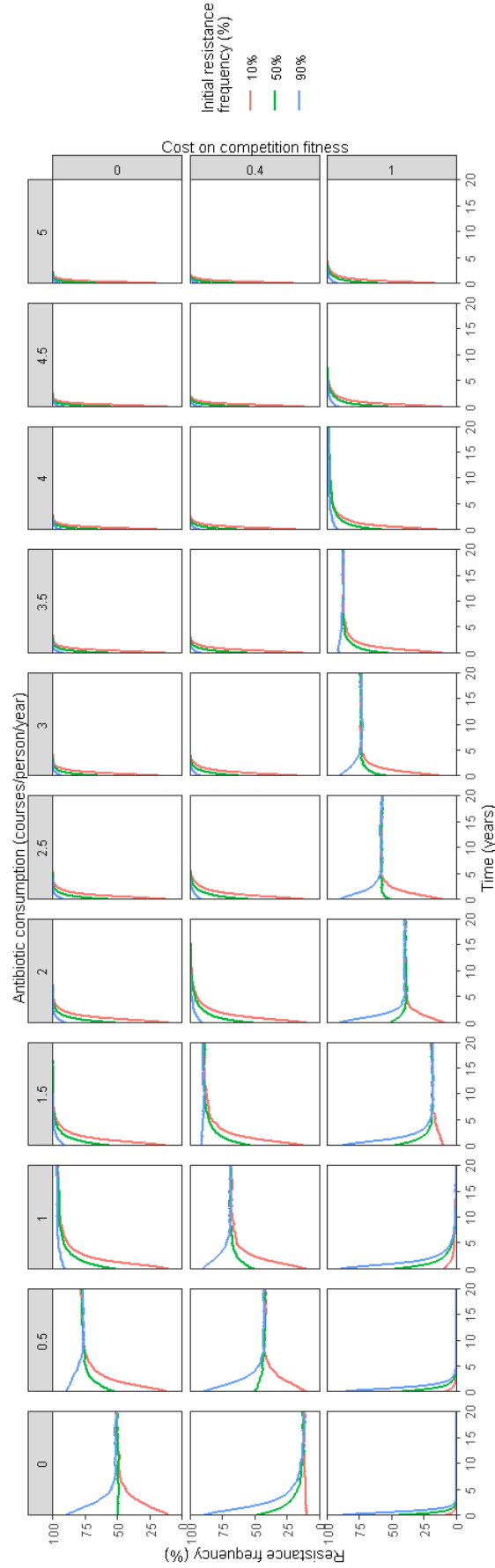


Figure 2.21: **Resistance frequency at equilibrium is determined by antibiotic consumption and cost.** Resistance frequency converges on the same value in each panel. Resistance associated with 50% of strains (A-E). Heterogeneity in strain epidemiological parameters, strains in matched pairs. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$.

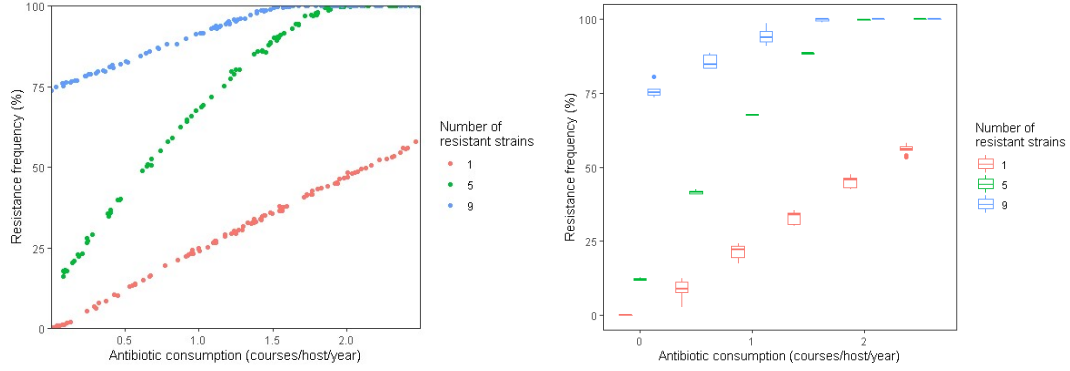


Figure 2.22: **Resistance frequency at equilibrium is determined by the proportion of strains associated with resistance.** Left, resistance associated with 1 (C), 5 (A-E) or 9 (A,B, D-J) strains. Right, results of 10 simulations at each parameter combination, resistance in different combinations of strains (see section 2.2.5.2). Heterogeneity in strain epidemiological parameters. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$.

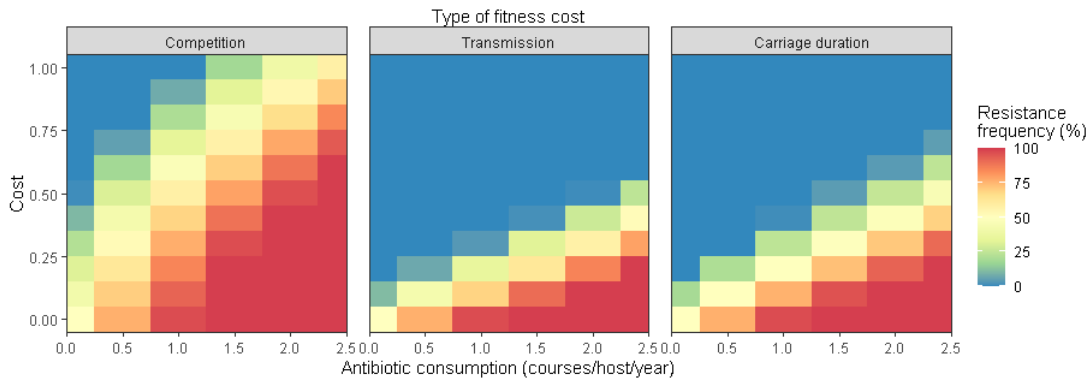


Figure 2.23: **Effect of different types and magnitude of cost on coexistence.** Cost on competition fitness (left), transmission (middle) and duration of carriage (right). Resistance associated with 50% of strains (A-E). Heterogeneity in strain epidemiological parameters, strains in matched pairs. $n = 10$, $\bar{R}_0 = 3$, $p_w = 1$, $p_b = 0.1$.

acute, and coexistence is only achieved in a narrow parameter window.

This is because the conditions required to maintain strain diversity (within-strain competition exceeding between-strain competition [197, 383, 433]) act as balancing selection on resistance as well as on strains, when resistance is associated with particular strains. This association between resistance and other strain characteristics that experience balancing selection has been hypothesised as a mechanism of coexistence [385, 414]. An association between resistance and duration of carriage was shown to promote coexistence in *S. pneumoniae* [385], although in that model, coexistence was still limited to a relatively narrow parameter window. My results also show that the impact of heterogeneity in carriage duration on the occurrence of coexistence overall is small. Rather, it is the strain-based nature of competition in my model, and the association of resistance with unique strains - rather than the specific differences between those strains - that has the greatest impact on coexistence.

The balance between the number of resistant and sensitive strains at model initialisation also affects coexistence. When competition is strain-based, the range of antibiotic consumption within which coexistence is observed is determined by the proportion of resistant strains in the system, not the frequency of resistance in individual bacteria. When considering the full range of antibiotic consumption rates examined here, coexistence in a multi-strain system is maximised when resistance is confined to a single strain. However, when considering only the lower rates of consumption (e.g. 0-0.5 courses/host/year, which approximately encompasses the range of national consumption of penicillins in European populations [252, 429]), persistent coexistence is more frequent when resistance is present in 50% of strains. Generally, increasing the number of resistant strains in the model increases the frequency of resistance overall. In the lower-consumption space, when resistance is confined to one strain, elimination of resistant bacteria is more common than coexistence across the full range of costs on competition fitness. Thus, increasing the proportion of resistant strains enables resistant bacteria to persist under additional combinations of cost and (low) antibiotic consumption.

These results also show that a two-strain model, with a single resistant and a single sensitive strain, as has been used previously [383–386, 398, 400, 434], does not accurately represent the occurrence of coexistence in a multi-strain system. Here, a two-strain sys-

tem with continuous within-host competition considerably over-estimated the frequency of coexistence achieved in the multi-strain systems under equivalent parameters. This is due to the increased frequency of within-strain competition relative to between-strain competition when only two strains are present, which enhances balancing selection. (If strain prevalences are roughly equivalent, bacteria of one strain are much more likely to encounter - and potentially compete with - bacteria of the same strain when the number of unique strains is low.) Davies and colleagues were able to show robust coexistence of two strains (resistant and sensitive), but not of multiple strains [406]. This is because their model lacked strain-based competition; coexistence was only achieved with concurrent strain diversity by the inclusion of strain-specific immunity.

The strain-based competition modelled here also has ramifications for the impact of type and magnitude of the cost of resistance on resistance frequency. My results show that modelling a cost on competition fitness achieves coexistence across a larger parameter space than when cost is imposed on the rates of transmission or natural clearance [383, 385]. When resistance imposes a cost on competition fitness, coexistence is a more frequent outcome of simulations where strains also vary in their inherent competition fitness, rather than their rates of transmission or natural clearance - although strain diversity is reduced. However, this difference is subject to the specific parameterisation of these simulations, and further exploration of the parameter space would be required to examine it more thoroughly. Independent of such quantitative differences in these variable values, the impact of strain heterogeneity on coexistence is small, compared to the impact of strain-based competition, and the association of resistance with a subset of strains (Figs. 2.13 and 2.18).

Strain-based competition enables resistant strains to persist in the absence of antibiotics, despite a non-negligible cost of resistance. This finding explains the not-uncommon persistence of resistant organisms in contexts where antibiotic consumption is minimal or absent [239, 316, 317, 319]. It also underlines the importance of biosecurity as a control measure for AMR [301–307, 319].

Although I have shown that strain-based competition means that antibiotic consumption is not necessary to maintain resistance in multi-strain populations, antibiotic use unequivocally promotes resistance in this model. The impact of antibiotic consumption

on the equilibrium frequency of resistance aligns with previous theoretical work and real-world observations (as outlined in the Introduction). This underlines the importance of antibiotic stewardship in controlling AMR [435].

While the model has been parameterised here for *S. pneumoniae*, it includes few biological specifics, making it appropriate for many different bacterial species, particularly as coexistence is seen across many different combinations of bacteria, antibiotics, and resistance mechanisms. Resident *E. coli* strains, for example, persist in the gut for months to years [436, 437]. My results show increasing the mean duration of carriage reduces coexistence; however, this is due to increased clearance by both competition and antibiotics reducing the number of bacterial agents in the system, and is easily remedied by increasing $\bar{R}_0$ accordingly. I have also not explored the impact of different rates of host entry and exit. Increasing this rate, as would be appropriate in livestock production systems, would modulate the impact of antibiotic consumption on resistance.

To summarise, the general features promoting coexistence in this model of strain-based competition are: conditions that promote strain diversity (high probability of within-strain competition, low probability of between-strain competition); strain-associated resistance; a cost of resistance imposed on competition fitness; and conditions that increase multi-strain infections (again, lower probability of between-strain competition, or higher R_0). To this can be added antibiotic consumption less than or equal to 1 course/host/year, as is true for consumption of individual antibiotic classes by humans across Europe [252], and moderate to low cost.

This model does not incorporate several features that could further promote coexistence. I have assumed random mixing of a homogenous host population. In reality, this assumption is not always appropriate: host populations are heterogeneous and structured according to contact networks [438–441]. Models of structured host populations have been shown to promote coexistence [386, 401], as has heterogeneity of antibiotic consumption [383, 401]; neither of these elements were modelled here. However, as outlined in the Introduction (section 1.6), these elements achieved relatively small improvements in the frequency of coexistence; their incorporation here is unlikely to have a major impact beyond that already achieved through continuous, strain-based competition. Strain-specific immunity, also not included here, has been shown to increase diversity in pneumococcal

serotypes [406], by providing balancing selection through indirect competition. However, this element is likely to have less of an impact on coexistence in my model, in which balancing selection is already achieved by strain-based competition.

The model developed here could potentially be improved by increasing the subtleties of interactions within hosts. The model developed contemporaneously by Davies *et al.* also used continuous competition to achieve frequent coexistence [406]. A competition event in my model results in complete elimination of the losing bacterial agent from that host. By contrast, Davies and colleagues modelled competitive success or loss via step-wise changes in the relative proportions of bacterial strains within that host. This more gradual elimination of the losing bacterial strain seems more biologically realistic, and - depending on the parameters - could theoretically increase co-colonisation of resistant and sensitive strains.

My results are generated under the simplifying assumption that genotypic resistance translates to phenotypic resistance in all cases, which is not realistic. Another element that warrants further exploration involves modelling resistance as a phenotypic continuum, rather than a binary characteristic. This would require modelling partial clearance of semi-resistant bacterial agents when treated with antibiotics, and could be facilitated by incorporation of explicit within-host growth. However, both these inclusions are programmatically more complicated than the current approach, and their relative impact is yet to be tested.

This model does not explicitly include strain-specific costs of resistance [442], although, when strains are heterogeneous, the impact of a given cost varies between strains. I have not modelled interaction between multiple resistance genes and/or different antibiotics, and therefore have not examined the role of co-selection. Nor have I explored heterogeneity in antibiotic consumption, or the effect of different treatment strategies [404, 443]. Previous work has shown that frequency of antibiotic treatment has a more substantial effect than duration of treatment [386, 401]. The distribution of consumption across the population can also affect resistance [444–448].

The effect of changing antibiotic consumption midway through a simulation can be anticipated from these results: resistance frequency will reach a new equilibrium, according to the combination of resistance distribution, cost, and the new rate of antibiotic consump-

tion. There is no suggestion in these results of long-term population restructuring that could help explain the different timescales at which changes in AMR have been observed in real-world systems. In Chapter 3, I will explore the role of antibiotic consumption in the emergence of resistance, as well as the impact of bacterial migration from other populations.

Here, I have shown the impact of the proportion of resistant strains in the model on the equilibrium frequency of resistance, and the presence of coexistence. This is likely to present a challenge when modelling mobile resistance, as HGT enables resistance to escape strain restrictions. Modelling HGT of resistance is explored further in the next chapter. The competition structure proposed here also has ramifications for the simulated duration of carriage of different strains. While the model is parameterised for a specified rate of natural clearance by the host γ_i , and thus a theoretical duration of carriage $D_i = 1/\gamma_i$ for each strain i , bacteria are often displaced by competing agents. Therefore, the actual duration of carriage in simulations D_i^* is less than D_i . A method for fitting strain-specific epidemiological parameters is developed in Chapter 4.

Chapter 3

Results: ABM with horizontal gene transfer (HGT)

3.1 Introduction

The strain-based model of competition developed in Chapter 2 demonstrated coexistence of resistant and sensitive bacteria over a wide parameter window. However, results were sensitive to the distribution of resistance within strains at model initialisation. This persistent coexistence relies on an association between resistance genes and strain identity, which could be disrupted by another key feature of AMR: HGT.

Analogous to the competition between bacteria for hosts is the competition between MGEs for bacterial cells. Many MGEs will target particular insertion sites through site-specific integrase genes, in order to integrate into a bacterial chromosome while only causing minimal disruption [449]. Each of these sites can be considered a niche for which a circulating set of MGEs compete. Although plasmids do not insert into integration sites, they similarly compete for bacterial hosts, because only one representative of each incompatibility group can be stably maintained by a host cell [450]. Different types of MGEs may also compete directly [325, 337]. While not MGE-mediated, uptake of sequences through transformation can also be competitive. At a given chromosomal locus, a single allele conferring (for example) either a sensitive or resistant phenotype may be present. These sequences can therefore also compete for host cells, with their mobilisation facilitated by transformation rather than MGEs [407].

Competition between MGEs can also be indirect, for example if they confer a similar benefit to the bacterial cell (e.g. by carrying resistance genes). In the Introduction, I explained the importance of MGEs such as ICEs and plasmids in transferring resistance and other cargo genes between bacteria. Other MGEs, such as phage, have been implicated less often [215, 217, 451]. This may be due to differences in the interactions between these elements and the host bacterial cell. Lytic phages reproduce rapidly then lyse the host cell, whereas temperate phages undergo lysogeny, where they integrate into the bacterial chromosome, becoming prophage [218, 452]. These prophage can later activate, excise, and kill the cell. This means that ICE and plasmids tend to have a longer-term association with the host cell than phage, and a potentially greater incentive to carry cargo resistance genes [453].

There are likely to be other differences in the types and magnitudes of costs imposed by different MGEs [221, 325, 326]. For example, ICEs require excision from the chromosome of the donor cell, and then integration into the chromosome of the recipient [218, 221]. Plasmids transfer between cells without this disruption, but may be restricted in their host range [218, 221]. Plasmid costs, as measured *in vivo*, vary considerably and are dependent on both their content and the bacterial host [325, 326, 328].

In this chapter, I address the possible role of competition in shaping MGE distributions, including associations with strains and resistance genes. I present the impact of HGT on the conditions promoting resistance, and establish the necessary criteria for modelling stable coexistence when resistance is mobile. This requires first understanding the emergence dynamics of resistance in new and existing strains. I also assess the effect of bacterial immigration on coexistence and the relationship between antibiotic consumption and resistance in the recipient population.

3.2 Methods

The model developed for Chapter 1 was utilised as described, with additions. All code is stored in the repository <https://github.com/tamsindewe/Thesis.git>.

3.2.1 Model components

The model was extended to explicitly incorporate a fourth category of agent: MGEs. MGEs were inserted in the model hierarchy between bacteria and genes. Conceptually, MGEs can transport genes between different bacterial agents colonising the same host. MGEs belong to distinct types, which represent an insertion site in the bacterial chromosome (e.g. for prophage or ICEs) or incompatibility group (e.g. plasmids). No more than one MGE of the same type can be carried by a given bacterial agent in a single time step, although MGEs of different types can be carried simultaneously. MGE types also have inherent properties such as the rate at which they transmit between bacterial agents, and whether they carry resistance genes or not. MGEs can impose a cost on transmission rate, duration of carriage, or the competition fitness of bacteria.

3.2.2 Model processes and concepts

Two new modules were added to the model: HGT and migration. In the HGT module, individual MGEs in a co-colonised host are allowed to transfer to other bacterial agents in random order within each time step. This occurs at rate β_{M_k} for each MGE type k . Once an MGE is established in a bacterial agent, it is retained, and MGEs are cloned along with the bacterial agent during transmission to new hosts. Similarly, any resistance genes carried by the MGE are retained once acquired, and co-transmitted during onwards infection. Immigration of bacterial agents into the host population occurs continuously and at a constant rate η . Hosts are seeded with incoming agents within each time step, following the initialisation procedure described in Chapter 1.

3.2.3 Scheduling

The scheduling process is the same as that described in Chapter 1, with the insertion of additional modules for MGE transfer and bacterial immigration (Fig. 3.1). The model is stochastic and runs in discrete time. For every process occurring at rate r , the probability p of it happening within a given time step is:

$$p = 1 - e^{-rt} = 1 - e^{-rl/365} \quad (3.1)$$

where t is time in years and l is the length of the chosen time step, in days. Occurrence of events within the time step is stochastic: the event is actualised if a random number drawn from a uniform distribution $U(0, 1)$ is less than the calculated value for p .

Within each time step, a series of processes occur, either internal to individual hosts or external (Fig. 3.1). Initial external processes are conducted first. The model then loops through every individual host to complete within-host processes, before performing additional external processes. These processes are summarised below, with the new additions in this chapter highlighted in bold.

1. External processes:

- (a) Seeding of any bacteria introduced at this time step;
- (b) **Implementation of bacterial immigration;**
- (c) Identification of hosts treated with antibiotics;
- (d) Identification of hosts leaving the population (e.g. through death or ageing-out);

2. Internal processes:

- (a) Identification and implementation of bacterial clearance:
 - i. Clearance by host immune system;
 - ii. Clearance by antibiotics;
 - iii. Clearance by competition;
 - iv. **Identification and implementation of HGT;**
- (b) Identification of new infections.

3. External processes:

- (a) Implementation of new infections;
- (b) Implementation of hosts leaving the population;
- (c) Implementation of new hosts entering the population.

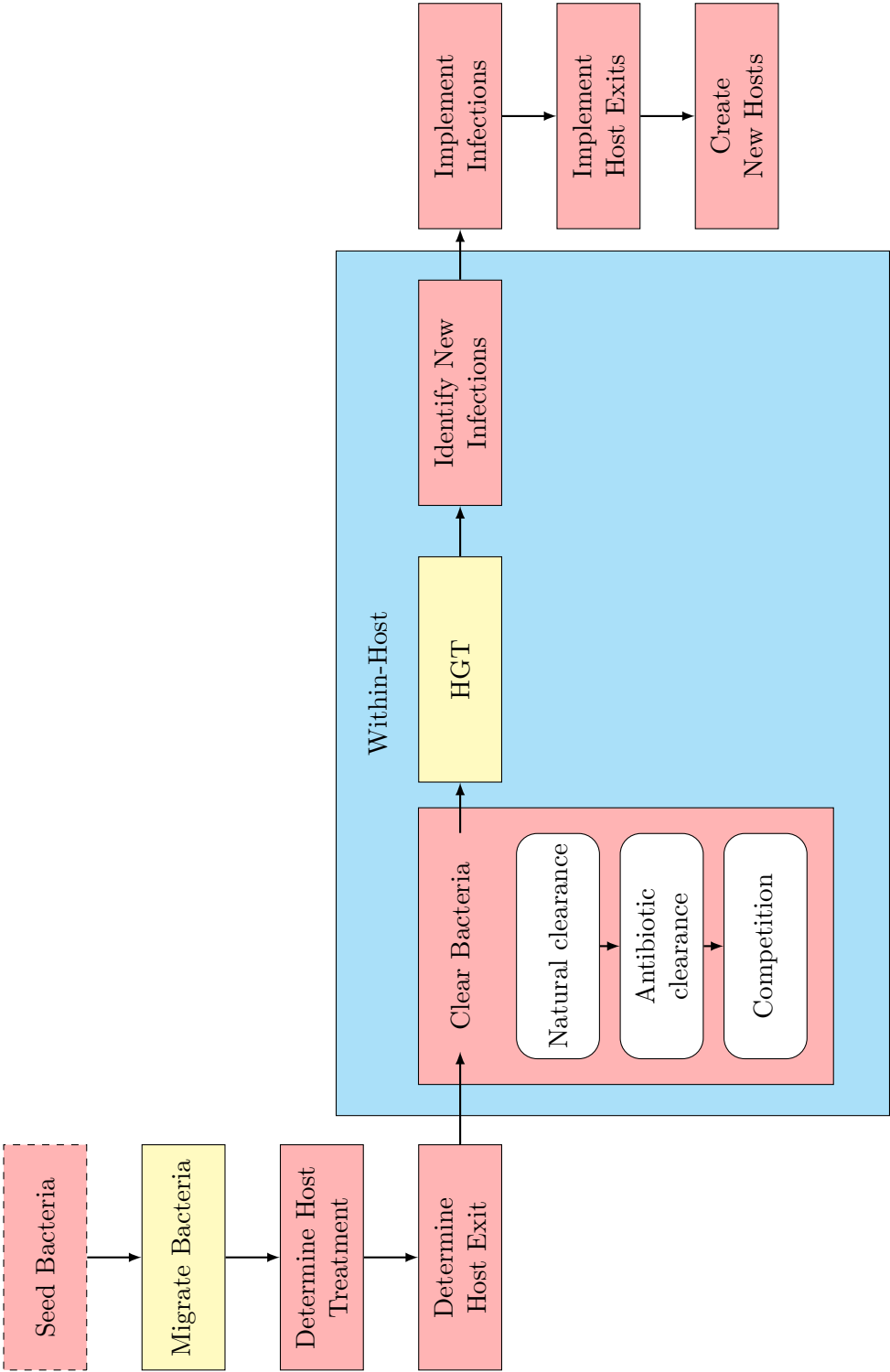


Figure 3.1: **Model workflow.** Processes performed during a single time step. New additions for this chapter in yellow.

3.2.4 Model initialisation

The model was initialised as described in Chapter 2. Distribution of resistance into bacterial agents at initialisation was expanded. Here, resistance was strain-associated or not strain-associated, as previously described. A third distribution, intermediate between the two, was also utilised. In this ‘strain-restricted’ distribution, resistance was seeded into bacterial agents belonging to specific strains (and not other strains), but only at a low frequency in each. MGEs were initialised according to one of the three distributions (Fig. 3.2).

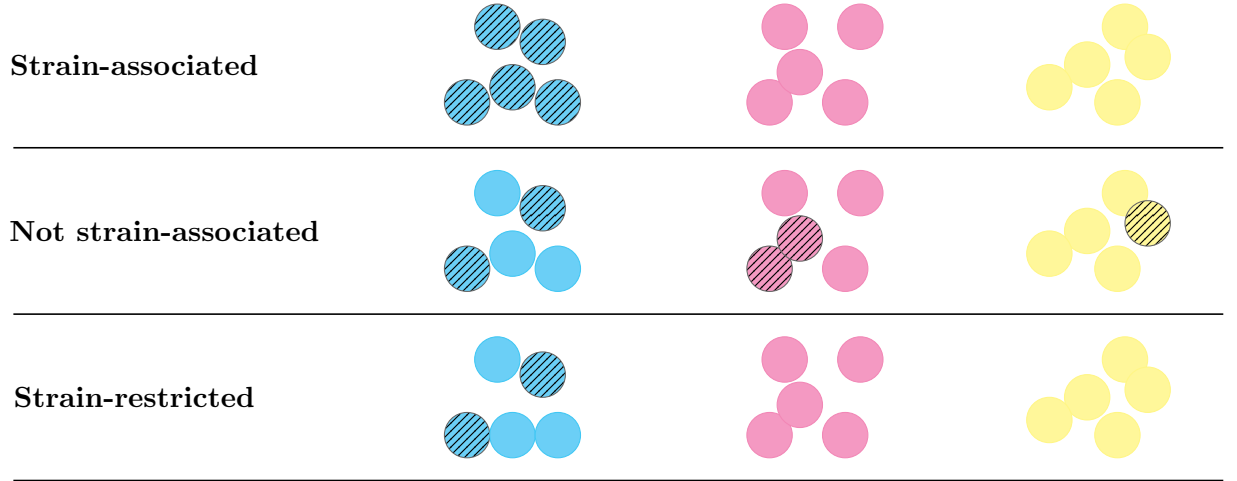


Figure 3.2: **Distribution of resistance in strains at model initialisation.** Bacterial agents (circles) belonging to different strains (colours) are seeded with resistance (shading) at model initialisation. Top: resistance is seeded within the blue strain only, and is fixed within that strain. Middle: resistance is seeded at low frequencies in all strains. Bottom: resistance is restricted to the blue strain, but is not at fixation within it.

3.2.5 Parameterisation

All simulations in this chapter involved 10 strains, with heterogeneity in epidemiological parameters ($n = 10$, $\bar{\gamma}_i = 6.05$, $\bar{R}_{0i} = 3.0$, $c_{fi} = 1$).

3.2.5.1 Strain-restricted resistance

Strains were parameterised for matched strains, as described in Chapter 2 (Table 3.1). Resistance was initially distributed in a strain-associated, non-strain-associated, or strain-restricted manner, as demonstrated in Fig. 3.2. This is detailed in Table 3.2.

Parameter	Strain	A, F	B, G	C, H	D, I	E, J
γ_i		4.036	5.045	6.054	7.063	8.072
R_{0_i}		3.250	3.125	3.000	2.875	2.750
f_i		1	1	1	1	1

Table 3.1: **Strain-specific parameters for 10 matched strains with heterogeneous carriage duration ($1/\gamma$) and R_0 .**

Distribution	Strains initialised with resistance	Initial prevalence of resistance within strain
Strain-associated (A-E)	A-E	1.0
Not strain-associated	A-J	0.1
Strain-restricted (A-E)	A-E	0.1

Table 3.2: **Distribution of resistance at initialisation.** All simulations involved strains A-J; only strains initialised with resistance genes are shown.

3.2.5.2 Emergence of resistance

The model was parameterised for unique strains, as previously described (Table 3.3). The model was initialised with fully-sensitive strains and allowed to reach equilibrium, before resistance was introduced. Two scenarios were tested: seeding resistance into an existing strain; and introducing a novel, fully-resistant strain. In both cases, approximately 100 resistant bacterial agents belonging to a single strain were introduced after 10 years of model run-time. The distribution of resistance at introduction was therefore: strain-restricted when resistance was introduced into an existing strain; and strain-associated when resistance was introduced along with a novel strain. Resistance was introduced into strains A, E, or J (longest-duration, mid-duration, and shortest-duration strains, respectively) under each condition.

Parameter	Strain	A	B	C	D	E	F	G	H	I	J
γ_i		4.036	4.485	4.933	5.381	5.830	6.278	6.727	7.175	7.624	8.072
R_{0_i}		3.250	3.194	3.139	3.083	3.028	2.972	2.917	2.861	2.806	2.750
f_i		1	1	1	1	1	1	1	1	1	1

Table 3.3: **Strain-specific parameters for 10 unique strains with heterogeneous carriage duration ($1/\gamma$) and R_0 .**

3.2.5.3 MGE dynamics

The effect of competition on the relationship between MGEs was explored by performing control simulations where mobility between strains was negligible. Here, the model was initialised with matched strains (Table 3.1). Two MGEs were introduced at initialisation, both bearing resistance genes. The overall distribution of resistance at initialisation remained constant (restricted to strains A-E), but the distribution of MGEs varied between scenarios (Table 3.4). MGEs were either strain-restricted or strain-associated, as illustrated in Fig. 3.2. Costs were chosen that produced roughly equivalent equilibrium frequencies of resistance in Chapter 2.

Scenario	Agent	Cost	Distribution	Strains initialised with agent	Initial prevalence of agent within strain
Identical MGEs	MGE ₁	$c_{f_{\text{MGE}_1}} = 0.4$	Strain-restricted	A-E	0.1
	MGE ₂	$c_{f_{\text{MGE}_2}} = 0.4$	Strain-restricted	A-E	0.1
Different competition costs	MGE ₁	$c_{f_{\text{MGE}_1}} = 0.1$	Strain-restricted	A-E	0.1
	MGE ₂	$c_{f_{\text{MGE}_2}} \in \{0.05, 0.1\}$	Strain-restricted	A-E	0.1
Different cost types (c_f and c_β)	MGE ₁	$0.1 \leq c_{f_{\text{MGE}_1}} \leq 0.5$	Strain-restricted	A-E	0.1
	MGE ₂	$c_{\beta_{\text{MGE}_2}} = 0.1$	Strain-restricted	A-E	0.1
Different cost types (c_f and c_γ)	MGE ₁	$0.1 \leq c_{f_{\text{MGE}_1}} \leq 0.5$	Strain-restricted	A-E	0.1
	MGE ₂	$c_{\gamma_{\text{MGE}_2}} = 0.1$	Strain-restricted	A-E	0.1
Strain-associated MGEs	MGE ₁	$0.1 \leq c_{f_{\text{MGE}_1}} \leq 0.5$	Strain-associated	A, C and E	1.0
	MGE ₂	$c_{\beta_{\text{MGE}_2}} = 0.1$	Strain-associated	B and D	1.0

Table 3.4: **Distribution of resistance and MGEs at initialisation, under different scenarios.** All simulations involved strains A-J; only strains initialised with resistance genes are specified here. Resistance was associated with 50% of strains (A-E) in all simulations. $\beta_{\text{MGE}_1} = \beta_{\text{MGE}_2} = 0$.

3.2.5.4 HGT

Single MGE Unique strains were parameterised as described in Table 3.3. First, control simulations were performed without any costs or antibiotic consumption ($c_f = c_\beta = c_\gamma = 0; \alpha = 0$). Resistance was borne by a single MGE, which was associated with a single strain at initialisation (strain A). The MGE transferred horizontally at rate β_{MGE} , where $\beta_{\text{MGE}} \in \{0.01, 0.05, 0.1, 0.5, 1\}$.

Antibiotic consumption and a cost on competition fitness, within the parameters indicated in Table 3.5, were then introduced.

	Parameter	Value
α	Rate of antibiotic consumption (courses/host/year)	0, 0.5, ..., 2.5
c_f	Cost on competition fitness	0.1, 0.4
β_{MGE}	Rate of HGT	$10^{-1}, 10^{-2}, \dots, 10^{-7}$

Table 3.5: **Model parameters.** Used for simulations involving a single MGE with HGT.

Two MGEs Simulations were performed with two circulating MGEs. At initialisation, these MGEs were strain associated: seeded in strains A and B, respectively. They could occupy the same or different sites within bacterial agents. Initial simulations were in the absence of costs or antibiotics. The MGEs transferred horizontally at rate β_{MGE} , where $\beta_{MGE} \in \{0.01, 0.05, 0.1, 0.5, 1\}$.

Additional simulation were then performed with antibiotics and costs. The first MGE (now MGE₁) retained a cost on competition fitness; the second MGE (MGE₂) conveyed a cost on carriage duration. MGE₂ was able to transfer horizontally at least as fast as MGE₁. This combination of parameters means these MGEs can be considered ‘ICE-like’ and ‘phage-like’, respectively [427]. Full parameters are provided in Table 3.6. Separate simulations were performed with the MGEs inserting at the same and at different sites within the bacterial agents.

	Parameter	Value
α	Rate of antibiotic consumption (courses/host/year)	0, 0.5, ..., 2.5
$c_{f_{MGE_1}}$	Cost on competition fitness	$0.1 \leq c_{f_{MGE_1}} \leq 0.5$
$c_{\gamma_{MGE_2}}$	Cost on carriage duration	0, 0.1, 0.5
β_{MGE_1}	Rate of HGT	$10^{-3}, 10^{-4}, 10^{-5}$
β_{MGE_2}	Rate of HGT	10^{-3}

Table 3.6: **Model parameters.** Used for simulations involving two MGEs with HGT.

3.2.5.5 Bacterial immigration

Strains were matched, as previously described (Table 3.1). Resistance was strain-associated (strains A-E). After equilibrium was reached, hosts were infected at random within each time step with immigrating bacterial agents at rate η , where $\eta \in \{10^2, 10^1, \dots, 10^{-3}\}$. The prevalence of resistance within the immigrating bacterial population was also varied (Table 3.7), in alignment with section 2.2.5.2 in the previous chapter.

Scenario	Individual strain frequency (A-E)	Individual strain frequency (F-J)	Overall resistance prevalence
1	0.02	0.18	≈ 0.1
2	0.10	0.10	≈ 0.5
3	0.18	0.02	≈ 0.9

Table 3.7: **Distribution of resistance in immigrating bacteria.** Strains A-E are fully resistant; strains F-J are fully susceptible. Individual strain frequency shows the proportion of all immigrating bacteria belonging to that strain.

3.3 Results

3.3.1 Strain-restricted resistance

Restricting resistance to a subset of strains, but initialising resistance in each of those strains at a low frequency, results in a relationship between antibiotic consumption and overall resistance frequency that pivots between those for strain-associated and not strain-associated resistance. In Fig. 3.3, the dose-response relationship at low rates of antibiotic consumption for strain-restricted resistance is similar to that of resistance that is not strain-associated. The initial dynamics are the fixing of resistance within strains. Once antibiotic consumption is high enough for resistance to become fixed in the strains into which it was introduced, it becomes strain-associated. From that point, the equilibrium frequencies of resistance are indistinguishable from those generated from simulations where resistance is strain-associated from the point of initialisation.

3.3.2 Emergence of resistance

The emergence of resistance was explored further by seeding low numbers of resistant bacterial agents into a fully-susceptible population (Fig. 3.4). These results show that resistance is able to persist at lower rates of antibiotic consumption when it is introduced at fixation within a new strain (i.e. is strain-associated), than when it is introduced at a low frequency into an existing strain (i.e. strain-restricted). The rate of antibiotic consumption at which resistance emerges successfully varies by strain; the difference between the two conditions is the least when resistance is introduced into the strain with the longest duration. This indicates that selection pressure for resistance is highest in this strain.

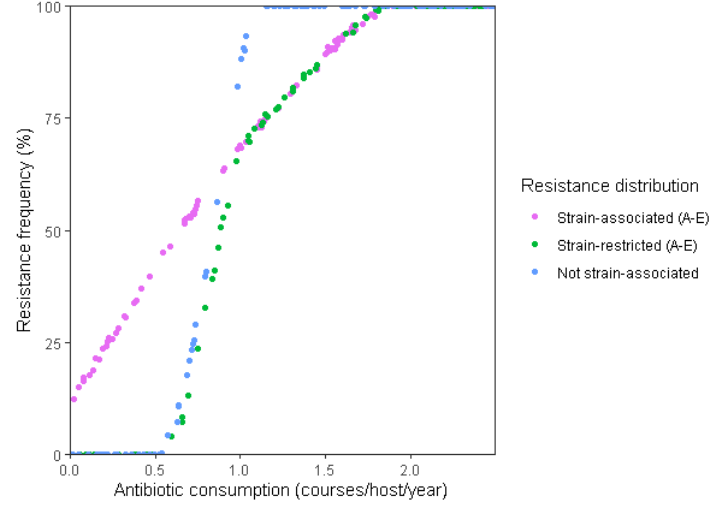


Figure 3.3: **Effect of resistance distribution in strains on equilibrium resistance frequency.** Distribution of resistance at initialisation as per Table 3.2. $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$.

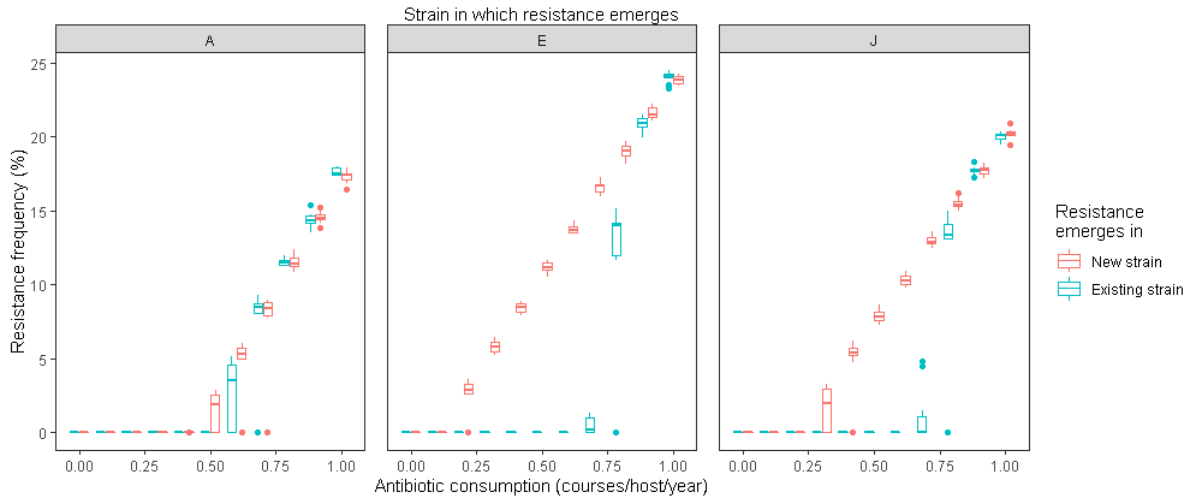


Figure 3.4: **Novel resistant strains become established in a susceptible population at lower rates of antibiotic consumption than resistance arising in an existing strain.** Approximately 100 bacterial agents were introduced into a fully-susceptible, multi-strain population, within an existing strain, or a novel one. Results of 10 runs. $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$. Strain parameters as per Table 3.3.

3.3.3 MGE dynamics

Strain-restricted MGEs Coexistence of two MGEs when HGT is negligible is dependent on the distribution of MGEs within strains. When MGEs are identical in all but name, but not fixed within strains, frequencies of each are approximately 50% of the overall resistance frequency, and demonstrate drift (Fig. 3.5, top panel). These dynamics mirror those of individual strains, as shown in Chapter 2. However, when costs are of the same type, but different magnitude, the more costly MGE is eventually excluded (Fig. 3.5, bottom panel). When MGEs carry resistance genes but different types of costs, competitive exclusion of one MGE tends to occur, and coexistence of both types is limited to a small parameter window (Fig. 3.6).

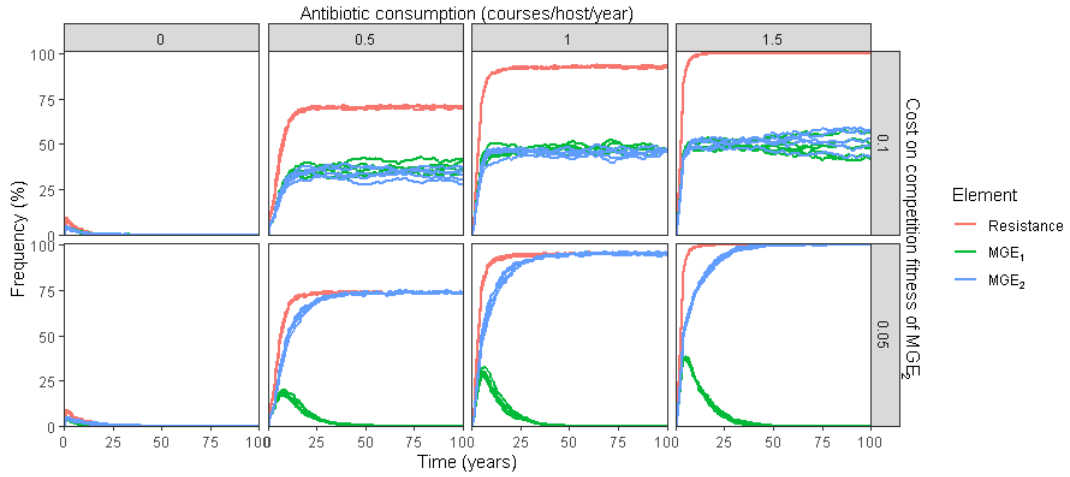


Figure 3.5: **Dynamics of two resistance-bearing MGEs with identical or differing costs on competition fitness.** Results of 5 simulations in which cost of MGE₁ is held constant ($c_{f_{\text{MGE}_1}} = 0.1$). Cost of MGE₂ varies (right axis). When both MGEs have the same cost (top), individual MGE frequencies oscillate around 50% of overall resistance, and show drift. When MGE₁ is twice as costly as MGE₂ (bottom), it is out-competed. $p_w = 1$, $p_b = 0.1$. Resistance is strain-restricted (strains A-E) and HGT is negligible. Results are similar for other types of cost.

Strain-associated MGEs When individual MGEs are fixed in a subset of strains, coexistence of both MGEs is achieved over a much wider parameter space. In Fig. 3.7, where MGEs are distributed differently from Fig. 3.6 (strain-associated, rather than strain-restricted), coexistence of both MGEs is achieved over a much wider parameter window.

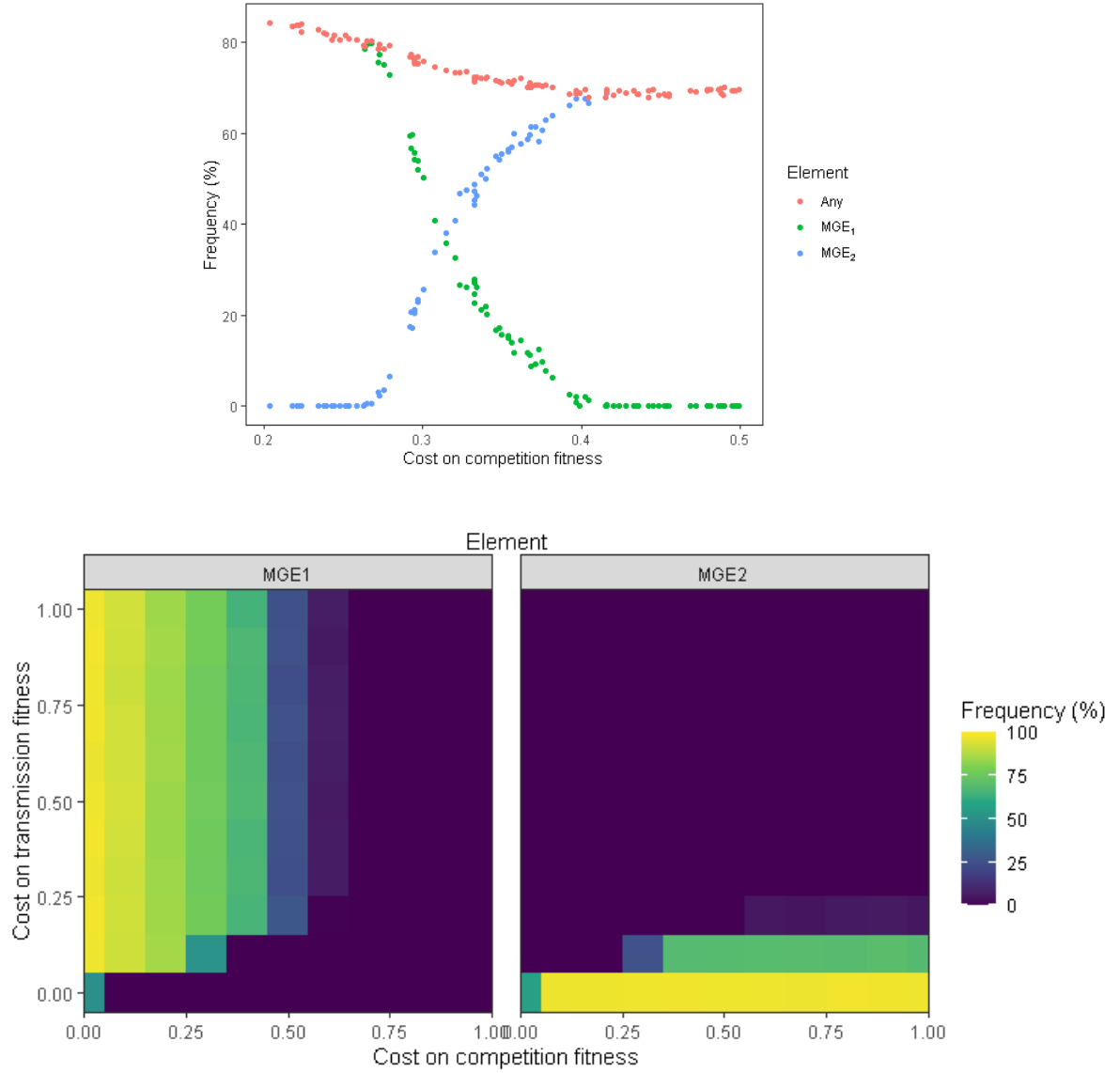


Figure 3.6: **Coexistence of MGEs with different cost types is limited if they are not strain-associated.** Top, dose-response curves for MGE₁ and MGE₂ when $c_{f_{MGE_1}}$ varies (x axis) and $c_{\beta_{MGE_2}} = 0.1$. Bottom, heat map showing relative frequencies of MGE₁ and MGE₂ at different values of $c_{f_{MGE_1}}$ (x axis) and $c_{\beta_{MGE_2}}$ (y axis). MGEs and resistance are strain-restricted (strains A-E) and HGT is negligible. Results are similar for cost on duration of carriage. $p_w = 1$, $p_b = 0.1$, $\alpha = 1$.

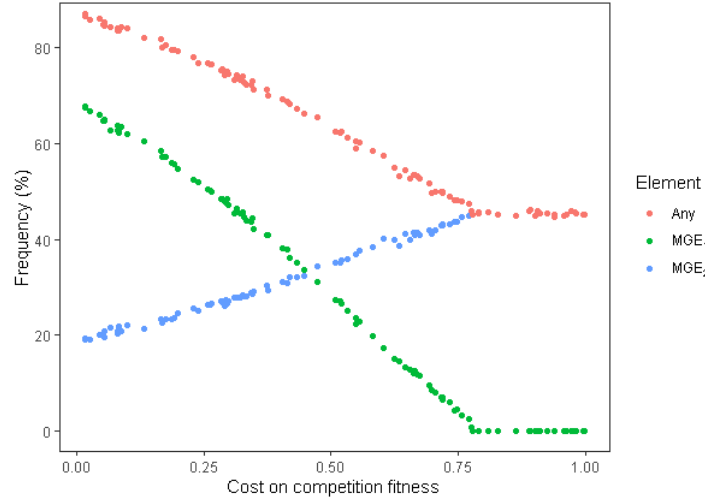


Figure 3.7: **Coexistence of MGEs with different cost types is much more frequent when they are strain-associated.** Dose-response curves for MGE_1 and MGE_2 when $c_{f_{\text{MGE}_1}}$ varies (x axis) and $c_{\beta_{\text{MGE}_2}} = 0.1$. MGEs are strain-associated as per Table 3.4, resistance is strain-associated (strains A-E), and HGT is negligible. Results are similar for cost on duration of carriage. $p_w = 1$, $p_b = 0.1$, $\alpha = 1$.

3.3.4 HGT

3.3.4.1 Single MGE

Without antibiotics or costs In the absence of cost, or antibiotic consumption, a single MGE with non-negligible rates of HGT transfers into new strains, and gradually increases in prevalence until it reaches fixation (Fig. 3.8).

With antibiotics and costs Successful HGT events do not always correspond to successful expansion of resistance into a new strain (Fig. 3.9). The relationship between antibiotic consumption and cost needs to be sufficiently favourable for expansion to be possible. This is foreshadowed by the results in section 3.3.2 for resistance emergence in existing strains.

However, if this balance is favourable, resistance can escape its association with specific strains. Fig. 3.10, top plot, shows the effect on resistance of varying rates of transfer of a single MGE type. When resistance is beneficial, HGT is followed by expansion of resistance in the new strain. Resistance ultimately becomes strain-associated (i.e. clonally-distributed), and a new equilibrium frequency of resistance is established. (This

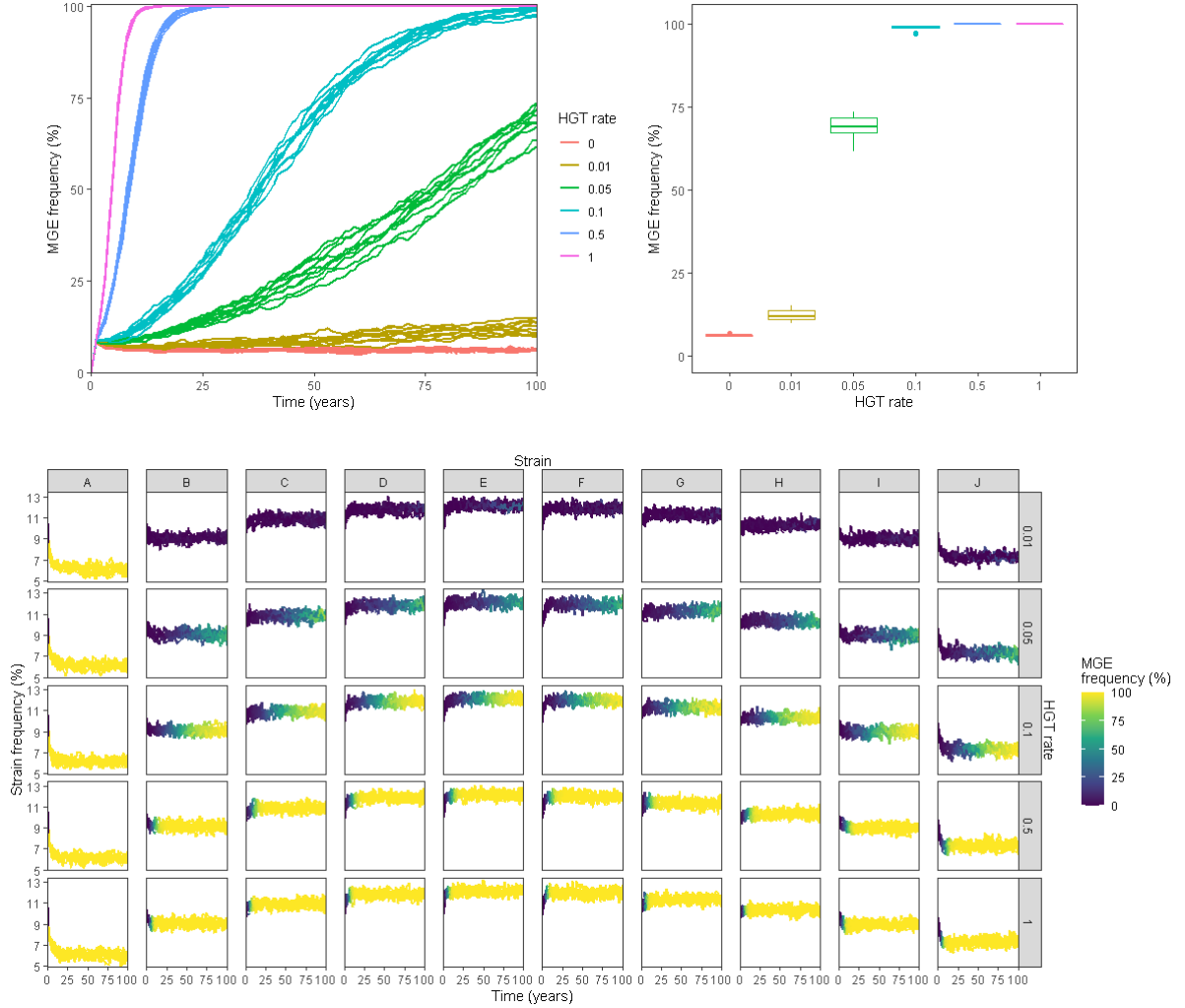


Figure 3.8: **HGT allows an MGE to move through multi-strain populations, gradually increasing in prevalence.** Top, overall MGE frequency in the population over time (left) and after 100 years (right), for different rates of HGT. Bottom, frequency of individual strains and MGEs within each strain. All plots show results of 10 simulations. $c_f = c_\beta = c_\gamma = 0$; $\alpha = 0$. Strain parameters as per Table 3.3.

is consistent with the results in Chapter 2, 2.3.2.3, where overall resistance frequency was determined by the proportion of resistant strains along with antibiotic consumption.) This process is repeated for additional strains and ultimately, resistance goes to fixation in the population (again, except for those parameter combinations where the costs/consumption balance is unfavourable). The rate of HGT transfer therefore affects the rate at which fixation is achieved (and this can take more than 100 years of model simulation time), but not the final result: fixation of resistance is inevitable.

Fig. 3.10, bottom plot, also demonstrates the interplay between HGT, resistance, and strain diversity. When HGT is negligible ($\beta_{\text{MGE}} = 0$), complete strain diversity is almost always maintained. This is because resistance remains associated with a single strain, and strain-based competition maintains diversity. When HGT rates are high ($\beta_{\text{MGE}} = 0.01$), strain diversity is also maintained, because all strains quickly become resistant; again, strain-based competition maintains diversity. However, for intermediate rates of HGT shown here, strain diversity is reduced considerably as antibiotic consumption increases. This is because acquisition of resistance by new strains is occurring sequentially; not all strains have the opportunity to acquire resistance before they are eliminated by high rates of antibiotic consumption.

3.3.4.2 Two MGEs

Identical MGEs Two MGEs with identical parameters and non-negligible rates of HGT move through the population as previously described. They can either insert at the same location in bacterial agents - i.e., competing directly - or at different sites. When MGEs insert at the same site within bacterial agents, total MGE frequency is similar to that of the single MGE in section 3.3.4.1 (Fig. 3.8, top), but each MGE represents approximately 50% of the MGE population.

When MGEs are located on different sites within bacterial agents, the total MGE frequency is higher (Fig. 3.11), but the dynamics for each individual MGE are similar to those observed for the single MGE in section 3.3.4.1.

Differing MGEs, one carrying resistance When MGEs have differing qualities, the dynamics change. Fig. 3.12 shows the results of simulations with one resistance-bearing,

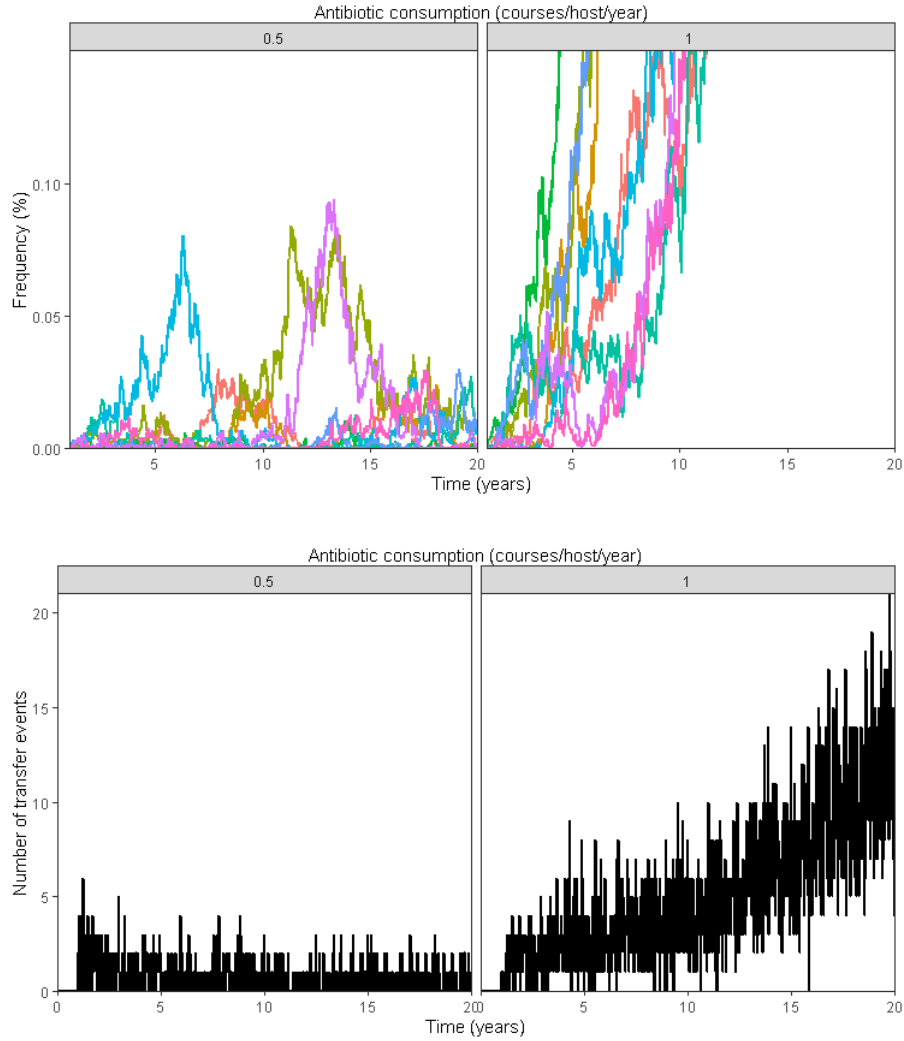


Figure 3.9: **Emergence of resistance in a new strain after an HGT event is affected by rates of antibiotic consumption.** Top, carriage of resistance (and MGEs) by initially-susceptible bacterial strains (expressed as a percentage of all bacteria). Bottom, number of HGT events per time step. Results shown for two different rates of antibiotic consumption (top axis); expansion of resistance/MGE in the new strains occurs at $\alpha = 1.0$ but not at $\alpha = 0.5$. Top right plot cropped for illustrative purposes. $p_w = 1$, $p_b = 0.1$, $\beta_{\text{MGE}} = 0.01$. Results from one simulation.

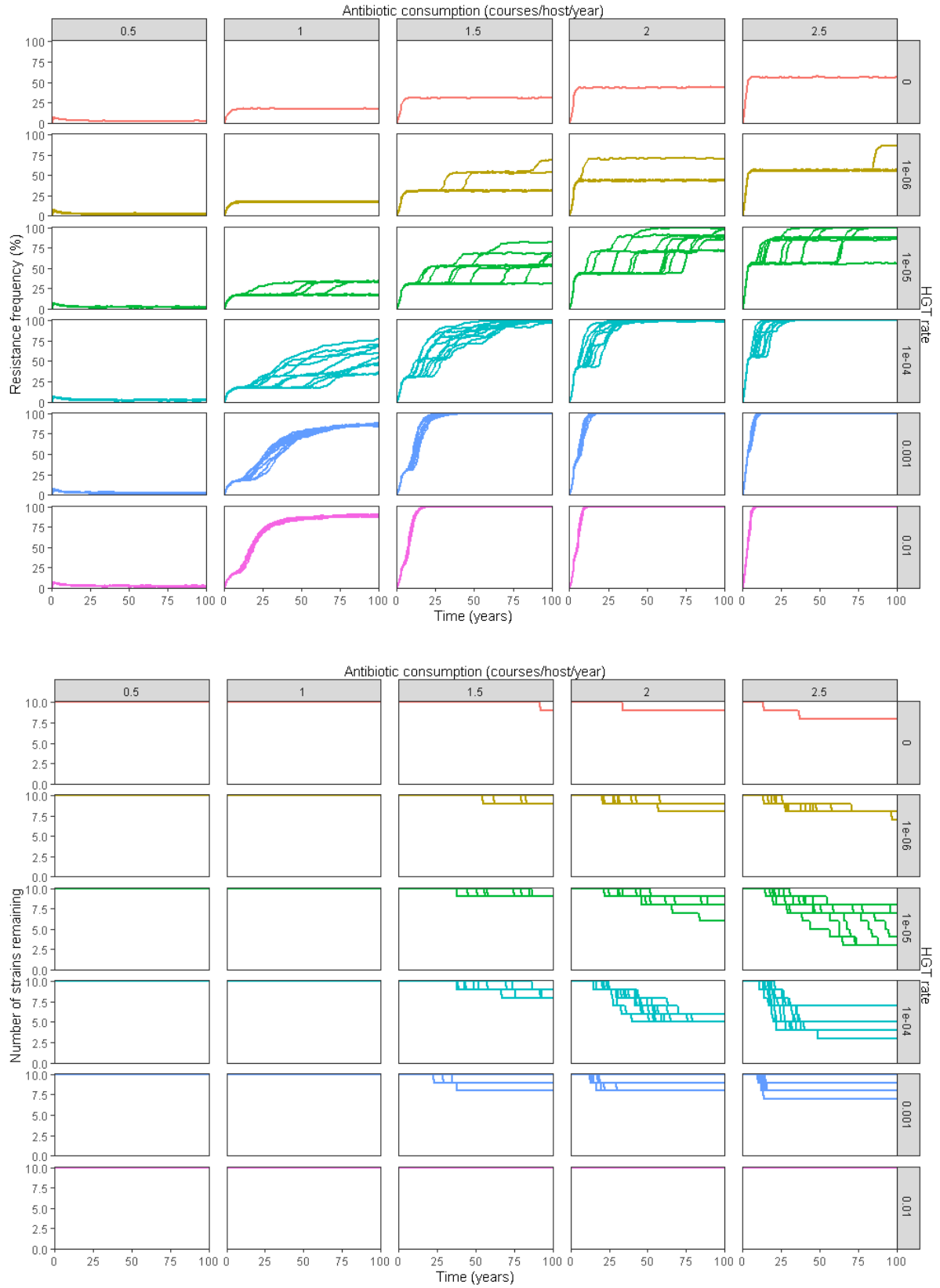


Figure 3.10: **Effect of HGT on resistance frequency (top) and strain diversity (bottom).** Resistance initialised in a single strain. Results of 10 runs shown; each line represents a single run. $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$. Strain parameters as per Table 3.3

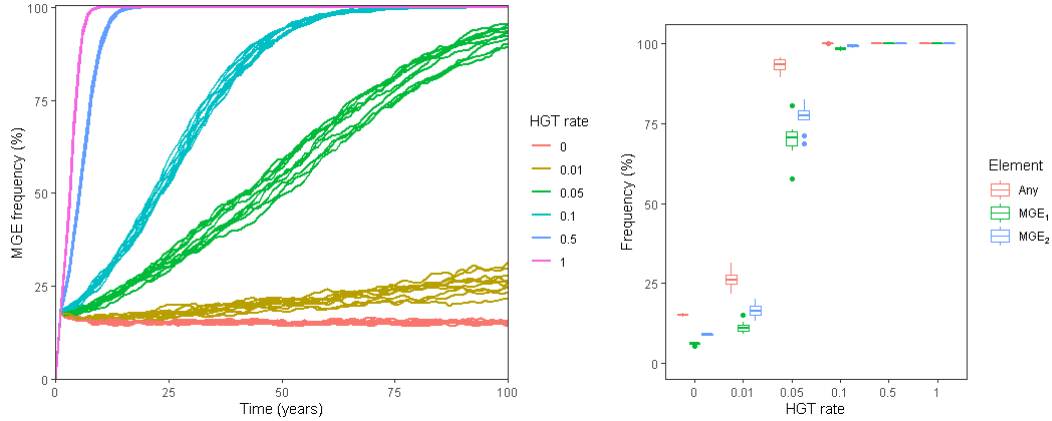


Figure 3.11: **HGT allows two MGEs to move through multi-strain populations, gradually increasing in prevalence.** Top, overall MGE frequency in the population over time (left) and after 100 years (right), for different rates of HGT. Frequencies of individual MGEs are also shown on the right. MGEs are associated with different sites. All plots show results of 10 simulations. $c_f = c_\beta = c_\gamma = 0$; $\alpha = 0$.

ICE-like MGE (MGE₁), and one phage-like MGE (MGE₂). As antibiotic consumption increases, resistance-bearing MGE₁ transfers into other strains, and expands to fixation. Dynamics of this MGE appear unaffected by it inserting at the same site within bacterial agents as MGE₂ (top). This implies that direct competition between bacterial agents for a physical location within bacterial genomes is not altering resistance dynamics.

By contrast, MGE₂ - which is not carrying resistance genes - is sensitive to competition for the same site as MGE₁. When MGEs target the same site, and MGE₁ is expanding due to selection pressure for resistance, MGE₂ is suppressed. This occurs irrespectively of whether the two MGEs undergo HGT at the same rate (top figure, bottom panel), or MGE₂ transfers more quickly (including by several orders of magnitude). These findings are reproducible over multiple simulations.

Differing MGEs, both carrying resistance Results are quite different when both MGEs carry resistance. In Fig. 3.13 (top), ICE-like MGE₁ with a moderate cost on competition fitness ($c_{f_{\text{MGE}_1}} = 0.4$, the same as in Fig. 3.12) is out-competed by resistance-carrying phage-like MGE₂, even when MGE₂ imposes a cost on carriage duration ($c_{\gamma_{\text{MGE}_2}} = 0.1$). These results are highly reproducible between simulations, and vary little if MGEs insert at the same, or different, sites.

By contrast, simulations where MGE₁ has a smaller cost ($c_{f_{\text{MGE}_1}} = 0.1$) are much more

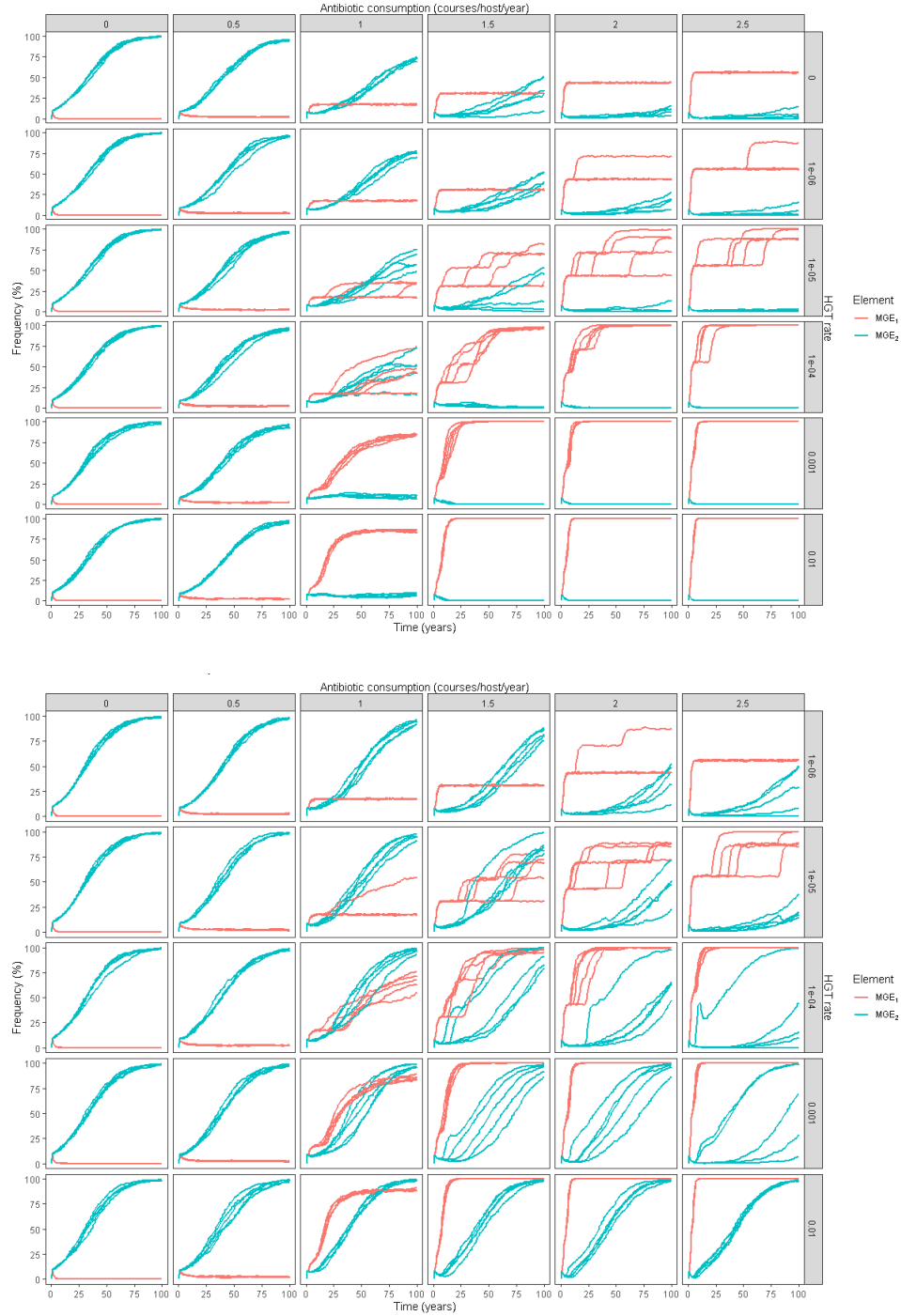


Figure 3.12: **Competition between an ICE-like MGE (MGE₁) and a phage-like MGE (MGE₂).** Top, MGEs located at the same site within the bacterial agent; bottom, MGEs located at different sites. Only MGE₁ carries resistance. β_{MGE_1} shown on right axis; $\beta_{\text{MGE}_2} = 0.01$. $c_{f_{\text{MGE}_1}} = 0.4$, no other costs. Results from 5 simulations.

variable. In Fig. 3.13 (bottom), equilibrium frequencies of different MGEs vary when their rates of HGT differ. Here, successful transfer of the ICE-like MGE₁ into a new strain incurs a corresponding decrease in frequency of phage-like MGE₂. This is illustrated in Fig. 3.14, where the phage-like MGE₂ almost always reaches fixation in individual strains (bottom plot, top panel). However, emergence of the ICE-like MGE₁ within a strain results in competitive exclusion of MGE₂.

Results from these simulations can be compared to those where MGEs have different costs, but HGT is negligible, as shown in Fig. 3.15 (top). In this plot, it is clear that when the cost on competition fitness $c_{f_{\text{MGE}_1}}$ is less than about 0.18, MGE₁ out-competes MGE₂, which bears a cost on duration of carriage $c_{\gamma_{\text{MGE}_2}} = 0.1$. However, when MGE₂ transmits horizontally at faster rates than MGE₁, it is able to persist in the population despite low costs on MGE₁ (bottom plot, top two panels). (Note that overall resistance goes to fixation under these conditions; i.e. resistant/sensitive coexistence is not maintained.) Additionally, there appears to be little difference in results when the two MGEs are directly competing for the same site within the bacterial agent, or not.

3.3.5 Bacterial immigration

The sensitivity of the equilibrium frequency of resistance to population antibiotic consumption is reduced with increasing rates of bacterial immigration from an external population (Fig. 3.16). Under the parameter conditions tested here, the relative impact of immigration compared to antibiotic consumption increases substantially between the orders of magnitude $\eta = 0.1$ and $\eta = 10$. As immigration rate increases, resistance frequency in the recipient population approaches the resistance frequency in the donor population (Fig. 3.17).

3.4 Discussion

I showed in Chapter 2 that coexistence in a multi-strain system is promoted by resistance being associated with some strains, but not others, and that the equilibrium frequency of resistance is determined by both the proportion of resistant strains, and the rates of antibiotic consumption. However, these relationships are threatened by the ability of

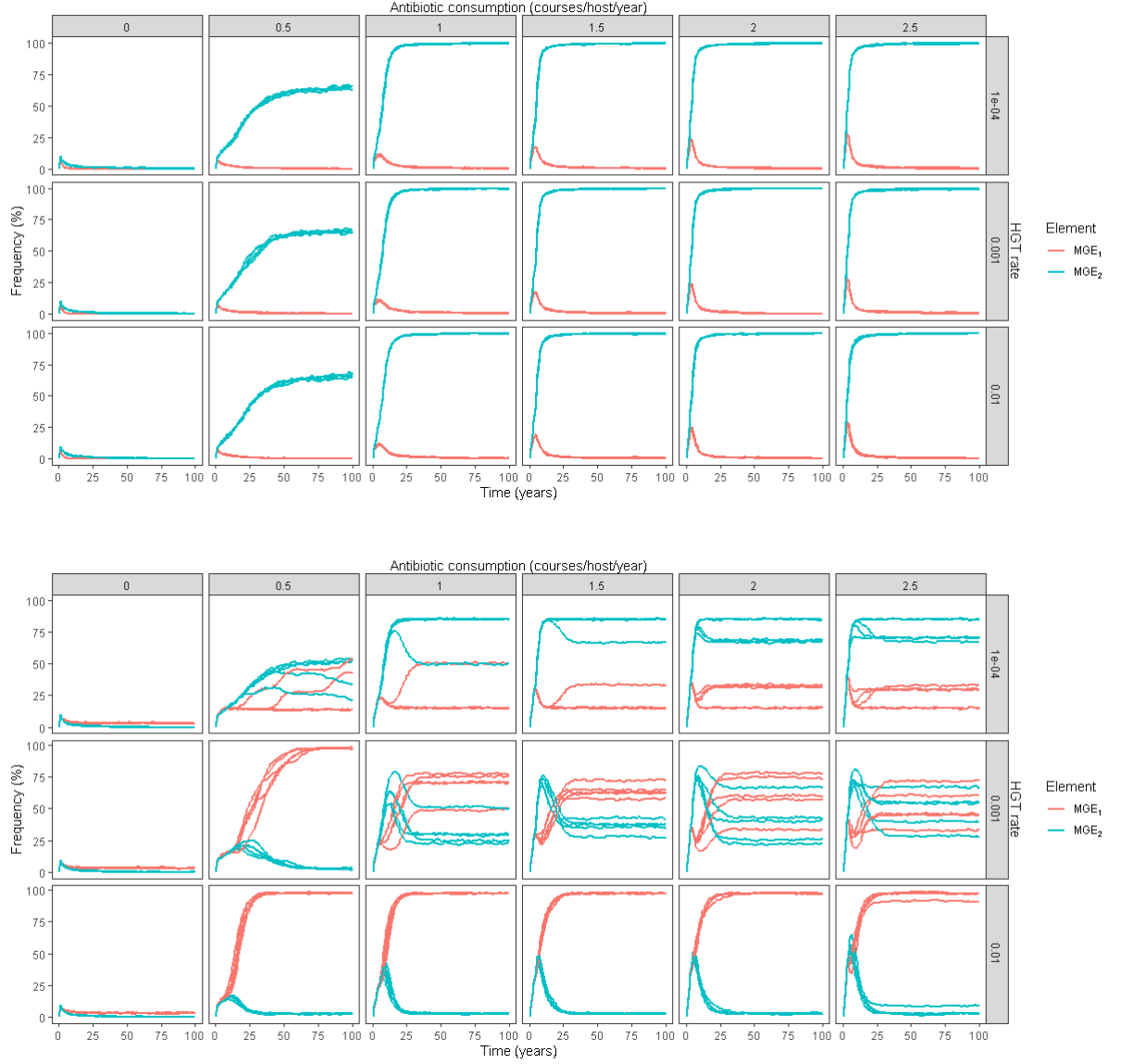


Figure 3.13: **Competition between an ICE-like MGE (MGE_1) and a phage-like MGE (MGE_2), when both carry resistance.** Top, MGE_1 has the same cost as previously shown (Fig. 3.12), $c_{f_{MGE_1}} = 0.4$; bottom, a smaller cost ($c_{f_{MGE_1}} = 0.1$). $c_{\gamma_{MGE_2}} = 0.1$ in both plots. β_{MGE_1} shown on right axis; $\beta_{MGE_2} = 0.01$. MGEs located at the same site; results are similar when located at different sites. Results of 5 simulations.

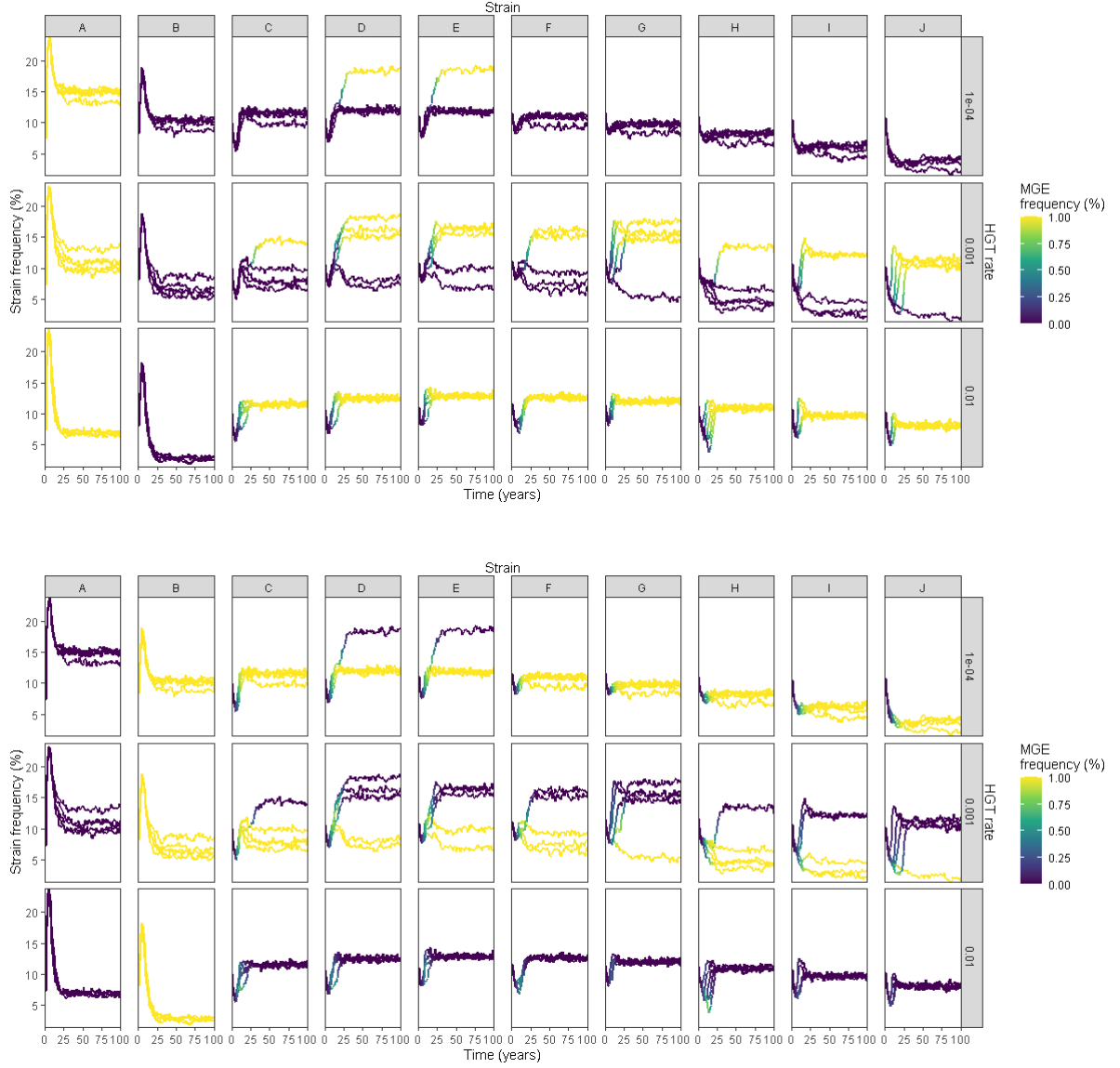


Figure 3.14: **Competition between an ICE-like MGE (MGE_1) and a phage-like MGE (MGE_2), when both carry resistance.** Individual strain frequencies (y axis) and MGE frequency (colour) within each strain, for MGE_1 (top) and MGE_2 (bottom). $c_{f_{MGE_1}} = c_{\gamma_{MGE_2}} = 0.1$ in both plots. β_{MGE_1} shown on right axis; $\beta_{MGE_2} = 0.01$. $\alpha = 1.0$. MGEs located at the same site; results are similar when located at different sites. Results of 5 simulations.

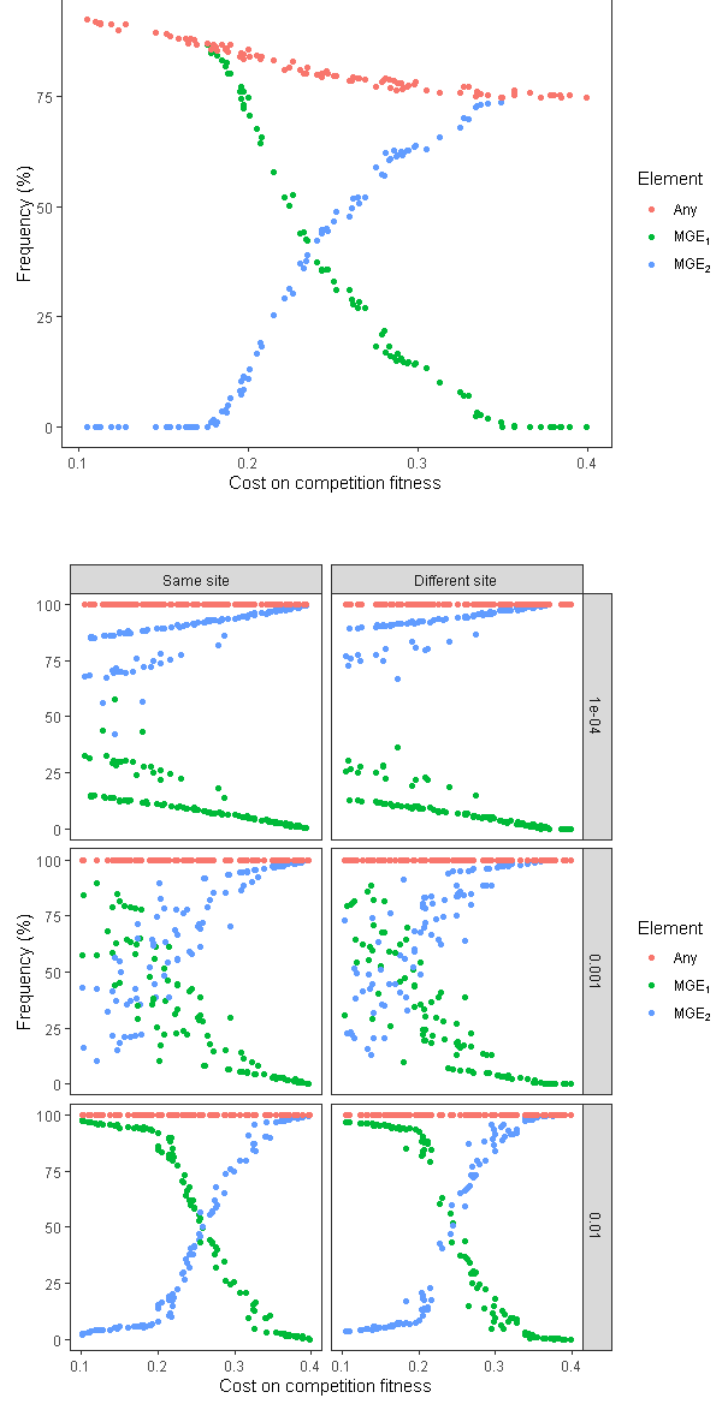


Figure 3.15: **Coexistence of MGEs with different cost types is facilitated by differing rates of HGT.** Top, dose-response curves for MGE₁ and MGE₂ when $c_{\gamma_{\text{MGE}_2}} = 0.1$ and HGT is negligible. Bottom, dose-response curves when rates of HGT are non-negligible. β_{MGE_1} shown on right axis; $\beta_{\text{MGE}_2} = 0.01$. $p_w = 1$, $p_b = 0.1$, $\alpha = 1$.

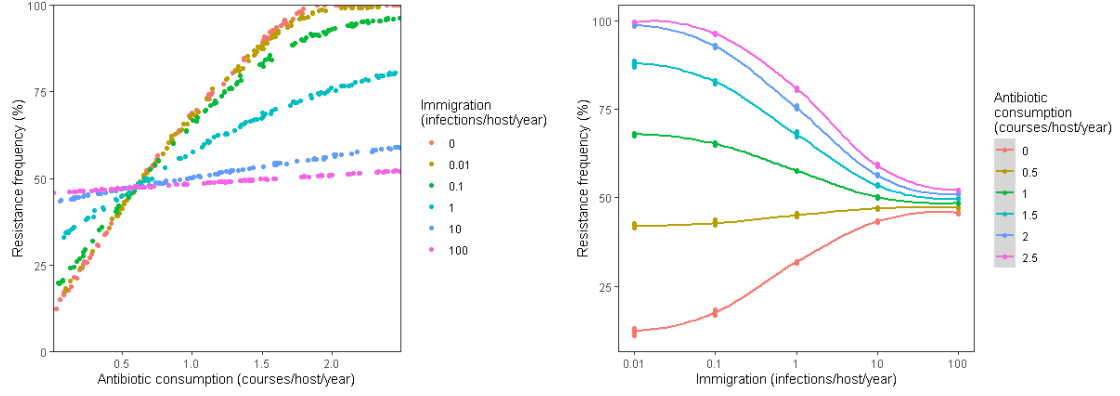


Figure 3.16: **Impact of bacterial immigration on the relationship between antibiotic consumption and resistance frequency in the recipient population.** Left, population dose-response curves for varying antibiotic consumption (x axis) and immigration rates (colour). Right, smoothed lines showing decreasing impact of recipient antibiotic consumption (colour) with increasing immigration rate (x axis). Resistance frequency in donor population = 50%. $n = 10$, $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$. Strain parameters as per Table 3.1. Results from 1 run (left); smoothed results from 10 runs (right) per parameter combination.

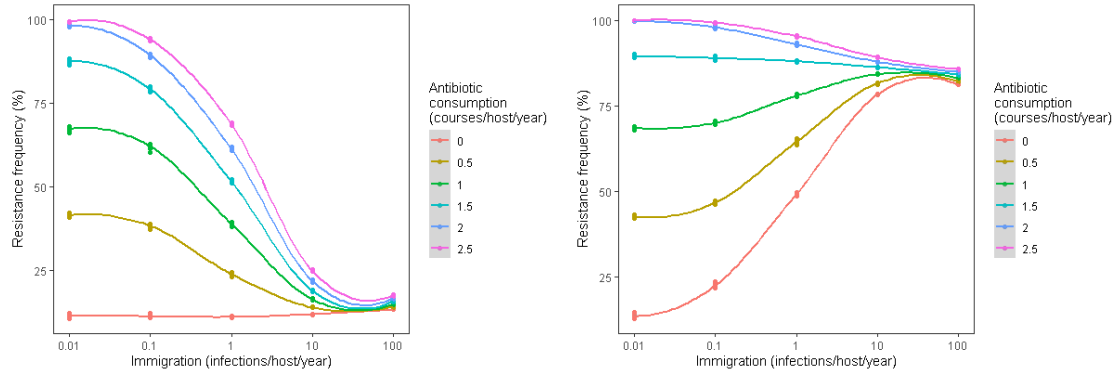


Figure 3.17: **Impact of resistance frequency in the donor population and immigration rates on resistance in the recipient population.** Both plots show resistance frequency in the recipient population on the y axis, immigration rate on the x axis, and antibiotic consumption. Donor population with 10% resistance (left) and 90% resistance (right). Resistance in the recipient population approaches the resistance frequency in the recipient population, as immigration rates increase. $n = 10$, $p_w = 1$, $p_b = 0.1$, $c_f = 0.4$. Strain parameters as per Table 3.1. Results of 10 runs per parameter combination.

resistance genes to transfer horizontally to other strains. In this chapter, the impact of different qualities of MGEs, and different rates of transmission between bacterial cells, was investigated.

Before allowing resistance to mobilise, it was necessary to understand the impact of strain-based competition on the expansion of resistance in a previously susceptible strain (and population). I introduced a new distribution of resistance at model initialisation, ‘strain-restricted’ resistance, where resistance genes were limited to specific bacterial strains, but were seeded into these strains at low prevalence. I showed in section 3.3.1 that the initial dynamics under these circumstances were fixing of resistance within strains (or elimination, if the balance of cost vs. antibiotic consumption was not favourable).

Emergence dynamics were explored further by introducing low numbers of resistant bacteria into stable, fully-susceptible, multi-strain populations. Results in section 3.3.2 show that introducing a small number of resistant bacterial agents from a novel strain establishes resistance in the population at lower rates of antibiotic consumption than introducing newly-resistant cells into a pre-existing (hitherto fully susceptible) strain. Furthermore, the difference between these two rates of antibiotic consumption varies by strain, and is smallest for the longest-duration strain (strain A). This is because this strain experiences the greatest selection pressure for resistance, and is consistent with previous work [385].

These two scenarios (a new resistant strain and newly-resistant cells within an existing strain) could plausibly represent acquisition of resistance via HGT or *de novo* mutation. HGT events are not restricted to resistance genes alone, and lateral transfer of large amounts of DNA can alter other phenotypic characteristics of bacterial cells, thus redefining their ecological niche [454, 455]. In a modelling context, this would warrant designation of a new (resistant) strain. *De novo* mutations conferring resistance could perhaps be better represented by emergence of partial resistance within a strain. To become robust to changes in antibiotic consumption, resistance would need to become fixed within that strain.

These emergence dynamics could also partly explain the varying timescales over which changes in antibiotic consumption are reflected in AMR prevalence. In Chapter 2, I hypothesised that strain-based competition could be facilitating persistence of resistant

strains despite reductions in antibiotic use. When antibiotic consumption is sufficient for new resistant strains to emerge and expand in a population, as I showed in section 3.3.2 above, the proportion of resistant strains increases. Subsequent reductions in antibiotic use will result in an equilibrium frequency of resistance that is determined by the interplay between consumption and this new proportion of resistant strains.

The results demonstrating competitive elimination of one of two resistance-bearing MGEs, when HGT is negligible, are consistent with the results of competition between resistant and sensitive bacteria in Chapter 2. Under this strain-based model of competition, MGEs, like resistance genes, need to be associated with specific strains in order to coexist in a way that is insensitive to small variations in cost and antibiotic consumption (Fig. 3.7). Otherwise, competition between MGEs means selection for the lowest fitness cost of resistance. This association increases the resilience of both MGE and strain and could explain why MGEs including ICEs [217, 331, 332] and plasmids [325] are associated with some bacterial strains, but not others.

It is difficult to parameterise rates of HGT in complex systems. Quantification of HGT in *in vitro* experiments does not necessarily translate to transfer *in vivo* [399]; even less so in real-world contexts. Exposure to antibiotics, for example, has been shown to induce up-regulation of mobilisable resistance genes [456, 457]. However, by simulating HGT rates across several orders of magnitude, I have shown that the impact of HGT on coexistence in this model is robust to large changes in the rate itself. If there is sufficient opportunity for resistance genes to transfer horizontally into previously susceptible strains (which is dependent on the frequency of strain co-colonisation, as well as the rate of HGT between cells), and conditions are favourable to resistance emerging in previously susceptible strains, the proportion of strains with which resistance is associated (and thus, the overall frequency of resistance) will increase. Fixation of resistance is then inevitable, except for the limited parameter window where cost and antibiotics are balanced. This outcome would be further exacerbated by any compensatory mutations that reduce the fitness costs of resistance, as has been shown to occur [458, 459].

I tested several scenarios involving competition between MGEs that could have potentially balanced the impacts of HGT of resistance. However, it is clear from the results presented in Figs. 3.12 that a slow-moving, resistance-bearing ICE-like MGE is not impeded

by a fast-moving, susceptible phage-like MGE, including when MGEs directly compete for the same location in the bacterial cell, and when the difference in rates of HGT is several orders of magnitude. The ICE-like MGE can be out-competed by a faster, resistance-bearing MGE, as I showed in Fig. 3.13, but under these circumstances, coexistence of resistant and sensitive bacteria is limited.

Taken together, the results in Figs. 3.13-3.15 provide important insights into competition between MGEs within this model. It is clear from these results that competition between resistance-carrying MGEs is not driven by direct competition within bacterial agents for a specific site; instead, the competition appears to occur within strains. Resistance-carrying MGEs that can enter new strains first, and expand within those strains, have an advantage. This allows fast-moving MGEs, even those carrying a cost on bacteria that would result in their competitive exclusion in the absence of HGT, to persist in the population. However, it is also clear from Fig. 3.14 that these fast-moving MGEs can be excluded from individual strains (and ultimately the population) if less costly, but slower-moving, competitors emerge. This implies a trade-off between vertical and horizontal modes of transmission [460], with the winning ‘strategy’ dependent on context, as has been suggested elsewhere [453].

My results also show that the relationship between antibiotic consumption and resistance frequency in a population can be affected by migration of bacteria from elsewhere. However, consistent with previous work [386], my results show that high rates of immigration are required to make the resident bacterial population insensitive to consumption. I have also assumed that the immigrating bacteria are equally well-adapted to onwards transmission as the resident bacteria, which is conservative. In reality, this would vary according to differences between hosts and the affinity of the bacterial species. A host-specialist bacteria such as *S. pneumoniae* immigrating between two demographically-similar human populations can be assumed to be equally well-adapted. However, this is not necessarily true for a specialist lineage of *E. coli* or *Salmonella* transmitting between animal and human hosts, for example [346, 370].

The results shown here present choices to modellers wishing to use a strain-based model of competition with HGT. In Chapter 2, I showed that robust coexistence in a closed system requires strain-associated resistance. Allowing resistance to transfer horizontally

between strains means that it can escape these restrictions. The observation that resistance is frequently strain-associated [325, 442], despite its ability to move horizontally between strains, is not easily explained. The framework developed here can help address that.

Models to understand the biological mechanisms shaping coexistence should be structurally neutral [384]. Given that the proportion of resistant strains in the current system is a key variable impacting equilibrium frequencies of resistance, models of strain-based competition that include HGT of resistance genes need to include some other mechanism to counteract the unidirectional acquisition of resistance (but not loss) by strains.

One possible option could be to allow for deletion of resistance elements [218, 274, 454, 459], for example, plasmid loss via segregation [461], as has been incorporated in other models [447, 462, 463]. However, it is clear from the results in this chapter that *ad hoc* deletion of resistance elements, for example excision of MGEs during recombination [464], will be insufficient as a strain-balancing mechanism in this model. This is because vertical transmission in this model is a stronger driver of resistant expansion than horizontal transfer. The influence of HGT in the presence of antibiotic consumption in this model is to introduce resistance genes into new strains, thereby allowing them to escape the balancing selection of strain-based competition. Thus, deletion of resistant elements, when the environment is favourable to resistance expansion, will be ineffective in reducing the proportion of resistance strains (because it is unable to delete resistance in the entire strain).

An attractive option is the implementation of interactions specific to each combination of strain and MGE [459], which would prevent successful establishment of resistance in previously susceptible strains. Mechanisms include: blocking the ability of certain genes or MGEs to transfer into some strains; or imposing strain-specific costs of MGEs, which would cause them to be selected against in some strains but not others. Both of these are biologically justifiable. Bacterial defences against MGE infection, such as restriction-modification systems [333–335], can preclude inter-species or inter-strain transfer of resistance genes and/or MGEs [217, 221, 336, 337]. HGT rates, and stability in the recipient bacteria, may differ depending on the MGE and the relatedness of the recipient strain to the donor [221, 459, 465]. Other authors have demonstrated varying costs of MGEs in different bacterial strains, including plasmids [221, 327, 328, 461, 466, 467] and ICE [221].

Recent work by Alonso-del Valle and colleagues measured the cost of an OXA-48 plasmid (conferring carbapenem resistance) in *Klebsiella* spp. and *E. coli* isolated from gut contents of human patients [328]. They showed plasmid costs to be highly variable, and normally distributed between positive and negative fitness effects.

Immigration of susceptible strains is a possible balancing mechanism, as my results indicate. High immigration rates are required to substantially modulate the relationship between antibiotic consumption and resistance in the recipient population (here, in the order of 10 colonisations/host/year). This may be realistic in some contexts (e.g. people who travel frequently [304], or work in close contact with animals [343]), but it is difficult to see how they could be generalisable across human populations. This level of immigration may be more relevant to models of gastrointestinal commensals such as *E. coli* in livestock, with bacterial immigration occurring from an environmental reservoir, or animals brought in from another source [434]. However, it could be effective as a means of introducing new, susceptible strains into the resident population to mitigate the inexorability of HGT in this model.

Alternatively, the model could incorporate continuous evolution of new sensitive strains, for example after MGE acquisition or mutation. This is biologically appropriate, and, while programmatically complex, would be achievable for an unlimited number of strains under an ABM approach.

The main limitations of this model are outlined in Chapter 2. There are additional biological realities of HGT within bacterial populations that are not captured here. I have not incorporated deletion of genes, which occurs continuously [454], or loss of MGEs. However, as discussed above, while inclusion of gene loss within this model would improve its biological accuracy, it is unlikely to affect the overall dynamics in a major way. I do not account for direct interactions between MGEs, which can be collaborative or antagonistic [337]. Most critical to sustained coexistence in the presence of HGT is likely to be interactions specific to each combination of strain and MGE, and/or the immigration or evolution of new susceptible strains. Both of these approaches could balance HGT-mediated fixation of resistance in all strains, as explained above.

Chapter 4

Results: Fitting to data using likelihood-free inference (LFI)

4.1 Introduction

Fitting the agent-based model (ABM) developed in Chapter 2 necessitated likelihood-free inference (LFI). LFI techniques measure the difference between observed and simulated data, and use this to approximate posterior distributions for parameters from which the simulated data is generated. Methodological options for LFI include approximate Bayesian computation (ABC) [468–470] and the more efficient sequential Monte Carlo (SMC) ABC [471, 472].

In ABC techniques, a sample θ^* is drawn from the prior distributions of the parameters θ , and model simulations are run. If the simulated output data x^* differ from the observed data x_{obs} by less than a given threshold ϵ , θ^* is accepted. The process is repeated many times to approximate the posterior distribution for θ .

Bayesian optimisation for likelihood-free inference (BOLFI) [473] is an alternative method of LFI. Rather than simply accepting or rejecting θ^* according to the distance between the observed and simulated data being within the threshold ϵ , BOLFI uses a Gaussian process to model the outputs of some initial simulations, and thereby generate a surrogate model of the relationship between a given parameter set and the resultant discrepancies between x^* and x_{obs} . Posterior estimates for each parameter correspond to the minimum discrepancy in this surrogate model and credibility intervals can be estimated

by Markov chain Monte Carlo (MCMC) sampling of the surrogate model surface.

BOLFI was selected because it offered order-of-magnitude reductions in computational time compared to other methods [473]. It was also accessible, having already been implemented within a Python library, Engine for LFI (ELFI) [474].

The observed x_{obs} and simulated x^* data used for LFI can be the full data, but typically, summary statistics encapsulating key features of the data are used instead [468, 469, 475, 476]. Summary statistics are usually not statistically sufficient for a given parameter θ [470], which means that their use involves a loss of information compared to the full data.

Identifying appropriate summary statistics on which to perform statistical inference is not necessarily intuitive, as a single statistic is unlikely to be sufficient for a given parameter set [477, 478]. BOLFI generates the probability surface from the discrepancy between a single target value and a simulated value; thus, the most informative summary statistic, or combination of statistics, and the most meaningful way to calculate the discrepancy, needed to be determined.

In this chapter, I describe the procedure I developed to identify the most suitable combination of summary statistics and discrepancy metrics to fit an ABM to existing data using BOLFI, including for strain-specific data, and present the results of this fitting.

4.2 Methods

4.2.1 Data set

Data on *S. pneumoniae* prevalence were drawn from a large survey in the Maela refugee camp near the Thailand/Myanmar border [426]. In this survey, nasopharyngeal swabs were taken from mother-infant pairs at monthly intervals from birth until the age of two years. Pneumococci were isolated from these swabs and characterised by serotype. Advanced diagnostic techniques such as microarray were used to determine the prevalence of multi-serotype carriage in these individuals [425]. Lees *et al.* subsequently estimated the duration of carriage of each serotype using a set of hidden Markov models [427].

4.2.2 Identifying summary statistics

The procedure I developed to identify suitable summary statistics and discrepancy metrics is summarised in Table 4.1.

-
1. Identify candidate summary statistics by examining the data.
 2. Create a grid of input parameter values and run simulations over all combinations of these values.
 3. Calculate candidate summary statistics and plot them against the parameter values.
 4. Refine list of candidate summary statistics to those with a clear relationship to parameter values.
 5. Identify candidate discrepancy metrics, including transformations.
 6. Select test parameter sets, run model simulations, and calculate the value of the resulting summary statistics and candidate discrepancies (both individual and combined, transformed and non-transformed).
 7. Use BOLFI to fit the model to the target discrepancy values.
 8. Assess the ability of the different discrepancy metrics to recover the test parameter set.
-

Procedure 4.1: Identifying optimal summary statistics and discrepancy metrics when fitting an ABM using BOLFI.

First, candidate summary statistics, such as prevalence of infection, were identified from the existing literature and by examination of the available data. I then conducted a systematic exploration of the model’s parameter space by generating a set of values at regular intervals across the value range for each input parameter (Table 4.1).

Initially, the model was populated with ten identical strains, each strain i with a natural clearance rate γ_i calculated from the duration of carriage of serotype 6A/C in the Maela dataset [427] (D_{6AC} , $\gamma_i = 1/D_{6AC}$, see Table 4.2). Competition was continuous and antibiotic consumption was zero.

For every combination of values from individual parameter sets, I ran the model simulation 10 times and calculated the value of the candidate summary statistics. The relationship between different parameter values and values of candidate SSs was assessed visually (Fig. 4.1). The list of candidate SSs was reduced to those where the SS value

	Parameter	Fixed or explored	Value
n	Number of strains	Fixed	10
γ	Rate of natural clearance	Fixed	6.13
R_0	Basic reproduction number	Explored	2.0 - 20.0
β	Transmission parameter	Explored	$R_0\gamma$
p_w	Probability of within-strain competition	Explored	0.0 - 1.0
p_b	Probability of between-strain competition	Explored	0.0 - 1.0

Table 4.1: **Parameter values systematically explored for the identification of summary statistics.** Homogenous duration of carriage and continuous competition.

generated from dissimilar parameter values varied widely (and predictably), but SS values generated from similar parameter values varied little (i.e., displayed a small inter-quartile range (IQR)).

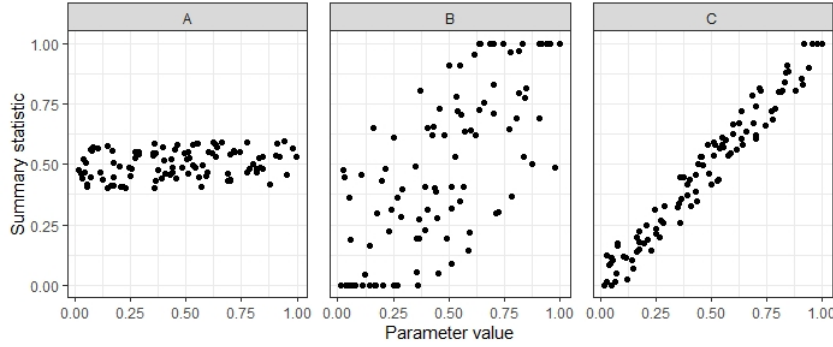


Figure 4.1: **Identifying suitable summary statistics.** Schematic showing relationships between values for a given hypothetical parameter and candidate summary statistics (SSs). A, not suitable: little variation in SS generated from dissimilar parameter values. B, suitable: SS varies with changing parameter values in a predictable way. C, most suitable: less variation in SS generated from similar parameter values than B.

4.2.3 Calculating discrepancy metrics

Different discrepancy metrics were then calculated from the candidate summary statistics.

4.2.3.1 Population summary statistics

The absolute discrepancy d_X between the observed value x and the simulated value x^* of a population-level summary statistic X was calculated:

$$d_X = |x - x^*| \quad (4.1)$$

The relative discrepancy d'_X between the same observed and simulated values was determined by:

$$d'_X = \frac{d_X}{x} = \frac{|x - x^*|}{x} \quad (4.2)$$

4.2.3.2 Strain-specific summary statistics

Some summary statistics were specific to individual strains (e.g. the duration of carriage of bacteria belonging to that strain). The discrepancy metric d_Y for a strain-specific summary statistic Y was calculated as:

$$d_Y = \sum_{i=1}^n \frac{|y_i - y_i^*|}{n} \quad (4.3)$$

where y_i and y_i^* are the observed and simulated values, respectively, of the summary statistic specific to strain i , and n is the number of strains.

4.2.4 Recovering parameter values

Finally, these candidate summary statistics and possible discrepancy metrics were tested for their ability to recover known values for the parameters R_0 , p_w and p_b . I chose reasonable values for the input parameters, ran the model simulation, and calculated the value of the summary statistics. I then generated the resultant discrepancies on both standard and log scales. Combinations of two or more summary statistics were combined by summing the untransformed or log-transformed values. This set of discrepancy values then became the target values for BOLFI inference. I used ELFI to perform 10 fits of the BOLFI model to each target value, then compared the inferred parameter values from each set of fits to the true parameter values.

4.2.5 Fitting to data

4.2.5.1 Population parameters

First, the model was fitted to population-level data. Prior distributions were determined from the grid exploration of the parameter space. To increase computational efficiency, the prior for R_0 was restricted to a narrower range (see Eqn. 4.4). Combinations of the probabilities of within- and between-strain competition were restricted to those necessary

for maintaining strain diversity, as defined and identified in Chapter 2. That is, the probability of within-strain competition occurring exceeded the probability of between-strain competition ($p_w > p_b$).

Bounds were used to set priors such that:

$$\begin{aligned} R_0 &\sim U(2, 10) \\ p_b &\sim U(0, 1) \\ p_w &\sim U(p_b, 1) \end{aligned} \tag{4.4}$$

The ABM was fitted to a ten-strain system. While initial parameter exploration and identification of summary statistics (steps 1-6 in 4.1) utilised $\gamma_i = 1/D_{6AC}$ for each strain i , for the purposes of model fitting, each strain was assigned a duration of carriage equivalent to the average duration of the Maela serotypes. That is, $\gamma_i = 1/\bar{D}$, where:

$$\bar{D} = \frac{1}{10} \sum_{m=1}^{10} D_m \tag{4.5}$$

and m represents each serotype detailed in Table 4.2.

The model was fitted to the selected population-level SS using the BOLFI method within the ELFI package [473, 474]. Parameter values were estimated according to the minimum discrepancy in this surrogate model (median of 10 fits). Credible intervals were obtained from 10,000 MCMC samples of the surrogate model surface; otherwise, posterior samples from 10 fits were pooled and regions of highest posterior density (HPD) for each parameter were identified using the *hdr.den* function in the **hdrcde** package in R [479].

To test the plausibility of these results, an additional 10 simulations were performed using the fitted parameter values, and the generated SS values were then compared to those observed in Maela.

4.2.5.2 Strain-specific parameters

The population-level parameters were then fixed to the inferred values, and the process was repeated for strain-specific rates of natural clearance and transmission. Due to computational limitations, fitting to strain-specific parameters was performed in a stepwise fashion, rather than for all parameters simultaneously. First, the average rate of natural

clearance $\bar{\gamma}$ for all strains i through n was inferred, using the prior distribution:

$$\bar{\gamma} \sim U(0, \frac{1}{D_{6AC}}) \quad (4.6)$$

Next, γ_i and R_{0_i} for each individual strain were inferred sequentially, in descending order of γ_i , as described in Procedure 4.2. Prior distributions for each strain i through n were as follows:

$$\begin{aligned} \gamma_i &\sim U(\frac{1}{D_{6AC}}, \frac{1}{D_i}) \\ R_{0_i} &\sim U(1.5, 4) \end{aligned} \quad (4.7)$$

Where carriage duration D_i is equivalent to the carriage duration of the corresponding serotype in the Maela dataset D_m (Table 4.2).

-
1. Let $i = 1$.
 2. Allow $\gamma_j = \bar{\gamma}$ and $R_{0_j} = R_0$, where R_0 is the result of the population-level inference, for all strains $j = i + 1$ through n .
 3. Fit for γ_i and R_{0_i} .
 4. Fix γ_i and R_{0_i} to values obtained in 3., then let $i = i + 1$.
 5. Repeat steps 2.-4. until $i > n$.
-

Procedure 4.2: Sequential fitting of strain-specific ABM parameters using BOLFI.

Estimates and credible intervals were obtained as before. Simulations using the resulting inferred values were performed and their results compared against the observed values. Simulations were repeated with the maximum value for p_w , $p_w = 1$, as identified in Chapter 2.

Antibiotic consumption and resistance were then incorporated into additional simulations using the inferred parameter values. Rate of antibiotic consumption α was set equivalent to consumption in Maela. Resistance was strain-restricted (as defined in Chapter 3, section 3.2.4), according to the distribution of resistance in the Maela serotypes. For comparison, additional simulations were performed where resistance was associated with strain A only. Costs associated with resistance were imposed on the competition fitness f ($c_f \in \{0, 0.1, \dots, 1\}$) and/or transmission parameter β ($c_\beta \in \{0, 0.1, \dots, 1\}$).

4.3 Results

4.3.1 Data

The prevalence of pneumococcal infection of children in Maela was 0.75 [425]. The proportion of infected individuals carrying one unique serotype was 0.54 [425]. The rate of antibiotic consumption was equivalent to $\alpha = 0.95$ (N. Croucher, unpublished data).

The ten most prevalent serotypes [426] for which estimates of carriage duration were available [427] are shown in Table 4.2, each of which were represented in the model by a different strain (nominally, A-J). Model fitting was initially performed using the average duration of carriage of these serotypes, such that $\gamma_i = 1/\bar{D} = 5.67$.

Serotype	Prevalence (%) [426]	Duration of carriage (days) [427]	Resistance (%) [N. Croucher, unpublished data]
19F	16.2	106.4	97.4
23F	12.9	80.5	80.7
6B	9.2	75.7	38.9
14	5.6	66.7	74.7
21	≈ 1	61.1	0.0
6A	5.0	59.5	12.4
6C	3.2	59.5	0.0
19B	≈ 1	59.4	0.0
18C	≈ 1	57.6	0.0
NT	10.5	47.2	79.4

Table 4.2: **Properties of pneumococcal serotypes in the Maela data set.** Table shows the ten most prevalent serotypes for which estimates of carriage duration are available.

4.3.2 Candidate summary statistics and discrepancy metrics

Candidate SSs identified from the Maela dataset included population-level measures such as the prevalence of infection and the proportion of infected hosts carrying a given number of uniquely identifiable strains, and strain-level measures such as the average duration of carriage of bacteria belonging to each strain (Table 4.3).

Notation	Summary statistic
I	Prevalence of infection
U_1	Proportion of infected hosts colonised with a single strain
U_2	Proportion of infected hosts colonised with two unique strains
U_3	Proportion of infected hosts colonised with three or more unique strains
D_i	Duration of carriage of strain i

Table 4.3: **Candidate summary statistics observed in the Maela data set.**

Grid exploration of input parameter values allowed us to identify which of these SSs provided the most information on the parameters, as outlined in 4.1.

The relationship between the parameters R_0 , p_w , and p_b and three of the candidate SSs are shown in Fig. 4.2. In this figure, the top left panel shows the small IQR of infection prevalence I generated from a single value of R_0 , over the full range of competition parameters. By contrast, the values for I at a given value of p_w or p_b have a large IQR. The top left panel also shows a clear relationship between increasing R_0 and I , which is not demonstrable for other parameters in this row.

I then used a fixed value of R_0 , and imposed the condition $p_w > p_b$ to explore the relationship between the remaining parameters and the candidate SSs. This showed a more clearly defined relationship between the competition parameters and summary statistics (Fig. 4.3). Here, the IQRs for each value of p_b were very small, indicating that this parameter should be clearly identifiable at a given value of R_0 .

4.3.3 Recoverability of parameter values

A parameter set that generated simulated data with summary statistics similar to those obtainable from the Maela data was selected (Table 4.4).

Parameter	Value
R_0	2.60
p_w	0.80
p_b	0.30

Table 4.4: **Parameter set used to test recoverability of known values.**

The model was fitted to d_I and the log of d_I . The minimum discrepancy values from the simulations used to generate the BOLFI surrogate model were more distinguishable when

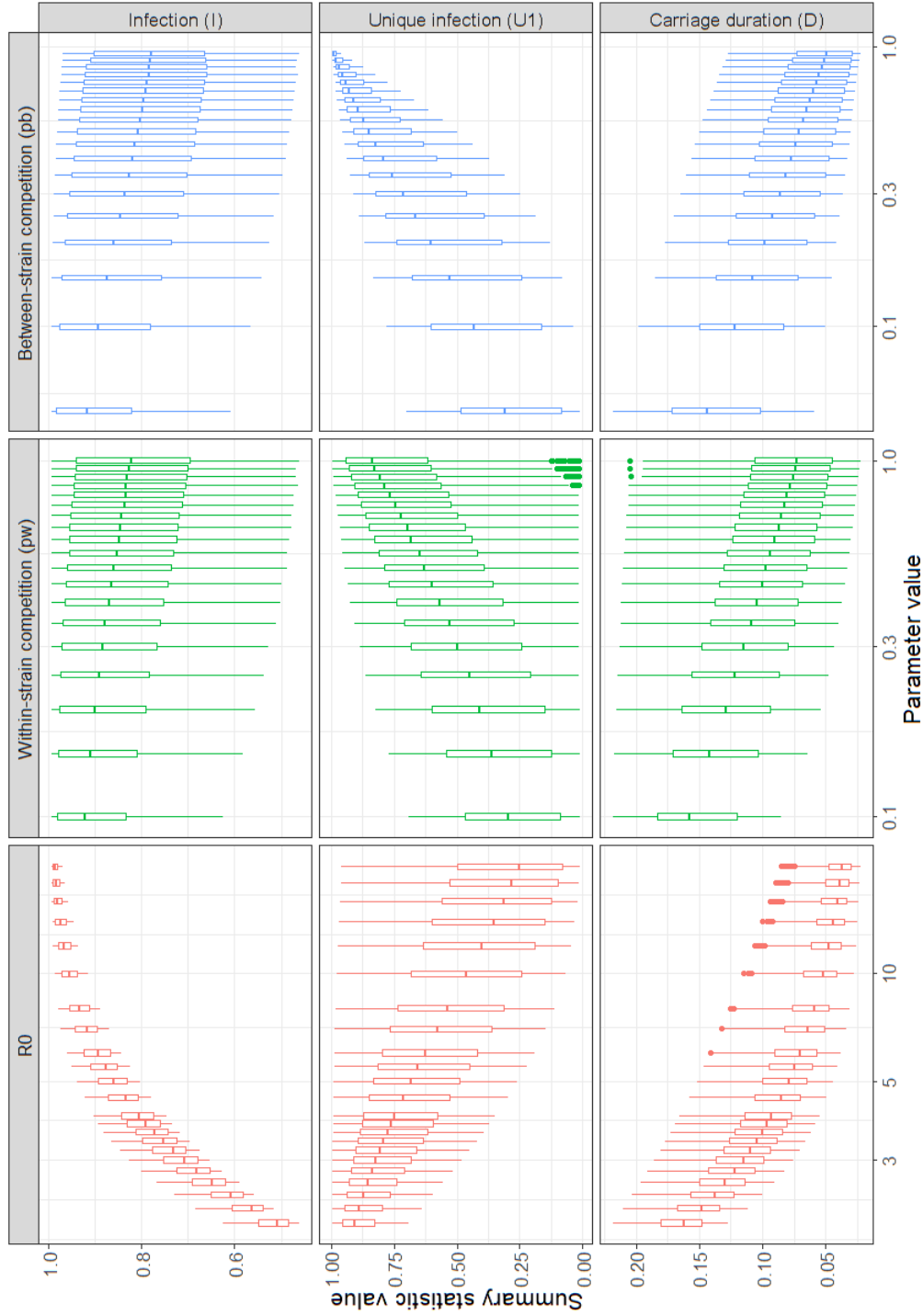


Figure 4.2: Variation in summary statistics (rows) for different input parameters (columns) during grid exploration of the parameter space. Results for the proportion of infected hosts carrying 2 or 3+ unique strains (U_2 and U_3) were similar to those shown for single-strain infection (U_1).

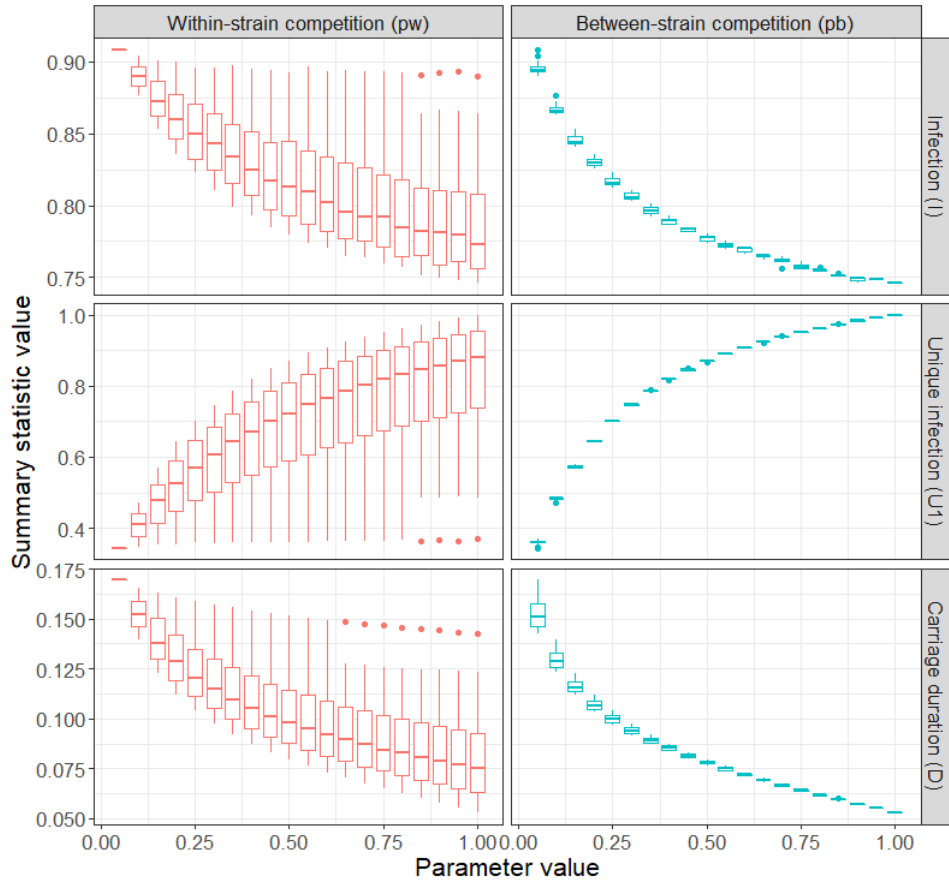


Figure 4.3: **Variation in summary statistics (rows) for different input parameters (columns) during grid exploration of the parameter space, when R_0 is fixed.** Results for the proportion of infected hosts carrying 2 or 3+ unique strains (U_2 and U_3) were similar to those shown for single-strain infection (U_1). $R_0 = 4.0$.

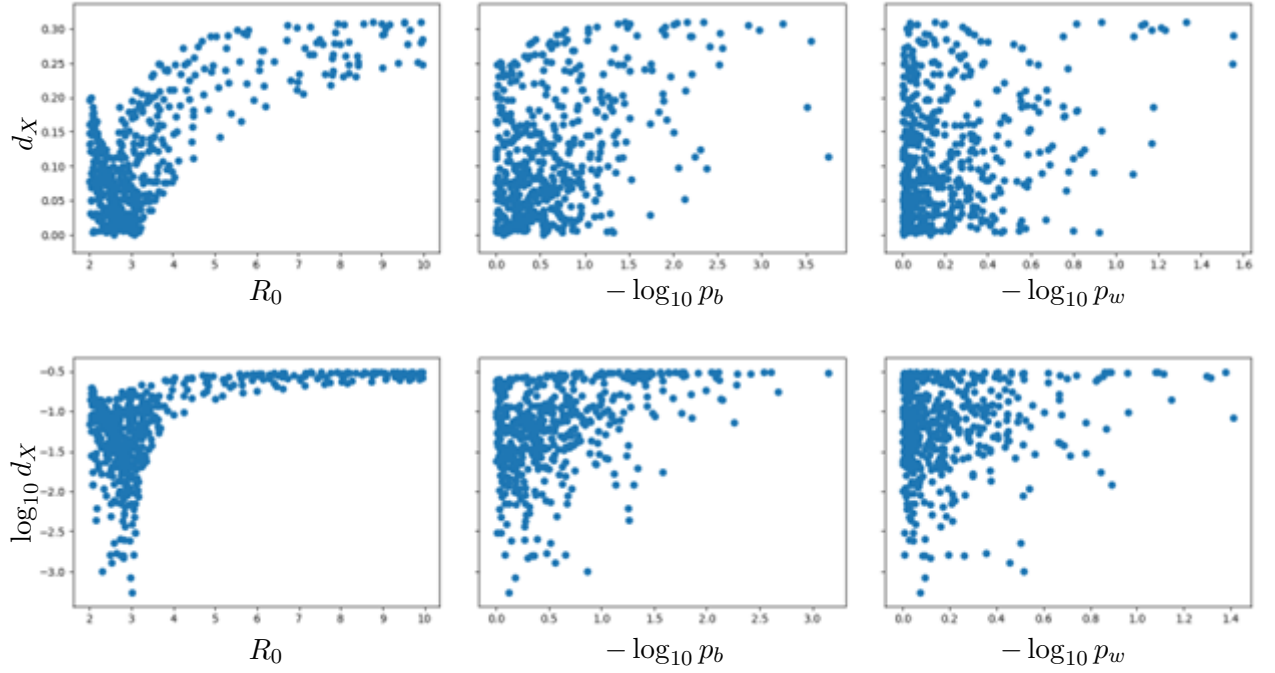


Figure 4.4: **Parameter values generating the smallest discrepancy between observed and simulated data are easier to identify using BOLFI when discrepancies are log-transformed.** BOLFI parameter estimation using the absolute discrepancy between observed and simulated values of infection prevalence (top, see also Eqn. 4.1) and log-transformation of the same (bottom). d_X , discrepancy (see Eqn. 4.1); p_b , probability of between-strain competition; p_w , probability of within-strain competition.

a log transformation was used (Fig. 4.4). Consequently, fitting to the log-transformed discrepancy inferred values much closer to the true values for all three parameters.

The model was then fitted multiple times to the log-transformed discrepancy d_I alone, and to the sum of the logs of d_I and d_{U_1} . The results showed that the parameter values inferred by the combined metric were both more accurate and more precise for R_0 and p_b (Fig. 4.5). The inferred value for p_w appeared more accurate using d_I alone, but estimates using either metric varied. The value of this parameter was repeatedly under-estimated.

4.3.4 Fitting to data

4.3.4.1 Population parameters

Example outputs from a single BOLFI fit are shown in Fig. 4.6. The parameter values inferred from fits to ten average-duration strains, using the sum of the logs of d_I and d_{U_1} , are shown in Fig. 4.7A.

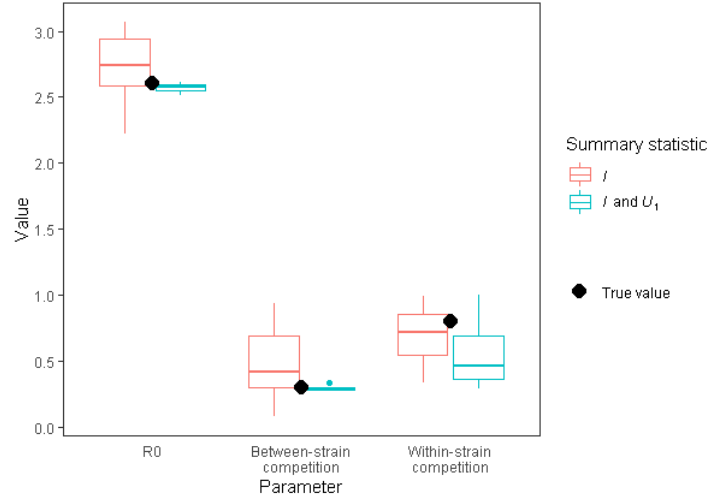


Figure 4.5: **Recoverability of true parameter values from different summary statistics using BOLFI.** Boxplots show results of simulations using parameter values inferred from the log-transformed discrepancy of infection (I) alone, or summed with the log-transformed proportion of unique infections (U_1). Points show true values. Results of 10 simulations.

Follow-up simulations using homogenous strain characteristics and the inferred population-level parameter values produced values for the summary statistics I and U_1 that were slightly lower than those observed in the Maela data, although variation was small (Fig. 4.7B).

4.3.4.2 Strain-specific parameters

Results of strain-specific parameter inference are shown in Fig. 4.8.

The results of simulations using the inferred values are shown in Fig. 4.9. Strain diversity was maintained, with strains persisting at different intermediate frequencies. Prevalence of infection and strain-specific carriage duration D_i^* produced by these simulations both aligned well with the Maela data. Sensitivity analysis using $p_w = 1$, rather than the inferred value $p_w = 0.15$, showed that the higher parameter value for p_w resulted in lower simulated carriage durations D_i^* for strains A-J.

The proportion of unique infections, U_1 , arising from simulations after strain-specific parameters had been fitted was less accurate than when the model was fitted to population-level parameters alone (compare Fig. 4.8B to Fig. 4.7B).

Incorporating Maela-like antibiotic consumption and resistance into these simulations showed that resistant and sensitive strains were able to coexist when there was sufficient

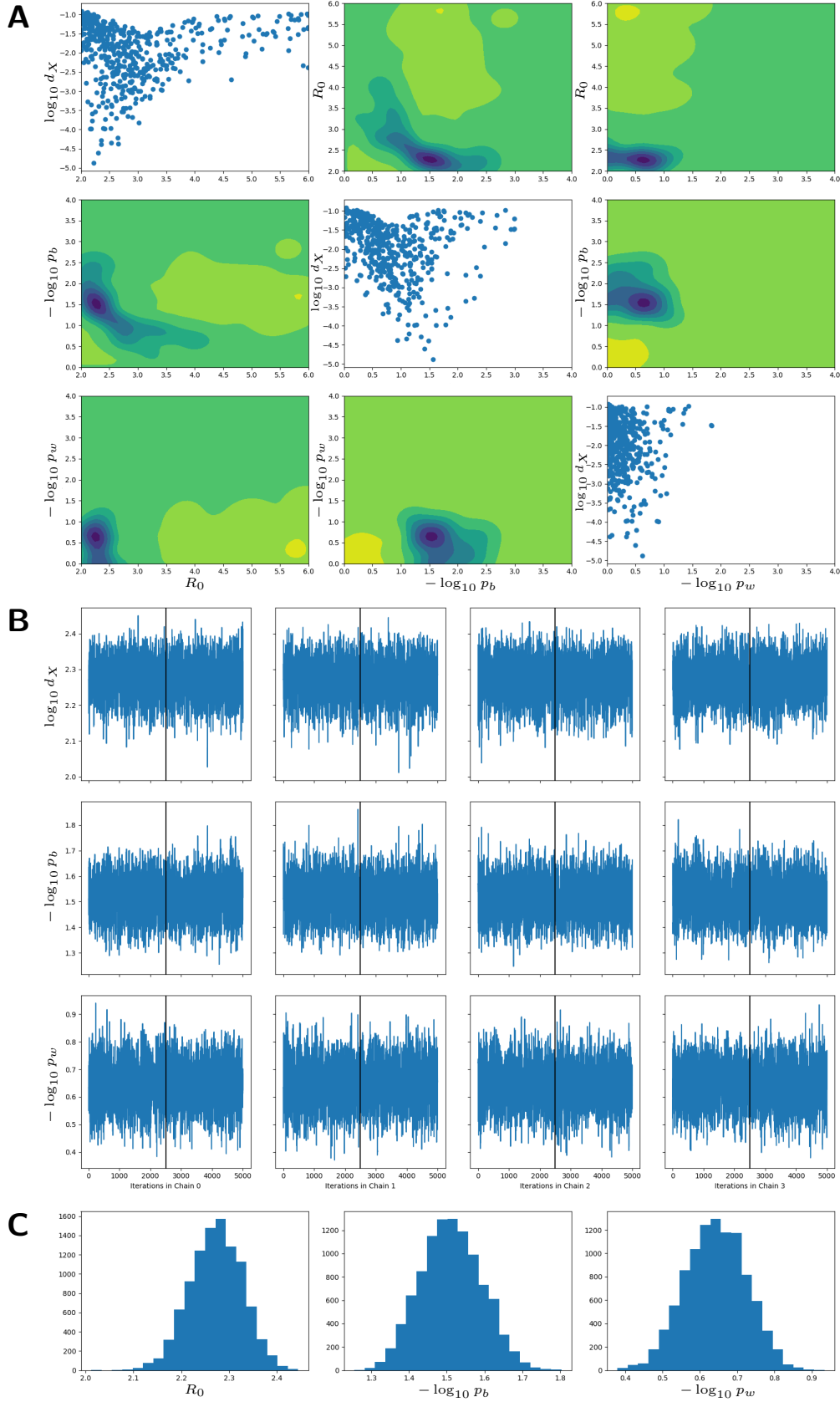


Figure 4.6: **Outputs from BOLFI model fit.** **A:** contour plots showing relative discrepancies (dark blue, smallest; yellow, largest) for combinations of parameter values, and dot plots showing discrepancies for each parameter, generated by the surrogate model. **B:** MCMC traces for each parameter. **C:** posterior distributions from MCMC sampling of the surrogate model surface. d_X , discrepancy (see Eqn. 4.1); p_b , probability of between-strain competition; p_w , probability of within-strain competition. Results representative of 10 fits.

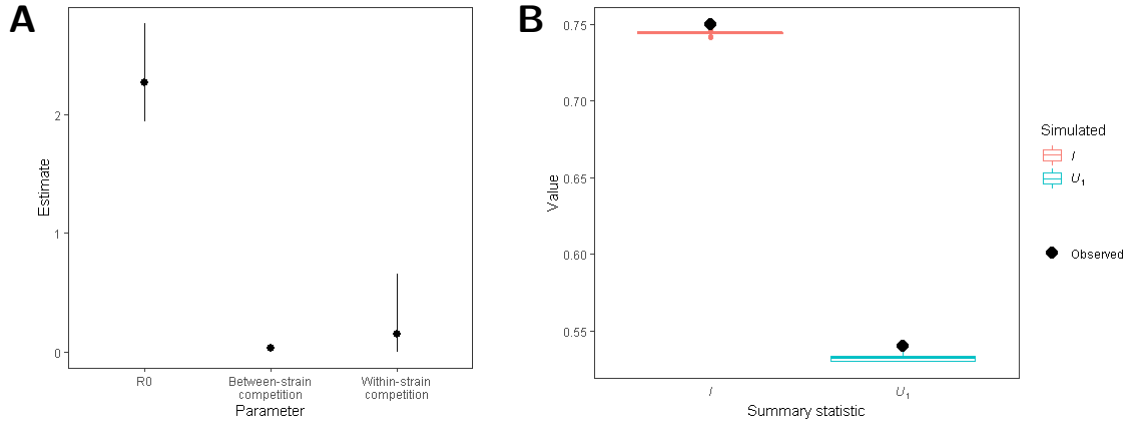


Figure 4.7: **Inferring parameter values from population summary statistics using BOLFI.** **A:** median estimate of parameter values (points) and 89% credible intervals (lines) from 10 BOLFI fits. **B:** summary statistic values generated from 10 simulations using the parameter values inferred in **A** (boxplots), and values observed in the Maela dataset (points). I , infection prevalence; U_1 , proportion of unique infections.

cost associated with resistance (Fig. 4.10). However, full strain diversity was unable to be maintained under these conditions using the inferred value for within-strain competition ($p_w = 0.15$); all 10 strains could be stably maintained when within-strain competition was higher ($p_w = 1$).

4.4 Discussion

Fitting the model to data was not straightforward. LFI methods such as the BOLFI technique used here require identification of informative summary statistics and discrepancy measures, through a largely empirical process. I identified candidate summary statistics from their availability in the data, then simulated values of these statistics via grid exploration of the parameter space. To test whether the best results were achieved from single or combination statistics, and absolute or transformed discrepancies, recoverability of known input parameters was assessed.

The most suitable statistic was infection prevalence, which had the clearest association with values of one parameter (R_0). Taken together, these results (Figs. 4.2 & 4.3) indicate that fitting to I , possibly in addition to another SS, should enable reasonable estimates of R_0 and p_b . I showed that good estimates of known values of R_0 and p_b could be achieved by fitting to the sum of the logs of the prevalence of infection and the proportion of hosts

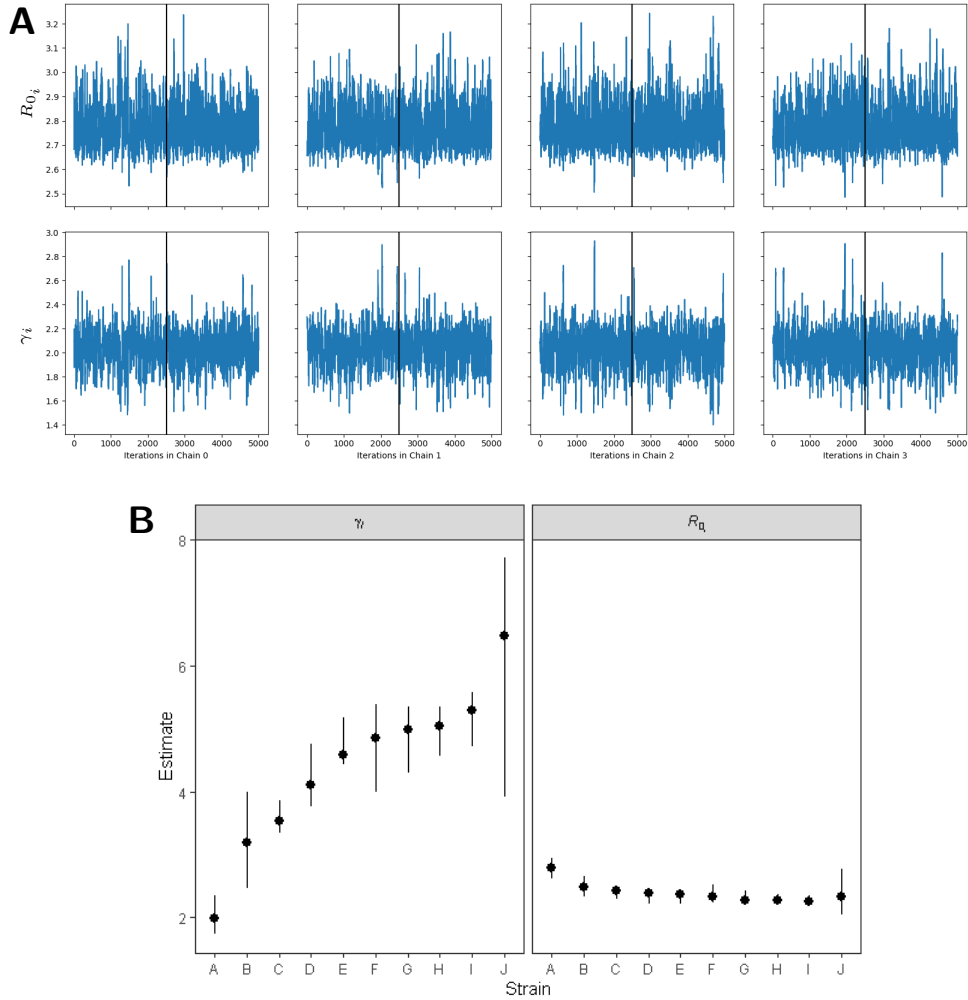


Figure 4.8: **Inferring strain-specific parameter values using BOLFI.** **A:** example of MCMC traces for strain-specific parameters (strain A). Results representative of 10 fits. **B:** median estimate of parameter values (points) and 89% credible intervals (lines) from 10 BOLFI fits. γ_i , strain-specific rate of natural clearance; R_{0_i} , strain-specific basic reproduction number.

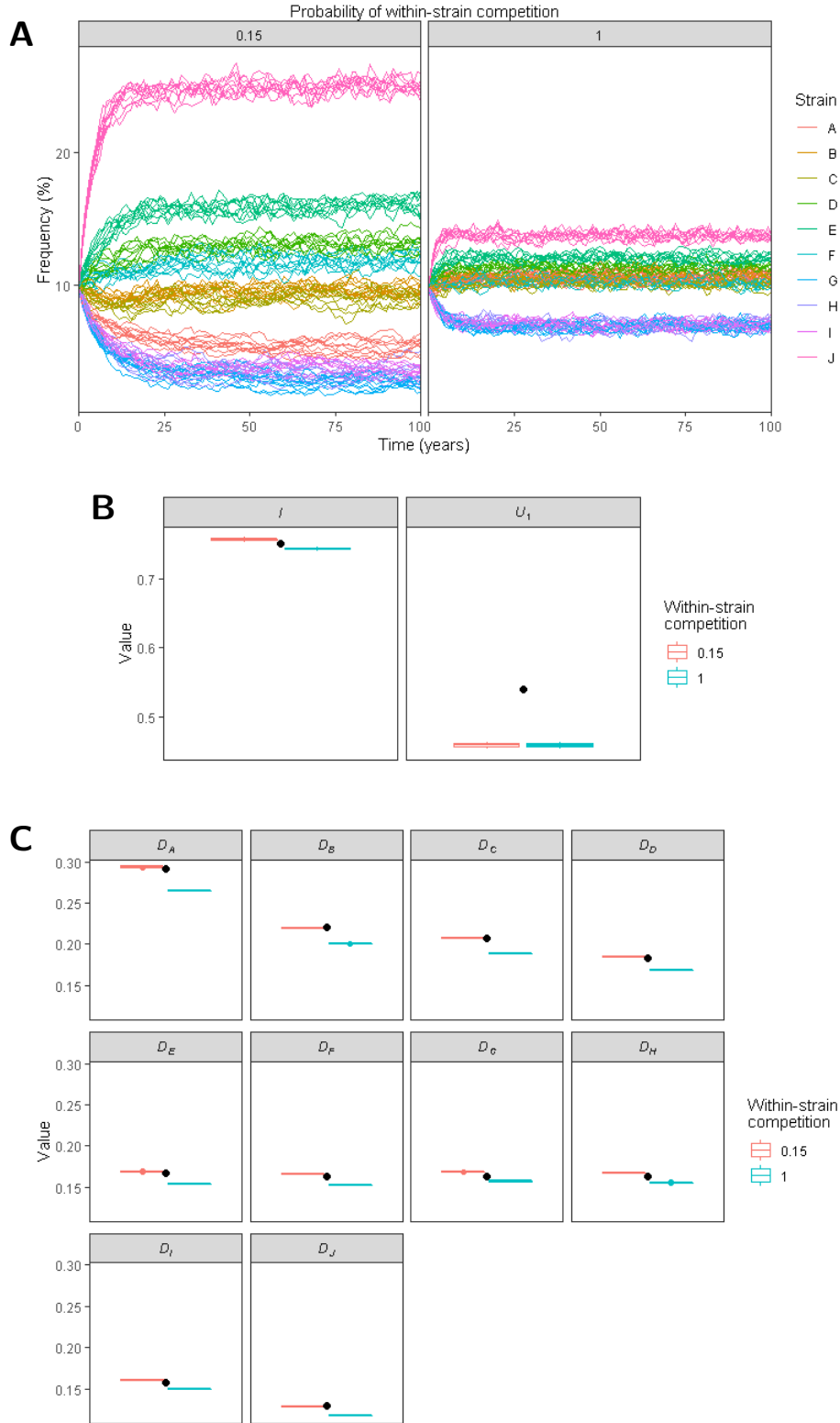


Figure 4.9: Effect of inferred strain-specific parameter values on strain frequencies, and resulting summary statistics. Results from 10 simulations using inferred parameter values (shown in Figs. 4.7A and 4.8B); simulations with the probability of within-strain competition $p_w = 1$ shown for comparison. **A**: strains stably coexist at different frequencies. **B**, **C**: boxplots show summary statistics for the population and for specific strains, generated from these simulations. Points show values observed in the Maela dataset. I , infection prevalence; U_1 , proportion of unique infections; D_i^* , simulated carriage duration for strain i .

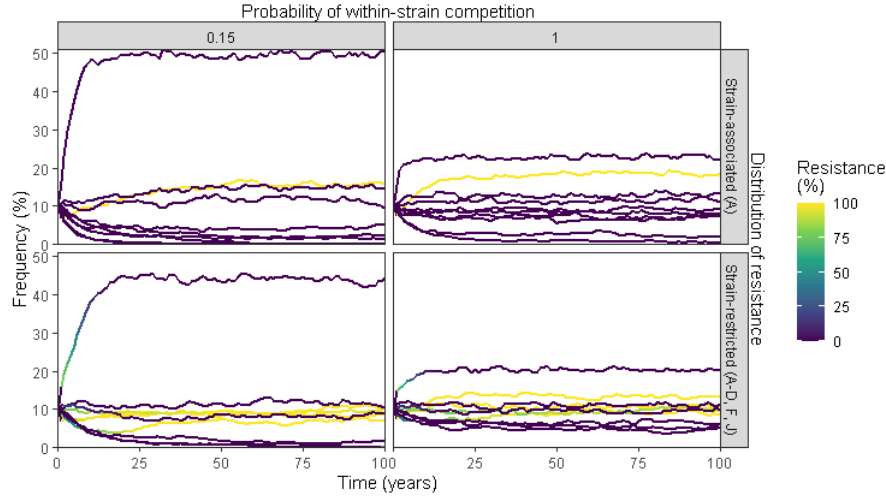


Figure 4.10: **Strain dynamics using inferred parameter values, after the introduction of antibiotic consumption and resistance.** Each panel shows the results of a single simulation; each line represents an individual strain, with its frequency on the y axis, and proportion of resistant cells in each strain denoted by colour. Initial resistance distribution either strain-associated (top) or strain-restricted, according to resistance in Maela serotypes (Table 4.2), and HGT is negligible. $\alpha = 0.95$, $c_f = 0.2$, $c_\beta = 0.2$. Inferred values as shown in Figs. 4.7A and 4.8B. Results are representative of 10 simulations.

infected with a single unique strain, from a simulated dataset. Use of log-transformed discrepancy metrics inferred parameter values much closer to the true values than non-transformed metrics.

As indicated from the grid exploration of the parameter space, within-strain competition could not be precisely estimated using the candidate summary statistics obtainable from the Maela data. This is supported by the results shown in Fig. 4.5 and the inferred value for p_w , which is surprisingly low, given the importance of a high p_w in maintaining strain diversity (as shown in Chapter 2). This is most likely because in a ten-strain model, bacteria are much more likely to encounter bacteria of other strains than their own strain, hence the relative insensitivity of BOLFI inference to the values of within-strain competition. In Chapter 2, I presented results showing that increasing R_0 and decreasing competition probabilities each increase the prevalence of infection amongst hosts, which is in turn correlated with unique infections.

When fitting the model to the Maela data using an average duration of carriage, there was more variation in the fitted values than during the parameter exploration exercise. This could be due to the difference in carriage duration affecting the intersection of values

of R_0 and between-strain competition at which the simulated summary statistics resembled those observed. This is because prevalence of infection increases with increasing R_0 but also with decreasing between-strain competition. Additionally, the point estimate taken from the poor fit to within-strain competition could be affecting the results.

Fig. 4.9 shows that sequential fitting of strain-specific parameters was effective in reproducing durations of carriage for each strain that are similar to those observed in the data. The wide range of inferred transmission values is notable, given that pneumococci should be under strong selection to transmit as quickly as possible (and persist as long as possible). This implies some kind of trade-off, although it may also involve costs and benefits of resistance.

Fig. 4.9 figure also shows that altering the probability of within-strain competition so that $p_w = 1$ does not improve these results. Notably, simulations using both the inferred and the assumed value for p_w do not closely replicate the proportion of unique infections observed in the data. This result, generated after strain-specific rates of transmission and natural clearance had been inferred, departs from the closer fit achieved for homogeneous strains, and is likely to be a consequence of fitting population-level parameters separately from strain-specific ones.

I used an absolute discrepancy between observed and simulated summary statistics, although relative or normalised discrepancies could also be considered. These were not necessary in this instance, due to the population-level summary statistics representing proportions, and being of the same order of magnitude. We showed that log transformation of the discrepancies achieved better results, due to the improved ability of the BOLFI model to distinguish the correct minimum of the Gaussian process. Multiple discrepancies can be combined in various ways, for example by taking a sum, product, or average, or through selection algorithms such as those utilised in ABC [478, 480].

The main limitation of this work was the ELFI package's requirement for a single summary value, around which to construct the surrogate model. In a complex system such as that contained within this ABM, successful parameter inference is likely to require multiple data points. While performing LFI on the full data is probably unnecessary, combining summary statistics into a single value may mean that too much resolution is lost for successful inference. Other techniques which measure discrepancies between

observed and simulated values, but allow the impact of parameter variation on *individual* SSs to be distinguished (e.g. ABC), may be more appropriate.

Other limitations of the BOLFI method of LFI, through the ELFI package, are largely computational. BOLFI was chosen for this work because it decreases computational time significantly compared to SMC-ABC, which is an alternative approach. I intended to infer rates of natural clearance for each individual strain simultaneously, but the dimensionality of a 10-parameter surrogate model exceeded the memory capacity of the available computing cluster. To address this, BOLFI was used to fit strain-specific clearance rates sequentially, thereby negating the major advantage of using this technique.

Chapter 5

Results: Generalised linear mixed models (GLMMs)

5.1 Introduction

Regression analyses have demonstrated positive associations between antibiotic consumption and antibiotic resistance at country level in both human [97, 245–247] and animal [249, 250, 418, 419] systems. These findings are consistent with antibiotic consumption being the primary driver of AMR within those system. However, there are large variations in the detected frequencies of resistance to individual antibiotics in different countries [250, 417–419], which do not necessarily align with consumption in those countries. Additionally, these analyses are restricted to single-year snapshots of resistance and do not account for non-independence of year-on-year sampling.

Data from Europe illustrates this point. Fig. 5.1 demonstrates the relationship between national penicillin consumption and penicillin non-susceptibility in *Streptococcus pneumoniae*. A positive, statistically significant relationship for Europe as a whole was demonstrated using linear regression on data from individual years in our previous paper [97], as well as by others [245]. Here, data points have been updated to span multiple years, and it is clear that this relationship holds at continental level (Fig. 5.1, left). However, when regression is performed individually for different countries, over multiple years, the relationship between penicillin consumption and resistance varies widely (Fig. 5.1, right). This finding, which applies to other combinations of bacterial pathogens and an-

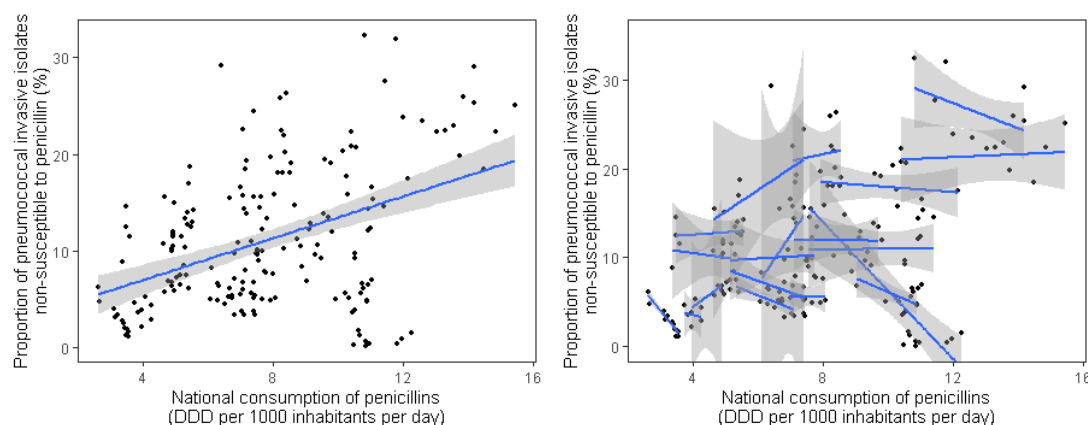


Figure 5.1: **Relationship between national penicillin consumption and penicillin non-susceptibility in *Streptococcus pneumoniae* in Europe, 2010-2021**. Blue lines show linear regression on data from all reporting countries, pooled (left) and individually (right). Shading shows 95% confidence intervals. Consumption data from [252, 482], resistance data from [483].

tibiotics in Europe [252, 481], indicates that alternative statistical methods for examining the relationship between antibiotic consumption and resistance are warranted.

Mixed-effects modelling is a relatively recent statistical technique that accounts for both population- and group-level effects on the dependent variable (commonly referred to as ‘fixed’ and ‘random’ effects, respectively) [484–487]. Mixed effect models are multivariate, thereby enabling the effect of several predictors to be tested simultaneously. Generalised linear mixed models (GLMMs) are extensions to both generalised linear models and linear mixed models, and can be applied to non-normal data. These techniques can explicitly account for non-independence of data points, such as repeated measurements of resistance within a population.

In this chapter, the relationships between antibiotic usage in people and animals, and resistance in isolates causing disease in people, were assessed by a series of GLMMs. First, I aimed to determine whether explicitly modelling group-level effects for individual countries improved models of resistance. Secondly, given the role of bacterial population dynamics in shaping resistance distributions, the impact of time was explicitly included in these models. Finally, I compared the effect of antibiotic consumption in hospital, community, and veterinary settings on resistance.

5.2 Methods

5.2.1 Data

Data for this analysis were obtained from publicly-available records of antibiotic consumption in animal and human populations, and resistance in clinical isolates from human patients, in European countries between 2010 and 2021.

5.2.1.1 Antibiotic consumption in the veterinary sector

Data on antibiotic consumption in food-producing animals were obtained from the European Surveillance of Veterinary Antibiotic Consumption (ESVAC) database [266] and reports [28, 488–497]. Data are expressed as total milligrams of active ingredient per population-corrected unit (mg/PCU). PCU is a proxy for the size of the animal population, calculated by multiplying the number of animals of each species by the assumed weight at the most likely time of treatment [498]. This enables direct comparison of consumption between animal species with very different body sizes.

Antibiotic class-specific mg/PCU were obtained from the ESVAC reports [28, 488–497]. From 2015 onwards, very low consumption of individual antibiotic classes is reported as “< 0.1” mg/PCU, rather than the precise number. In these instances, dummy variables were drawn from a uniform distribution $U(0, 0.1)$. All absolute values for mg/PCU were converted to percentage of total consumption. In some cases, due to the use of dummy variables and/or rounding errors, the sum of these percentages did not equal 100, in which case all percentages were scaled accordingly. Data in the reports are subject to amendment after publication, and so all percentages were multiplied by the 2021 total mg/PCU figure for each country, as obtained from the online database [266].

5.2.1.2 Antibiotic consumption in hospital and community sectors

Data on human antibiotic consumption were obtained from the European Centre for Disease Prevention and Control (ECDC). Data for available antibiotic classes and subclasses, spanning the years 2012–2021, were obtained from the most recent ECDC report [252]; additional data points, including those from 2010–2011, were downloaded from the Eu-

ropean Surveillance of Antimicrobial Consumption Network (ESAC-Net) database [482]. These data were expressed as the defined daily dose (DDD) per 1000 inhabitants per day (DDD/1000/day), which provides an estimate of the proportion of the national population treated with antibiotics [252, 499]. Data were retrieved from primary care (‘community’) and secondary/tertiary care (‘hospital’) settings for all classes and subclasses categorised within the WHO Collaborating Centre for Drug Statistics Methodology’s Anatomical Therapeutic Chemical (ATC) classification J01 [500].

5.2.1.3 Resistance in medical isolates

Data on the prevalence of resistance in human-associated bacteria were obtained from ECDC sources. These data were based on routine antimicrobial susceptibility testing of human clinical isolates. Information on *Acinetobacter* spp., *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. pneumoniae* resistance was retrieved from the Surveillance Atlas of Infectious Diseases [483]. Data on resistance in *Salmonella* and *Campylobacter* spp. were extracted from annual summary reports [154, 481, 501–505]. Resistance was reported as the number of isolates of each bacterial species tested against individual antibiotics (or antibiotic classes), and number of isolates clinically resistant, according to EUCAST methodology [106].

5.2.2 Data cleaning

All data cleaning and analysis was conducted in R [431].

5.2.2.1 Consumption data

Data points obtained prior to substantial changes in individual countries’ reporting methodologies, as noted in the reports [28, 252, 488–497], were excluded.

To ensure comparability of consumption results from veterinary and human sectors, totals for equivalent antibiotic classes (or subclasses) were generated where appropriate, as shown in Table 5.1. For example, consumption of cephalosporins in veterinary medicine is reported in the pooled categories ‘first- and second- generation cephalosporins’ and ‘third-

and fourth-generation cephalosporins', whereas in human medicine, individual generations are reported separately. New pooled categories were therefore generated for human medicine in this instance, by summing the results of consumption from individual generations.

Individual and pooled categories arising from each sector were assessed for correlation using Spearman's correlation coefficient, and highly correlated consumption variables were excluded, where clinically appropriate.

5.2.2.2 Resistance data

Resistance data were assessed visually for consistency of monitoring. For some combinations of bacterial species and antibiotics in some countries, the number of isolates tested varied widely year-on-year. Results from these species were excluded from further analysis.

5.2.3 Generalised linear mixed-effect models (GLMMs)

The R package **lme4** [506] was used throughout to construct logistic mixed effects models, which are a special case of GLMM. The logistic function is a standard dose-response curve for fractions in the range $[0, 1]$; here, it specifies the link between the expected value of the model response, and the explanatory variables, thus allowing regression analyses on non-normal data. GLMM models in **lme4** are constructed from formulae denoting fixed (population-level) and random (group-level) effects. Random effects can be applied to the intercept or slope of the regression curve, or both. The formulae used in this chapter take the general forms:

$$y \sim 1 + (1|g) \tag{5.1}$$

$$y \sim x + (1|g) \tag{5.2}$$

$$y \sim x + (x|g) \tag{5.3}$$

Where x and y are the explanatory and response variables, respectively, and g is the grouping variable. These formulae denote: random intercepts for each group g (Eqn. 5.1); fixed effect x and random intercepts for each group g (Eqn. 5.2); fixed effect x , and random slopes for x and random intercepts for each group g (Eqn. 5.3).

For all model fits performed here, the response variable y was set to the proportion of resistant isolates: the number of resistant isolates (successes, k) divided by the number of tested isolates (trials, n), for each country and year. Explanatory variables included different combinations of consumption variables and/or time, as outlined below. In all cases, time t was expressed as years since 2010 ($t = \text{Year} - 2010$), and each consumption variable x was standardised and scaled within the relevant data set, such that each individual data point x_i was converted to x'_i . That is:

$$x'_i = \frac{x_i - \mu(x)}{\sigma(x)}, \quad (5.4)$$

where $\mu(x)$ and $\sigma(x)$ are the mean and standard deviation, respectively, of all the values in x .

5.2.3.1 Model selection

Model selection was performed for each bacterial species-antibiotic combination that had sufficient data. Complete data points were defined as each country/year combination where at least 100 isolates were tested for resistance to a given antibiotic, and where consumption data was also available for the antibiotics (individual or pooled) within the same antibiotic class, in each sector. Sufficiency for model fitting was defined by at least five complete data points, from at least five different countries [487].

Inclusion of country-specific random effects First, the inclusion of country-specific random effects was validated. A baseline generalised linear model (GLM, no random effects), and a baseline GLMM (random effects grouped by country) were fitted. The model formulae for these models were, respectively:

$$k/n \sim 1 \quad (5.5)$$

$$k/n \sim (1|\text{Country}) \quad (5.6)$$

GLMs were constructed using the *glm* function in base R, and GLMMs were built using *glmer* from **lme4**. Models were weighted by n and utilised the logit link function (binomial family). The resulting fits were assessed by a likelihood ratio test, performed

with the *anova.merMod* function in **lme4**.

Inclusion of time as an explanatory variable Next, the validity of including time as an explanatory variable was tested. The intention here was to capture the effects of other theoretical, but not quantified, variables, affecting resistance independently of antibiotic consumption. To validate the inclusion of time as an explanatory variable, logistic mixed-effect models were fitted independently of consumption variables. Additional GLMMs were constructed with either random intercepts, or random intercepts and slopes, for the relationship between time and resistance. These modelled $y = k/n$ as a function of time t , with either the intercept, or both intercept and slope (i.e. the size and direction of the relationship), varying by country. The model formulae for these relationships were, respectively:

$$k/n \sim t + (1|\text{Country}) \quad (5.7)$$

$$k/n \sim t + (t|\text{Country}). \quad (5.8)$$

These GLMMs were constructed as described above. All models with non-convergence, singularity, or multicollinearity were rejected. Multicollinearity was identified by a variance inflation factor (VIF) of any term ≥ 3 [507]). Models were compared to the baseline GLMM and to each other using a likelihood ratio test, as before. Values for Aikake Information Criterion (AIC) and Bayesian Information Criterion (BIC) were also generated and compared.

Inclusion of antibiotic consumption as explanatory variables The effect of time as an explanatory variable in the model was then compared to measures of antibiotic consumption. GLMMs incorporating consumption variables only, and both consumption *and* time as explanatory variables, were assessed for goodness of fit.

A set of all possible combinations of the relevant consumption variables, S , was generated. Relevant consumption variables were defined as those belonging to the same antibiotic class as the antibiotic for which resistance was measured, and total consumption, in all three sectors. Model formulae were constructed to incorporate fixed effects of s , and

random effects by country, for each combination $s \in S$, for both random intercepts:

$$k/n \sim s + (1|\text{Country}) \quad (5.9)$$

and random intercepts and slopes:

$$k/n \sim s + (s|\text{Country}) \quad (5.10)$$

$$k/n \sim s + (s||\text{Country}) \quad (5.11)$$

(In **lme4**, $||$ is used to denote uncorrelated intercepts and slopes.)

Additional models were created where time t was combined with each element $s \in S$, again with fixed effects and intercept-only and intercept-and-slope random effects:

$$k/n \sim t + s + (s|\text{Country}) \quad (5.12)$$

$$k/n \sim t + s + (t|\text{Country}) \quad (5.13)$$

$$k/n \sim t + s + (t + s|\text{Country}) \quad (5.14)$$

$$k/n \sim t + s + (t + s||\text{Country}). \quad (5.15)$$

All GLMMs were fitted, and invalid models rejected, as before.

Identifying the best-fitting model Model fits were assessed using AIC and BIC. When there was disagreement between the models chosen using AIC and BIC, the model with the smallest BIC was selected as the best-fitting model, if the effect on model performance was minimal, and model assumptions were not violated. To assess this, posterior predictive distributions were generated by performing 1000 simulations of the best-fitting model, using the *simulate.merMod* function in **lme4**, and extracting distributions of summary statistics of resistance (median, inter-quartile range, minimum and maximum values). These distributions were compared against the observed values to ensure that the latter were not extreme [508].

To further confirm the choice of the best-fitting model, point estimates for predicted values were obtained from the *predict* function in **lme4**. The 95% confidence intervals (CI) around model predictions were obtained by bootstrapping predictions 200 times using the

bootMer function from **lme4**. Results of these predictions were assessed against the data and against the accuracy of the alternative model.

5.2.3.2 Model interpretation

Likelihood ratio tests were used to confirm the inclusion of each term (fixed effects and random slopes) in best-fitting models. Likelihood ratio tests were performed on each variable separately, by comparing nested models (i.e. the full model compared to the full model without one variable), as before. The values of the fixed and random effects were also examined.

Fixed effects Log-odds estimates of the fixed effects of the best-fitting model for each bacteria-antibiotic combination were obtained directly from the **lme4** model output. Likelihood profile confidence intervals (CI) around the fixed effect estimates were generated using the *confint.merMod* function within **lme4**. Variables were considered statistically significant if the 95% CI of the fixed effect did not overlap 0 (or 1 on the odds ratio scale, which was used for visualisation).

Random effects The random intercepts, and the standard deviations of the random slopes, from each best-fitting model were obtained directly from the **lme4** model output. Likelihood profile CIs around these standard deviations were generated using the *confint.merMod* function within **lme4**. The percentage of total random effect variance attributable to each random effect term was also determined.

5.2.3.3 Effect of influential points

Influential points were identified via the R package **influence.ME** [509]. Influential countries were identified using Cook's distance, a summary measure of the influence of a single grouping factor (here, country) on all parameter estimates simultaneously [509]. Countries with a Cook's distance above the threshold $4/c$, where c is the total number of countries in the dataset, were considered influential. The impact of these countries was assessed by comparing the significance, size, and direction of the fixed effects of the original model, with those generated once individual influential points had been excluded.

For some bacteria-antibiotic combinations, to further assess the effect of influential countries on model selection, models were refitted after all influential points were removed from the data set [510]. The data set was then re-standardised to the smaller sample size, and models were refitted, according to the procedure described above. Predicted values and 95% CIs were generated, as before, for these new fits.

5.3 Results

5.3.1 Consumption data overview

Antibiotic consumption data were obtained for 8 antibiotic classes in the medical sectors, and for 9 classes in the veterinary sector (Table 5.1).

Consumption of the pooled antibiotic class ‘macrolides, lincosamides & streptogramins’ was strongly correlated with consumption of ‘macrolides & lincosamides’, in both hospital and community settings (Spearman’s correlation coefficient = 0.988 and 0.986, respectively). This combined class including streptogramins was therefore excluded from further analyses. Consumption of fluoroquinolones in hospital and community settings was perfectly correlated with overall quinolone consumption (Spearman’s correlation coefficient = 1.0, in both cases). The total quinolone consumption for these sectors was therefore excluded from further analyses, as was consumption of other quinolones, which was negligible in both sectors (but not in food-producing animals). Also excluded from further analyses due to limited data were ‘other cephalosporins and penems’ and ‘unclassified beta-lactams’, in both hospital and community settings, and carbapenems consumed in the community.

Antibiotic consumption in the veterinary and hospital sectors is right-skewed, and there are clear differences in the distribution of consumption in community and hospital settings (Fig. 5.2). For most antibiotics, community consumption exceeds hospital consumption, but there are some exceptions (e.g. carbapenems). Between-country variation in amounts of antibiotic consumed usually exceeds within-country variation over different years, a phenomenon observed across all three sectors (Figs. 5.3 and 5.4).



Figure 5.2: **Consumption of different antibiotics in the veterinary and medical sectors.** Density of annual antibiotic consumption in food-producing animals (top), and in hospital and community settings (bottom), in Europe in 2010-2021. Individual antibiotics, antibiotic classes, and relevant subclasses and pooled classes. Note differing metrics (x axis). ML = macrolides & lincosamides; gen.ceph = generation cephalosporins; TMPS = trimethoprim-sulfonamide.

Antibiotic class	Medical consumption	Veterinary consumption
Aminoglycosides	Aminoglycosides	Aminoglycosides
Amphenicols	Amphenicols	Amphenicols
Beta-lactams	Penicillins	Penicillins
	First/second generation cephalosporins	First/second generation cephalosporins
	Third/fourth generation cephalosporins	Third/fourth generation cephalosporins
	Carbapenems	-
	Other cephalosporins & penems	-
	Monobactams	-
	Unclassified beta-lactams	-
Macrolides, lincosamides & streptogramins	Macrolides	Macrolides
	Lincosamides	Lincosamides
	Streptogramins	-
	Macrolides & lincosamides	Macrolides & lincosamides
Pleuromutilins	-	Pleuromutilins
Polymyxins	Polymyxins*	Polymyxins
Quinolones	Fluoroquinolones	Fluoroquinolones
	Other quinolones	Other quinolones
Sulfonamides & trimethoprim	Sulfonamides	Sulfonamides
	Trimethoprim	Trimethoprim
	Sulfonamide/trimethoprim combinations	-
Tetracyclines	Tetracyclines	Tetracyclines
Total	Total	Total

Table 5.1: **Antibiotic classes.** The antibiotic classes, and individual antibiotics, for which consumption data were obtained. Medical consumption data were obtained for both hospital and community settings, except where indicated (*, hospital sector only).

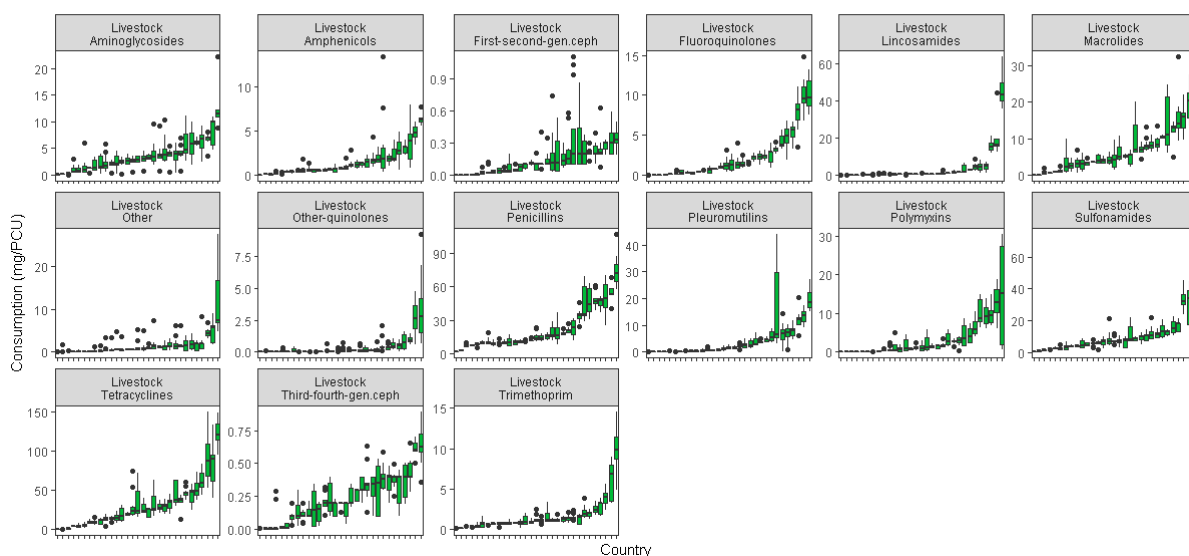


Figure 5.3: **Within- and between-country variation in consumption of different antibiotics in the veterinary sector.** Distribution of annual antibiotic consumption in food-producing animals in individual European countries in 2010-2021. Each boxplot represents a single country, ordered by median value. Gen.ceph = generation cephalosporins.

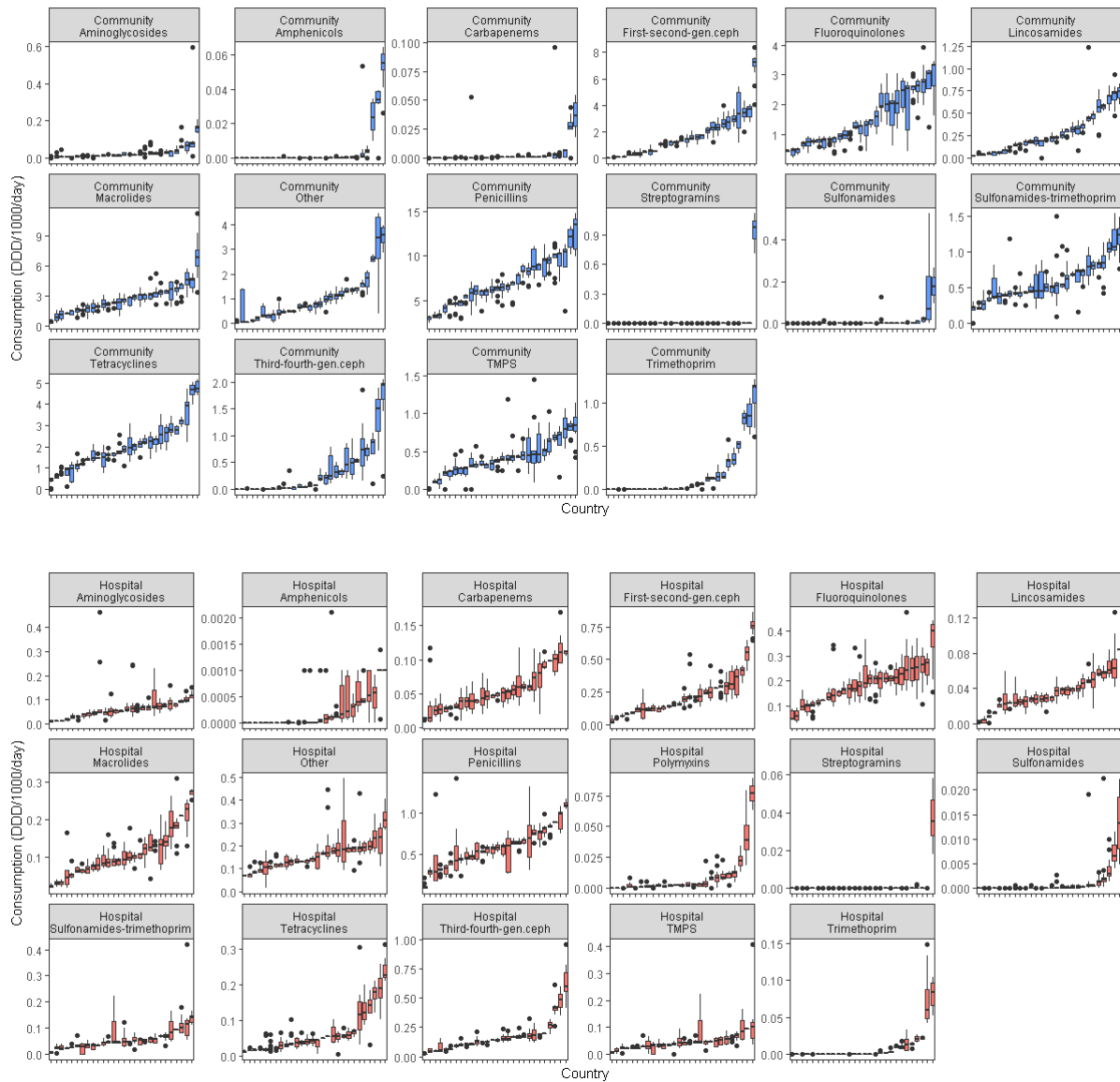


Figure 5.4: **Within- and between-country variation in consumption of different antibiotics in the medical sectors.** Distribution of annual antibiotic consumption in human community (top) and hospital (bottom) settings, in individual European countries in 2010-2021. Each boxplot represents a single country, ordered by median value. Gen.ceph = generation cephalosporins; TMPS = trimethoprim-sulfonamide.

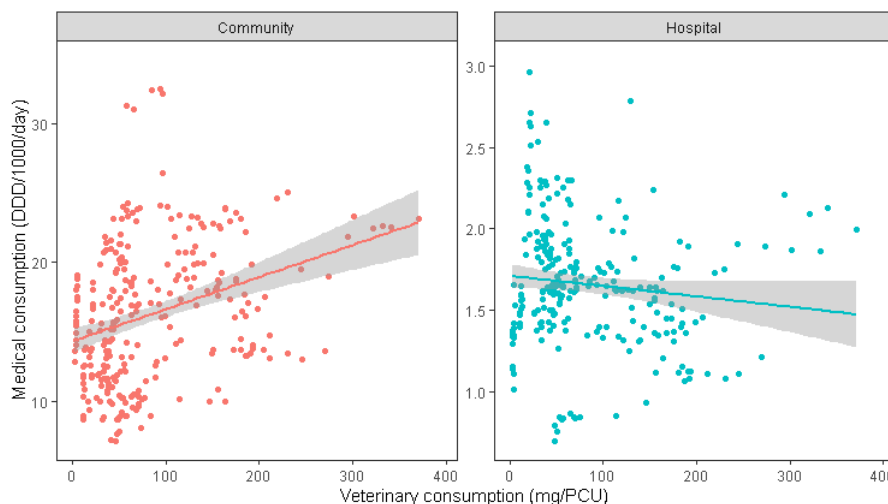


Figure 5.5: **Total antibiotic consumption in the veterinary and medical sectors.** Each point represents total antibiotic consumption in a European country in a single year, 2010-2021. Shading shows 95% confidence intervals. Note different metrics on x and y axes.

Total antibiotic consumption in the livestock sector was positively correlated with total consumption in the community, but negatively correlated with total consumption in hospital settings (Fig. 5.5). Total community consumption was positively correlated with hospital consumption. These seemingly contradictory relationships can be explained by the correlations of individual antibiotic classes and subclasses between different sectors. Antibiotic consumption in community and hospital settings is positively correlated for individual antibiotics (Fig. 5.6), whereas correlations between consumption in the veterinary sector and in human medical settings vary by antibiotic (Fig. 5.7).

5.3.2 Resistance data overview

AMR surveillance results were obtained for 11 bacterial species and 3 serovars of *Salmonella enterica* ssp. *enterica* (Table 5.2). These species can be broadly categorised according to key features of their epidemiology, host specialisation, and natural history: commensal organisms (and opportunistic pathogens); organisms that persist in the environment and cause opportunistic infections, especially in healthcare settings; and foodborne pathogens. Commensal organisms included those from the gastrointestinal tract (*Enterococcus* spp., *E. coli*, and *K. pneumoniae*); the upper respiratory tract (*K. pneumoniae* and *S. pneumoniae*); and the integument (*S. aureus*). All are potentially zoonotic, with the exception

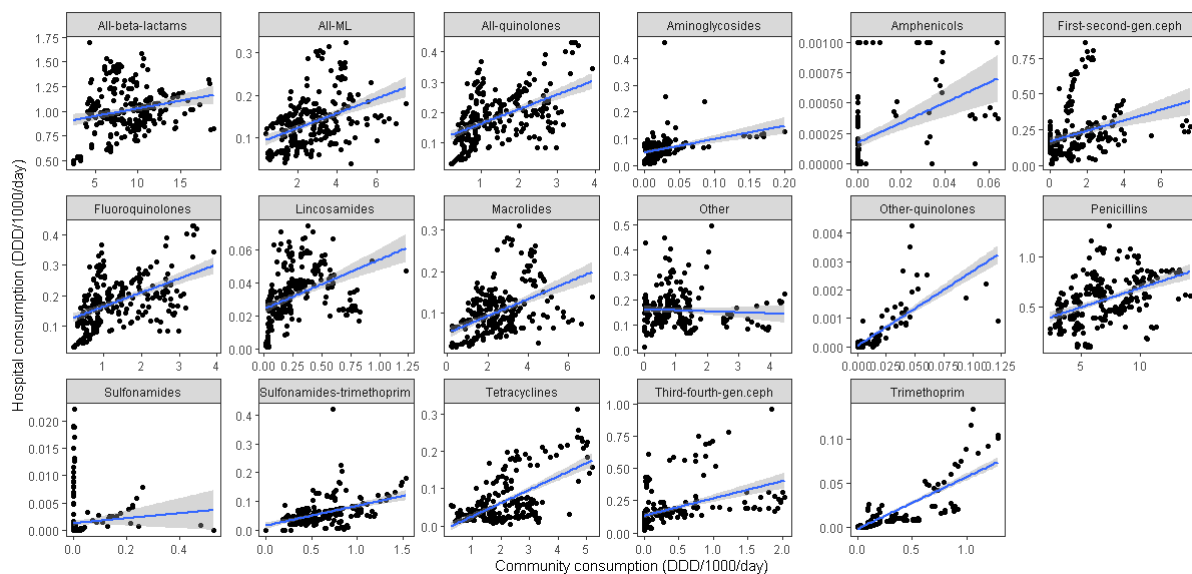


Figure 5.6: **Consumption of different antibiotic classes and sub-classes in community and hospital settings.** Each point represents total antibiotic consumption in a European country in a single year, 2010-2021. Shading shows 95% confidence intervals. ML = macrolides & lincosamides; gen.ceph = generation cephalosporins.

of *S. pneumoniae*, although animals are not always an important component of the epidemiology of human disease [162, 165, 359, 360, 370].

AMR results for these bacteria included testing for resistance against a total of 31 different antibiotics, from 9 antibiotic classes: aminoglycosides, amphenicols, beta-lactams, glycopeptides, macrolides/lincosamides, quinolones, polymyxins, sulfonamides/trimethoprim, and tetracyclines. There were 51 combinations of bacterial species and antibiotics where at least 100 isolates were tested annually by at least one country (Figs. 5.8 and 5.9). The total number of isolates tested varied widely: the median number of isolates of a given species tested against a single antibiotic by individual countries was 547, but in some cases, over 30,000 isolates were tested.

Resistance was detected to all antibiotics, and coexistence of resistant and sensitive isolates was very common. The proportion of resistant isolates was zero in $6.6 \times 10^{-2}\%$ of data points, and 1 in $4.0 \times 10^{-3}\%$ of data points. For most combinations of bacterial species and antibiotics against which resistance in clinical isolates were tested, the data were not normally distributed (Fig. 5.8). In many cases, there was also more variation in resistance frequency between countries than within countries (Fig. 5.9).

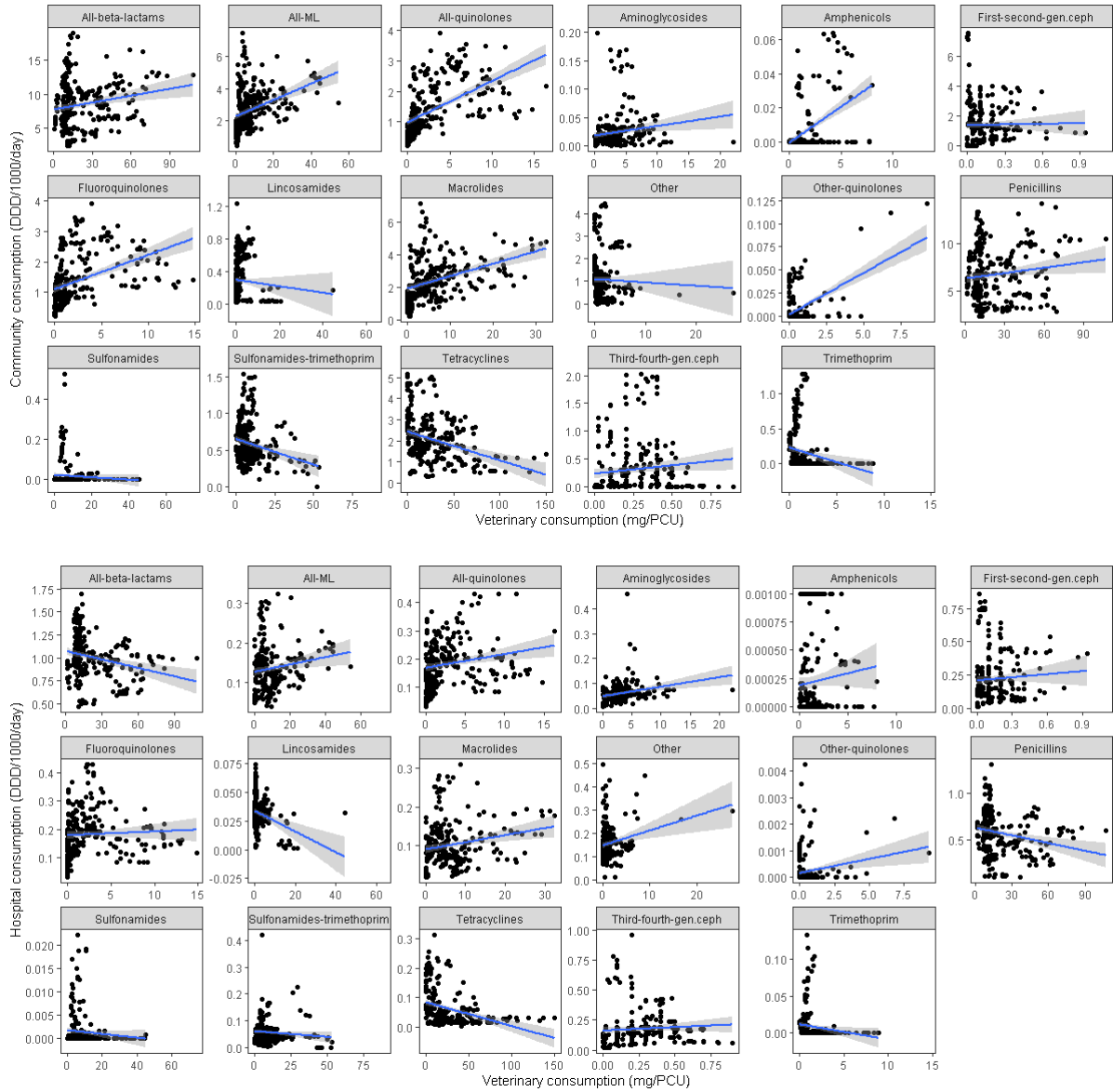


Figure 5.7: Consumption of different antibiotic classes and sub-classes in veterinary and medical settings. Each point represents total antibiotic consumption in a European country in a single year, 2010-2021. Consumption of individual antibiotics and antibiotic classes in livestock, compared to consumption in community (top) and hospital (bottom) settings. Data from Europe, 2010-2021. Shading shows 95% confidence intervals. Note different metrics on x and y axes. ML = macrolides & lincosamides; gen.ceph = generation cephalosporins.

Categorisation	Bacterial species +/– serovar	Zoonotic
Commensal organisms	<i>Enterococcus faecalis</i>	Yes
	<i>Enterococcus faecium</i>	Yes
	<i>Escherichia coli</i>	Yes
	<i>Klebsiella pneumoniae</i>	Yes
	<i>Staphylococcus aureus</i>	Yes
	<i>Streptococcus pneumoniae</i>	No
Environmental organisms	<i>Acinetobacter baumannii</i>	Yes
	<i>Klebsiella pneumoniae</i>	Yes
	<i>Pseudomonas aeruginosa</i>	Yes
Foodborne pathogens	<i>Campylobacter coli</i>	Yes
	<i>Campylobacter jejuni</i>	Yes
	<i>Salmonella</i> spp.	Yes
	<i>Salmonella</i> Enteritidis	Yes
	<i>Salmonella</i> Infantis	Yes
	<i>Salmonella</i> Typhimurium	Yes

Table 5.2: **Bacterial species and serovars for which resistance data was obtained.**

5.3.3 Data cleaning

Consumption data were available for 8 of the antibiotic classes against which resistance was tested (Table 5.1), but not for glycopeptides (vancomycin). Models for resistance to this antibiotic were therefore tested against total consumption only. There were 44 combinations of bacterial species and antibiotic resistance that contained sufficient data for fitting mixed models (Fig. 5.10).

5.3.4 GLMM overview

Of the 44 bacteria-antibiotic combinations with sufficient data for fitting, 43 resulted in valid GLMM fits. Attempts to fit meropenem resistance in *Salmonella* spp. resulted in singular fits in all cases. This was due to a high proportion of susceptible isolates (96%, 134/140, see Fig. 5.8).

Measures of model fit using AIC and BIC were in agreement in 44% of cases (19/43). When they were not in agreement, model performance was assessed. The percentage of observed values within 95% CIs of the predicted values was higher for the model selected

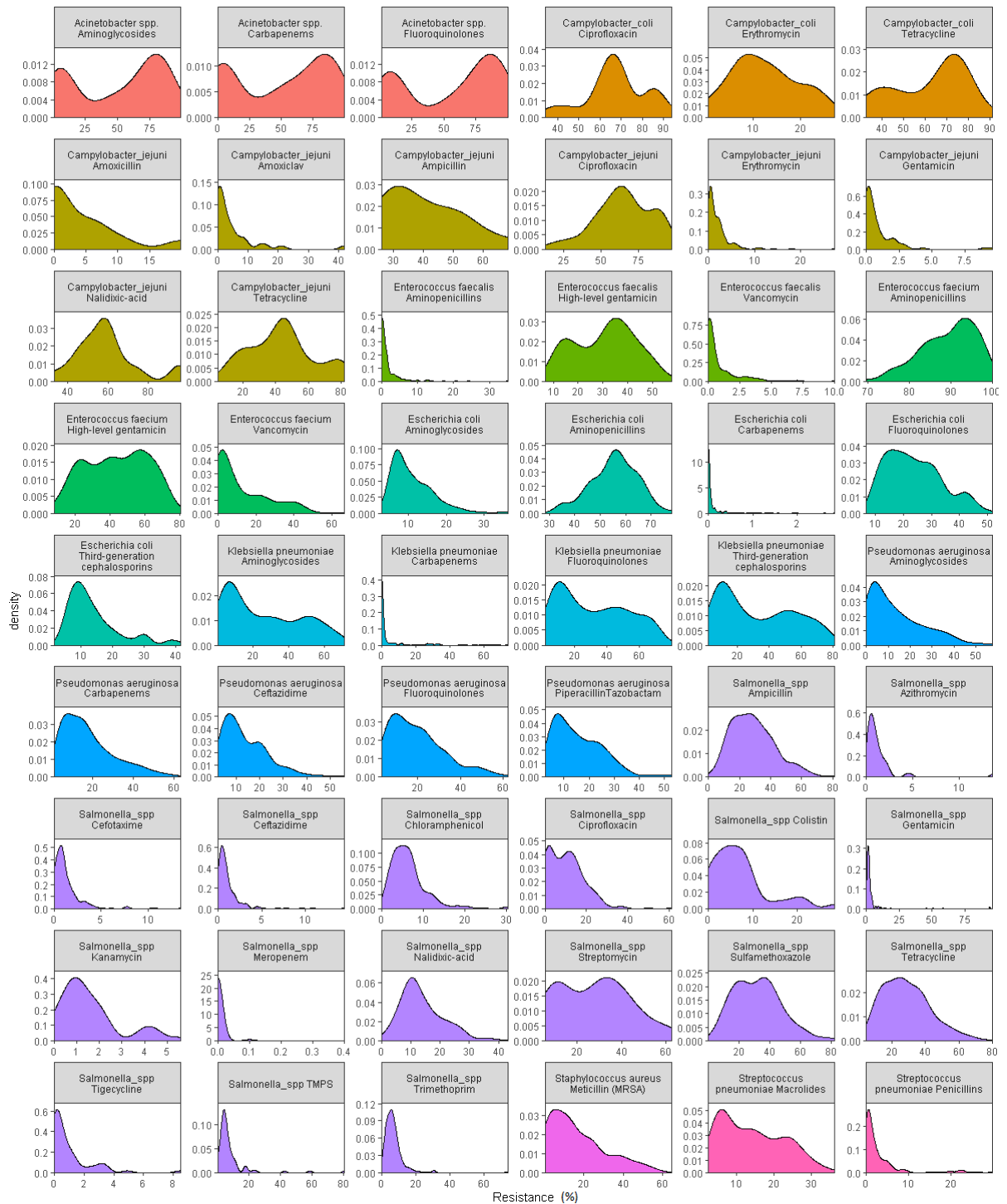


Figure 5.8: **Antibiotic resistance in clinical isolates from human patients.** Density of resistance of medical isolates to different antibiotics (identified in facet labels) in Europe in 2010-2021. Only data points where at least 100 isolates were tested are shown. Colours signify bacterial species. Amoxiclav = amoxicillin/clavulanic acid; TMPs = trimethoprim/sulfamethoxazole.

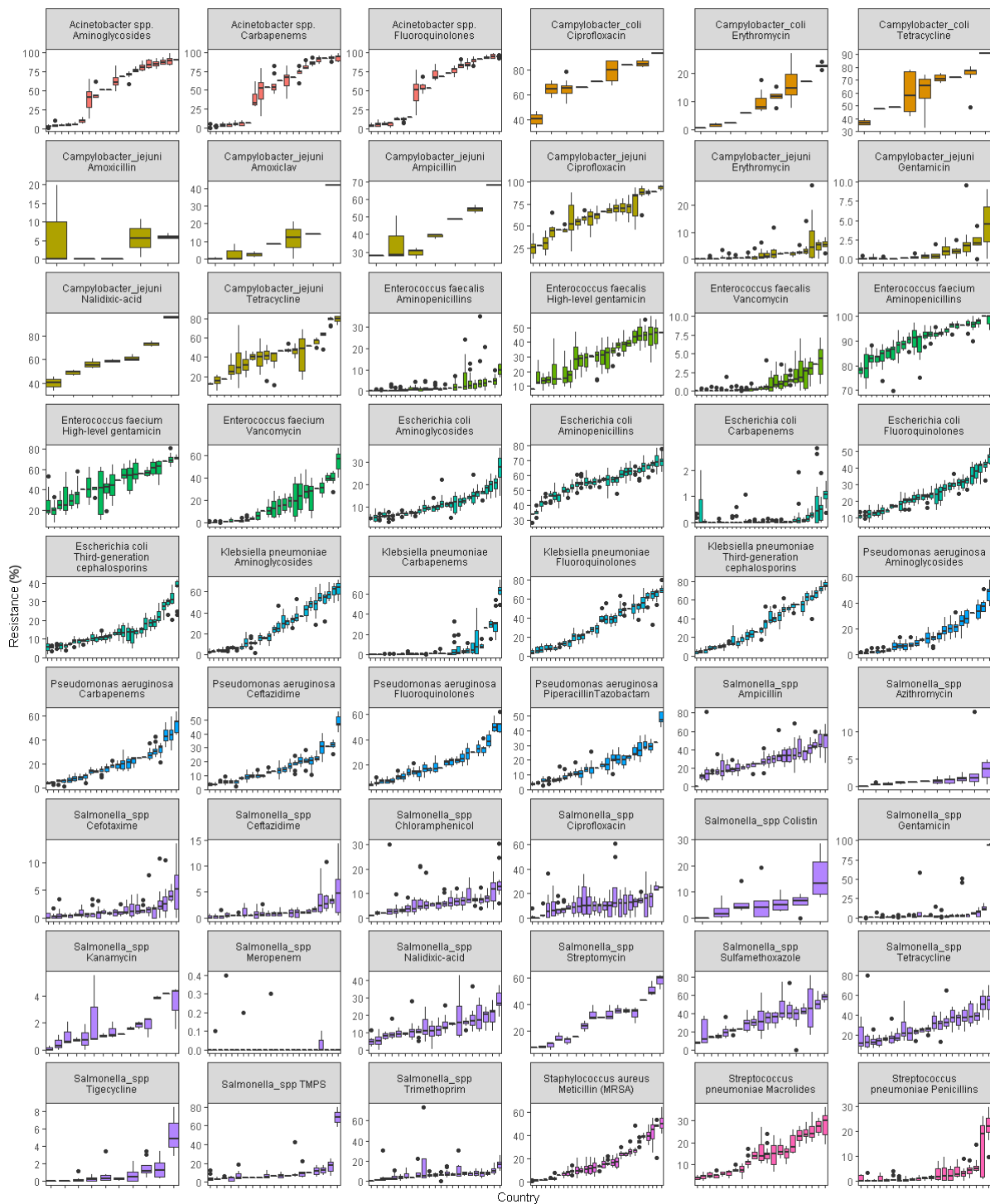


Figure 5.9: **Within- and between-country variation in antibiotic resistance in clinical isolates from human patients.** Resistance of medical isolates to antibiotics (identified in facet labels) in European countries in 2010-2021. Each individual boxplot represents a single country, ordered by median value. Only data points where at least 100 isolates were tested are shown. Colours signify bacterial species. Amoxiclav = amoxicillin/clavulanic acid; TMPs = trimethoprim/sulfamethoxazole.

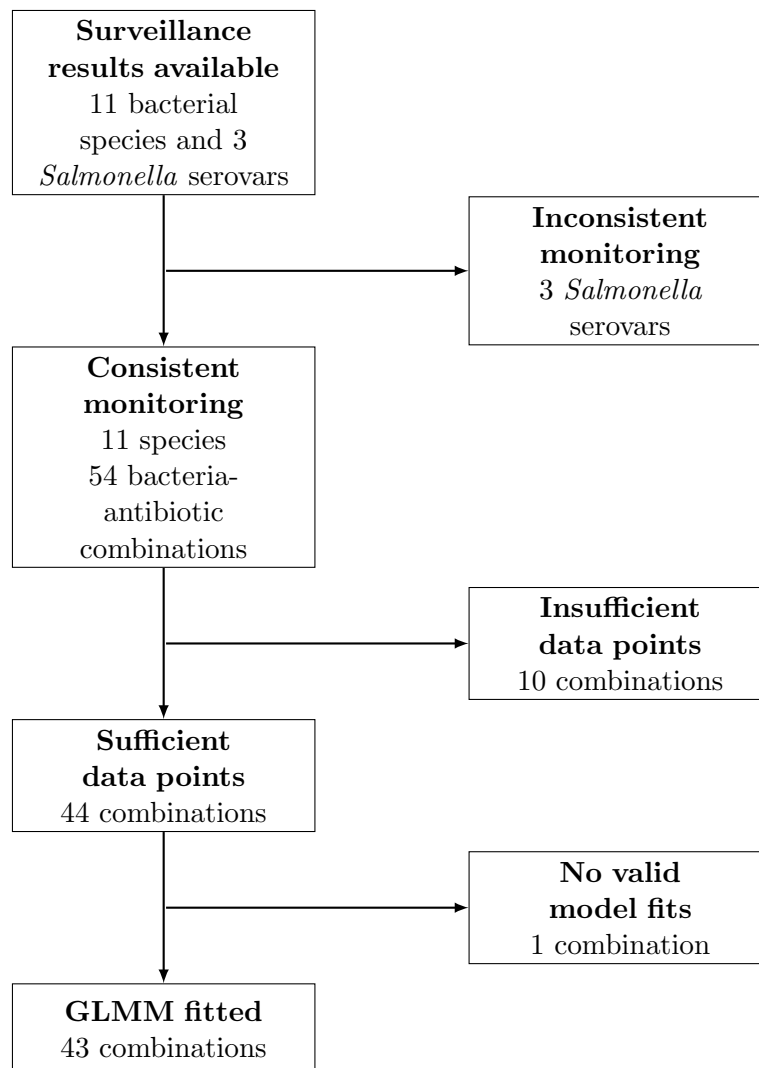


Figure 5.10: Combinations of bacterial species and antibiotic subject to analysis.

by AIC in 42% of these (10/24). In all cases, the difference in performance was within 5 percentage points, and there was no suggestion from the plots of posterior predictive values that the BIC-selected model should be ruled out. Thus, the best-fitting model for each combination was determined by the lowest BIC value. Two examples of best-fitting models, fitted to third-generation cephalosporin resistance in *E. coli* and methicillin resistance in *S. aureus*, are shown in Figs. 5.11 and 5.12, respectively.

5.3.4.1 Inclusion of country-specific random effects

All of the 43 combinations that could be fitted showed a statistically significant effect of incorporating random (group-level) effects for individual countries, compared to a baseline GLM, using the likelihood ratio test ($p < 0.05$).

5.3.4.2 Inclusion of time as an explanatory variable

Incorporating time as an explanatory variable within the GLMMs improved model fit in 95% of cases (41/43). For these 41 combinations, model fit was significantly improved (as measured by likelihood ratio test, AIC, and BIC) when time was modelled as a fixed effect with both random intercepts and random slopes for individual countries (Eqn. 5.8).

5.3.4.3 Inclusion of antibiotic consumption as explanatory variables

Inclusion of antibiotic consumption terms as explanatory variables significantly improved model fits from the baseline GLMM in 95% of combinations (41/43), as measured by likelihood ratio tests, AIC and BIC. For 4.7% of bacteria-antibiotic combinations (2/43), consumption-only models were a significantly better fit than those incorporating time. In 91% of combinations (39/43), the best-fitting model incorporated consumption terms as well as time as explanatory variables; all of these models were a statistically significant improvement on the corresponding time-only GLMMs (again, as measured by likelihood ratio tests, AIC, and BIC).

Best-fitting models were identified by BIC, after cross-checking model assumptions were not violated (section 5.2.3.1). Of the 41 models incorporating consumption terms, 34 included consumption in the hospital sector (79% of best-fitting models); 32 included community consumption terms (74%), and 31 included consumption in animals (72%).

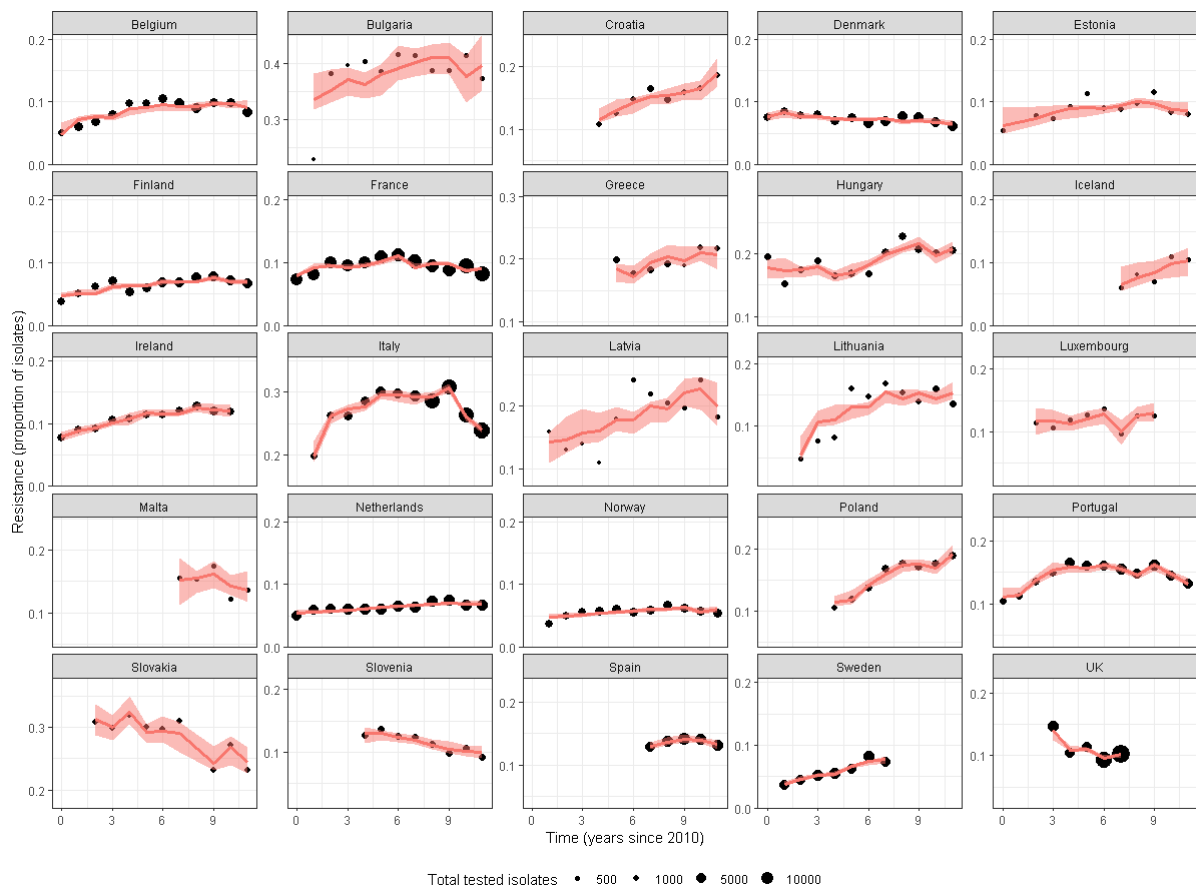


Figure 5.11: **Best-fitting generalised linear mixed model (GLMM) of third-generation cephalosporin resistance in *E. coli*.** Estimates (line) and 95% confidence intervals (shading) of predicted values, compared to observed data (points). Model fit weighted by total number of isolates tested (point size). See table 5.4 for model details.

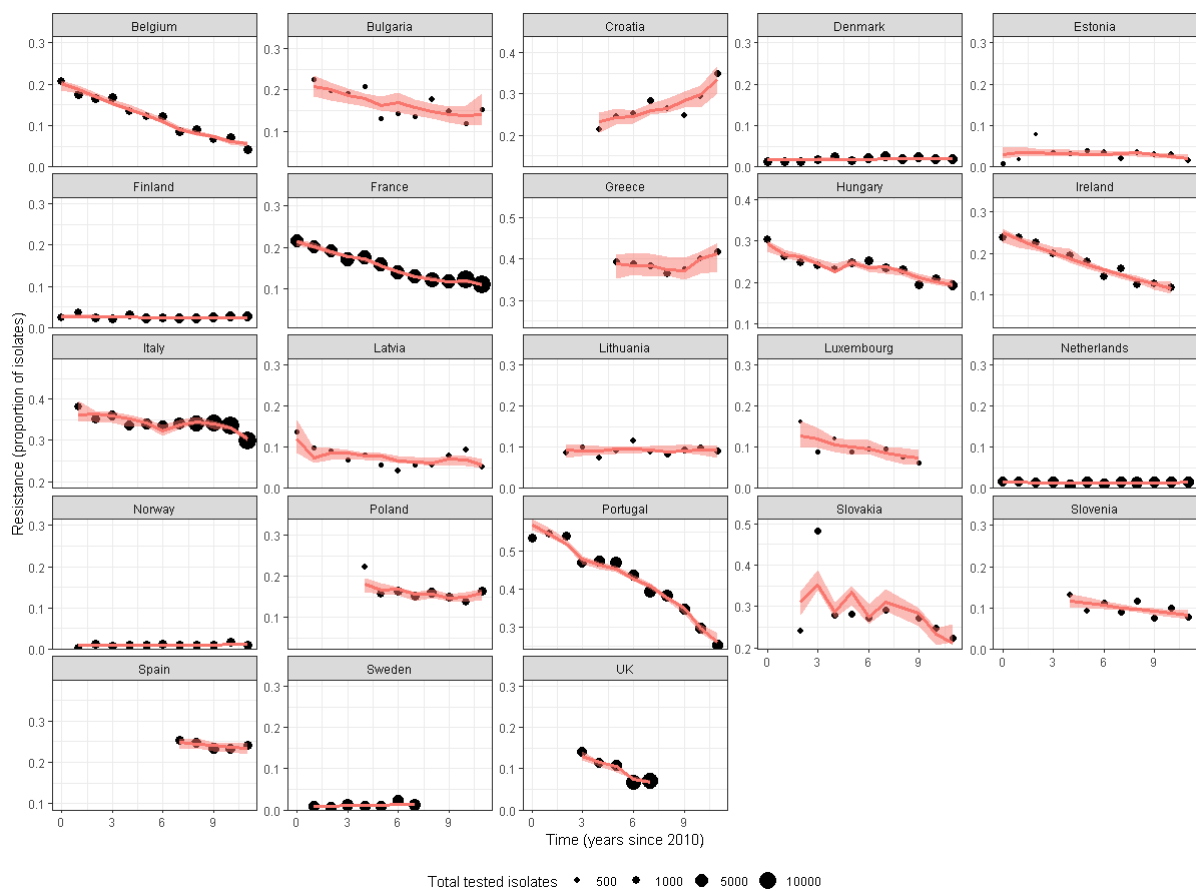


Figure 5.12: **Best-fitting generalised linear mixed model (GLMM) of methicillin resistance in *S. aureus*.** Estimates (line) and 95% confidence intervals (shading) of predicted values, compared to observed data (points). Model fit weighted by total number of isolates tested (point size). See table 5.6 for model details.

Total consumption in one or more sectors was included in the best-fitting model in 22 cases (51% of best-fitting models): total consumption in hospital and community settings were each included in 15 of the best-fitting models (35%); total consumption in the animal sector was included in 10 models (23%). Of the models including total consumption variables, 14 (33%) contained total measures only, and eight also included consumption of specific antibiotics or antibiotic classes. For 19 combinations (44%), the best-fitting models included measures of consumption of specific antibiotics or antibiotic classes only.

5.3.5 Commensal organisms

GLMMs were successfully fitted to 18 commensal-antibiotic combinations: three each for *Enterococcus* spp., five for *E. coli*, four for *K. pneumoniae*, one for *S. aureus*, and two for *S. pneumoniae* (Tables 5.3-5.6). All models incorporated random intercepts for country, and both fixed effects and random slopes for the effect of time on resistance. In 89% (16/18) of combinations, the best-fitting model also incorporated fixed effects and random slopes for at least one consumption variable. In 5.6% of cases (1/18; macrolide resistance in *S. pneumoniae*), the best-fitting model did not contain random slopes for one consumption variable. In another 5.6% of cases (1/18; vancomycin resistance in *E. faecalis*), the best-fitting model did not include any consumption variables. Inclusion of each individual variable in each best-fitting model had a statistically significant impact on model fit (using the likelihood ratio test on nested models). Results are summarised by antibiotic class in Figs. 5.14 to 5.18.

5.3.5.1 Fixed effects

Fixed effect estimates and measures of uncertainty for selected combinations are shown in Fig. 5.13, and estimates are summarised in Figs. 5.14 to 5.18, top plots. The fixed

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>E. faecalis</i>	Aminopenicillins	20	$t + s + (t + s g)$	Total (community) Penicillins (animal) First/second generation cephalosporins (animal) Third/fourth generation cephalosporins (animal)
	High-level gentamicin	15	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)
	Vancomycin	20	$t + (t g)$	-
<i>E. faecium</i>	Aminopenicillins	17	$t + s + (t + s g)$	Total (hospital) First/second generation cephalosporins (animal)
	High-level gentamicin	11	$t + s + (t + s g)$	Aminoglycosides (hospital) Aminoglycosides (community)
	Vancomycin	17	$t + s + (t + s g)$	Total (community) Total (animal)

Table 5.3: **Best-fitting GLMMs for resistance in *Enterococcus* spp.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>E. coli</i>	Aminoglycosides	23	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)
	Aminopenicillins	24	$t + s + (t + s g)$	Total (hospital) Penicillins (community) Third/fourth generation cephalosporins (community)
	Carbapenems	24	$t + s + (t + s g)$	Third/fourth generation cephalosporins (community) Total (animal)
	Fluoroquinolones	22	$t + s + (t + s g)$	Fluoroquinolones (hospital) Fluoroquinolones (community) Other quinolones (animal)
	Third-generation cephalosporins	23	$t + s + (t + s g)$	Penicillins (hospital) Third/fourth generation cephalosporins (hospital) Carbapenems (hospital) Penicillins (community) Third/fourth generation cephalosporins (community) First/second generation cephalosporins (animal) Thirds/fourth generation cephalosporins (animal)

Table 5.4: **Best-fitting GLMMs for resistance in *Escherichia coli*.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>K. pneumoniae</i>	Aminoglycosides	21	$t + s + (t + s g)$	Aminoglycosides (hospital) Aminoglycosides (community) Aminoglycosides (animal)
	Carbapenems	23	$t + s + (t + s g)$	Penicillins (hospital) First/second generation cephalosporins (hospital) Third/fourth generation cephalosporins (hospital) All beta-lactams (community) Third/fourth generation cephalosporins (animal)
	Fluoroquinolones	22	$t + s + (t + s g)$	Total (hospital) Total (community)
	Third-generation cephalosporins	23	$t + s + (t + s g)$	Third/fourth generation cephalosporins (hospital) Penicillins (community) First/second generation cephalosporins (community) Penicillins (animal) First/second generation cephalosporins (animal)

Table 5.5: **Best-fitting GLMMs for resistance in *Klebsiella pneumoniae*.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>S. aureus</i>	Methicillin	23	$t + s + (t + s g)$	Third/fourth generation cephalosporins (hospital) Total (community) Third/fourth generation cephalosporins (animal)
<i>S. pneumoniae</i>	Macrolides	16	$t + x + (t g)$	Total (community)
	Penicillins	17	$t + s + (t + s g)$	Third/fourth generation cephalosporins (hospital) First/second generation cephalosporins (community) Third/fourth generation cephalosporins (community) Third/fourth generation cephalosporins (animal)

Table 5.6: **Best-fitting GLMMs of resistance in *Staphylococcus* and *Streptococcus* spp.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

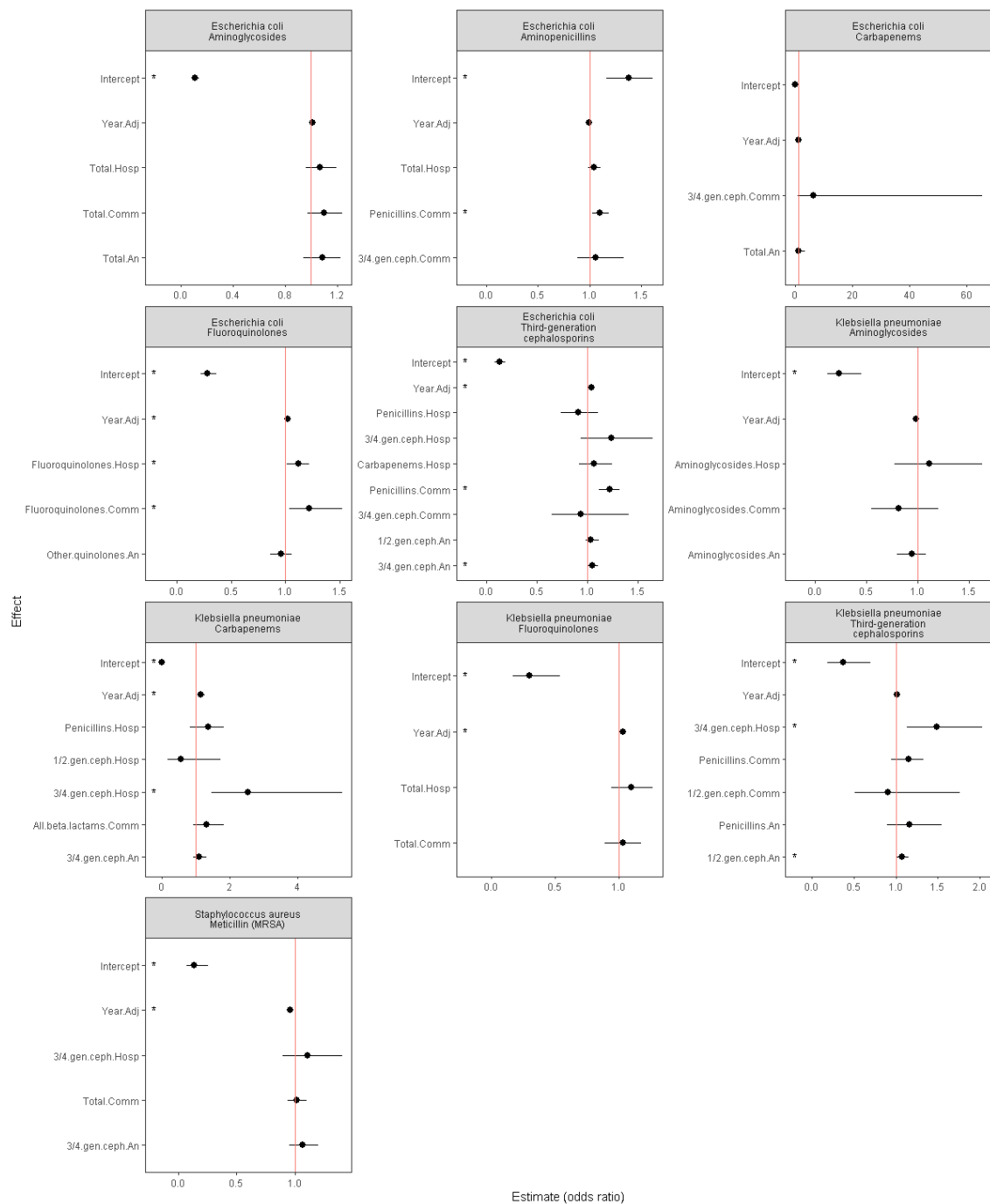


Figure 5.13: Results of best-fitting generalised linear mixed models (GLMMs) of resistance in commensal organisms: fixed effects. Estimates (points) and 95% confidence intervals (lines) for each variable. * denotes significance. Selected commensal-antibiotic combinations shown (number of countries for which data was available > 20). Year.Adj = year - 2010 = t . Hosp = hospital sector; Comm=community sector, An = animal sector.

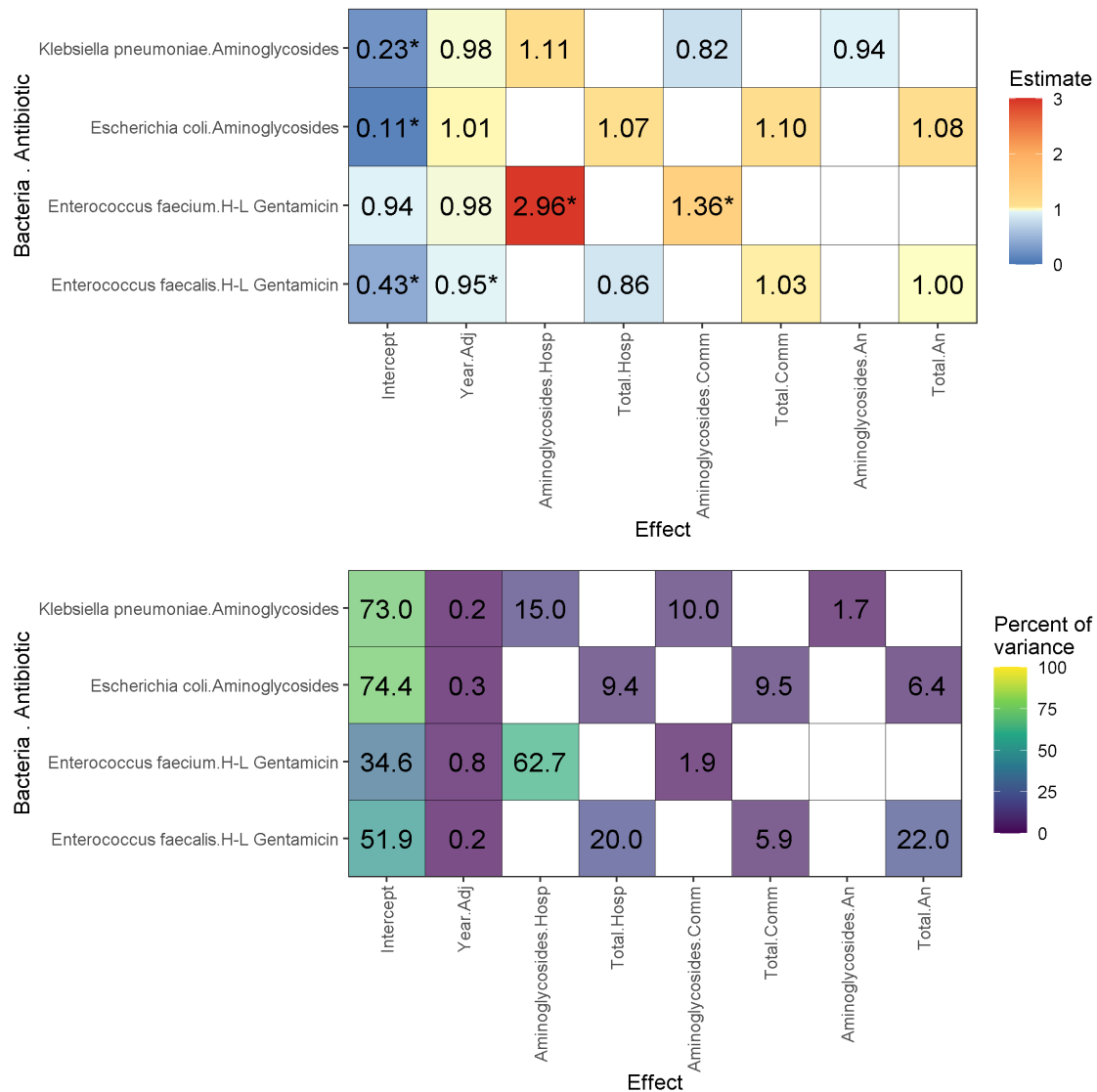


Figure 5.14: **Results of best-fitting models of resistance to aminoglycoside antibiotics in commensal organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance ($p < 0.05$). Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector. H-L, high-level.

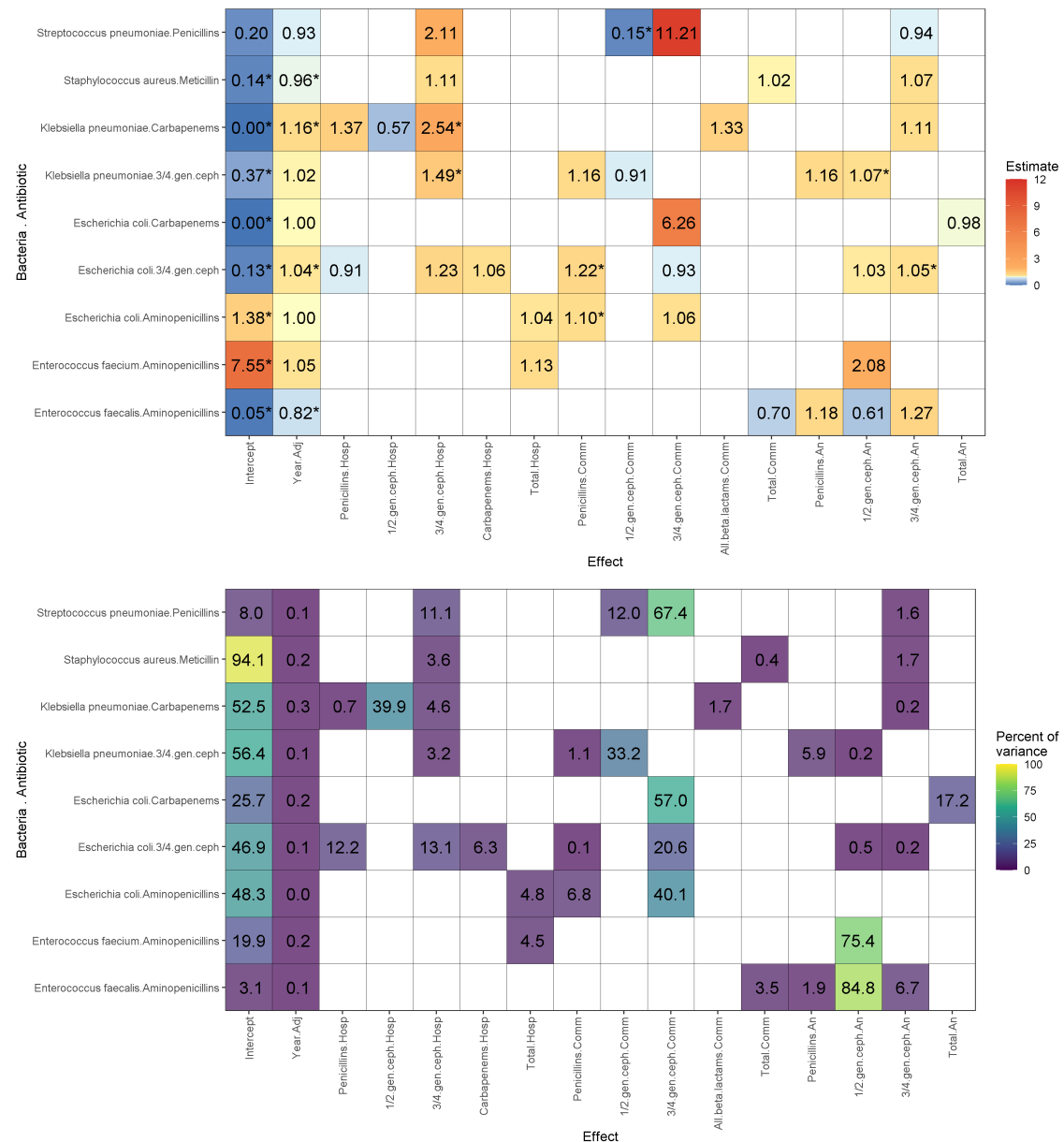


Figure 5.15: **Results of best-fitting models of resistance to beta-lactam antibiotics in commensal organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance ($p < 0.05$). Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector. Gen.ceph, generation cephalosporins.

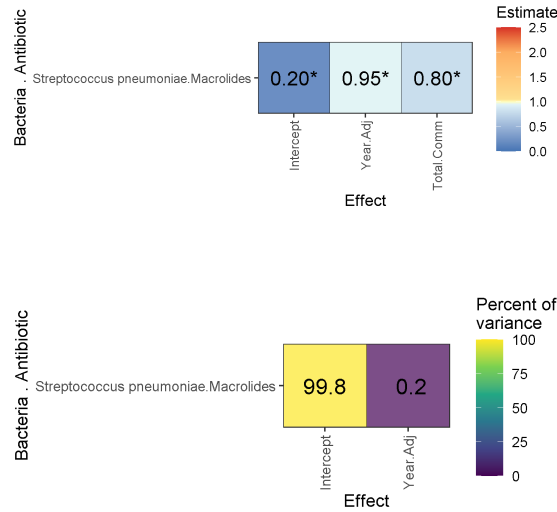


Figure 5.16: **Results of best-fitting models of resistance to macrolide antibiotics in commensal organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance ($p < 0.05$). Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year - 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

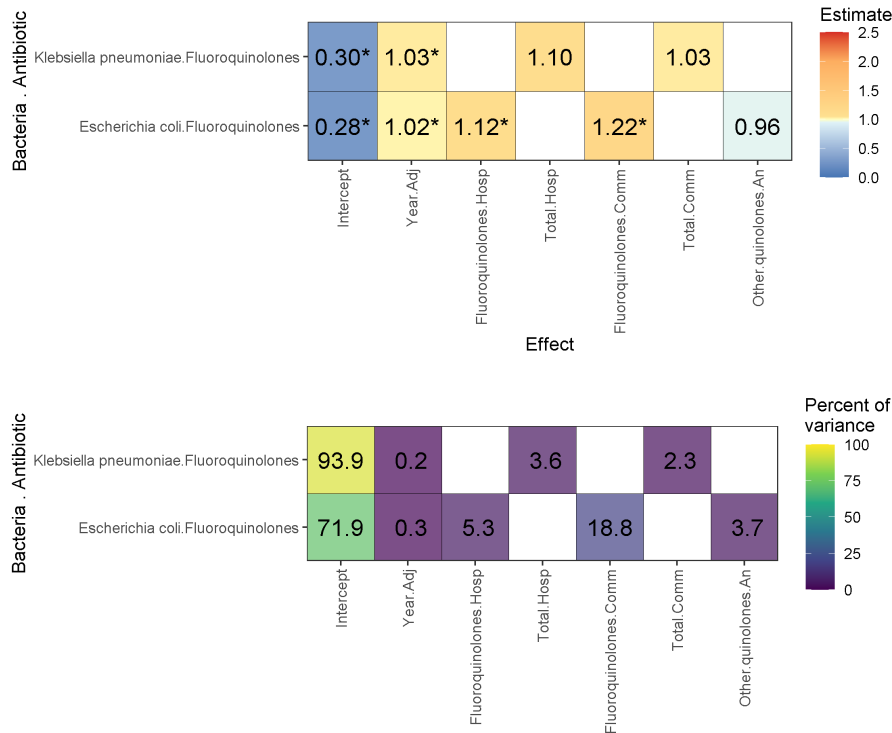


Figure 5.17: **Results of best-fitting models of resistance to quinolone antibiotics in commensal organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance ($p < 0.05$). Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year - 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

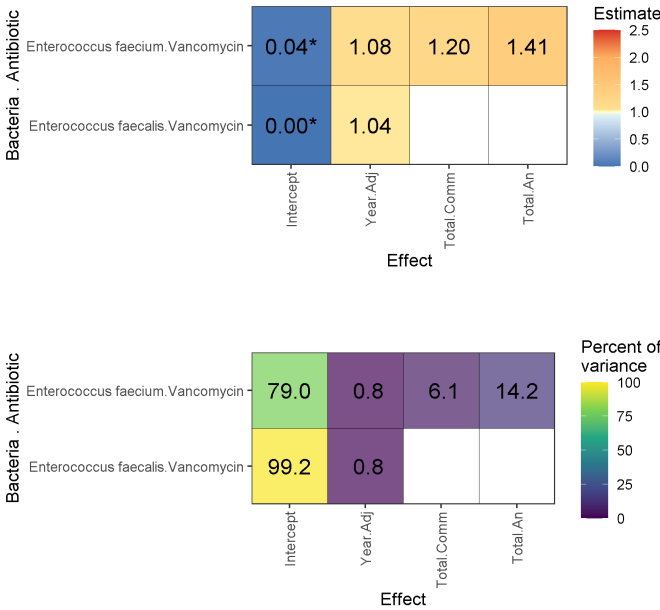


Figure 5.18: **Results of best-fitting models of resistance to glycopeptide antibiotics in commensal organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance ($p < 0.05$). Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

effect of time t was statistically significant (using 95% confidence intervals on the estimate) in 44% of cases (8/18). Fixed effects of at least one antibiotic consumption variable were statistically significant in 44% of best fits (8/18). These eight combinations with statistically-significant Europe-wide effects of consumption included 12 different consumption variables: 50% of these involved antibiotic consumption in the community (6/12, of which two had a negative effect); 33% (4/12) were from hospital settings; and 17% (2/12) were from the agricultural sector.

The largest fixed effects estimates were for the impact of third- and fourth-generation cephalosporin consumption in community settings, on penicillin resistance in *S. pneumoniae* and carbapenem resistance in *E. coli* (Fig. 5.15), although neither of these figures were statistically significant. Consumption of third- and fourth-generation cephalosporins in any sector was the most frequently-included antibiotic, and had significant positive effects in three models: hospital consumption, for carbapenem and third- and fourth-generation cephalosporin resistance in *K. pneumoniae*; and consumption in food-producing animals for third- and fourth-generation cephalosporin resistance in *E. coli*. Consumption

of third- and fourth-generation cephalosporins in hospital settings had positive fixed effects estimates in all cases, whereas effect estimates of consumption of this antibiotic in the veterinary sector tended to be lower.

Consumption variables in the best-fitting models of resistance in enterococci varied between species. In the beta-lactam class, three measures of consumption in animals were included in the best-fitting model of aminopenicillin resistance in *E. faecalis* (Fig. 5.15). Two of these had positive fixed effects estimates. Magnitude and direction of consumption variables often differed between enterococcal species. In *E. faecium*, high-level gentamicin resistance showed a positive, statistically significant fixed effect of aminoglycoside consumption in both hospital and community settings; but not in *E. faecalis* (Fig. 5.14).

The amount of uncertainty around estimates of fixed effects varied between bacteria-antibiotic combinations, and for specific variables (Fig. 5.13). Uncertainty around the estimates of the fixed effects of consumption variables was usually larger than for time.

5.3.5.2 Random effects

An example of a random effects output is provided in Fig. 5.19. The percentage of the total random effect variance attributable to each term in the best-fitting model are summarised in Figs. 5.14 to 5.18, bottom plots. Random intercepts varied between commensal-antibiotic combinations (left-hand column in these plots). For commensal organisms, the proportion of random effects variance attributable to the intercept (i.e. baseline between-country variation) varied from 3.1% (*E. faecalis* resistance to aminopenicillins) to almost 100% (*S. pneumoniae* resistance to macrolides). Strong between-country differences (> 90% of the random effects variance), independent of antibiotic consumption, were evident for: macrolide resistance in *S. pneumoniae*; vancomycin resistance in *E. faecalis*; fluoroquinolone resistance in *K. pneumoniae*; and methicillin resistance in *S. aureus*. The relationship between resistance and time explained less than 1% of the random effects variance for all combinations.

There was also considerable variation in the random slopes of resistance in commensal organisms against different consumption variables. In Fig. 5.19, for example, variation between countries in the random slopes for cephalosporin consumption in animals, and penicillins consumed in the community, is small. Random slopes for the other consump-

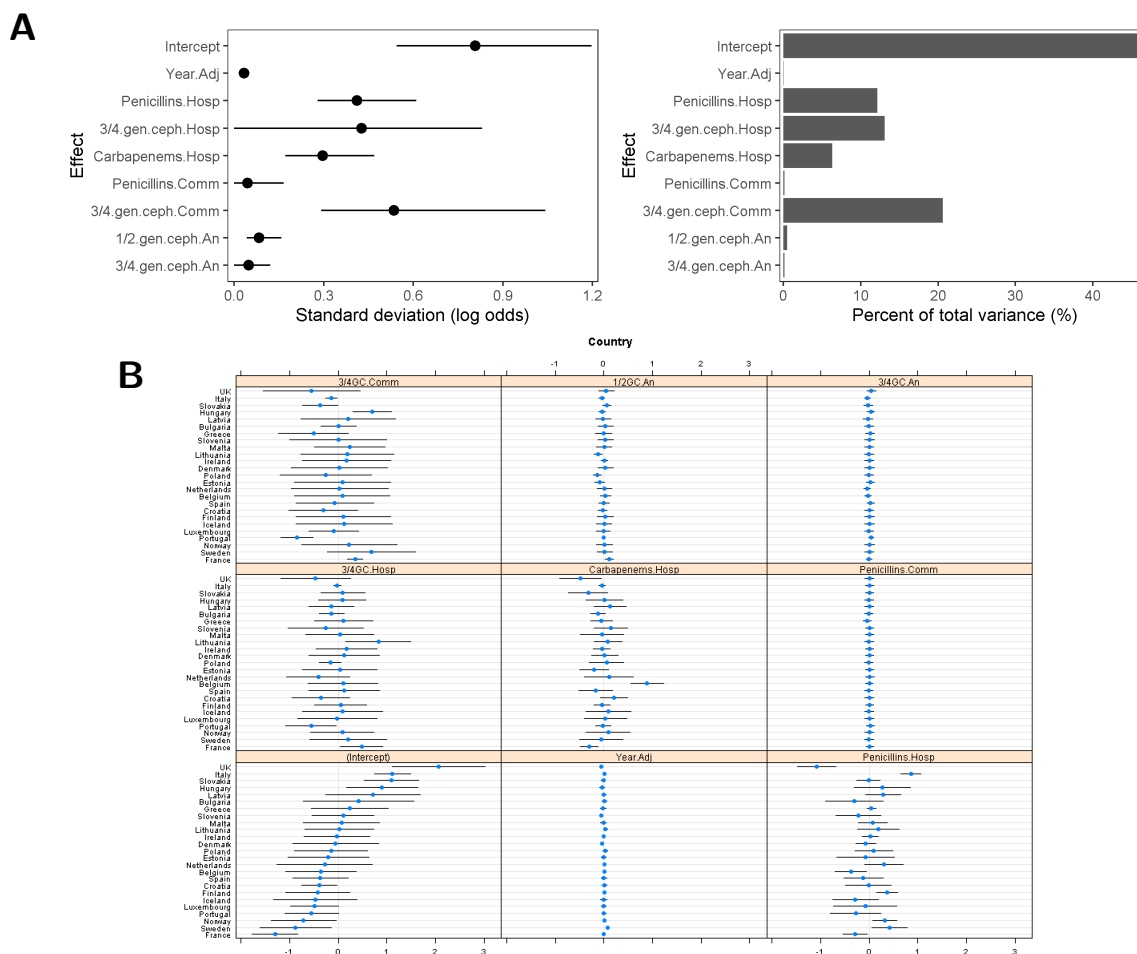


Figure 5.19: Results of best-fitting GLMM of resistance to third- and fourth-generation cephalosporins in *E. coli*: random effects. **A**: left, estimates (points) and 95% confidence intervals (lines) of the standard deviation of the random effect slopes. Right, percentage of overall variation among countries explained by each variable. **B**: estimates (points) and 95% confidence intervals of the random effect estimate for individual countries.

tion terms explain a larger proportion of the overall random effects variance (Fig. 5.19, top right). Random slopes for consumption variables explaining $> 50\%$ of the random effect variance in GLMMs of resistance in commensal organisms are: third- and fourth-generation cephalosporin consumption in community settings (penicillin resistance in *S. pneumoniae* and carbapenem resistance in *E. coli*); first- and second-generation cephalosporin consumption in the veterinary sector (aminopenicillin resistance in *E. faecalis* and *E. faecium*); and aminoglycoside consumption in hospitals (high-level gentamicin resistance in *E. faecium*).

5.3.5.3 Effect of influential points

Influential points, as determined by Cook's distance, were present in 16 of 18 best-fitting models of resistance in commensal organisms. Significance of antibiotic consumption fixed effects were not affected by influential points in seven of these models. In one case (vancomycin resistance in *E. faecium*), excluding the most influential country resulted in an additional consumption variable (total consumption in the community) becoming a statistically significant fixed effect (Fig. 5.20). Refitting this model to the reduced dataset produced an alternative best-fitting model, without random slopes for consumption. In five cases, excluding the most influential point produced a singular fit. In another three cases, excluding the most influential country did not make a difference to the statistical significance of fixed effects, but excluding the second- or third-most influential country did.

Across commensal bacteria, excluding influential countries resulted in three additional consumption variables becoming statistically significant, for three different bacteria-antibiotic combinations. These variables were aminoglycoside consumption in hospitals (aminoglycoside resistance in *K. pneumoniae*) and total beta-lactam consumption in the community (carbapenem resistance in *K. pneumoniae*), in addition to total community consumption (vancomycin resistance in *E. faecium*), as mentioned.

5.3.5.4 Summary of results for commensal organisms

These results show that the best-fitting GLMMs of antibiotic resistance in commensal organisms involve both consumption-specific and temporal explanatory variables. It is clear from the fixed effects estimates that there is a detectable influence of time on resistance in commensal organisms, sometimes statistically significant, and random effects vary by a small amount. By contrast, the specific antibiotic consumption variables included in the best-fitting models, and the magnitude of their effects, vary between different combinations of commensals and antibiotic resistance. Estimates of fixed effects of antibiotic consumption variables for gastrointestinal commensal organisms are summarised in Fig. 5.21.

Some of the individual combinations of commensal organism and antibiotic resistance are notable. *S. pneumoniae* resistance to penicillins and *E. coli* resistance to carbapen-

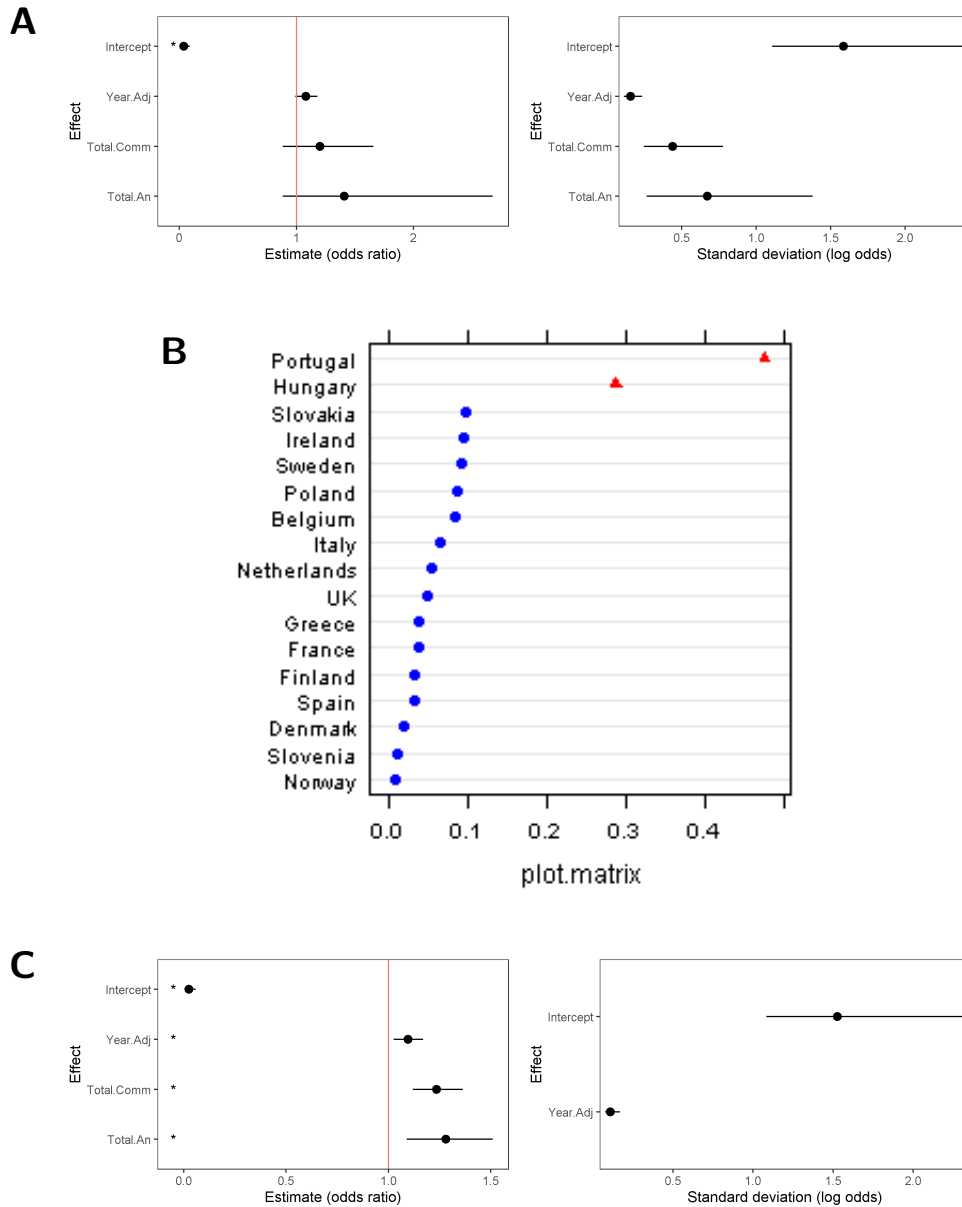


Figure 5.20: **Impact of influential points on the best-fitting GLMM for vancomycin resistance in *Enterococcus faecium*.** **A:** Results from best-fitting model. Left, estimates (points) and 95% confidence intervals (lines) of fixed effects. * denotes significance. Right, estimates (points) and 95% confidence intervals (lines) of the standard deviation of the random effect slopes. **B:** Cook's distance for each country, with influential countries shown in red. **C:** Results from best-fitting model after influential points excluded and model refitted. This model has random slopes for time only. Fixed effects (left) and standard deviations of random slopes (right), and 95% confidence intervals. Year.Adj = year - 2010 = t . Comm, community sector; An, animal sector.

ems both showed very high fixed effects attributable to third- and fourth-generation cephalosporin consumption in community settings (Figs. 5.13 and 5.15). For these combinations, a high proportion of the random effects variance (67% and 57%, respectively) was attributable to variation in the random slopes between countries. Thus, between-country differences in the relationship between third- and fourth-generation cephalosporin consumption, and these resistances, have a large effect. By contrast, MRSA is associated with positive fixed effects of third- and fourth-generation cephalosporins in hospital and animal settings, but the majority of the random effects variance for this resistance is attributable to random intercepts, i.e. between-country variation.

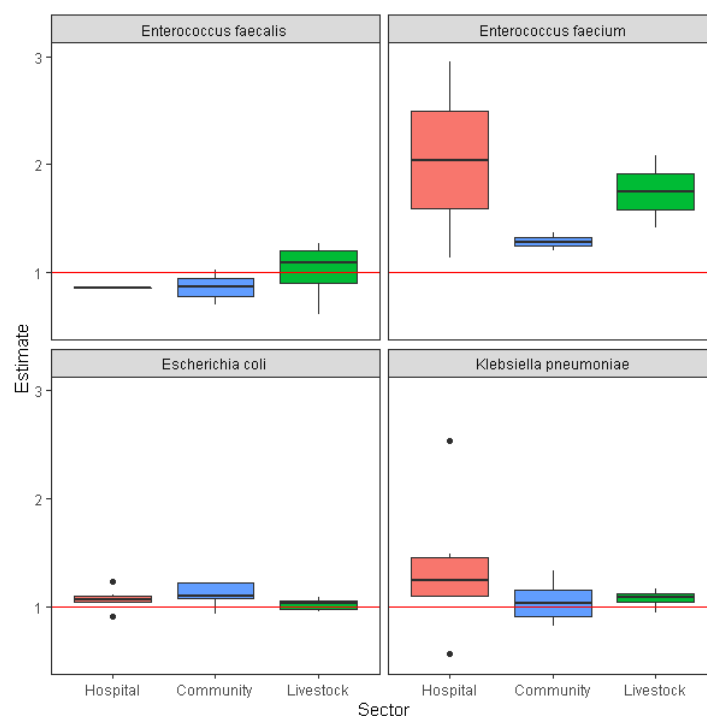


Figure 5.21: **Pooled fixed effect estimates for antibiotic consumption variables in best-fitting models of resistance in gastrointestinal commensals.** Models detailed in Tables 5.3-5.6. Fixed effect estimates for individual consumption variables categorised by sector. Odds ratio scale. Outliers cropped for visualisation purposes.

5.3.6 Environmental organisms

Best-fitting GLMMs were obtained for 12 combinations of environmental organisms and antibiotic resistance: three for *Acinetobacter* spp., four for *K. pneumoniae*, and five for *P. aeruginosa*. Best-fitting models are detailed in Tables 5.7 and 5.8, and Table 5.5 in the

Bacteria	Antibiotic	Countries	Model form	Consumption variables in x
<i>Acinetobacter</i> spp.	Aminoglycosides	9	$t + x + (t g)$	Aminoglycosides (hospital)
	Carbapenems	11	$t + x + (t + x g)$	Total (hospital) First/second generation cephalosporins (community) Penicillins (animal) Third/fourth generation cephalosporins (animal)
	Fluoroquinolones	11	$t + x + (t + x g)$	Fluoroquinolones (hospital) Fluoroquinolones (community) All quinolones (animal)

Table 5.7: **Best-fitting GLMMs for resistance in *Acinetobacter* spp.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

Bacteria	Antibiotic	Countries	Model form	Consumption variables in x
<i>P. aeruginosa</i>	Aminoglycosides	17	$t + x + (t + x g)$	Total (animal)
	Carbapenems	18	$t + x + (t + x g)$	Third/fourth generation cephalosporins (hospital) First/second generation cephalosporins (animal)
	Ceftazidime	18	$t + x + (t + x g)$	All beta-lactams (hospital) Third/fourth generation cephalosporins (community) First/second generation cephalosporins (animal)
	Fluoroquinolones	18	$t + (t g)$	-
	Piperacillin/tazobactam	18	$t + x + (t + x g)$	Penicillins (community) Third/fourth generation cephalosporins (community) First/second generation cephalosporins (animal) Third/fourth generation cephalosporins (animal)

Table 5.8: **Best-fitting GLMMs for resistance in *Pseudomonas aeruginosa*.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

previous section.

All of the best fits incorporated random intercepts for individual countries, and fixed effects and random slopes for time. Of these, 83% (10/12) also incorporated fixed effects and random slopes for at least one antibiotic consumption variable. Inclusion of each individual variable in each best-fitting model had a statistically significant impact on model fit (using likelihood ratio tests on nested models).

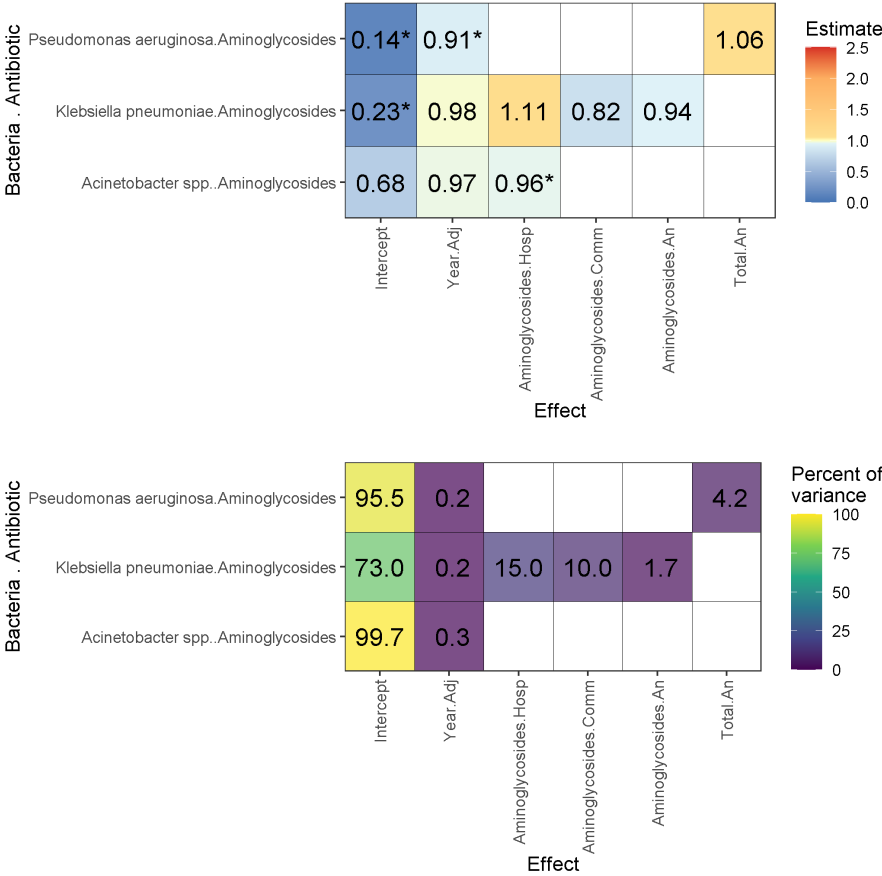


Figure 5.22: **Results of best-fitting models of resistance to aminoglycoside antibiotics in environmental organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

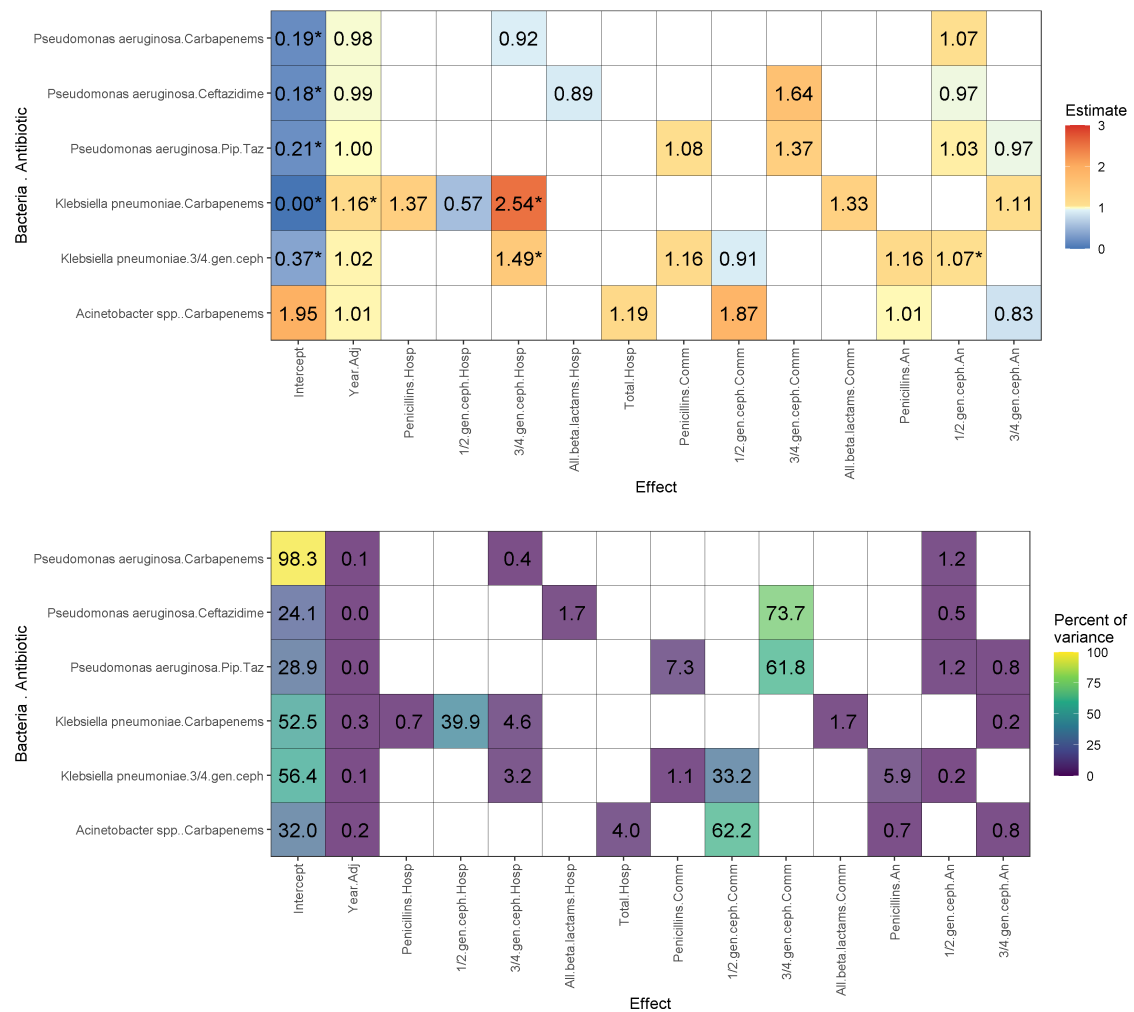


Figure 5.23: Results of best-fitting models of resistance to beta-lactam antibiotics in environmental organisms. Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector. Gen.ceph = generation cephalosporins, Pip.Taz = piperacillin/tazobactam.

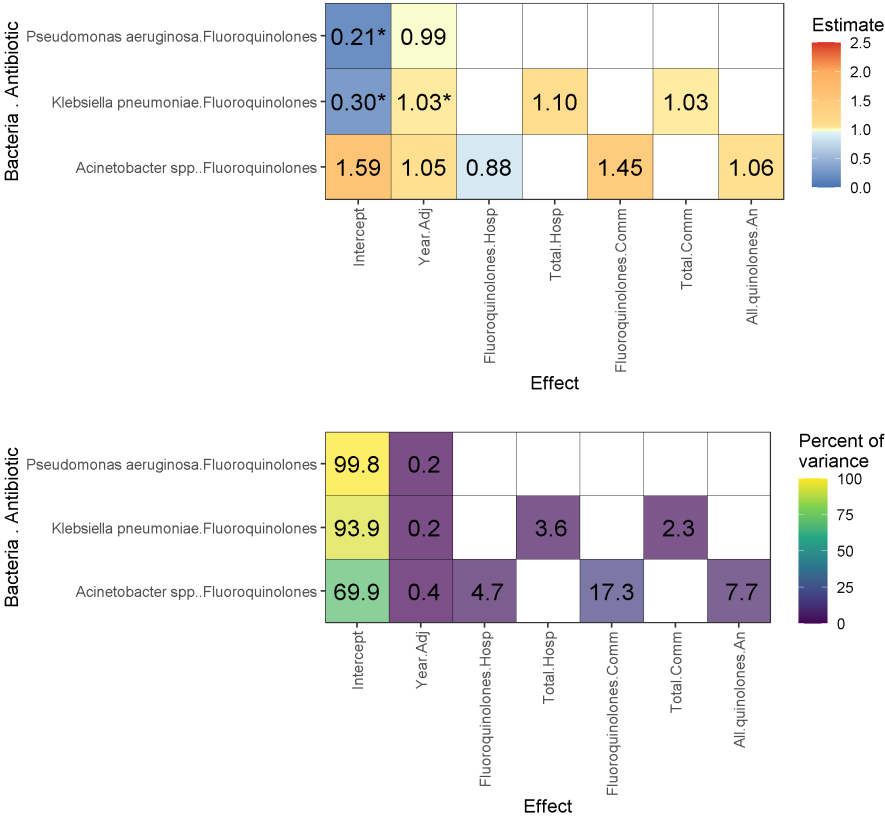


Figure 5.24: **Results of best-fitting models of resistance to quinolone antibiotics in environmental organisms.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

5.3.6.1 Fixed effects

Of the 12 best-fitting models, 11 included fixed effects for at least one antibiotic consumption variable: nine (75%) contained measures of hospital consumption; eight (67%) contained community consumption variables; nine (75%) contained veterinary variables (Figs. 5.22-5.24, top plots). The fixed effect of time t was statistically significant in three cases. At least one antibiotic consumption variable had a statistically significant fixed effect across Europe in three of the best-fitting models, according to 95% confidence intervals on the estimate (Figs. 5.22 to 5.24). These three models included four separate significant consumption variables: three from hospitals (one of which was negative) and one from the veterinary sector.

The largest fixed effects estimates for environmental organisms were for resistance to beta-lactams (Fig. 5.23): third- and fourth-generation cephalosporin consumption in hospitals (resistance to carbapenems in *K. pneumoniae*, as previously described); first- and second-generation cephalosporins in community settings (carbapenem resistance in *Acinetobacter* spp.); and third- and fourth-generation cephalosporins in communities (cef-tazidime resistance in *P. aeruginosa*). Beta-lactam consumption in hospital settings was rarely implicated in best-fitting models of resistance in *Acinetobacter* spp. and *P. aeruginosa*.

Consumption of aminoglycosides had a negative fixed effects estimate in 3 out of 4 best-fitting models of resistance in environmental organisms; the exception was aminoglycosides in hospitals (aminoglycoside resistance in *K. pneumoniae*.)

5.3.6.2 Random effects

The percentage of the total random effect variance attributable to each term in the best-fitting models are summarised in Figs. 5.22-5.24, bottom plots. For environmental organisms, the proportion of random effects variance attributable to the intercept (i.e. baseline between-country variation) was higher for resistance to antibiotics in the aminoglycoside (Fig. 5.22) and quinolone (Fig. 5.24) classes than for beta-lactam resistance (with the exception of carbapenem resistance in *P. aeruginosa*, Fig. 5.23). Over 90% of the between-country random variation was explained by differences in random intercepts in models of:

aminoglycoside resistance in *Acinetobacter* spp. and *P. aeruginosa*; carbapenem resistance in *P. aeruginosa*; and fluoroquinolone resistance in *K. pneumoniae* and *P. aeruginosa*.

By contrast, > 50% of the random-effect variance were attributable to random slopes for individual consumption variables in 3 combinations of environmental organisms and resistance: all cephalosporin consumption in the community. The variation among countries in the relationship between resistance and time explained less than 1% of the variance for all combinations.

5.3.6.3 Effect of influential points

Influential points, as determined by Cook's distance, were present in 10 of 12 best-fitting models. Significance of antibiotic consumption fixed effects were not affected by influential points in four of these models. Excluding the most influential country resulted in additional statistically significant fixed effects of consumption variables in three cases (two from the animal sector, one from the hospital sector); all involved beta-lactam consumption, and all were negative. These variables were: beta-lactam consumption in hospitals (ceftazidime resistance in *P. aeruginosa*); and third- and fourth-generation cephalosporin consumption in animal (carbapenem resistance in *Acinetobacter* spp. and piperacillin/tazobactam resistance in *P. aeruginosa*). In two cases, excluding the most influential country had no effect or produced a singular fit, but excluding the second- or third-most influential points made two additional consumption variables significant (and positive): aminoglycoside consumption in hospitals and beta-lactam consumption in communities (affecting aminoglycoside and carbapenem resistance in *K. pneumoniae*, respectively).

Greece, Hungary, and Portugal were each identified twice as influential countries affecting the significance of fixed effects in models of environmental resistance.

5.3.6.4 Summary of results for environmental organisms

There were clear species differences between environmental organisms. More antibiotic consumption variables were included in best-fitting models of resistance in *K. pneumoniae* than the other environmental organisms. The magnitude of the fixed effect variables are summarised in Fig. 5.25. The largest fixed-effect estimates for consumption variables in models of resistance in environmental organisms were all in the beta-lactam class

(Fig. 5.23). Models involving this antibiotic class also included the highest proportions of between-country variation in random effects that was attributable to random slopes.

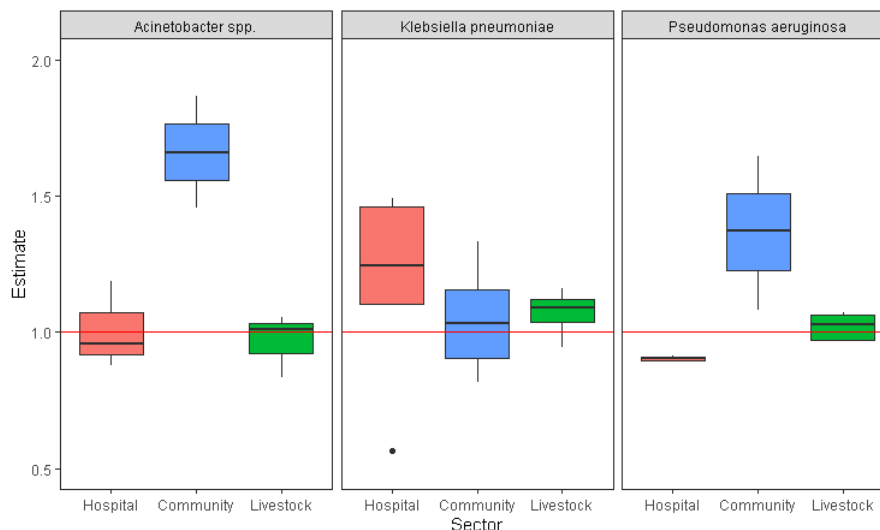


Figure 5.25: **Pooled fixed effect estimates for antibiotic consumption variables in models of resistance to environmental organisms.** Models detailed in Tables 5.5, 5.7, 5.8. Fixed effect estimates for individual consumption variables categorised by sector. Odds ratio scale. Outliers cropped for visualisation purposes.

5.3.7 Foodborne pathogens

There were 10 combinations of foodborne pathogens and antibiotic resistance for which GLMMs were not fitted, due to insufficient data: five involving *C. jejuni*, three for *C. coli*, and two involving *Salmonella* spp. (Tables 5.9 and 5.10). GLMMs were successfully fitted to 17 combinations of foodborne pathogens and antibiotic resistance: three for *C. jejuni*, and 14 for *Salmonella* spp. A valid GLMM could not be obtained for meropenem resistance in *Salmonella* spp., due to singular fits (resistance data is zero-inflated, see Fig. 5.8).

All best-fitting models incorporated random intercepts for country, and 88% (15/17) also incorporated fixed effects and random slopes for time t . The best-fitting models for azithromycin and tigecycline resistance in *Salmonella* spp. did not include a time component. Of the 15 best fits incorporating fixed and random effects for time, 93% (14/15;

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>C. coli</i>	Ciprofloxacin	4	Insufficient data	-
	Erythromycin	4	Insufficient data	-
	Tetracycline	4	Insufficient data	-
<i>C. jejuni</i>	Amoxicillin	0	Insufficient data	-
	Amoxicillin/clavulanic acid	3	Insufficient data	-
	Ampicillin	0	Insufficient data	-
	Ciprofloxacin	12	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)
	Erythromycin	13	$t + s + (t + s g)$	Total (hospital) Total (community)
	Gentamicin	3	Insufficient data	-
	Nalidixic acid	0	Insufficient data	-
	Tetracycline	11	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)

Table 5.9: **Best-fitting GLMMs for resistance in *Campylobacter* spp.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

Bacteria	Antibiotic	Countries	Model form	Consumption variables in s
<i>Salmonella</i> spp.	Ampicillin	18	$t + s + (t + s g)$	Penicillins (hospital) Third/fourth generation cephalosporins (hospital) Carbapenems (hospital) Total (community) Penicillins (animal) First/second generation cephalosporins (animal) Third/fourth generation cephalosporins (animal)
	Azithromycin	5	$s + (s g)$	Lincosamides (hospital) All macrolides & lincosamides (community)
	Cefotaxime	18	$t + s + (t + s g)$	First/second generation cephalosporins (community) Penicillins (animal) First/second generation cephalosporins (animal) Third/fourth generation cephalosporins (animal)
	Ceftazidime	14	$t + s + (t + s g)$	Total (hospital) Penicillins (animal)
	Chloramphenicol	15	$t + s + (t + s g)$	Total (hospital) Total (community)
	Ciprofloxacin	17	$t + s + (t + s g)$	Fluoroquinolones (hospital) Fluoroquinolones (community) Fluoroquinolones (animal) Other quinolones (animal)
	Colistin	5	$t + s + (t + s g)$	Polymyxins (hospital) Polymyxins (animal)
	Gentamicin	15	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)
	Kanamycin		Insufficient data	-
	Meropenem		No valid model	-
	Nalidixic acid	11	$t + s + (t + s g)$	Fluoroquinolones (hospital) Fluoroquinolones (animal) Other quinolones (animal)
	Streptomycin		Insufficient data	-
	Sulfamethoxazole	12	$t + s + (t + s g)$	Sulfonamides (hospital) Trimethoprim (hospital) Sulfonamide/trimethoprim combinations (hospital) Trimethoprim (community) Trimethoprim (animals)
	Sulfonamide/trimethoprim combinations	5	$t + s + (t g)$	Total (hospital)
	Tetracycline	17	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)
	Tigecycline	6	$s + (1 g)$	Tetracyclines (hospital)
	Trimethoprim	15	$t + s + (t + s g)$	Total (hospital) Total (community) Total (animal)

Table 5.10: **Best-fitting GLMMs for resistance in *Salmonella* spp.** Number of countries with sufficient data for model fitting. Model form as described in Section 5.2.3.

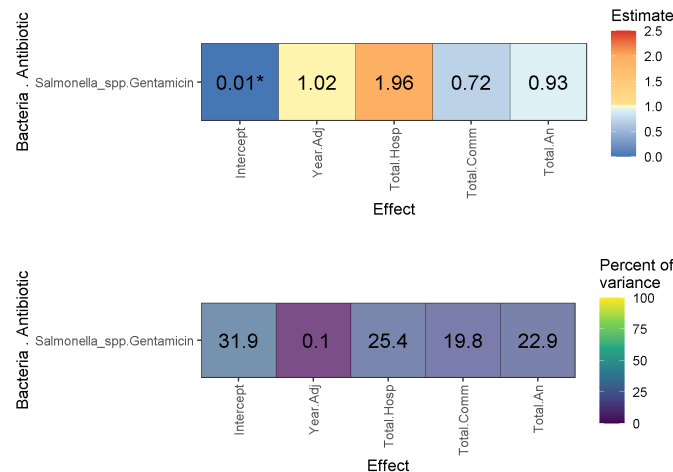


Figure 5.26: **Results of best-fitting models of resistance to aminoglycoside antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

82% of the total foodborne combinations) also included fixed effects and random slopes for at least one consumption variable. The best-fitting model for one combination included a fixed effect of consumption, but no random slope. These results are summarised in Figs. 5.26-5.33.

5.3.7.1 Fixed effects

All 17 best-fitting models of resistance in foodborne pathogens included fixed effects of at least one consumption variable (Figs. 5.26-5.32). Of these, 16 (94%) included fixed effects of hospital consumption, 12 (71%) included community consumption, and 12 (71%) included veterinary consumption variables. Of the best-fitting GLMMs, 15/17 (88%) included fixed effects of time.

The fixed effect of time t was statistically significant in 12% of the best-fitting GLMMs for foodborne pathogens (2/17). One antibiotic consumption variable had a statistically significant fixed effect in 41% of the best-fitting models (7/17), according to 95% confidence intervals on the estimate. Of these variables, four were positive on the log-odds scale (i.e. had odds ratios greater than one). Total antibiotic consumption was a statistically significant fixed effect in five cases, of which three were positive, all involving resistance in

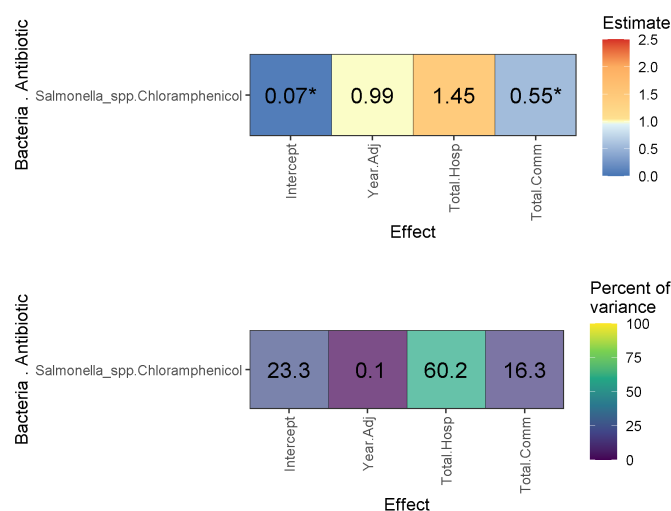


Figure 5.27: **Results of best-fitting models of resistance to amphenicol antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

C. jejuni: total community consumption (ciprofloxacin and erythromycin resistance) and total consumption in food-producing animals (tetracycline consumption).

Total antibiotic consumption in hospital settings had a positive fixed effect in six different foodborne pathogen-antibiotic resistance combinations: resistance to chloramphenicol, gentamicin, tetracycline, and trimethoprim in *Salmonella* spp.; and resistance to erythromycin and tetracycline in *C. jejuni*. Total community consumption produced positive fixed effect estimates in three models (*C. jejuni* resistance to ciprofloxacin, erythromycin, and tetracycline). Total community consumption produced negative estimates of fixed effects in 5/14 models of resistance in *Salmonella* spp. (36%): ampicillin, chloramphenicol, gentamicin, tetracycline, and trimethoprim. Total antibiotic consumption in food-producing animals had a positive fixed effect in four models: ciprofloxacin and tetracycline resistance in *C. jejuni*, and tetracycline and trimethoprim resistance in *Salmonella* spp.

Models of resistance to beta-lactam antibiotics in *Salmonella* spp. included seven consumption variables from food-producing animals (Fig. 5.28); 86% of these variables (6/7) had negative fixed effect estimates. The highest fixed effect estimates for resistance

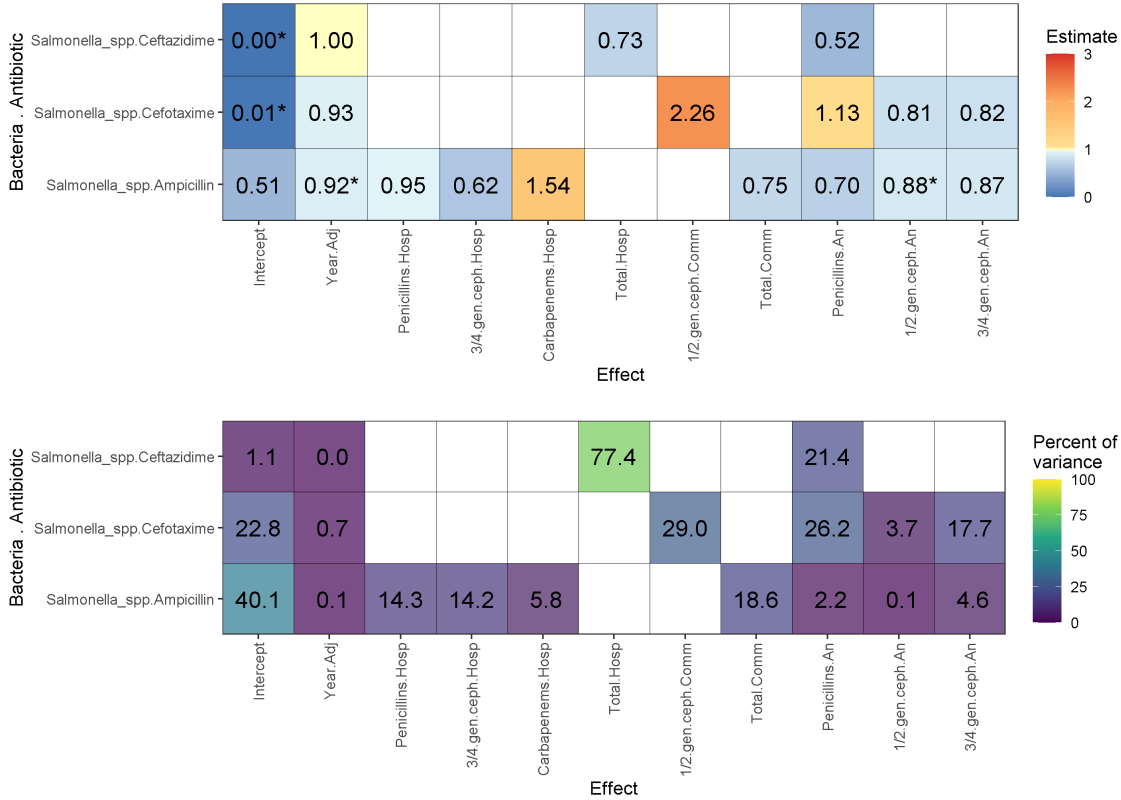


Figure 5.28: **Results of best-fitting models of resistance to beta-lactam antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector. Gen.ceph = generation cephalosporin.

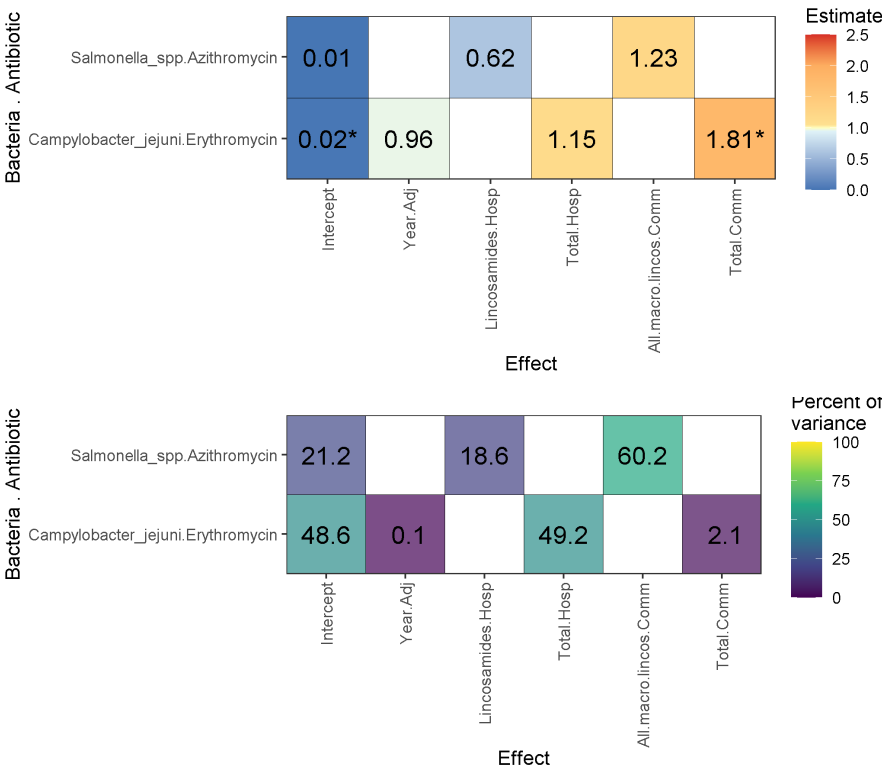


Figure 5.29: **Results of best-fitting models of resistance to macrolide & lincosamide antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

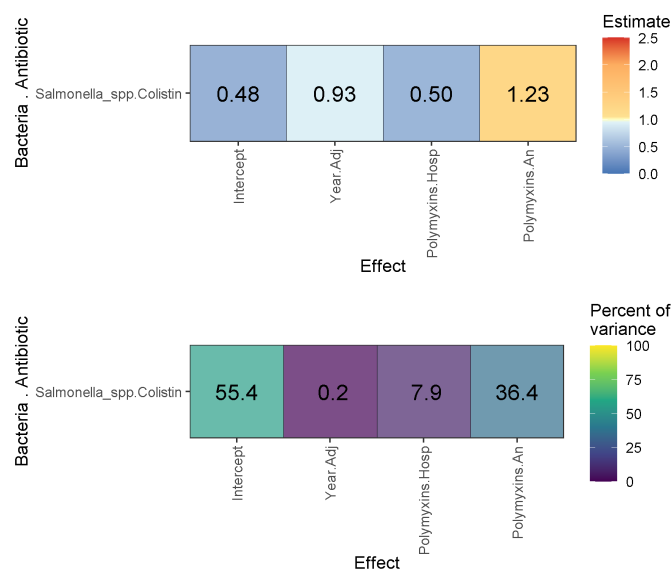


Figure 5.30: **Results of best-fitting models of resistance to polymyxin antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

to beta-lactams were attributed to first- and second-generation cephalosporin consumption in the community, and carbapenem usage in hospitals. Models of macrolide resistance in both *C. jejuni* and *Salmonella* spp. included antibiotic consumption variables in the hospital and community sectors only.

Several antibiotic consumption variables had large fixed effects for resistance to quinolone antibiotics, across both veterinary and medical sectors (Fig. 5.31). Resistance to tetracycline in both *C. jejuni* and *Salmonella* spp. were both associated with measures of total antibiotic consumption in all three sectors (Fig. 5.33); 83% of these (but not total community consumption and tetracycline resistance) were positive. By contrast, fixed effects arising from models of *Salmonella* resistance to sulfonamides & trimethoprim differed (Fig. 5.32). Of these, the largest fixed effect estimates were associated with total consumption in animals and hospitals (trimethoprim resistance) and trimethoprim consumption in the community (sulfamethoxazole resistance).

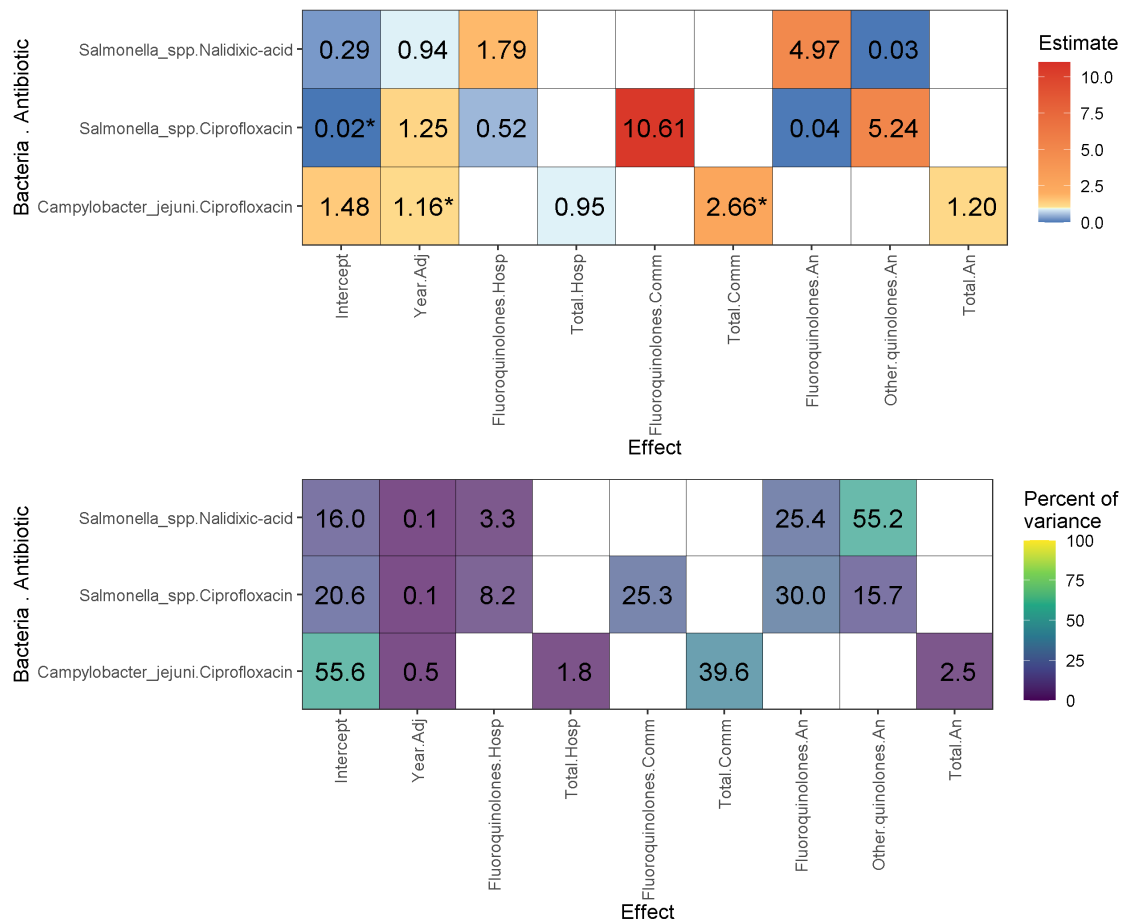


Figure 5.31: **Results of best-fitting models of resistance to quinolone antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

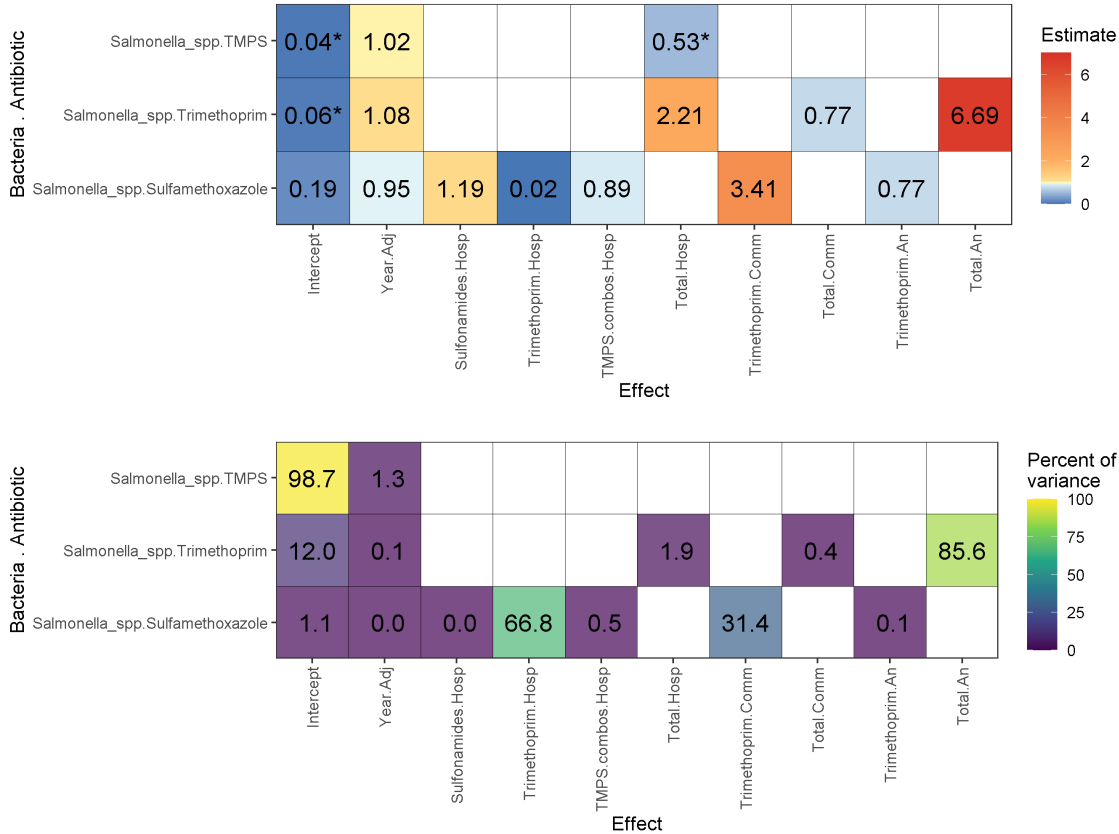


Figure 5.32: **Results of best-fitting models of resistance to sulfonamide & trimethoprim antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector. TMPS = trimethoprim/sulfamethoxazole.

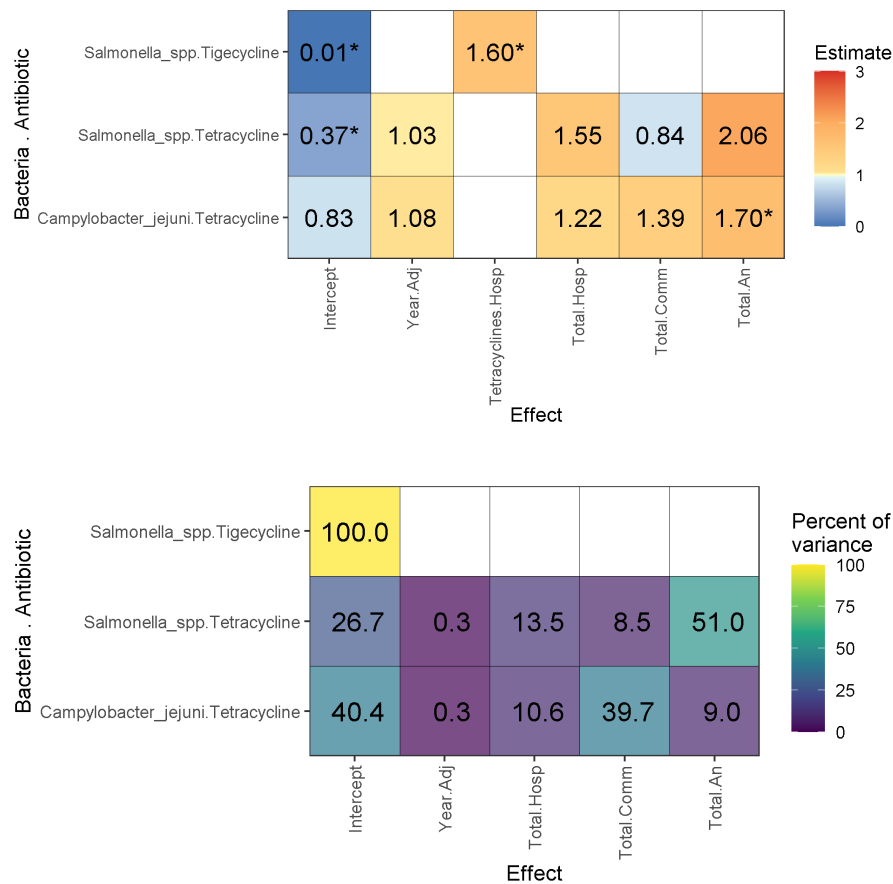


Figure 5.33: **Results of best-fitting models of resistance to tetracycline and tetracycline-derivative antibiotics in foodborne pathogens.** Top, estimates (as odds ratios) of fixed effects; * denotes significance. Bottom, percentage of the random effects variance attributable to each variable. Bacteria-antibiotic combinations on y axis. Year.Adj = year – 2010 = t . Hosp = hospital sector; Comm = community sector, An = animal sector.

5.3.7.2 Random effects

The percentage of the total random effect variance attributable to each term in the best-fitting models of resistance in foodborne pathogens are summarised in Figs. 5.26-5.33, bottom plots. Consumption variables explaining $> 50\%$ of the random effect variance between countries were only observed in models of resistance to *Salmonella*: total hospital consumption (chloramphenicol and ceftazidime resistance); trimethoprim consumption in hospitals (sulfamethoxazole resistance); macrolides & lincosamide consumption in the community (azithromycin resistance); total veterinary consumption (tetracycline and trimethoprim resistance); and veterinary use of other quinolones (nalidixic acid).

5.3.7.3 Effect of influential points

Influential points did not affect the significance of fixed effects in six models of resistance in foodborne pathogens (35%). In five cases, removing influential points caused fixed effects of one consumption variable to become statistically insignificant. In one instance (cefotaxime resistance in *Salmonella* spp.), removing the most influential country produced a singular fit, but removing the second-most influential country caused an additional consumption variable (first/second generation cephalosporins in the community) to become significant. In two cases (resistance to ceftazidime and sulfamethoxazole in *Salmonella* spp.), removing influential countries resulted in singular fits. In three cases, removing influential countries meant that the data set was no longer sufficient to fit GLMMs (number of countries < 5).

Hungary was identified in three different bacteria-antibiotic combinations as affecting the significance of fixed effects of consumption variables in models of foodborne resistance.

5.3.7.4 Summary of results for foodborne pathogens

Results from models of resistance in foodborne pathogens varied between bacterial species, antibiotic class, and sometimes, individual antibiotics. Species-specific differences were notable (Fig. 5.34), with greater variation in the fixed effects of consumption in models of *Salmonella* compared to *Campylobacter*. Consumption variables from all sectors were represented.

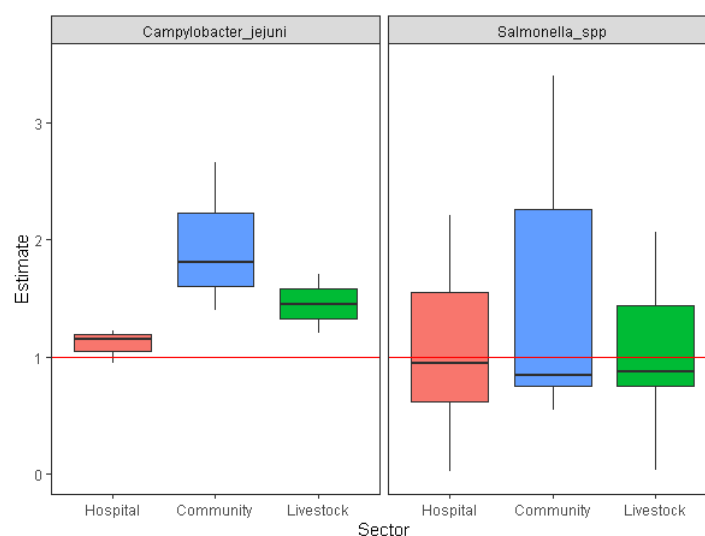


Figure 5.34: **Pooled fixed effect estimates for antibiotic consumption variables in models of resistance to foodborne pathogens.** Models detailed in Tables 5.9-5.10. Fixed effect estimates for individual consumption variables categorised by sector. Odds ratio scale. Outliers cropped for visualisation purposes.

5.3.8 Summary of GLMM results

Almost all best-fitting models of resistance in commensal, environmental, and foodborne organisms included random intercepts, random slopes, and fixed effects for both temporal and antibiotic consumption variables. However, the specific antibiotic consumption variables included in each model, and the magnitude of the resultant fixed effects, varied. Taken together, this suggests a persistent role for antibiotic consumption across a wide range of bacterial organisms and resistances.

Overall results are summarised in Fig. 5.35. Here, for clarity, *K. pneumoniae* is represented in the commensal category only. Fixed effects of antibiotics consumed in hospital settings on resistance in commensals tend to be larger than those those from community or animal settings, although differences are small. By contrast, estimates of the fixed effects of community antibiotic consumption on environmental organisms *Acinetobacter* spp. and *P. aeruginosa* are considerably larger than those from the other sectors. Large amounts of variation are evident in fixed effects estimates of resistance in foodborne pathogens, but again, the median of these estimates is higher for antibiotics consumed in community settings.

Results are also summarised by the antibiotics to which resistance was measured

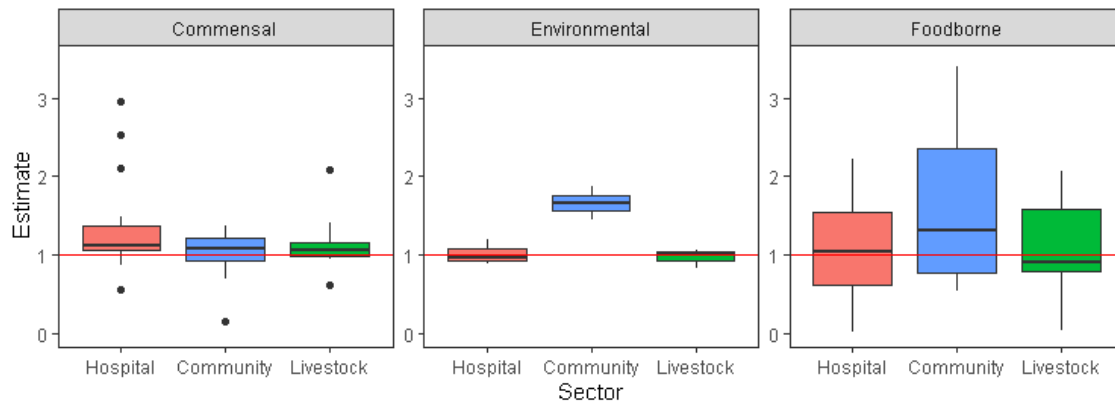


Figure 5.35: **The impact of antibiotic consumption in different sectors on fixed effect estimates from models of resistance in commensal and environmental organisms, and foodborne pathogens.** Models detailed in Tables 5.3-5.10. *K. pneumoniae* represented in commensal category only. Fixed effect estimates for individual consumption variables categorised by sector. Odds ratio scale. Outliers cropped for visualisation purposes.

(Fig. 5.36). Antibiotic consumption in hospitals had the highest median pooled fixed effect in models of resistance to aminoglycosides (including high-level gentamicin resistance), which are predominantly used in hospitals (Fig. 5.2), and third-generation cephalosporins. Consumption in community settings had the highest median fixed effects in models of resistance to carbapenems, ceftazidime, and fluoroquinolones (including ciprofloxacin). Antibiotic use in food-producing animals had the highest median fixed effects in models of resistance to aminopenicillins and tetracycline.

Statistically significant, positive fixed effects of antibiotic consumption variables are summarised graphically in Fig. 5.37.

The variation in random effects is related to the number of countries used for model-fitting, as shown in Fig. 5.38.

5.4 Discussion

The results presented here confirm the presence of strong country-to-country differences in antibiotic resistance in Europe. Incorporation of group-level (random) effects for individual countries in models of resistance in clinical isolates significantly improved model fits for all tested combinations of bacterial species and antibiotics. In many cases, much of the overall

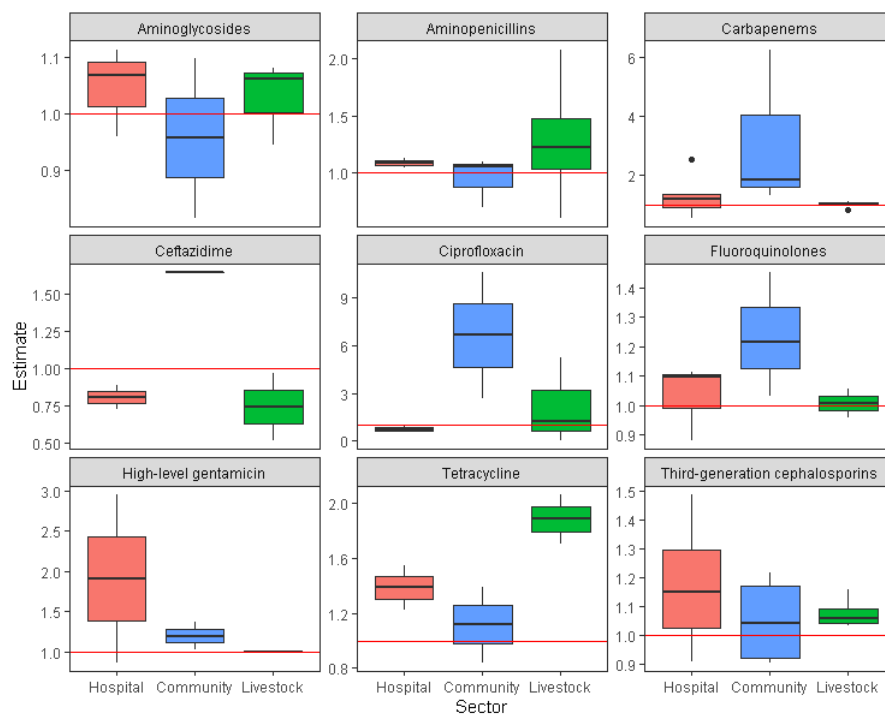


Figure 5.36: **Pooled fixed effect estimates for consumption variables in models of resistance to different antibiotics.** Models detailed in Tables 5.3-5.10. Results include resistance in commensal, environmental, and foodborne organisms, for at least 2 model fits. Fixed effect estimates for individual consumption variables categorised by sector. Odds ratio scale.

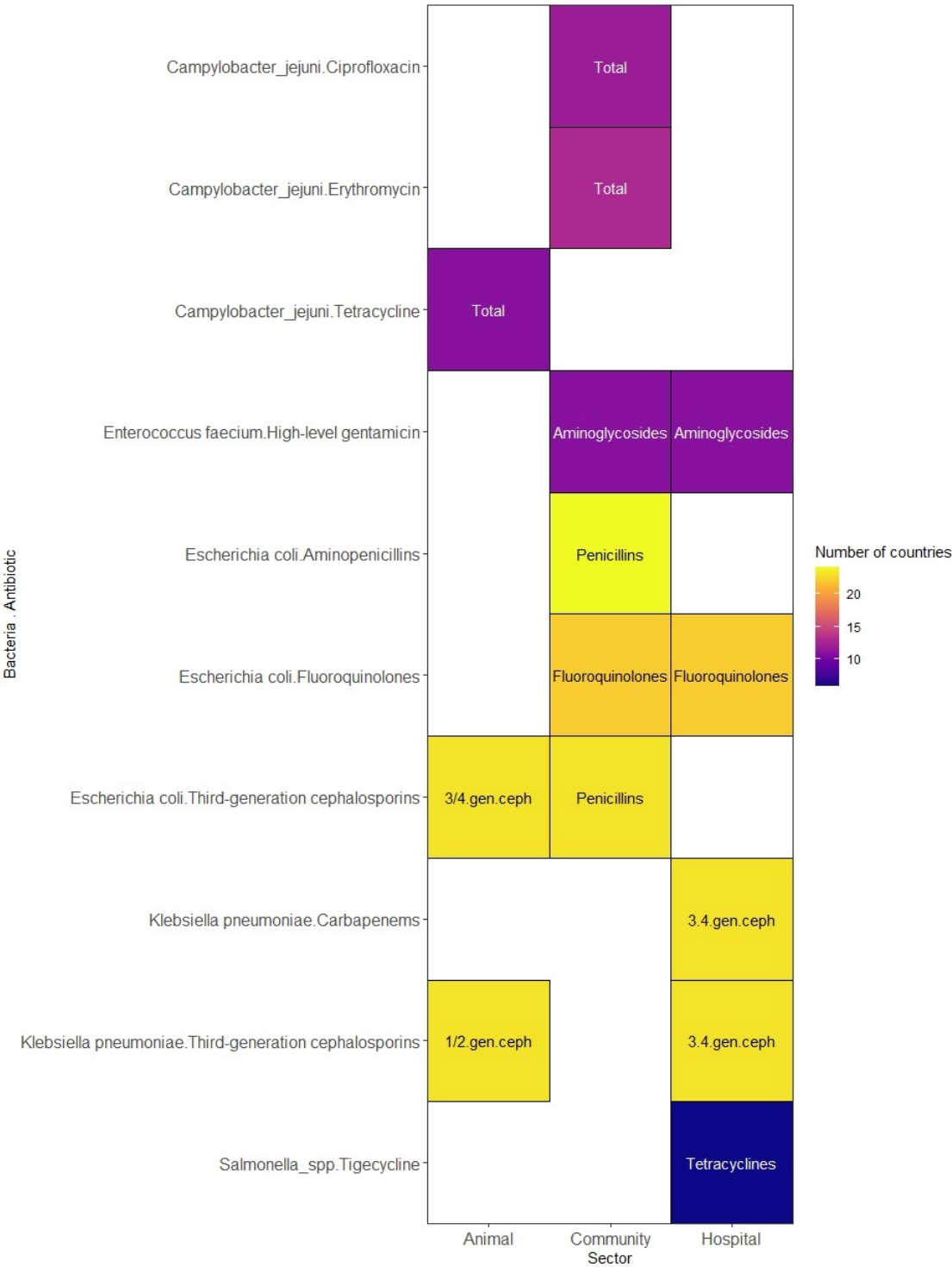


Figure 5.37: **Summary of significant, positive fixed effects across all best-fitting GLMMS.** Results shown by sector (x axis) and bacteria-antibiotic resistance combinations (y axis).

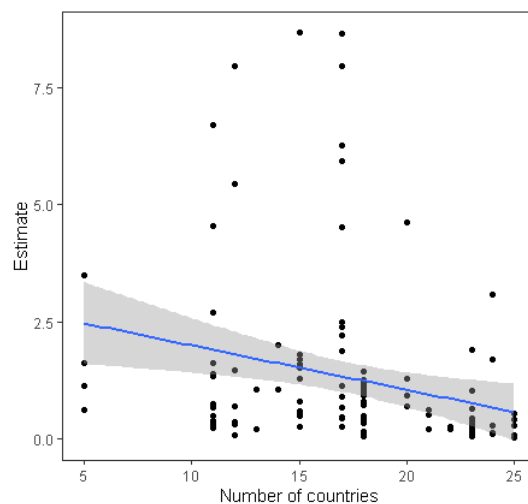


Figure 5.38: **The impact of the number of countries in GLMMs of resistance in commensal and environmental organisms, and foodborne pathogens, on the variation in random effects.** Models detailed in Tables 5.3-5.10. Number of countries used for model-fitting on the x axis; standard deviation of individual random effects on the y axis. Log-odds scale. Shading shows 95% confidence intervals.

random effect variance was attributable to country-level random intercepts. This finding is consistent with previous models demonstrating country-level differences in resistance in clinical isolates [247, 417–419].

However, previous studies (including our own [97]) modelling the relationship between consumption and resistance focus on results from isolated years, and do not account for non-independence of results generated in successive years. Given what we know about bacterial population structure and evolution (as outlined in Chapter 1), assuming independence of repeated samples seems unrealistic. Here, I used time as an explanatory variable in a series of GLMMs, as a proxy for other factors shaping resistance, independently of antibiotic consumption. In 98% of the bacteria-antibiotic combinations tested, model fit was significantly improved by explicitly incorporating time as an explanatory variable; and time was included in the best-fitting model in 95% of cases. While almost all best-fitting models included time as a fixed effect and random slope, the majority of these fixed effects were not statistically significant. Additionally, the proportion of overall random effects variance attributable to time random slopes was consistently low, across all combinations of bacterial species and antibiotic tested here. Taken together, these findings are suggestive of small but persistent differences in the prevalence of AMR over

time, in different countries, independently of antibiotic consumption. This supports a role for the underlying bacterial population - in the community and/or within hospitals - in shaping resistance at national level.

Antibiotic consumption was also influential. Most of the best-fitting models (95%) for the bacteria-antibiotic combinations tested here also included consumption variables, and addition of consumption variables to time-only GLMMs significantly improved model fits in almost all cases. Likelihood ratio tests on nested models showed that the addition of each consumption variable for these bacteria-antibiotic combinations significantly improved model fits in all cases.

The magnitude of the effect of consumption tended to be higher than that of time. However, there was considerable variation in the specific antibiotic consumption variables included in the best-fitting models for each bacteria-antibiotic combination, and the size of their effect. Uncertainty surrounding these estimates was usually higher than for estimates of the effect of time. This was most marked in models of resistance in *Salmonella* spp., which could be explained by specific features of the epidemiology of this species, namely the occurrence of outbreaks, and the influence of the rise and fall of different serovars on resistance results [511].

There were also differences in the effect of consumption between models of resistance in commensal, environmental, and foodborne organisms. Pooled fixed effect estimates of consumption variables in models of resistance in commensal organisms were slightly higher for in-hospital consumption compared to antibiotic use in community and hospital settings, but the differences between consumption variables arising from each sector were fairly small (Fig. 5.21). By contrast, models of resistance in environmental organisms *Acinetobacter* spp. and *P. aeruginosa* produced much higher fixed effect estimates for consumption variables compared to the other sectors (Fig. 5.35). Pooled fixed effects of antibiotic consumption in models of resistance in foodborne pathogens were also higher for community consumption; however, there was more uncertainty around these estimates.

The overall similarities between the fixed effects associated with antibiotic consumption in different sectors for commensal organisms break down when effects are examined at species level (Fig. 5.21). Here, the largest fixed effects for resistance in *E. coli* are attributable to community antibiotic consumption, which is consistent with previous findings

[512], and aligns with other evidence demonstrating a limited contribution of animal-origin ESBL-producing *E. coli* to resistant infections in people [346, 359, 360]. Differences in the contributions of consumption in different settings to models of resistance extend to closely related species (*E. faecalis* and *E. faecium*), and to a lesser extent, to those that freely exchange genetic material, including resistance genes, between species (*E. coli* and *K. pneumoniae*).

Environmental organisms such as *Acinetobacter* spp. and *P. aeruginosa* are able to persist in natural environments, but when causing disease, are most commonly associated with nosocomial (i.e. hospital-acquired) infections in people. The strong signal implicating community antibiotic consumption in resistance in these organisms is therefore unexpected. Carbapenem resistance in *A. baumannii* for example, has repeatedly been associated with carbapenem use in hospitals [513, 514]. Recent work by Diaz and colleagues offers an explanation for this effect in *P. aeruginosa* [515]. In this study, approximately 1/3 of intensive care patients were colonised by multiple *P. aeruginosa* strains simultaneously, and antibiotic therapy resulted in rapid selection of resistant strains within this mixed population. It is therefore feasible that the prevalence of resistance in the colonising population could be shaped by antibiotic consumption in the wider community, who are then entering hospitals (either as patients or as staff), and contributing to resistance there.

Consumption terms included measures of total consumption in one or more sectors in over 50% of bacteria-antibiotic combinations, although consumption of specific antibiotics were also frequently included in best-fitting GLMMs. This is consistent with recent literature showing that total antibiotic consumption in medical and veterinary sectors are each significantly associated with AMR in important human pathogens [516]. The presence of antibiotic use in hospitals as a predictor of resistance in several bacteria-antibiotic combinations is not surprising, given high levels of resistance reported in hospital-acquired infections [114]. Hospital consumption is not always explicitly included in models of AMR [97, 406], due to the majority of antibiotic use occurring outside these settings. However, the results presented here make a strong case for including hospital consumption when modelling associations between consumption and resistance in clinical isolates.

Several of the fixed effect estimates are consistent with antibiotic use, when pooled by antibiotic, and compared between settings. For example, median fixed effect esti-

mates for aminoglycoside resistance are > 1 for both hospital and veterinary consumption (Fig. 5.36), which is where these antibiotics are most used. Median estimates from the animal sector are higher than those for other sectors for both aminopenicillin and tetracycline resistance; these are the two most-used antibiotic classes in farm animal medicine [108, 417]. Community consumption of fluoroquinolones is also high (Fig. 5.2), and corresponds to the largest fixed effects of resistance being attributable to this class. Conversely, the greatest effect on carbapenem resistance is also attributable to consumption in the community, where these antibiotics are almost never used (Fig. 5.2). However, resistance to this antibiotic could be driven by use of other beta-lactams, including penicillins, which are extensively used in community settings [417].

The overall (i.e. Europe-wide) fixed effect of individual consumption variables was not always statistically significant. However, twenty different consumption variables had statistically significant fixed effects. Fourteen of these were positive predictors (Fig. 5.37); six were negative. Of the positive predictors, five (36%) involved antibiotic consumption in hospital settings: aminoglycosides, fluoroquinolones, third/fourth generation cephalosporins (twice) and tetracyclines. Six (43%) involved community settings: aminoglycosides, fluoroquinolones, penicillins (twice), and total consumption (twice); three (21%) were from the veterinary sector (first/second generation cephalosporins, third/fourth generation cephalosporins, and total consumption). All of these are plausible targets for further investigation and interventions at European level.

Three measures of antibiotic consumption in animals had positive, statistically significant fixed effects across countries: third/fourth and first/second generation cephalosporin consumption (for third-generation cephalosporin resistance in *E. coli* and *K. pneumoniae*, respectively), and total consumption (for tetracycline resistance in *C. jejuni*). The role of cephalosporin consumption warrants further investigation, especially of the highest-priority critically-important antibiotics (HP-CIAs) third/fourth generation cephalosporins, as their use in food-producing animals is discouraged in Europe [1]. This is despite previous studies showing that interspecies transmission of extended-spectrum beta-lactamase-producing *E. coli* (ESBLs), which are resistant to third/fourth generation cephalosporins as well as other beta-lactams, is infrequent [346, 360]. There may be additional factors confounding these results, but it is also possible that the models may be detecting a small

but persistent contribution to resistance in people from animal sources [360], for example through horizontal gene transfer (HGT) [346]. The positive fixed effect of total veterinary consumption on tetracycline-resistant *C. jejuni* is consistent with both tetracyclines being one of the main antibiotic classes used in food-producing animals [28, 108]; and a known association between resistant *C. jejuni* in broiler chickens and in people [517].

Not all of the consumption variables with a statistically significant fixed effect across Europe are easily explained. For example, total antibiotic consumption and use of first/second generation cephalosporins in the community both had significant negative fixed effects on resistance of *S. pneumoniae* to macrolides and penicillins, respectively. This result could be artefactual (resistance is low in many countries, which affects model fitting), or may reflect between-country differences in healthcare systems, for example, around collection of diagnostic samples and reporting of AMR results [114]. It could also be confounded by routine vaccination against *S. pneumoniae*, which makes it unique amongst the bacteria examined here. However, this model was affected by the impact of influential points; excluding these removed the significance of cephalosporins as a fixed effect.

Random slopes for the relationship between antibiotic consumption and resistance, in addition to random intercepts varying by country, were a persistent feature of the best-fitting models identified here. Again, this could reflect differences in bacterial population structure in different countries. Bacterial population structure and strain dynamics can in turn can be affected by historical antibiotic consumption, as well as other phenomena. Random slopes could also be indicative of a range of heterogeneities affecting these relationships, including host population structure [386, 401, 445] and differing treatment regimes [444]. Other systemic differences between countries, which may include waste management [518–520], farm management practices [237, 241, 312, 434], or healthcare infrastructure and management [352, 521], could also be impacting bacterial populations and in turn, the distribution of AMR [54, 251].

An alternative explanation for the variability in the contribution of antibiotic consumption to these results could be the process used for model selection. In several cases, there was more than one model that fitted well to the data, with little difference in goodness of fit between the best model, and the best-performing alternatives. This may be partially attributable to the large number of consumption variables tested, including measures of

consumption of individual antibiotics, classes, and sub-classes. Simplifying model choices to major antibiotic classes only, and/or measures of total consumption, could help alleviate this. Conversely, model fitting was limited to consumption of antibiotics within the same antibiotic class (or pooled class) to which resistance was tested. Expanding consumption variables to other antibiotic classes would allow for examination of the role of co-selection on resistance [512].

There were some other limitations associated with the technique used here. GLMMs are a powerful statistical tool and are recognised as the best means of analysing non-normal data with random effects [508, 522], but model selection could potentially be improved by model averaging [522]. Additionally, the results generated here were sensitive to the number of countries with sufficient data. Although five is considered the minimum number of grouping factors required for successful mixed effect modelling [522], I found that GLMMs fitted to 5-6 countries were particularly affected by influential points. Some of my other criteria for minimum data sets, such as the number of isolates or complete data points required, were fairly stringent, but I wanted to ensure that surveillance programmes in individual countries were well-established.

There are some caveats around consumption data. The data on antibiotic consumption in the veterinary sector used here is limited to food-producing animals, and does not include pets. Close contact between pets and their owners is a feasible means of AMR transmission, and use of HP-CIAs such as cephalosporins are also more frequent in these species [108, 523]. However, national-level reporting of consumption is not widely established. One additional possible limitation is the lack of a universally recognised metric to compare antibiotic consumption data between human and animal populations [524]. However, both the human and animal metrics used here are recognised by their respective sectors, and both correct for the size of the underlying population [524]. Additionally, all consumption metrics were centred and scaled before fitting GLMMs, which ensures comparability [487].

There are also inherent limitations in using clinical resistance data of this kind. AMR in disease-causing organisms is affected by factors such as virulence [452, 525, 526], and is not representative of AMR carriage in the general population, which is where most antibiotic exposures happen [235]. AMR surveillance based on clinical submissions is passive, non-

representative, and subject to bias, especially in terms of diagnostic practices [114]. Year-on-year variation in resistance levels could be influenced by changes in clinical practices or surveillance guidelines in individual countries [114, 253]. Resistance is also measured using clinical breakpoints (CBPs), which, while clinically relevant, are a less sensitive measure of resistance emergence than epidemiological cut-off values (see Chapter 1, 1.2.4). Interventions to reduce AMR in hospital settings, such as screening patients and staff, or general IPC measures, may also be influencing results. This work also does not take into account non-domestic factors such as international travel, which have also been shown to influence AMR results [301, 304].

The results presented in this chapter have ramifications for public health professionals and policy-makers. These results underline the contribution of antibiotic consumption to resistance in a range of bacterial species. Additionally, the identification of several consumption variables with statistically significant effects on resistance, even once country-level variation has been taken into account, could provide direction for antibiotic stewardship interventions at national or continental level. The repeated inclusion of consumption variables in models spanning a range of bacterial species and antibiotic resistances, supports policies to reduce AMR by optimising antibiotic use in human and veterinary medicine generally, as my colleagues and I have advocated [435].

However, international variation in the relationships between consumption and resistance, as well as a clear role for between-country differences that can not be explained by antibiotic consumption, emphasise that other factors are at play. These results also indicate that specific interventions would be better identified within-country. Further modelling work for public health purposes should be specific to individual countries and incorporate antibiotic consumption in hospital settings. Other variables, such as interventions to reduce AMR, could also be included in these models. A useful explanatory variable that is not currently available at national level, but would be helpful to input into such models, would be AMR carriage in human populations. This would bridge the gap between consumption and resistance in isolates causing disease.

Chapter 6

Conclusion

6.1 Aims of this work

The motivation behind this work was to better understand the relationship between antibiotic consumption and resistance in heterogeneous populations. To do this, I aimed to develop a robust model of coexistence of resistant and sensitive bacterial strains. Coexistence is a widespread phenomenon that has been observed across a wide range of bacterial species, antibiotics, mechanisms of resistance, and host populations [53, 102, 216, 217, 244, 245, 249, 268–273, 407–412, 483], but is not well-captured by existing models [383, 385, 386, 398, 401].

I then sought to understand the impact of other biological factors that shape the distribution of antimicrobial resistance (AMR), and could affect the relationship between antibiotic consumption and resistance. These include phenomena such as bacterial competition [383, 404, 406], horizontal gene transfer (HGT) [178, 210–212, 216–221, 223, 224, 283, 286, 322, 323, 328, 346, 359], and transmission between different host populations [173, 338–345, 354].

In doing so, I hoped to explain some of the other features of AMR repeatedly observed in real-world systems. For example, the persistence of presumably costly resistance genotypes and phenotypes in antibiotic-free environments [316–320]. Other phenomena include changes in resistance frequencies, associated with changes in antibiotic consumption, occurring over disparate timescales: from weeks [227, 237, 255, 258, 259] to years [260–262, 264, 265, 267]. Large variations in resistance between different countries have also been re-

ported, despite similarities in antibiotic consumption, geographic location, and regulatory environment [246, 248–250, 317, 318, 417–419].

6.2 Approach

For this work, I developed an agent-based model (ABM) of AMR in multi-strain bacterial populations. I took a novel approach to modelling competition between bacterial agents: as a function of strain identity, rather than resistance status. In this model, competition occurred continuously, allowing costs to be imposed on the competition fitness of resistant bacteria, in addition to epidemiological parameters.

The model was extended to incorporate additional agents and functions, which allowed me to explore the dynamics of mobile genetic elements (MGEs), including competition between them, and their role in shaping distributions of resistance. I also used this model to investigate the conditions under which novel resistance can emerge.

To facilitate application of the model to real-world scenarios, I developed an algorithm for fitting it to observed data using likelihood-free inference (LFI).

I complemented this work with a statistical modelling approach, in which I fitted a series of generalised linear mixed models (GLMMs) to international data on antibiotic consumption in hospital, community and veterinary settings, and antibiotic resistance in human clinical isolates.

6.3 Main findings

6.3.1 Strain-based competition enables robust coexistence of multiple resistant and sensitive bacterial strains

I was able to achieve robust coexistence in a multi-strain bacterial system, across a range of costs and rates of antibiotic consumption exceeding that reported globally [114, 429]. As I argued in Chapter 2, competition between bacteria should be modelled according to how biologically similar they are [185–188], and therefore how likely they are to compete for resources, not their resistance status. This view is shared by others [414], but to my knowledge, this is the first time competition has been modelled as a function of strain,

independently of resistance status, to achieve coexistence.

Strain-based competition also allowed the persistence of resistant strains - even those bearing a cost - in the absence of antibiotic consumption, thereby replicating a phenomenon observed in multiple farming systems [238, 239, 241, 317, 527]. Of course, strain-based competition is unlikely to be the sole mechanism maintaining non-zero prevalences of resistance in antibiotic-free environments. AMR persistence in the absence of antibiotic consumption could be promoted by continual reintroduction of AMR from external sources [302, 307].

As I showed in Chapter 2, this model of strain-based competition requires resistance to be associated with some strains but not others. This is consistent with previous work, which hypothesised that coexistence could be achieved by an association between resistance and other strain characteristics that experience balancing selection [385, 414]. This is because negative frequency-dependent selection (NFDS) is acting on resistance, as well as on strains, when this association holds. Relatively high within-strain (or within-species) competition, compared to between-strain (-species) competition has long been recognised as an essential stabilising mechanism to maintain diversity [197, 383, 433].

6.3.2 Two-strain models do not accurately represent multi-strain coexistence

My results also show that two-strain models (with one resistant and one sensitive strain), which dominate previous work [383–386, 398, 400, 406, 434, 445], considerably overestimate the frequency of coexistence achieved in multi-strain systems using the same parameters. This is explained, again, by balancing selection: bacterial agents are more likely to encounter others of the same strain (and compete with them) when there are only two strains in the system.

6.3.3 Novel resistant strains become established in a susceptible population at lower rates of antibiotic consumption than resistance arising in an existing strain

In my model of strain-based competition, the dynamics of resistance emergence differed depending on the distribution of resistance and strains within the system. I introduced

a small number of resistant bacterial agents into fully-susceptible populations, either as a novel resistant strain, or within existing strains. Novel resistant strains become established in fully-susceptible multi-strain populations at lower rates of antibiotic consumption than resistance arising in an existing strain. This is consistent with other results from Chapter 2, which show how strain-based competition, and an association between resistance and individual strains, promotes coexistence. The emergence study also shows that the difference between the two scenarios varies according to the characteristics of the strain into which resistance arises. When resistance first appears in the longest-duration strain (i.e. the strain which stands to benefit most from the acquisition of resistance [385]), its successful establishment in the population is relatively insensitive to its initial distribution within strains.

6.3.4 HGT disrupts the association between resistance and individual strains

As explained above, maintaining robust coexistence in my model is dependent on resistance being associated with some strains, but not others. However, this relationship is disrupted by HGT. If there is sufficient opportunity for resistance genes to transfer into fully-susceptible strains, and conditions are favourable to the emergence of resistance, these strains will become resistant. Fixation of resistance is then inevitable; the HGT rate parameter affects the rate at which this occurs, but not the final equilibrium.

This finding is not consistent with the persistent coexistence of resistant and sensitive strains in diverse systems, as outlined above; nor with the distribution of MGEs such as ICE and plasmids, which are associated with some bacterial strains but not others [217, 325, 331, 332]. I showed in Chapter 2 that the equilibrium prevalence of resistance in my model is determined by the relative frequency of resistant and sensitive strains, as well as antibiotic consumption. Thus, for coexistence to remain stable when HGT is non-negligible, additional mechanisms need to be incorporated that maintain a balance between resistant and sensitive strains. Competition between MGEs is not enough, as I have shown; other results from Chapters 2 & 3 imply that deletion of resistance elements would also not be sufficient, unless new strains are formed. I have made specific suggestions for how to achieve this in Section 6.7 below.

6.3.5 Fitting a model of strain-based competition to data is not straightforward

Fitting my agent-based model (ABM) incorporating strain-based competition to real-world data required likelihood-free inference (LFI). I developed an algorithm to identify informative summary statistics (SSs) from my model, and appropriate measures of discrepancy between observed and simulated data points. I identified candidate SSs based on their availability in real-world data, and then assessed the performance of a range of discrepancy metrics generated from these SSs, by comparing the recoverability of known input parameters using Bayesian optimisation for LFI (BOLFI). By doing so, I was able to infer both population-level input parameters, such as the probability of competition between different bacterial strains, as well as strain-specific parameters such as rates of transmission and natural clearance.

6.3.6 The frequency of resistance in clinical isolates is partly explained by between-country differences

Statistical analyses comparing the fit of different GLMMs to antibiotic consumption and resistance data showed that temporal trends in resistance in human clinical isolates varied between European countries. This is consistent with previous work, as outlined above. However, my novel approach also demonstrated that these differences were only partly attributable to antibiotic usage. This signal, which persisted over almost all of the 44 combinations of bacterial species and antibiotic resistance examined, suggests that additional factors are shaping the frequency of resistance. These are likely to include the bacterial population structure within different countries [317], host population dynamics and transmission networks [386, 401], clinical differences including treatment coverage [444, 445], and surveillance considerations [114, 120].

6.3.7 Antibiotic consumption variables included in the best-fitting models of resistance were spread across sectors

Analysis of the best-fitting GLMMs for each combination of bacteria and antibiotics showed that the antibiotic consumption variables included in the best-fitting models of

resistance were evenly spread across hospital, community, and veterinary sectors. Notably, consumption of various antibiotics within hospitals - often excluded from population-level models of resistance [97, 406] - were repeatedly identified within the best-fitting models.

That antibiotic consumption across all three sectors was represented in these models is not unexpected [417–419, 516]. However, there were clear differences in the influence of antibiotic consumption, depending on the life cycle of the bacteria of interest. In models of resistance in commensal organisms, hospital consumption variables had the largest effect, whereas in environmental organisms, community consumption variables had the greatest influence. There was considerable variation in the impact of consumption variables on resistance in foodborne pathogens, which makes it difficult to draw firm conclusions. However, when the results were analysed by resistance to individual antibiotics, the largest effects tended to occur for sectors where that antibiotic is heavily used.

6.3.8 The relationship between antibiotic consumption and resistance also varies between countries

There were between-country differences in both the intercept and gradient of the relationship between antibiotic consumption variables and resistance, for the majority of the bacteria-antibiotic combinations tested. Despite this variation, some antibiotic consumption variables were significant predictors of clinical resistance, across Europe. These include usage of aminoglycosides, fluoroquinolones, and third/fourth generation cephalosporins in hospital settings, penicillin usage in the community, and cephalosporin use in animals. Total antibiotic consumption in community and veterinary settings were also identified as significant. These results are not surprising: penicillins are the most frequently-consumed antibiotics in the UK medical sector [115], for example. Reducing total antibiotic consumption has been a target of both medical and veterinary sectors for several years [528, 529].

6.4 Implications of this work

Taken together, the results presented here underline the importance of antibiotic consumption as a driver of AMR, but they also demonstrate the role of other factors in shaping

distributions of resistance in complex ecosystems [251]. I have showed that resistance distribution is affected by competition, and thus by bacterial population structure and dynamics. These are in turn shaped by antibiotic consumption, including historical consumption, but the nature of the relationship between consumption and resistance varies between ecosystems.

Generally, my results show that high-antibiotic environments make it easier for new resistant lineages to emerge and become established in the population. My results also offer a possible explanation for the different time scales over which changes in resistance have been observed, and the persistence of resistant strains in low- or no-antibiotic environments. This lends weight to the importance of biosecurity (e.g. on farms, in hospitals, or at borders) in controlling AMR [122, 303, 306, 308, 319, 320].

My identification of antibiotic consumption variables from hospital, community, and veterinary settings in the best-fitting models of resistance in Europe supports other work demonstrating the relatedness of consumption and resistance in different sectors [256, 417–419, 516]. However, large gaps in our knowledge still exist, including the role of the environment as a conduit to transmit antibiotic residues, resistance genes, or resistant organisms between different host populations [530], and the ways in which antibiotic consumption interacts with other factors to shape resistance in heterogeneous populations can be difficult to predict. More evidence is needed. Until these interactions are adequately understood, my findings emphasise the importance of a One Health approach to AMR control, including responsible antibiotic stewardship in all sectors [54, 435, 523].

6.5 Potential applications

Through this research, I have developed several methods that can be applied more widely. Firstly, my flexible, multi-strain model of strain-based competition could be applied to a range of bacterial systems, or used to enhance existing models of AMR (e.g. [434]) and/or HGT. The algorithm for identifying suitable summary statistics can also be applied to other agent-based models, within or outside this field. And finally, the statistical approach I adopted, where I used model selection to identify parameters most associated with resistance, can be applied to other data sets (see below).

6.6 Limitations

Specific limitations of this work are detailed in individual chapters. However, two major areas are worth highlighting. My agent-based model does not capture several complexities of within-host interactions that could expand the parameter range across which coexistence is observed, including heterogeneities in treatment coverage [401, 444, 445], and strain-specific effects of resistance genes and/or MGEs [217, 221, 328, 336, 459]. I also do not examine the effects of co-selection or multi-drug resistance, which can affect model outcomes [531].

The other main limitation of this work is the gap between carriage of resistance genes by commensal organisms, as modelled in Chapters 2-4, and resistance in organisms causing clinical disease, as modelled in Chapter 5. Commensal organisms are most commonly exposed to antibiotics when they are *not* the target organism [235], whereas pathogens are mostly exposed when they cause disease. AMR in pathogens can also not be considered representative of AMR carriage in the general population, due to biological differences such as virulence [351, 526, 532], as well as in methods of surveillance [108].

6.7 Recommendations for further work

Recommendations for further work include improving the within-host subtleties of my agent-based model, as outlined above. Of these, I would focus on MGE interactions. As I showed in Chapter 3, some means of balancing resistant and sensitive strains in the presence of HGT is needed. One possible option is to implement specific strain-MGE interactions, including varying fitness costs [221, 326–328, 459, 533–535] and blocking of inwards HGT to represent bacterial defence mechanisms [217, 221, 536]. The addition of specific MGE-MGE interactions could also facilitate this [337].

A more robust way to reconcile my findings in Chapter 2 with real-world data would be to utilise national-level surveillance of AMR in commensal organisms, rather than those causing disease, as utilised in Chapter 5. This type of surveillance is not established in human populations (although it is an area under development [537]), but it is in animals. Representative surveillance of AMR in several commensal organisms of the gastrointesti-

nal system has been conducted in European livestock since 2014 [154], including in the UK post-Brexit [108, 158]. This data is representative of large food-producing animal populations and is standardised across all participating countries [155, 156, 538, 539], although it is not readily accessible in full.

However, while this approach would better align with models of AMR carriage, it is unlikely to aid interventions to control AMR, without linkage to clinical data. Thus, until the gap between antibiotic consumption, resistance carriage, and resistance in organisms causing disease can be filled, further modelling and investigative work at national or sub-national level is likely to be most fruitful. Agent-based models, such as the one developed here, would be an appropriate approach for such work.

6.8 Concluding remarks

The research presented in this thesis reiterates the importance of antibiotic consumption as a driver for AMR. However, other mechanisms are at play, too, and the ways in which they interact are likely to be specific to different contexts. Agent-based models can help us understand these relationships, as they are able to simulate the complexities of AMR evolution within heterogeneous populations. This will help identify context-specific interventions, which are not necessarily transferable. Where knowledge gaps preclude identification of targeted interventions, my results show that reducing antibiotic consumption across sectors remains an essential strategy in the control of AMR.

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Appendix

This appendix contains additional materials from Chapter 2.

Two-strain system

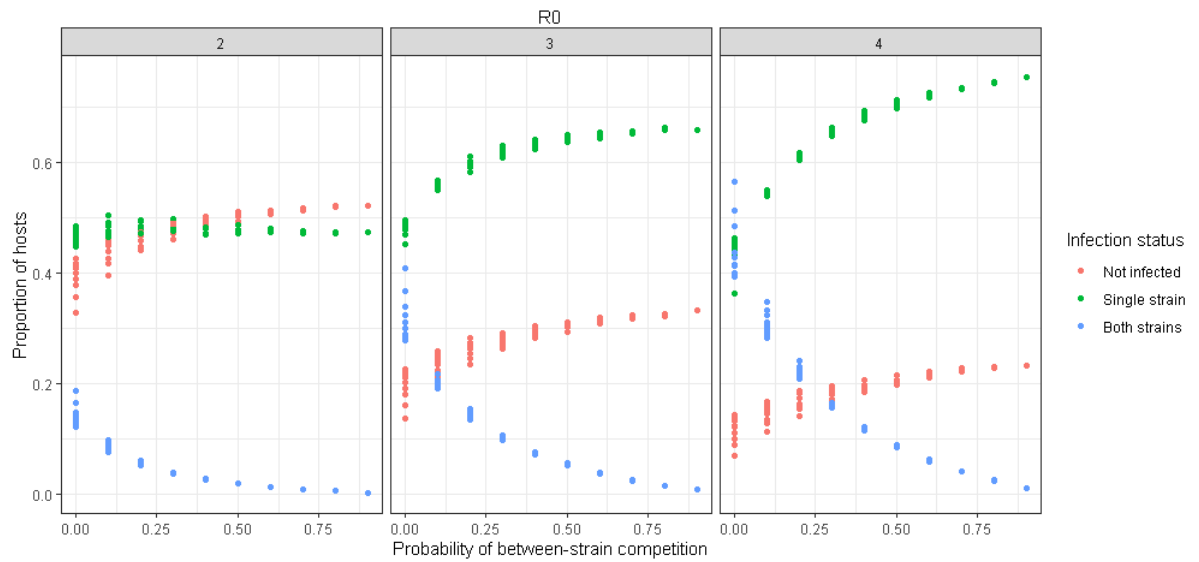


Figure 6.1: Effect of R_0 and the probability of between-strain competition on the proportion of hosts infected with 0, 1, or 2 strains, in a two-strain system. Dots show simulations across the range of within-strain competition probability ($p_b < p_w \leq 1$). $n = 2$, $\gamma = 6.05$. No antibiotics or cost.

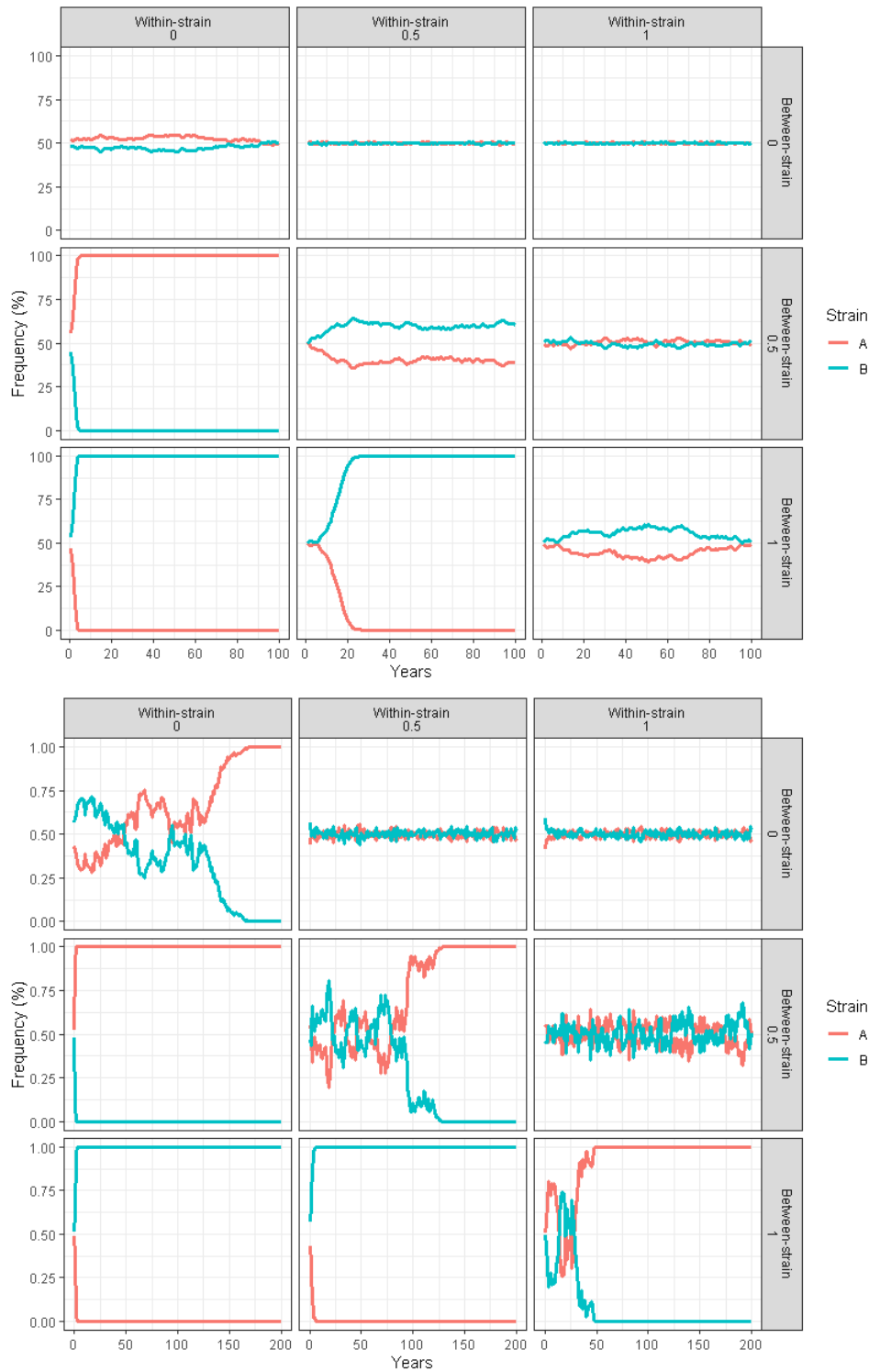


Figure 6.2: Dynamics of a homogenous two-strain bacterial system in host populations of 100,000 (top) and 10,000 (bottom). Frequencies of individual strains in a single simulation are shown. $n = 2$, $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.

Ten-strain system

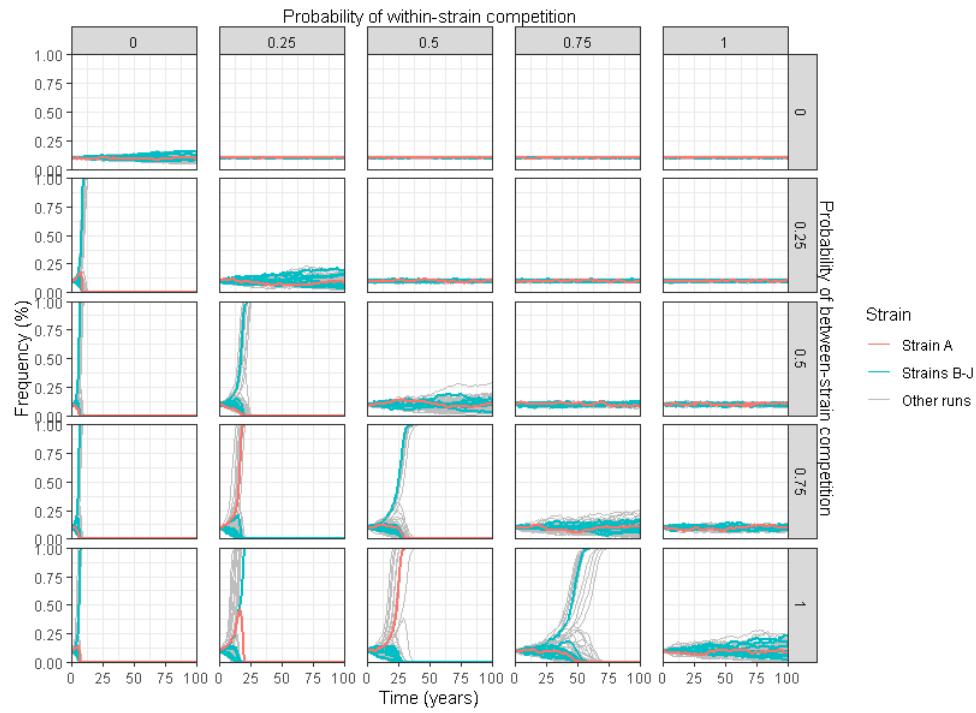


Figure 6.3: Dynamics of a homogenous ten-strain bacterial system in a host population of 100,000. Results of 10 simulations. Frequencies of individual strains in a single simulation are shown in colour. Frequencies of both strains in additional simulations are shown in grey. $n = 10$, $\gamma = 6.05$, $R_0 = 3$. No antibiotics or cost.