

**Modelling the spatial and temporal
epidemiology of antimicrobial resistant
Salmonella Typhi and Paratyphi A
infections**



Annie J. Browne
Linacre College
University of Oxford

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Abstract

Enteric fever caused by *Salmonella enterica* Typhi and Paratyphi A infers a large burden of disease in low- and middle-income countries (LMICs). Antimicrobial resistance (AMR) reduces our ability to treat these infections, increasing morbidity and mortality.

The aim of this thesis was to improve our understanding of the spatial and temporal trends in the prevalence of AMR in enteric fever and its drivers, such as antibiotic consumption, in order to guide policy and combat the growing threat of AMR.

A systematic review was performed to collate a contemporary dataset of multidrug resistance (MDR) and fluoroquinolone non-susceptibility (FQNS) in *S. Typhi* and *S. Paratyphi A*. A meta-analysis was used to assess broad scale trends in resistance.

Estimates of antibiotic usage in LMICs, and estimates of antibiotic consumption for 204 countries were produced for the years 2000 to 2018 using statistical models. These estimates were then combined with the systematic review data and additional covariates to model the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for each endemic country and year from 1990 to 2018.

Large national and subnational variations in antibiotic usage are identified. Antibiotic consumption was estimated to vary almost 10 fold, from 5.0 [4.8-5.3] defined daily doses (DDD) per 1,000 population per day in Philippines to 45.9 DDD/1,000/day in Greece.

The prevalence of MDR *S. Typhi* decreased between 1990 and 2018 in South and Southeast Asia, but increased greatly in sub-Saharan Africa. FQNS *S. Typhi* increased considerably in South and Southeast Asia over the study period and increased, albeit to a lesser extent, in sub-Saharan Africa. MDR *S. Paratyphi A* remained low throughout, but the prevalence of FQNS *S. Paratyphi A* increased.

This thesis identifies both high rates of antibiotic consumption and a lack of access to antibiotics in various locations. It provides detailed estimates of the prevalence of AMR in enteric fever and analyses the trends over space and time. These estimates can be used as a benchmark from which to measure future interventions and to guide policy implementation to tackle AMR.

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Statement of contributions

My supervisors, Christiane Dolecek, Simon Hay and Michael Chipeta provided methodological and scientific guidance throughout all stages of this work. Additional contributors for each chapter are listed below.

For Chapter Two, Christiane Dolecek designed the search strategy for the systematic review and academic librarian Eli Harris conducted the database searches. Joshua Longbottom and Bahar Kashef Hamadani acted as second reviewers, checking all extracted data.

For Chapter Three, Emmanuelle Kumaran and Bahar Kashef Hamadani provided help with extracting data from the household surveys. Base code for the model based geostatistical (MBG) model was provided by the Local Burden of Disease (LBD) team at the Institute of Health Metrics and Evaluation (IHME), and was adapted for the purpose of this analysis. Bobby Reiner and Ani Deshpande provided guidance for the tuning of the MBG model. A proportion of the work matching administrative districts to shapefiles was conducted before the commencement of this DPhil, by myself, Joshua Longbottom and additional members of the LBD team at IHME.

For Chapter Four, guidance of the interpretation of the IQVIA dataset was provided by Peter Stephens of IQVIA. The code for the spatial-temporal Gaussian process regression (ST-GPR) model was provided by IHME.

For Chapter Six, as with the previous chapters, the code for the ST-GPR and the basis for the MBG model was provided by IHME and adapted for the purpose of this analysis.

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List of abbreviations

ABS	Antibiotic stewardship
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
ATC	Anatomical therapeutic chemical
BSAC	British Society of Antimicrobial Chemotherapy
CDC	Center for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DCS	Decreased ciprofloxacin susceptibility
DDD	Defined daily doses
DHS	Demographic Health Surveys
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended spectrum beta-lactamase
EQA	External quality assessment
FQNS	Fluoroquinolone non-susceptibility
GBD	Global Burden of Disease
GLASS	Global antimicrobial resistance surveillance system
HIC	High-income country
INLA	Integrated nested Laplace approximations
ISO	International Organization for Standardization
LMIC	Low- and middle-income country
LRI	Lower respiratory tract infection
MBG	Model based geostatistics
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MICE	Multivariate imputation by chained equations
MICS	Multiple Indicator Cluster Surveys
NAP	National action plan
RDT	Rapid diagnostic test
SLIPTA	Stepwise laboratory quality improvement process towards accreditation
SLMTA	Strengthening laboratory management toward accreditation
SDG	Sustainable development goal
SDM	Species distribution modelling
SPDE	Stochastic partial differential equation
ST-GPR	Spatial temporal Gaussian process regression
WHO	World Health Organisation
XDR	Extensive drug resistance

Introduction

1.1 *Salmonella enterica* serovars Typhi and Paratyphi A

Salmonella enterica serovars Typhi and Paratyphi A (*S. Typhi* and *S. Paratyphi A*) are human-restricted bacterial pathogens which cause severe bloodstream infections, together termed enteric fever. They are transmitted faeco-orally, primarily through contaminated water and food. Symptoms of enteric fever include prolonged fever, headache, malaise, cough and abdominal pain. Left untreated, infection can lead to intestinal perforation, neurological symptoms and death.^{1,2} Traditionally, infections with *S. Paratyphi A* were considered to be less severe than those with *S. Typhi*, however, recent studies have found them to be clinically indistinguishable.³

Enteric fever is a major cause of febrile illness. The Global Burden of Disease (GBD) study estimated there to be 14.3 million cases and 135,000 deaths from typhoid and paratyphoid fevers in 2017,⁴ whilst the International Vaccine Institute (IVI) produced similar estimates of 11.9 million cases and 129,000 deaths in low- and middle-income countries (LMICs) in 2010⁵ and the Yale School of Public Health produced slightly higher estimates of 17.8 million cases per year in LMICs, using data from 1980 to 2014.⁶ The highest burden of disease is in South Asia, Southeast Asia and sub-Saharan Africa.⁴ *S. Typhi* is the aetiological agent of almost 30% of community acquired blood stream infections in Asia⁷ and 10% in Africa.⁸ *S. Paratyphi A* infections infer a lower burden of disease, however, *S. Paratyphi A* is an emerging threat in Asia and has recently been shown to be responsible for up to 35% of enteric fever episodes in India and Nepal, and over 60% in China.⁹ Interestingly, the incidence of *S. Paratyphi A* is poorly described in Africa and the Middle East, where the burden of disease is thought

to be negligible.¹⁰

Historically enteric fever caused a large burden of disease globally but economic development and vast improvements in drinking water and sanitation, as well as effective use of vaccination and antibiotics, have essentially eliminated locally acquired infections in many high-income countries (HICs). The majority of infections in HICs are now associated with travel to LMICs.¹¹ Discordant estimates of the burden of enteric fever in Latin and South America exist,^{4,6,12} and although studies of typhoid incidence in this region are sparse, successful interventions have been implemented to control locally acquired enteric fever infections.¹³

Enteric fever disproportionately affects children and young adults, with the highest incidence observed in children aged five to nine years old, and the incidence decreasing as age increases.^{4,5} Modelled estimates show that 56% of all cases occur in children under 15, and that case fatality rates are higher in children, compared to adults. Almost 60% of enteric fever fatalities are estimated to be in children under 15 years old.⁴

The gold standard diagnostic for enteric fever is to isolate the bacteria from blood cultures but this is expensive, slow to perform and has a low sensitivity rate (approximately 50%).¹⁴ Antibiotics are frequently taken prior to a person attending a healthcare facility and having blood drawn for culture, contributing to the low sensitivity of blood cultures.^{15–17} In such situations bone marrow cultures have been shown to have high sensitivity, although this procedure is more invasive, difficult to perform, and is therefore infrequently undertaken.^{1,18} Cultures are also frequently performed on stool isolates, although these too have limited sensitivity¹⁹ and may also indicate chronic carriage of *S. Typhi* or *S. Paratyphi A*, meaning that they should not be used to infer acute enteric fever infection. Due to the limitations in culture capabilities the Widal test is frequently used to diagnose enteric fever in LMICs, but this also is characterised by very low levels of sensitivity and specificity.²⁰

Enteric fever can be successfully treated with antibiotics. Chloramphenicol was found to be an effective treatment in the late 1940's²¹ and its introduction for the treatment of enteric fever aided in reducing the case fatality rate from over 15% in the pre-antibiotic

era to less than 1%.² Ampicillin and co-trimoxazole were shown to be effective treatments in the 1980's²² and added to the recommended treatment regimen. Fluoroquinolones were later identified as an inexpensive and effective treatment for enteric fever²³ in the early 1990's, and more recently clinical trials have identified azithromycin,²⁴ and third-generation cephalosporins (cefixime and ceftriaxone)²⁵ as treatment options. However, parenteral ceftriaxone requires hospitalisation and cefixime is clinically less effective than the alternatives.²⁶ Additionally, these antimicrobials are often unavailable or unaffordable in many resource limited settings where enteric fever is endemic.

Today, the World Health Organisation (WHO) recommends chloramphenicol, co-trimoxazole, ampicillin, fluoroquinolones, third generation cephalosporins (cefixime and ceftriaxone) or azithromycin for the treatment of enteric fever.¹⁴ However, antimicrobial resistance (AMR) is now widespread in enteric fever, threatening the effectiveness of established antimicrobial therapies and potentially leading to the resurgence of enteric fever across the world. AMR enteric fever infections are associated with poorer clinical outcomes and a higher rate of complications,^{27,28} potentially leading to longer hospital stays and increasing the burden of this disease on healthcare systems.

1.2 Antimicrobial resistance

The concept of antimicrobial resistance is not a new one. In his 1945 Nobel prize acceptance speech for the discovery of penicillin, Alexander Fleming stated that: *“The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant”*. It is evident that this warning was not heeded, and antimicrobial resistance is now considered one of the greatest threats to human health of the 21st century.

The WHO recognise and publicise the importance of AMR and released the “Global Action Plan on Antimicrobial Resistance” in 2015.²⁹ They highlight the importance of increasing awareness of AMR, improving knowledge through research and surveillance, optimising antibiotic usage, reducing the incidence of infectious diseases and increasing

economic investment in new diagnostics, medicine and vaccines. The current epidemiological situation regarding AMR is hugely concerning, and without action things are only going to deteriorate further.

The UK government commissioned a review on antimicrobial resistance which estimated there were 700,000 deaths per year due to AMR in the present day, with the danger that this could rise to 10 million by 2050 if no action was taken.³⁰ An analysis of the European Centre for Disease Prevention and Control (ECDC) AMR surveillance data estimated that over 670,000 infections with AMR bacteria and 33,000 attributable deaths occurred in the European Union (EU) and European Economic Area (EEA) in 2015.³¹ In 2018, over half of *Escherichia coli* (58.3%) and over a third of *Klebsiella pneumoniae* (37.2%) and *Pseudomonas aeruginosa* (31.2%) isolates reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net) were resistant to at least one of the antimicrobial groups under regular surveillance.³² In the USA, the Center for Disease Control and Prevention (CDC) estimated over 2.8 million AMR infections and over 35,000 deaths a year in 2019.³³ These estimates are shocking, and all pertain to HICs, with limited data and knowledge of AMR in LMICs where the highest burden of infectious diseases persists and where the impact of AMR will likely be the greatest.

Antibiotics work by killing bacteria in five main ways: inhibiting cell wall synthesis, disrupting the cell membrane structure, inhibiting protein synthesis, inhibiting nucleic acid synthesis and inhibiting metabolic pathways. Different classes of antibiotics work via different mechanisms; bacteria can have intrinsic resistance to antibiotics but also have an unrivalled ability to adapt to environmental pressures and become resistant to antibiotics. Bacteria can acquire resistance genes through both horizontal and vertical gene transfer, as well as by spontaneous mutations. Horizontal gene transfer allows for genetic material to be passed between strains, and even species, of bacteria living in the same environment, enabling the rapid development and spread of AMR across bacterial populations.³⁴

The mechanisms by which bacteria are resistant to antibiotics are categorised by four main modes of actions. Firstly, the bacteria can modify or destroy the antibiotic; this

generally involves the production of enzymes to alter the chemical structure of the antibiotic, leaving it functionally inert. A common example of this is the production of β -lactamases leading to β -lactam resistance, which was first noted in penicillin-resistant *Staphylococcus aureus* isolates in the 1940's³⁵ and has more recently caused concern with the presence of NDM-1 (New Delhi Metallo β -lactamase) inferring multiple resistances in *Enterobacteriaceae* which have spread rapidly around the world.^{36,37}

Most antibiotics have intracellular targets, and the second main mechanism for antibiotic resistance involves preventing the antibiotic from reaching the target. The first way of doing this is by preventing the antibiotic from entering the cell. Gram-negative bacteria have an outer membrane which can naturally protect them from some antibiotics (e.g. vancomycin). Additionally, bacteria are able to modify the permeability of the cell membranes, reducing the amount of antibiotic which is able to enter the cell. The third way of preventing antibiotics reaching their target is by activating efflux pumps. These work via multiple mechanisms to remove substances from the cell once they have entered,³⁶ leaving the bacteria resistant to the antibiotic.

Finally, bacteria can also become resistant to antibiotics by changing the target site of the antibiotic. This can be achieved by protecting or modifying the target site, either enzymatically or by mutations. An example of this mechanism is quinolone resistance, in susceptible bacteria quinolones would target and disrupt the enzymes DNA gyrase and topoisomerase IV. Resistance is mediated by mutations in the DNA gyrase and topoisomerase IV genes, leading to weakened interactions between the enzymes and the quinolones, decreasing quinolone efficacy.³⁸ Similarly to this, bacteria can also adapt their cell metabolic processes, essentially removing the binding site of the antibiotic.³⁹

Bacterial infections with resistance to multiple antibiotics are becoming increasingly common, particularly in hospital acquired infections. The global epidemic of methicillin resistant *Staphylococcus aureus* (MRSA) has been well publicised and highlights the threat posed by common pathogens with high rates of AMR, leading to large estimates of excess deaths and huge economic impacts on healthcare systems.⁴⁰ *Enterobacteriaceae* are highly susceptible to acquiring multiple resistances; *Escherichia*

coli and *Klebsiella pneumoniae* cause a large burden of disease, both in hospitals and the community, and are susceptible to acquiring fluoroquinolone, carbapenem and cephalosporin resistances.⁴¹ The emergence of *Neisseria gonorrhoeae* resistant to first line treatments and ceftriaxone has raised huge concerns over the potential spread of untreatable gonorrhoea,⁴² whilst the progress made in reducing the incidence of *Mycobacterium tuberculosis* over the last few decades may be severely hampered by the spread of both multidrug resistant and extensively drug resistant strains.⁴³

The situation with *S. Typhi* and *S. Paratyphi A* is equally concerning, as these pathogens are widely spread in LMICs and have been shown to easily acquire AMR. After the introduction of chloramphenicol for the treatment of enteric fever in the 1940's reports of resistance to this antibiotic were quick to emerge.^{44,45} However, it remained generally effective until multiple outbreaks of chloramphenicol resistant *S. Typhi* were identified in the 1970's. These outbreaks were characterised by the presence of resistance factors transferable between bacteria.⁴⁶⁻⁴⁸ With the introduction of ampicillin and co-trimoxazole for the treatment of enteric fever, concurrent resistance to all three of these antibiotics, termed multidrug resistance (MDR), was quick to arise.⁴⁹ MDR was identified to be encoded by plasmids of the H1 incompatibility group (IncH1); these plasmids are transmissible between multiple gram-negative bacteria in the laboratory⁵⁰ and have been identified in *S. Typhi*, *S. Paratyphi A* and *E. coli* in nature.⁵¹

By the mid 1990's *S. Typhi* with MDR inferred by the IncH1 plasmid had become the dominant phenotype in many regions.⁵² Whole genome sequence analysis identified that the dominant H58 lineage of *S. Typhi*, which is strongly associated with MDR, had spread throughout both Asia and Africa, replacing sensitive isolates.⁵²

Due to the increased prevalence of MDR, the use of fluoroquinolones to treat enteric fever increased greatly in the early 1990's, and mutations encoding for decreased fluoroquinolones susceptibility were quick to arise, shortly followed by complete resistance (isolates with either decreased fluoroquinolone susceptibility or fluoroquinolone resistance are together termed fluoroquinolone non-susceptible, FQNS). Unlike MDR, FQNS is usually inferred by point mutations in the quinolone resistance

determining region (QRDR) of the *gyrA*,⁵³ although plasmid-mediated fluoroquinolone resistance has also been noted.⁵⁴ These mutations are common in strains from South and Southeast Asia.⁵⁵

Third generation cephalosporins and azithromycin are now commonly used to treat enteric fever due to the high levels of MDR and FQNS in *S. Typhi* isolates; emerging resistance to these alternative antibiotics is of huge concern. Outbreaks of ceftriaxone resistant enteric fever have been reported in Pakistan, with these *S. Typhi* isolates typically harbouring extended spectrum β -lactamase (ESBL) genes, as well as fluoroquinolone resistance and MDR.⁵⁶ Together this is termed extensively drug resistant, XDR. Many of these XDR isolates also belong to the H58 clonal lineage.⁵⁶ One of the last remaining antibiotics in our arsenal for the treatment of enteric fever is azithromycin, however, resistance has been emerging in Nepal and Bangladesh, encoded by a mutation in the AcrB protein.^{57,58}

The drivers of AMR are complex, and are related to clinical, biological, socio-demographic, political and environmental factors. Inappropriate use of antibiotics in both human and animal populations is a well-established risk factor for AMR.^{59–62} Weak healthcare infrastructure, poor sanitation and unsafe drinking water, in combination with a high disease burden and unregulated antibiotic sales has led to many LMICs being hotbeds for the emergence and propagation of AMR infections.⁶¹

Exposing bacteria, both commensal and pathogenic, to antibiotics risks the development of AMR. Suboptimal levels of antibiotic are thought to induce mutations encoding AMR,⁶³ and whilst sensitive strains are killed off, there is the potential that selection of resistant organisms takes place. Inappropriate use of antibiotics, defined as either overuse or misuse, is common globally. Estimates of antibiotic consumption show huge increases in the levels of antibiotics used since the beginning of the century, particularly in LMICs.⁶⁴ Over the counter, unregulated sales of antibiotics are common in LMICs^{58,65} and are a particular risk factor for AMR.

Along with overuse of antibiotics in humans, high levels of antibiotic consumption in food animals is of concern. Antibiotics are frequently given to animals as growth

promoters, for disease prophylaxis, and less frequently for treatment of infections.⁶² The extreme use of antibiotics in animals allows for them to enter the environment and the human food chain, increasing the exposure of bacteria to antibiotics and driving resistance. Regulations on the use of antibiotics for growth promotion in animals have been implemented in numerous countries, alongside measures to reduce and restrict their prophylactic use in animals.⁶⁶ Although there is some evidence that this reduction in use reduces the prevalence of AMR,⁶⁷ there is still a long way to go in controlling antibiotic consumption in animals.

Concerns about the emergence and spread of AMR pathogens are compounded by the limited discovery of new antibiotics in recent years. Although there were a wealth of antibiotics discovered in the mid 1970's, the last new class of antibiotics discovered was daptomycin in 1986, meaning that new antibiotics reaching the market are all associations or improvements of existing compounds.⁶⁸ Whilst AMR cannot be tackled by the advent of new compounds alone, an effective pipeline of new antibiotics is an important pillar in the fight against AMR.

With established treatments of bacterial infections failing, and the lack of discovery of new treatments, it has never been more important to focus on improving sanitation and hygiene and investing in vaccine development to control communicable diseases.^{29,30} Improving sanitation and hygiene can both reduce the spread of AMR infections, and along with vaccination, can reduce the overall incidence of both bacterial and viral infections. Antibiotics are frequently taken inappropriately for viral respiratory tract infections, therefore decreasing the incidence of such infections should relate to a corresponding decrease in the inappropriate use of antibiotics.^{29,30} Waste water has been shown to contain a wealth of antibiotics, bacteria and antibiotic resistance genes and is thought to contribute to the emergence and spread of AMR.⁶⁹ Reducing the contamination of water is important for both limiting bacterial conjugation and the acquisition of AMR genes, ultimately reducing the spread of AMR pathogens.

The uncontrolled proliferation of AMR infections is estimated to have huge financial costs. The World Bank produced a “low-impact” estimate that AMR could cost the global

economy \$1 trillion (USD) per year by 2030, and a “high-impact” estimate of \$3.4 trillion per year, with LMICs disproportionately affected.⁷⁰ This highlights the urgency of the situation and why a call to action is imperative.

Surveillance of both antibiotic resistant organisms and antibiotic consumption are vitally important to tackle the threat of AMR. Both the WHO Global Action Plan on Antimicrobial resistance²⁹ and the UK governments Review on Antimicrobial Resistance³⁰ identify surveillance as the cornerstone to tackling AMR. Understanding the spatial and temporal trends in AMR is important to inform patient treatments, identify emerging threats, inform local and regional policies, and to monitor interventions. Gathering data and understanding the epidemiology of AMR pathogens must be considered a priority in the fight against AMR.

1.3 Spatial-temporal statistical modelling

Data on the prevalence of AMR is sparse, and our understanding of the spatial and temporal trends in resistance is poor, particularly in LMICs. High quality estimates of the prevalence of AMR and the utilisation of antibiotics are critical for improving our understanding of the distribution of AMR infections and informing policies to combat its development. When existing data is not adequate to fully understand the public health situation, statistical modelling techniques have proved to be effective tools for producing spatially and temporally refined estimates of the prevalence and burden of infectious diseases and health indicators, allowing for comprehensive analysis of historic, current, and potential future situations.

The vast improvement in computational capacity over the past few decades has enabled the development of new methodologies and opened the potential of using spatial and spatial-temporal statistical models to improve our understanding of disease incidence, spread and control. This coincides with huge increases in the availability of data. Surveillance networks and research institutes have greatly expanded the amount of publicly available infectious disease data in recent years, whilst the use of remote sensing has produced a wealth of spatially refined environmental covariates and the

availability of socio-demographic data is constantly increasing. This provides huge opportunities to produce quantitative models of real-life situations, and in the past 20 years spatial modelling of infectious diseases has become an exciting research field with the number of published studies increasing year on year.^{71,72}

A wide range of spatial modelling techniques have been implemented for various indicators. These can be based on point level data (data relating to precise locations), areal data (data linked to a specific area, often an administrative district), and even national level data, with a variety of modelling techniques utilised in different situations. Statistical modelling has been used for years in the field of ecology for species distribution modelling (SDM).⁷³ SDM has been used to estimate the spatial-distribution of various species including potential hosts⁷⁴ and disease vectors,^{75,76} and have been expanded on to estimate the distribution of diseases themselves.^{77,78}

These models are generally based on datasets of disease occurrence and provide a measure of environmental suitability for the disease, or the probability of occurrence. For the purposes of public health and policy implementation it is important to understand the incidence or prevalence of a disease (or health metric) in a certain area. For this a variety of spatial modelling techniques have been developed. One of the most popular methodologies for estimating the prevalence of such indicators at a fine spatial resolution is Model Based Geostatistics (MBG). MBG takes available data on disease prevalence, alongside geographically refined socio-demographic, environmental and biological covariates, and using the spatial pattern in the data to model the prevalence of disease in a Bayesian framework. This allows the production of quantitative estimates of disease prevalence for specific locations and time periods, as well as capturing the uncertainty of the model.

MBG provides robust estimations of disease prevalence and can be used to produce fine resolution maps based on limited records of disease. Highly informative estimates of malaria incidence,^{79,80} childhood mortality⁸¹ and child growth failure,⁸² amongst others, have been produced using this methodology. However, these models are very data hungry and require a selection of spatially and temporally refined informative covariates. For

many disease and health metrics data are often sparse and such fine scale estimates are not required to inform policy. In such situations there have been a range of spatial modelling techniques developed.

The Global Burden of Disease (GBD) study uses statistical models to estimate the prevalence and incidence of a large number of diseases in space and time across the globe, at a national level (with subnational districts modelled for selected countries).⁸³ A range of modelling strategies have been developed based on the metric being modelled and the data available, and are used to quantify large scale epidemiological trends in data, providing the most comprehensive collection of global estimates of disease burden. As part of this thesis, various statistical modelling strategies are investigated, with the aims of estimating antibiotic use and antibiotic resistance at the finest spatial resolution allowed by the data.

1.4 Thesis aims and structure

The overall aim of this thesis was to investigate the spatial and temporal epidemiology of AMR in enteric fever; utilising statistical models to produce comprehensive estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, at the finest spatial resolution possible, for each year from 1990 to 2018.

To achieve these aims a comprehensive dataset of the prevalence of AMR in enteric fever was compiled; in addition spatially and temporally refined estimates of antibiotic usage and consumption were produced, quantifying this important driver of AMR. The potential biases within the data were explored and these data used in statistical models to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*.

Chapter Two details a systematic review and meta-analysis. The purpose of this chapter was to gather quantitative data on the prevalence of AMR in *S. Typhi* and *S. Paratyphi A* isolates, with the aim of using this data to model the prevalence of MDR and FQNS in later chapters. A meta-analysis is used to assess the broad-scale spatial and temporal trends in AMR within the data, and an assessment of the evidence of emerging resistance to ceftriaxone and azithromycin undertaken. The characteristics of individual studies are assessed, and sensitivity analyses undertaken to investigate potential sources

of bias within the data.

Chapters Three and Four focus on producing quantitative estimates of antibiotic usage and consumption, identifying locations where there is a lack of access to essential antibiotics, and those where there is overuse of antibiotics, as well as presenting changes over time. Chapter Three focusses on antibiotic usage in LMICs and uses established MBG methodology to estimate the probability that children under five years old with symptoms of lower respiratory tract infection (LRI) receive antibiotics. Estimates are produced at a fine spatial resolution for each year, 2000 to 2018; and the national and subnational disparities in antibiotic usage explored in detail.

Chapter Four then expands on the estimates of Chapter Three. Quantitative data are gathered on the total amounts of antibiotics consumed in each country and year, in addition to the classes of antibiotics consumed. Statistical models are then employed to produce global estimates of total antibiotic consumption by country and year for 2000 to 2018. In addition, consumption of key antibiotic classes are presented in detail.

Chapter Five returns to the systematic review data and provides a narrative review of the potential biases within the data, and the challenges which arise when collecting data on the prevalence of AMR. The impact of these challenges and biases on estimating the prevalence of AMR are discussed in detail; these biases represent limiting factors to our ability to accurately estimate the burden of AMR.

In Chapter Six, statistical models are built to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, for each endemic country and year, from 1990 to 2018. This utilises both the systematic review data collected in Chapter Two, and the estimates of antibiotic usage and consumption from Chapters Three and Four. Results are interpreted in relation to the limitations discussed in Chapter Five.

Finally, Chapter Seven entails a general discussion and summation of the methodology and results of this thesis. The results of the preceding chapter are interpreted in relation to policy decisions, and future directions of this work are discussed with relation to the impending fight against AMR.

Drug resistant enteric fever, 1990 to 2018: A systematic review and meta-analysis

2.1 Introduction

Antimicrobial resistance in *S. Typhi* and *S. Paratyphi A* is of global concern, with the WHO identifying fluoroquinolone resistant *S. Typhi* as a high priority pathogen for the research and development of new antibiotics.⁸⁴ *S. Typhi* is highly transmissible and causes a high burden of disease in LMICs.⁴ This, alongside the propensity for isolates to acquire MDR and increasing reports of FQNS, has contributed to this WHO categorisation.⁸⁵ In spite of the importance of this pathogen in the landscape of AMR, at the time this chapter was constructed there were no comprehensive systematic reviews or datasets presenting a quantitative assessment of AMR in *S. Typhi* or *S. Paratyphi A* globally.

The WHO currently recommends treatment of enteric fever with chloramphenicol, co-trimoxazole, ampicillin, fluoroquinolones, third generation cephalosporins (cefixime and ceftriaxone) or azithromycin.¹⁴ It was deemed important to compile a dataset of the prevalence of resistance in *S. Typhi* and *S. Paratyphi A* to all clinically relevant antibiotics. An understanding of the spatial and temporal trends of AMR in enteric fever is essential for informing empirical treatment options and guiding policies tackling AMR. Surveillance systems reporting the prevalence of AMR in *Salmonella* infections are sparse in LMICs,⁸⁶ and published literature provides an important source of data for analysing the spatial and temporal trends in AMR.

S. Paratyphi A infections are clinically indistinguishable from *S. Typhi* and infer a

considerable burden of disease in Asia.⁹ With the role-out of the newly approved conjugate *S. Typhi* vaccine expected to drive down the incidence of *S. Typhi*⁸⁷ it is important to understand both the contribution of *S. Paratyphi* A (for which there is no approved vaccine) to the total burden of enteric fever, and to fully understand the AMR pattern for each individual serotype.

The gold standard diagnostic for enteric fever is blood culture, as this identifies invasive disease. Once the pathogen has been isolated from blood, antimicrobial susceptibility testing (AST) can be performed using several methods (including disk-diffusion, E-test and microdilution), providing information on which antibiotics will be effective against the infecting strain. Antibiotic therapy can then be prescribed or altered accordingly. However, the highest burden of enteric fever is in LMICs, where blood culturing and AST facilities are often limited due to financial and infrastructural constraints, meaning patients are often treated empirically without a confirmed diagnosis or AST results. This introduces a high risk of inappropriate antibiotic treatments and propagation of resistant strains. It is therefore important to have a broad understanding of the prevalence of AMR in enteric fever isolates in various locations to guide empirical antibiotic usage.

This chapter details a systematic review and meta-analysis of published literature performed to ascertain the prevalence of resistance in *S. Typhi* and *S. Paratyphi* A against recommended antimicrobials, and an evaluation of the spatial and temporal trends of AMR enteric fever. Data are collated and analysed by the GBD study regions (Figure A.1) and five-year time-periods. This chapter provides an extensive dataset of AMR in *S. Typhi* and *S. Paratyphi* and assesses the broad scale patterns and trends in resistance. This systematic review aimed to be extensive and exhaustive, expanding on existing knowledge and systematic reviews conducted in this area.

2.2 Methods

2.2.1 Overview

A systematic review on the prevalence of AMR in *S. Typhi* and *S. Paratyphi A* was conducted for 1990 to 2018. The aim of this work was to gain a comprehensive understanding of the existing literature; to compile a dataset of the prevalence of AMR in *S. Typhi* and *S. Paratyphi A* linked to the year and location of infection, and to develop a high-level understanding of both the levels and spatial-temporal trends of resistance to key antibiotics.

The systematic review followed the Preferred Reporting Items for Systematic reviews and Meta Analysis (PRISMA) guidelines (Table A.1)⁸⁸ and the protocol was registered with the international prospective register of systematic reviews (PROSPERO registration number: CRD42018029432). Seven databases were searched for published literature detailing antimicrobial resistance in *S. Typhi* or *S. Paratyphi A* between 1990 and 2018. Studies were screened at title, abstract and full text stage and data on the prevalence of resistance and accompanying study information extracted from included studies and entered in a predefined database.

The prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* isolates from individual studies were illustrated using forest plots and the spatial and temporal trends in resistance analysed using a random-effects meta-analysis, stratified by GBD region and five-year time period. Additionally, a descriptive analysis of resistance to ceftriaxone and azithromycin was undertaken. A sensitivity analysis was performed to investigate biases due to laboratory quality or reporting procedures.

2.2.2 Search strategy and selection criteria

Scopus, Web of Science, Lilacs, Medline, Embase, Global Health and Cochrane databases were searched using Medical Subject Headings (MeSH) and free text terms for the pathogens of interest (e.g. *Typhi*, *Paratyphi*, “enteric fever” etc.) combined with

terms for antimicrobial resistance (e.g. resistanc*, suscept*, surveil* etc. Table A.2). The search was limited to publications from 1990 onwards with no restrictions on language or study type. The initial search was conducted in October 2017 and updated in March 2019.

After de-duplicating the library all records were screened for inclusion at title, abstract and full text stages based on predefined inclusion and exclusion criteria (Table 2.1). For inclusion, studies were required to report quantifiable *in vitro* antimicrobial susceptibility results for at least ten representative *S. Typhi* and/or *S. Paratyphi A* isolates from blood culture and indicate the study location. Only *S. Typhi* and *S. Paratyphi A* isolated from patients from 1990 onwards were included. Studies on travellers diagnosed in HICs were excluded, as were studies with pooled *S. Typhi* and *S. Paratyphi A* susceptibility data.

Inclusion Criteria	Exclusion Criteria
Quantifiable in vitro antimicrobial susceptibility data for clinically relevant antibiotics	<10 isolates
Studies from 1990 onwards	Infections in travellers
Known country in which infection was acquired	Imported infections
Humans blood culture isolates	Environmental isolates

Table 2.1: Systematic review inclusion criteria

Prospective and retrospective studies were included if they met the inclusion criteria, as were hospital, community and laboratory-based studies. Review articles were scanned for relevant references. Antimicrobial susceptibility data were extracted for antimicrobials recommended for the treatment of enteric fever by WHO, including ampicillin/amoxicillin, chloramphenicol, co-trimoxazole, fluoroquinolones (e.g. ciprofloxacin, ofloxacin), third generation cephalosporins (e.g. cefixime, ceftriaxone) and azithromycin. The prevalence of MDR (defined as concurrent resistance to ampicillin/amoxicillin, chloramphenicol and co-trimoxazole) was recorded, along with nalidixic acid resistance, for use as a proxy marker for ciprofloxacin non-susceptibility.^{27,89}

Additional variables extracted included study start and end dates, patients' characteristics (age range, mean age, percentage of males, inpatients or outpatients),

study design, number of patients screened, AST method, number of persons with positive cultures for which AST was performed, and the number (or percentage) of resistant, intermediate and susceptible isolates out of the total number of isolates tested against each antimicrobial. Case fatality rates, clinical outcomes and pre-admission antibiotic use were recorded when available. The testing standard (e.g. Clinical and Laboratory Standards Institute, CLSI) and interpretive criteria (including version or year) used to define resistance were recorded, along with the use of internal quality controls and participation in external quality assessment (EQA) schemes. The study setting (hospital or community), precise study location, country and GBD study region were recorded for each study. Data were disaggregated by serotype and study location. All extracted data were checked by a second reviewer and an additional 10% checked by a third reviewer. Studies on duplicate isolates were excluded from the analysis.

2.2.3 Data analysis

This review analysed the prevalence of resistance in isolates from blood culture. Each study was assigned to the GBD study region in which infection was acquired and a five-year time-period based on the study mid-year (1990-1994; 1995-1999; 2000-2004; 2005-2009; 2010-2014; 2015-2018). If study dates were not provided, the mid-year was imputed as the publication year minus the median difference between the publication year and the study mid-year for the remaining studies in the dataset (three years). Analysis was limited to GBD regions deemed to be endemic for *S. Typhi* or *S. Paratyphi A*, defined as a typhoid or paratyphoid fever incidence of ten cases per 100,000 population or higher in 2017⁴ (Figure 2.1). This threshold was selected as there was a paucity of data in regions with under ten cases per 100,000 population, meaning that accurate estimates of the prevalence of resistance could not be ascertained from the available data.

During the study period the breakpoints for fluoroquinolone resistance were altered, effectively lowering the threshold for what is classified as intermediate and resistant (together termed “non-susceptible”). Prior to 2012, the CLSI classified isolates with a

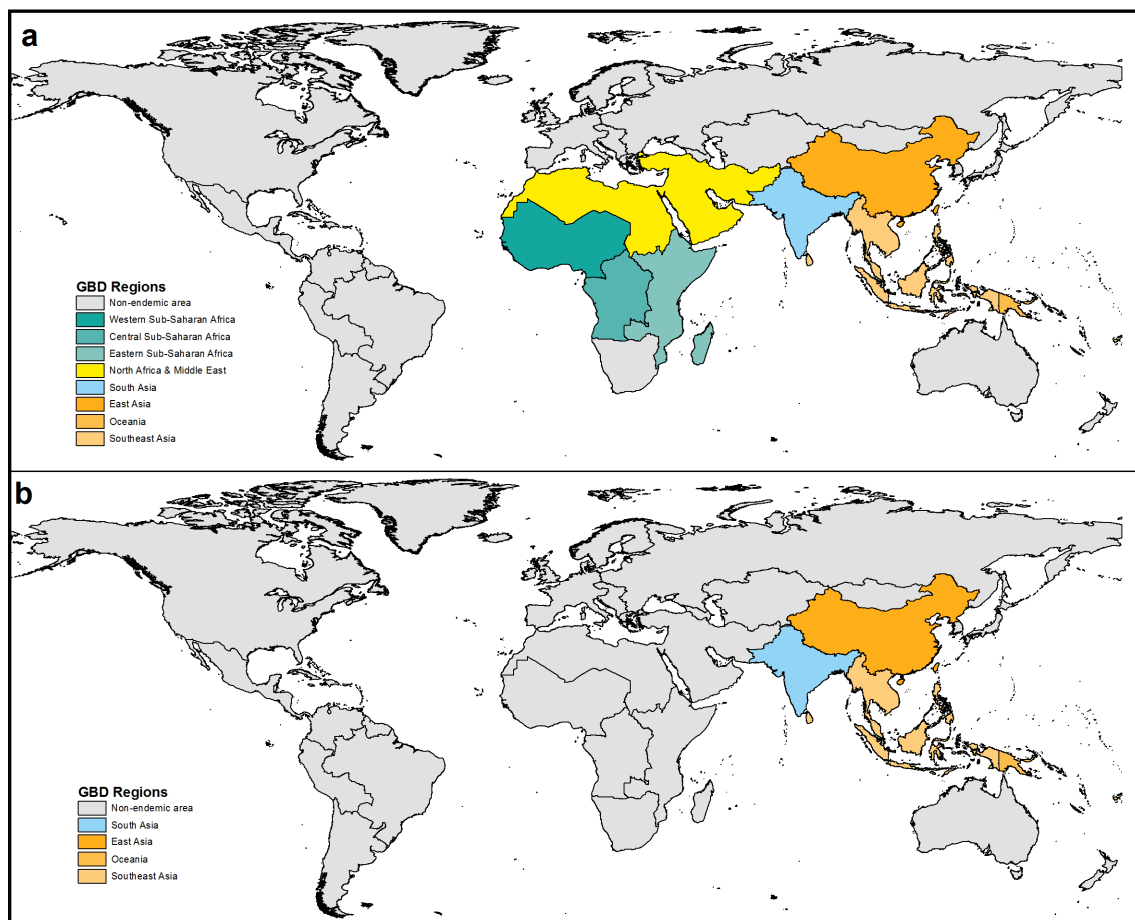


Figure 2.1: *S. Typhi* and *S. Paratyphi* endemic regions. Map of GBD study regions endemic for (a) *S. Typhi* , and (b) *S. Paratyphi* A (≥ 10 cases per 100,000 population in 2017⁴)

minimum inhibitory concentration (MIC) for ciprofloxacin (the most commonly used fluoroquinolone) of $2\mu\text{g}/\text{ml}$ to be intermediate, and those with a MIC of $4\mu\text{g}/\text{ml}$ or higher to be resistant. In 2012 these guidelines were updated to state that any isolate with a MIC of $0.125 - 0.5\mu\text{g}/\text{ml}$ was to be classified as intermediate, and those with an MIC of $1\mu\text{g}/\text{ml}$ or higher as resistant. To allow for the analysis of temporal trends in non-susceptibility, ciprofloxacin results were interpreted according to the updated CLSI 2012 criteria (i.e. isolates with an MIC of $0.125\mu\text{g}/\text{ml}$ or higher were classified as FQNS). Nalidixic acid resistance has been shown to be a suitable proxy marker for FQNS;^{27,89,90} in studies where the ciprofloxacin susceptibility results were not interpretable based on the CLSI 2012 breakpoints, the prevalence of nalidixic acid resistance was used as a proxy for the prevalence of FQNS. In addition, isolates reported as showing “decreased ciprofloxacin susceptibility” pre-2012 were also classified as FQNS.

Isolates with intermediate resistance were classified as resistant. The proportions of *S. Typhi* or *S. Paratyphi A* isolates resistant to each antimicrobial were determined for each study and the 95% confidence intervals calculated using the Agresti-Coull method.⁹¹ A double arcsine transformation was used to stabilise the variance of the proportions and a random-effects meta-analysis to calculate the pooled prevalence of MDR and FQNS for each GBD region and five-year time-period included in the analysis. Forest plots were used to visualise the proportion of resistance in individual studies, stratified by GBD region and five-year time-period. Heterogeneity was assessed visually using the forest plots and quantitatively using the I^2 statistic and its associated p-value. All statistical analyses were conducted at a 5% significance level using the statistical software package “metafor” in R (version 3.4.2).

An additional analysis of the temporal trends in ciprofloxacin MICs for *S. Typhi* was undertaken. Large studies from Delhi, India presenting the MIC values for each isolate were identified for three consecutive time periods (1995-1999, 2000-2004, 2005-2009). Stacked bar plots were used to illustrate changes in the proportion of isolates with full susceptibility (MIC $< 0.125\mu\text{g}/\text{ml}$); intermediate resistance (MIC $0.125 - 0.5\mu\text{g}/\text{ml}$);

low level resistance (MIC 1 – 2 μ g/ml) and high-level resistance (MIC $\geq$ 4 μ g/ml) to ciprofloxacin over the study period.

Ceftriaxone and azithromycin are recommended for the treatment of MDR and FQNS enteric fever.¹⁴ A descriptive analysis of ceftriaxone and azithromycin resistance was undertaken as part of this review. Azithromycin resistance breakpoints from CLSI (introduced in 2015)⁹² and the British Society of Antimicrobial Chemotherapy (BSAC)⁹³ differ. CLSI state that isolates with a disk-diffusion diameter of 12mm or lower, or an MIC of 32 μ g/ml or higher be classified as resistant, whilst BSAC state that those with a disk-diffusion diameter of 18mm or lower, or an MIC higher than 16 μ g/ml are considered resistant. This study used the BSAC guidelines for the definition of azithromycin resistance.

The use of predefined inclusion and exclusion criteria aimed to control for biases and allow for comparison across studies. However, variations in the quality of laboratory testing were likely to persist within the dataset. To investigate whether laboratory quality impacted the reported prevalence of AMR a series of sensitivity analyses were conducted. Four measures of study quality were investigated for their impact on the pooled prevalence of resistance, these were: a) clearly stated AST methods; b) clearly stated use of a control strain; c) clearly stated resistance breakpoints; and d) sample size of 50 or more isolates. For each measure, data not meeting the stated criteria were held out and a random-effects meta-analysis on the prevalence of MDR and of FQNS in *S. Typhi* from South Asia (as this subgroup had the largest quantity of data) was performed for each time period. The results of each meta-analysis were compared to the full meta-analysis results visually using scatter plots and statistically using Welch's two sample t-test.

A risk of bias tool was adapted from Arndt¹⁰ to combine multiple measures of laboratory quality. Studies were assigned a "quality assessment score", ranking them from one to three, with one being the highest quality and three being the lowest. Studies conducted in International Organization for Standardization (ISO) 15189 accredited laboratories were deemed to be of the highest quality (level one). Lists of ISO accredited laboratories were accessed from multiple sources (accrediting bodies identified from

<https://ilac.org/signatory-search/> and lists of accredited laboratories identified from the relevant bodies) and matched to the extracted data. Studies not clearly conducted in ISO accredited laboratories, but clearly stating the AST methods, use of a control strain and the guidelines or resistance breakpoints used for interpretation were deemed to be of medium quality (level two). Studies not meeting the criteria of level one or two were deemed to be of low quality (level three). Meta-analyses were performed on the level one quality studies, and for the level one and two quality studies, the results were compared to the full meta-analysis visually using scatter plots and statistically using Welch's two sample t-test. In the final analysis no studies were excluded based on the risk of bias assessments and sensitivity analyses.

Finally, due to high heterogeneity in results, it was investigated whether the median prevalence of resistance would be a more suitable metric to pool the results of individual studies. These results are displayed and compared to those of the meta-analysis using a scatter plot and the correlation coefficient (Figure A.2).

2.3 Results

2.3.1 Study selection

Online database searches identified in 4,557 articles post de-duplication, with an additional 22 obtained through reference tracking. A total of 3,112 studies were excluded at title and abstract screening and 1,445 at full text review. The final dataset included data from 384 articles representing antibiotic susceptibility information for 124,347 isolates; 94,616 *S. Typhi* and 29,731 *S. Paratyphi A*. Each study could contribute multiple data points to the analysis due to reporting on multiple antibiotics, serotypes or locations. There were a total of 199 data points for MDR *S. Typhi*, 185 for FQNS *S. Typhi*, 74 for MDR *S. Paratyphi A* and 78 for FQNS *S. Paratyphi A* (Figure 2.2a; Tables A.3-A.4).

The majority of studies were from the South Asia region (India, Pakistan, Bangladesh and Nepal), with 45% (173/384) of studies presenting data from India. A large amount

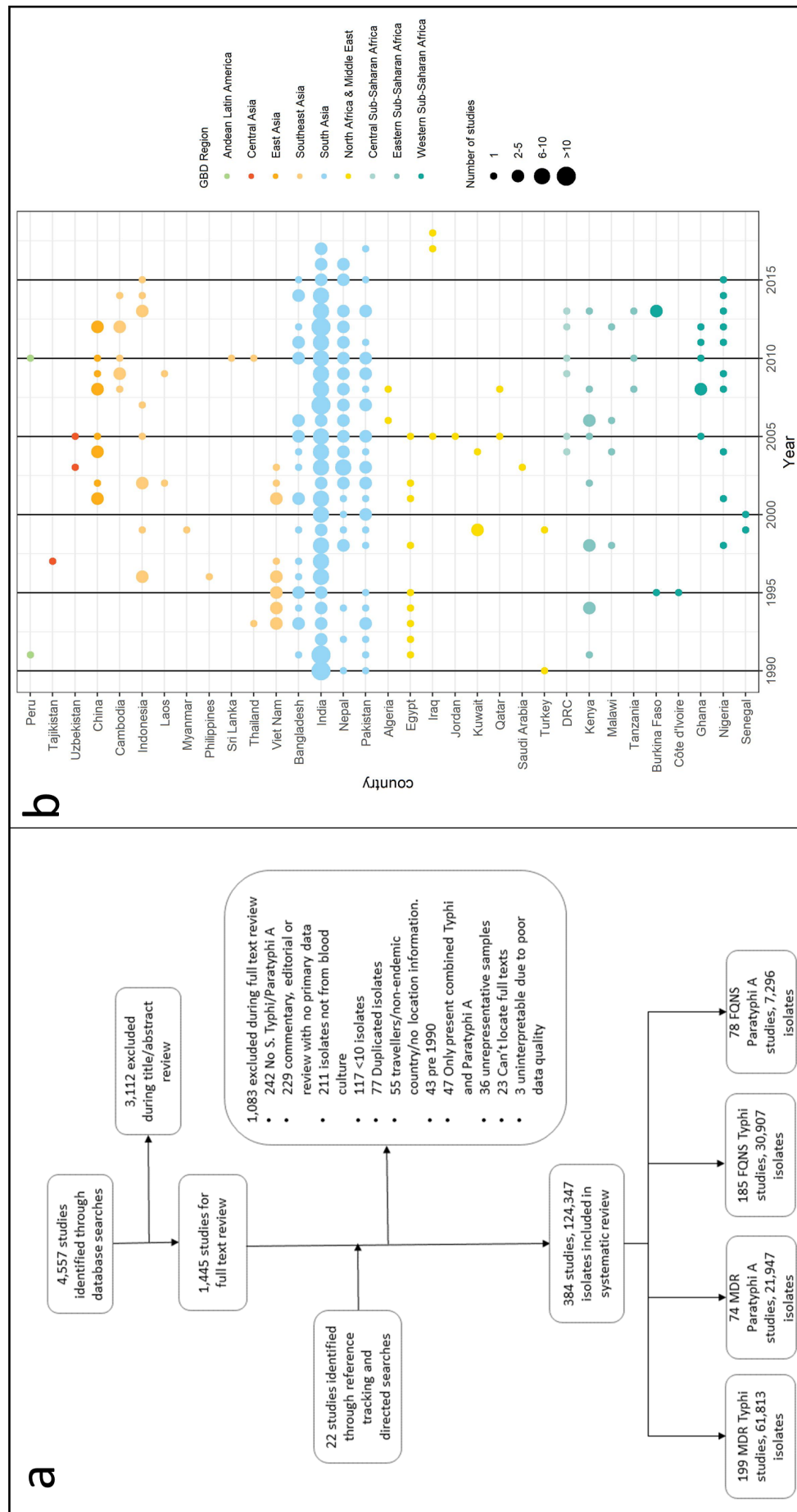


Figure 2.2: Systematic review study selection and data availability. a) PRISMA Flow chart depicting study screening and selection; approximately 150 studies were received from Bodleian Library/British National Library; b) Data availability plotted by year (x-axis) and country (y-axis), grouped by GBD region. The number of studies for each country-year is depicted by the size of the point.

of data were also identified from Southeast Asia (40 studies, 8,679 isolates), whilst data from North Africa and the Middle East and sub-Saharan Africa were sparse and no studies were identified from Oceania. Extracted Data covered every year of the study but were sparser in the earliest time-period (1990-1994) and the most recent time-period (2015-2018) (Figure 2.2b; Table A.5).

2.3.2 Study characteristics

The majority of studies (60%; 230/384) were retrospective and were conducted in hospitals. The number of *S. Typhi* isolates per study ranged from 10 to 13,532 with a median of 70.5 (interquartile range 33.25-175.25). There were fewer *S. Paratyphi A* isolates per study with a range of 10-6,028 isolates, and a median of 50 (IQR 25-107.5). AST methods were widely reported (82% of studies; 329/384) and were primarily Kirby-Bauer disk diffusion. Testing standards were reported in 57% (218/384) of studies, with CLSI the most used. However, the interpretive criteria (i.e. version or year of the testing standards used to interpret resistance) were only reported in 44% (168/384) of studies. Use of an internal quality control was stated in 32% (122/384) of studies and was most frequently *E. coli* ATC 25922. Participation in an EQA scheme was only reported in seven studies and 23 studies reported that results were confirmed by a national or international surveillance laboratory. The majority of studies included both adults and children (81%, 311/384), whilst the use of antibiotics prior to AST and case fatality rates were infrequently reported. When reported, case fatality rates were low, with 67% of studies (61/91) reporting no fatalities and 23% (21/91) reporting case fatality rates of between one and five percent (Table 2.2-2.3).

Study Characteristics	Number of studies
Region of study¹	
Andean Latin America	2
Central Asia	3
East Asia	12
North Africa & Middle East	16
South Asia	275
Southeast Asia	38
Sub-Saharan Africa, Central	6
Sub-Saharan Africa, Eastern	14
Sub-Saharan Africa, Western	21
Number of blood cultures screened	
0-99	13
100-499	43
500-999	30
1000-4999	49
5000+	59
Not stated	190
Specific age groups	
Adults only	5
Children only	68
No specified age restrictions/Adults and children	311
Reported pre-admission antibiotic use (proportion of patients in the study)²	
0	19
1-25%	15
26-50%	13
51-75%	7
76-100%	7
Not stated	329
Reported case fatality rate	
0	61
1-5%	24
6-10%	2
11-15%	1
16-20%	2
21-25%	1
Not stated	293
Patient type³	
Inpatients	73
Outpatients	14
Outpatients & Emergency department	6
Inpatients & Outpatients	44
Community	7
Not specified	241

Table 2.2: Study Characteristics – population characteristics. ¹Three studies reported isolates from multiple regions. ²Six studies reported the proportion of participants using antibiotics prior to testing separately for different sites or for persons infected with *S. Typhi* and *S. Paratyphi A* separately. ³One study consisted of two separate parts, one was community based and the other in outpatients.

Study Quality Characteristics		Number of studies
Study design¹		
	Clinical trial	16
	Prospective	139
	Retrospective	230
Sample size		
	10-29	63
	30-99	137
	≥100	184
Method of antimicrobial susceptibility testing		
	Disk-diffusion	183
	Disk-diffusion & MIC determination	98
	Microdilution	26
	E-test	9
	Automated methods ²	7
	Multiple MIC determination methods	6
	Not stated	55
Guidelines for antimicrobial susceptibility testing³		
	BSAC/EUCAST	11
	CLSI/NCCLS	200
	Other	12
	Not stated	166
Version of antimicrobial susceptibility testing guidelines stated		
	Stated	168
	Not stated	216
Internal quality control reported		
	Yes	122
	No	262
External quality assessment (EQA) participation reported		
	International EQA	5
	National EQA	2
Results confirmed by national or international surveillance laboratory		
	Not stated	23
		354

Table 2.3: Study Characteristics – quality assessment. ¹One article combined the report of a retrospective and a prospective study. ²Automated systems include VITEK 2, Phoenix 100 and Rapid ATB tests. ³Six studies used the CLSI/NCCLS and the BSAC/EUCAST guidelines for different antibiotics so contribute to the numbers twice.

2.3.3 Pooled prevalence of resistance: Meta-analysis results

Overview

The pooled prevalence of MDR and FQNS were calculated for each five-year time-period and GBD region endemic for *S. Typhi* and *S. Paratyphi A* and are displayed alongside the 95% confidence intervals in Table 2.4. Heterogeneity was high ($I^2 > 80\%$) within most subgroups, therefore the median prevalence of resistance for MDR and FQNS was calculated for each subgroup as a more conservative metric. Strong correlation between the median prevalence and meta-analysis estimates ($r^2 = 0.97$, $p < 0.001$; Figure A.2) confirmed the suitability of a meta-analysis despite the high heterogeneity. It is noted that in some subgroups, primarily in sub-Saharan Africa, the 95% confidence intervals presented are very wide due to limited data. In such cases the results are interpreted with caution. In cases where data are severely limited (defined as less than three studies in a subgroup), interpretation of the meta-analyses results has been deemed inappropriate and data from individual studies have been analysed instead.

GBD Region	Pooled prevalence of resistance (%) [95% confidence interval]					
	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2018
Multidrug resistant <i>S. Typhi</i>						
East Asia	-	-	0% [0-3]	0% [0-7]*	-	-
North Africa & Middle East	75% [68-81]*	19% [0-69]	44% [5-88]	8% [1-20]	-	-
South Asia	63% [54-71]	38% [24-52]	34% [24-44]	12% [6-20]	7% [4-12]	9% [0-32]
Southeast Asia	67% [47-83]	62% [29-90]	22% [2-53]	77% [63-88]	64% [21-97]	0% [0-4]*
Sub-Saharan Africa, Central	-	-	-	71% [1-100]	36% [29-43]	-
Sub-Saharan Africa, Eastern	5% [0-24]	24% [0-92]	82% [74-89]*	76% [61-88]	59% [27-87]	-
Sub-Saharan Africa, Western	-	14% [0-63]	-	66% [56-76]*	39% [0-96]	-
Fluoroquinolone non-susceptible <i>S. Typhi</i>						
East Asia	-	-	7% [0-26]	44% [11-81]	-	-
North Africa & Middle East	-	-	90% [59-100]	31% [16-48]	-	6% [1-13]
South Asia	2% [0-14]	49% [26-72]	62% [47-77]	76% [69-83]	82% [75-89]	70% [38-94]
Southeast Asia	-	6% [0-17]	20% [0-57]	65% [26-95]	51% [3-98]	-
Sub-Saharan Africa, Central	-	-	7% [0-20]*	8% [0-27]	37% [30-45]	-
Sub-Saharan Africa, Eastern	0% [0-4]*	11% [8-14]*	45% [35-56]	28% [3-64]	16% [1-43]	-
Sub-Saharan Africa, Western	-	8% [0-20]	3% [0-13]	13% [0-71]	7% [0-42]	-
Multidrug resistant <i>S. Paratyphi</i>						
East Asia	-	-	0% [0-1]	0% [0-2]	-	-
South Asia	0% [0-0]	10% [1-28]	3% [0-8]	1% [0-4]	0% [0-1]	2% [0-15]
Southeast Asia	0% [0-11]*	0% [0-9]*	0% [0-7]*	-	0% [0-1]	-
Fluoroquinolone non-susceptible <i>S. Paratyphi</i>						
East Asia	-	-	89% [84-93]*	91% [85-96]	94% [63-100]	-
South Asia	-	62% [52-72]*	67% [44-86]	95% [90-98]	94% [87-99]	53% [0-100]
Southeast Asia	-	-	0% [0-4]*	-	4% [0-22]	-

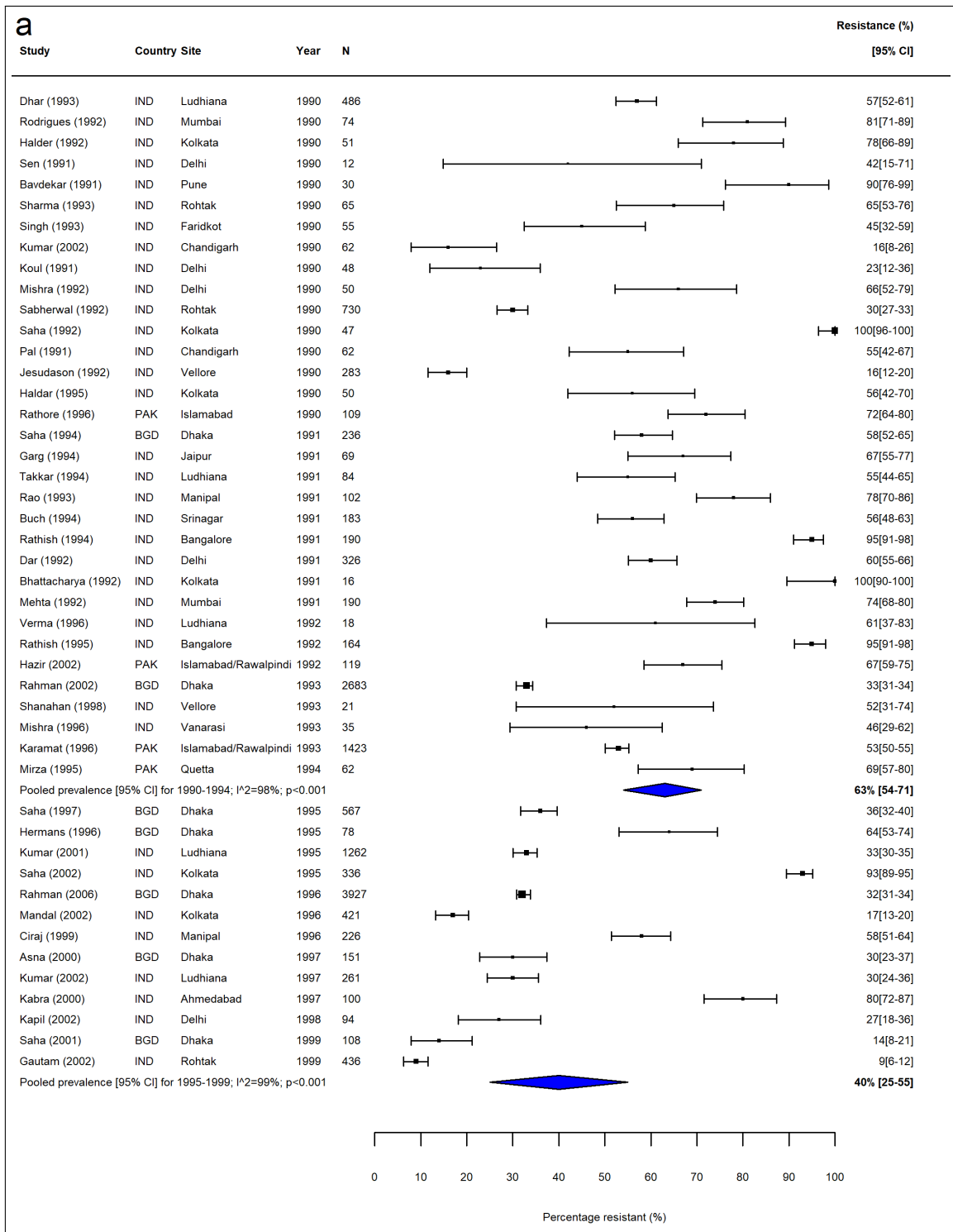
Table 2.4: Pooled prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*. Meta-analysis results [95% confidence intervals] are displayed by GBD region and five-year time-period. *Indicates subgroups with only one study, for which prevalence and calculated 95% confidence intervals are displayed.

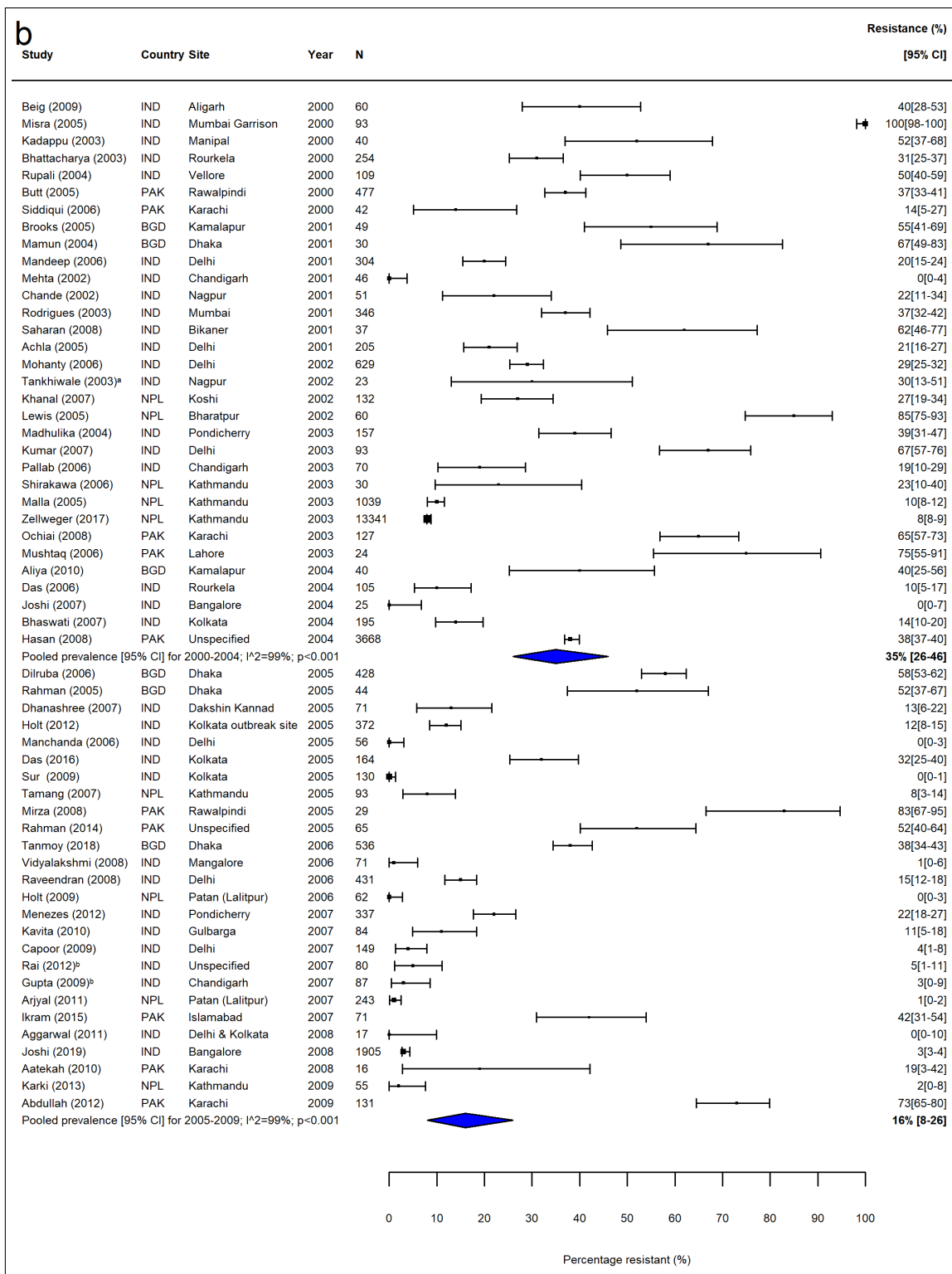
Multidrug resistant *Salmonella* Typhi

The proportion of *S. Typhi* isolates with MDR showed a high degree of variation in the seven GBD regions endemic for *S. Typhi*. In South Asia, which includes the high burden countries India, Bangladesh, Pakistan and Nepal, despite high heterogeneity there is a clear downward trend in MDR throughout our study period, with a pooled prevalence of MDR of 63% [95% confidence interval 54-71%] in 1990-1994 dropping to 9% [0-32%] in 2015-2018 (Figure 2.3; Table 2.4). Resistance rates varied between countries. In 2015-2018 all studies from India and Nepal report less than five percent resistance, whilst those in Pakistan and Bangladesh report 57% and 39% MDR respectively.^{94,95} In 2010-2014, although the pooled prevalence of resistance is 9% [4-14%], three studies from Pakistan reported over 45% of isolates to be MDR.⁹⁶⁻⁹⁸

In contrast, the levels of MDR in Southeast Asia remained consistently high throughout the study period, with the pooled prevalence of MDR remaining over 60% in most time-periods (Table 2.4). Variability in resistance within this region was also high, with Indonesia and Philippines reporting virtually no resistance⁹⁹⁻¹⁰² whilst studies from Vietnam and Cambodia reported a considerably higher prevalence of MDR (Figure 2.4). Interestingly, only one study was identified from Laos, which neighbours Vietnam and Cambodia, and this reported only low levels of MDR (11% of 161 isolates).¹⁰³ Only four studies were identified from East Asia. All were from China and all reported 0% of MDR in *S. Typhi* (Figure 2.5).

A large variability of MDR in *S. Typhi* was observed in North Africa and the Middle East. High levels of MDR were reported in Egypt in 1992 (75%, 165 isolates)¹⁰⁴ and 1998 (67%, 45 isolates),¹⁰⁵ Saudi Arabia in 2003 (100%, 12 isolates),¹⁰⁶ Kuwait in 2004 (42%, 136 isolates)¹⁰⁷ and Iraq in 2005 (67% 45 isolates).¹⁰⁸ The pooled prevalence of MDR *S. Typhi* decreased from 44% [5-88%] in 2000-2004 to 8% [1-20%] in 2005-2009, mainly due to a multi-centre study conducted in 2005 reporting relatively low levels of MDR.¹⁰⁸ Studies from Turkey and Algeria reported no isolates to be MDR^{109,110} (Figure 2.6).





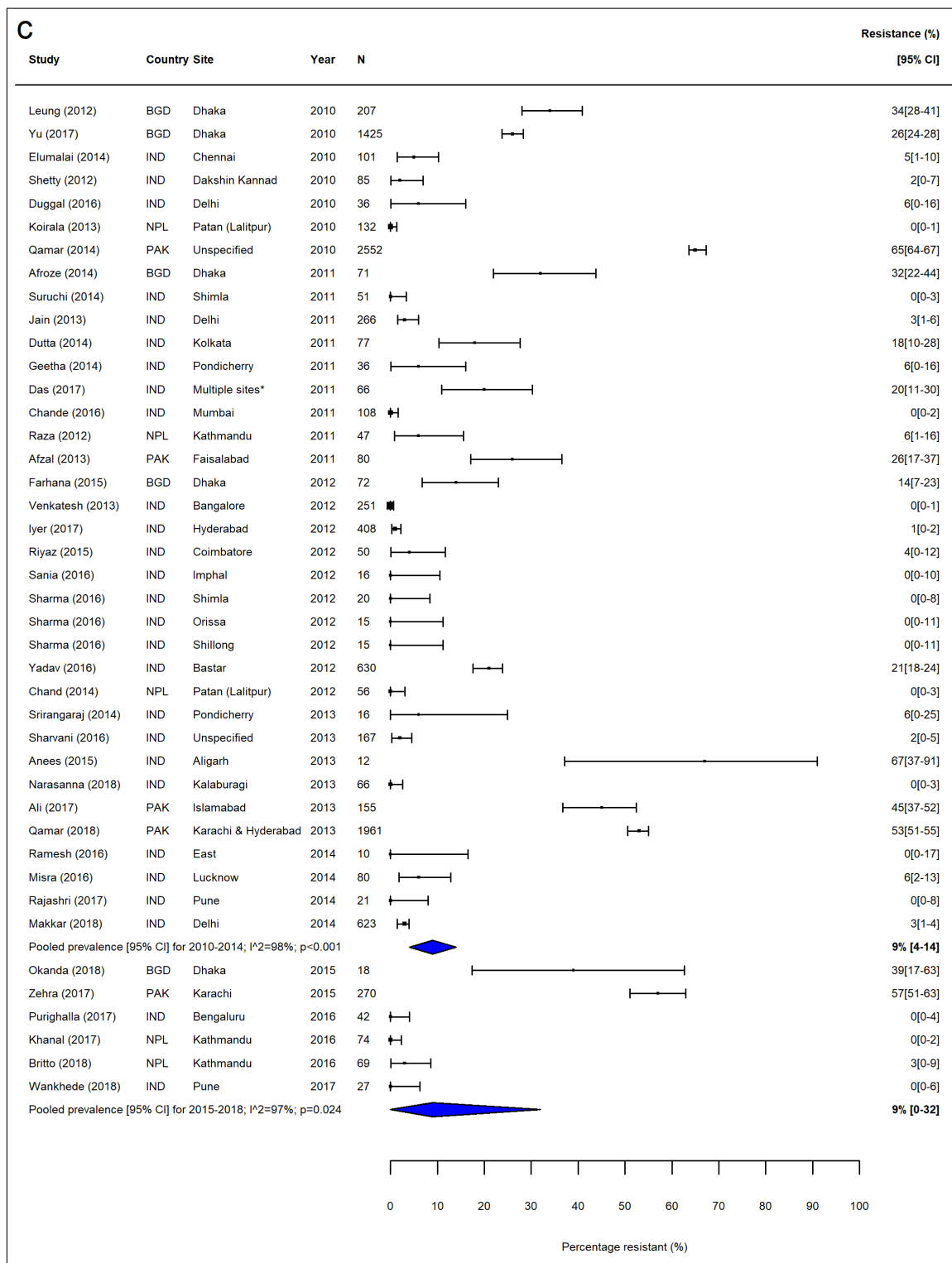


Figure 2.3: Forest plot of MDR *S. Typhi* in South Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup is represented by the blue diamond: (a) 1990-1999; (b) 2000-2009; (c) 2010-2018.

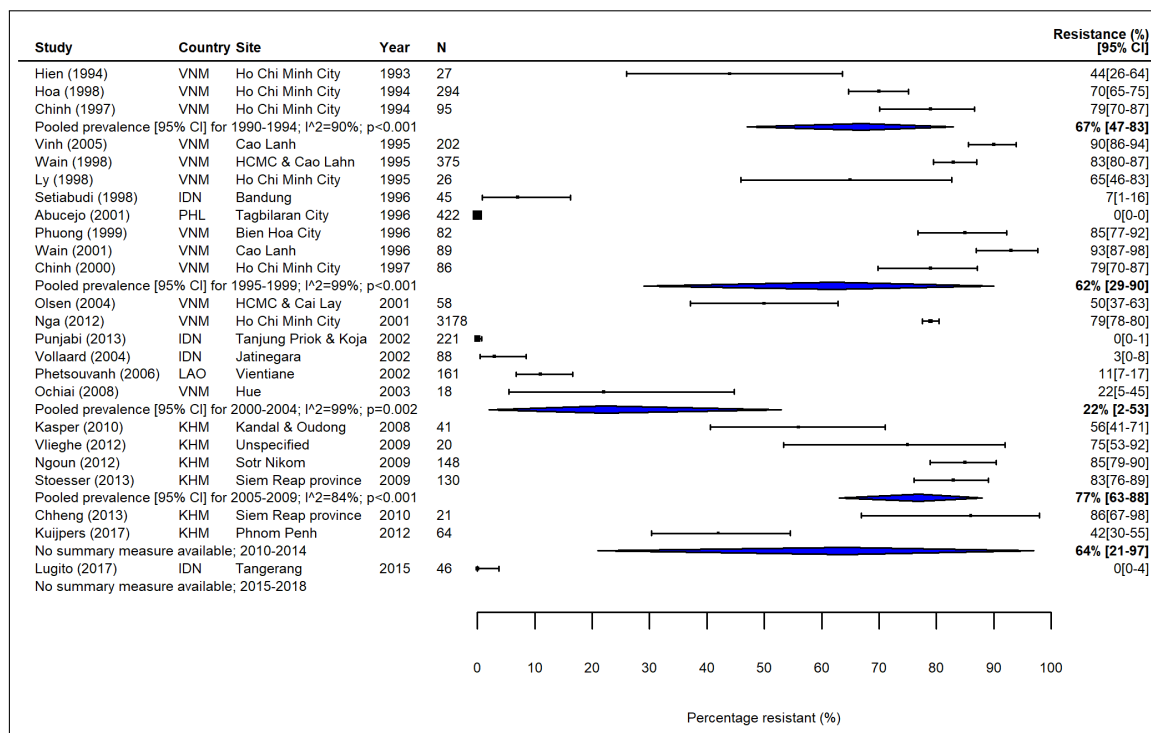


Figure 2.4: Forest plot of MDR *S. Typhi* in Southeast Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95% CI] for each subgroup is represented by the blue diamond.

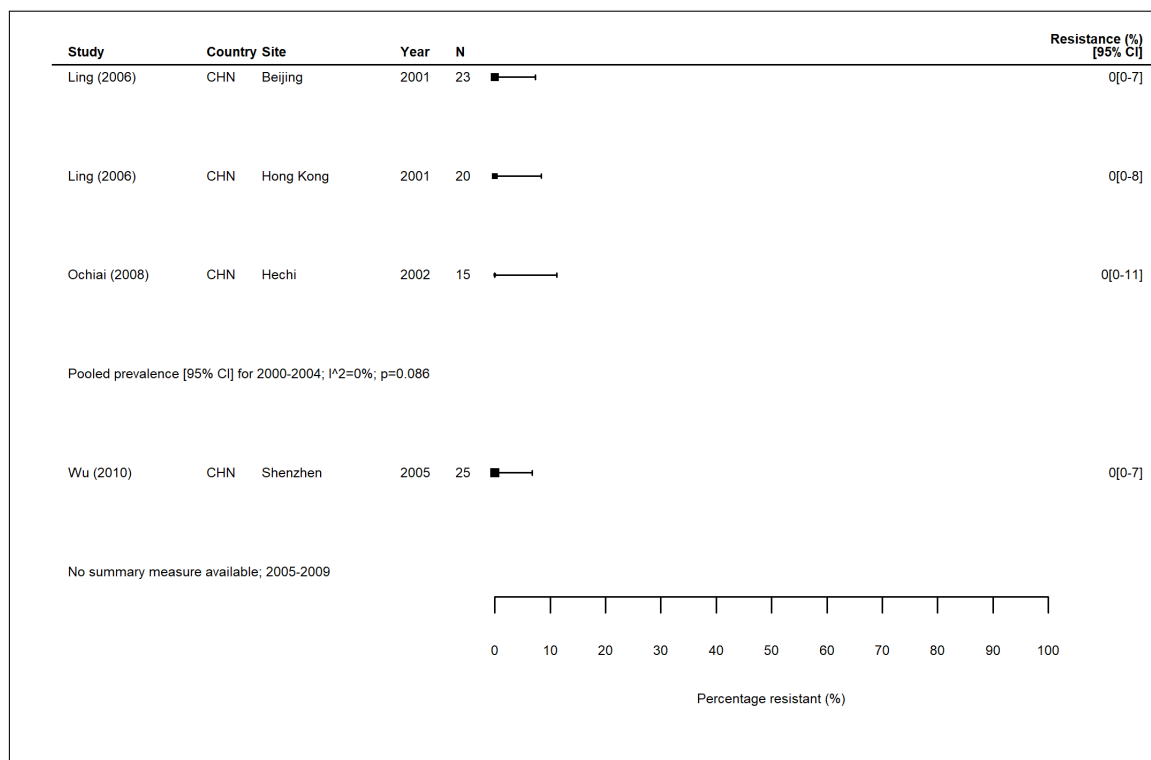


Figure 2.5: Forest plot of MDR *S. Typhi* in East Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95% CI] for each subgroup is represented by the blue diamond.

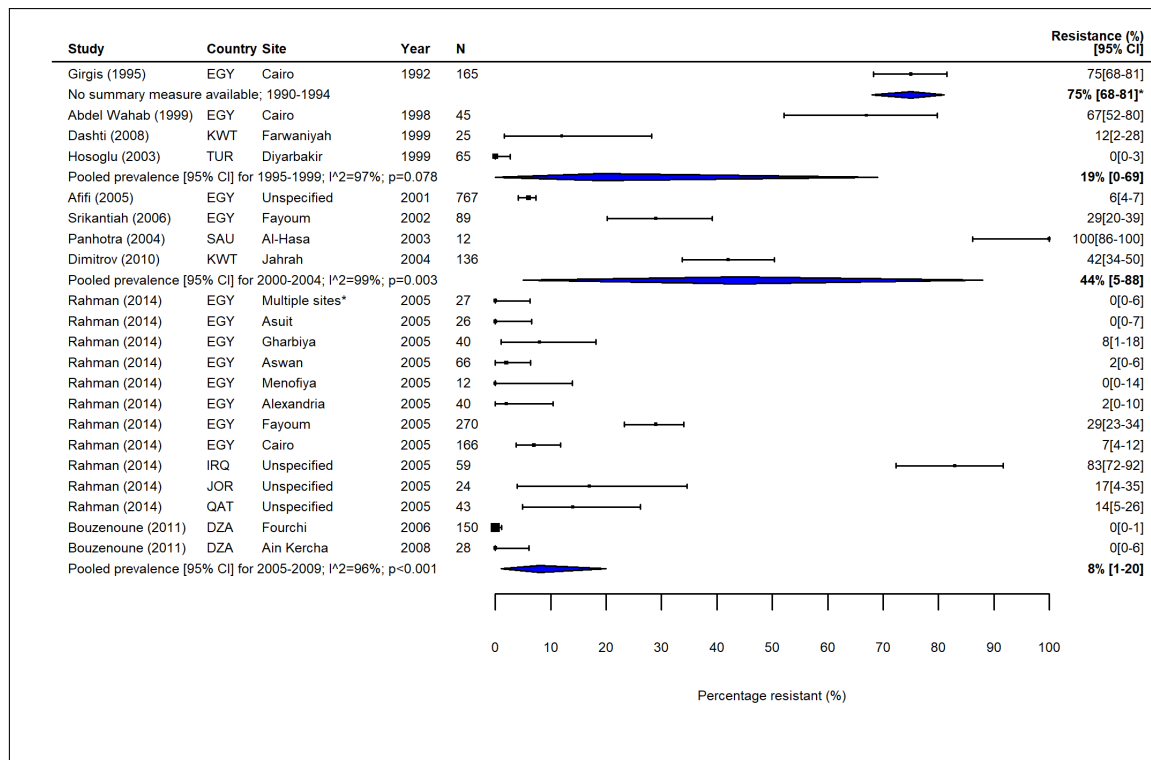


Figure 2.6: Forest plot of MDR *S. Typhi* in North Africa and the Middle East. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95% CI] for each subgroup is represented by the blue diamond.

In sub-Saharan Africa data were sparse, with only 21 studies reporting the prevalence of MDR in *S. Typhi* identified. In Eastern sub-Saharan Africa, despite few data points, the prevalence of MDR increased throughout the study period. In 1990-1995 two studies from Kenya reported 0% (24 isolates)¹¹¹ and 13% (38 isolates)¹¹² of *S. Typhi* isolates to be MDR. In the following time-periods MDR in Kenya ranged from 60-82%. In Malawi, no MDR was reported in 1995-1999 (12 isolates),¹¹³ whilst 88% (2054 isolates) were MDR in 2005-2009¹¹⁴ (Figure 2.7).

For Central sub-Saharan Africa five studies were identified, all from the Democratic Republic of the Congo. Four of these studies reported a mid-level prevalence of MDR (24-38%); three of which were from 2010-2014 equating to a pooled prevalence of MDR of 36% [29-43%] for this time period. The final study reported 100% of 11 isolates to be MDR in 2005-2009 (Figure 2.7). This study was conducted during an outbreak of enteric fever, during which there was also high levels of peritonitis and intestinal perforation.¹¹⁵ Comprehensive and carefully conducted household transmission studies in endemic settings have shown that despite *S. Typhi* being a genetically monomorphic pathogen,

different genotypes circulate within the same household.¹¹⁶ In enteric fever, single source outbreaks might be associated with clonal nature and could introduce bias when estimating prevalence of resistance, therefore the prevalence of resistance from such studies should be interpreted with care.

In Western sub-Saharan Africa six studies (seven data points) were available. The results varied greatly with no MDR reported from Burkina Faso,^{117–119} whilst in neighbouring Nigeria MDR increased from 37% (68 isolates)¹²⁰ in 1998 to 100% (58 isolates)¹²¹ in 2014. High levels of MDR, 66% (89 isolates)¹²² and 63% (30 isolates),¹¹⁸ were also reported from Ghana in 2008 and 2011 respectively (Figure 2.7).

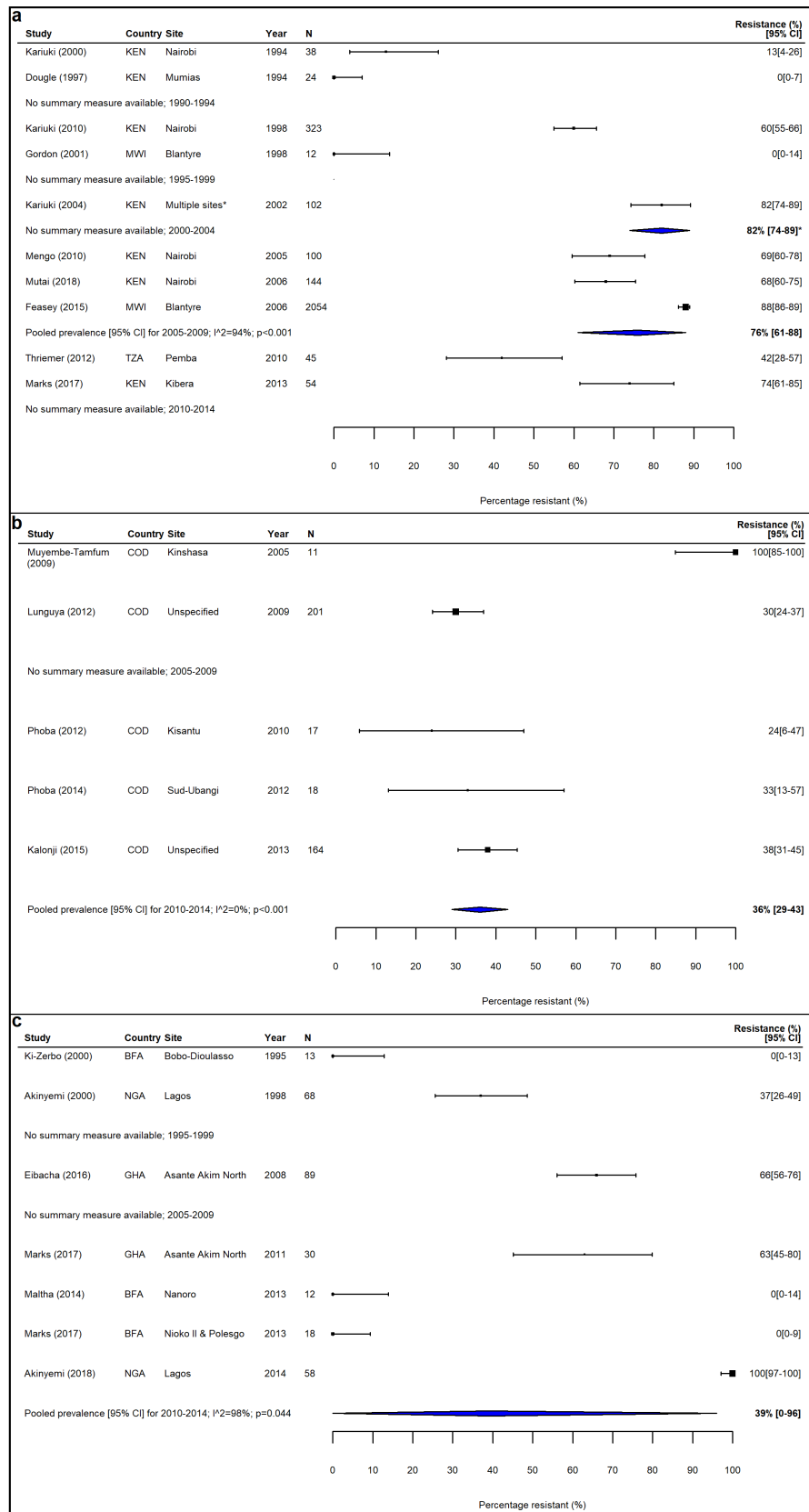


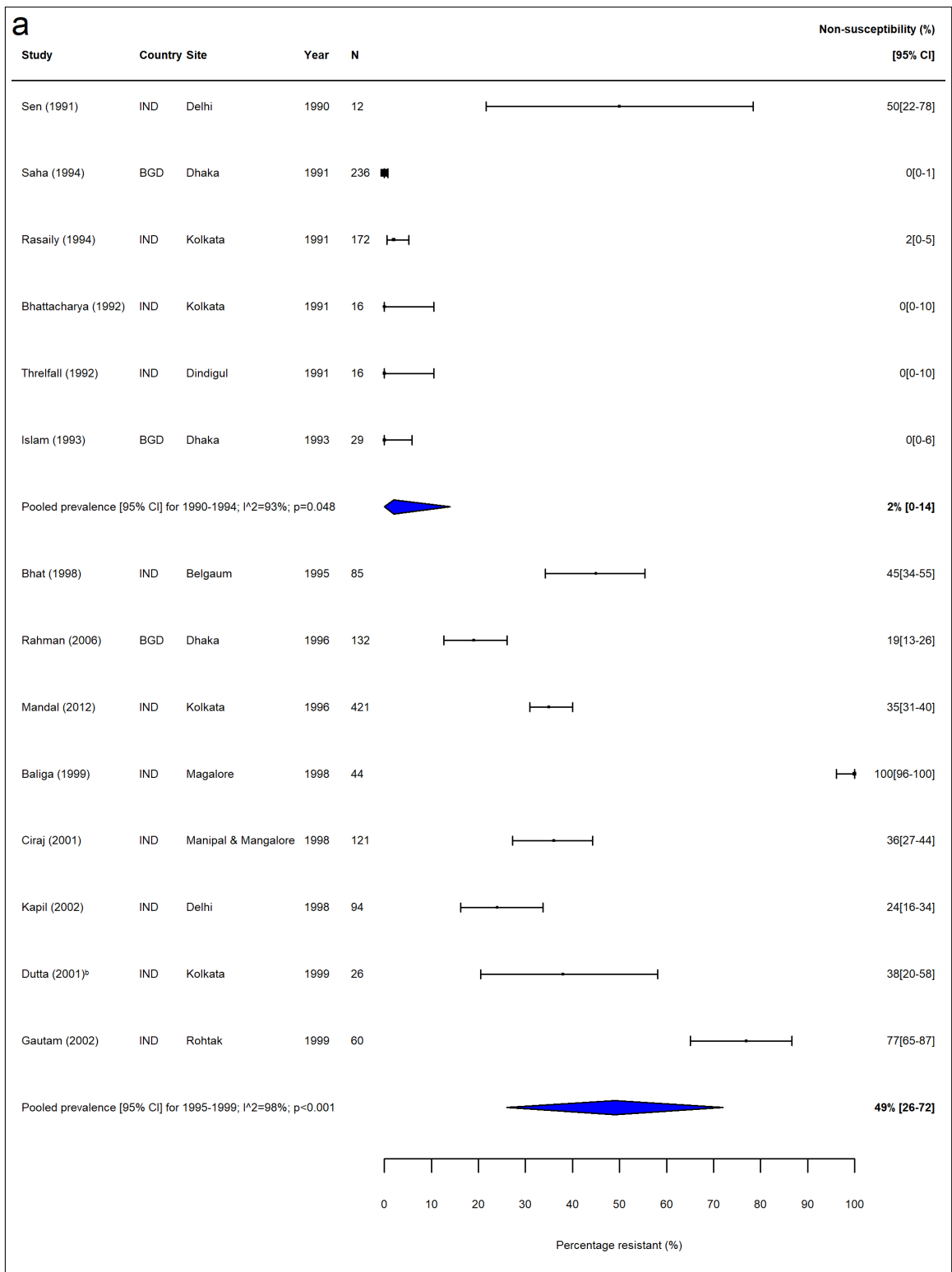
Figure 2.7: Forest plots of MDR *S. Typhi* in sub-Saharan Africa. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup is represented by the blue diamond: (a) Eastern sSA; (b) Central sSA; (c) Western sSA.

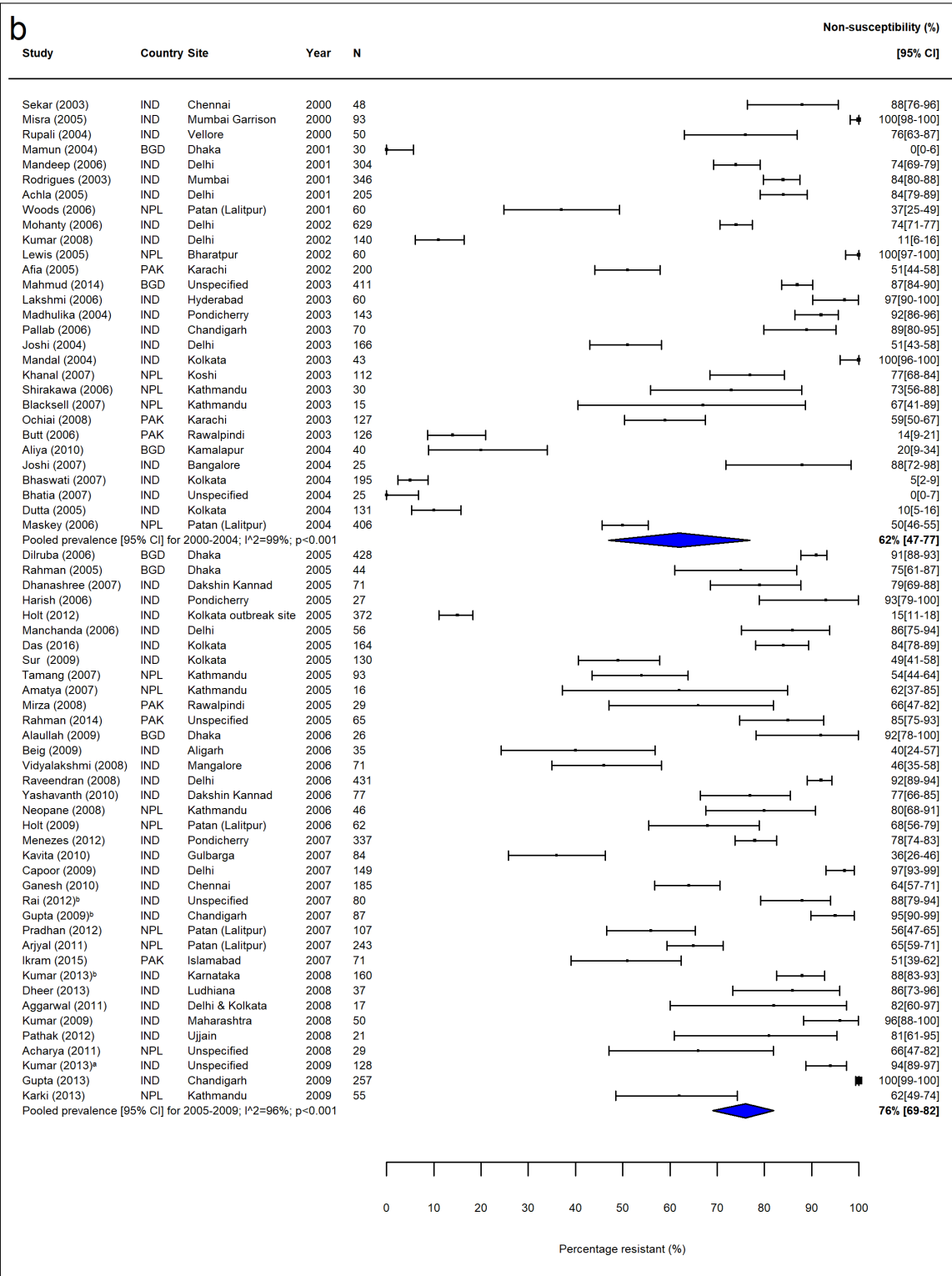
Fluoroquinolone non-susceptible *Salmonella* Typhi

Trends in FQNS differed to those in MDR across all regions, most likely reflecting changes in antimicrobial use and prescribing patterns over the study period. In South Asia there was a clear increase in FQNS with the pooled prevalence increasing from 2% [0-14%] in 1990-1994 to 82% [75-89%] in 2010-2014 (Table 2.4). Despite high heterogeneity, by 2010-2014 just seven of 46 identified studies reported less than 50% of isolates to be FQNS (Figure 2.8).¹²³⁻¹²⁹

In Southeast Asia, similarly to in South Asia, there was a clear increase in FQNS across the study period, from 6% [0-17%] in 1995-1999 to 51% [3-98%] in 2010-2014. As with MDR, the confidence intervals are very large for Southeast Asia, representing high heterogeneity within this region. As with MDR, a lower prevalence of FQNS was reported in Indonesia and Laos (Figure 2.9).

In North Africa and the Middle East data were sparse and no temporal trends were identified. High levels of FQNS *S. Typhi* were reported in Saudi Arabia in 2003 (100% of 12 isolates)¹⁰⁶ and Kuwait in 2004 (77% of 136 isolates),¹⁰⁷ whilst a multicentre study undertaken in 2005 reported rates of 12-81% in various location in Egypt, 81% in Iraq, 62% in Jordan and 65% in Qatar.¹⁰⁸ Two additional studies in 2017 and 2018 reported only low levels of FQNS in Iraq (8% of 39 isolates and 3% of 30 isolates respectively).^{130,131} As with MDR, no FQNS was reported in Algeria (Figure 2.10).¹¹⁰





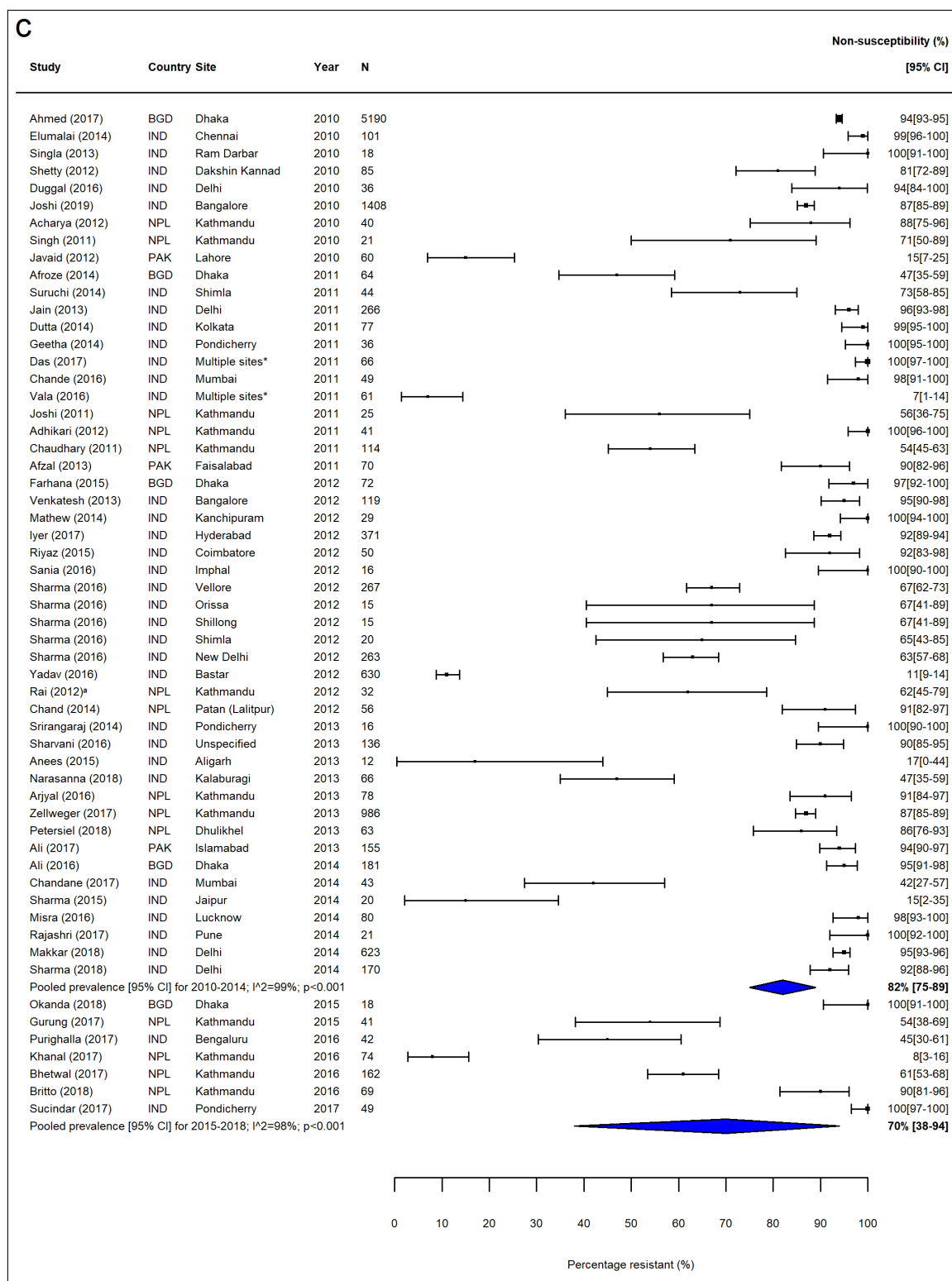


Figure 2.8: Forest plots of FQNS *S. Typhi* in South Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds: (a) 1990-1999; (b) 2000-2009; (c) 2010-2018.

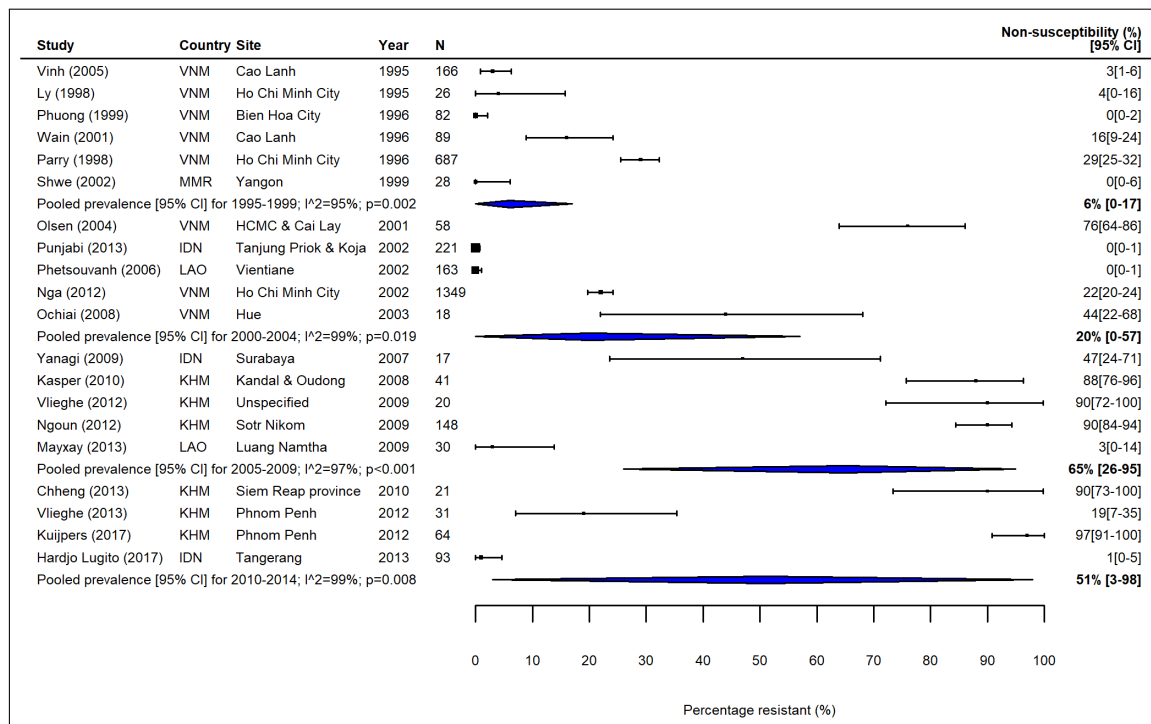


Figure 2.9: Forest plot of FQNS *S. Typhi* in Southeast Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

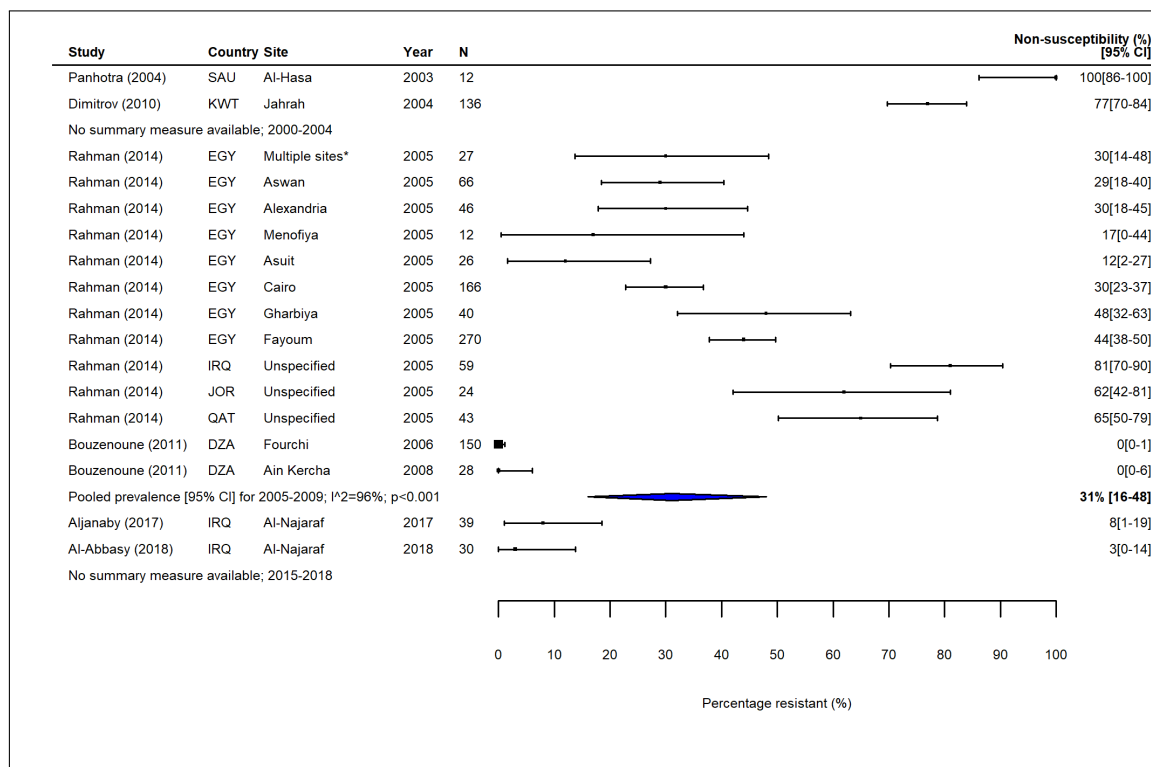


Figure 2.10: Forest plot of FQNS *S. Typhi* in North Africa and the Middle East. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

In sub-Saharan Africa, few studies were available and the prevalence of FQNS varied greatly. Generally, the prevalence of FQNS was lower than that identified in South or Southeast Asia. In Eastern sub-Saharan Africa the prevalence of FQNS was low in Kenya in 1990-1994 (0%, 38 isolates)¹¹² and 1995-1999 (11%, 323 isolates),¹³² but increased to a 45% (85 isolates) and 50% (14 isolates) in 2000-2004.¹¹² For 2005-2009 and 2010-2014 the prevalence of FQNS was highly variable, ranging from 0% (17 isolates) in Korogwe, Tanzania¹³³ to 76% (144 isolates) in Nairobi, Kenya.¹³⁴ In Central sub-Saharan Africa only five studies were identified, all from the Democratic Republic of the Congo; these showed a clear increase in FQNS over time, from 7% (29 isolates) in 2004¹³⁵ to 37% (164 isolates) in 2013.¹³⁶ In Western sub-Saharan Africa the majority of studies reported very low levels of FQNS throughout the study period. Data from Nigeria varied greatly with a seemingly steady upward trend in resistance from 13% in 1998 (68 isolates)¹²⁰ to 82% (39 isolates) in 2012.¹³⁷ However, this is contrasted by a report of 0% FQNS in 230 isolates in 2013¹²¹ (Figure 2.11).

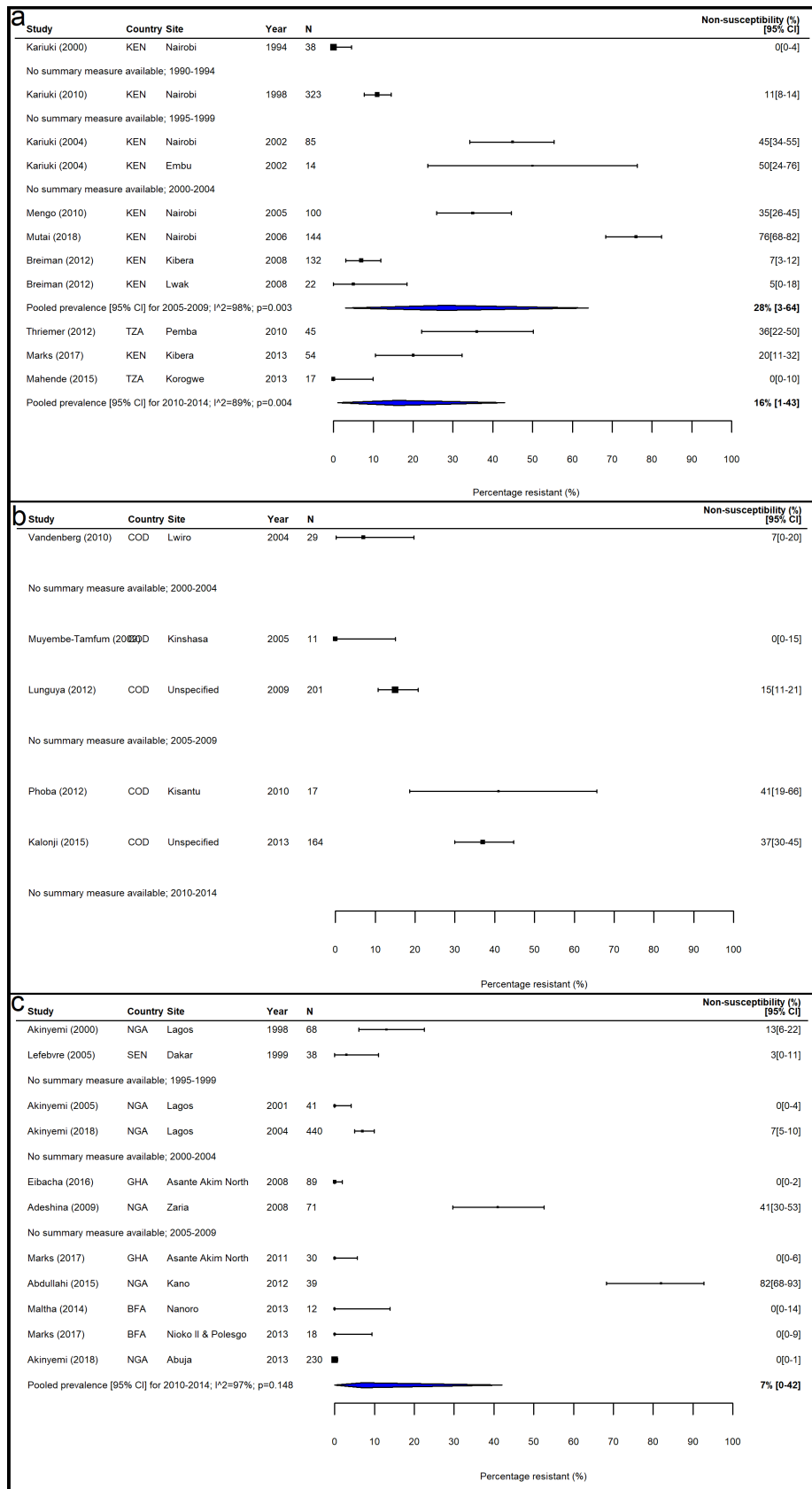


Figure 2.11: Forest plots of FQNS *S. Typhi* in sub-Saharan Africa. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup is represented by the blue diamond: (a) Eastern sSA; (b) Central sSA; (c) Western sSA.

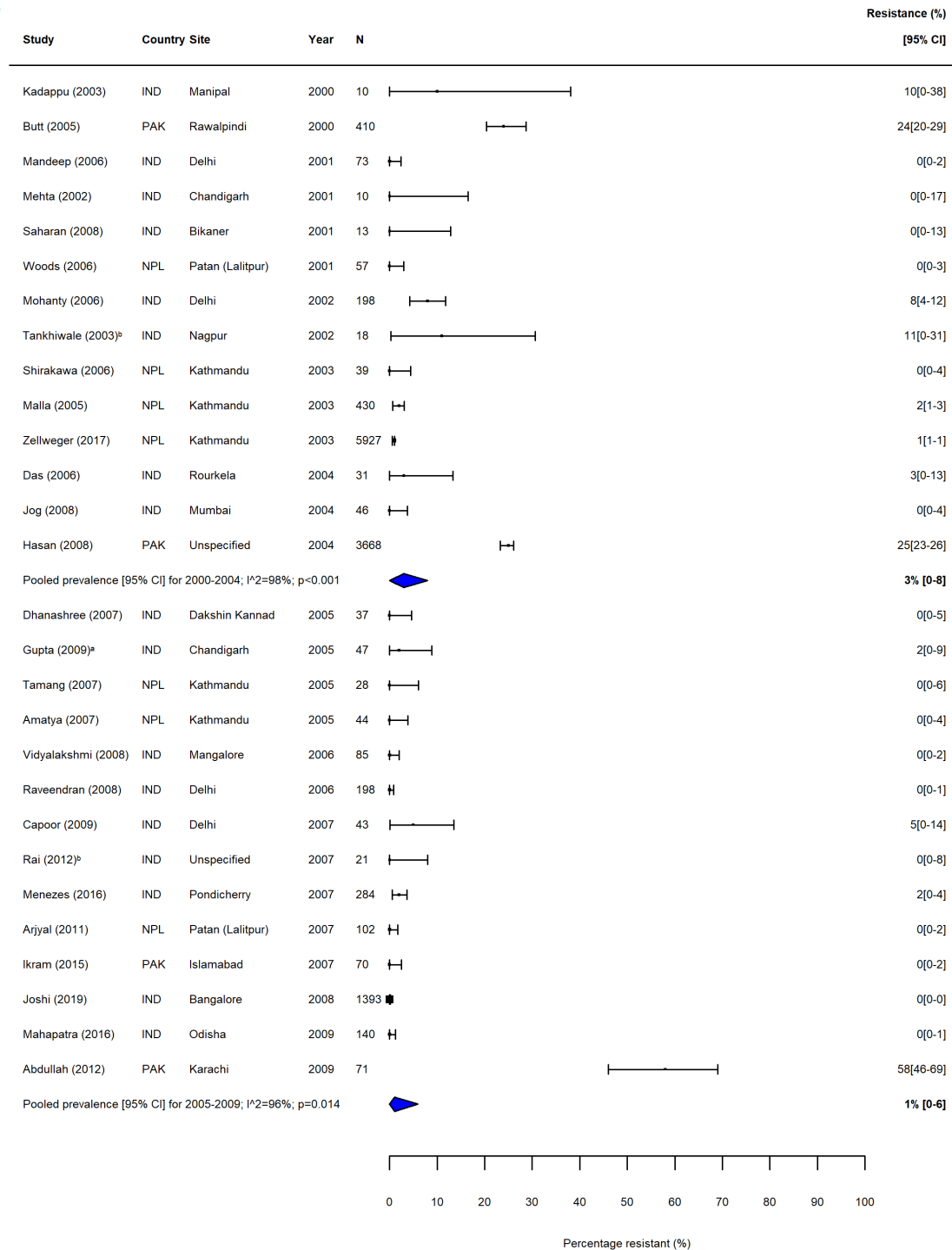
Multidrug resistant *Salmonella* Paratyphi A

The patterns of MDR in *S. Paratyphi* A differed greatly to those in *S. Typhi*. In South Asia the majority of studies reported no, or low levels of MDR in all time periods. Just six of the 63 studies reported over 20% of isolates as MDR.^{94,126,138–141} Five of the studies reporting high levels of MDR were from Pakistan^{94,138–141} and one was from India.¹²⁶ Of 14 studies and 6,928 isolates identified from Nepal just two studies reported any MDR. Malla *et al.*¹⁴² identified 2% of isolates to be MDR (7/430) in 2002 whilst Zellweger *et al.*¹⁴³ found just 44 of 5,927 (1%) *S. Paratyphi* A isolates were MDR over a 23 year study period. Two studies from Bangladesh were identified, one included 1,061 *S. Paratyphi* A isolates, none of which were found to be MDR,¹⁴⁴ the other reported three of 26 isolates (12%) as MDR¹²⁴ (Table 2.4; Figure 2.12).

In Southeast Asia five studies investigating MDR *S. Paratyphi* A were identified,^{99,100,145–147} with a total of 304 isolates. None of these studies identified any MDR in *S. Paratyphi* A (Table 2.4, Figure 2.13). Similarly, in East Asia of the four studies and 4,422 isolates identified,^{148–151} just one was MDR¹⁴⁸ (Table 2.4, Figure 2.14).



b



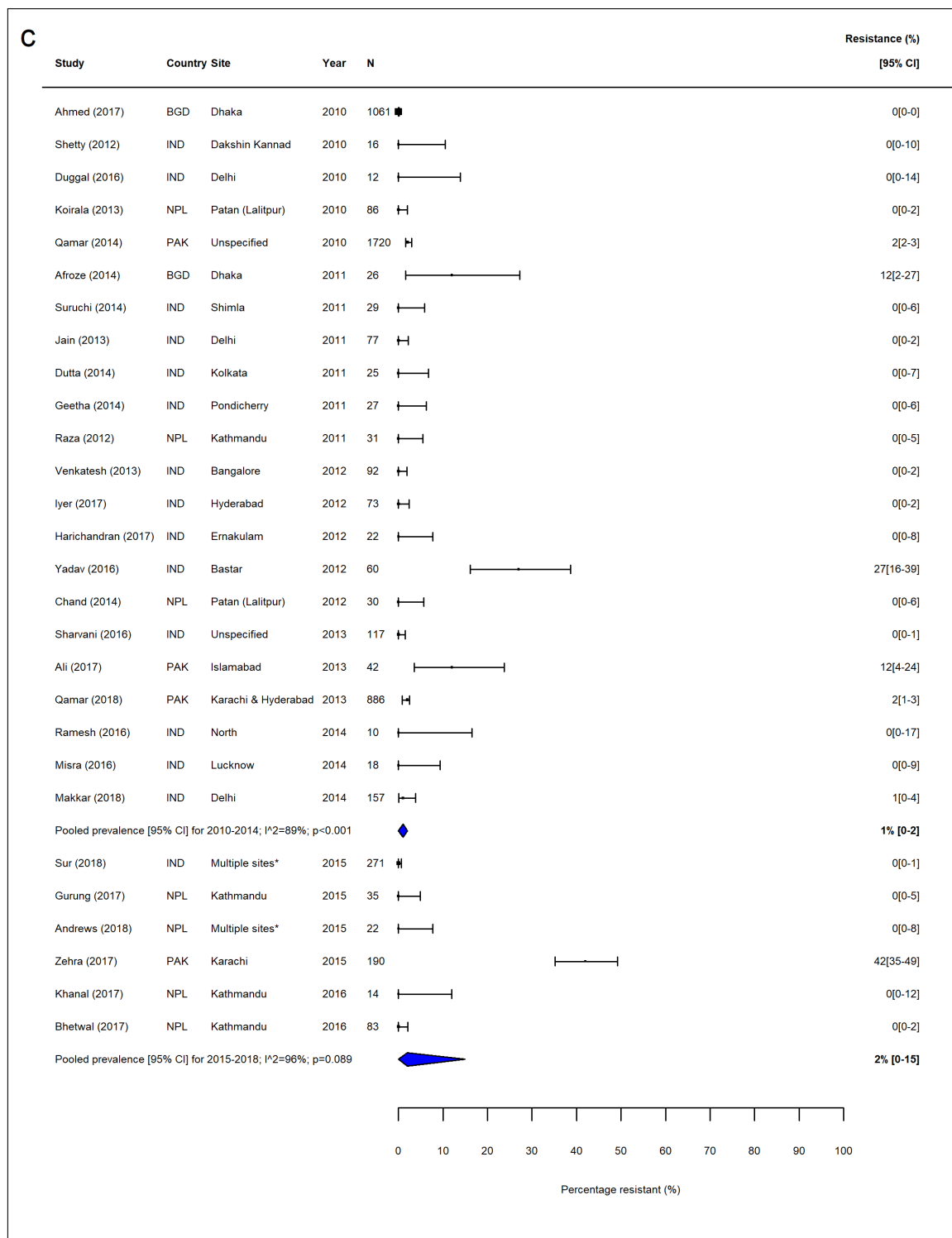


Figure 2.12: Forest plots of MDR *S. Paratyphi A* in South Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup is represented by the blue diamond: (a) 1990-1999; (b) 2000-2009; (c) 2010-2018.

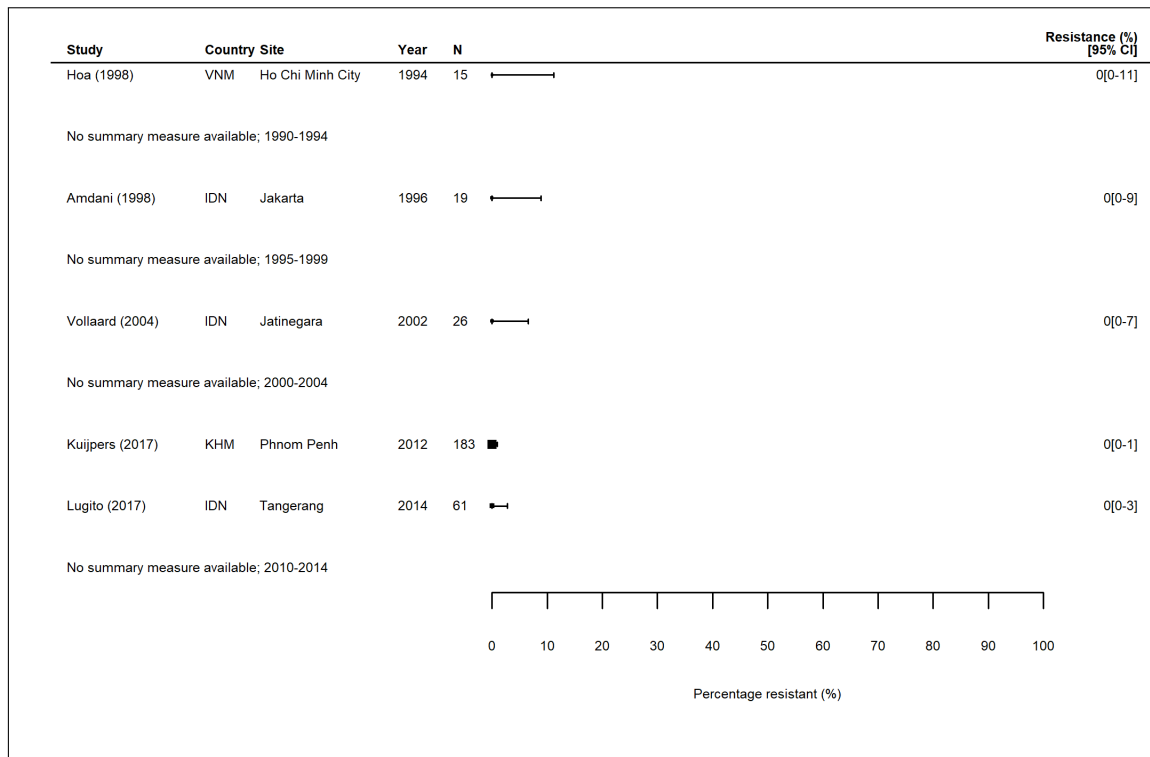


Figure 2.13: Forest plot of MDR *S. Paratyphi A* in Southeast Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

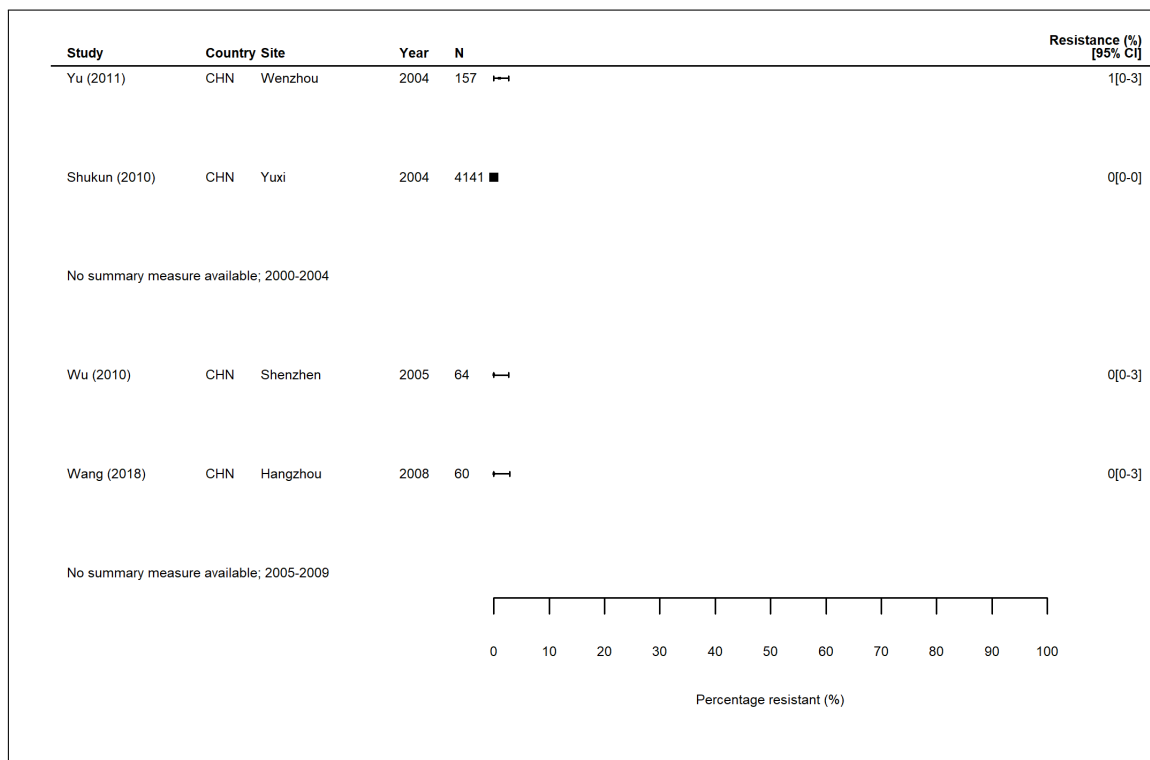


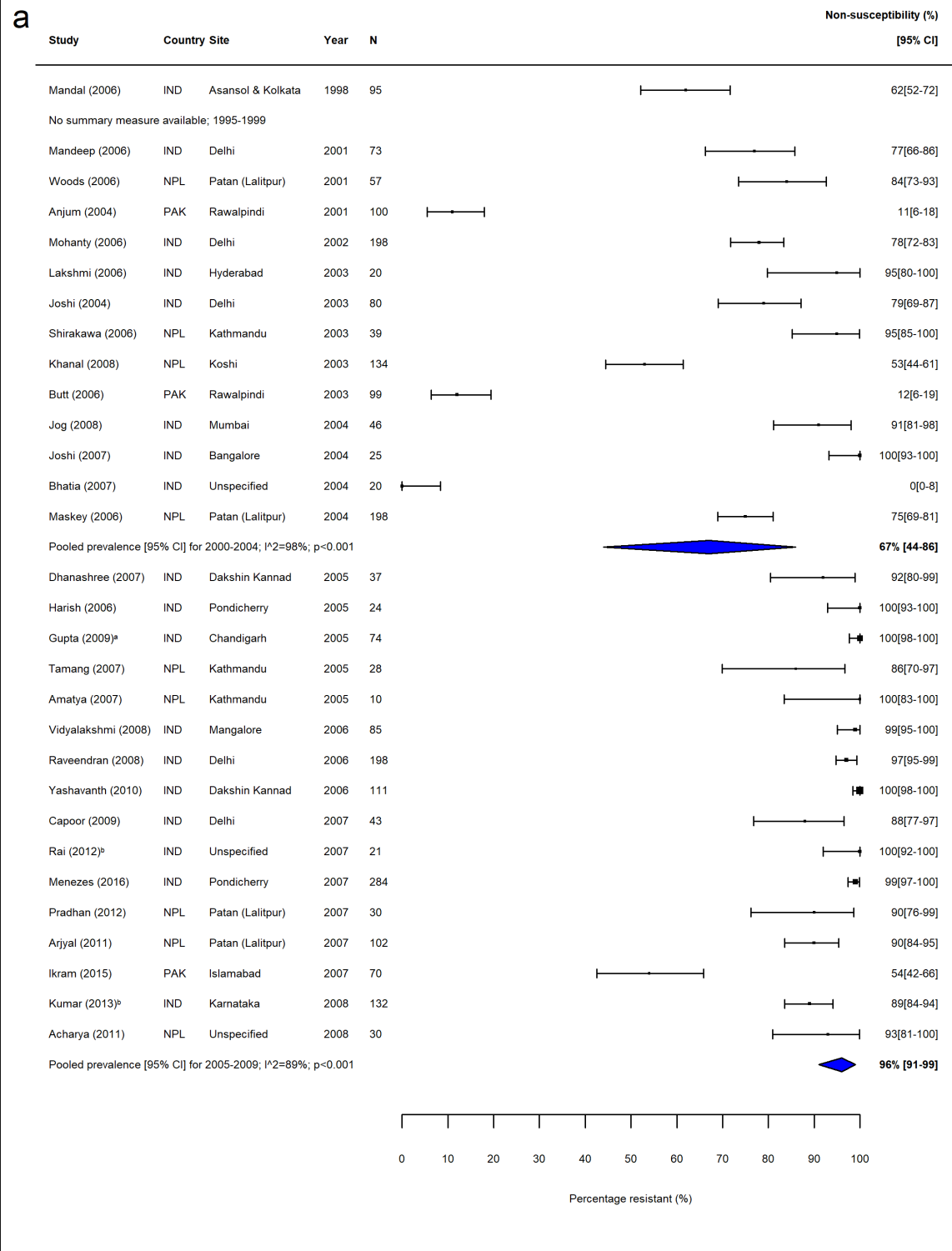
Figure 2.14: Forest plot of FQNS *S. Paratyphi* in East Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

Fluoroquinolone non-susceptible *Salmonella* Paratyphi A

The trends in FQNS *S. Paratyphi* A different greatly to those for MDR. In South Asia the vast majority of studies reported high levels of FQNS in every time-period of the study, with all time periods having a pooled prevalence of FQNS over 50% (Table 2.4, Figure 2.15).

In Southeast Asia, only three studies on FQNS *S. Paratyphi* A were identified, and all showed low levels of FQNS with 0% reported in Indonesia in 2002¹⁰¹ and 2013,¹⁵² and 11% (183 isolates) reported from Cambodia in 2012.¹⁴⁷ These are contrary to the results displayed from South Asia, however, data are sparse in this region and two of the three studies were from Indonesia where no/low levels of MDR and FQNS were also reported for *S. Typhi* (Figure 2.16).

In East Asia, five studies (six data points) were identified, all reporting high levels of FQNS. One study reported 63% (of 126 isolates)¹⁵³ as FQNS, three studies reported 88-94% FQNS,^{149,150,154} and the final study reported 100% FQNS from two separate locations in China.¹⁵⁵



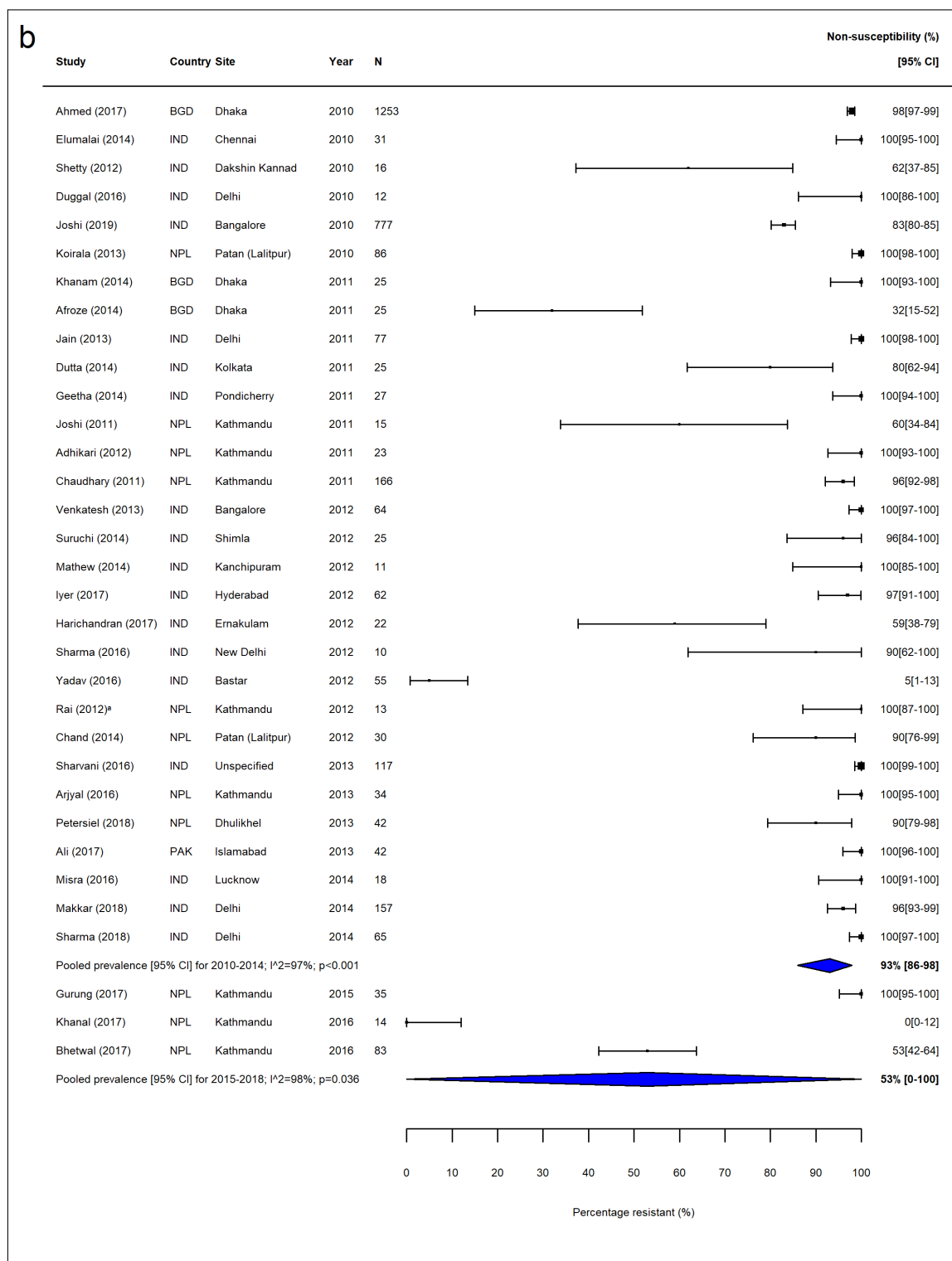


Figure 2.15: Forest plots of FQNS *S. Paratyphi A* in South Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup is represented by the blue diamond: (a) 1990-2009; (b) 2010-2018.

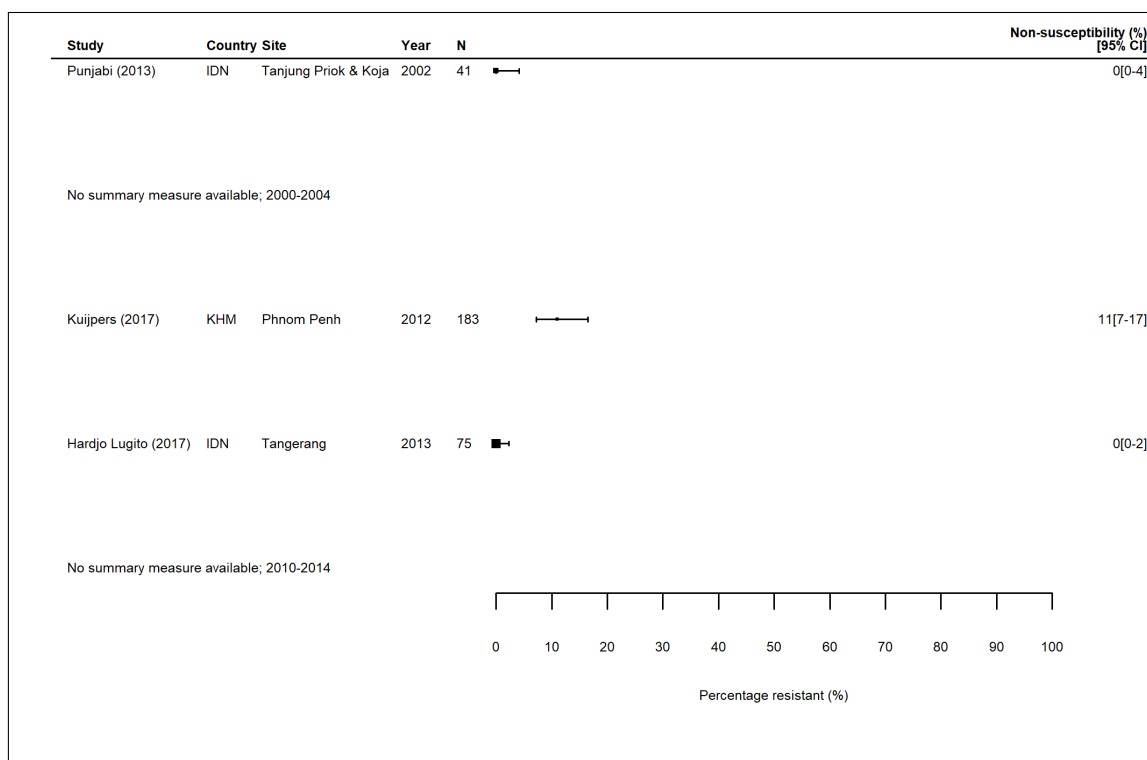


Figure 2.16: Forest plot of FQNS *S. Paratyphi A* in Southeast Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

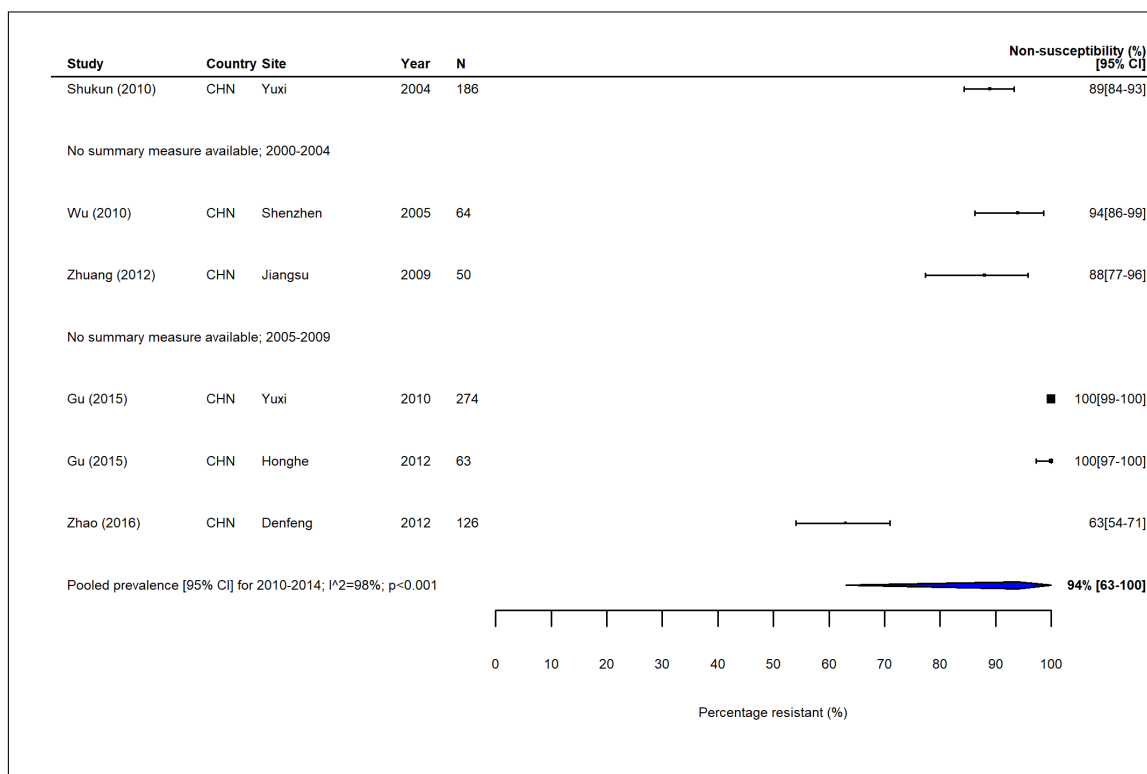


Figure 2.17: Forest plot of FQNS *S. Paratyphi A* in East Asia. Individual study results are displayed with 95% confidence intervals grouped by five-year time-periods, the pooled prevalence [95%CI] for each subgroup are represented by the blue diamonds.

2.3.4 Temporal analysis of ciprofloxacin minimum inhibitory concentrations

Three large studies from Delhi, India^{89,156,157} from different time periods were identified to investigate temporal changes in ciprofloxacin MICs for *S. Typhi*. These showed a huge variation in the distribution of MICs over the study period. In 1995-1999 the majority of isolates (75%) were fully susceptible to ciprofloxacin (MIC < 0.125 µg/ml); the remaining 25% had decreased ciprofloxacin susceptibility (DCS; MIC 0.125 – 0.99 µg/ml), with no isolates in this time period fully resistant to ciprofloxacin.⁸⁹ In 2000-2004 the proportion of isolates with DCS increased and isolates with both low level resistance (MIC 1 – 3.9 µg/ml) and high level resistance (MIC ≥ 4 µg/ml) emerged.¹⁵⁷ By 2005-2009 only 3.4% of isolates were fully susceptible to ciprofloxacin; the majority were DCS (59.7%); 10.7% and 26.2% exhibited low-level and high-level resistance respectively (Figure 2.18).¹⁵⁶

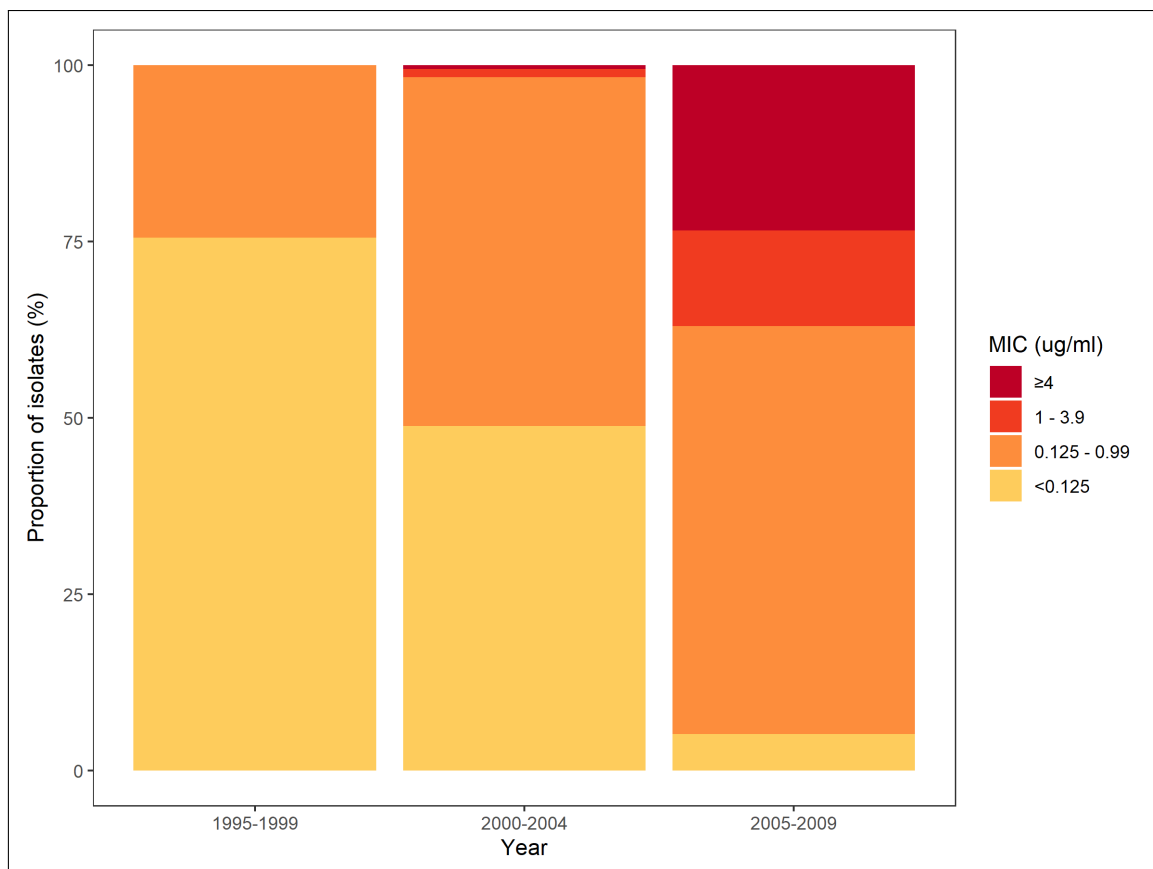


Figure 2.18: *S. Typhi* ciprofloxacin MIC distributions. A stacked bar chart showing the distribution of ciprofloxacin MICs for *S. Typhi* isolates from three selected studies with sample sizes of more than 90 strains performed in Delhi, India^{89,156,157}

2.3.5 Ceftriaxone and azithromycin resistant *Salmonella* Typhi and Paratyphi A

Ceftriaxone susceptibility data were extracted from 198 studies (221 data points) for *S. Typhi* and 91 studies (91 data points) for *S. Paratyphi A*. As with MDR and FQNS, the majority of data points (74%, 232/312) were from South Asia, with a paucity of data from sub-Saharan Africa (20 data points) (Table 2.5).

GBD Region	<i>S. Paratyphi A</i>	<i>S. Typhi A</i>	Total
Andean Latin America	0 (0)	1 (33)	1 (33)
Central Asia	0 (0)	1 (123)	1 (123)
Central Sub-Saharan Africa	0 (0)	1 (29)	1 (29)
East Asia	2 (119)	5 (98)	7 (217)
Eastern Sub-Saharan Africa	0 (0)	9 (835)	9 (835)
North Africa & Middle East	0 (0)	21 (2,995)	21 (2,995)
South Asia	79 (9,992)	153 (36,535)	232 (46,527)
Southeast Asia	9 (590)	21 (2,305)	30 (2895)
Western Sub-Saharan Africa	1 (10)	9 (466)	10 (476)
Total	91 (10,711)	221 (43,419)	312 (54,130)

Table 2.5: Ceftriaxone data availability. Number of studies (isolates) reporting antimicrobial susceptibility to ceftriaxone for *S. Typhi* and *S. Paratyphi A*

Ceftriaxone resistance was similar in *S. Typhi* and *S. Paratyphi A*, with over 70% of data points reporting all isolates to be fully susceptible to ceftriaxone for both serotypes. Fifteen percent of *S. Typhi* and 18% of *S. Paratyphi A* data points reported ceftriaxone resistance in one to five percent of isolates and 11% of *S. Typhi* and 7% of *S. Paratyphi A* reported resistance in over five but less than 20% of isolates. Less than five percent of studies identified 20% ceftriaxone resistance or higher for either serotype (Figure 2.19).

Of the seven studies reporting 20% ceftriaxone resistance or higher for *S. Typhi*, all were from 2010 onwards. Four of these were from South Asia with 45% (of 31 isolates) ceftriaxone resistance in Kathmandu, Nepal;¹⁵⁸ 44% (of 80 isolates) in Faisalabad, Pakistan;¹⁵⁹ 37% (of 66 isolates) in Kalaburagi, India¹⁶⁰ and 26% (of 220 isolates) in Allahabad, India.¹⁶¹ Additionally, studies in Iraq,¹³¹ Nigeria¹⁶² and Tanzania¹³³ reported 33%, 29% and 24% resistance (of 30, 35 and 17 isolates) respectively. All seven studies reporting over 5% of *S. Paratyphi A* isolates to be ceftriaxone resistant were from South

Asia, and from 2001 onwards.^{126,163–168} Studies reporting no resistance or low levels of resistance ($\leq 5\%$) in *S. Typhi* and *S. Paratyphi A* were identified from all endemic regions and for all time-periods, indicating the emerging nature of ceftriaxone resistance.

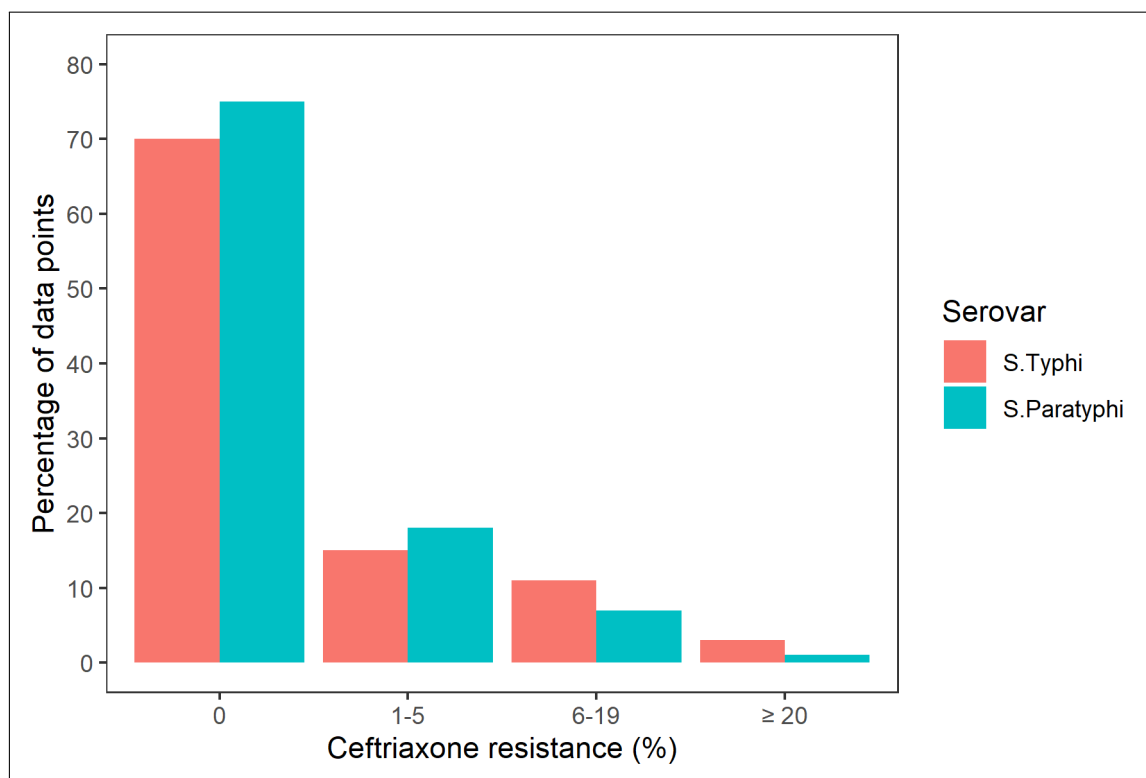


Figure 2.19: Ceftriaxone resistance in *S. Typhi* and *S. Paratyphi A*. A bar chart showing the proportion of identified studies reporting 0%, 1-5%, 6-19% and $\geq 20\%$ resistance to ceftriaxone for *S. Typhi* and *S. Paratyphi A*.

Standardised data on azithromycin resistance in *S. Typhi* were identified from 22 studies. Two studies identified high levels of azithromycin resistance ($\geq 20\%$) with 34% (of 80 isolates) and 85% (of 71 isolates) identified as resistant from India¹⁶⁹ and Pakistan,¹⁷⁰ in 2007 and 2001 respectively. Four studies identified medium levels of resistance ($\geq 5\%$; $< 20\%$), three were from India^{171–173} and one from Cambodia.¹⁷⁴ These studies were all conducted between 2007 and 2013. Two studies^{156,175} reported one isolate to be resistant and the remaining 14 reported no resistance (Table 2.6).

Author (Year)	Country	Testing Method	Number examined (N)	Resistant, N (%)
Chinh (2000)	Viet Nam	Agar dilution	86	0 (0)
Capoor (2009)	India	E-test	149	1 (1)
Kasper (2010)	Cambodia	E-test	41	0 (0)
Ngoun (2012)	Cambodia	E-test	102	0 (0)
Rai ^b (2012)	India	E-test	80	27 (34)
Vlieghe (2012)	Cambodia	E-test	20	1 (5)
Garg (2013)	India	E-test	17	3 (7)
Jain (2013)	India	Disk-diffusion	266	17 (6)
Koirala (2013)	Nepal	E-test	218	0 (0)
Venkatesh (2013)	India	E-test	132	0 (0)
Dutta (2014)	India	E-test	77	0 (0)
Maltha (2014)	Burkina Faso	E-test	12	0 (0)
Phoba (2014)	DRC	E-test	18	0 (0)
Srirangaraj (2014)	India	E-test	16	2 (13)
Ikram (2015)	Pakistan	Disk-diffusion	71	60 (85)
Chande (2016)	India	E-test	108	0 (0)
Iyer (2017)	India	E-test	217	0 (0)
Khanal (2017)	Nepal	Agar dilution	74	0 (0)
Kuijpers (2017)	Cambodia	E-test	64	0 (0)
Okanda (2018)	Bangladesh	Microdilution	18	0 (0)
Sharma (2018)	India	E-test	95	1 (1)
Joshi (2019)	India	E-test	300	0 (0)

Table 2.6: Azithromycin resistance in *S. Typhi*. Resistant isolates are defined as having an MIC $>16\mu\text{g/ml}$ (EUCAST 2014, BSAC 2012) or by disk diffusion, a zone diameter $\leq 18\text{mm}$ (BSAC 2012). Sensitive isolates have an MIC $\leq 16\mu\text{g/ml}$ or zone diameter $\geq 19\text{mm}$.

2.3.6 Risk of bias and sensitivity analysis

The sensitivity analysis undertaken shows that none of the investigated conditions significantly impacted the pooled prevalence of resistance (Figure 2.20, Table A.6). The risk of bias assessment tool identified 92 studies to be of the highest quality (level one), 170 studies to be of medium quality (level two), and 122 studies to be of low quality (level three). This also had no impact on the pooled prevalence of resistance estimates (Figures 2.20c-d). These results supported the decision not to exclude any studies based on the risk of bias assessment, and all studies were used to calculate the pooled prevalence of MDR and FQNS for each region and time period.

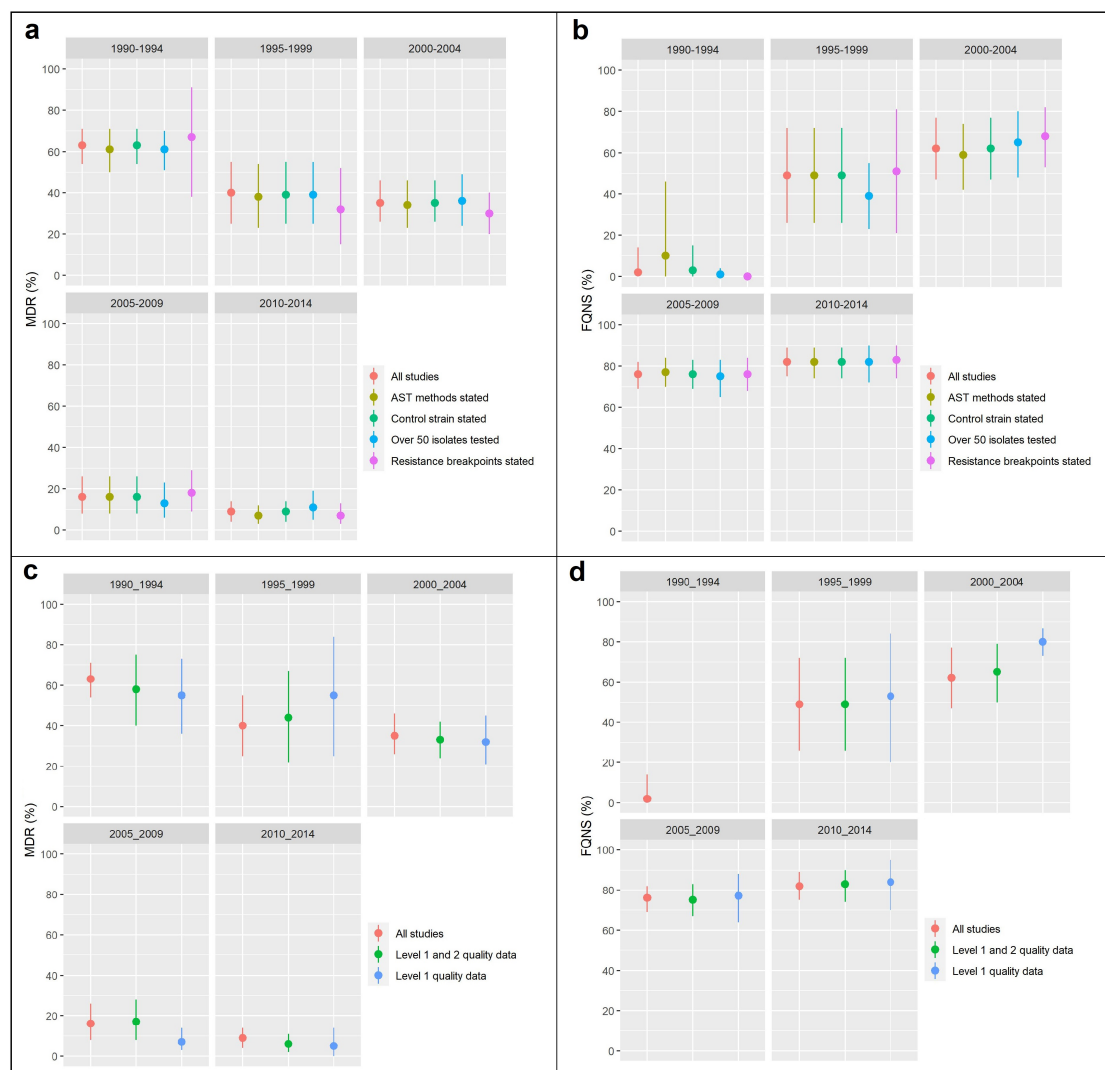


Figure 2.20: Sensitivity analysis. Comparison of random effects meta-analysis results for studies on *S. Typhi* from South Asia by five-year time-period. Plots (a) and (b) compare the pooled prevalence of resistance results for MDR and FQNS respectively for all studies (red) with only those reporting AST methods used (olive); internal control strain reported (green), and those on over 50 isolates of *S. Typhi* (blue), resistance breakpoints clearly stated (pink). Plots (c) and (d) compare the pooled prevalence of resistance results for MDR and FQNS respectively for all studies (red), to those of quality level one and two (green), and quality level one only (blue).

2.4 Discussion

2.4.1 Overview

This systematic review and meta-analysis provides a comprehensive overview of the prevalence of AMR in *S. Typhi* and *S. Paratyphi A*, identifying large scale spatial and temporal trends in the prevalence of AMR across the endemic area. A contemporary geolocated dataset was assembled, containing data from 384 studies reporting the prevalence of AMR in 94,616 *S. Typhi* and 29,731 *S. Paratyphi A* blood culture isolates, from 33 countries and 19 years. Detailed trends in FQNS and MDR were explored across the study period and evidence of the emerging threat of resistance to alternative antimicrobial classes was collated and analysed.

The start of this review period coincides with the height of the MDR *S. Typhi* epidemic in South and Southeast Asia and this review identified MDR as the predominant *S. Typhi* phenotype at this time. The rise in MDR necessitated a switch in treatment regimen away from the former first line drugs to fluoroquinolones.¹⁴ This change in treatment is followed by a notable decrease in the prevalence of MDR *S. Typhi* in South Asia (excluding Pakistan) over the study period, and a corresponding introduction and rapid increase in the prevalence of FQNS *S. Typhi*, resulting in FQNS being the dominant phenotype in South and Southeast Asia by the end of the study period.

FQNS typhoidal *Salmonella* infections are associated with poorer clinical outcomes and a higher rate of complications than susceptible strains.^{27,28} As the prevalence of FQNS increased third generation cephalosporins and azithromycin were recommended by the WHO for treatment of MDR and FQNS enteric fever.¹⁴ This review found evidence of emerging resistance to these second line therapies, with isolates in Pakistan recently identified with MDR, FQNS and third generation cephalosporin resistance (XDR).⁵⁶ With resistance to these various antimicrobial classes evident, treatment options for enteric fever may become very limited.

Spatial variation in the prevalence of AMR in *S. Typhi* between regions was identified.

Although MDR decreased in South and Southeast Asia throughout the study period, there was an introduction and expansion in the prevalence of MDR *S. Typhi* in sub-Saharan Africa, with some countries identifying virtually all *S. Typhi* isolates as MDR by the later years. Additionally, high levels of FQNS *S. Typhi* were identified in all regions of Africa and the Middle East. Although levels have not reached the heights identified in South, Southeast and East Asia this is cause for significant concern as it severely limits treatment options.

The prevalence of MDR and FQNS *S. Typhi* were identified to vary within GBD regions, showing that a more detailed spatial analysis is required to fully understand the epidemiology of these drug resistant infections. In Southeast Asia high rates of MDR and FQNS were identified in Vietnam and Cambodia, whilst only limited levels of resistance were found in Indonesia. In India the levels of MDR *S. Typhi* decreased largely towards the end of the study period, whilst rates in Pakistan remained high. This observed variation is likely due to multiple factors, including the limited availability and quality of microbiology facilities negatively effecting the ability to detect and report AMR, whilst differences in economic development, medical infrastructure and antimicrobial consumption could also affect transmission and impact the circulating strains.

Stark difference in the patterns of drug resistance in *S. Paratyphi A* compared to *S. Typhi* were identified by this review. Although MDR *S. Typhi* was prevalent in all regions, only very low levels of MDR were identified in *S. Paratyphi A*, with most isolates remaining sensitive to these antimicrobials throughout the whole study period. Conversely, FQNS was found to be highly prevalent in *S. Paratyphi A*, with the prevalence often exceeding that in *S. Typhi* in South and East Asia. This highlights the contribution of *S. Paratyphi A* to the problem of AMR in enteric fever. There is currently no vaccine for *S. Paratyphi A* so the development of AMR is of particular concern, especially in countries such as China where this serotype causes a large proportion of enteric infections.⁹

S. Typhi and *S. Paratyphi* have distinct genomic evolution and epidemiology.¹⁷⁶ The

majority of MDR isolates in *S. Typhi* are due to the presence of the IncH1 plasmid.⁵² Genomic studies on *S. Paratyphi A* are sparser than those for *S. Typhi*, with limited evidence of plasmid mediated MDR in this serotype.¹⁷⁷ Despite reports of multiple introduction events,¹⁷⁸ plasmid-mediated resistance has not become prevalent in *S. Paratyphi*, this might be due to the fitness costs associated with the plasmid.

Several studies reporting a high prevalence of ceftriaxone and azithromycin resistance in *S. Typhi* were identified. This was inconsistent with findings of another recent, smaller review of AMR *S. Typhi*¹⁷⁹ and may imply methodological weaknesses in the identified studies. The majority of studies reporting a high prevalence of ceftriaxone resistance performed their AST using only the disk-diffusion method and failed to confirm resistance with MIC testing or investigate further with ESBL-testing. Although this is compliant with the current CLSI guidelines, it brings into question the reliability of these results as several studies have been identified reporting conflicting AST results from disk-diffusion and MIC testing methods.^{126,165,180} This issue is highlighted as one study reported 100% of isolates to have intermediate resistance to ceftriaxone without further ESBL-testing and failed to discuss these results further.¹⁶³

The majority of data were from South and Southeast Asia where the burden of enteric fever is highest, whilst there was a paucity of data from South America, sub-Saharan Africa and Oceania. The lack of data from South America and Southern sub-Saharan Africa is consistent with the low disease burden observed in these regions.^{181,182} Economic development and improvements in water safety and sanitation have driven the decrease in enteric fever incidence, meaning that these regions no longer fit this study's definition of endemic. However, data were also sparse in Western, Central and Eastern sub-Saharan Africa where *S. Typhi* still causes a large burden of disease.¹¹⁸ *S. Typhi* data were only identified from 10 countries in sub-Saharan Africa, and from a limited number of institutions, indicating a paucity of microbiology facilities undertaking AST in these regions. Routine surveillance of AMR is vital to inform local and national treatment guidelines and policies for combatting AMR.¹⁸³ This limited availability of AST risks negatively affecting health outcomes due to enteric fever and

other infectious diseases. Although no official guidelines for enteric fever exist, it is generally considered that when the prevalence of resistance to a specific antimicrobial exceeds 20% a change in treatment regimen is required. Surveillance of local resistance rates is vital to ensure recommended antimicrobial treatments remain below this threshold and to drive change if this threshold is exceeded.

In spite of the high incidence rates of enteric fever in South Asia, Southeast Asia and sub-Saharan Africa,⁴ the majority of identified studies had small sample sizes. This likely reflects the low rates of treatment seeking behaviour, self-treatment with antimicrobials prior to attending a healthcare facility and the low sensitivity of blood culture for detecting typhoidal *Salmonella* infections.^{55,184} These are just some of the challenges in identifying invasive *Salmonella* infections and conducting AST in LMICs and are investigated further in Chapter Five, along with their impact on the reported prevalence of resistance.

This review identified variations in the quality and reporting of AST data. Variables were extracted in an attempt to assess the quality of the AST undertaken by the study (use of laboratory testing guidelines, specification of resistance breakpoints, use of a control strain and participation in an EQA scheme). However, reporting of these metrics was incomplete or absent in a large number of studies with less than a third describing their use of an internal control. This is consistent with figures seen in other systematic reviews, with use of an internal control reported at 48% by Tadesse¹⁸⁵ and 47% by Leopold.¹⁸⁶ This may reflect variations and weaknesses in the testing methodology across LMICs but also highlights the lack of international guidelines for the undertaking and reporting of AST. It is questionable whether the absence of statements regarding the use of guidelines or internal and external controls in published literature indicates that these measures were not undertaken, or whether they were just not reported (several studies from well-resourced laboratories known to participate in EQA schemes failed to report this in the methodology). Additionally, when stated that specific guidelines were followed (e.g. “AST was conducted following CLSI 2015 guidelines”) it is unclear whether all facets of that guidance were followed and to what degree. This leads to difficulties in interpreting the quality of data extracted from the studies, explaining some

of the observed heterogeneity in this review.

With increases in AMR evident in this study, it is important to understand the relationship between excessive and inappropriate use of antimicrobials and the corresponding impact on AMR. The use of antimicrobials to treat preventable infections has been contributing to the increase in antibiotic consumption over the last two decades,¹⁸⁷ driving resistance in both the target and bystander organisms.¹⁸⁸ With the threat of AMR well established, vaccines and non-pharmaceutical interventions are essential to tackle the threat of AMR *S. Typhi* and *S. Paratyphi A* by decreasing the burden of enteric fever.

2.4.2 Limitations

This study entailed an exhaustive search of published literature and synthesised all relevant articles on AMR in *S. Typhi* and *S. Paratyphi A*. There were a large number of studies from various locations and years with different patient selection and blood sampling criteria identified, along with large variations in methodological robustness resulting in high heterogeneity in most subgroups of this study. Sensitivity analyses performed identified no impact of sampling and laboratory quality indicators on the reported prevalence of resistance but high heterogeneity persisted. Strong correlation between the meta-analysis results and the more conservative metric of median prevalence of resistance confirmed the appropriateness of the meta-analysis for this analysis.

The high heterogeneity is translated into large confidence intervals around some of the pooled prevalence of resistance estimates, particularly in Africa where there was a paucity of data and studies were often characterised by small sample sizes. The majority of studies were undertaken in hospitals in LMICs, where the policy for taking blood cultures and performing AST is varied and not consistently implemented, introducing the risk of selection and sampling biases, potentially contributing to the observed heterogeneity. The majority of studies did not report clinical information or the numbers of blood cultures screened annually (as recommended by WHO GLASS¹⁸³) and in many

cases it is likely that only the most serious cases had blood cultures performed, risking the over-representation of resistant isolates in hospital data.¹⁸⁹ These factors and their potential impact on prevalence of resistance estimates are discussed further in Chapter Five, and methods for accounting for them investigated.

Most studies were undertaken in urban centres where there is a higher availability of antimicrobial drugs. The prevalence of resistance may differ between urban and rural locations, however, due to the rapid expansion of the H58 haplotype⁵² susceptible to acquisition of MDR and FQNS genes, it is reasonable to predict that resistance would be reasonably evenly distributed across fine scale locations. This study identified spatial variations in the prevalence of resistance within the GBD regions analysed. Although part of this variation is likely due to the specified limitations, actual variations in the prevalence of MDR and FQNS *S. Typhi* and *S. Paratyphi A* may persist within the GBD regions. To investigate this further a finer scale spatial analysis of the prevalence of resistance is conducted in Chapter Six.

Interpretative breakpoints for resistance to two antimicrobial classes were changed during the study period, impacting the ability to analyse the data. Breakpoints for the third generation cephalosporins were lowered in 2010¹⁹⁰ and fluoroquinolone breakpoints lowered in 2012.¹⁹¹ In the early part of this study ceftriaxone resistance was rare therefore the change in breakpoints is not thought to have affected the results of this systematic review. To interpret FQNS, nalidixic acid was used as a proxy marker for resistance. Although this is often considered an appropriate proxy measure, nalidixic acid testing does not capture all mechanisms of FQNS therefore this study may slightly underestimate the prevalence of FQNS.²⁷ There was a wide range in degrees of resistance to fluoroquinolones with MICs for ciprofloxacin ranging from below 0.06 μ g/ml to above 32 μ g/ml. This study has not quantified the degree of resistance, and for isolates with low level resistance an increased dose of fluoroquinolones may still be an effective cure for enteric fever.²⁷

2.4.3 Conclusions

Despite the discussed limitations the overall trends highlighted by this meta-analysis are corroborated by large longitudinal studies with standardised patient recruitment and testing procedures, conducted by high quality microbiology laboratories participating in EQA schemes or ISO accreditation.^{25,27,98,134,144,174,178,192,193} This study provides evidence of the worsening landscape of AMR in *S. Typhi* and *S. Paratyphi A*; although there are decreasing trends in MDR in some regions the extent of FQNS and emergence of third generation cephalosporin resistance is of major concern, leaving limited treatment options for enteric fever.

The establishment of international reporting guidelines, such as the recently published MICRO guidelines,¹⁹⁴ will be helpful in improving the quality of data coming out of laboratories in LMICs. Improving the quality and capacity of microbiology facilities is vital in establishing a high level of AMR surveillance. Understanding the AMR landscape is important for identifying effective treatments, informing policy and understanding the scale of the issue. This study provides a broad understanding of AMR in *S. Typhi* and *S. Paratyphi A* but further investigations are required to fully comprehend this issue. The later chapters of this thesis further explore the issues identified by this review, establishing knowledge on the trends of antibiotic use; investigating the challenges and biases related to estimating AMR, and utilising statistical models to produce comprehensive estimates of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for all countries within the endemic region for 1990-2018.

Antibiotic usage in children with lower respiratory tract infections in low- and middle-income countries, 2000 to 2018

3.1 Introduction

Bacterial infections are a major cause of morbidity and mortality worldwide. Antibiotics have been hugely successful in improving health outcomes, and alongside improvements in nutrition, clean water, sanitation, and vaccination provision, have aided in the global reduction of under-five mortality from 216 deaths per 1000 live births in 1950 to 39 deaths per 1000 live births in 2017, as well as an increase in male life expectancy from 48 to 71 years within the same time period.^{195,196} The positive impact of antibiotics, however, is threatened by increasing levels of AMR worldwide and hampered by the lack of access to essential antibiotics in many LMICs.^{30,197}

The link between inappropriate antibiotic use and the development and spread of AMR is well documented.⁵⁹ AMR limits treatment options, especially in LMICs where second line antibiotics are often too expensive and difficult to access.¹⁹⁸ A full understanding of antibiotic usage in each geographic context is vital to inform national action plans aimed at promoting judicious antibiotic use and limiting the spread of AMR.²⁹

Sustainable Development Goal (SDG) 3-8 specifies a need for “access to safe, effective, quality and affordable essential medicines and vaccines for all”.¹⁹⁹ Limited access to essential antibiotics is a major cause of morbidity and mortality;¹⁹⁷ not only

does this leave bacterial infections untreated, but sub-optimal dosing and poor pharmaceutical quality can contribute to the development and propagation of AMR.²⁰⁰ Improving access to antibiotics is vital to improving health outcomes, particularly in LMICs where financial and infrastructure constraints negatively impact access to quality healthcare and essential medicines.¹⁹⁸

Understanding spatial and temporal trends in the use of antibiotics is important for both improving access to essential medicines and establishing control programmes to prevent their overuse. In LMICs, national surveillance of antibiotic sales and consumption is sparse,²⁰¹ and the primary source of spatially and temporally comparable data on antibiotics is from household surveys. These surveys provide a measure of “antibiotic usage”, referring to the use of antibiotics by individual persons, by questioning caregivers of children under five years old with symptoms of lower respiratory tract infection (LRI) on whether they received antibiotics or not.

Such surveys have previously been utilised as input data for geostatistical models estimating the prevalence of infectious diseases and health related indicators.^{82,202–204} This chapter assimilates data from numerous spatially and temporally diverse household surveys and estimates the prevalence of antibiotic usage in time and space. Established geostatistical modelling techniques are used to estimate the prevalence of antibiotic usage in LMICs, at a high spatial resolution for the years 2000 to 2018. From these estimates comparisons of antibiotic usage both between and within countries are made, as well an assessment of the temporal trends of antibiotic usage in LMICs.

3.2 Methods

3.2.1 Overview

Two large household survey series from USAID’s Demographic Health Surveys (DHS) and UNICEF’s Multiple Indicator Cluster Surveys (MICS) were identified as the main source of internationally comparable data on antibiotic usage in LMICs. These surveys are nationally representative and are frequently conducted in numerous developing

countries, asking standardised questions on antibiotic usage. Caregivers of children aged under five years are asked whether each child had suffered from symptoms of LRI (assessed as cough, fever, difficulty or rapid breathing and chest symptoms) in the previous two weeks and whether the child received antibiotics for this illness. Although these data are only for children, they provide a wealth of internationally comparable data on antibiotic usage, allowing for comparisons across space and time.

The proportion of children under five years with symptoms of LRI in the previous two weeks who received antibiotics was extracted from all identified surveys, for the years 2000 to 2018. These data were then linked to the smallest geographic location and input into a two stage Bayesian binomial hierarchical model to estimate the prevalence of antibiotic usage in children under-five with LRI for every 5x5km pixel in the study area (LMICs) for each year from 2000 to 2018 (Figure 3.1). Results were then aggregated to the administrative level two (district), administrative level one (province), and country resolutions.

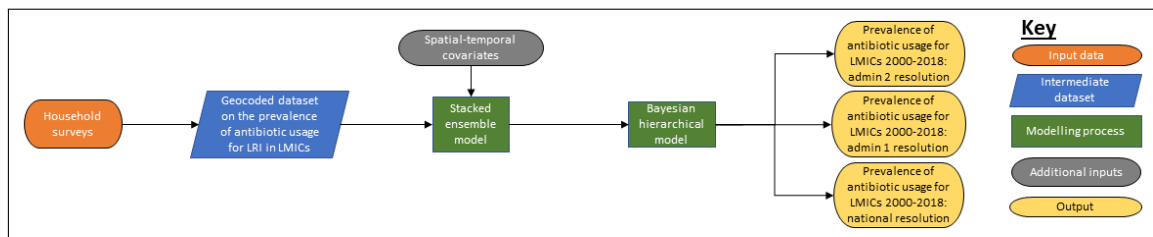


Figure 3.1: Antibiotic usage model schematic. A schematic diagram for the modelling of antibiotic usage in LMICs using household survey data.

3.2.2 Antibiotic usage data

Data extraction and processing

All household surveys conducted by DHS and MICS in LMICs from 1990 onwards were screened for inclusion. To be included in the dataset a child had to have had a caregiver reported cough in the previous two weeks, for information on whether antibiotics were taken for this illness to be available (binary indicator, “yes” or “no”), and for the residence of the child to be linkable to a subnational location (either latitudes and longitudes or administrative divisions). Additional data were extracted where available, this included

variables on whether the child had difficulty or rapid breathing, chest symptoms or fever associated with this illness, along with whether treatment was sought for the child, and where treatment was sought from (e.g. a pharmacy, clinic or hospital).

The residence of the child was linked to the smallest location available, either point locations (latitudes and longitudes of the household cluster) or administrative divisions (districts or provinces). Where the location was an administrative division this was then linked to a polygon within a shapefile representing the administrative division boundaries. Shapefiles were obtained from the Food and Agriculture Organisations Global Administrative Unit Layers (GAUL; <http://www.fao.org/geonetwork/>), the Database of Global Administrative Areas (GADM; <https://gadm.org/>), and additional freely available sources online. When required, shapefiles were edited using R and ArcGIS to represent the district boundaries at the time the survey was conducted.

LRI was defined as caregiver reported symptoms of cough, rapid/difficulty breathing, chest symptoms and fever in the previous two weeks. The reporting of such symptoms varied between surveys and included children with the following combinations of symptoms: a) cough, b) cough and rapid/difficulty breathing, c) cough and fever, d) cough and rapid/difficulty breathing and fever, and e) cough, rapid/difficulty breathing and chest symptoms. To ensure that all surveys were comparable and to maximise the amount of data available, the proportion of antibiotic usage in children with each of the sets of symptoms were adjusted to be the equivalent of the given LRI definition. For surveys containing variables on all available symptoms, the proportion of antibiotic usage was calculated for each of these sets of symptoms and linear regression models fit between antibiotic usage for LRI, and antibiotic usage for the other sets of symptoms. The coefficients of these models were then used to adjust the survey data to represent antibiotic usage for LRI (Table 3.1).

Data from each survey was aggregated to one observation per location (at the finest resolution possible within the data, either household cluster or administrative division) and year, calculating the proportion of antibiotic usage in each location and year using the number of children reported to have received antibiotics as the numerator, and the

number of children with symptoms of LRI (as defined by that survey) as the denominator. Children missing data on antibiotic usage were considered to be missing at random and were excluded from the analysis at this point. The proportion of antibiotic usage was then adjusted for the given LRI definition, using the coefficients specified in Table 3.1, and any surveys conducted prior to 2000 were assigned the year 2000 (this included only surveys conducted in DHS phase four, 1997-2003). Data for all surveys were combined into one dataset containing data from 209 surveys and 284,045 children, covering 101 countries and 19 years (Figure 3.2).

Coefficient	Cough	Cough, rapid/ difficulty breathing & chest symptoms	Denominator Cough, rapid/ difficulty breathing & fever	Cough, rapid/ difficulty breathing	Cough & fever
Correlation (r^2)	0.89	0.93	0.91	0.92	0.9
Intercept	0.053	-0.002	0.036	0.021	0.038
Beta coefficient (β)	1.107	1.018	1.077	1.086	1.05

Table 3.1: LRI adjustment model coefficients. Correlation coefficient and linear regression coefficients for the relationship between the proportion of antibiotic usage in children with LRI (standard definition) to those with differing combinations of symptoms.

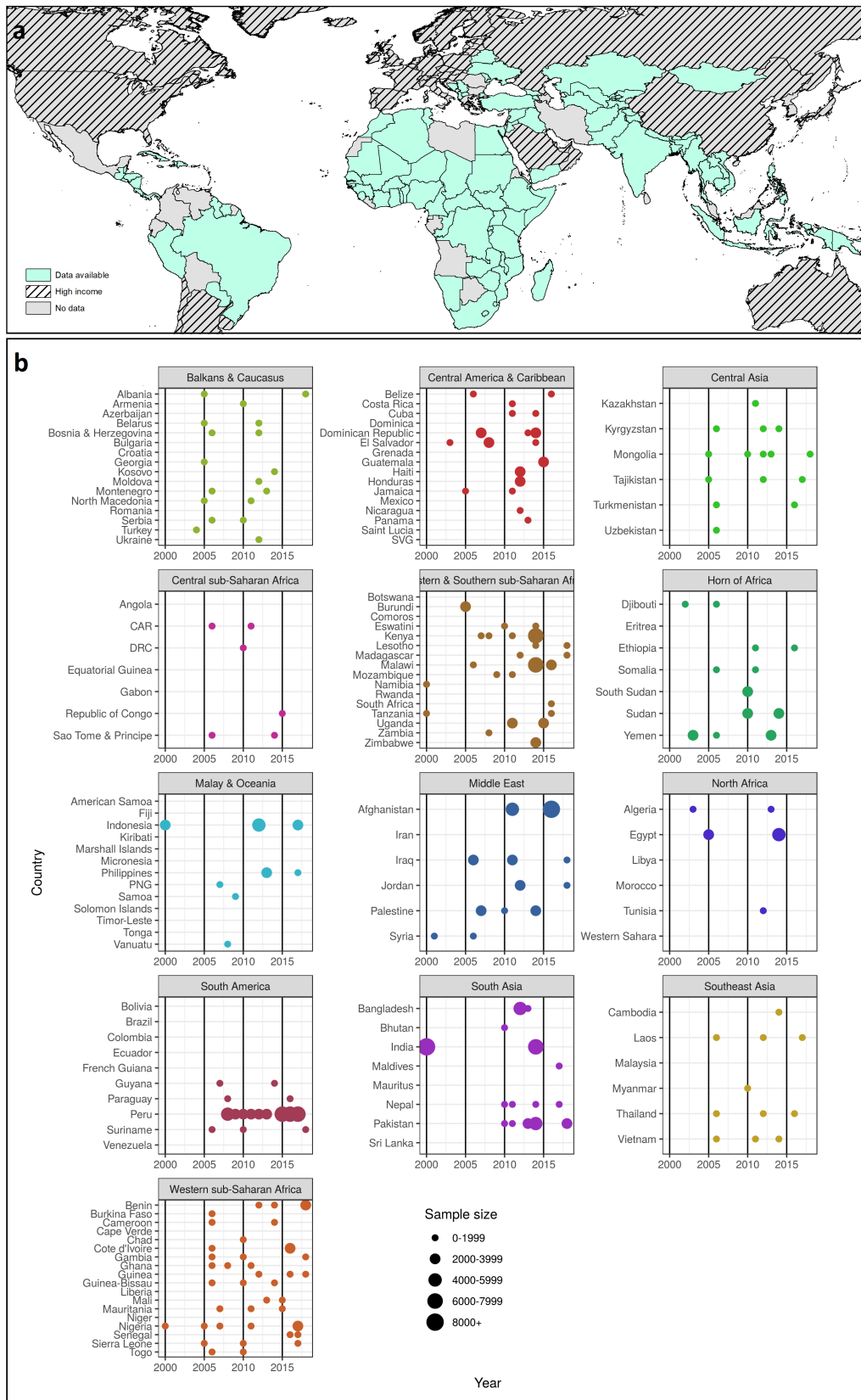


Figure 3.2: Antibiotic usage data availability map and plot. a) Data availability map; low- and middle-income countries with household surveys contributing to the model are in turquoise, countries with no data are in grey, whilst high-income countries (which are not being modelled) are shown in grey with diagonal stripes. b) Data availability plot; the x-axis indicates the year and the y-axis the country in which the survey was undertaken. The size of the points is representative of the number of children with LRI in each survey, and the colour indicates the modelling region to which that country belongs.

Polygon resampling

Data linked to point locations were used directly in the geostatistical model. To incorporate the data linked to administrative districts, the matched polygons were resampled to point locations prior to modelling, using a population weighted κ -means clustering methodology.⁸¹ For each polygon a raster of the WorldPop 2010 global population estimates,²⁰⁵ with a cell area of 5x5km at the equator, was cropped to the polygon boundary (Figure 3.3a). Ten thousand point locations (latitude and longitude of the cell centroid) were sampled from each polygon, with a sampling probability proportional to the cell population (Figure 3.3b). κ -means clustering was then applied to the point locations, resulting in one cluster per 1,000 raster cells within the polygon. Each cluster was then assigned an integration weight proportional to the number of points in the cluster (Figure 3.3c), with the weights constrained to sum to one. The resampled, weighted point locations were combined with the actual point data (which were assigned weights of one), and this dataset used as input data for the geostatistical model.

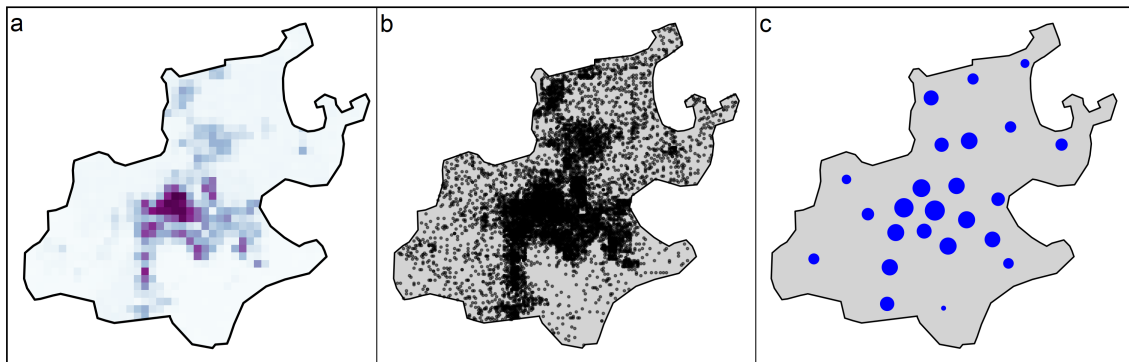


Figure 3.3: Polygon resampling. A pictorial representation of polygon resampling methods using κ -means clustering; a) a raster of the total population cropped to the polygon to be resampled; b) 10,000 point locations sampled with a probability based on the cell population; c) κ -means clustering applied to the point locations in (b), the size of the point represents the integration weight.

3.2.3 Antibiotic usage model

Overview

A two stage Bayesian binomial hierarchical model, established for fine scale mapping of health outcomes,^{81,82,196,203,206} was fit to estimate the probability of antibiotic usage for

each 5x5km pixel of the study area, and each year from 2000 to 2018. In the first stage, a stacked ensemble model was fit to the data using socio-demographic, health and environmental covariates. The estimates from these models were then used as the explanatory covariates to fit a spatially and temporally explicit binomial Bayesian generalised linear model to predict the probability of antibiotic usage.

The study area consisted of 127 LMICs. All countries defined as low or middle income by the World Bank,²⁰⁷ excluding Russia, China and Lebanon. Russia and China were excluded as they cover vast geographic areas and no data on antibiotic usage were available, meaning it was deemed inappropriate to extrapolate the model to these locations. Lebanon was excluded, as although it is classified as upper-middle income, it was deemed to be epidemiologically dissimilar to neighbouring countries such as Syria, and more epidemiologically similar to high-income countries in the region such as Israel.

The study area was split into 13 regions (Figure 3.4). Separate models were fit for each region to improve computational stability, incorporate epidemiological differences between regions, and to allow for differing effects and powers of covariates within the models. Predictions of antibiotic usage from the geostatistical model were not drawn for the Maldives, Mauritius, North Korea, Nauru, Tuvalu, American Samoa and Dominica. It was deemed unsuitable to extrapolate from the geostatistical model for these countries/territories as they either have small populations and limited data (Nauru, Tuvalu, Dominica and American Samoa have populations of less than 100,000 people); are located a long distance from neighbouring countries (Maldives and Mauritius), or had limited accurate covariate data (North Korea). For these countries the median of the national estimates of antibiotic usage for the corresponding region were calculated and assigned to that country/territory.

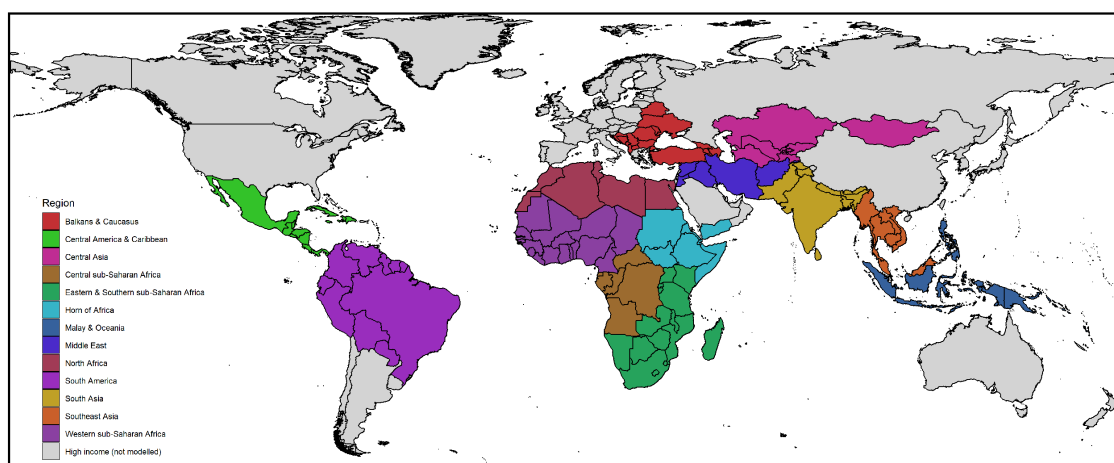


Figure 3.4: Epidemiological regions for the antibiotic usage model. Although not modelled, North Korea was considered part of Southeast Asia; American Samoa, Nauru and Tuvalu as part of Malay and Oceania, Dominica as part of Central America and the Caribbean, and Mauritius and the Maldives as part of South Asia.

Covariate selection

A selection of socio-demographic and health related covariates thought to impact the prevalence of antibiotic usage were considered for inclusion. When pairs of covariates were highly correlated ($r^2 > 0.8$) then one of the pair was dropped; covariates were then normalised by subtracting the mean from each value and dividing them by the standard deviation, resulting in all covariates having a mean of 0 and a standard deviation of 1. This is considered important in penalised regression models as it puts the regression coefficient onto the same scale and allows for the comparisons of covariates. Covariate selection was then based on their importance in the child models and their impact on the predictive performance of the model. The covariates used for each of the modelling regions are shown in Figure 3.5 and the sources detailed in Table B.1.

Covariate	Modelling region												
	South America	Central America & Caribbean	Balkans & Caucasus	Middle East	North Africa	Western sSA	Central sSA	Horn of Africa	Eastern & Southern sSA	Central Asia	South Asia	Southeast Asia	Malay & Oceania
Access to cities													
Ratio of children to adults													
Distance to rivers and lakes													
DPT3 coverage													
Mean maternal education													
Outdoor air pollution													
Population													
Mean temperature													
Malaria incidence													
Stunting													
Fertility													
GDP													
Normalised vegetation index													
Urbanicity													
Latitude													
Health access and quality indicator													

Figure 3.5: Antibiotic usage model covariates. Covariates are specified for each modelling region.

Stacked ensemble model

A stacked ensemble model was fit to the data and the selected explanatory covariates, following the methodology of Bhatt *et al.*²⁰⁸ The input data were matched to covariate values by latitude, longitude and year (for temporally varying covariates). For each region three child models were then fit: boosted regression trees (BRT), generalised additive models (GAM) and elastic net (E-net). The BRT, a decision tree-based machine learning algorithm, was fit using “xgboost” and the “caret” package version 6.085. A grid of potential model parameters were trialled and the final model fit on parameters resulting in superior predictive performance. The GAM models were fit using the “mgcv” package version 1.8.31, with smoothing terms applied to the covariates using thin plate regression splines. E-net is a penalised regression model which incorporates both Lasso (L1) and Ridge (L2) regularisation as was fit using the “glmnet” package version 3.0.2, setting the α to 0.5 and the λ to the minimum value within one standard deviation of the λ of the best performing model. This modelling was all conducted in R version 3.5.0.

All child models were fit using five-fold cross-validation. The out-of-sample predictions were then used as the explanatory covariates in the subsequent geostatistical model. This modelling strategy is beneficial as it aids in covariate selection, captures potential non-linear effects and accounts for interactions between covariates, improving the predictive power over single models alone.²⁰⁸

Geostatistical model

A spatially and temporally explicit binomial Bayesian generalised linear model (GLM), with a logit link function, was then used to estimate the proportion of antibiotic usage in each 5x5km pixel in the study area, and each year, 2000-2018. The logit probability of antibiotic usage (p_i) was estimated using a GLM with the out-of-sample predictions from the stacked ensemble model (χ_i) used as the explanatory covariates with the coefficients (β) constrained to sum up to one. An independent nugget effect (ϵ_i) was included alongside a Gaussian country level random effect (ϵ_C) to allow for differences in antibiotic provision, policies and political effects. A Gaussian survey level random effect

(ϵ_S) was included to account for biases and reporting inaccuracies; this was included in model fitting but not in prediction. Finally, a term was included for the residual spatial and temporal error (ϵ_{GP}), which was modelled as a three-dimensional Gaussian process with a covariance matrix as the Kronecker product of spatial covariance ($\mathbf{K}_{space}$), and temporal covariance ($\mathbf{K}_{time}$). Spatial covariance was modelled as a stationary Matérn function and temporal covariance as an autoregressive function of order 1 (AR1).⁸¹

$$\begin{aligned}
N_i^+ &\sim \text{Binomial}(p_i, N_i) \\
\text{logit}(p_i) &= \alpha + \boldsymbol{\chi}_i \boldsymbol{\beta} + \epsilon_{GP} + \epsilon_i + \epsilon_C + \epsilon_S \\
\epsilon_{GP} &\sim GP(0, \mathbf{K}_{space} \otimes \mathbf{K}_{time}) \\
\mathbf{K}_{space} &= (2^{\nu-1} \Gamma(\nu))^{-1} (\kappa \mathbf{D})^\nu K_\nu(\kappa \mathbf{D}) \\
\mathbf{K}_{time} &= p^{|t_\kappa - t_l|}
\end{aligned}$$

Priors were included on each of the random effects. These varied slightly by region and were selected to produce the models with the best predictive performance. The priors on ϵ_C , ϵ_i and ϵ_s were set to $\text{loggamma}(\alpha = 2, \gamma = 0.05)$ for all regions except North Africa and Malay and Oceania in which ϵ_C was changed to $\text{loggamma}(\alpha = 2, \gamma = 1)$ and Western sub-Saharan African in which ϵ_C was changed to $\text{loggamma}(\alpha = 2, \gamma = 4)$. Additionally a prior was placed on the spatial autocorrelation of $\theta_2 t = N(\mu = 2.5, \sigma^2 = 1/1.21)$. For the spatial hyperparameters (κ and τ), uncorrelated normal priors were automatically selected by R-INLA, based on the finite elements spatial mesh.

The models were fit using the Stochastic partial differential equation (SPDE)²⁰⁹ approach in Integrated Nested Laplace Approximations (R-INLA version 18.07.12), R version 3.5.0. A finite element spatial mesh was required to fit the SPDE approximation. Smoothed polygon boundaries for the modelled regions were used as templates for the meshes and inner triangles for the mesh were created with a maximum edge length set to 0.25 degrees and outer buffer triangles with a maximum edge length set to 5 degrees. The spatial mesh was uniform for all modelled regions (example for Western

sub-Saharan Africa in Figure 3.6). Laplace Approximations were then used to model the spatial-temporal error. One thousand draws of the model posterior were taken and the mean and 95% uncertainty intervals calculated for the probability of antibiotic usage for each pixel. Population weighted estimates of antibiotic usage at the district, province and national level were then calculated from the model draws using population estimates for each year of the study.²⁰⁵ The mean and 95% uncertainty intervals were calculated for each spatial aggregation and year estimated.

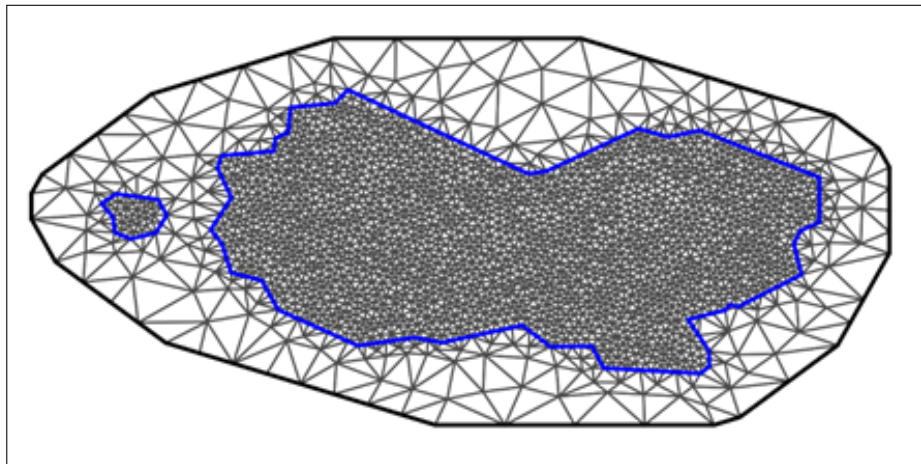


Figure 3.6: Finite element mesh for the Western sub-Saharan Africa region.

Model Validation

To assess the predictive validity of the model (i.e. understanding how well the model predicts to locations with no data) all models were run with five-fold cross-validation. The root mean square error (RMSE) and the R^2 were calculated between the input data and the out-of-sample predictions to assess model fit. The dataset contained relatively rare binomial outcomes with small sample sizes, meaning there are an abundance of antibiotic usage proportions in the survey data at 0, 0.5 and 1 relative to other values. The overabundance of antibiotic usage estimates at these proportions is not realistic, but an artefact of the small sample sizes. To stabilise the proportions and satisfactorily validate the model, the validation metrics were calculated on the data aggregated to the national level.

Analysis of model results

To analyse the spatial and temporal trends in antibiotic usage, the results were plotted on maps at the pixel, district, province and national resolutions for each year of the study, alongside the uncertainty intervals. To assess within country variation in antibiotic usage, the mean relative deviation of district level antibiotic usage was calculated for each country. The district level antibiotic usage was subtracted from, and divided by the country level usage value, and the mean of this calculated for each country. Comparisons were made between the relative deviation in antibiotic usage for each country in the years 2000 and 2018.

Antibiotic usage was then compared between urban and rural populations within each country and year. Each pixel of the study areas was designated urban or rural based on the Global Human Settlement layer,²¹⁰ and the population weighted mean antibiotic usage calculated for all urban and rural pixels within each country from 1,000 draws of the model posterior. The variation of antibiotic usage was assessed by calculating the relative percentage difference between the proportion of antibiotic usage in rural populations to that in urban populations, for each country and year.

Finally, antibiotic usage was analysed by the type of treatment sought for the child. For each survey included in the study that provided information on treatment seeking behaviour, a binary indicator was created on whether treatment or advice was sought from a formal healthcare setting (defined as a healthcare clinic or hospital) for children who received antibiotics. Although this is not a direct indicator of where antibiotics were obtained from, it provides an indication of the availability of healthcare in these settings and a proxy for where the antibiotics were obtained. These data were then summarised at the region and super-region level.

3.3 Results

3.3.1 Spatial and temporal variation in antibiotic usage

The model results showed high spatial and temporal variation in antibiotic usage across the study area. In 2018 antibiotic usage was highest in the GBD super-region of Central Europe, Eastern Europe and Central Asia, where the median national antibiotic usage was 72% [interquartile range 60-78%]. Antibiotic usage was lowest in sub-Saharan Africa, with a median national usage of 42% [IQR 35-49%] in 2018 (Figure 3.7). Between 2000 and 2018 antibiotic usage increased in all super-regions except for Southeast Asia, East Asia and Oceania where the median antibiotic usage remained the same.

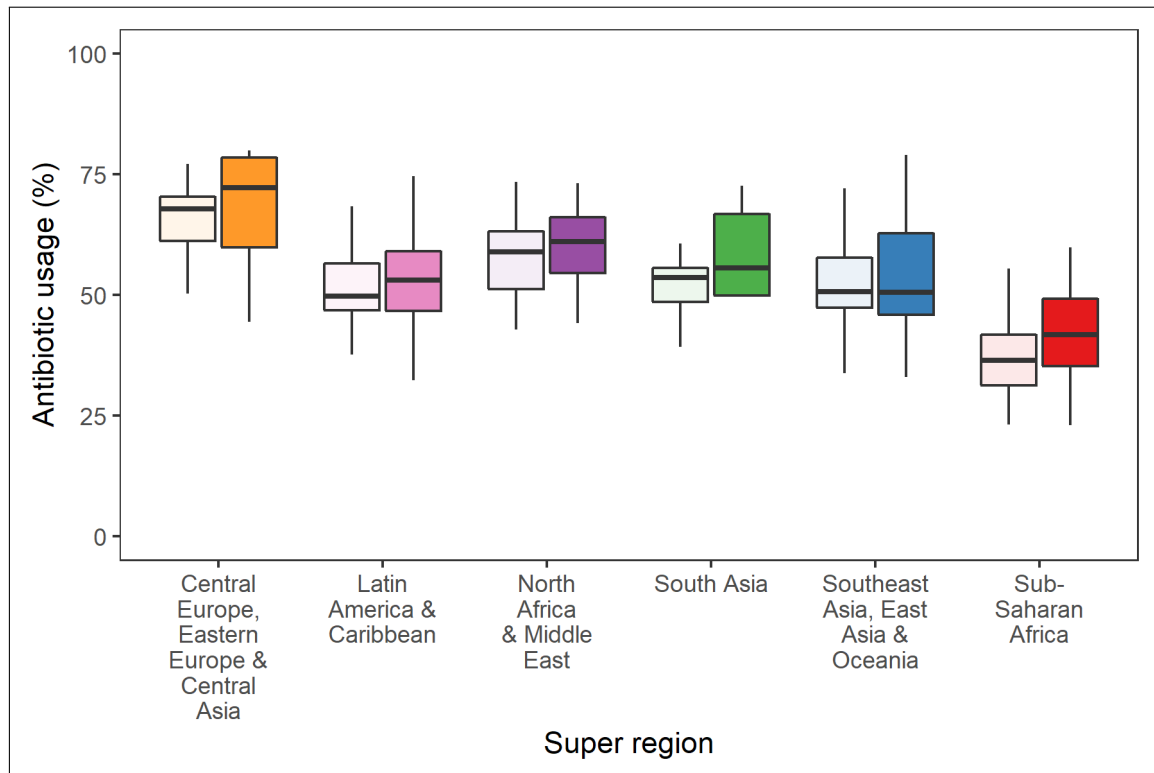


Figure 3.7: Box and whisker plots of the median antibiotic usage by GBD super-region for 2000 and 2018. The median and interquartile range of caregiver reported antibiotic usage for children under-five with symptoms of LRI for LMICs by GBD super-regions are shown for the year 2000 (faded colours), and 2018 (block colours).

National estimates of antibiotic usage (in children under-five with LRI) in 2018 were highest in Ukraine (80% [95% uncertainty interval 68-89%]), Kazakhstan (80% [74-85%]), Bosnia and Herzegovina (79% [73-85%]), and Belarus (79% [73-84%]); the lowest antibiotic usage was estimated in Mauritania (23% [15-32%]), Guinea-Bissau

(24% [15-34%]) and Ethiopia (28% [24-31%]). Eighty-one percent (34/41) of countries in sub-Saharan Africa had national antibiotic usage of 50% or less. Interestingly, antibiotic usage was considerably higher in Sudan (73% [69-77%]) compared to the rest of the Sahel region (Figure 3.8).

In Southeast Asia antibiotic usage was very diverse in 2018. The median usage was 51% [IQR 46-63%] and was highest in Vietnam (74% [68-80%]) and Malaysia (79% [71%-85%]), whilst Papua New Guinea, Philippines and Indonesia were notably lower at 33% [16-54%], 42% [28-56%] and 43% [33-54%] respectively. In South Asia, antibiotic usage was reasonably high in India (67% [58-74%]) and Bangladesh (73% [59-84%]), with slightly lower rates in neighbouring Pakistan (50% [41-60%]) and Nepal (50% [35-65%]).

In Latin America and the Caribbean there was a large spatial variation in antibiotic usage in 2018, with low rates in Nicaragua (46% [39-53%]) and El Salvador (47% [40-53%]) differing greatly from high rates in Panama (75% [67-81%]) and Cuba (67% [60-73%]) (Figure 3.8).

In general, between 2000 to 2018 there were only small increases in the prevalence of antibiotic usage, with levels in sub-Saharan Africa consistently being the lowest and Central Europe, Eastern Europe and Central Asia the highest (Figure 3.7). Some countries did experience large increases in antibiotic usage, most notably Sudan (49% [45-55%] to 73% [69-77%]), India (48% [41-56%] to 67% [58-74%]), Vietnam (59% [50-67%] to 74% [68-80%]) and Cambodia (63% [51-73%] to 77% [70-84%]). Antibiotic usage also increased greatly in Mexico from 44% [35-52%] in 2000 to 65% [57-73%] in 2018, whilst most other countries in Latin America and the Caribbean remained stable across the study period (Figure 3.9).

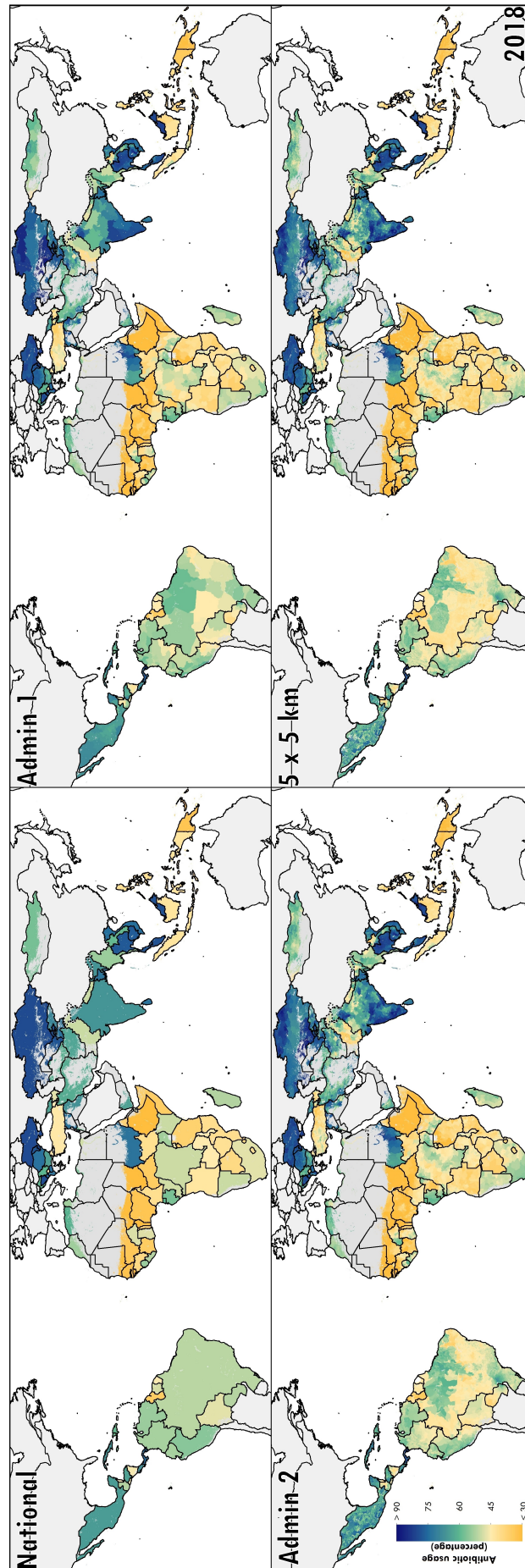


Figure 3.8: Antibiotic usage in children under-five with symptoms of LRI in 2018. Modelled estimates of the percentage of children under five years old with symptoms of LRI with caregiver reported antibiotic usage in LMICs, in 2018, shown by: a) national level aggregation; b) level 1 administrative divisions; c) level 2 administrative divisions; and d) 5 x 5km pixels. High income countries and pixels (1x1 km) with populations of less than 10 are shown in grey.

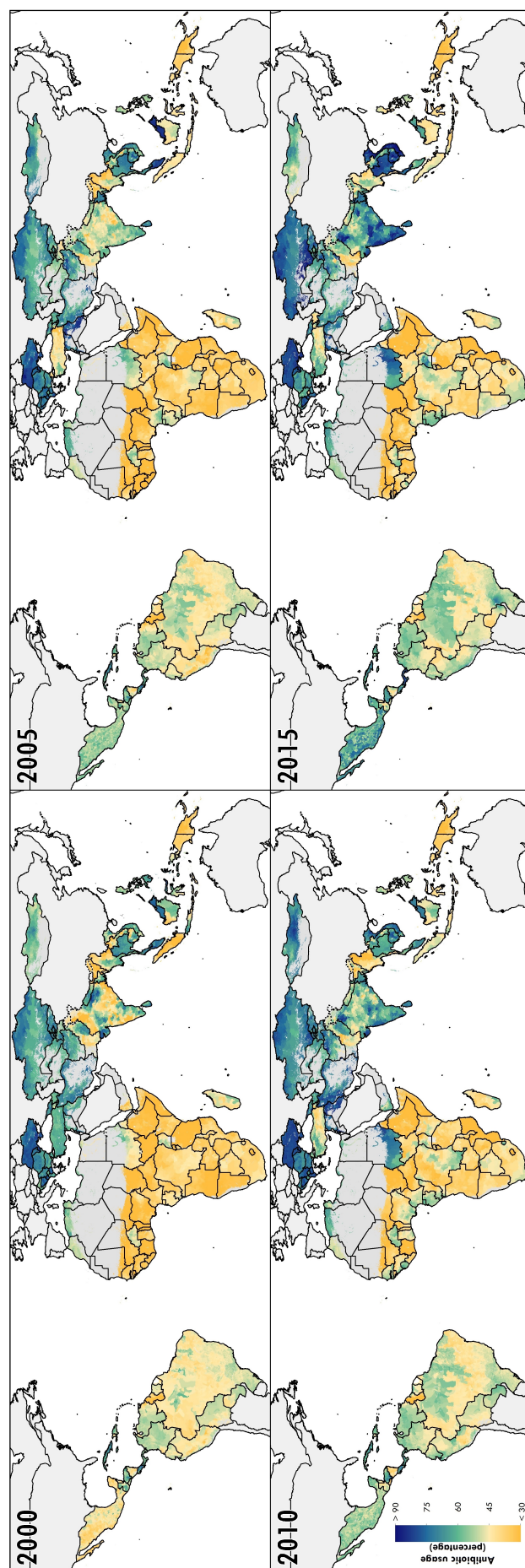


Figure 3.9: Five yearly estimates of antibiotic usage in children under-five with symptoms of LRI. Modelled estimates of the percentage of children under five years old with symptoms of LRI with caregiver reported antibiotic usage in LMICs, displayed by second level administrative divisions for the years: (a) 2000; (b) 2005; (c) 2010; (d) 2015; (e) 2020. 1x1 km pixels with a population of less than 10 people have been masked.

The relative uncertainty of the antibiotic usage estimates were calculated for each district (the ratio of the uncertainty intervals to the proportion of antibiotic usage) and displayed in relation to the proportion of antibiotic usage (Figure 3.10). Antibiotic usage estimates in Latin America and the Caribbean varied greatly in 2018 (32-75%); however, uncertainty in this region was high, due to a paucity of data. In India and Central Asia the high estimates of antibiotic usage were accompanied by low relative uncertainty. Conversely, the low estimates of antibiotic usage in Indonesia, Philippines and Papua New Guinea have a high relative uncertainty. In sub-Saharan Africa, low relative uncertainty was estimated in many countries (Ethiopia, South Sudan, Central African Republic, Nigeria, Benin, Côte d'Ivoire). However, uncertainty was high in locations lacking data such as Botswana, Mozambique, and Niger (Figure 3.10). High uncertainty indicates locations where more data are required to improve the model estimates. Maps of the upper and lower 95% uncertainty interval estimates of antibiotic usage are shown in the appendices.

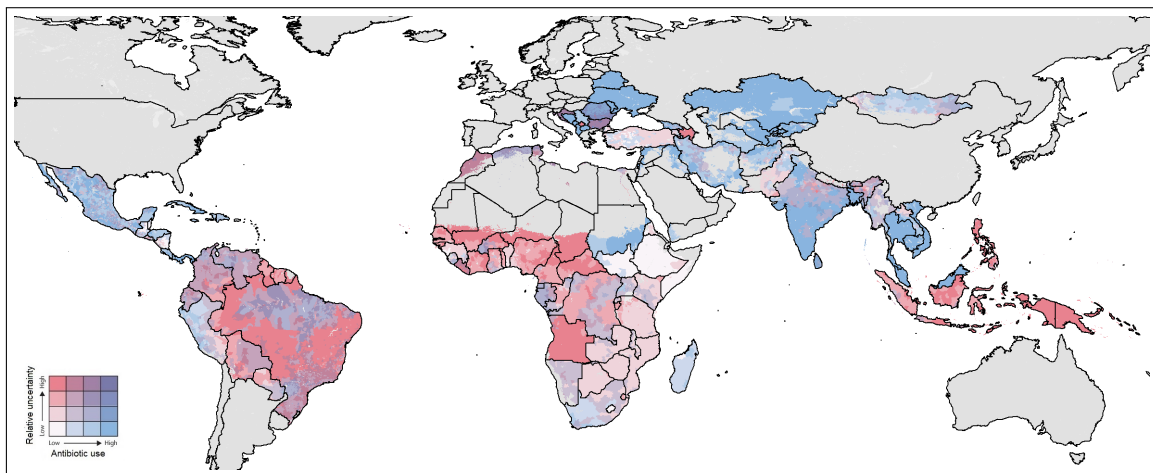


Figure 3.10: Relative uncertainty of antibiotic usage in children under-five with symptoms of LRI. Estimates are shown by second level administrative divisions. Relative uncertainty was defined as the ratio of the 95% uncertainty intervals to the proportion of antibiotic use. Data were coloured based on the quartile of the proportion of antibiotic use and the quartile of relative uncertainty to which they belonged. Pixels (1x1km) with populations less than 10 persons are masked in grey.

3.3.2 Subnational variation in antibiotic usage

High inequalities in antibiotic usage within countries were identified throughout the study period. In 2018 the mean relative deviation of antibiotic usage (of admin 2 districts from the country value) was highest in Western sub-Saharan Africa, most notably in

Benin, Mauritania, and Mali (25%, 23% and 22% respectively). Large variations were also noted in India (14%) and Laos (18%) (Figure 3.11). In India the mean relative deviation of antibiotic usage decreased greatly between 2000 and 2018 (from 28% to 14%) as antibiotic usage become more ubiquitous. Large reductions were also evident in Indonesia (23% to 10%) and Tanzania (24% to 12%). Interestingly, antibiotic usage became more unequal over the study period in numerous countries in sub-Saharan Africa; with the mean relative deviation increasing between 2000 and 2018 in Niger, Benin, Guinea, Mali, Burkina Faso and Ethiopia, amongst others (Figure 3.11).

Antibiotic usage was lower in rural than urban locations for most countries in 2018. The largest disparities were seen in sub-Saharan Africa where the relative percentage difference of rural to urban antibiotic usage in Mauritania, Somalia and Benin were -38%, -29% and -28% respectively. The largest urban-rural disparity in Southeast Asia, East Asia and Oceania was seen in Laos (-16%). There were some countries where antibiotic usage in rural location exceeded that in urban locations. In sub-Saharan Africa and the Caribbean these tended to be smaller island nations such as Sao Tome and Principe, Comoros, Dominican Republic and Grenada (Figure 3.12).

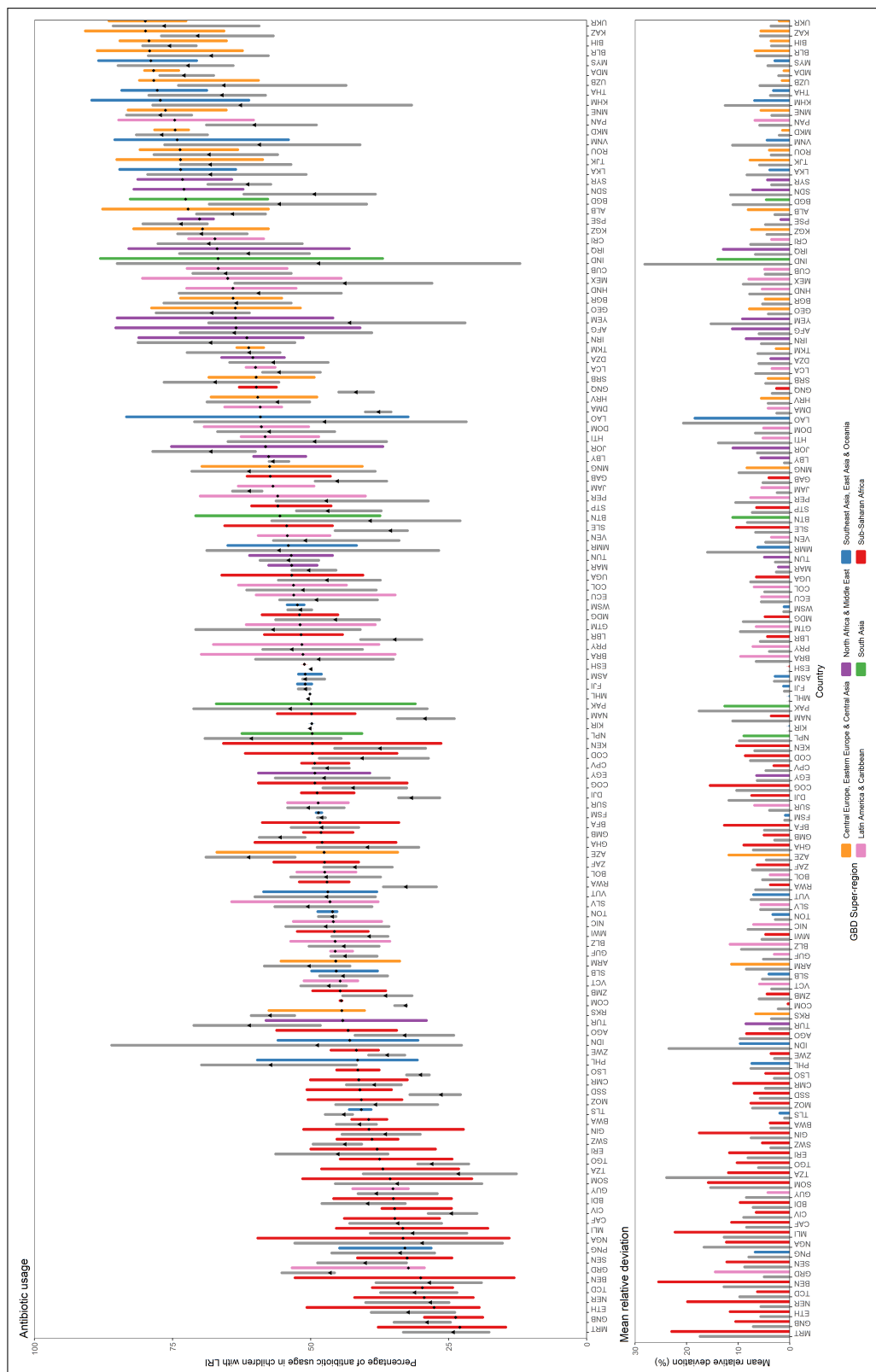


Figure 3.11: Within-country variation in antibiotic usage in 2000 and 2018. (a) Bars show the range in antibiotic usage for districts within each country; coloured bars represent estimates from 2018 and grey bars represent estimates from 2000. Points represent the mean percentage of antibiotic usage (y-axis) for each country (x-axis), with diamonds representing 2018 and triangles 2000. (b) The bars show the mean relative deviation of antibiotic usage in admin 2 districts from the country level antibiotic usage; the coloured bars represent estimates from 2018 and the grey bars represent estimates from 2000. The 2018 colours are based on the GBD super-region to which the country belongs and countries are ordered (on the x-axis) based on the mean antibiotic usage in 2018 (ascending). Countries are labelled using the ISO codes, available from <https://www.iban.com/country-codes> (last accessed 30/06/2020).

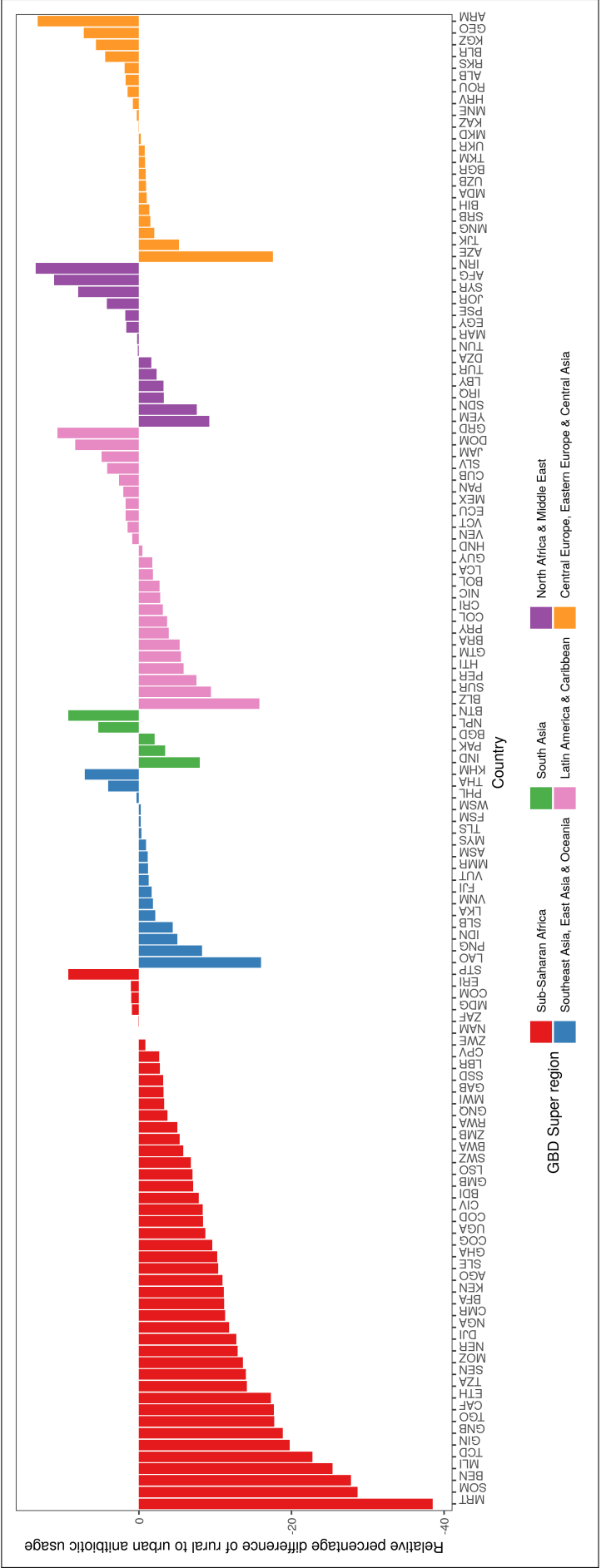


Figure 3.12: Relative percentage difference of antibiotic usage in rural and urban locations. The colours are based on the GBD super-region to which the country belongs. Countries are labelled using the ISO codes, available from <https://www.iban.com/country-codes> (last accessed 30/06/2020).

3.3.3 Antibiotic usage and treatment seeking behaviour

The proportion of children with LRI receiving antibiotics, who sought formal healthcare (caregiver reported) was calculated for each household survey. This does not directly show where the antibiotics were obtained from, but provides an indication of whether medically trained personnel were involved in the child's treatment, and therefore increases the likelihood that antibiotics were correctly prescribed, and of good quality. In Central and Eastern Europe, on average (median of all surveys), formal healthcare was sought for over 80% [interquartile range 74-92%] of children receiving antibiotics. Interestingly, this percentage was also very high in Southeast Asia (76% [IQR 71-84%]) and North Africa and the Middle East (80% [IQR 74-82%]). In sub-Saharan Africa formal healthcare was sought for far fewer children, indicating antimicrobials were likely obtained from other, unregulated sources. This was most evident in Central sub-Saharan Africa (46% [IQR 44-53%]), with slightly higher values in Eastern and Western sub-Saharan Africa (68% [IQR 52-79%] and 63% [IQR 53-82%] respectively; Table 3.2).

Super-region (Region)	Median [IQR] percentage of children with LRI using antibiotics seeking formal healthcare	Number of surveys
North Africa & Middle East	80 [74-82]	14
North Africa & Middle East	80 [74-82]	14
Sub-Saharan Africa	68 [52-84]	64
Central Sub-Saharan Africa	46 [44-53]	4
Eastern Sub-Saharan Africa	68 [52-82]	18
Western Sub-Saharan Africa	63 [53-82]	36
Southern Sub-Saharan Africa	80 [78-85]	6
South Asia	59 [42-70]	9
South Asia	59 [42-70]	9
Central Europe, Eastern Europe & Central Asia	82 [74-82]	14
Central Europe	96 [92-96]	7
Eastern Europe	92 [89-93]	4
Central Asia	74 [69-80]	13
Latin America & Caribbean	76 [71-85]	33
Caribbean	82 [71-90]	16
Andean Latin America	76 [75-78]	10
Tropical Latin America	91 [91-91]	1
Central Latin America	72 [64-81]	6
Southeast Asia, East Asia & Oceania	79 [71-84]	16
Southeast Asia	79 [71-84]	15
Oceania	71 [71-71]	1

Table 3.2: Formal healthcare seeking behaviour in children with LRI using antibiotics. The proportion of children under five years old with LRI who received antibiotics, for whom treatment was sought from a formal healthcare setting (defined as a hospital or clinic) was calculated for all identified household surveys reporting antibiotic usage in at least 20 children. The median and interquartile range of this percentage is displayed for each GBD super-region and region of the study area.

3.3.4 Model validation

Validation metrics were calculated between the out-of-sample predictions of the model (aggregated to the national level) and the total proportion of antibiotic usage from each survey in the input dataset. The full model was correlated to the input data with an R^2 of 0.53 and had an RMSE of 0.14 (Figure 3.13). The predictive validity varied by region with the model performing best in the African regions, South America and the Balkans and Caucasus, but with lower predictive validity in Asia (Table B.2; Figure B.2). This is partially due to a paucity of survey data in these regions, but also indicates that complex drivers of antibiotic usage might not be captured by the covariates or spatial-temporal trends. The proportion of held-out data points for which the model predictions were within the 95% uncertainty intervals of the model was 83%.

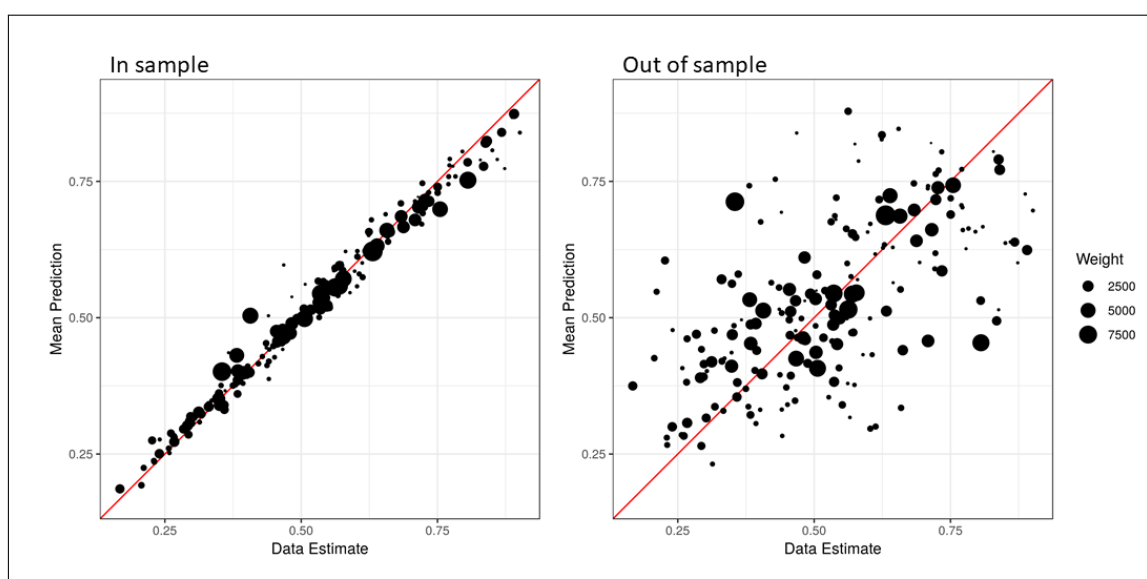


Figure 3.13: Validation plots for the model of antibiotic usage. Scatter plots representing the mean proportion of antibiotic use for each survey, at the national level, compared to: a) the in-sample, and b) the out-of-sample model predictions for that country-year. Out-of-sample estimates were based on five-fold cross validation.

3.4 Discussion

3.4.1 Overview

This study enhances the understanding of community level antibiotic usage by providing the first detailed subnational estimates of antibiotic usage in children under five with LRI

in LMICs. Large variations in the proportions of antibiotic usage in children were identified, with the highest levels in 2018 estimated in Central Europe Eastern Europe and Central Asia (median antibiotic usage of 72%), and the lowest levels in sub-Saharan Africa (median antibiotic usage 43%). In general, antibiotic usage increased over the study period and became more equitable within countries, however, large inequalities in antibiotic usage within countries persisted, particularly in sub-Saharan Africa.

A recent study²¹¹ also utilised household surveys to estimate antibiotic usage in children with fever, diarrhoea and LRI across LMICs, and provided estimates grouped by WHO and World Bank regions. Similar to our results, the highest antibiotic usage was estimated for European LMICs. However, whilst our estimates showed the lowest antibiotic usage to be in sub-Saharan Africa, Allwell-Brown *et al.* estimated the lowest usage to be in Southeast Asia.²¹¹ Similar to our results, they estimated modest increases in antibiotic usage between 2005 and 2017.²¹¹ The results are not directly comparable due to differing methodologies, and it is hypothesised that the observed differences may be partially due to regional estimates of antibiotic usage disguising spatial and temporal trends, as well as the differing rates of antibiotic usage between the illnesses modelled. The covariates used in this model were selected to improve the models out-of-sample predictive performance and not to infer causation or determine associations with antibiotic usage. The covariates used differed from those used by Allwell-Brown *et al.* and may explain some of the variations in estimates of antibiotic usage between the studies. Although these factors may contribute to the different results, it was not possible to determine the reasons for these differences from the provided data at this time.

The prevalence of antibiotic usage in countries that stood out as having extreme values (either low or high compared to neighbouring countries) were investigated further. Sudan was estimated to have high levels of antibiotic usage in recent years. This was corroborated by studies reporting high levels of non-prescription antibiotic use in two separate adult population in Khartoum state.^{58,212} High levels of antibiotic usage were also evident in Southeast Asia towards the end of the study period, particularly in Vietnam. Vietnam is an emerging economy with a high burden of infectious disease and

easy, unrestricted access to antibiotics.⁵⁸ In recent years access to antibiotics has increased greatly (indicated by the reduction in the relative deviation of antibiotic usage). Although there are regulatory frameworks in place, these are often not enforced leading to both excessive and inappropriate use of antibiotics.^{58,213}

Subnational estimates from this study highlight inequalities in antibiotic usage within countries, underlining issues of access to healthcare, particularly in poor and rural communities. Controlling antibiotic usage is a balancing act between improving the lack of access to antibiotics and preventing their overuse. Although the inequalities have generally decreased over the past two decades, these disparities still remain very high, and have even increased in the poorer countries of the world where clear disparities in urban and rural antibiotic usage persist. Whilst in emerging economies, where the focus has previously been on improving access to antibiotics, this needs to be implemented in conjunction with improving regulations and decreasing inappropriate use of essential antibiotics.

In European LMICs the vast majority of children with LRI who received antibiotics sought formal healthcare. Although it is difficult to infer appropriate use of antibiotics from this data, this does indicate that medical professionals were involved in the decision to treat with antibiotics and that antibiotics were likely obtained from a formal source. In sub-Saharan Africa the proportion seeking formal healthcare was far lower, indicating a high likelihood of inappropriate use of unregulated medicines. Falsified and substandard medicines are common in LMICs, and with little regulation their use will both fail to cure treatable infectious diseases and propagate AMR.^{214,215}

3.4.2 Limitations

The main limitations of this study are centred around data quality and availability. Whilst DHS and MICS surveys are the only sources of representative and comparable data, they contain multiple biases. Firstly, small sample sizes restrict the ability to accurately determine the prevalence of disease and prevalence of antibiotic usage from the sample;²¹⁶ secondly, the use of caregiver reported symptoms is inaccurate in

determining disease occurrence (the denominator); thirdly, the inaccuracy of caregiver identification of antibiotics introduces further uncertainty.²¹⁶

It is not possible to identify the types of antibiotics taken from the household surveys (due to the style of question), or to ascertain whether the antibiotics were used for the correct indication and at the correct dosage. Due to these factors, it is not possible to calculate the overall amounts of antibiotic consumed in LMICs from these data. There are currently limited alternatives to these surveys in LMICs, and if interpreted with care, they provide a useful resource for comparing spatial and temporal trends in antibiotic usage.^{216,217}

These surveys only provide data on antibiotic usage in children under five years; there is limited standardisable data for other age groups. Although there is some evidence that antibiotic usage in children exceeds that in adults in LMICs,²¹⁸ healthcare utilisation surveys revealed a strong tendency for adults to self-medicate with over-the-counter antibiotics in LMICs,^{65,219,220} therefore the modelled metric was deemed suitable to provide spatial-temporal comparisons on the prevalence of antibiotic usage, as long as the limitations are taken into consideration when interpreting the absolute values.

3.4.3 Conclusions

In conclusion, this study provides the first spatially and temporally refined estimates of antibiotic usage in children under five in LMICs. Although there is a paucity of data and the discussed limitations need to be taken into consideration, this provides an overview of how the prevalence of antibiotic usage differs both between and within countries, and how this has evolved over the previous two decades. Understanding where there is a lack of access to antibiotics and where there is an overuse of antibiotics is essential for improving their utility in treating infectious diseases, as well as safeguarding them against overuse and the consequent establishment and propagation of AMR.

Finally, although this does not provide quantitative estimates for the amount of antibiotic consumption in each country and year, it provides an interesting metric to

assess the use of antibiotics in LMICs and can be expanded on further to fully assess global antibiotic consumption.

Global antibiotic consumption, 2000 to 2018

4.1 Introduction

Surveillance of antibiotic consumption is a vital component of strategies combatting the growing threat of AMR.^{30,221} Estimates of antibiotic consumption, comparable between countries and over time, are important for informing policy on increasing access to, or restricting the overuse of essential antibiotics. They can provide a benchmark from which to measure the effectiveness of interventions and to monitor the trends in antibiotic consumption.

A comprehensive understanding of the quantity and types of antibiotics used in certain locations is important for ensuring the availability and implementation of effective antibiotic therapies for bacterial infections, as well as for monitoring the emergence and propagation of AMR infections. Access to broad spectrum antibiotics is essential for treating common bacterial infections. At the same time, appropriate use of antibiotics is critical to maintain their efficacy and fight the growing threat of AMR.

The WHO implemented the Access, Watch, Reserve (AWaRe) categorisation system for antibiotics in 2017,²²² updated in 2019.²²³ “Access” antibiotics are identified as those with a wide range of activity against common bacterial infections and should be widely available (and affordable) for use as first or second line treatments. The “watch” category is defined as those antibiotics with a higher potential for the development of resistance and states that their use should be limited and carefully monitored by surveillance programmes. Finally, “reserve” antibiotics are those which should only be used as a last resort for the treatment of life-threatening drug-resistant infections.²²² This

provides a useful resource for monitoring consumption of antibiotics, guiding policy to increase availability of access antibiotics whilst preventing the overuse of watch and reserve antibiotics, preserving their efficacy for future use.

High quality surveillance systems on antibiotic consumption exist in HICs^{224,225} and the WHO plays an important role in promoting national surveillance of antibiotic consumption.²⁰¹ WHO published their first report on antibiotic consumption surveillance in 2018,²⁰¹ and although data from 65 countries and territories were compiled, the data were only for 2015/16 and the majority came from HICs, primarily in Europe. Antibiotic consumption data are sparse in LMICs and only a few studies have been undertaken to quantify antibiotic consumption for multiple countries.^{64,187,226} These studies are based on a single data source, namely the IQVIA dataset, and do not provide estimates for individual countries not covered by the dataset.

Chapter Three provides a useful spatial and temporal analysis of antibiotic usage in LMICs. However, this analysis does not cover HICs or provide measures of the amount or type of antibiotics consumed within a country and year. It is important to understand the global situation and enable comparisons between HICs and LMICs over time. Additionally, a comprehensive, quantitative understanding of both the amounts and types of antibiotics used in locations is important for directing policy. This chapter aims to combine multiple data sources and use spatial-temporal statistical models to estimate total antibiotic consumption for 204 countries for 2000 to 2018, alongside analyses of antibiotic consumption by Anatomical Therapeutic Chemical (ATC) classifications and WHO AWaRe category.

4.2 Methods

4.2.1 Overview

In this chapter a dataset of antibiotic consumption was compiled for all countries for 2000 to 2018. Antibiotic consumption refers to an aggregate measure of the total amount of various antibiotics consumed by a country and is generally derived from national

sales, import and reimbursement databases.²⁰¹ The total antibiotic consumption for 204 countries over a period of 19 years was estimated using separate models for HICs and LMICs, incorporating the antibiotic usage estimates from Chapter Three, alongside additional health and socio-demographic related covariates for the latter model (Figure 4.1).

The ATC classification system groups drugs into various categories at five levels, based on the organ or system on which they act and the pharmacological and therapeutic properties of the drug. This chapter focusses on antibacterials for systemic use, which are defined by the ATC level two code J01. Further analysis was then undertaken to estimate consumption rates of groups of antibiotics classified by the ATC level three and level four categories, along with analysis by the AWaRe categories. The spatial and temporal trends in antibiotic consumption were analysed at the country, super-region and global level for all indicators.

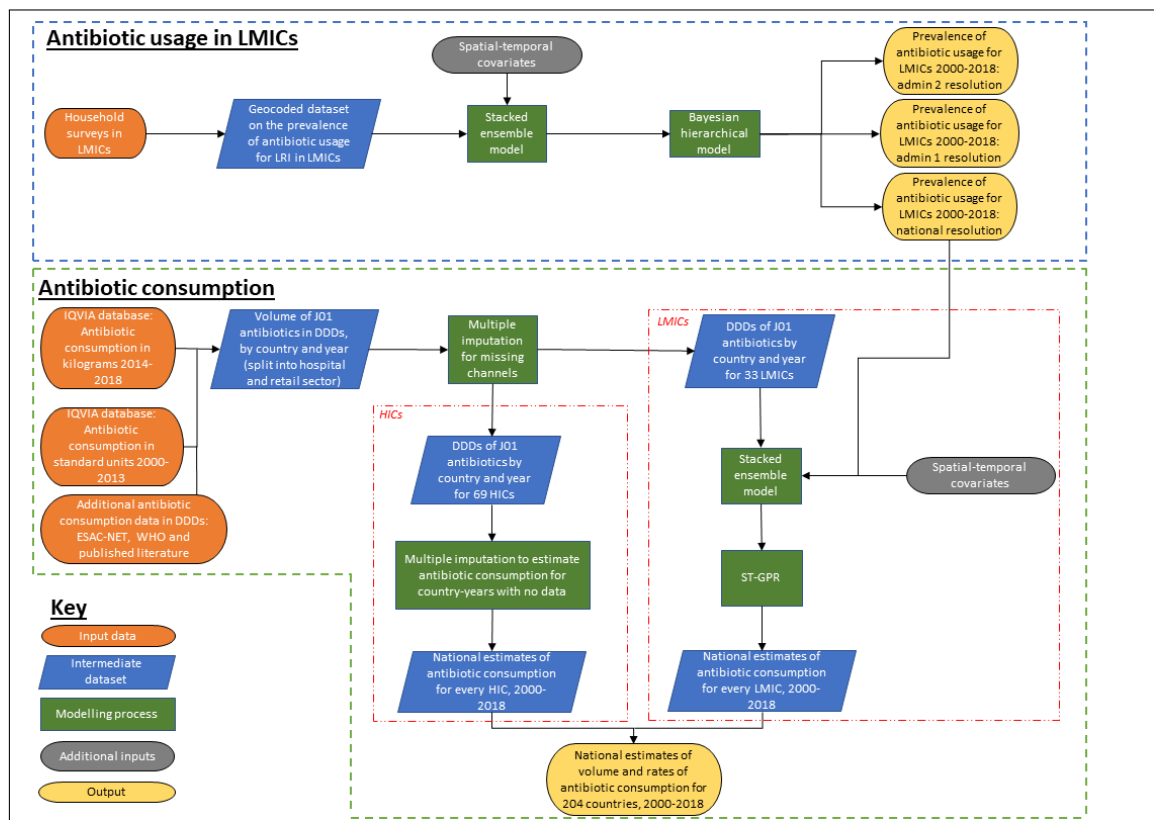


Figure 4.1: Antibiotic consumption model schematic. A schematic diagram for the modelling of antibiotic usage and consumption.

4.2.2 Antibiotic consumption data

Data measuring human consumption of antibiotics for systemic use (ATC classification J01) were accessed from multiple sources and compiled into a dataset reporting antibiotic consumption in defined daily doses (DDDs) by country and year. DDDs are a standardised WHO metric defined as “the assumed average maintenance dose per day for a drug used for its main indication in adults”,²²⁷ and were calculated using the 2019 version of the ATC/DDD guidelines and index.^{227,228} This allowed for a standardised comparison of antibiotic consumption between countries and years. Antibiotic consumption data were accessed from IQVIA, WHO, European Surveillance of Antimicrobial Consumption Network (ESAC-Net) and published literature. Data from each of the sources were analysed and compared to each other for consistency and duplication, prior to being combined into one dataset.

IQVIA data

The primary source of antibiotic consumption data is a proprietary dataset from IQVIA. They conduct national surveys and audits to calculate the total sales of each antibiotic preparation by country and year. They provided two datasets, one for 2000-2013 and one for 2014-2018, with antibiotic consumption data for 76 countries and territories. Data were aggregated for Central America (Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) and French West Africa (Benin, Burkina Faso, Côte d’Ivoire, Cameroon, Congo, Gabon, Guinea, Mali, Senegal and Togo); whilst data for China and Hong Kong were provided separately. Data were disaggregated by the “channel” in which they were sold, hospital or retail.

For the 2000-2013 dataset antibiotic sales were provided in “standard units” for each preparation of each antibiotic (i.e. one record for each available brand/product of each antibiotic). A standard unit is defined as one unit of the preparation and is often one tablet/capsule or one 1ml or 5ml dose of parenteral antibiotic. The dataset was limited to J01 antibiotics, excluding any antibiotics deemed to have primary uses as topical agents or for veterinary use, and each preparation was matched to the ATC level 5 code (indicating

the actual chemical substance, i.e. one code per antibiotic) based on the molecule name.²²⁸ The total weight of antibiotic, in grams, in each preparation was calculated by multiplying the weight of antibiotic (provided in the IQVIA dataset) by the number of standard units. This was aggregated to the total quantity, in kilograms, of each antibiotic by country, year and channel.

For the 2014-2018 dataset, data were provided as the total weight (in kilograms) of antibiotic sales by antibiotic, channel, country and year. The dataset was limited to J01 antibiotics, each substance matched to its ATC level 5 code and was then combined with the 2000-2013 dataset. For antibiotics combined with salts, diluents or probiotics, the additional substances were considered to have a negligible contribution to the total weight.

The number of DDDs consumed for each antibiotic was calculated using the WHO methodology.^{227,228} This methodology provides a value, in grams, which is considered the DDD for each antibiotic (Table C.1). The weight of each antibiotic was converted from kilograms to grams and divided by the corresponding DDD value to get the total number of DDDs consumed for each antibiotic by country, year and channel. For combination antibiotics without specific ATC level 5 codes and DDD values, the DDDs were calculated and split between the component antibiotics, based on the standard preparations of the combination substances. In cases where the oral and parenteral DDD values differed expert opinion was used to select the suitable DDD value (Table C.2). The rate of antibiotic consumption was then calculated as DDDs per 1,000 population per year (DDD/1,000/year) for each antibiotic using the population estimates from the GBD 2019 study (available from <http://ghdx.healthdata.org/>). For French West Africa and Central America, the sum of the populations from all included countries were used to calculate the DDD/1,000/year, and this value assigned to all countries in the territory to enable estimates for individual countries to be made.

For the majority of countries data were available from both hospital and retail channels, however, some countries had retail data only. Multiple imputation was used to fill in these missing values, using the classification and regression trees (CART) method from the “mice” package version 3.7.0.²²⁹ MICE, Multivariate Imputation by Chained

Equations, is a method for dealing with missing data which is based on Fully Conditional Specification (FCS).²³⁰ Each incomplete variable is imputed by a separate model with multiple iterations generated by Gibbs sampling, producing plausible values for each target variable based on a set of predictor variables within the dataset. The predictor variables were super-region, region, country, year, ATC3 code, ATC4 code and retail DDD/1,000/year. The classification and regression trees algorithm was selected for use as this produced the most realistic values when trialling imputation methods.

The multiple imputation algorithm was run 10 times, with 100 iterations to impute the missing DDD/1,000/year values. The imputed values were squeezed to be within the existing range of values for each ATC level three group of antibiotics. The total consumption of J01 antibiotics was then calculated in DDD/1,000/year for each country and year, as well as the total DDD/1,000/year for each ATC level three class of antibiotics (J01A tetracyclines; J01B amphenicols; J01C beta-lactam antibacterials - penicillins; J01D other beta-lactam antibacterials; J01E sulfonamides and trimethoprim; J01F macrolides, lincosamides and streptogramins; J01G aminoglycosides; J01M quinolones), selected ATC level four classes (J01CA broad spectrum penicillins; J01DD third-generation cephalosporins; J01DH carbapenems; J01MA fluoroquinolones), and for each AWaRe category (Access, Watch, Reserve, the designation of antibiotics to each of these categories is shown in Table C.3).²²³

WHO data

The “WHO report on surveillance of antibiotic consumption”²⁰¹ contains estimates of total antibiotic consumption (J01 antibiotics), and antibiotic consumption by ATC level three class, in DDD/1,000/day for 65 countries, for either 2015 or 2016. Data for 40 countries overlapped with the IQVIA datasets; similar rates of antibiotic consumption were estimated by WHO and IQVIA, with a correlation of $r^2 = 0.83$ between the two datasets (Figure C.1), which increased to $r^2 = 0.9$ when excluding the non-representative data points from the WHO data (Austria, Czech Republic, Germany, New Zealand and Spain only contained antibiotic consumption data from the community; Jordan covered

less than 70% of the population and was not population adjusted). Large amounts of data were from the same primary sources; to avoid duplication the IQVIA dataset was deemed to be the gold standard (as it covered a larger number of countries and years, provided disaggregated data, and contributed data to WHO), and data from WHO was only included for countries not covered by the IQVIA dataset. The exception to this was Brazil, where the WHO estimate of antibiotic consumption was considerably higher than that from the IQVIA dataset. These data came from different sources, with IQVIA being from an audit of retail antibiotic sales and WHO data being from manufacturers. No reasons for this difference were identified, so due to this inconsistency data from both WHO and IQVIA were included in the final dataset.

ESAC-NET data

ESAC-Net, managed by the European Centre for Disease Prevention and Control (ECDC), compiled data on antimicrobial consumption for 27 countries in the European Union (EU) and European Economic Area (EEA) for selected years between 1997 and 2018. Antibiotic consumption data were provided disaggregated by channel (hospital or community), and were available for the total J01 class antimicrobials and at the ATC level three.²²⁴ Not all countries reported both hospital and community channel antimicrobial consumption for all years. Data missing from either the hospital or retail sector was imputed using the multiple imputation method, as described for the IQVIA dataset. If data were missing from both sectors for a specific country/year this was excluded from the analysis.

A comparison between the ESAC-NET and IQVIA datasets showed a strong correlation between the reported antibiotic consumption ($r^2 = 0.93$ for all years, and $r^2 = 0.89$ for 2015 only). The IQVIA dataset reported higher antibiotic consumption for Spain and Greece than the data for ESAC-NET. Reasons for this difference are not explored in existing publications. The data sources were deemed comparable and ESAC-NET data for countries not covered by IQVIA were added to the dataset.

Additional sources

Published literature was searched for additional antibiotic surveillance systems or research studies publishing nationally representative antibiotic consumption data in DDDs (PubMed was searched using for articles with “antibiotic” OR “antimicrobial” AND “defined daily dose” in the title or abstract). Two further data sources were identified which were research studies from Samoa,²³¹ and Kenya.²³² These were added to the dataset which consisted of antibiotic consumption data for 112 countries and territories, covering 47 out of 69 (68%) HICs and 65 out of 135 (48%) LMICs (Figure 4.2).

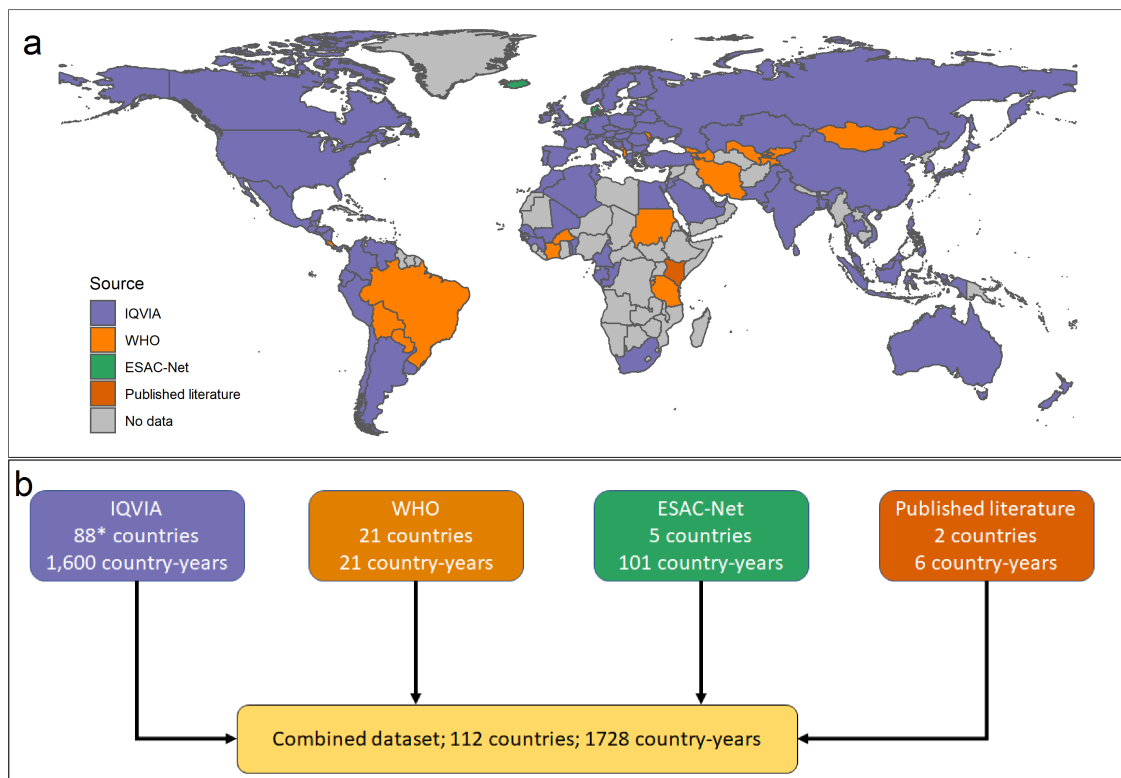


Figure 4.2: Antibiotic consumption data availability. a) A map of countries with data available by source; b) A flow chart of the number of countries and country-years from each data source and overall. *IQVIA is stated to cover 88 countries here as the Central America and French West Africa regions have been disaggregated into their individual countries.

4.2.3 Antibiotic consumption model

Separate modelling strategies were used for HICs and LMICs due to variations in data coverage, different patterns of antibiotic consumption and varying covariate effects

between these regions. HICs were classified as those defined as high-income by the World Bank,²⁰⁷ plus China, Russia and Lebanon. China, Russia and Lebanon were modelled as part of the HICs as they had a large amount of data available from IQVIA, but were lacking in data on antibiotic usage (Chapter Three) and were considered to be irregular for the GBD regions in which they are located.

Low- and middle-income countries

For LMICs, data coverage was sparse (data covered 65 out of 135 countries and 78.3% of the population), but spatial and temporal trends in antibiotic consumption were evident. To leverage these effects, utilise the estimates of antibiotic usage from Chapter Three, and to incorporate health and socio-demographic covariates, a two-stage spatial-temporal modelling framework was implemented to estimate antibiotic consumption (in DDD/1,000/year) for each LMIC from 2000 to 2018. This modelling strategy used a stacked ensemble model to capture covariate effects, then a Spatial Temporal Gaussian Process Regression (ST-GPR) model to smooth the estimates in time and space, borrowing strength from countries and years with more data to inform estimates for country/years without data.

Twenty-nine covariates with biologically plausible associations with antibiotic consumption were identified from the GBD study database (<http://ghdx.healthdata.org/>). A Lasso regression model was fit between the log of the antibiotic consumption rate (DDD/1,000/year) and the covariates to select the most informative covariates without increasing model complexity. This ensures that the model is not overly complex and reduces over-fitting. The final covariates included in the model were: antibiotic usage (the nationally aggregated estimates from Chapter Three), antenatal care coverage, hospital beds per 1,000 persons, mean temperature, outdoor air pollution, sanitation, physicians per capita, health access and quality index, socio-demographic index. Various transformations of the covariates were trialled to identity any more predictive associations with the data. The only improvements to the model whilst fitting were by taking the log of hospital beds per 1,000 persons, therefore all other covariates were used

with no transformations.

A stacked ensemble model²⁰⁸ was fit between the antibiotic consumption data and covariates matched to the input data by country and year. Eight child models were considered for inclusion: boosted regression trees (BRT), generalised linear models (GAM), neural nets, random forest, cubist and penalised regression (elastic net (e-net), ridge and lasso) models. Each model was fit with five-fold cross validation and the best performing, non-correlated ($r^2 < 0.8$) child models were selected for inclusion in the ensemble. The included child models were BRT, GAM, ridge, neural net, random forest and cubist. The predictive performance of the models was judged on the correlation (R^2) and root mean squared error (RMSE) between the input data and out-of-sample estimates.

The child models were combined using a stacking model, various options of which were trialled. These included linear regression, constrained weighted mean, BRT, neural net and GAM. Based on superior out-of-sample performance, a constrained weighted mean algorithm was used to stack the child models. The weights for each child model were calculated using quadratic programming and constrained to sum to one. The weighted mean of the child models was then calculated, estimating antibiotic consumption in DDD/1,000/year for each LMIC for 2000 to 2018. These out-of-sample estimates were taken forward to the next stage of the model and will hereafter be referred to as the stage one model. Models were fit in R version 3.6.1.

The second stage of the model is the ST-GPR, which is a flexible modelling framework developed by the GBD study.²³³ The code for this model was provided by the Institute of Health Metrics and Evaluation (IHME) and is run in Python. This is a stochastic model which easily captures non-linear trends and borrows strength from data in space and time. It firstly smooths the stage one estimates in space and time by applying spatial and temporal weights to the residuals, then fits the trend using a Gaussian Process Regression (GPR).

Temporal weights determine how quickly the correlation between datapoints diminishes over time and were based on the scaled distance (in time) between two

observations. Temporal weights are controlled by the parameter lambda, λ ; with a lower λ indicating high temporal correlation, higher temporal weights, and therefore smoother trends in time.

$$W_i = \frac{1}{e^{\lambda|t - t^0|}}$$

Spatial smoothing was determined by the GBD location hierarchy (Table C.6). A single constant was calculated based on a vector of weights for each level of the location hierarchy, controlled by the parameter zeta, ζ . Varying weights were assigned based on the country's location in the hierarchy:

- Weights of residuals in the same country: ζ^0
- Weights of residuals in different countries in the same region: ζ^1
- Weights of residuals in different regions in the same super-region: ζ^2
- Weights of residuals from other super-regions: ζ^3

These weights work in a scalar fashion and ζ can be interpreted as the amount to down-weight residuals from different countries within the same region by, compared to datapoints from the same country. For example, when applying spatial weights to datapoint from Tanzania, if $\zeta = 0.01$, then residuals from data in Kenya will be weighted 1/100 of the weight of those in Tanzania, a datapoint in Nigeria will be given 1/10,000 of the weight of those in Tanzania, whilst data from India will be given 1/1,000,000 of the weight. This means that a higher ζ translates to more spatial smoothing across locations more distant in the location hierarchy.

The spatial and temporal weights were combined by multiplying them together and summing them across each level in the location hierarchy. The weights were then constrained to sum to one for each time period and multiplied by the residuals which are then added back into the stage one estimates to smooth them in space and time.

$$Stage2 = Stage1 + \sum residual_j * w_{i,j}$$

For the final model the smoothing parameters were varied based on the amount of data within each country. For locations with no data the smoothing parameters were set to $\lambda = 0.05$; $\zeta = 0.1$; for locations with one datapoint $\lambda = 0.05$; $\zeta = 0.05$; and for locations with more than one datapoint as $\lambda = 0.005$; $\zeta = 0.5$, indicating that as the data density increased less spatial and temporal smoothing was required. The model for the Oceania region was run with increased smoothing, $\lambda = 0.05$; $\zeta = 0.1$ as there was only one datapoint in the region which was from published literature instead of the gold standard datasets, and therefore less weight was assigned to this.

The GPR was then fit to the stage two model. This assumes the trend follows a Gaussian distribution defined by a mean function, $m(\cdot)$, and a covariance function $Cov(\cdot)$, with this model using a Matérn-Euclidean covariance function. The GPR was controlled by setting the scale and amplitude to five, meaning there was correlation over five years and the estimates could vary up to five times the median absolute deviation of the input data from the stage two estimates. One thousand draws of the GPR were taken and the mean antibiotic consumption for each year and country in the study period taken, alongside the 2.5th and 97.5th percentiles to define the 95% uncertainty intervals of the estimates.

The full model (stacked ensemble plus ST-GPR) was validated using five-fold cross validation, holding out all the data from 20% of the countries from each fold and predicting the antibiotic consumption for the held-out data. All the data from each country were held out within the same fold, as it all tended to be from the same source, providing a rigorous test of the model's predictive validity. The root mean squared error (RMSE) and correlation (R^2) for the input data versus the out-of-sample predictions were calculated to quantify the models predictive power.

High-income countries

Data coverage for HICs was far higher than for LMICs. Countries with missing data were generally small in terms of both area and population (data were not available for Andorra, Antigua and Barbuda, Bahamas, Bahrain, Barbados, Trinidad and Tobago, Bermuda, Cook Islands, Greenland, Guam, Israel, Monaco, Northern Mariana Islands, Niue, Oman, Palau, Qatar, San Marino, Seychelles, Saint Kitts and Nevis, Tokelau, and US Virgin Islands), meaning that the input data covered 99.2% of the HICs population.

The suitability of a spatial-temporal model was investigated to estimate antibiotic consumption in HICs. No covariates, or combinations of covariates, were identified to satisfactorily predict the spatial-temporal trends in antibiotic consumption in HICs (details in appendix C). A stacked ensemble model (as described in section 4.2.3) was trialled but resulted in limited predictive capabilities, deeming the methodology implemented for LMICs unsuitable for HICs. Due to the small amount of missing data, it was decided to use the CART multiple imputation method as detailed in section 4.2.2 to estimate antibiotic consumption in DDD/1,000/year for the few HICs missing data.

Global estimates

Global estimates of antibiotic consumption were produced by combining the models from HICs and LMICs, calculating both the total DDDs and the DDD/1,000/day (interpreted as the number of people per 1,000 population taking antibiotics each day), for 204 countries from 2000 to 2018. Estimates for LMICs are presented with 95% uncertainty intervals calculated from 1,000 draws of the GPR model. Estimates for HICs are directly from the input data or from multiple imputations and therefore do not have uncertainty intervals associated with the predictions. Due to the combination of models, when aggregating estimates to the regional, super-regional, or global level the 95% uncertainty intervals are calculated using the single estimate of antibiotic consumption for HICs, in addition to the uncertainty intervals from the LMIC model.

Consumption by antibiotic classes

As with HICs, a spatial-temporal modelling strategy was deemed unsuitable to estimate antibiotic consumption by ATC level three (details in appendix C), ATC level four and AWaRe category, due to a lack of clear spatial and temporal patterns and informative covariates. Antibiotic consumption in DDD/1,000/day were calculated for each ATC level three and select ATC level four classes by firstly calculating the proportion of the total antibiotics consumed for each class from the input data, then applying these proportions to the modelled estimates of total antibiotic consumption by country and year. For country/years with no data for these categories, the median proportion for the corresponding GBD region was calculated, and applied to the total antibiotic consumption estimates for each country/year.

Antibiotic consumption by AWaRe category was only available from the IQVIA dataset. The proportions of antibiotics consumed by “access”, “watch”, “reserve” and “other” categories were calculated for each country and year in the cleaned dataset and are presented in the results.

4.3 Results

4.3.1 Total antibiotic consumption

Global antibiotic consumption was estimated at 40.1 [95% uncertainty interval 37.2-43.7] billion DDDs in 2018, equating to a rate of 14.1 [13.2-15.6] DDD/1,000/day. Antibiotic consumption was inequitable between HICs and LMICs with respective rates of 20.6 and 13.1 [11.8-14.6] DDD/1,000/day in 2018 (Figure 4.3). South Asia (India, Pakistan, Nepal, Bangladesh, and Bhutan), with a population of 1.8 billion, consumed over a quarter (25.2%) of all antibiotics in 2018, whilst China alone (1.4 billion population) consumed 10%. Antibiotic consumption rates were highest in North Africa and the Middle East (23.6 [20.4-27.5] DDD/1,000/day) and lowest in Central sub-Saharan Africa with 8.2 [5.9-11.3] DDD/1,000/day (Table 4.1).

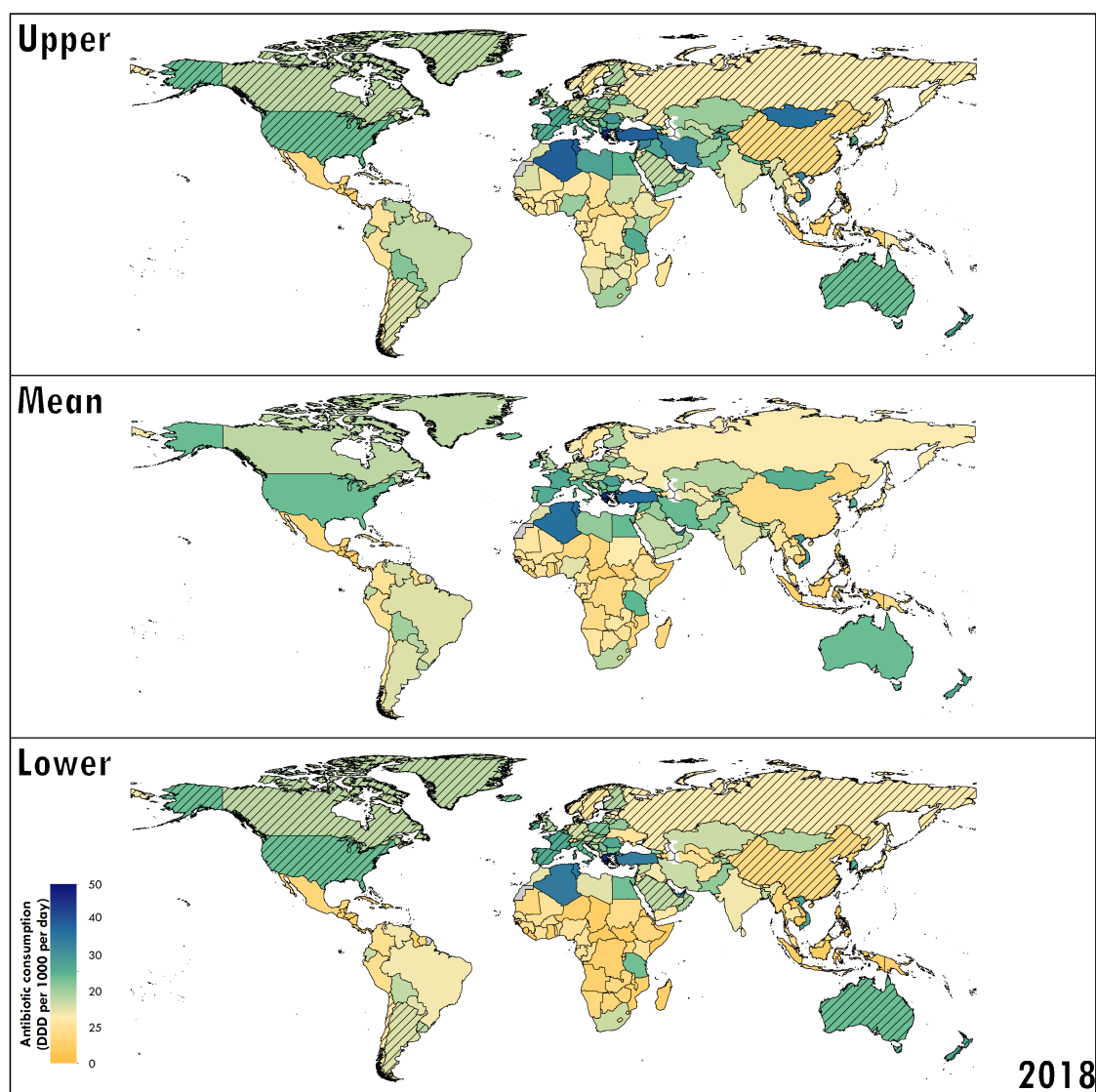


Figure 4.3: Total antibiotic consumption rates for the year 2018, with uncertainty intervals. Estimates of antibiotic consumption rates in defined daily doses (DDDs) per 1,000 population per day. The mean map combines the modelled estimates for LMICs, and the imputed dataset for HICs; the upper and lower represent the 95% uncertainty interval values for the LMIC model with HICs shown in grey shading.

Super-region (Region)	Volume of total antibiotic consumption (Million DDDs) [95% UI]	Percentage of global antibiotic consumption	Rate of total antibiotic consumption DDD/1000/day [95% UI]
Southeast Asia, East Asia, and Oceania	7,048 [6,717-7,437]*	17.5	9.0 [8.5-9.5]*
East Asia	4,413 [4,383-4,454]*	11.0	8.2 [8.2-8.3]*
Southeast Asia	2,592 [2,305-2,926]	6.5	10.6 [9.4-12]
Oceania	42 [29-57]	0.1	9.3 [6.5-12.6]
Central Europe, Eastern Europe, and Central Asia	2,525 [2,333-2,752]*	6.3	16.5 [15.3-18]
Central Asia	546 [433-682]	1.4	16.2 [12.8-20.2]
Central Europe	951 [922-984]	2.4	22.5 [21.8-23.3]
Eastern Europe	1,028 [977-1,086]*	2.6	13.4 [12.8-14.2]*
High-income	8,196*	20.4	20.8*
High-income Asia Pacific	1,142*	2.8	16.7*
Australasia	257*	0.6	24.5*
Western Europe	3,364*	8.4	21.1*
Southern Latin America	342*	0.9	14.2*
High-income North America	3,092*	7.7	23.4*
Latin America and Caribbean	2,477 [2,226-2,758]	6.2	11.6 [10.5-13]
Caribbean	166 [135-207]	0.4	10.2 [8.3-12.7]
Andean Latin America	319 [296-343]	0.8	13.6 [12.6-14.6]
Central Latin America	792 [733-856]	2.0	8.5 [7.8-9.1]
Tropical Latin America	1,200 [1,062-1,352]	3.0	15.1 [13.4-17]
North Africa and Middle East	5,229 [4,515-6,095]	13.0	23.6 [20.4-27.5]
North Africa and Middle East	5,229 [4,515-6,095]	13.0	23.6 [20.4-27.5]
South Asia	10,104 [9,650-10,598]	25.2	15.5 [14.8-16.2]
South Asia	10,104 [9,650-10,598]	25.2	15.5 [14.8-16.2]
Sub-Saharan Africa	4,586 [3,564-5,905]	11.4	11.8 [9.2-15.2]
Central Sub-Saharan Africa	387 [276-533]	1.0	8.2 [5.9-11.3]
Eastern Sub-Saharan Africa	1,788 [1,374-2,324]	4.5	12.2 [9.4-15.9]
Southern Sub-Saharan Africa	445 [391-509]	1.1	15.6 [13.7-17.9]
Western Sub-Saharan Africa	1,966 [1,524-2,538]	4.9	11.9 [9.2-15.4]
Global	40,165	100	14.3 [13.2-15.6]*
	[37,200-43,740]*		

Table 4.1: Antibiotic consumption estimates by GBD super-region and GBD region, for the year 2018. DDDs = defined daily doses. UI = uncertainty intervals. *Uncertainty intervals were not available for high-income countries, Russia, Lebanon and China, for regions/super-regions including these countries the aggregated upper and lower uncertainty intervals are calculated using the single estimate of antibiotic consumption in those countries.

Large spatial disparities in the rates of antibiotic consumption were identified, with the rates in Greece (45.9 DDD/1,000/day) and Tunisia (38.0 [36.0-40.2] DDD/1,000/day) almost ten times higher than the lowest rates found in countries such as Philippines (5.0 [4.8-5.3] DDD/1,000/day), Honduras (5.6 [5.4-5.9]), Burundi (5.3 [4.3-6.4] DDD/1,000/day), and Central African Republic (5.7 [4.0-8.0] DDD/1,000/day) (Figure 4.3). Rates of antibiotic consumption differed notably between neighbouring countries, with Mongolia (26.1 [18.3-36.9] DDD/1,000/day) surpassing consumption rates in China (8.0 DDD/1,000/day) and Sudan having noticeably higher consumption (12.3 [8.4-16.8] DDD/1,000/day) than neighbouring South Sudan and Chad (6.5 [4.5-9.1] and 6.9 [4.8-9.8] DDD/1,000/day respectively). Similarly, low consumption was estimated in Indonesia and Philippines (5.3 [4.5-6.2] and 5.0 [4.8-5.3] DDD/1,000/day respectively) in stark contrast to high consumption in Vietnam, Thailand, and Malaysia (30.0 [27.7-32.5], 12.4 [11.8-13.2] and 9.9 [9.4-10.5] DDD/1,000/day, respectively).

Global antibiotic consumption rates increased from 9.8 [9.2-10.5] DDD/1,000/day in 2000 to 14.3 [13.2-15.6] DDD/1,000/day in 2018. This differed greatly between HICs and LMICs; whilst consumption rates remained relatively stable in HICs across the study period (20.8 DDD/1,000/day in 2000 to 20.6 DDD/1,000/day in 2018), there was a 76% increase observed in LMICs (7.4 [6.6-8.3] DDD/1,000/day in 2000 to 13.1 [11.8-14.6] DDD/1,000/day in 2018). The largest increases in antibiotic consumption were seen in the North Africa and Middle East region (111% increase from 11.2 [9.2-13.7] DDD/1,000/day in 2000 to 23.6 [20.4-27.5] DDD/1,000/day in 2018) and South Asia (116% increase from 7.2 [6.6-7.8] DDD/1,000/day in 2000 to 15.5 [14.8-16.2] DDD/1,000/day in 2018); for all other regions (excluding the high-income region which decreased slightly) more modest increases in consumption were seen (22-100% increases, Figure 4.4).

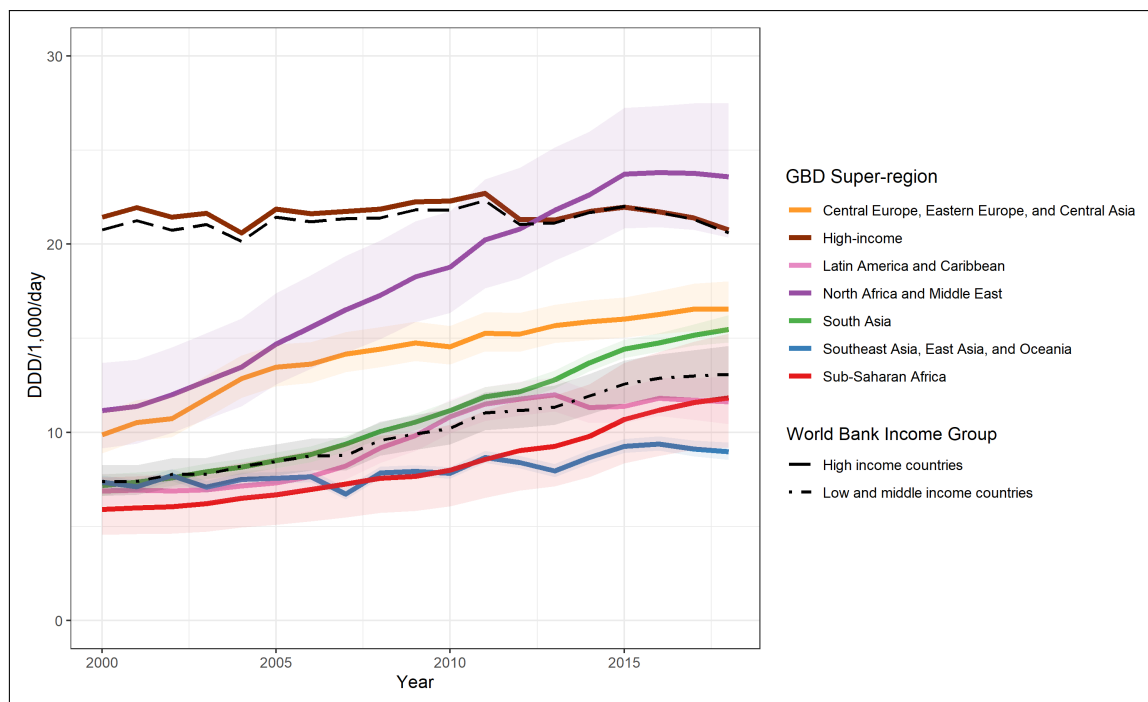


Figure 4.4: Temporal trends in total antibiotic consumption rates for GBD super-regions and World Bank income groups. Modelled estimates of antibiotic consumption rates in defined daily doses (DDDs) per 1000 population per day for 2000 to 2018; estimates are plotted as the total for each GBD super-region (solid lines) and each World Bank income group (dashed lines), with the 95% uncertainty intervals represented by the coloured ribbons (not available for HICs).

Antibiotic consumption rates were remarkably high in North America and Western Europe in 2018 (23.4 and 21.1 DDD/1,000/day respectively), although lower rates were observed in Switzerland (11.4 DDD/1,000/day) Netherlands (9.7 DDD/1,000/day) Sweden (10.8 DDD/1,000/day) and Norway (12.9 DDD/1,000/day). Sweden reduced antibiotic consumption by 26% from 2000 to 2018 (14.6 to 10.8 DDD/1,000/day), whilst in the USA there was a 14% reduction over the same time period (27.8 to 24.0 DDD/1,000/day). Some of the largest increases in antibiotic consumption over the study period were seen in Vietnam (374%, 6.3 [4.9-8.1] to 30.0 [27.7-32.5] DDD/1,000/day), Brazil (238%, 4.4 [3.5-5.5] to 15.0 [13.3-16.9] DDD/1,000/day) and UAE (223%, 3.8 to 12.2 DDD/1,000/day) (Figure 4.5).

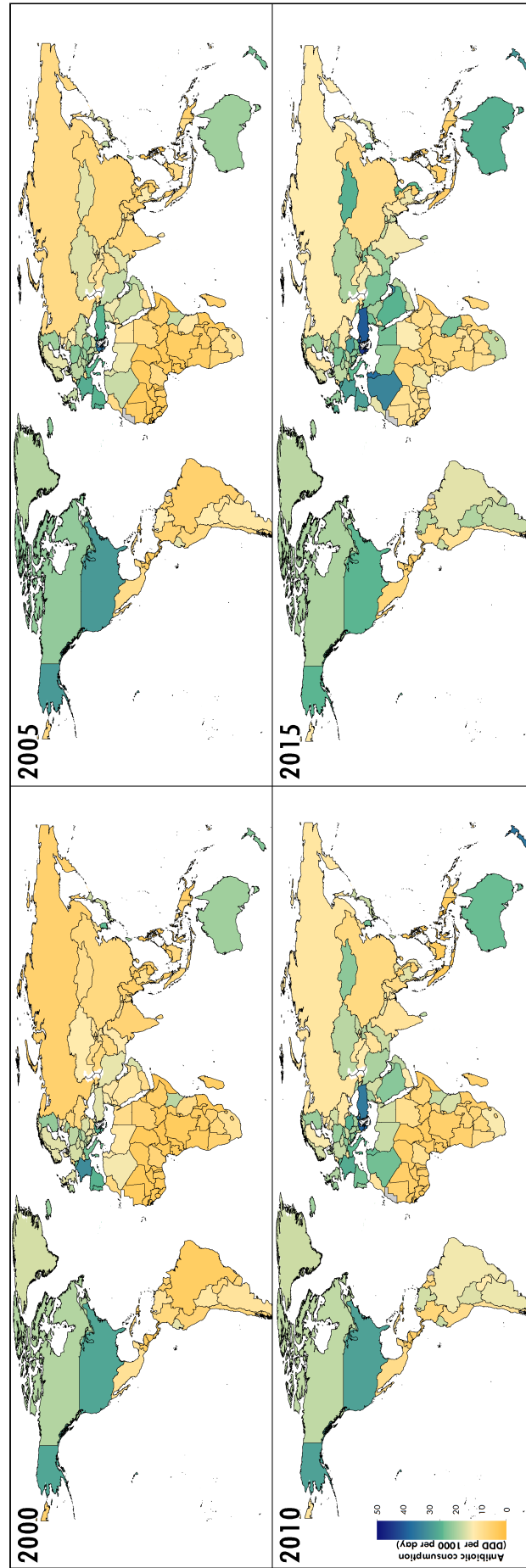


Figure 4.5: Five yearly estimates of total antibiotic consumption rates from 2000 to 2015. Estimates of antibiotic consumption rates in defined daily doses (DDDs) per 1000 population per day for the years 2000; 2005; 2010; and 2015.

4.3.2 Consumption by antibiotic classes

The rates of antibiotic consumption varied greatly by antibiotic class. Of the ATC level three classes, consumption was highest in penicillins (J01C) for all years and increased from 2.8 DDD/1,000/day in 2000 to 4.8 DDD/1,000/day in 2018. The antibiotic class with the lowest consumption globally was amphenicols (J01B) with less than 0.1 DDD/1,000/day for all years. Consumption of most classes of antibiotic increased or stayed consistent over the study period, except for sulfonamides and trimethoprim (J01E) which decreased from 1.4 DDD/1,000/day in 2000 to 0.5 DDD/1,000/day in 2018 (Figure 4.6).

Spatial variation in the consumption of different antibiotic classes is evident with sulfonamides and trimethoprim consumption highest in southern and eastern sub-Saharan Africa whilst penicillins and other beta-lactams are far higher in Europe and North Africa (Figure 4.7).

Examining key ATC level four antibiotic classes in select GBD super-regions showed stable rates of broad-spectrum penicillin (J01CA) consumption over the study period, with the highest rates in 2018 in the High-Income super-region (3.9 DDD/1,000/day), and lowest in South Asia (0.8 DDD/1,000/day) (Figure 4.8). Carbapenem (J01DH) consumption was highest in the High-Income super-region, where it increased from 0.05 to 0.09 DDD/1,000/day from 2000 to 2018. Fluoroquinolone consumption increased in all super-regions and in 2018 was highest in North Africa and the Middle East (3.2 DDD/1,000/day), and South Asia (2.9 DDD/1,000/day). The consumption of third-generation cephalosporins also increased greatly in North Africa and the Middle East (from 0.2 DDD/1,000/day in 2000 to 2.8 DDD/1,000/day in 2018) and South Asia (from 0.2 DDD/1,000/day in 2000 to 3.3 DDD/1,000/day in 2018), but remained lower in the High-Income and sub-Saharan Africa super-regions (1.1 and 1.0 DDD/1,000/day in 2018 respectively; Figure 4.8).

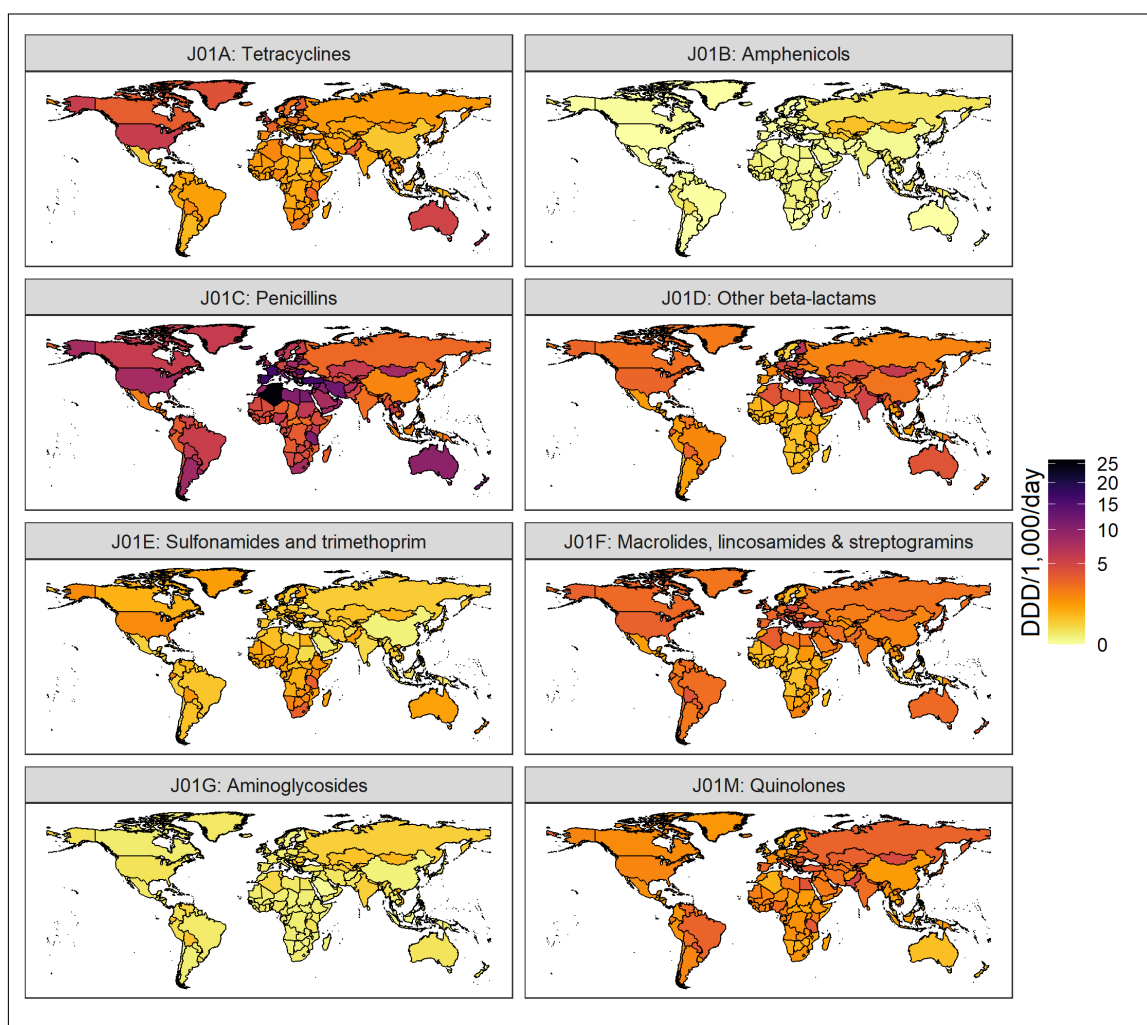


Figure 4.6: Antibiotic consumption by ATC level three class for 2018. The proportions of consumption of each antibiotic class were calculated from IQVIA, WHO and ESAC-Net data and applied to the modelled total antibiotic consumption for each country and expressed as DDDs per 1000 population per day on a square root transformed scale.

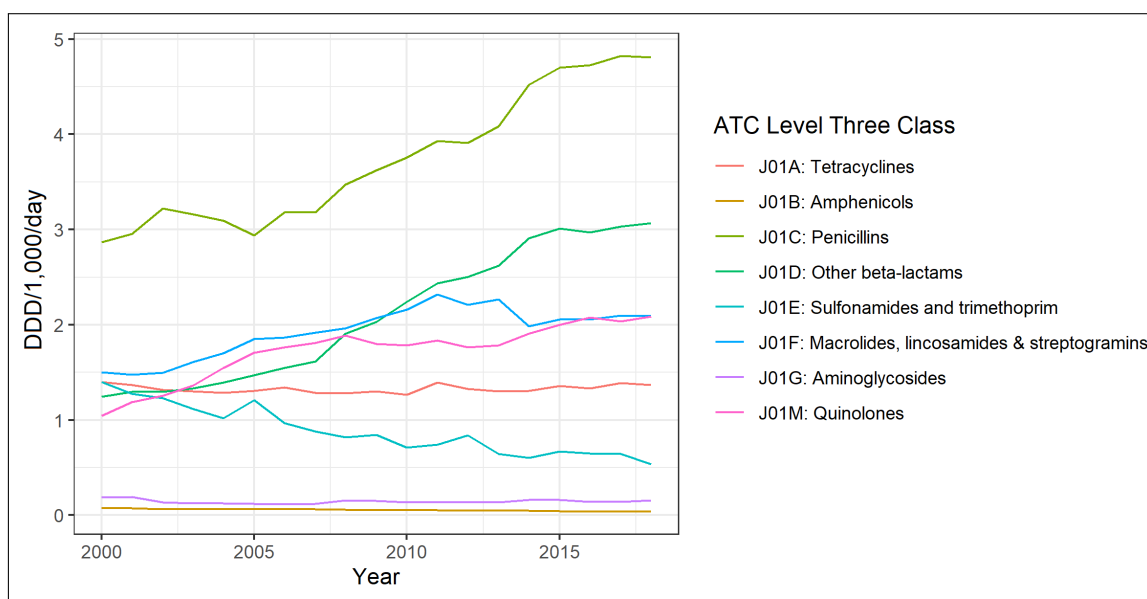


Figure 4.7: Temporal trends in antibiotic consumption rates by ATC level three class for 2000-2018. The proportions of consumption of each antibiotic class were calculated from IQVIA, WHO and ESAC-Net data and applied to the modelled total antibiotic consumption for each country and expressed as DDDs per 1000 population per day.

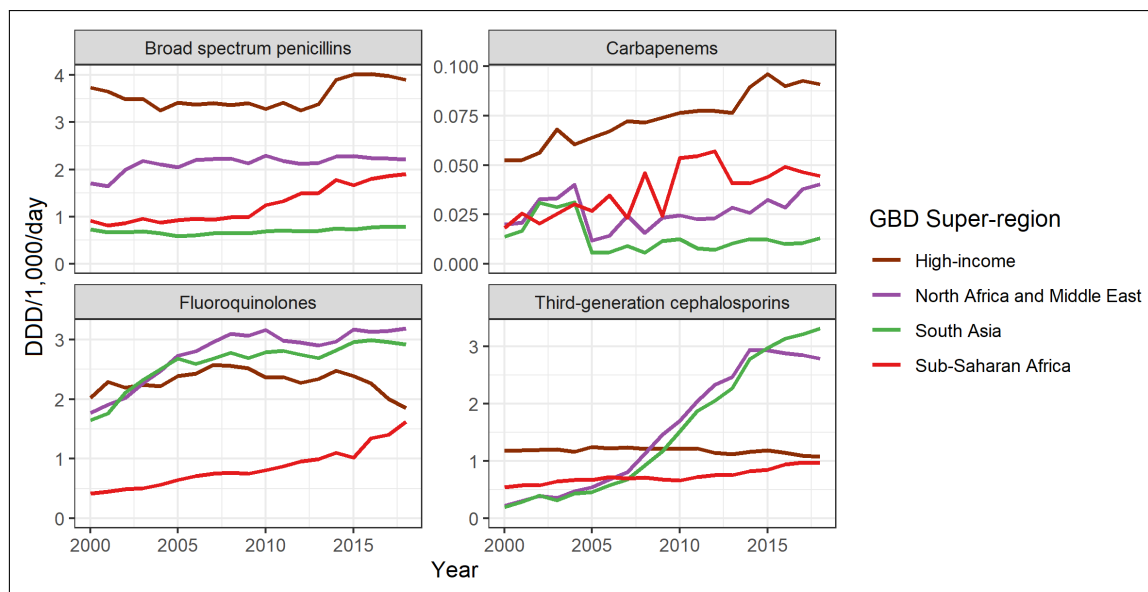


Figure 4.8: Temporal trends in consumption of selected ATC level 4 antibiotic classes, displayed for four selected GBD super-regions. The proportion of each antibiotic class (ATC level 4) was calculated from the IQVIA dataset and applied to the modelled estimates of total antibiotic consumption for each country and aggregated to the High-Income; North Africa and the Middle East; South Asia; and Sub-Saharan Africa super-regions. Estimates are displayed for: a) broad spectrum penicillins, J01CA; b) carbapenems, J01DH; c) Fluoroquinolones, J01MA; and d) third generation cephalosporins, J01DD.

The proportion of antibiotics consumed based on the WHO AWaRe categories were calculated based on the IQVIA dataset for each country/territory and year available. In 2018, access antibiotics accounted for 63.6% of the total antibiotic consumption. Whilst watch and reserve antibiotics accounted for 34.6% and 0.7% of consumption respectively. This varied greatly by country, in 2000 the proportion of access antibiotic consumption was highest in Indonesia (90.3%) but this decreased over time (60.1% in 2018) as more watch antibiotics were used. Japan had the lowest proportional use of access antibiotics in both 2000 and 2018 (11.3% and 18.1% respective), and mainly consumed watch antibiotics. WHO recommend that at least 60% of a country's antibiotic consumption should be access antibiotics;^{64,223} in 2000, 42 out of the 68 (61.8%) countries/territories in the IQVIA dataset met this criteria; in 2018 this number was 35 out of 74 (47.3%) countries/territories (Figure 4.9).

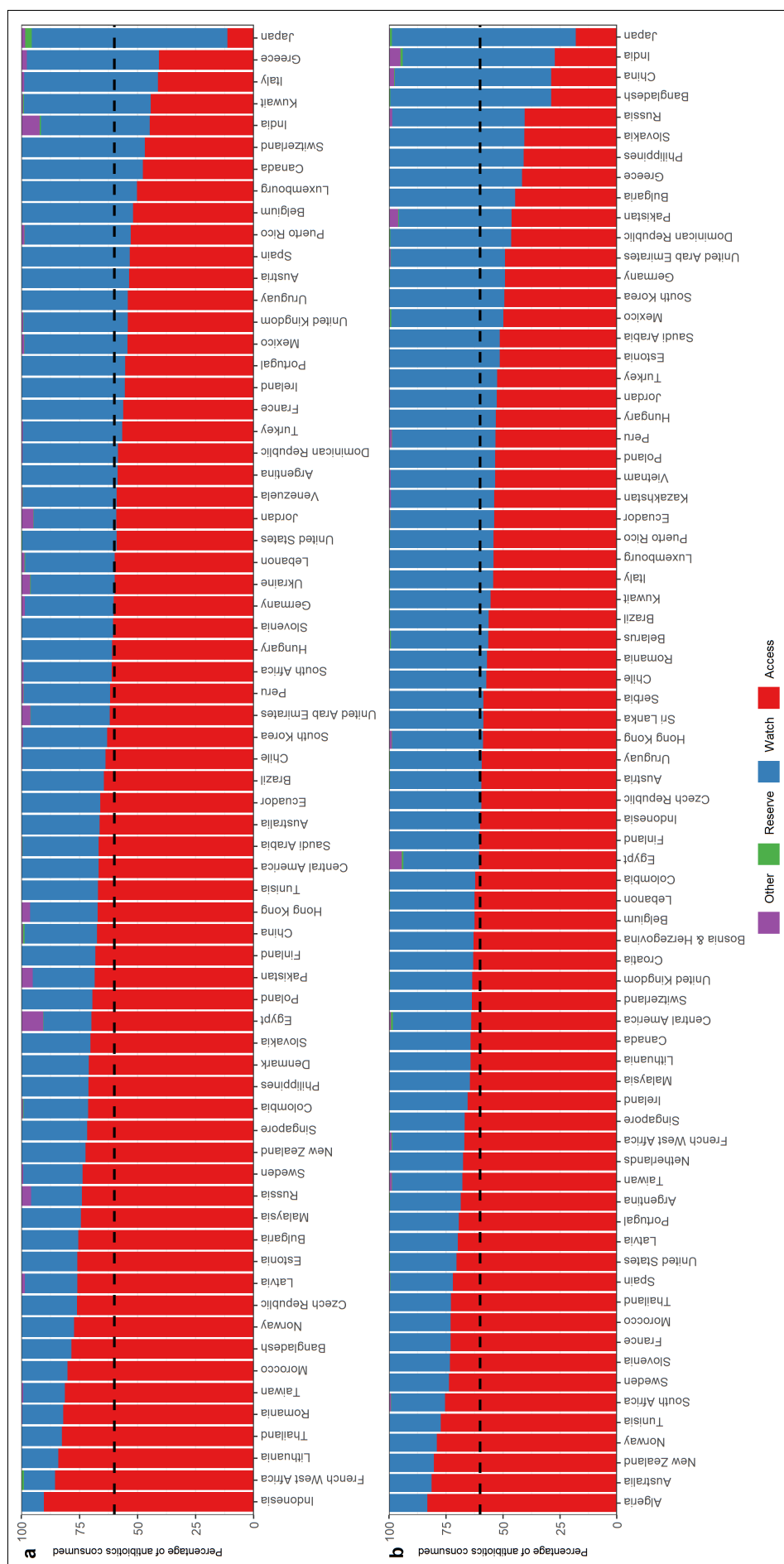


Figure 4.9: Proportion of antibiotic consumption by AWaRe category, 2000 and 2018. Proportions are based on IQVIA data and the WHO AWaRe classification. Estimates are not available for all countries in 2000 and 2018. Names and borders of countries and territories are based on IQVIA's designation. a) The proportion of antibiotic consumed by AWaRe categorisation by country for the year 2000; b) The proportion of antibiotic consumed by AWaRe categorisation by country for the year 2018

4.3.3 Model validation

Overall the model had relatively good predictive validity with correlation between the input data and out-of-sample-estimates of $R^2 = 0.53$ and an RMSE of 2000 (DDD/1,000/year; Figure 4.10). The proportion of held-out data points for which the model predictions were within the 95% uncertainty intervals of the model was 65%.

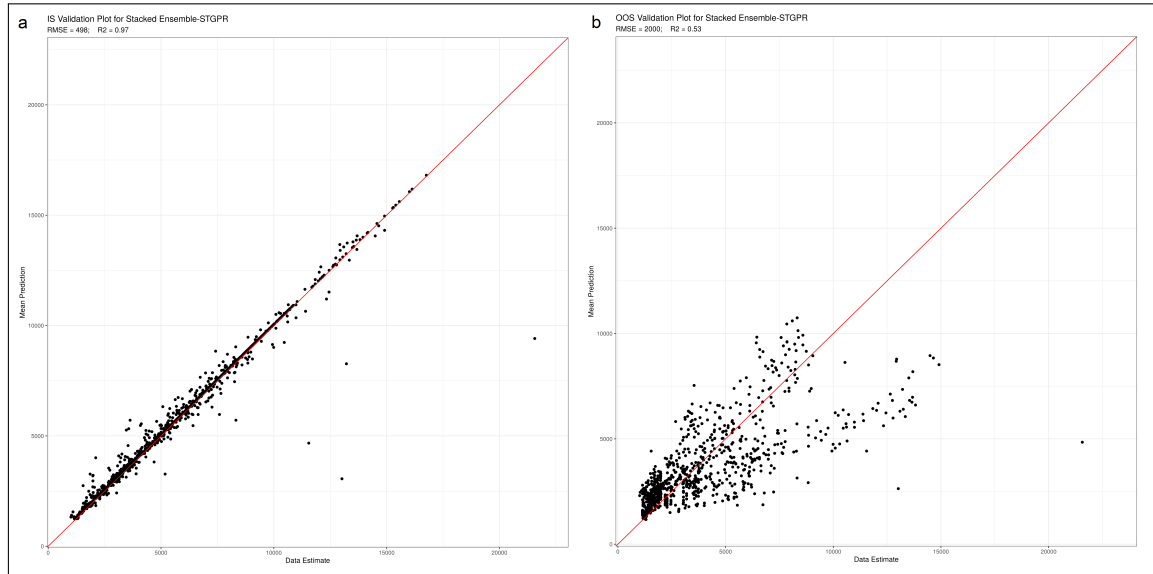


Figure 4.10: Validation plots for the model of antibiotic consumption. a) Scatter plot presenting the antibiotic consumption of J01 antibiotics in DDD/1,000/year for each country-year, against the in-sample predicted antibiotic consumption for that country-year; b) Scatter plot of the antibiotic consumption of J01 antibiotics in DDD/1,000/year for each country-year, against the out-of-sample predicted antibiotic consumption for that country based on five-fold cross validation

4.4 Discussion

4.4.1 Overview

This study provides the first contemporary estimates of human antibiotic consumption for 204 countries from 2000 to 2018. It incorporates data from multiple sources and utilises a spatial-temporal modelling methodology to estimate antibiotic consumption for countries lacking surveillance data. The results highlight important spatial disparities in antibiotic consumption with high rates of consumption identified in high- and upper-middle-income countries in North America, Europe and the Middle East, contrasted by very low rates of consumption in sub-Saharan Africa and parts of

Southeast Asia. Total antibiotic consumption rates showed almost a ten-fold variation, from a low of 5.0 DDD/1,000/day in the Philippines to 45.9 DDD/1,000/day in Greece. In addition, this is the first global longitudinal analysis of antibiotic consumption that applies the higher 2019 DDD values.^{228,234}

Few studies exist analysing antibiotic consumption on a global scale.^{64,187,226,235} Klein *et al.*¹⁸⁷ provided the most comprehensive estimates, predicting global antibiotic consumption for 2000 to 2015 using the ATC/DDD methodology. However, they relied exclusively on the IQVIA dataset and derived estimates for countries with no data by extrapolating from countries within the same income group, not reporting estimates for individual countries. Klein *et al.* estimated global antibiotic consumption to be 42.3 billion DDDs (15.7 DDD/1,000/day) in 2015, whereas this study produced a lower estimate of 38.3 [36.1-41.2] billion DDDs (14.1 [13.3-15.2] DDD/1,000/day) for the same year. The differences were most apparent in HICs where the estimates of this study (20.6 DDD/1,000/day in 2018) were considerably lower than those of Klein *et al.* (25.7 DDD/1,000/day in 2015). This difference is partially explained by use of the updated DDD values^{228,234} for key antibiotics such as ampicillin and amoxicillin, which are frequently used in HICs. Analysing the IQVIA dataset using the historic DDD values would result in consumption rates of 24.4 DDD/1,000/day in HICs for 2018. Interestingly, this difference was not observed in LMICs (Table C.7), likely reflecting the different classes of antibiotics used in these countries.

Large spatial and temporal variations in the classes of antibiotics consumed were identified by this study. In South Asia, fluoroquinolone and third-generation cephalosporin consumption notably increased over the study period (1.8 fold and 17 fold increase respectively); comparatively, broad spectrum penicillin use was consistently highest in the high-income region. These differences reflect variations in treatment guidelines and indicate that non-adherence and self-medication may be more common in South Asia, as fluoroquinolones and third generation cephalosporins tend to be more commonly available in tablet form over the counter. Appropriate antibiotic treatments for different clinical indications is vital, and local AMR profiles must be taken into

consideration. Improved surveillance of antibiotic consumption is required, especially in LMICs, to correctly ascertain which classes of antibiotics are used where, so that resources can be better directed to ensure the availability of appropriate antibiotics, whilst maintaining that last line drugs are saved for essential use only.

The rate of antibiotic consumption both increased and decreased in different countries around the world throughout this study period. Sweden showed particular success in reducing its high rates of antibiotic consumption, and in 2018 had one of the lowest antibiotic consumption rates in Europe (10.8 DDD/1,000/day). This example shows how the implementation of a well-funded national action plan focussing on the surveillance of antibiotic use and of AMR, national prescription targets, treatment guidelines, infection control, public engagement and education can be successful at reducing the overuse of antibiotics,¹² and helping to tackle the threat of AMR. At the other end of the spectrum, antibiotic consumption increased almost 400% over the study period in Vietnam. Economic development has led to an increase in the availability of antibiotics in Vietnam, and although national regulations on prescriptions are in place, these are infrequently enforced leading to high rates of self-medication and overuse of antibiotics in both community and hospital settings.^{58,213}

Estimates of antibiotic consumption in Indonesia were some of the lowest globally in 2018. This may be considered an outlier as this differs greatly from other countries in the region such as Malaysia and Vietnam where antibiotic consumption is estimated to be far higher (Indonesia was 5.3 DDD/1,000/day, Malaysia 9.9 DDD/1,000/day and Vietnam 30.0 DDD/1,000/day). This variation stems from the IQVIA dataset where there is a noticeable drop in antibiotic consumption in Indonesia between 2007 and 2008 (Figure C.3). Personal communication with IQVIA identified updates to their methodology around this time, but no reason for the inconsistencies in the antibiotic consumption data from Indonesia were provided. There is no comparable data available on antibiotic consumption to validate the IQVIA dataset. Chapter Three showed lower estimates of antibiotic usage in Indonesia (42.9% [32.8-53.5%]) compared to Malaysia (79.0 [70.9-85.5%]) and Vietnam (74.2% [67.9-80.2%]), but estimates for sub-Saharan Africa

were far lower than this. The low rates of antibiotic consumption reported in Indonesia may be related to suboptimal data collection, high consumption of informal antibiotics not captured by the IQVIA dataset, or national rates masking high disparities between urban and rural antibiotic consumption in this country. Further research is required to accurately quantify antibiotic consumption in Indonesia and improve estimates in this region.

AWaRe categorisation of antibiotics provides useful guidance on which antibiotics should be easily accessible and which should be reserved for emergency use. There are large disparities in the types of antibiotic used, and action is required in countries with high use of watch and reserve antibiotics, such as China, India and Japan, to reduce their consumption and preserve the efficacy of these antibiotics. In 2018, the majority of countries failed to meet the WHO target of 60% of antibiotic consumption to be access antibiotics. Surveillance and regulation of the consumption of watch and reserve antibiotics is vital to ensure their efficacy and maintain treatment options for the growing number of AMR pathogens.

Both inappropriate use of antibiotics and lack of access to antibiotics are significant public health problems²³⁶ highlighted by this study. Using the global incidence of sepsis as an indicator for the need of antibiotics, a striking disconnect between the requirement for antibiotics and the actual volume of antibiotics consumed becomes evident. In 2017, 34% (16.6 million out of 48.9 million) of global sepsis cases occurred in sub-Saharan Africa but only 11.0% (4 million DDDs) of global antibiotic consumption, whilst in the GBD high-income region 7% (3.4 million out of 48.9 million) of sepsis cases occurred but 21.1% (8.4 million DDDs) of antibiotic consumption.²³⁷ Lack of access and delayed access to antibiotics is an important contributing factor to the high mortality in children under-five years in LMICs.^{197,238}

Previous publications have shown GDP to be significantly associated with antibiotic consumption in LMICs,¹⁸⁷ and a suite of socio-demographic and health-related covariates which improved the predictive performance of the model of antibiotic consumption were identified. However, in HICs these covariates had no association with

the observed patterns in consumption and failed to improve prediction. Drivers of antibiotic use in HICs were identified to be related to cultural and behavioural attitudes of the populations,^{239–241} as well as attitudes of prescribers, and awareness of antibiotic efficacy and resistance.^{239,242} This resulted in few identifiable spatial patterns in antibiotic consumption in HICs, precluding the use of spatial-temporal modelling to estimate total antibiotic consumption. The modelling strategy implemented in this chapter was focussed on prediction, the use of the same covariates for various stages of the model precludes the ability to infer causation at any stage.

Inappropriate use of antibiotics is a major risk factor for the development of AMR⁵⁹ and effective antibiotic stewardship programmes (ABS) are essential for combatting this threat.²⁹ ABS needs to be directed to the requirements of that location, whether this is increasing access to essential medicines or reducing overuse, with baseline antibiotic consumption rates informing these policies. Implementing national action plans that combine ABS with infection prevention and control measures, such as wide-spread vaccinations and improvement in water and sanitation, is essential for combatting AMR.²⁹

4.4.2 Limitations

The IQVIA dataset provides the largest international dataset on antibiotic consumption, but it is not without its limitations. Robust reporting systems are required to collect this data, meaning data from LMICs are severely limited (coverage included 40 high-, 22 upper middle-, 13 lower middle- and one low-income countries/territories). There is limited availability of information to validate the IQVIA dataset. Where available these data were compared to WHO and ECDC antibiotic consumption data and were assumed to be an accurate representation of antibiotic consumption. The IQVIA dataset is a proprietary dataset, limiting access and analysis. Only formal channels of antibiotic sales are included, therefore sales of antibiotics from informal markets may be absent from the dataset. In LMICs, informal sellers are common and can make up large proportions of antibiotic sales,^{218,243} meaning these results may be an underestimate of total

consumption.

A major limitation of this work is the lack of antibiotic consumption data in LMICs; the combined dataset only covered 48% of LMICs, impacting the robustness of the estimates and increasing the uncertainty intervals around them. This highlights the urgent need for the establishment and promotion of antibiotic consumption surveillance programmes, especially in LMICs. The sparse data also affected the estimates of consumption of antibiotic classes. For countries lacking data, the median proportions from the relevant GBD region were used to calculate consumption by antibiotic class. This means that variations within the region will not be fully captured by these estimates.

The definition of DDDs used in this study is an adult “maintenance” dose of antibiotic. As antibiotic doses for children are substantially lower than for adults, this could lead to an underestimation of antibiotic consumption in countries with a young demographic.²²⁷ Antibiotic sales and consumption data do not necessarily equate to antibiotic exposure; a lack of patients’ compliance to treatment protocols, and the storage or loss of antibiotics is not represented within this data.

Lastly, limited data exists on the sales and use of suboptimal (substandard or falsified) antibiotics and therefore has not been incorporated into this analysis.^{214,244} These can make up a considerable proportion of antibiotic sales in LMICs²¹⁴ and have a major impact on health outcomes, leading to treatment failure as well as promoting AMR.²⁴⁴ It is vital to address the use of suboptimal antibiotics within ABS programmes in LMICs.

4.4.3 Conclusions

This study provides an important benchmark of antibiotic consumption for 204 countries, highlighting spatial disparities in antibiotic consumption. Countries with high antibiotic consumption and those with low antibiotic consumption are identified, highlighting locations where national action plans need to focus on increasing access to essential antibiotics, or on decreasing their overuse. This study also shows the need for the establishment and improvement of antibiotic consumption surveillance programmes,

especially in LMICs, to improve estimates of consumption and aid in the implementation of ABS. Understanding the local AMR patterns of key pathogens is vital and needs to be taken into consideration alongside antibiotic consumption data to increase effective use and reduce inappropriate use of antibiotics, ultimately reducing morbidity and mortality due to bacterial infections.

Challenges and biases in the use of passive surveillance data for estimating resistance

5.1 Introduction

The emergence and spread of AMR causes difficulties in the treatment and control of enteric fever. Accurate estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* are vital to inform analysis of the spatial and temporal trends in resistance, and to guide interventions combatting AMR.

There are many challenges which arise when estimating the burden of AMR.^{245,246} The most notable of these are the lack of data, laboratory quality and biases in the available data. AMR data often comes from a wide range of sources with many organisations and individuals undertaking testing. This results in different methods for sampling, testing and reporting. Even when contributing to national or supranational surveillance systems there is limited guidance for conducting standardised AST and quality control is left up to contributing organisations and laboratories,¹⁸³ resulting in highly heterogeneous data.

To accurately determine the “true” prevalence of AMR enteric fever in a community, an understanding of the limitations and potential biases in the data are required. This chapter is a narrative review investigating the challenges and biases related to estimating the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, for all endemic countries between 1990 and 2018. Firstly, the limitations due to data availability are discussed, followed by the effect of laboratory quality on the identification of AMR in enteric fever. Potential biases in data reporting the prevalence of AMR are then identified

and discussed in turn. Finally, methods of adjusting for identified biases when estimating the prevalence of AMR in enteric fever are discussed, along with their suitability for implementation within the scope of this thesis.

5.2 Data availability

Enteric fever is primarily a disease of LMICs.⁴ A recent review of supranational surveillance networks identified 44 networks reporting on AMR in bacteria, but only four of these covered *Salmonella spp.*.⁸⁶ This shows that continuous surveillance data on AMR in enteric fever is sparse, and where data are available there is a notable lack of standardisation in sampling, testing and reporting protocols.²⁴⁷ In lieu of high quality surveillance data, estimates of AMR in enteric fever are reliant on data from academia. However, academic research can provide a good source of high-quality AMR data.²⁴⁸

Longitudinal, multicentre studies such as Typhoid Surveillance in Africa (TSAP)¹⁸⁴ and the Strategic Typhoid Alliance across Africa and Asia (STRATAA)²⁴⁹ provide high quality data on AMR in enteric fever. They are conducted to rigorous standards, provide a high level of detail on the study populations and include additional clinical and demographic variables. However, studies such as these are expensive and time consuming to conduct, meaning that their spatial and temporal coverage is often limited to a few countries and years. Although these studies provide an excellent source of data to estimate location- and time-period-specific prevalence of AMR in enteric fever, to expand such estimates to all endemic countries and a wider time period requires a more extensive dataset. Additional data is therefore only available from studies conducted by individual hospitals and academic institutes, and accessed through personal communication and systematic review.

When considering all available sources of data reporting the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* (see Chapter Two and Chapter Six), there were a total of 560 non-duplicate sources reporting AST results for five or more isolates between 1990 and 2018 in endemic countries. This equated to approximately 158,995 isolates of *S. Typhi* and *S. Paratyphi A*, covering 45 countries over 29 years (Figure 5.1). This is only

a tiny fraction of the global enteric fever cases which were estimated at over 14 million cases in 2017.⁴ The majority of data are from South Asia, with a paucity of data in central sub-Saharan Africa and no studies identified in Oceania. Data covers most years between 1990 and 2018 but is sparse in the most recent years due to a delay between studies being conducted and published in peer review journals (with a median of three years between a study being conducted and publication).

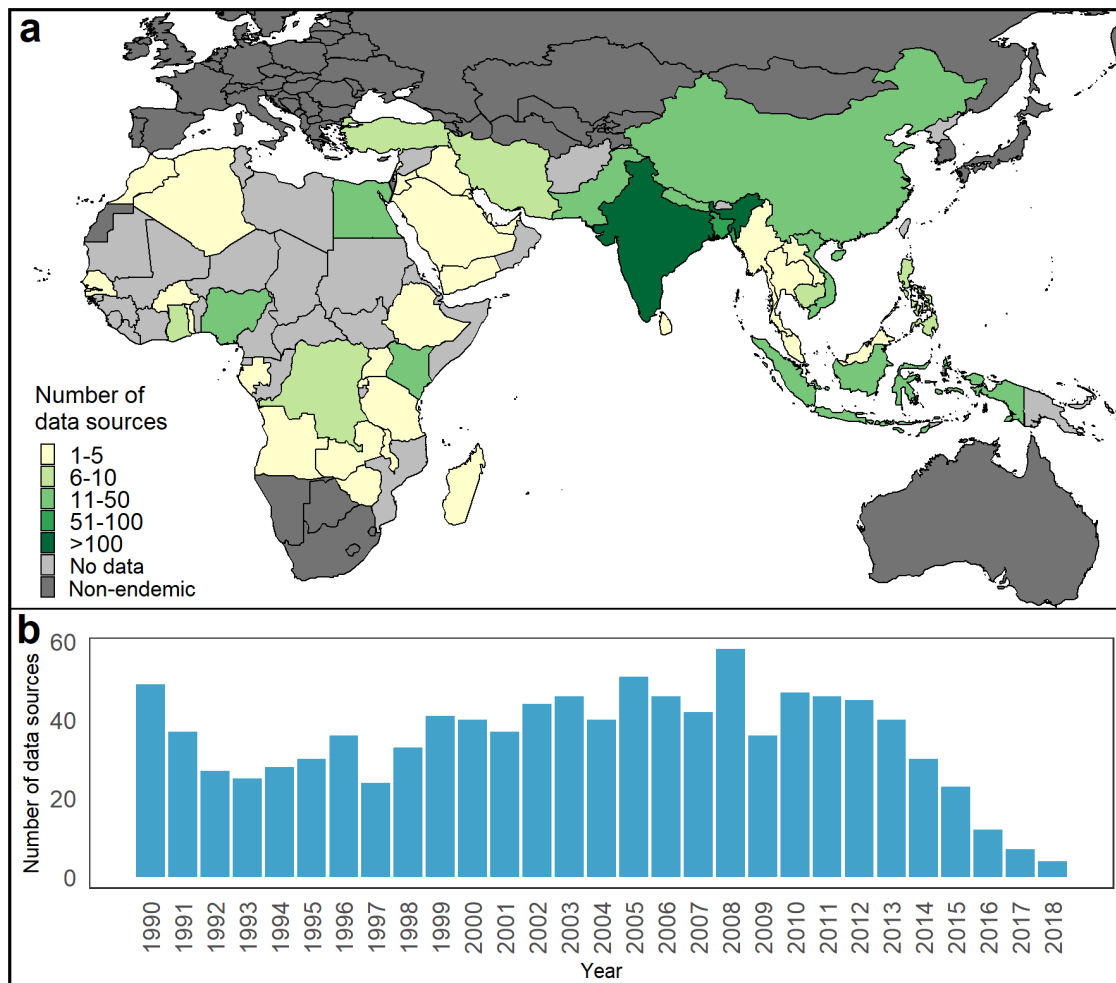


Figure 5.1: Data available on MDR and FQNS *S. Typhi* and *S. Paratyphi A*. a) A map showing the number of data sources reporting the prevalence of MDR or FQNS in *S. Typhi* or *S. Paratyphi A* for each endemic country for all years 1990 to 2018, b) A bar chart showing the number of studies reporting the prevalence of MDR or FQNS in *S. Typhi* or *S. Paratyphi A* for all endemic countries for each year 1990 to 2018

Sparse data provides difficulties in estimating the prevalence of AMR across large study areas and time periods. Spatial modelling methodologies are popular for estimating the incidence and prevalence of infectious diseases and health indicators in locations and years lacking data,^{81,196,203,204} but these models are data hungry and results are highly influenced by the quality of the input data, with estimates of prevalence or incidence

accompanied by large uncertainty in locations and years where data are sparse.

The need for high quality AMR surveillance data has been recognised by the WHO. The Global Antimicrobial Resistance Surveillance System (GLASS)¹⁸³ was established in 2015, aiming to collate and disseminate internationally comparable AMR data. Contributing organisations undertake their own AMR surveillance, including quality assessments, and report their AMR data for priority pathogens (including *S. Typhi*). GLASS provides guidance for setting up and implementing national surveillance systems,²⁵⁰ but highly functional testing and reporting facilities are required in-country to contribute to GLASS. As of April 2020, 82 countries were enrolled in the GLASS-AMR surveillance, however just 28 reported the number of patients sampled and the antimicrobial susceptibility testing (AST) results.²⁵¹ These were disproportionately made up of high- and upper-middle-income countries. Additionally, GLASS has not embarked on retrospective data collection, meaning its utility for estimating temporal trends in AMR is currently limited. Investment is required to set up high quality AMR surveillance in LMICs. The Fleming Fund from the UK Department of Health and Social Care has developed a roadmap for increasing the participation of LMICs in GLASS,²⁵² along with investing in both retrospective and prospective collection of AMR data in LMICs.²⁵³ An increase in the amount of high-quality AMR data from LMICs would be incredibly beneficial for estimating both the prevalence and burden of AMR.

5.3 Laboratory quality

The gold standard for diagnosing *S. Typhi* or *S. Paratyphi A* infections and the corresponding AMR profiles is to grow the pathogen from blood culture, identify the pathogen and then determine the phenotypic resistance profile using disk-diffusion, E-test, broth microdilution or automated methods.²⁵⁴ These procedures can only be undertaken in an established high quality microbiology laboratory, with appropriate equipment and stocks of required reagents, alongside well-trained staff. A lack of access to such laboratories provides an obvious limitation in the detection of *S. Typhi* and *S.*

Paratyphi A and restricts the amount of susceptibility testing data available to determine the corresponding prevalence of AMR.

In HICs there are high numbers of ISO 15189 accredited microbiology laboratories with good infrastructure, established supply chains and well trained experienced staff adhering to professional standards.²⁵⁵ In LMICs, where enteric fever is highly endemic, this is often not the case.²⁵⁶ Accreditation provides a measure of quality, and ensures the reliability of data coming from that laboratory. This lack of accredited microbiology laboratory capacity is reflected by the lack of data available from sub-Saharan Africa and parts of Southeast Asia such as Myanmar (Figure 5.1). The high heterogeneity in the prevalence of resistance data reported from such locations (observed in Chapter Two) might also be a reflection of the variations in laboratory quality within regions.

Participation in external quality assessment schemes (EQA) is an important tool for assessing the performance of diagnostic and microbiology laboratories. An EQA study of 81 African laboratories from 42 countries showed that just 75% had “acceptable” identification of *S. Typhi* isolates and just 62% performed “acceptable” AST for *S. Typhi* in 2013.²⁵⁷ Many laboratories have additionally been identified to lack the capacity to determine the correct serotypes of *Salmonella* isolates.²⁵⁸ This not only impacts the quantity and reliability of AMR data but could have a major impact on the treatment prescribed to patients and the resulting patient outcomes.

The use of control strains in microbiology laboratories is critical for both bacterial diagnosis and AST. A lack of access to control strains is frequently cited as a limiting factor for performing AST^{254,259} and can negatively impact the reliability of results. The systematic review conducted in Chapter Two showed that only 32% of studies reporting AMR in *S. Typhi* or *S. Paratyphi A* clearly reported use of a control strain.

A lack of laboratory capacity results not only in a risk of improper treatment of patients, but also in limited availability and reliability of data from that location. Investment in the establishment and maintenance of high-quality microbiology laboratories in LMICs is essential for building complete datasets of the prevalence of AMR, estimating the global prevalence of AMR, and fully understanding the extent of

the threat posed by drug resistant pathogens.

5.4 Biases in the data

Overview

Surveillance of AMR in enteric fever is often conducted in the form of passive surveillance. This means that cases are required to attend a formal healthcare setting with access to an established microbiology laboratory. Once a person has attended such a facility, a decision is made whether to draw blood for culture or not. If a positive blood culture is returned, there is then a decision to conduct AST. Those results need to be reported into a surveillance system or ongoing study to be included in a dataset of AMR. There are many steps in this process and at each step there is the potential for biases to be introduced. Passive surveillance only captures the tip of the iceberg when identifying AMR in enteric fever and Figure 5.2 identifies the steps involved and the potential biases introduced at each stage. Each of these biases is discussed in turn and the potential direction and magnitude of the bias assessed.

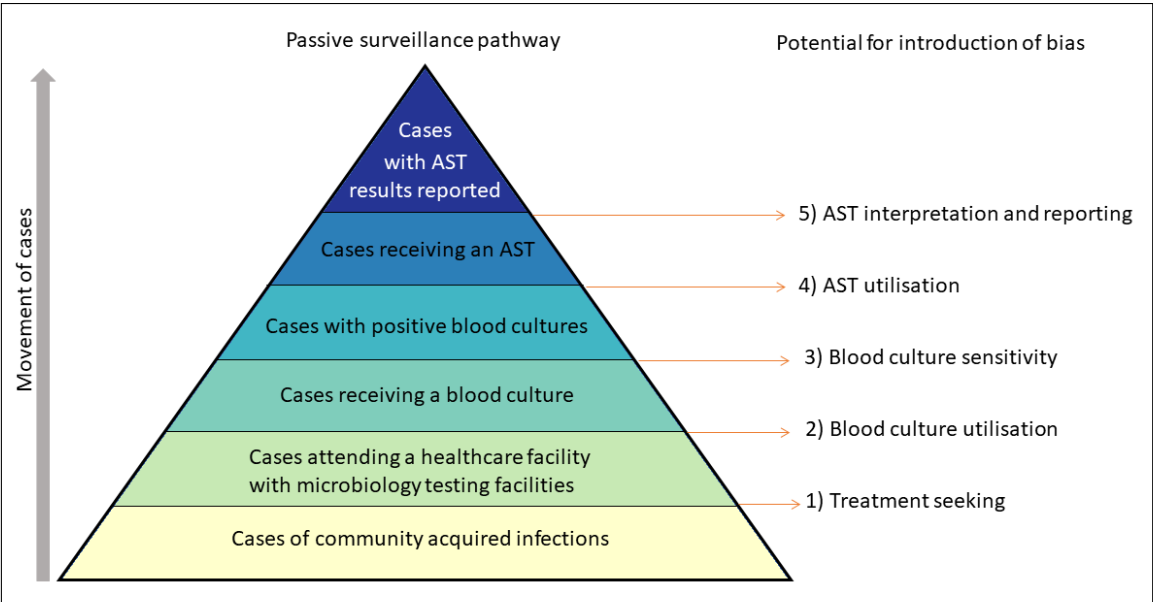


Figure 5.2: Bias pathway for passive surveillance data. The left side of the diagram describes the patient pathway from acquiring a pathogen in the community, to the AST results being reported by the hospital. The right side of the diagram identifies the stages where the sample could be biased to the over- or under-identification of resistant strains

Treatment seeking

Enteric fever is a community acquired infection, meaning that the first stage in identifying cases is for the person to attend a formal healthcare facility. In HICs the proportion of cases seeking formal healthcare is generally high, due to well established healthcare systems which are often free at the point of entry. In LMICs treatment seeking rates are generally lower, with treatment seeking for children under-five with fever (the main symptom of enteric fever) estimated to vary from around 25% in Ethiopia and Chad to over 80% in Uganda, Indonesia and Vietnam.²⁶⁰

The use of formal healthcare is often limited by large out-of-pocket expenses with the perceived severity of the disease,²⁶¹ availability and proximity to healthcare facilities,^{261–264} socio-economic status of the caregiver^{261,264,265} and failure of alternative treatment²⁶⁴ largely impacting the decision to seek formal healthcare. Instead, treatment is often sought from pharmacies and unregistered doctors or alternative healers,^{261,262,265} with large proportions of people receiving antibiotics before attending a formal healthcare setting.^{261–263,265} Low rates of formal treatment seeking results in only a small proportion of enteric fever cases being identified by formal healthcare, even in countries with a high burden of disease, and introduces potential bias into the prevalence of AMR in reported data. It is likely that AMR will be over-represented in the cases of enteric fever who attend formal healthcare facilities as this population will be disproportionately made up of those with persistent, severe infections and those not cured by antibiotics from alternate sources.

Blood culturing utilisation

Once a person has attended a healthcare facility blood cultures need to be performed to identify the infecting pathogen. The utilisation rate of blood cultures is often low in LMICs due to a lack of facilities (such as sterile venipuncture kits, well equipped microbiology laboratories and trained staff), time delays in receiving results, low positivity and high contamination rates, and clinicians perceiving limited utility in the results.^{118,266} Low blood culturing rates are frequently cited to underestimate the

prevalence of AMR.^{17,183,189} When resources are limited blood culturing will be prioritised for patients not responding to empirical antibiotics, resulting in both limited data availability and the reported prevalence of resistance being biased towards resistant cases.

Blood culture sensitivity

S. Typhi blood cultures have low sensitivity, estimated to be around 50%.¹⁴ Low volumes of blood, limited access to automated blood culturing methods, and a lack of experienced staff and training can negatively impact the yield of positive blood cultures.²⁶⁷ Furthermore, use of antibiotics prior to blood cultures being taken has been shown to substantially lower the sensitivity of blood cultures.^{15–17}

In populations with high pre-admission antibiotic usage, or when blood cultures are drawn after the commencement of antibiotic therapy, this negatively impacts the ability to undertake AMR surveillance as it not only provides a challenge in identifying pathogens, but introduces further potential for bias. It is likely that drug susceptible strains will not grow in cultures if the person has taken antibiotics prior to the blood being drawn, whilst drug resistant strains may still grow, leading to the overestimation of AMR.

Antimicrobial susceptibility testing utilisation

As with blood cultures, the determination of resistance profiles can have limited accuracy. Disk-diffusion is the most commonly used AST method in LMICs (due to its simplicity to perform and interpret, and lower cost compared to E-tests). Testing should be undertaken using a control strain to ensure internal validity, however, the availability of both control strains and suitable antibiotic disks are frequently cited as limiting factors for performing AST^{254,259} and can substantially limit the reliability of results. Limited resources introduce the same biases as with blood culture because resources will be prioritised for more severe, persistent cases, biasing the results to overestimating the prevalence of AMR.

Antimicrobial Susceptibility Testing interpretation and reporting

AST results need to be interpreted based on defined breakpoints to establish susceptibility to the specified antibiotic. Guidelines for AST methods and breakpoints for interpretation of susceptibility are defined by the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST). Both of these are regularly updated and are only disseminated in English.²⁵⁵ It is recommended that when reporting AST results the guidelines followed, including the version/year, should be reported.¹⁹⁴ CLSI guidelines need to be purchased, therefore they are often unavailable or out-of-date in LMICs.^{254,256,259}

Using out-of-date breakpoints can majorly impact the prevalence of resistance reported. The CLSI breakpoints for ciprofloxacin resistance in *Salmonella spp.* were lowered in 2012 (a four-fold change from $\geq 4\mu\text{g/mL}$ to $\geq 1\mu\text{g/mL}$ for full resistance, and a 16-fold change from $\geq 2\mu\text{g/mL}$ to $\geq 0.125\mu\text{g/mL}$ for non-susceptibility), dramatically increasing the proportion of isolates which should be classified as non-susceptible. Using data from Capoor *et al.* 2006¹⁵⁷ as an example, interpreting the minimum inhibitory concentration (MIC) results of 178 *S. Typhi* isolates using the CLSI 2010 breakpoints¹⁹⁰ 98% of isolates would be classified as susceptible and 2% as intermediate or resistant. Interpreting the results for the same isolates based on the CLSI 2012¹⁹¹ breakpoints 49% would be susceptible and 51% intermediate or resistant. The lack of proper, up-to-date guidelines and the limited reporting of which guidelines were used in published literature (Chapter Two) limits the reliability and utility of the data. The majority of data sources do not provide the raw MIC or disk diffusion results, and if the guidelines used for interpretation are not reported, this precludes the use of that data to analyse the temporal trends in AMR for antibiotics with breakpoint changes. This also may bias the data as the majority of changes in CLSI or EUCAST breakpoints relate to lowering the threshold for resistance which would result in studies underestimating the prevalence of resistance when using out-of-date guidelines.

Once the AMR profile of a pathogen has been determined a surveillance or reporting system is required for this to be incorporated into a dataset. Electronic reporting systems

are highly beneficial for the collection, analysis and dissemination of AMR data. These can not only improve the ease of data entry, but allow for standardisation and validation of data, as well as quick analysis of temporal trends in resistance and the identification of local or national outbreaks. Few LMICs have established electronic reporting systems with both infrastructure and personnel constraints (lack of a trained IT workforce) limiting its implementation.²⁹ There is little evidence for the introduction of biases when reporting AMR profiles. It is possible that there are negative connotations to a hospital reporting high AMR rates, such as decreased reputation and loss of funding sources. There is no clear evidence that this leads to inaccurate reporting of AMR.²⁶⁸

5.5 Approaches for data adjustment

This review identifies treatment seeking, blood culture and AST utilisation rates (combined into one bias as the underlying reasons are similar) and blood culture sensitivity as important factors contributing towards the potential over-estimation of the prevalence of AMR in passive surveillance data. The biases related to AST interpretation are not included in this discussion of bias adjustments, as this can be accounted for by including only data that specifies interpretation based on up-to-date breakpoints. Potential approaches to dealing with the identified biases were investigated and are discussed in detail, with reference to estimating the prevalence of resistance in *Salmonella* Typhi from data collected in Chapter Two.

5.5.1 Direct adjustment of passive surveillance data

Firstly, the potential for a direct adjustment of passive surveillance data was investigated. Studies using active or augmented surveillance to estimate the prevalence of MDR and FQNS in *S. Typhi* infections were identified from the systematic review in Chapter Two. Active surveillance involves staff identifying all suspected cases of disease in the population under surveillance, whilst augmented surveillance is a study conducted in a healthcare facility which also involves staff going into the community under surveillance

and encouraging or incentivising suspected cases to attend the healthcare facility. The active/augmented surveillance studies would represent the “expected” or “true” prevalence of resistance in the community, and these were matched to the equivalent hospital based (passive surveillance) studies, which represent the “observed” prevalence of resistance. Due to the lack of data, exact location and year-based matches between the active and passive surveillance studies could not be made, so the active surveillance studies were matched to the median prevalence of resistance from passive surveillance studies in the same country and five-year time-period.

Eighteen data-points (from 13 studies) reporting the prevalence of MDR in *S. Typhi* were identified from active/augmented surveillance^{118,269–279} and 13 data-points from eight studies for FQNS.^{118,270,272–276,279} The prevalence of resistance in passive surveillance (observed, x-axis) was plotted against the prevalence of resistance from active/augmented surveillance (expected, y-axis) in Figure 5.3. Ideally, a curve could then be fit between the observed and expected data, and the fitted equation then used to adjust the remaining observed data-points.

This comparison shows largely inconclusive results; studies using active or augmented surveillance are extremely sparse, have small sample sizes (for MDR median sample size of 45.5, for FQNS 40), and are from select locations in few countries (India, Pakistan, Bangladesh and Kenya), therefore it is uncertain how representative they are of community AMR rates in those countries. Additionally, there is a large variation in the observed prevalence of resistance within the studies grouped to provide the passive surveillance estimates (as displayed by the interquartile ranges in Figure 5.3). A two-sample t-test showed no significant difference between the prevalence of MDR ($p = 0.62$) or FQNS ($p = 0.38$) between the active/augmented surveillance studies and the median prevalence of resistance from the matched passive surveillance studies. These limitations mean that fitting the relationship between passive and active surveillance data and calculating adjustment factors was deemed inappropriate and adjustments could not be made for the purposes of this analysis. Further identification of active/augmented surveillance studies would be required to fully investigate the effects

of surveillance strategy on the prevalence of resistance reported.

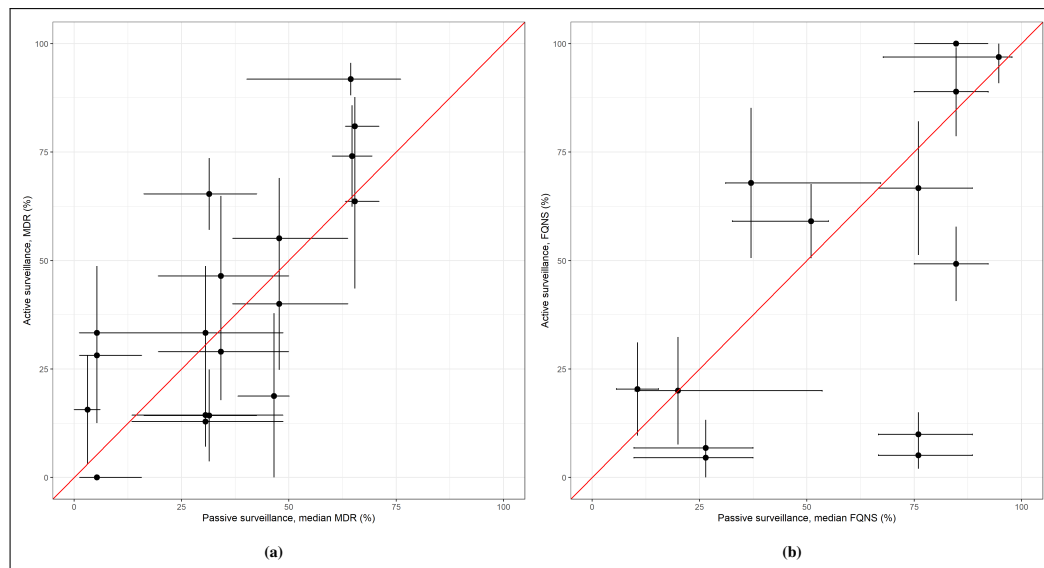


Figure 5.3: Comparison of *S. Typhi* MDR and FQNS in augmented or active surveillance and passive surveillance. Studies estimating the prevalence of resistance from augmented or active surveillance were identified from the systematic review (x-axis, with calculated 95% confidence intervals), these were then matched to the median prevalence of resistance from passive surveillance studies identified for the same country and five-year time period (y-axis, with interquartile range). a) displays the results for MDR *S. Typhi* (Sinha *et al* 1999²⁷⁷ uses chloramphenicol as MDR was not presented); b) displays the results for FQNS *S. Typhi*

5.5.2 Modelling of identified biases

The second potential approach to account for the identified biases is to model the effect of each bias in turn, calculating factors for which to adjust the prevalence of resistance estimates from passive surveillance studies to be the equivalent of that in the community. No published studies modelling the biases for prevalence of resistance estimates were identified, however, the Strategic Typhoid Alliance across Africa and Asia (STRATAA) study²⁴⁹ used a Bayesian modelling framework to estimate the incidence of typhoid fever from passive surveillance data, adjusting the estimates for treatment seeking behaviour, blood culturing rate and blood culture sensitivity.²⁸⁰

This study firstly estimated the impact of blood culturing rates by modelling the probability of “true” typhoid in persons not receiving blood culture (compared to those receiving blood culture) based on the age, duration of fever and temperature of the patient at presentation. The impact of blood culture sensitivity was then accounted for by modelling the probability of “true” typhoid in blood culture negative cases (compared to blood culture positive) based on the volume of blood drawn and antibiotic usage prior to

admission. Finally, the probability of “true” typhoid in those not seeking healthcare was modelled based on healthcare utilisation surveys and the prevalence of location specific typhoid risk factors.²⁸⁰ These factors were then combined into a Bayesian model to estimate adjustment factors for the rates of blood culturing, blood culture sensitivity and treatment seeking. Estimates for the incidence of typhoid were then calculated based on these adjustment factors.

For the STRATAA study, the variables used to adjust typhoid incidence estimates were collected in the study, producing a large, high-quality, patient level dataset including both positive and negative cases. To model the effects of bias on AMR prevalence in passive surveillance data, measures of factors influencing the bias are required. No data containing such variables were identified as part of this thesis, therefore investigation of such a modelling strategy could not be expanded on. Additionally, the data collected from the systematic review does not contain variables driving the bias (i.e. rates of treatment seeking and blood culturing, volume of blood drawn and patients’ symptoms). This limits the application of calculated adjustment factors, as without knowing the values of such variables in the collected data it is difficult to adjust for the biases related to these factors.

5.6 Discussion

5.6.1 Overview

This chapter discusses the limitations and biases related to the use of passive surveillance data to estimate the prevalence of AMR in *S. Typhi* and *S. Paratyphi A*. High quality surveillance of AMR is limited in many LMICs, primarily due to a lack of availability of high-quality microbiology laboratories. This provides major challenges in conducting AMR surveillance and introduces the potential for bias in the reported results. Bias in passive surveillance is primarily due to low rates of treatment seeking, blood culture utilisation, blood culture sensitivity (or high contamination) and AST utilisation. It is hypothesised that these factors lead to an overestimation of the prevalence of AMR in passive surveillance data,¹⁸⁹ however, the magnitude and significance of these biases

could not be satisfactorily ascertained at this time.

Large scale national (or supranational) continuous surveillance conducted in ISO accredited laboratories participating in regular EQA schemes is the gold standard for AMR surveillance.²⁵⁰ Although there is clear evidence of what is required to establish high quality surveillance of AMR, the limitations discussed in this chapter highlight the difficulties in establishing such systems in many LMICs. Establishing sentinel surveillance sites has been identified as a vital first step in implementing AMR surveillance, building on existing capacity to establish such sites then expanding this to create a comprehensive AMR surveillance network.²⁵² Enabling representative testing and reporting of AMR in LMICs is vital and would contribute significantly to efforts estimating and monitoring the prevalence and burden of AMR.

Restricted access to healthcare is a major limitation in implementing AMR surveillance, severely effecting the amount of data available to estimate the prevalence of AMR. Increasing healthcare capacity in LMICs should greatly increase the proportion of the population with enteric fever attending formal healthcare facilities and enable more effective identification, treatment and surveillance of both drug resistant and drug sensitive infections.

Improvements in the numbers, capacity and quality of microbiology laboratories is essential for establishing and improving AMR surveillance across LMICs.²⁵² Laboratory capacity was identified as a major limitation for conducting high quality AMR surveillance. The establishment of secure supply chains, training of laboratory personnel and investment in automated blood culturing facilities would greatly improve the ability of laboratories to identify invasive pathogens and their AMR profiles. Accreditation is an important measure of laboratory quality and the WHO Regional Office for Africa developed the Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA) checklist, now operated by the African Society for Laboratory Medicine (ASLM) in conjunction with their Strengthening Laboratory Management Toward Accreditation (SLMTA) programme.²⁵⁶ These provide guidance on improving laboratory quality with the aim of achieving accreditation. From the launch of the SLIPTA

programme in August 2014 the number of accredited laboratories in Africa increased by 50%,²⁵⁶ representing a large increase in laboratory quality. Improvements and investments are still required to establish sufficient access to high quality laboratories in many LMICs, particularly across sub-Saharan Africa.²⁸¹ Additionally, participation in EQA schemes can greatly improve laboratory quality, identifying issues and providing guidance on how to improve pathogen identification and AST.²⁸²

The availability of globally standardised guidelines for the testing, interpretation and reporting of AMR would be hugely beneficial in ensuring standardised estimates from different countries and regions across the globe. Firstly, a harmonisation of EUCAST and CLSI guidelines would aid in standardised methodology and interpretation of results; ensuring free, easy access to these guidelines in LMICs is essential and providing translations of key documentation into local languages would likely improve their uptake. Additional reporting measures are required to ensure the representativeness and reliability of AST results. Adherence to international reporting guidelines, such as Microbiology Investigation Criteria for Reporting Objectively (MICRO) guidelines,¹⁹⁴ would be beneficial in standardising AMR surveillance and research, greatly aiding in the production of accurate estimates of the prevalence of AMR in enteric fever and allowing for the adjustment of biases and assessment of data quality.

The establishment of well-functioning electronic reporting systems could improve the collection and dissemination of AMR surveillance data in LMICs. They could be utilised to improve communications between local and national stakeholders, improve linkage of clinical, demographic and microbiology data and can increase the ability for timely analysis of trends and identification of outbreaks.²⁸³ However, investment in both the infrastructure and training of personnel is required to make this a reality.

Whilst the existing challenges and limitations in conducting AMR surveillance in LMICs persist, currently the only option for estimating the prevalence of AMR in enteric fever globally is to combine data from multiple sources and attempt to account for the biases and heterogeneity within the data. This chapter investigated potential methods for bias adjustment, both using a direct comparison between active and passive surveillance

data, and by modelling the effects of established biases at each stage. Unfortunately, a paucity of data has deemed both of these potential methods currently inadequate to quantify and adjust for the potential biases. This means that estimates of the prevalence of AMR in *S. Typhi* and *S. Paratyphi* are produced based on unadjusted passive surveillance data and therefore need to be interpreted with an understanding that these biases may contribute towards an overestimation of AMR in enteric fever cases in the community.

Future focus for this research would be to either increase the undertaking of active surveillance studies on AMR in enteric fever (although these methods are prohibitively expensive and time consuming), or to access high quality patient level datasets reporting both positive and negative culture and AST results alongside measurements of variables hypothesised to drive the identified biases (e.g. volume of blood drawn, prior antibiotic use, severity of disease). A modelling framework could then be developed to accurately quantify and adjust for the established biases. No such datasets were available for use in this thesis, limiting the potential for bias adjustment at this stage. This poses an interesting research question that could be explored in the future.

5.6.2 Conclusions

There are many challenges and biases relating to estimating the prevalence of AMR in enteric fever. The availability of data in LMICs is limited, stemming from the lack of well established, well-funded healthcare systems with adequate laboratory capacity. Improvements in such systems are vital for establishing AMR surveillance. The majority of AMR data comes from passive surveillance and there is a considerable potential for bias in such data, primarily due to variations in the rates of treatment seeking, blood culturing, blood culture sensitivity and AST. Based on these factors, the potential for the over-representation of drug resistant pathogens in AMR passive surveillance data is high. Further work is required to fully investigate and quantify these biases, and to enable the adjustment of passive surveillance AMR data to be representative of the community. In the meantime, this must be considered as a limitation when using passive surveillance

data to estimate the prevalence of AMR in enteric fever, particularly in resource limited settings. In spite of this, passive surveillance data are still a useful resource for identifying the magnitude and trends in the prevalence of resistance, if interpreted with care.

The prevalence of antimicrobial resistance in *Salmonella enterica* serovars Typhi and Paratyphi A infections, in endemic countries, 1990 to 2018

6.1 Introduction

Antimicrobial resistance in *S. Typhi* and *S. Paratyphi A* is a growing concern as infections are common in parts of the world with limited access to alternative antibiotics. Ineffective antibiotic treatment can lead to an increase in treatment failure, with a higher rate of complications and death, along with an increased risk of onwards transmission.^{27,284} Consequentially the World Health Organisation (WHO) has listed *S. Typhi* as a high-priority pathogen for the development of new antimicrobial therapies.⁸⁴

Enteric fever is prevalent in LMICs⁴ with limited laboratory capacity, meaning empirical treatment with antibiotics, without access to AST results, is common. It is therefore important to understand the prevalence of AMR in *S. Typhi* and *S. Paratyphi A* within a country in order to help guide antibiotic prescribing and improve treatment outcomes. Additionally, knowledge of the trends of AMR can inform ABS measures, especially in locations where there are now limited options for the treatment of common bacterial infections such as enteric fever.

The systematic review conducted in Chapter Two provides a broad overview of the spatial and temporal trends of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, however,

this does not provide detailed estimates of the prevalence of resistance for specific countries and years, which are important to fully understand the development and spread of AMR in enteric fever.

Statistical modelling has been shown to be an effective method for estimating the burden of both infectious diseases and health indicators for locations where data are lacking.^{81,82,203} In this chapter an extensive dataset of geolocated studies on the prevalence of AMR in *S. Typhi* and *S. Paratyphi A* is taken and statistical modelling techniques utilised to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for each country with endemic disease for each year, 1990 to 2018. These estimates are then applied to the GBD study estimates of disease incidence⁸³ to calculate the overall numbers of drug resistant and drug sensitive enteric fever infections per year. All estimates were produced with accompanying uncertainty intervals and all models are fully validated.

6.2 Methods

6.2.1 Overview

The systematic review in Chapter Two provided an initial dataset for the prevalence of AMR in *S. Typhi* and *S. Paratyphi A*. This systematic review was expanded on to include data from studies reporting MDR and FQNS in *S. Typhi* and *S. Paratyphi A* isolates from sites other than blood culture, and those reporting for five or more isolates (the initial inclusion criteria was ten or more isolates). Data were extracted and processed according to the methodology stated in Chapter Two and disaggregated to provide data linked to the year the isolate was cultured and tested. Where disaggregation was not possible, data were matched to the study mid-year. The modified inclusion criteria were assessed and deemed to cause no bias to the prevalence of AMR reported by the studies. Additionally, data were included from public health surveillance reports and datasets from researchers in the field to produce an extensive dataset of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, linked to the year and location that infection was acquired

(Figure 6.1).

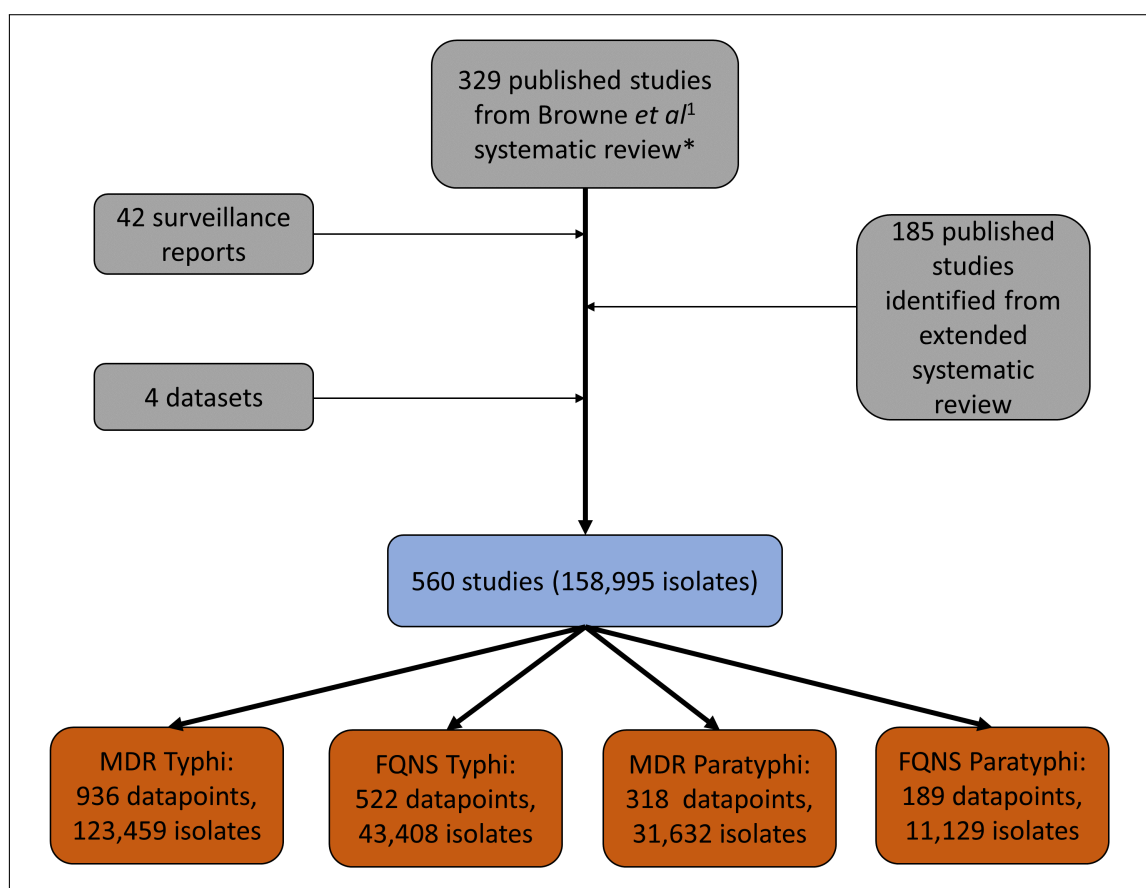


Figure 6.1: Flowchart displaying data sources for the models of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*. *Two of the studies identified by the systematic review were replaced by line level datasets provided by the researchers, one study was deemed a duplicate, four were published in 1993 and did not provide study dates and were deemed to likely be from pre-1990, the remaining studies not included from the systematic review did not provide MDR or FQNS data for *S. Typhi* or *S. Paratyphi A*

These datasets were then used to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* infections, for each endemic country and year for 1990 to 2018. In keeping with the definitions used in Chapter Two, MDR was defined as concurrent resistance to ampicillin, chloramphenicol and co-trimoxazole; FQNS as isolates with a ciprofloxacin minimum inhibitory concentration of $\geq 0.125\mu\text{g/ml}$, or nalidixic acid resistance if the former was not available. Endemic countries were defined as those with an incidence rate of at least 10 cases per 100,000 population in 2017.⁴ Zimbabwe was added to the list of endemic countries, due to recent outbreaks of typhoid fever and evidence of endemicity^{285,286} it was deemed important to characterise the prevalence of AMR in this country. For isolates with AST results for only two of the three antibiotics defining MDR, MDR was assumed (Appendix D.2). Resistance to ampicillin, chloramphenicol and co-trimoxazole is generally encoded on the same IncH1 plasmid,⁵¹

AST results for two of the three antibiotics would indicate the presence of the plasmid.

A stacked ensemble followed by an ST-GPR model, as described in Chapter Four, was used to produce national estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi* for each endemic country and year from 1990 to 2018. These estimates were then combined with estimates of the incidence of *S. Typhi* and *S. Paratyphi* from the GBD study⁸³ to calculate the total numbers of MDR and FQNS *S. Typhi* and *S. Paratyphi* A infections for each year of the study (Figure 6.2).

Additionally, subnational estimates of the prevalence of MDR in *S. Typhi* were produced for each administrative level two district of the study area for 1990 to 2018, using the model based geostatistics methodology described in Chapter Three. This provides both a comparison of estimates from different modelling strategies and an assessment of the subnational variation in MDR *S. Typhi*.

6.2.2 National estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi* A

A two-stage spatial-temporal modelling framework was used to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi* A infections for each endemic country and year for 1990 to 2018. Models were fit separately for each indicator, following the methodology described in section 4.2.3. Firstly, a range of spatially and temporally explicit health and socio-demographic related covariates, with biologically plausible associations to the prevalence of AMR in *S. Typhi* and *S. Paratyphi* A were selected from the GBD database (<http://ghdx.healthdata.org>). These covariates were considered for inclusion, alongside the estimates of antibiotic consumption from Chapter Four. This list of covariates was narrowed down by fitting a lasso penalised regression model between the data and covariates for each dataset and selecting the most influential covariates for each indicator to be taken forward.

Due to high heterogeneity in the input datasets, datapoints found to have the most extreme values for the prevalence of resistance were outliered. A generalised linear mixed-effects model (GLMM) was fit to the data and selected covariates to capture the

crude spatial-temporal trends - the prevalence of resistance within a country could realistically vary from 0% to 100% over the study period. Datapoints which lay outside of two times the median absolute deviation (MAD) of the input data from the modelled estimate for each country were determined to be outliers and removed from the dataset. The GLMM was fit with nested random effects based on the GBD super-region, region and country to capture the spatial effects, and was fit using the “lme4” package version 1.1-21. This resulted in approximately 10% of the most extreme data points being classified as outliers.

The datasets excluding significant outliers were used to fit spatial-temporal statistical models of the prevalence of AMR for each indicator; a stacked ensemble model was fit to leverage covariate effects, followed by an ST-GPR to smooth the data in time and space. For MDR *S. Typhi* the stacked ensemble models were fit separately for sub-Saharan Africa and the remaining regions, and for FQNS *S. Paratyphi A* was fit separately for South Asia and the remaining regions. This enabled the model to capture differing spatial trends and associations between covariates and data in these regions. The covariates were lagged by two years to capture the delayed effects of covariates on the prevalence of AMR. Various time lags were trialled and the two year lag was selected due to superior model predictive performance. As the estimates of antibiotic consumption only covered the years 2000 to 2018, for years prior to this the values from 2000 were used. The covariates and child models selected for each indicator are displayed in Figure 6.3, and the parameters used for the ST-GPR are displayed in the appendices (Table D.2), with more spatial and temporal smoothing applied to locations with sparser data. The mean and 95% uncertainty intervals of the prevalence of AMR were calculated from 1,000 draws of the GPR for all endemic countries and 29 years for each indicator.

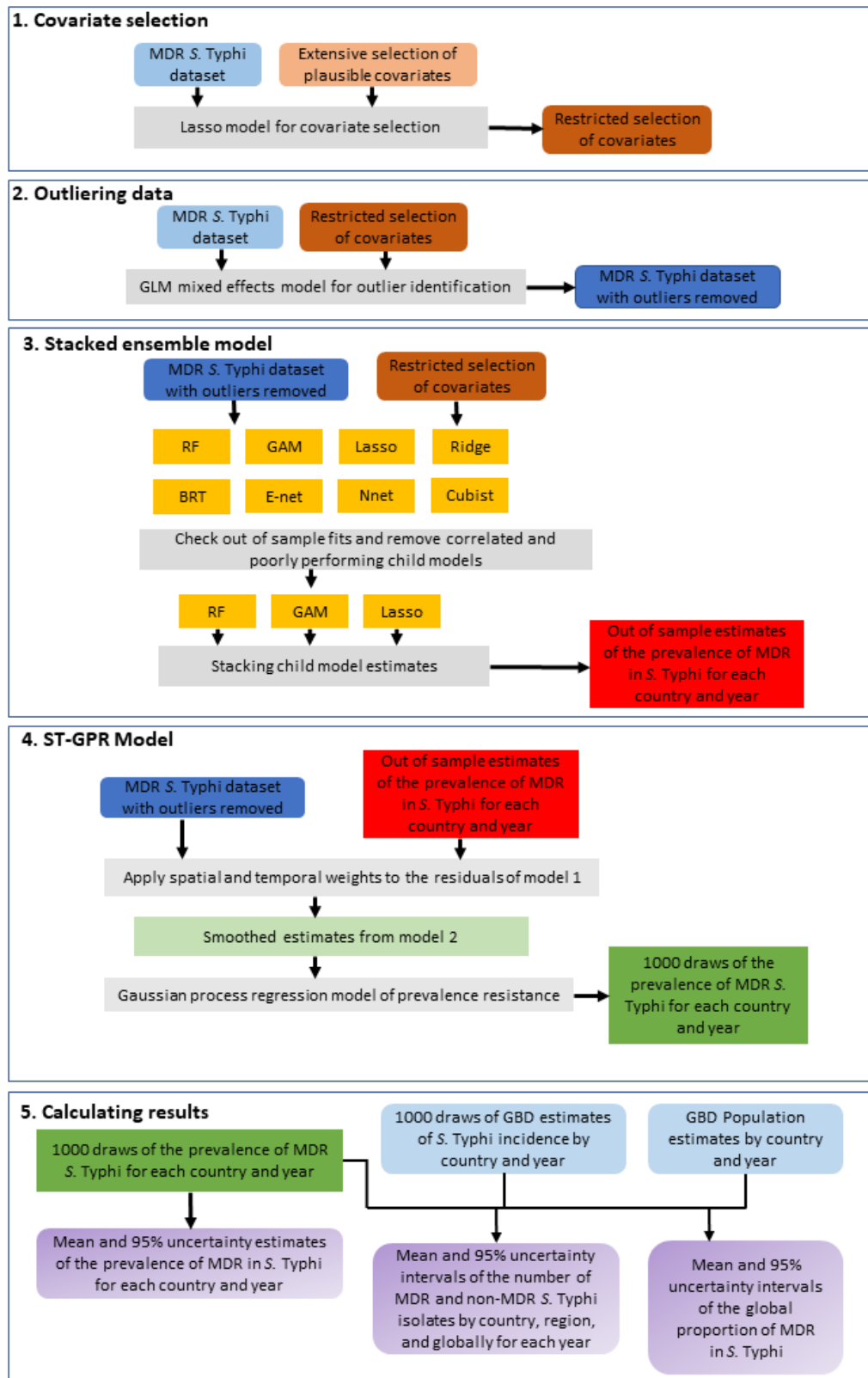


Figure 6.2: Model schematic for national estimates of MDR and FQNS S. Typhi and S. Paratyphi A. This uses MDR S. Typhi as an example, the same modelling strategy was used for all indicators.

Covariate Name	MDR <i>S. Typhi</i>	FQNS <i>S. Typhi</i>	MDR <i>S. Paratyphi A</i>	FQNS <i>S. Paratyphi A</i>
Antenatal care				
DPT coverage				
Hospital beds/1000 population				
Mean temperature				
Total fertility rate				
Improved sanitation				
Improved water				
Antimalarial effectiveness				
Childhood stunting				
Typhoid incidence				
Paratyphoid incidence				
Total antibiotic consumption				
J01C antibiotic consumption				
J01M antibiotic consumption				
Antibiotic usage proportion				
Lag distributed income				
Outdoor air pollution				
Diabetes				
HIV incidence				
Quality assessment				
Child models				
GAM				
Random forest				
Lasso				
Ridge				
Cubist				
Neural network				

Figure 6.3: Stacked ensemble model specifications. For each indicator the covariates and child models used in the stacked ensemble model are shown by the coloured cells. For the MDR *S. Typhi* the stacked ensemble model was fit separately for sub-Saharan Africa and all other regions; covariates and child models used in both models are shown in solid colour, those for just sub-Saharan Africa with horizontal hatching and those for the other regions in vertical hatching. For FQNS *S. Paratyphi* the stacked ensemble model was fit separately for the South Asia super-region and the Southeast Asia, East Asia and Oceania super-region; covariates and child models used in both models are shown in solid colour, those for just South Asia with horizontal hatching and those for just Southeast Asia, East Asia and Oceania in vertical hatching.

6.2.3 Estimating the total number of MDR and FQNS *S. Typhi* and *S. Paratyphi A* infections

The total number of MDR and FQNS *S. Typhi* and *S. Paratyphi A* infections were calculated based on the estimates of disease incidence from the GBD study.⁸³ The incidence of *S. Typhi* and *S. Paratyphi A* for each location and year was modelled previously using a Bayesian meta-regression tool, DisMod-MR. This modelling strategy estimated disease incidence from 1,000 draws of the model posterior distribution; these draws were provided by the Institute of Health Metrics and Evaluation (IHME) and used in the following calculations. The number of *S. Typhi* and *S. Paratyphi A* infections, by endemic country and year, were calculated by multiplying the incidence rate by the population. The total number of MDR and FQNS *S. Typhi* and *S. Paratyphi* infections were then calculated by multiplying these infection numbers by the estimates of the prevalence of resistance produced by this study. These estimates were aggregated up to regional, super-regional and global (all endemic countries) levels for each year of the study. All calculations were conducted at the draw level to propagate uncertainty through the calculations, with the mean and 95% uncertainty intervals calculated at each stage.

6.2.4 Subnational estimates of the prevalence of MDR *S. Typhi*

To investigate alternative modelling strategies and to assess potential subnational variation in the prevalence of AMR, a two stage Bayesian binomial hierarchical modelling framework was used to estimate the prevalence of MDR *S. Typhi* for each 5x5km pixel of the study area, following the methodology described in section 3.2.3. Estimates were then aggregated to the admin level two, admin level one and national level. This modelling strategy requires a large amount of data and satisfactory models could not be fit for the other indicators due to a paucity of data.

Firstly, each study in the dataset was matched to the location where it was undertaken, be this a hospital or a study site within the community. The coordinates (latitude and longitude) of the study site were then identified from either information

within the published study, information provided by collaborators, GoogleMaps or additional ad hoc internet searches. The majority of data were from hospital studies (see Chapter Two), meaning that this is not the precise location where the infection was acquired. To gain a representative sample of where the patients likely acquired the infection, each hospital was matched to the administrative level two district in which it was located and the district was resampled to point locations based on the population weighted κ -means clustering method described in Chapter Three.

The endemic area was split into six regions (Figure D.3) and a stacked ensemble model fit for each region between the data and a selection of spatially and temporally refined covariates hypothesised to impact the prevalence of MDR in *S. Typhi* (Figure 6.4). The estimates of the child models (GAM and Ridge) were then used as covariates in the full geostatistical model (Model priors are shown in Table D.3). One thousand draws of the model posterior were taken and the mean and 95% uncertainty estimates of the prevalence of MDR in *S. Typhi* calculated for each administrative level two district, admin level one province and country.

Covariate	Modelling region					
	South Asia	Oceania	Southeast Asia	North Africa & Middle East	Eastern sSA	Western sSA
DPT3 coverage						
GDP						
Mean temperature						
Childhood stunting						
Outdoor air pollution						
Access to cities						
Urbanicity						
Total health expenditure						
Hospital beds/1000 population						
Total antibiotic consumption						
Improved sanitation						

Figure 6.4: MDR *S. Typhi* subnational model covariates. Covariates are specified for each modelling region.

6.2.5 Model Validation

All models were validated using ten-fold cross validation, and the RMSE and R^2 between the input data and the out-of-sample estimates were calculated to assess the models' predictive performance.

6.3 Results

6.3.1 Data availability

Data were included from 560 sources and approximately 158,995 isolates of *S. Typhi* and *S. Paratyphi A*, covering 45 countries over 29 years (Figure 6.5). The amount of data varied between each of the indicators, with MDR *S. Typhi* having the most data (936 data points, 123,459 isolates), followed by FQNS *S. Typhi* (522 data points, 43,408 isolates), MDR *S. Paratyphi A* (318 data points, 31,632 isolates) and finally FQNS *S. Paratyphi A* (189 data points, 11,129 isolates), reflecting the different geographic distributions of the serotypes. The number of datapoints outnumbers the number of data sources as data were disaggregated by location, year and serotype where possible.

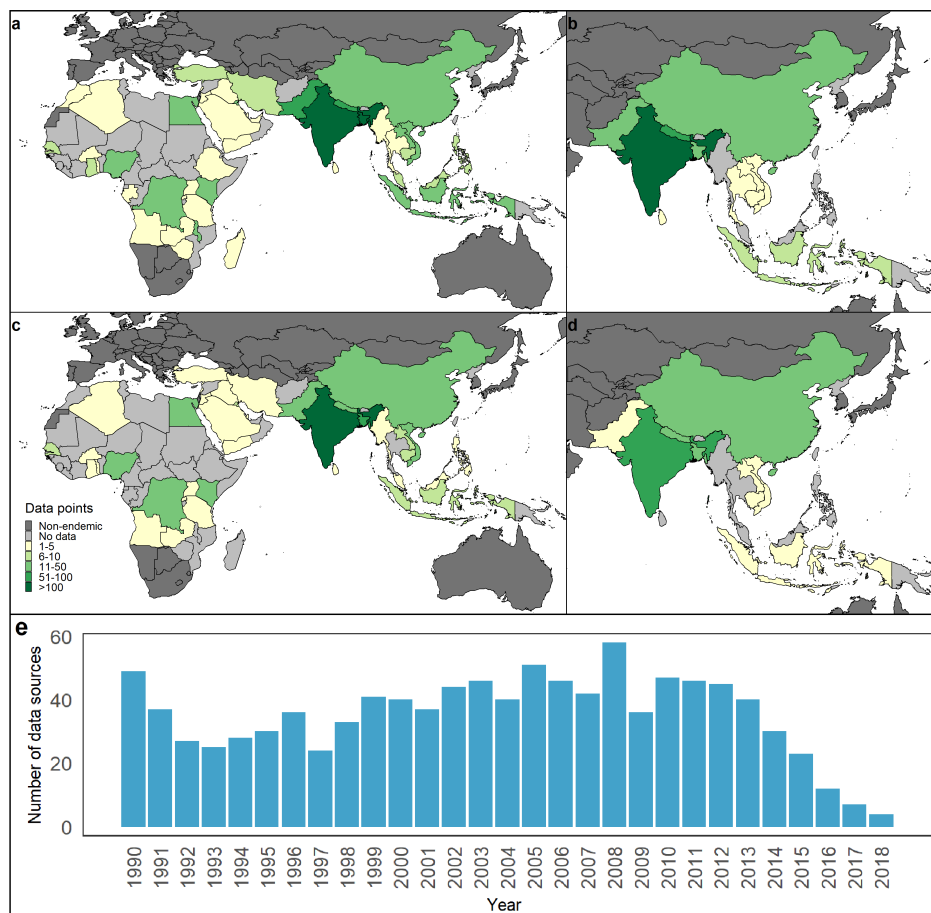


Figure 6.5: MDR and FQNS *S. Typhi* and *S. Paratyphi A* model input data. Maps showing the number of input data points for each endemic country for all years 1990 to 2018, for a) MDR *S. Typhi*; b) MDR *S. Paratyphi A*; c) FQNS *S. Typhi*; and d) FQNS *S. Paratyphi A*

6.3.2 National prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*

MDR *S. Typhi*

The prevalence of MDR in *S. Typhi* varied greatly both over time and by location. In the early 1990's the prevalence of MDR was high in South Asia and this steadily decreased over time from 45.3% [95% uncertainty interval 43.0-47.7%] in 1990 to 17.9% [13.5-22.7%] in 2018 (Figure 6.6). In India, where the incidence of typhoid fever is very high, 45.7% [43.2-48.1%] of isolates were MDR in 1990, but over the study period the majority of isolates regained sensitivity to the first line drugs by 2010 (11.0% [9.4-12.7%] MDR). Spatial variation in the prevalence of MDR in the region is evident, as only modest declines were estimated in neighbouring Pakistan with MDR at 52.1% [42.0-62.3%] in 1990; 32.3% [26.2-39.6%] in 2000; and 33.6% [22.3-45.6%] in 2018 (Figure 6.7).

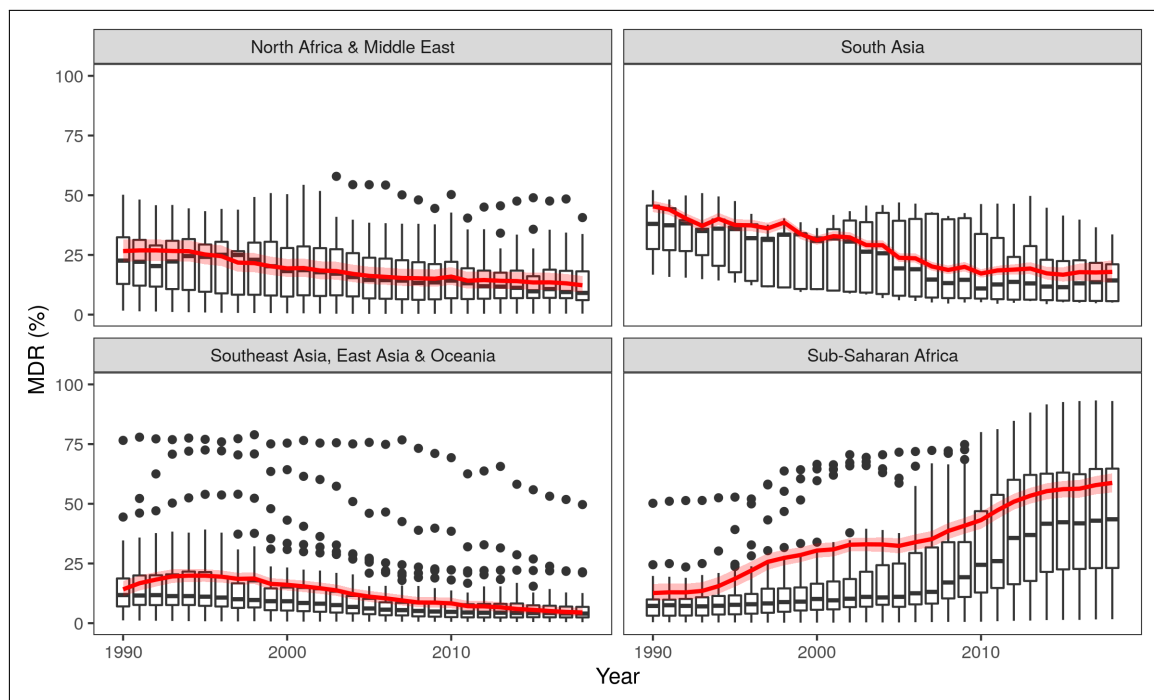


Figure 6.6: Estimates of MDR in *S. Typhi* by super-region, 1990 to 2018. Box plots show the median and interquartile ranges of the national estimates of the prevalence of MDR in *S. Typhi* in each region and year, with outliers shown by the black points. The red line shows the aggregated prevalence of MDR estimates for each region and year with the associated 95% uncertainty intervals, weighted by the number of *S. Typhi* isolates estimated by the GBD study.⁸³

In the Southeast Asia, East Asia and Oceania region the prevalence of MDR also

decreased over time, peaking at 19.9% [17.3-22.7%] in 1994 before decreasing to 4.5% [3.1-6.4%] in 2018 (Figure 6.6). However, the prevalence of MDR varied greatly in this region. In Cambodia and Vietnam extremely high level of MDR were estimated in the early 1990's, reaching heights of 77.5% [57.7-91.1%] and 72.0% [61.8-81.0%] respectively in 1994 before decreasing over time. Conversely, in Indonesia and Philippines the prevalence of MDR remained under 4% for all years of the study (Figure 6.7).

In sub-Saharan Africa the temporal trends of the prevalence of MDR in *S. Typhi* differed greatly to that in Asia with the overall prevalence of MDR increasing from 12.6% [9.4-16.4%] in 1990 to 58.8% [54.7-62.7%] in 2018 (Figure 6.6). There were pockets of high resistance in 1990, with Ghana (50.2% [25.5-72.7%]) standing out. However, levels were generally far lower than in South and Southeast Asia and were accompanied by large uncertainty intervals. By the year 2000, high levels of MDR had emerged in Kenya (59.7% [51.3-68.1%]), Nigeria (66.5% [55.9%-75.9%]) and Ghana (64.6% [48.4%-77.8%]), and in the following years MDR *S. Typhi* spread across most of sub-Saharan Africa. The prevalence of MDR *S. Typhi* in Malawi increased from just 3.1% [1.1-7.5%] in 1990 to 93.0% [87.0-97.0%] in 2018, and in Nigeria from 24.5% [12.7-39.8%] to 93.0% [85.2-97.4%] over the same time period (Figure 6.7).

Finally, in North Africa and the Middle East data were sparse and the prevalence of MDR quite varied. Overall the prevalence of MDR decreased from 26.6% [22.1-31.5%] in 1990 to 12.3% [9.3-16.2%] in 2018. MDR was notably high in Sudan (50.2% [23.0-78.6%]) and Egypt (43.0% [36.2-49.9%]) in 1990. High rates of MDR persisted in Iraq throughout the study period (40.6% [25.0-58.4%] in 2018).

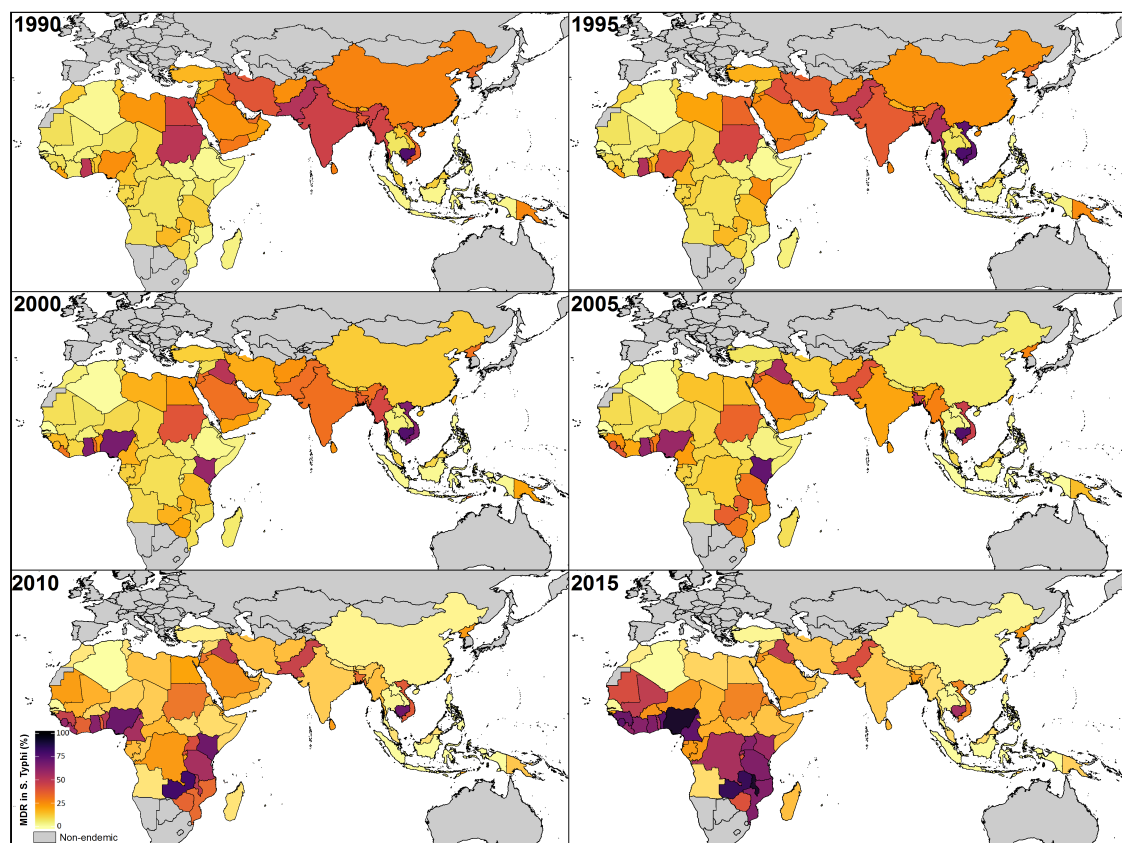


Figure 6.7: Model estimates for the prevalence of MDR in *S. Typhi* in all endemic countries for 1990, 1995, 2000, 2005, 2010 and 2015. Mean of 1,000 draws of the stacked ensemble plus ST-GPR model. Estimates were produced only for endemic countries, with non-endemic countries presented in grey.

FQNS *S. Typhi*

The spatial and temporal trends in the prevalence of FQNS in *S. Typhi* differed greatly to those of MDR. Virtually all isolates across the study area were estimated to be susceptible to fluoroquinolones in 1990, with the prevalence of FQNS increasing throughout the study period. In South Asia the overall prevalence of FQNS increased from 17.0% [7.3-32.2%] in 1990 to 64.5% [45.5-80.6%] in 2018. In the other super-regions the increase was less pronounced but the prevalence of FQNS in *S. Typhi* still increased between 1990 and 2018 from 2.2% [1.2-4.2%] to 14.7% [9.7-22.0%] in Southeast Asia, East Asia and Oceania; 3.9% [2.0-7.2%] to 24.8% [18.5-32.5%] in sub-Saharan Africa; and 3.2% [1.8-5.9%] to 31.8% [23.5-41.5%] in North Africa and the Middle East (Figure 6.8).

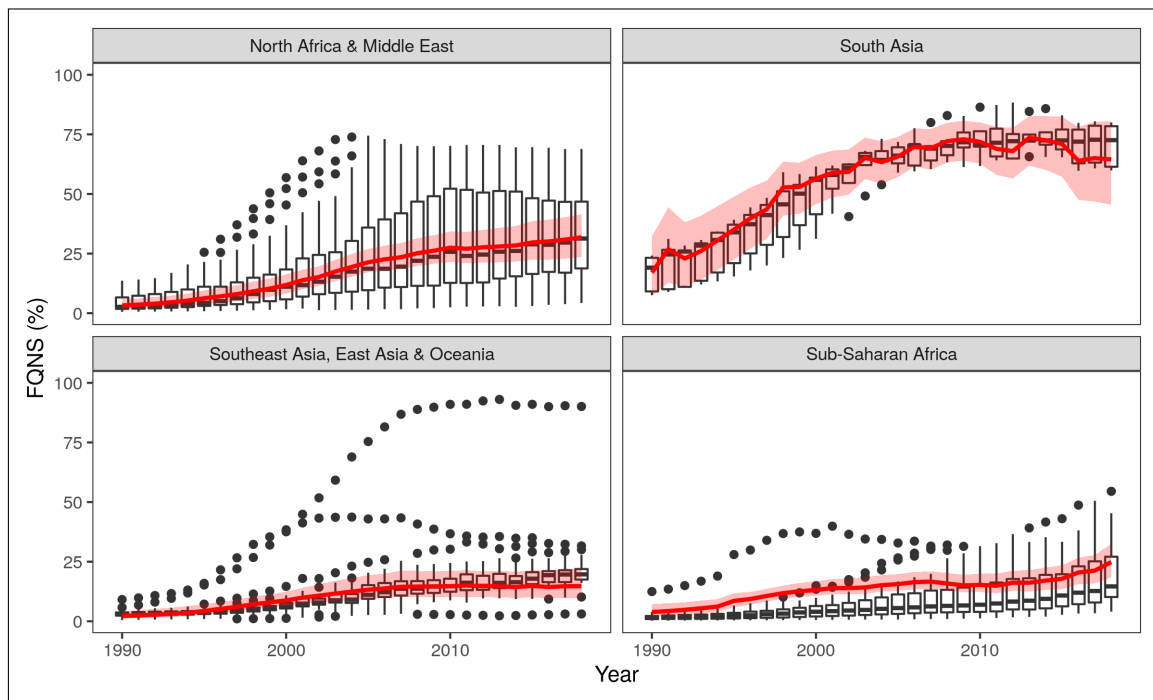


Figure 6.8: Estimates of FQNS in *S. Typhi* by super-region, 1990 to 2018. Box plots show the median and interquartile ranges of the national estimates of the prevalence of FQNS in *S. Typhi* in each region and year, with outliers shown by the black points. The red line shows the aggregated prevalence of MDR estimates for each region and year with the associated 95% uncertainty intervals, weighted by the number of *S. Typhi* isolates estimated by the GBD study.⁸³

In South Asia the prevalence of FQNS reached 69.9% [35.1-81.0%] of isolates in India and 78.4% [50.2-94.7%] in Pakistan in 2018. In Southeast Asia the estimated prevalence of FQNS varied greatly between countries. The most notable increase was estimated in Cambodia where the prevalence of FQNS increased from 9.1% [1.1-31.6%] in 1990 to 90.1% [75.9-97.4%]. As with MDR, virtually all isolates remained

susceptible in Indonesia (3% [0%-13%] in 2018; Figure 6.9).

In sub-Saharan Africa, the prevalence of FQNS in *S. Typhi* was lower than in South and Southeast Asia at all time points, but there was still a steady increase in FQNS throughout the study period. In 2018 the prevalence of FQNS reached 54.5% [33.5-74.4%] in the Democratic Republic of the Congo, and 45.3% [24.6-69.2%] in Kenya. In Western sub-Saharan Africa most isolates remained susceptible to fluoroquinolones, although data were sparse and estimates accompanied by wide uncertainty intervals (e.g. 4% [0%-19%] FQNS in Ghana in 2018), and higher levels of FQNS were noted in Nigeria (33% [15%-54%] in 2018; Figure 6.9). The prevalence of FQNS in *S. Typhi* also increased in the Middle East, however, data were very sparse in this region and model uncertainty is therefore very high.

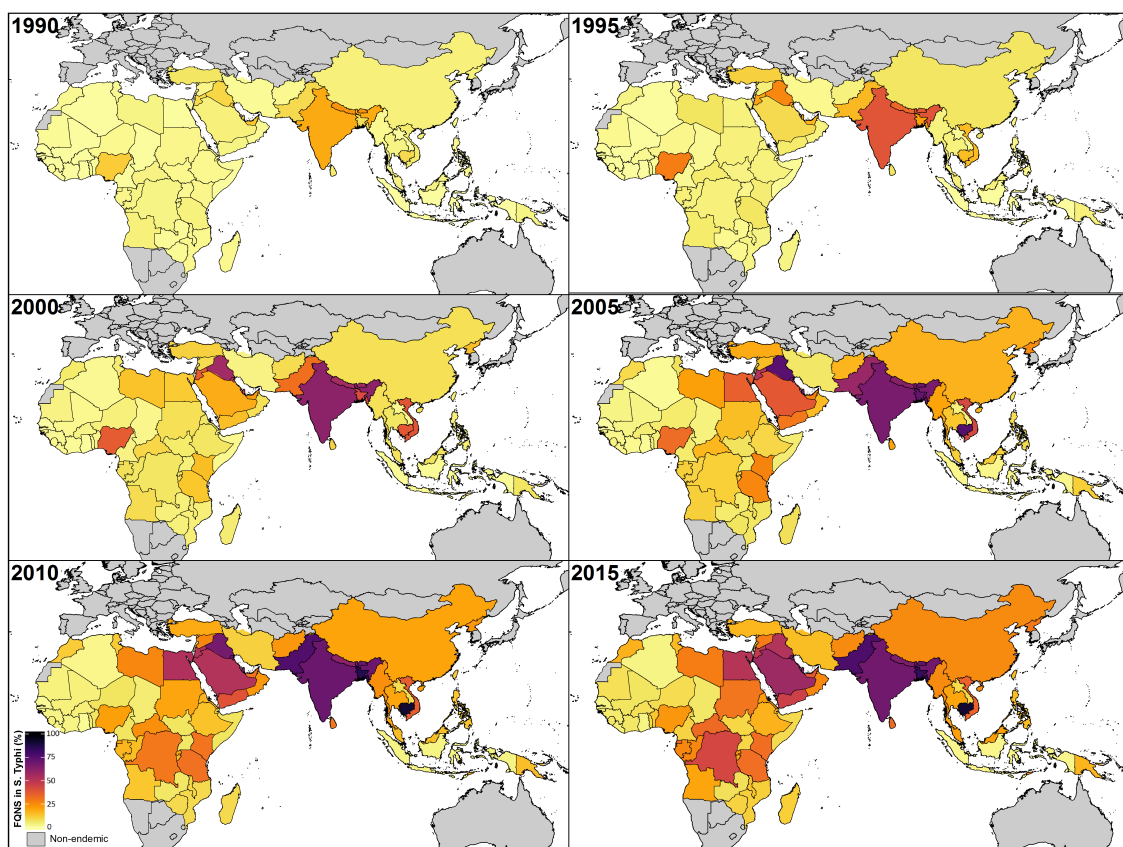


Figure 6.9: Model estimates for the prevalence of FQNS in *S. Typhi* in all endemic countries for 1990, 1995, 2000, 2005, 2010 and 2015. Mean of 1,000 draws of the stacked ensemble plus ST-GPR model. Estimates were produced only for endemic countries, with non-endemic countries presented in grey.

MDR and FQNS *S. Paratyphi* A

The prevalence of MDR in *S. Paratyphi* A was estimated to remain low in all countries across the study period (Figure 6.10). In 2000 this reached a height of 24.5% [22.3-26.6%] in Pakistan, however, this did not expand throughout the region and levels decreased again in the following years (Figure 6.12).

Estimates of FQNS in *S. Paratyphi* A differed greatly from those for MDR. The prevalence of FQNS was quite low in all countries in 1990, but this increased considerably across the study period resulting in the majority of isolates in South, Southeast and East Asia estimated to be FQNS in 2018 (85.9% [75.0-93.0%] in India; 48.2% [20.0-78.3%] in Vietnam, and 86.3% [76.8-92.8%] in China). Estimates in Indonesia remained low throughout the study period ((Figure 6.11), Figure 6.13).

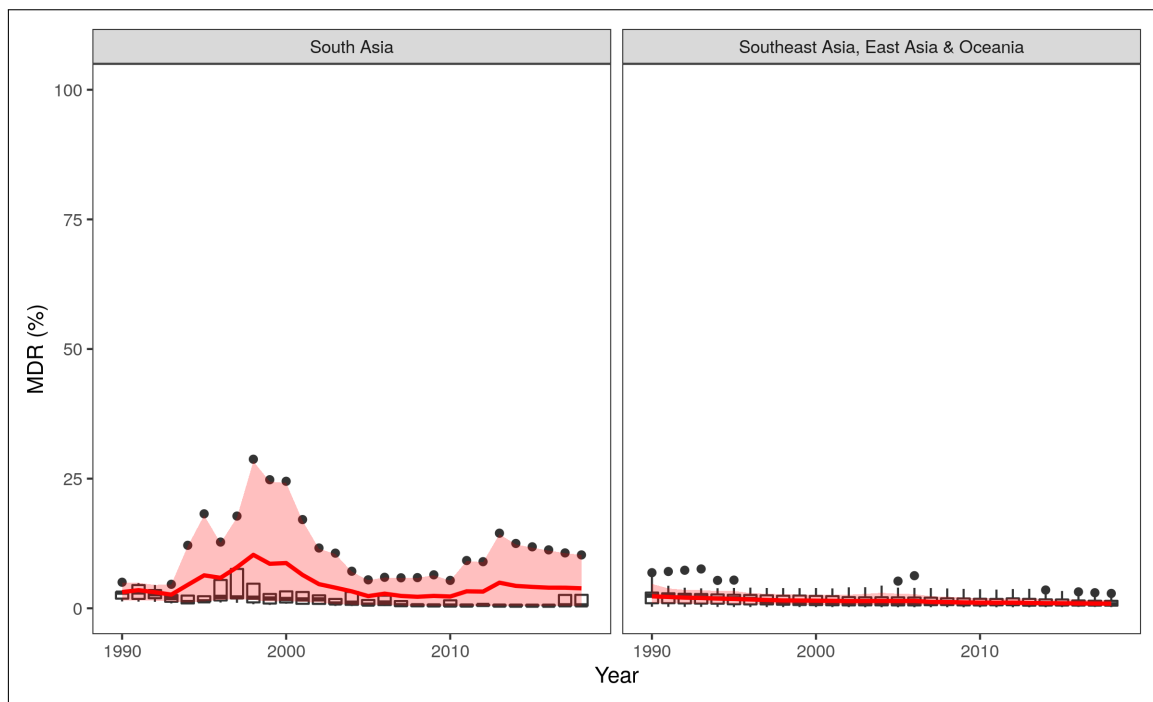


Figure 6.10: Estimates of MDR in *S. Paratyphi* by super-region, 1990 to 2018. Box plots show the median and interquartile ranges of the national estimates of the prevalence of MDR in *S. Paratyphi* in each region and year, with outliers shown by the black points. The red line shows the aggregated prevalence of MDR estimates for each region and year with the associated 95% uncertainty intervals, weighted by the number of *S. Paratyphi* isolates estimated by the GBD study.⁸³

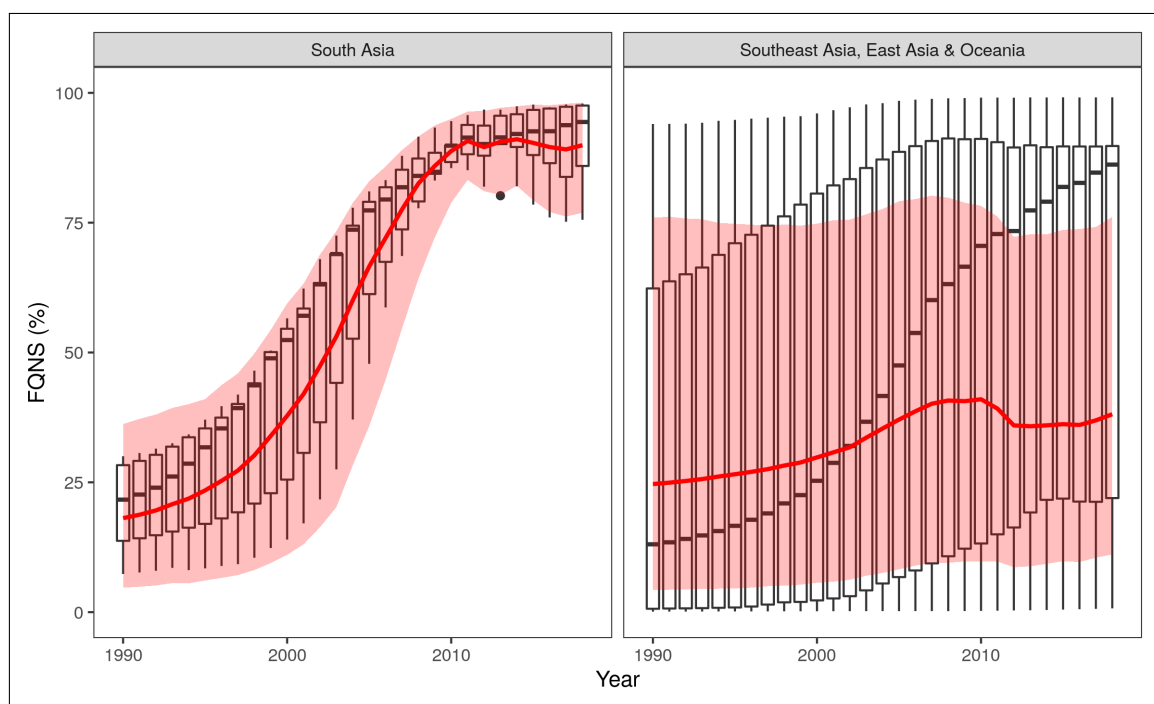


Figure 6.11: Estimates of FQNS in *S. Paratyphi* by super-region, 1990 to 2018. Box plots show the median and interquartile ranges of the national estimates of the prevalence of FQNS in *S. Paratyphi* in each region and year, with outliers shown by the black points. The red line shows the aggregated prevalence of MDR estimates for each region and year with the associated 95% uncertainty intervals, weighted by the number of *S. Paratyphi* isolates estimated by the GBD study.⁸³

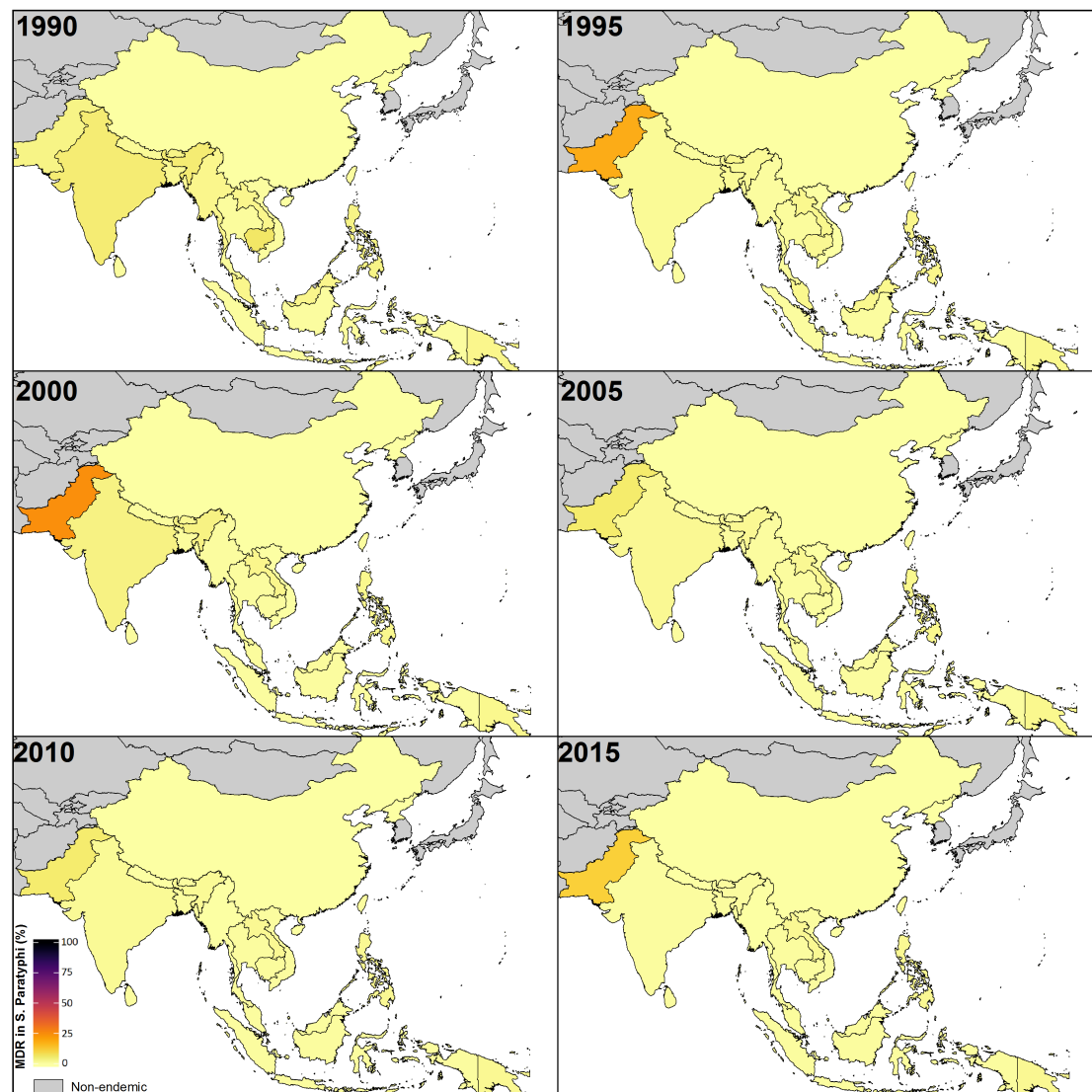


Figure 6.12: Model estimates for the prevalence of MDR resistance in *S. Paratyphi A* in all endemic countries for 1990, 1995, 2000, 2005, 2010 and 2015. Mean of 1,000 draws of the stacked ensemble plus ST-GPR model. Estimates were produced only for endemic countries, with non-endemic countries presented in grey.

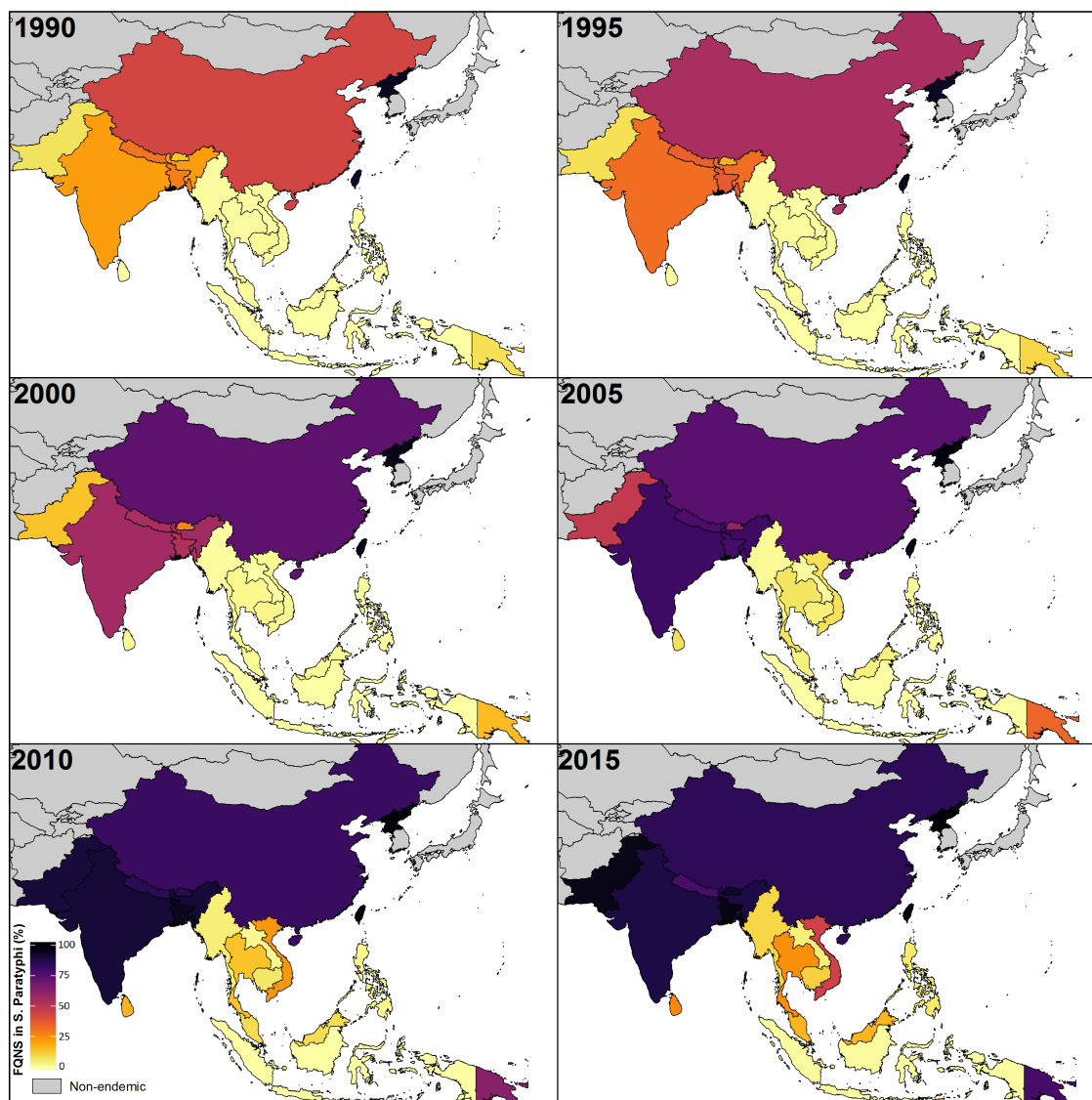


Figure 6.13: Model estimates for the prevalence of FQNS in *S. Paratyphi* A in all endemic countries for 1990, 1995, 2000, 2005, 2010 and 2015. Mean of 1,000 draws of the stacked ensemble plus ST-GPR model. Estimates were produced only for endemic countries, with non-endemic countries presented in grey.

Model uncertainty

Model uncertainty varied greatly between countries and indicators. The relative uncertainty for the 2018 modelled estimates was calculated as the ratio of the uncertainty interval to the prevalence of AMR. Each country was then assigned to the quartile of AMR prevalence and of relative uncertainty to which they belonged and this was plotted on a map (Figure 6.14). This indicates that for MDR in *S. Typhi* in 2018 the high estimates of resistance in Eastern, Central and Western sub-Saharan Africa were generally accompanied by low relative uncertainty; although Chad, South Sudan, Central African Republic, Ethiopia and Senegal had high relative uncertainty. There was low relative uncertainty around the MDR *S. Typhi* estimates in South Asia (India, Pakistan, Bangladesh and Nepal); but China, Myanmar and Thailand, as well as Indonesia and Philippines had high relative uncertainty. For the model of MDR in *S. Paratyphi A* there was similarly low relative uncertainty in India and Pakistan but high relative uncertainty in Myanmar and Indonesia.

For the model of FQNS in *S. Typhi* there was notably high relative uncertainty in North Africa and Western sub-Saharan Africa, excluding Nigeria. There was much lower uncertainty around the high estimates of FQNS in parts of Eastern sub-Saharan Africa (Kenya and Tanzania) as well as South and Southeast Asia. As with the model of MDR in *S. Typhi* there was high relative uncertainty in Myanmar and Indonesia. The relative uncertainty of the FQNS *S. Paratyphi A* model followed the same pattern with low uncertainty in South Asia, compared to far higher relative uncertainty in Southeast Asia and Oceania.

The level of uncertainty reflects the amount of data available within a country and indicates where additional data could greatly improve the model estimates. Maps of the upper and lower uncertainty intervals for each indicator are shown in the appendices.

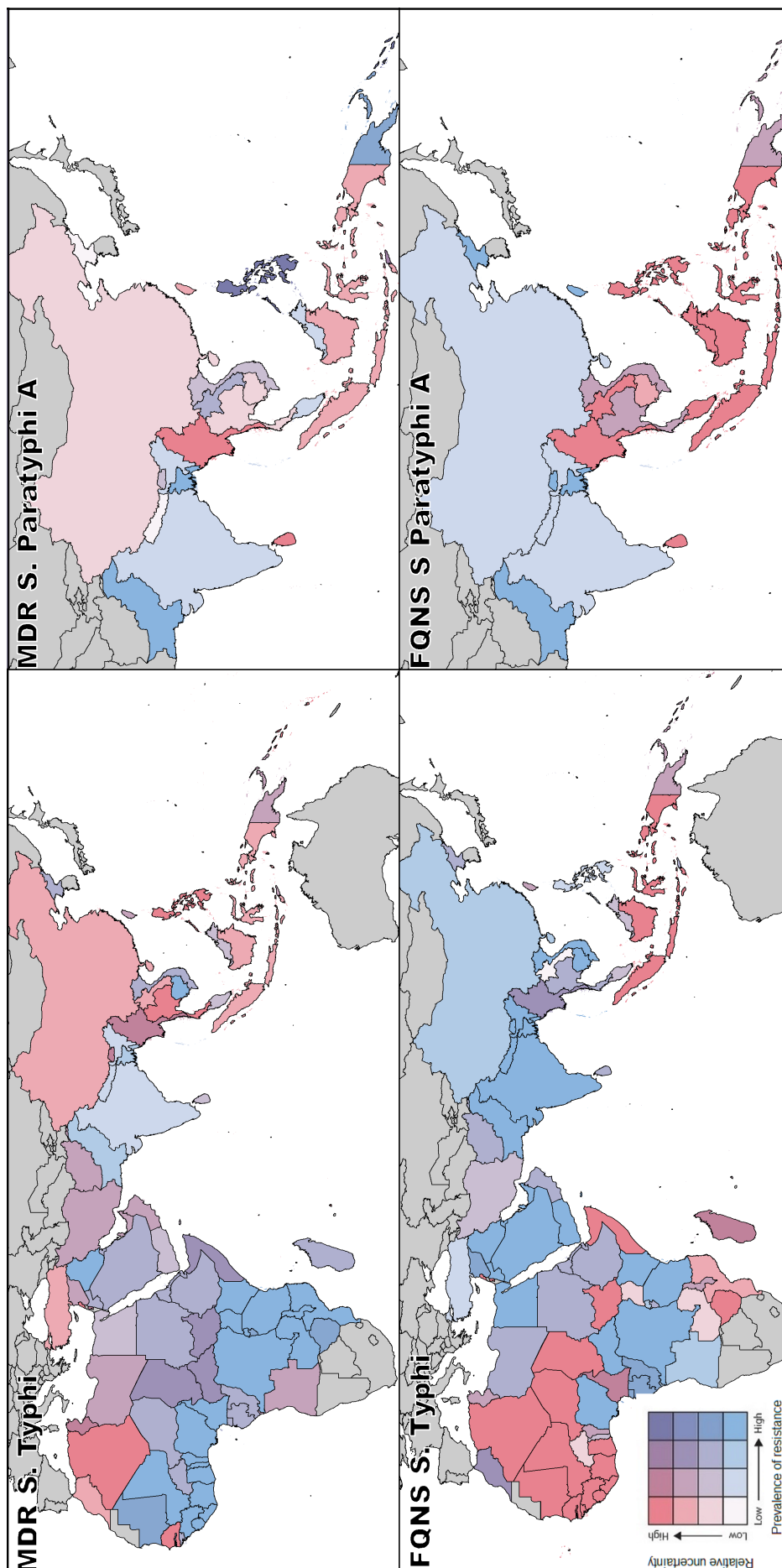


Figure 6.14: Relative uncertainty of modelled estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* in 2018. Estimates are provided for each country. Relative uncertainty was defined as the ratio of the 95% uncertainty intervals to the proportion of AMR. Data were coloured based on the quartile of the proportion of AMR and the quartile of relative uncertainty to which they belonged. Countries not endemic for *S. Typhi* or *S. Paratyphi A* are coloured in grey.

6.3.3 Total numbers of AMR *S. Typhi* and *S. Paratyphi A*

In 1990, of the 18.7 million [11.8-28.9 million] *S. Typhi* infections estimated in all endemic countries by the GBD study,⁸³ 7.1 million [4.4-11.0 million] (37.7% [35.6-39.7%]) were calculated to be MDR and 2.5 million [1.0-5.5 million] (13.5% [6.6-24.6%]) to be FQNS. In 2018, the number of *S. Typhi* infections had almost halved to 9.6 million [6.2-14.7 million] and the number of MDR infections reduced to 2.1 million [1.3-3.2 million] (21.5% [18.2-25.1%]). However, the number of FQNS *S. Typhi* infections had almost doubled over the study period with an estimated 5.0 million [3.0-8.1 million] (52.3% [38.4-63.9%]) infections in 2018. These trends in the numbers of MDR and FQNS infections differed greatly by GBD super-region, reflecting the differing patterns in the prevalence of AMR (Figure 6.15; Figure 6.16).

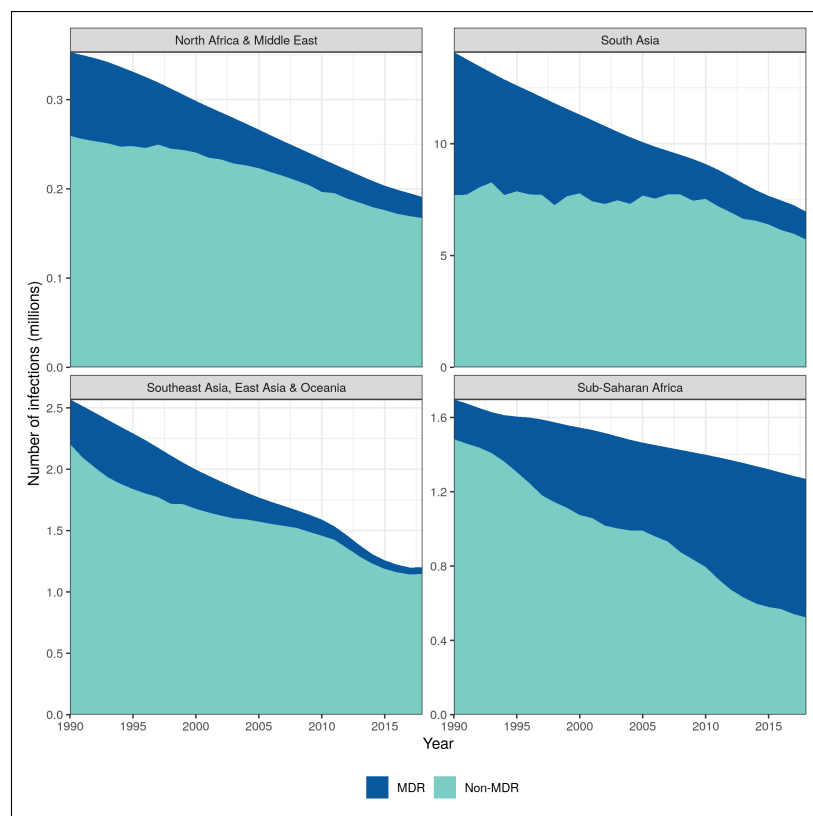


Figure 6.15: Number of MDR, and non-MDR *S. Typhi* isolates by GBD super-region and year, 1990 to 2018. Calculations are based on incidence estimates from the GBD study⁸³ and the modelled estimates of the prevalence of resistance from this chapter.

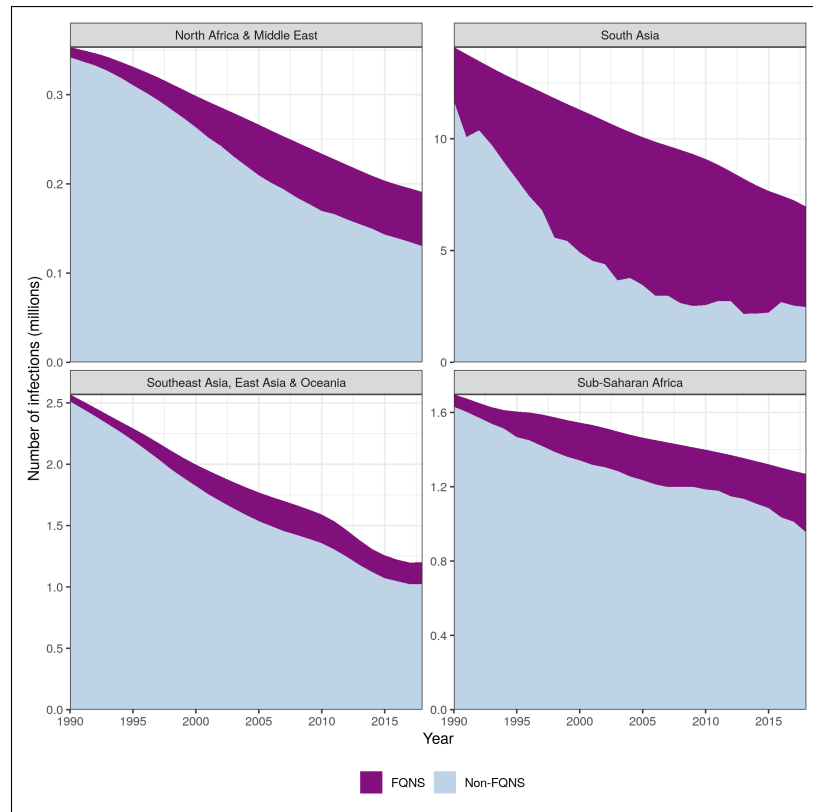


Figure 6.16: Number of FQNS, and fluoroquinolone susceptible *S. Typhi* isolates by GBD super-region and year, 1990 to 2018. Calculations are based on incidence estimates from the GBD study⁸³ and the modelled estimates of the prevalence of resistance from this chapter.

For *S. Paratyphi* A, of 1.2 million [189,792-6.8 million] infections estimated by the GBD study for 1990,⁸³ just 34,284 [4,549-260,521] (2.7% [1.4-4.6%]) were MDR whereas 235,787 [29,465-1.3 million] (22.5% [5.6-74.8%]) were FQNS. In 2018, out of 662,699 [111,178-3.5 million] *S. Paratyphi* A infections, the numbers with MDR stayed constant at 21,385 [1,052-1.0 million] (2.3% [0.5-8.7%]), whilst FQNS increased to 451,553 [63,437-3.0 million] (61.2% [20.8-92.7%]).

6.3.4 Subnational estimates of the prevalence of MDR *S. Typhi*

Results of the subnational model of MDR in *S. Typhi* were similar to that of the stacked ensemble plus ST-GPR. The prevalence of MDR in *S. Typhi* was still estimated to be very high in South Asia in 1990 and decreased throughout the study period, with 41.4% [29.7-54.4%] of *S. Typhi* isolates estimated to be MDR in India in 1990, reducing to 2.6% [1.1-5.6%] in 2018. The modelled estimates supported the local persistence of MDR in Pakistan with numerous districts in the southern provinces of Balochistan and Sindh

having over 20% MDR in 2018 (Figure 6.17).

The prevalence of MDR continued to rise in mainland Southeast Asia between 1990 and 1995, before steadily decreasing throughout the region. MDR *S. Typhi* peaked in Vietnam in 1995 at 75.0% [57.0-89.3%] before decreasing to just 0.9% [0.0-7.5%] in 2018. The prevalence of MDR in *S. Typhi* was low across the island nations of Indonesia and the Philippines over the whole study period. The estimates of MDR in China differed to those of the ST-GPR model, with only low levels of MDR estimated for all years. However, there was considerable subnational variation, in 1995 MDR varied from 0% to 44.7% in various districts of China (Figure 6.17).

The subnational estimates show a clear spread of MDR *S. Typhi* through sub-Saharan Africa over the study period, with the prevalence of MDR increasing from 22.0% [5.9-41.4%] in 1990 to 65.0% [33.5-96.4%] in 2018 in Kenya. From here, MDR *Typhi* spread south through Tanzania, Mozambique, Zambia and Malawi where it increased from 5.9% [0.1-28.0%] in 1990 to 85.1% [50.5-98.9%] in 2018. There is also a clear spread of MDR north into Ethiopia, Somalia and South Sudan. In Western sub-Saharan Africa, again there is an introduction of MDR *S. Typhi* into Nigeria, with higher levels in the more populous south, from where it spreads throughout the region (31.2% [5.5-70.6%] in 1990 to 78.1% [48.4-97.1%] in 2018; Figure 6.17).

Little subnational variation in MDR *S. Typhi* is identified by these estimates. It appears that once MDR *S. Typhi* isolates are introduced into an area they quickly become dominant. This is shown by comparing the situation in Southeast Asia in 1990 and 1995. In 1990 there are a selection of districts with high rates of MDR, but by 1995 this phenotype dominates the whole region. Similarly, in the earlier years of the study MDR is much higher in the eastern districts of Kenya and Tanzania than in the rest of the country; however, these resistant isolates then spread and become dominant across the region.

All estimates were calculated with corresponding 95% uncertainty intervals. These estimates are shown in the appendices.

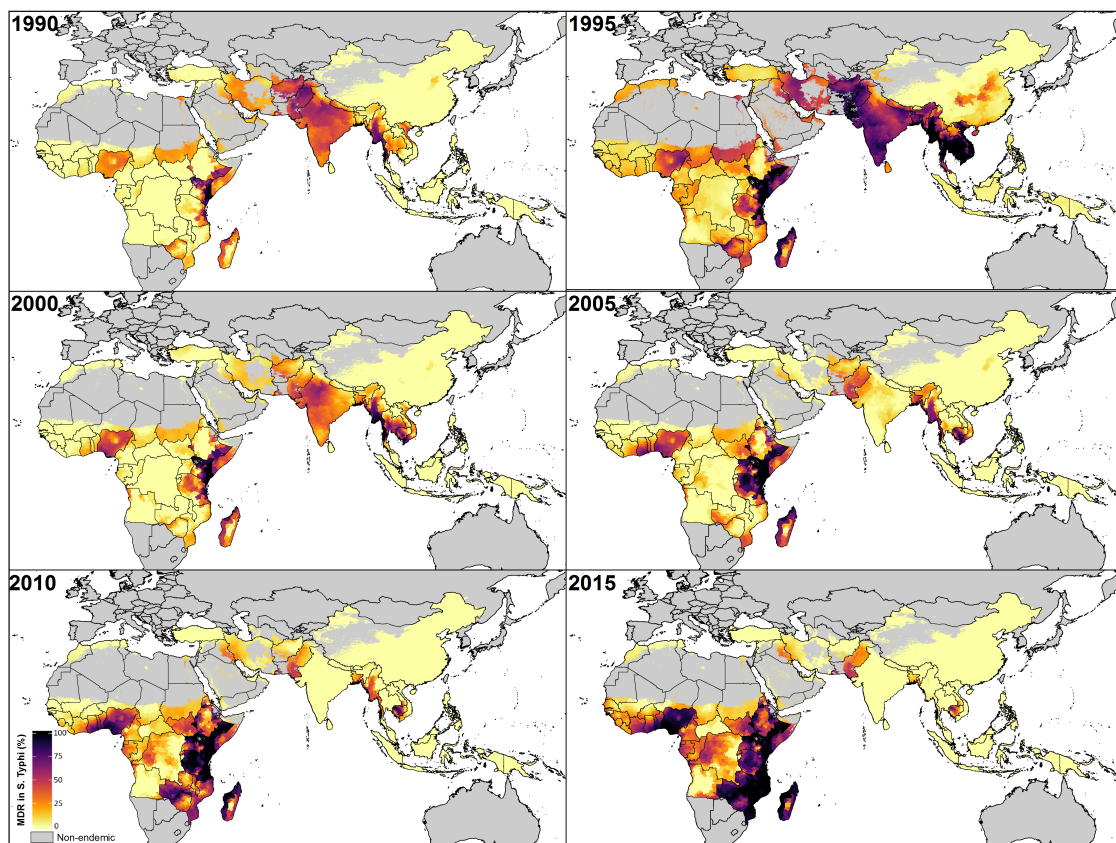


Figure 6.17: Subnational estimates of the prevalence of MDR in *S. Typhi* for all endemic countries, 1990, 1995, 2000, 2005, 2010 and 2015. Estimates from the MBG model are displayed for each administrative level two district. All 1x1km pixels with a population of less than ten are coloured in grey.

6.3.5 Model validation

The model validation statistics show that the models produced reasonable out-of-sample estimates (Table 6.1, Figure 6.18). The relatively high RMSE values for both the in- and out-of-sample estimates correspond to the high heterogeneity in the model input data, but the similarity between the in- and out-of-sample statistics confirms the validity of the models for predicting to country-years without data. For MDR *S. Typhi*, the out-of-sample predictive performance was the similar for both models tested. The stacked-ensemble plus ST-GPR model was less computationally intensive and was therefore selected to produce national estimates for all indicators.

Model	In Sample		Out-of-sample	
	RMSE	R^2	RMSE	R^2
Stacked ensemble/ST-GPR				
MDR <i>S. Typhi</i>	0.14	0.77	0.16	0.68
FQNS <i>S. Typhi</i>	0.22	0.69	0.24	0.62
MDR <i>S. Paratyphi</i>	0.08	0.45	0.09	0.32
FQNS <i>S. Paratyphi</i>	0.19	0.55	0.26	0.30
MBG				
MDR <i>S. Typhi</i>	0.07	0.93	0.15	0.74

Table 6.1: MDR and FQNS *S. Typhi* and *S. Paratyphi* A model validation statistics. In- and out-of-sample validation metrics of RMSE and R^2 for each of the five models.

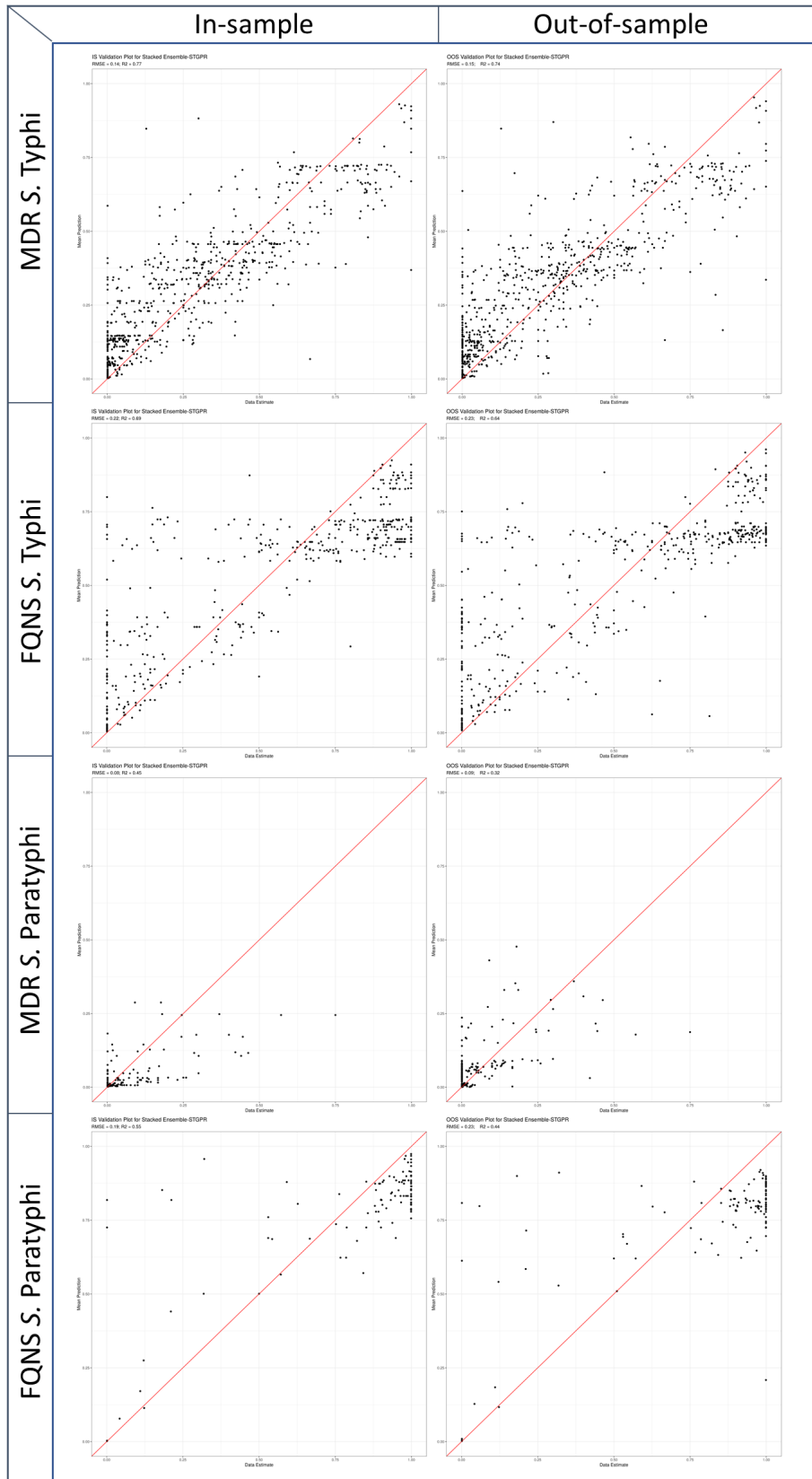


Figure 6.18: Validation plots for the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*. For MDR *S. Typhi*, FQNS *S. Typhi*, MDR *S. Paratyphi A* and FQNS *S. Paratyphi A*, the results of the in- (left) and out-of-sample (right) model predictions are shown on the y-axis, against the estimate from the input data (x-axis).

6.4 Discussion

6.4.1 Overview

This study provides the first detailed estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for countries where enteric fever is endemic, from 1990 to 2018. At the beginning of the study period MDR *S. Typhi* was highly prevalent in South and Southeast Asia. This was followed by a clear decrease in the MDR prevalence in these regions over the following decades. Contrasting this, MDR was virtually non-existent in sub-Saharan Africa in the 1990's, but from the year 2000 onwards there was a dramatic increase in the prevalence of MDR, as it spread across the continent.

Due to the high prevalence of MDR in certain regions, fluoroquinolones became the first choice for the treatment of enteric fever in the 1990's.¹⁴ In the early 1990's almost all *S. Typhi* isolates were susceptible to fluoroquinolones across the endemic region, but with the change in treatment FQNS was quick to arise. The prevalence of FQNS started to increase in South Asia in the late 1990's and spread across many countries in Southeast Asia, East Asia and the Middle East. Of particular concern is the recent increase in the prevalence of FQNS in sub-Saharan Africa, where MDR is also highly prevalent. The pattern of resistance in *S. Paratyphi A* was shown to differ greatly to that in *S. Typhi*, with only low rates of MDR, but extremely high FQNS across the endemic area.

The observed decrease of MDR in South Asia is consistent with reports from India stating a clear re-emergence of chloramphenicol susceptible *S. Typhi*.^{103,104} MDR in *S. Typhi* is typically encoded by the IncHI1 plasmid,⁵² which is easily transmissible between isolates but incurs a large “fitness cost” to the pathogen.⁵⁵ With the reduced use of the older first line antibiotics in certain regions, the selective pressures exerted on *S. Typhi* isolates have decreased. This has resulted in IncHI1 plasmids no longer being maintained in *S. Typhi* populations,⁵¹ resulting in a reduction in the prevalence of MDR.

A whole genome sequencing study that examined the population structure of *S. Typhi* in Africa revealed the recent introduction of clades H58 into Eastern sub-Saharan Africa,⁵² and H56 into Western sub-Saharan Africa.²⁸⁷ Both these clades are highly

susceptible to acquiring mutations or mobile genetic elements encoding both MDR and FQNS. The estimates of this study are consistent with these findings, showing the introduction of MDR *S. Typhi* into Eastern and Western sub-Saharan Africa around the years 2000 and 2005 respectively, followed by a subsequent dominance of the MDR phenotype.

FQNS is encoded by chromosomal mutations in the *gyrA* gene of the DNA gyrase region, and genomic studies show that recent *S. Typhi* isolates in Africa have MDR genes incorporated into the chromosome. This means that both of these mutations may be stable and result in resistance to a larger number of antibiotics.⁵⁵ Concurrent MDR and FQNS in *S. Typhi* is of great concern. Although cephalosporins (e.g. cefixime and ceftriaxone) and azithromycin are effective treatments for drug resistant enteric fever,¹⁴ there have been recent reports of extensively drug resistant *S. Typhi* (XDR; defined as MDR, FQNS, and ceftriaxone resistance) in Pakistan,⁵⁶ and azithromycin resistant *S. Typhi* in Nepal and Bangladesh.^{57,288} This leaves limited treatment options, and highlights the importance of AMR surveillance.

Some countries stood out with levels of resistance differing from that in surrounding areas. Both MDR and FQNS *S. Typhi* were estimated to be low in Indonesia and the Philippines. This is consistent with the lower rates of antibiotic consumption estimated in these countries in Chapter Four. The data coverage for these countries was fair with 18 data points from Indonesia and six from the Philippines for MDR *S. Typhi* and 10 and five for FQNS *S. Typhi*, with little heterogeneity noted. However, as these are highly populous countries, larger and more geographically diverse datasets on the prevalence of AMR would be beneficial for producing accurate estimates of the prevalence of AMR. Estimates for Pakistan show consistently high levels of MDR *S. Typhi* throughout the study period, this is in part due to an ongoing national outbreak of XDR *S. Typhi* in this country.²⁸⁹ In Cambodia, MDR *S. Typhi* was also persistently high. In this case, all data points were from two hospitals (Angkor Children's Hospital and Sihanouk Hospital) with all studies from 2010 onwards having sample sizes of less than 40 isolates. FQNS *S. Typhi* in Laos was notably low throughout. Six of the nine data points were from one

hospital and all studies had sample sizes of 30 isolates or fewer. Small sample sizes and a limited number of sampling locations indicates a low representativeness of the data and highlights the need for establishing robust networks of AMR testing and reporting.

The emergence of cephalosporin and azithromycin resistant *S. Typhi* strains is concerning, as strains with multiple resistances leave very limited treatment options. This study shows the importance of determining the *Salmonella enterica* serotypes for informing treatment decisions, however this can be challenging in many laboratories in LMICs,²⁵⁸ highlighting the importance of building laboratory capacity and training.

The introduction of the new typhoid conjugate vaccine is predicted to play an important role in reducing the incidence of *S. Typhi*.²⁹⁰ However, there is currently no vaccine for *S. Paratyphi A*, which has already been shown to be the predominant serotype in parts of Asia.⁹ The proportion of enteric fever cases caused by *S. Paratyphi A* is only likely to increase as vaccination drives down the incidence of *S. Typhi*. Focus on improvements in sanitation and clean water, alongside vaccination and maintaining access to effective antibiotics are essential for continuing to successfully treat enteric fever infections. Surveillance of AMR is essential for monitoring the trends in resistance and determining appropriate treatment options for enteric fever, reducing morbidity and mortality.

6.4.2 Limitations

The main limitations of this study surround the paucity and high heterogeneity of the input data. Data were particularly sparse in Oceania, parts of sub-Saharan Africa and the Middle East; this is reflected by the large uncertainty intervals around the estimates in these regions. This study identifies locations with no data on AMR *S. Typhi* and *S. Paratyphi A*, where investments in AMR research and surveillance are fundamentally required.

The Clinical Laboratory and Standards institute (CLSI) changed the recommended breakpoints for FQNS¹⁹¹ during the study period, in order to analyse the temporal trends FQNS was defined as ciprofloxacin non-susceptibility (based on CLSI 2019 breakpoints)

or nalidixic acid resistance. This severely limited the amount of data available as many studies did not specify the breakpoints used to interpret ciprofloxacin resistance. Although nalidixic acid is frequently used as a marker of FQNS it does not identify all mechanisms of resistance.²⁹¹ CLSI recently changed their recommendations to using pefloxacin disk diffusion to detect FQNS in *Salmonella* infections.²⁹² Unfortunately this was not tested for historically and could not be incorporated into the analysis.

The input data from systematic review had limited information on the laboratory quality, microbiological methods used, sampling strategies implemented and potential biases in the data. To account for the effect of study quality on the reported prevalence of resistance, a measure of study quality (the “quality assessment score” defined in Chapter Two) was included in the stacked ensemble model, and a term for non-sampling variance included in the Gaussian process regression model. This enabled the models to decipher the spatial and temporal trends in AMR amongst noisy data, however, the impact of the high heterogeneity is reflected by the validation metrics of the models. Good quality input data are essential to model the prevalence of AMR; the establishment and expansion of AMR surveillance networks in LMICs is vital to obtain reliable microbiology data and successfully monitor trends in AMR.

The vast majority of AMR data were collected through passive surveillance. This may lead to an over-representation of AMR, as the samples will disproportionately be made up of persons with persistent or severe infections seeking formal healthcare and receiving diagnostics (discussed in detail in Chapter Five). It was not possible to quantify the impact of passive surveillance on the prevalence of resistance reported at this time, and is therefore important to consider this limitation when interpreting the results.

The input data were primarily aggregate data and was limited for alternative antibiotics. This meant that it was not possible to model the prevalence of concurrent MDR and FQNS, or the emergence of cephalosporin or azithromycin resistance. It is vitally important to continue monitoring MDR and FQNS in *S. Typhi* and *S. Paratyphi* A, as well as the emergence of XDR and azithromycin resistant strains.

6.4.3 Conclusions

This study provides useful information on the trends of MDR and FQNS in *S. Typhi* and *S. Paratyphi A*, highlighting the re-emergence of non-MDR *S. Typhi* strains in parts of South and Southeast Asia and the subsequent emergence and spread of FQNS isolates. It provides a useful resource to inform treatment options and a benchmark from which to measure future changes in AMR *S. Typhi* and *S. Paratyphi A*. AMR surveillance data are sparse in LMICs and the establishment and maintenance of high-quality microbiology laboratories in these regions is essential for conducting AMR surveillance and fully understanding the threat posed by drug resistant infections.

General conclusions

7.1 Overview

This thesis aimed to investigate the prevalence of AMR in enteric fever. In the process of this investigation a contemporary dataset of AMR *S. Typhi* and *S. Paratyphi A*, linked to the location and year of infection was compiled. Spatial-temporal maps of antibiotic usage and consumption were produced to quantify this key driver of resistance. The potential challenges and biases related to gathering AMR data and estimating the prevalence of AMR were discussed, and finally, statistical models were used to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for each endemic country and year, from 1990 to 2018.

Here, each chapter is summarised and the key findings discussed in relation to the thesis aims, their implications to the global burden of AMR, and the subsequent policy decisions to tackle AMR. The results and limitations of the thesis are put into context and directions for future work are identified.

7.2 Summary of chapters

In Chapter Two a systematic review and meta-analysis were undertaken to assess the prevalence of AMR in blood culture isolates of *S. Typhi* and *S. Paratyphi A*. An extensive dataset of the prevalence of resistance in these isolates was compiled and a meta-analysis conducted to assess the pooled prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* separately for each GBD region and five-year time period from 1990 to 2018. An assessment of emerging ceftriaxone and azithromycin resistance was drawn from the available data.

Chapter Three focussed on estimating the probability of antibiotic usage in children under-five with symptoms of LRI in LMICs, as a proxy for general antibiotic usage in the community. Data were extracted from household surveys and a Bayesian hierarchical model used to produce estimates of antibiotic usage for each 5x5km pixel of the study area, for each year from 2000 to 2018. Analysis of the spatial and temporal trends in antibiotic usage was conducted at both the national and subnational level.

Chapter Four expands on the estimates from Chapter Three; data on antibiotic consumption were accessed from multiple sources and a stacked ensemble model was fit, followed by an ST-GPR model to estimate the total antibiotic consumption in defined daily doses per 1,000 population per day for 204 countries and 19 years, from 2000 to 2018. The consumption of various classes of antibiotics were calculated for each country and year, alongside an analysis of consumption by the WHO AWaRe categories.

Chapter Five contains a narrative review of the challenges and biases associated with AMR data and the estimation of the prevalence of AMR. Issues of limited data availability in LMICs were discussed, along with the lack of laboratory capacity, quality control, and the potential biases that may arise from passive surveillance data. Low rates of treatment seeking behaviour, a lack of blood culture capacity, limited blood culture sensitivity and limited AST capacity were identified as key stages at which bias could be introduced, leading to the potential overestimation of AMR in passive surveillance data. Possible methods for accounting for these biases were discussed, however, these were found to not be actionable in the scope of this thesis and were identified as a future area requiring research.

In Chapter Six, work from the previous four chapters was pulled together and used to estimate the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* for each country and year, from 1990 to 2018. The dataset from Chapter Two was used alongside the estimates of antibiotic consumption from Chapter Four and statistical models were implemented to produce estimates of the prevalence of AMR with uncertainty. These estimates were then applied to contemporary estimates of enteric fever incidence from the GBD study to calculate the overall number of MDR and FQNS *S. Typhi* and *S. Paratyphi*

A infections for each year of the study.

7.3 Key findings

The thesis identified a paucity of AMR surveillance data in many LMICs. Established high quality surveillance networks reporting the AMR profiles of enteric fever isolates in LMICs are sparse,⁸⁶ the analysis was therefore largely reliant on data gathered from systematic review. Data from systematic review were associated with high levels of heterogeneity and limited accompanying information to assess the sampling strategies or the quality of data. Compliance to reporting guidelines such as the recently published Microbiology Investigation Criteria for Reporting Objectively (MICRO) guidelines¹⁹⁴ will be beneficial in ensuring the quality of data in published literature is high and easily assessable by those reading the studies.

Data were particularly sparse in Oceania and parts of sub-Saharan Africa. Limited laboratory capacity was identified as the primary reason for the lack of data from these regions. Investment in improving capacity is vital to ensure a high standard of diagnostics to improve both patient outcomes and the surveillance of AMR. The Fleming Fund, set up by the UK government to tackle antimicrobial resistance globally, has produced a document: “AMR surveillance in low- and middle-income countries: a roadmap for participation in the global antimicrobial surveillance system (GLASS)”,²⁵² citing key areas where further work is required to improve AMR surveillance. The Fleming Fund currently works with 24 countries across sub-Saharan Africa, South Asia and Southeast Asia to improve their laboratory capacity and AMR surveillance,²⁵³ but further investment across LMICs is essential to monitor and control AMR in these regions.

Quantifying the amounts of antibiotics used in various locations and how this has changed over time was deemed critical to improve our understanding of AMR. Chapter Three showed large variations in individual level antibiotic usage both within and between LMICs. Antibiotic usage was highest in Central Europe, Eastern Europe and Central Asia, and lowest in sub-Saharan Africa. Large within country disparities in antibiotic usage

were identified, with usage in urban locations greatly outweighing that in rural settings, most notably in Western and Central sub-Saharan Africa. These areas should be the focus for policy makers to ensure equitable access to essential antibiotics, in order to achieve SDG 3.2.

Expanding this analysis to estimate antibiotic consumption (in DDD/1,000 population/day) in 204 countries/territories revealed extreme antibiotic consumption rates in some countries, representing overuse and lack of access to antibiotics. An almost 10-fold variation in national rates of antibiotic consumption was identified, with the highest rates in Southeast Europe (Greece, Turkey), North Africa (Algeria, Tunisia), and parts of Southeast Asia (Malaysia, Vietnam) and the lowest rates in sub-Saharan Africa (Central African Republic, Burundi) and other parts of Southeast Asia (Indonesia, Philippines). In spite of antibiotic consumption remaining stable in HICs and almost doubling in LMICs between 2000 and 2018, antibiotic consumption in LMICs still lags far behind that in HICs. Interventions are required on both ends of the spectrum to improve treatment of bacterial infections and reduce the pressures exerted on pathogens resulting in the development and propagation of AMR.

Detailed estimates of the prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* were produced for each endemic country and year between 1990 and 2018. The prevalence of MDR in *S. Typhi* was extremely high in South and Southeast Asia in the early 1990's, but isolates have largely regained sensitivity to the older first line drugs in recent years. High levels of MDR in *S. Typhi* still persist, most notably in Pakistan. In sub-Saharan Africa the picture was very different. MDR *S. Typhi* was introduced into Eastern and Western sub-Saharan Africa around the year 2000 and has spread rapidly across the continent. This is in part due to the clonal dominance of the H58 haplotype susceptible to acquiring MDR mutations.⁵⁵

Only a very small proportion of *S. Typhi* isolates were identified as FQNS globally in 1990, this increased greatly throughout the study period. Evidence suggests that FQNS first emerged in South Asia and spread through Southeast Asia, then later it was introduced in sub-Saharan Africa. Currently, the majority of *S. Typhi* isolates globally

are FQNS.

The susceptibility pattern of *S. Paratyphi* A was shown to differ greatly to that of *S. Typhi*, with isolates generally remaining susceptible to all first-line drugs in all locations throughout the study period. Conversely, high FQNS in *S. Paratyphi* A was identified across South, Southeast and East Asia, increasing in prevalence throughout the study period. MDR is commonly inferred by the IncH1 plasmid in *S. Typhi*.⁵² Although this plasmid has been identified in *S. Paratyphi* A,¹⁷⁸ it has not become dominant, indicating that there might be fitness costs associated with plasmid carriage. FQNS differs as this is commonly encoded by a point mutation in the quinolone resistance determining region (QRDR), which has been frequently identified in both *S. Typhi* and *S. Paratyphi* A,⁵⁵ corresponding to the high levels of FQNS estimated in both serotypes.

The trends in resistance observed in *S. Typhi* are largely in line with the estimates of antibiotic consumption from Chapter Four. Figure 4.8 shows a huge increase in fluoroquinolone consumption in South Asia between 2000 and 2010; this is clearly reflected by the increase of FQNS in *S. Typhi* and *S. Paratyphi* A in South Asia over this time period. Although fluoroquinolone consumption also increased in sub-Saharan Africa, this increase was seen later in the study period, from 2010 onwards with a steeper increase from 2015. This is reflected by the later introduction of FQNS *S. Typhi* into sub-Saharan Africa. Based on data for the consumption of individual antibiotics from the IQVIA dataset and using India and French West Africa as examples for the situation in South Asia and sub-Saharan Africa; the consumption of chloramphenicol, co-trimoxazole and ampicillin all decreased in India over the study period, whilst in French West Africa consumption of these antibiotics remained relatively constant or increased (Figure C.4). This is mirrored by the prevalence of MDR decreasing in most of Asia over the study period, whilst increases in MDR *S. Typhi* were estimated for sub-Saharan Africa.

With the high rates of MDR and FQNS identified in *S. Typhi*, the emergence of third generation cephalosporin and azithromycin resistance is of major concern. The majority of studies identified reporting the emergence of resistance to third generation

cephalosporins (ceftriaxone) were from South Asia (Chapter Two), where consumption of this antibiotic class increased over 16-fold between 2000 and 2018 (Figure 4.8). More recently, reports of azithromycin resistance have emerged in South Asia,^{57,169–173,288} where the IQVIA dataset showed huge increases in azithromycin consumption between 2000 and 2018 (Figure C.4).

7.4 Implications to policy

This work highlights the growing threat of AMR enteric fever. High levels of both MDR and FQNS, alongside emerging ceftriaxone and azithromycin resistance are concerning. The temporal analysis of resistance shows that once resistant strains are introduced into a location they quickly become dominant. We are rapidly running out of efficacious antibiotics for the treatment of enteric fever, the WHO classified *Salmonella* as a high priority pathogen for the research and development of new antimicrobial therapies.

Chloramphenicol is a cheap and effective treatment for drug-sensitive enteric fever.²⁹³ The re-emergence of chloramphenicol susceptible *S. Typhi* provides potential for it to once again be used as a first line treatment for enteric fever in parts of South and Southeast Asia. However, there is limited knowledge as to how quickly *S. Typhi* isolates may regain IncH1 plasmids encoding for MDR if once again exposed to chloramphenicol. Use of this antibiotic would need to be implemented alongside AST and careful monitoring of the prevalence of resistance. Additionally, there are reports of MDR encoded by genes incorporated into the bacterial chromosome. These mutations are likely to be more stable than plasmid acquired resistance and sensitivity is less likely to re-emerge when antibiotic exposure is reduced.⁵⁵

With the abundance of FQNS in *S. Typhi* and *S. Paratyphi*, higher doses of fluoroquinolones and use of the alternative antibiotics ceftriaxone and azithromycin are vital for treating enteric fever. The consumption of fluoroquinolones and third generations cephalosporins, along with azithromycin (estimates from the IQVIA dataset), increased considerably in South Asia between 2000 and 2018. This is a major driver of resistance and use of these antibiotics needs to be carefully monitored to ensure

their continued efficacy.

The spread of FQNS *S. Typhi* and the emergence of ceftriaxone and azithromycin resistance in sub-Saharan Africa is lagging behind that in South and Southeast Asia. Antibiotic consumption in sub-Saharan Africa is lower than that in Asia, with a substantial lack of availability of alternative antimicrobial therapies. Consequentially, it is vitally important to monitor the prevalence of AMR in this region and to improve access to effective antibiotics, whilst also reducing their inappropriate use. Access to healthcare and increases in laboratory capacity have got to be considered key to such strategies.

With the efficacy of antibiotic therapies for enteric fever waning it is more important than ever to have a multi-faceted approach to reducing the incidence of both drug-sensitive and drug-resistant infections. Improving hygiene and sanitation are the corner stones of infectious disease control and their importance in fighting enteric fever can not be emphasised enough.¹⁴ HICs have essentially eliminated enteric fever as a public health problem. *S. Typhi* and *S. Paratyphi A* are transmitted through contaminated food and water, therefore increasing provisions of clean water and improved sanitation would greatly reduce the incidence of enteric fever in LMICs.

The development of real time rapid diagnostic tests (RDTs) to identify bacterial infections and distinguish them from viral infections is considered important for AMR research.²⁹ Antibiotics are often inappropriately used either over the counter or with clinicians prescribing antibiotics for common upper respiratory infections. The availability of inexpensive and effective RDTs could greatly reduce empirical antibiotic use, and reduce the pressure this exerts on bacterial pathogens.²⁹⁴

Vaccines play a key role in controlling infectious diseases, and the new conjugate *S. Typhi* vaccine pre-qualified by the WHO has been shown to be highly effective, confer long lasting immunity, and be suitable for children under two years old (which previous vaccines were not).⁸⁷ This vaccine is currently being introduced into many highly endemic LMICs and should play an important role in reducing the incidence of typhoid fever, with GAVI having invested heavily in its dissemination.²⁹⁵ There are currently no

S. Paratyphi A vaccines approved for humans use, and few in the development pipeline.²⁹⁶ A vaccine effective against *S. Paratyphi A* has got to be considered a research priority for tackling enteric fever. The lack of such a vaccine highlights the importance of disease surveillance, effective antibiotic treatments and non-pharmaceutical interventions to reduce the incidence of this disease.

Surveillance of both antibiotic consumption and antibiotic resistance are essential. To achieve this, large investments in laboratory infrastructure and capacity are required in LMICs. This work identifies large gaps in the availability of data and can guide decisions on where investment is most required and will likely be the most effective. Whilst surveillance systems are being built up, statistical modelling provides a useful tool for estimating both the prevalence of resistance and the consumption of antibiotics in locations lacking data. Statistical models are an efficient tool for estimating disease burden, they can be run with limited infrastructure and can produce estimates of disease incidence and prevalence to inform policy decisions. Employing statistical models to guide policy in LMICs may be a useful component of strategies to tackle AMR.²⁹⁷

7.5 Future research areas

Although the statistical models implemented in this thesis provide informative estimates of the prevalence of AMR in *S. Typhi* and *S. Paratyphi A*, the drivers of AMR were not assessed. A range of socio-demographic and health related covariates were included in the models and found to be important, but the methodology utilised was built to maximise prediction of the prevalence of AMR and not to infer causation. The drivers of AMR are very complex, with a mixture of social, environmental, political and health related variables thought to impact the prevalence of AMR. Although a range of variables have been identified to impact AMR at the individual level, limited evidence exists on what drives AMR at the population level.^{63,298,299} This is an important area of research and the establishment of large datasets on the prevalence of AMR in selected pathogens, as produced here, can provide a vital starting point for detailed epidemiological analyses. A full, distinct understanding of the drivers of AMR will be important for designing and

implementing interventions.

Chapter Five identified the potential biases and overestimation which arise when using passive surveillance to estimate the prevalence of AMR. Although the biases were identified, it was not possible to quantify or account for them within the estimation of the prevalence of resistance at this time. Further investigations are required to gather high quality surveillance data on AMR, with additional information to quantify the potential overestimation of AMR in passive surveillance studies. This is an interesting research area which should be pursued and applied to future estimates of the prevalence of AMR.

This thesis provided detailed estimates of antibiotic consumption, but did not incorporate estimates of suboptimal, falsified or counterfeit antimicrobials and their effect on AMR. Such medicines make up a considerable proportion of sales in LMICs²¹⁴ and are thought to have a considerable impact on AMR.²⁴⁴ Quantifying the scale of this problem would be an important first step in tackling it, improving both patient outcomes and reducing the potential development of AMR infections.

The prevalence of AMR is a valuable metric for understanding the development and spread of AMR, however, corresponding estimates of morbidity and mortality due to AMR enteric fever would be highly informative and provide a complete picture of the burden of AMR enteric fever. In this thesis the number of MDR and FQNS *S. Typhi* and *S. Paratyphi A* infections were calculated based on incidence rates estimated by the GBD study. The next stages of this research would be to estimate the relative risk of mortality and morbidity due to AMR in enteric fever, and use this to quantify the overall number of deaths and disability adjusted life years (DALYs) due to MDR and FQNS in *S. Typhi* and *S. Paratyphi A*. The Global Research on Antimicrobial Resistance (GRAM) group at the University of Oxford is currently working in collaboration with the Institute of Health Metrics and Evaluation (IHME) to develop this methodology,²⁹⁷ and to include such estimates in the Global Burden of Disease study.

Whilst the modelling strategy implemented in this thesis produces estimates of the prevalence of AMR with reasonable predictive validity, alternative spatial-temporal modelling strategies are available. A Bayesian hierarchical spatial-temporal

geostatistical model was fit to the MDR *S. Typhi* data in Chapter Six. However, this was found to be highly computationally intensive and provided little improvement in predictive performance over the stacked ensemble plus ST-GPR model. Alternative Bayesian hierarchical spatial-temporal models are available and could be investigated for the purposes of estimating the prevalence of AMR. Two methods worth investigating would be fitting spatial-temporal conditional autoregressive models, either in a fully Bayesian framework using Markov Chain Monte Carlo (MCMC) in WinBugs,³⁰⁰ or in R-INLA.³⁰¹ Both models are fit on areal data using a spatial adjacency matrix, and can be fit on the national or subnational (administrative divisions) level data. Implementing such models would provide a complete model comparison and may result models with superior predictive performance.

This thesis provides a framework for estimating the prevalence of AMR in bacterial pathogens. It establishes statistical models with high predictive validity when estimating the spatial and temporal trends of AMR and provides estimates of antibiotic consumption to be used in such models. The estimates of antibiotic consumption can also be used in future studies to investigate drivers of AMR and the further impacts of antibiotic consumption. Future research should also involve extending this methodology to additional pathogens to fully understand the burden of AMR.

7.6 Conclusions

This thesis provides a detailed understanding of the spatial and temporal trends of AMR in enteric fever. Estimates of the current prevalence of MDR and FQNS in *S. Typhi* and *S. Paratyphi A* were produced, alongside the trends in resistance for the past three decades. A comprehensive geolocated dataset on AMR in enteric fever was compiled, and evidence of emerging ceftriaxone and azithromycin resistance established. Detailed maps of antibiotic usage in LMICs and antibiotic consumption globally were produced for 2000 to 2018, providing a wealth of information on the overuse and lack of access to antibiotics in different countries and how this has changed over time.

The estimates of antibiotic usage and consumption can be used alongside those of the

prevalence of AMR to guide policy, and as a benchmark from which to measure change. A paucity of data on AMR were identified from many LMICs, highlighting the importance of improving laboratory capacity and establishing AMR surveillance in LMICs. AMR surveillance, along with the promotion of judicious antibiotic use, improvements in hygiene and sanitation, vaccination, and the research and development of new antibiotics and diagnostics are essential components for combatting AMR. This thesis summarises the worsening landscape of AMR in enteric fever and highlights why immediate action is essential to tackle this growing threat, and ensure that antibiotics remain the effective life saving resource they are today.

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Appendices

High-income

- Southern Latin America
- Western Europe
- High-income North America
- Australasia
- High-income Asia Pacific

Latin America and Caribbean

- Caribbean
- Central Latin America
- Tropical Latin America
- Andean Latin America

Sub-Saharan Africa

- Southern sub-Saharan Africa
- Western sub-Saharan Africa
- Central sub-Saharan Africa
- Eastern sub-Saharan Africa

North Africa and Middle East

- North Africa and Middle East

South Asia

- South Asia

Southeast Asia, East Asia, and Oceania

- East Asia
- Southeast Asia
- Oceania

Central Europe, Eastern Europe, and Central Asia

- Central Asia
- Eastern Europe
- Central Europe

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Section/topic	#	Checklist item	Reported
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	☒
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	☒
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	☒
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	☒
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	☒
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	☒
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	☒
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	☒
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	☒
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	☒
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	☒
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	☒
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	☒
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I ²) for each meta-analysis.	☒
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	☒
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	☒
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	☒
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	☒
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	☒
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	☒
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	☒
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	☒
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	☒
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	☒
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	☒
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	☒
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	☒

Table A.1: PRISMA Checklist. Completed checklist adapted from Moher *et al.*

Search number	Search term
1	Typhoid Fever/ (11123)
2	typhoid*.ti,ab. (12200)
3	paratyphoid*.ti,ab. (1674)
4	"enteric fever*".ti,ab. (1511)
5	"salmonella typhi".ti,ab. (4311)
6	"salmonella paratyphi".ti,ab. (821)
7	paratyphoid fever/ (2380)
8	1 or 2 or 3 or 4 or 5 or 6 or 7(20567)
9	exp Drug Resistance, Bacterial/ (78649)
10	exp Microbial Sensitivity Tests/ (116858)
11	"antibacterial resistan*".ti,ab. (617)
12	"antibacterial drug resistan*".ti,ab. (33)
13	"bacterial drug resistan*".ti,ab. (208)
14	"antimicrobial resistan*".ti,ab. (14949)
15	"antibiotic drug resistan*".ti,ab. (67)
16	"bacterial resistan*".ti,ab. (5266)
17	"antibiotics resistan*".ti,ab. (634)
18	"bacterial surveillan*".ti,ab. (40)
19	"antibiotic surveillan*".ti,ab. (36)
20	"antibacterial surveillan*".ti,ab. (1)
21	"antimicrobial surveillan*".ti,ab. (393)
22	"antimicrobial susceptib*".ti,ab. (11401)
23	exp Bacteremia/ (27328)
24	bacteraemia*.ti,ab. (5772)
25	bacteremia*.ti,ab. (21957)
26	("blood culture*" adj3 result*).ti,ab. (1319)
27	(antibacter* adj3 surveillan*).ti,ab. (29)
28	9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 (215387)
29	8 and 28 (2202)
30	29 (2202)
31	limit 29 to yr="1990 -Current" (1930)

Table A.2: Systematic review database search strategy. Strategy designed in MedLine and altered for other databases

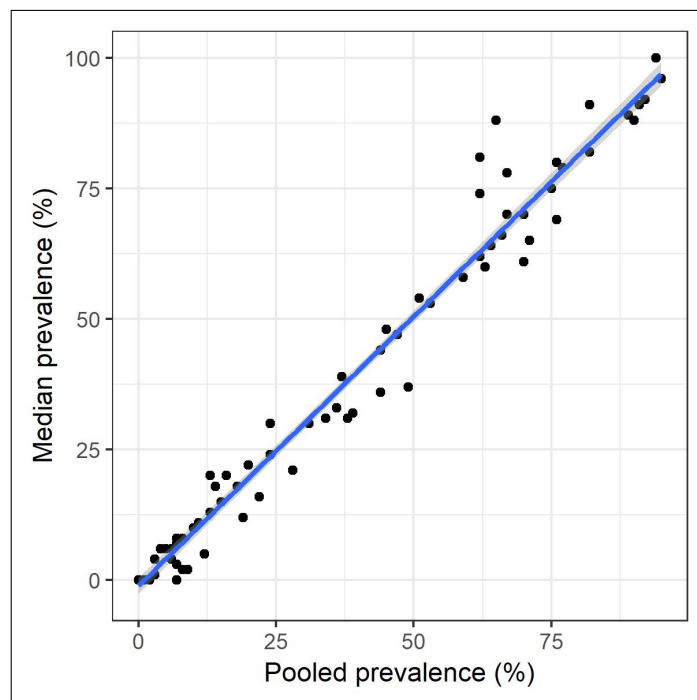


Figure A.2: Comparison between the meta-analysis and median prevalence results. A scatter plot to compare the meta-analysis results (pooled prevalence of resistance) and the median prevalence of resistance for each subgroup of the analysis.

GBD Region	Number of studies (Number of isolates)						Total
	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2018	
Andean Latin America	0(0)	0(0)	0(0)	0(0)	1(33)	0(0)	1(33)
Australasia	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Caribbean	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Asia	0(0)	1(34)	1(41)	1(123)	0(0)	0(0)	3(198)
Central Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Sub-Saharan Africa	0(0)	0(0)	1(29)	2(410)	3(363)	0(0)	6(802)
East Asia	0(0)	0(0)	5(618)	2(164)	0(0)	0(0)	7(782)
Eastern Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Eastern Sub-Saharan Africa	2(62)	3(357)	2(345)	6(2,504)	4(254)	0(0)	17(3,522)
High-income Asia Pacific	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
High-income North America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
North Africa & Middle East	4(643)	5(1,218)	4(1,004)	3(996)	0(0)	2(69)	18(3,930)
Oceania	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
South Asia	57(13,136)	31(17,472)	55(12,726)	55(10,530)	62(19,581)	13(2,274)	273(75,719)
Southeast Asia	5(761)	12(2,260)	6(3,728)	7(421)	7(689)	1(46)	38(7,905)
Southern Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Southern Sub-Saharan Africa	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Tropical Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Sub-Saharan Africa	0(0)	4(176)	3(513)	7(431)	7(570)	1(35)	22(1,725)
Total	68(14,602)	56(21,517)	77(19,004)	83(15,579)	84(21,490)	17(2,424)	*385(94,616)

Table A.3: Number of included studies and isolates for *S. Typhi*. Displayed by GBD region and five-year time-period. *Some studies provide data on multiple sites and/or time periods therefore the total number of studies displayed here is higher than the total number of studies included in this review.

GBD Region	Number of studies (Number of isolates)						Total
	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2018	
Andean Latin America	1(97)	0(0)	0(0)	0(0)	0(0)	0(0)	1(97)
Australasia	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Caribbean	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Asia	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Sub-Saharan Africa	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
East Asia	0(0)	0(0)	2(4,298)	4(279)	2(463)	0(0)	8(5,040)
Eastern Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Eastern Sub-Saharan Africa	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
High-income Asia Pacific	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
High-income North America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
North Africa & Middle East	0(0)	0(0)	1(40)	0(0)	0(0)	0(0)	1(40)
Oceania	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
South Asia	5(715)	9(5,781)	28(6,404)	30(3,858)	40(6,305)	7(663)	119(23,726)
Southeast Asia	3(144)	1(19)	2(67)	0(0)	6(544)	0(0)	12(774)
Southern Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Southern Sub-Saharan Africa	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Tropical Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Sub-Saharan Africa	0(0)	1(17)	0(0)	1(15)	2(22)	0(0)	4(54)
Total	9(956)	11(5,817)	33(10,809)	35(4,152)	50(7,334)	7(663)	*145(29,731)

Table A.4: Number of included studies and isolates for *S. Paratyphi A*. Displayed by GBD region and five-year time-period. *Some studies provide data on multiple sites and/or time periods therefore the total number of studies displayed here is higher than the total number of studies included in this review.

GBD Region	Number of data points (Number of isolates)						Total
	1990-1994	1995-1999	2000-2004	2005-2009	2010-2014	2015-2018	
Andean Latin America	1(97)	0(0)	0(0)	0(0)	1(33)	0(0)	2(130)
Australasia	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Caribbean	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Asia	0(0)	1(34)	1(41)	1(123)	0(0)	0(0)	3(198)
Central Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Central Sub-Saharan Africa	0(0)	0(0)	1(29)	2(410)	3(363)	0(0)	6(802)
East Asia	0(0)	0(0)	7(4,916)	4(443)	2(463)	0(0)	13(5,822)
Eastern Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Eastern Sub-Saharan Africa	2(62)	3(357)	2(345)	6(2,504)	4(254)	0(0)	17(3,522)
High-income Asia Pacific	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
High-income North America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
North Africa & Middle East	4(643)	5(1,218)	4(1,044)	3(996)	0(0)	2(69)	18(3,970)
Oceania	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
South Asia	57(13,851)	35(23,253)	59(19,130)	60(14,388)	64(25,886)	13(2,937)	288(99,445)
Southeast Asia	5(905)	12(2,279)	6(3,795)	7(421)	8(1,233)	1(46)	39(8,679)
Southern Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Southern Sub-Saharan Africa	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Tropical Latin America	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Europe	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Western Sub-Saharan Africa	0(0)	4(193)	3(513)	7(446)	7(592)	1(35)	22(1,779)
Total	69(15,558)	60(27,334)	83(29,813)	90(19,731)	89(28,824)	17(3,087)	*408(124,347)

Table A.5: Total number of included data points and isolates. Displayed are the total number of data points and isolates included by GBD region and five-year time-period. *Some studies provide data on multiple sites and/or time periods therefore the total number of data points displayed here is higher than the total number of studies included in this review.

Time Period	MDR S. Typhi			FQNS S. Typhi		
	Studies (N)	Pooled prevalence [95% CI]	p-value	Studies (N)	Pooled prevalence [95% CI]	p-value
All studies						
1990-1994	33	63% [54-71]		6	2% [0-14]	
1995-1999	13	40% [25-55]		8	49% [26-72]	
2000-2004	32	35% [26-46]		29	62% [47-77]	
2005-2009	26	16% [8-26]		37	76% [69-82]	
2010-2014	36	9% [4-14]		50	82% [75-89]	
2015-2018	6	9% [0-32]		7	70% [38-94]	
Studies with resistance breakpoints			0.917			0.992
1990-1994	5	67% [38-91]		1	0% [0-0]	
1995-1999	6	32% [15-52]		6	51% [21-81]	
2000-2004	19	30% [20-40]		22	68% [53-82]	
2005-2009	20	18% [9-29]		30	76% [68-84]	
2010-2014	32	7% [3-13]		45	83% [74-90]	
2015-2018	6	10% [0-32]		6	62% [30-89]	
Studies with AST methods stated			0.936			0.952
1990-1994	25	61% [50-71]		3	10% [0-46]	
1995-1999	11	38% [23-54]		8	49% [26-72]	
2000-2004	26	34% [23-46]		26	59% [42-74]	
2005-2009	26	16% [8-26]		35	77% [70-84]	
2010-2014	33	7% [3-12]		48	82% [74-89]	
2015-2018	6	10% [0-32]		7	70% [38-94]	
Studies with control strain stated			1.000			0.992
1990-1994	33	63% [54-71]		6	3% [0-15]	
1995-1999	13	39% [25-55]		8	49% [26-72]	
2000-2004	32	35% [26-46]		29	62% [47-77]	
2005-2009	26	16% [8-26]		37	76% [69-83]	
2010-2014	36	9% [4-14]		50	82% [74-89]	
2015-2018	6	10% [0-32]		7	70% [38-94]	
Studies with over 50 isolates			0.989			0.804
1990-1994	23	61% [51-70]		2	1% [0-4]	
1995-1999	13	39% [25-55]		6	39% [23-55]	
2000-2004	21	36% [24-49]		20	65% [48-80]	
2005-2009	22	13% [6-23]		25	75% [65-83]	
2010-2014	24	11% [5-19]		29	82% [72-90]	
2015-2018	3	13% [0-57]		3	53% [7-95]	
Quality level 1			0.883			0.264
1990-1994	10	55% [36-73]		0		
1995-1999	3	55% [25-84]		5	53% [20-84]	
2000-2004	11	32% [21-45]		10	80% [73-87]	
2005-2009	7	7% [3-14]		10	77% [64-88]	
2010-2014	10	5% [0-14]		15	84% [70-95]	
2015-2018	0					
Quality level 1 and 2			0.947			0.468
1990-1994	13	58% [40-75]		0		
1995-1999	6	44% [22-67]		8	49% [26-72]	
2000-2004	23	33% [24-42]		24	66% [51-80]	
2005-2009	21	17% [8-28]		32	75% [67-83]	
2010-2014	30	6% [2-11]		45	83% [74-90]	
2015-2018	6	9% [0-32]		6	62% [30-89]	

Table A.6: Sensitivity Analysis. Results of the sensitivity analysis comparing the full random-effects meta-analysis results to that with a) studies clearly stating the resistance breakpoints, b) studies clearly stating the AST methods, c) studies clearly stating the control strain used and d) studies with over 50 isolates, e) studies defined as quality level one and f) studies defined as quality level one or two. The *p*-value represent the results of Welch's two sample t-tests identifying significant differences between the results of each sub meta-analysis and the full meta-analysis

Appendix to Chapter Three

This section contains additional tables and figures relating to Chapter Three: Antibiotic usage in children with lower respiratory tract infections in low- and middle-income countries, 2000 to 2018. This includes information on model covariates, additional plots of model uncertainty and model validation statistics.

Covariate name	Covariate Source
Travel time to nearest settlement >50,000 inhabitants	Weiss DJ, Nelson A, Gibson HS, et al. A global map of travel time to cities to assess inequalities in accessibility in 2015. <i>Nature</i> 2018; 533(7688):333-336. doi: 10.1038/nature25181
Ratio of dependants to working-age adults	Lloyd CT, Sorichetta A, Tatem AJ. High resolution global gridded data for use in population studies. <i>Sci Data</i> 2017; 4(170001). doi: 10.1038/sdata.2017.1. Available from: http://www.worldpop.org.uk/data/get_data/
Distance to rivers and lakes	Natural Earth. Rivers and lake centerlines dataset. Available at: http://www.naturalearthdata.com/downloads/10mphysical-vectors/10m-rivers-lake-centerlines/ . (Accessed: 24th July 2017)
DPT3 vaccine coverage	Produced by the local burden of disease team at the Institute of Health Metrics and evaluation, contact Simon Hay (sihay@uw.edu) for further information
Malaria incidence	Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on <i>Plasmodium falciparum</i> in Africa between 2000 and 2015. <i>Nature</i> 2015; 526(7572):207–211. doi: 10.1038/nature15535
Gross domestic product (GDP) per pixel	Kummu, M., Taka, M., & Guillaume, J. H. A. Data Descriptor: Gridded global datasets for Gross Domestic Product and Human Development Index over 1990–2015. <i>Sci Data</i> 2018; 6(5):180004. doi: 10.1038/sdata.2018.4.
Urbanicity	Pesaresi M, Ehrlich D, Ferri S et al. Operating procedure for the production of the Global Human Settlement Layer from Landsat data of the epochs 1975, 1990, 2000, and 2014. JRC Technical Report EUR 27741 EN. doi:10.2788/253582 (online)
Years of maternal education	Graetz N, Friedman J, Osgood-Zimmerman A. et al. Mapping local variation in educational attainment across Africa. <i>Nature</i> 2018; 555:48–53. https://doi.org/10.1038/nature25761 (2018).
Ambient air pollution, particulate matter 2.5	Data integration model available at: https://arxiv.org/abs/1609.00141
Population of children under 5	Lloyd C, Sorichetta A, & Tatem A. High resolution global gridded data for use in population studies. <i>Sci Data</i> 2017; 4(170001). doi: 10.1038/sdata.2017.1. Available at: http://www.worldpop.org.uk/data/get_data/
Mean temperature	Harris I, Jones PD, Osborn TJ, Lister DH. Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 dataset. <i>Int J Climatol</i> 2014; 34: 623–642
Childhood stunting	Produced by the local burden of disease team at the Institute of Health Metrics and evaluation, contact Simon Hay (sihay@uw.edu) for further information
Normalised difference vegetation index	NASA & NOAA. Advanced Very High Resolution Radiometer (AVHRR) Normalized Difference Vegetation Index (NDVI) dataset. Available at: https://nex.nasa.gov/nex/projects/1349/ . (Accessed: 25th July 2017)
Latitude	The latitude of each pixel calculated by the local burden of disease team at the Institute of Health Metrics and evaluation.
Health Access and Quality Index (HAQI)	Barber, RM. et al. Healthcare Access and Quality Index based on mortality from causes amenable to personal health care in 195 countries and territories, 1990–2015: a novel analysis from the Global Burden of Disease Study 2015. <i>Lancet</i> 2017; 390(10091): 231 – 266. doi: 10.1016/S0140-6736(17)30818-8

Table B.1: Covariates included in the model of antibiotic usage.

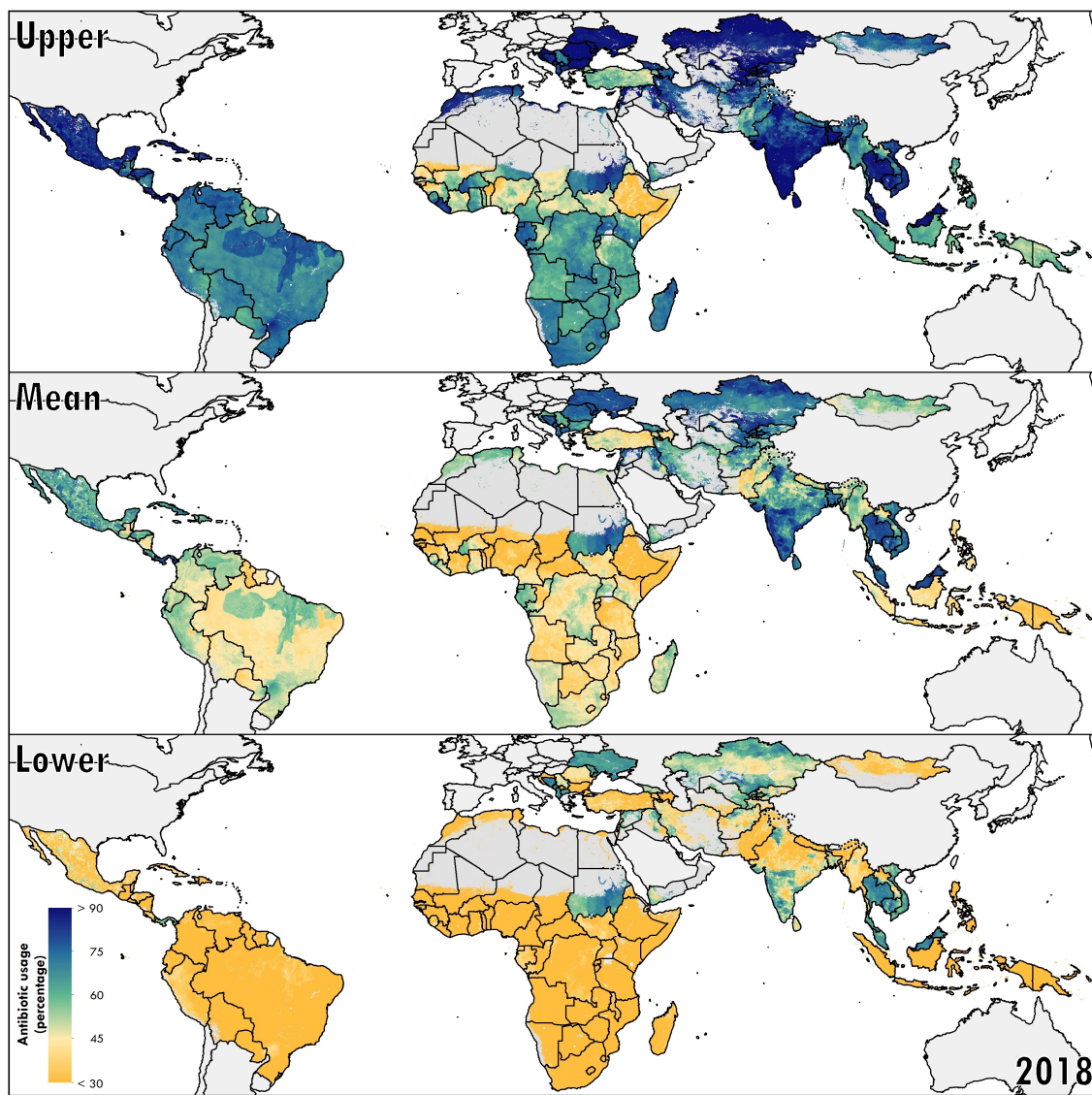


Figure B.1: Antibiotic usage in 2018, model uncertainty. Modelled estimates of the proportion of children under-five with LRI with care-giver reported antibiotic usage. The mean, upper and lower 95% uncertainty intervals are shown for each administrative level two district for 2018. Pixels (1x1km) with a population less than 10 persons are masked in grey.

Region	In sample		Out-of-sample	
	RMSE	Correlation (R^2)	RMSE	Correlation (R^2)
OVERALL	0.03	0.99	0.14	0.53
Balkans & Caucasus	0.02	1.00	0.14	0.73
Central America & Caribbean	0.02	1.00	0.15	-0.09
Central Asia	0.03	0.98	0.09	0.46
Central sub-Saharan Africa	0.02	0.98	0.07	0.70
Eastern & Southern sub-Saharan Africa	0.01	1.00	0.11	0.54
Horn of Africa	0.01	1.00	0.09	0.89
Malay & Oceania	0.01	0.99	0.11	-0.8
Middle East	0.02	0.99	0.11	-0.05
North Africa	0.01	1.00	0.09	0.21
South America	0.02	0.97	0.07	0.14
South Asia	0.05	0.99	0.24	-0.20
Southeast Asia	0.03	0.99	0.23	-0.44
Western sub-Saharan Africa	0.02	0.99	0.11	0.24

Table B.2: In- and out-of-sample metrics for the five-fold cross validation models on antibiotic usage. Metrics were calculated between the nationally aggregated estimates of the model and the input data.

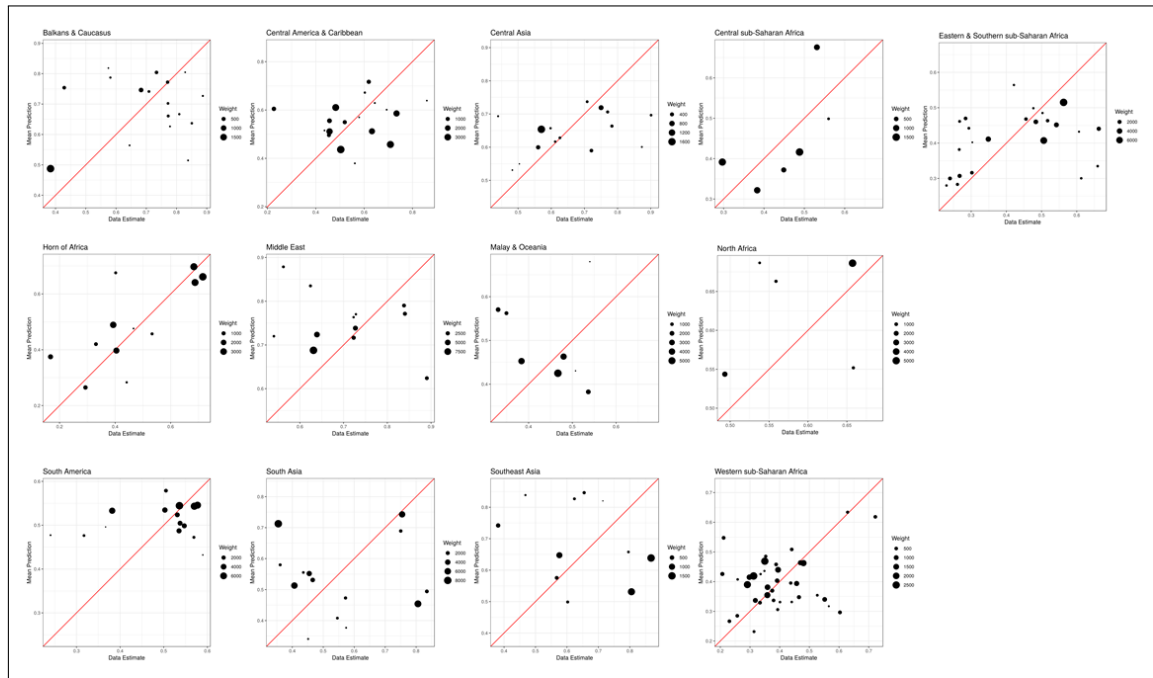


Figure B.2: Validation plots for the model of antibiotic use for each modelling region. Each plot shows the mean proportion of antibiotic use from each survey, at the national level, against the out-of-sample predicted proportion of antibiotic use for that country-year based on five-fold cross-validation. a) Balkans & Caucasus; b) Central America & Caribbean; c) Central Asia; d) Central sub-Saharan Africa; e) Eastern & Southern sub-Saharan Africa; f) Horn of Africa; g) Malay & Oceania; h) Middle East; i) North Africa; j) South America; k) South Asia; l) Southeast Asia; m) Western sub-Saharan Africa.

Appendix to Chapter Four

This section contains additional tables and figures relating to Chapter Four: Global antibiotic consumption, 2000 to 2018. This includes information pertaining to the calculate of DDDs; comparisons of data from different sources and additional model information.

Antibiotic	Daily Defined Dose (grams)	Antibiotic	Daily Defined Dose (grams)
acetylspiramycin	3	flomoxef	2
amikacin	1	flucloxacillin	3
amoxicillin	1.5	flumequine	1.2
amoxicillin & beta-lactamase inhibitor	1.5	fosfomycin	3
ampicillin	6	fusidic acid	1.5
ampicillin & beta-lactamase inhibitor	6	garenoxacin	0.4
arbekacin	0.2	gatifloxacin	0.4
azidocillin	1.5	gemifloxacin	0.32
azithromycin	0.3	gentamicin	0.24
azlocillin	12	imipenem & cilastatin	2
aztreonam	4	isepamicin	0.4
bacampicillin	1.2	josamycin	2
benzathine	2	kanamycin	1
phenoxymethylpenicillin			

biapenem	1.2	latamoxef	4
carbenicillin	12	levofloxacin	0.5
cefaclor	1	lincomycin	1.8
cefadroxil	2	linezolid	1.2
cefalexin	2	lomefloxacin	0.4
cefalotin	4	lymecycline	0.6
cefamandole nafate	6	meropenem	3
cefatrizine	1	meropenem & vaborbactam	3
cefazedone	3	metacycline	0.6
cefazolin	3	metronidazole	1.5
cefbuperazone	2	mezlocillin	6
cefcapene pivoxil	0.45	midecamycin	1
cefdinir	0.6	minocycline	0.2
cefditoren pivoxil	0.4	moxifloxacin	0.4
cefepime	4	nafcillin	3
cefetamet pivoxil	1	neomycin	1
cefixime	0.4	netilmicin	0.35
cefmenoxime	2	norfloxacin	0.8
cefmetazole	4	ofloxacin	0.4
cefminox	4	oleandomycin	1
cefodizime	2	ornidazole	1
cefonicid	1	oxacillin	2
cefoperazone	4	oxytetracycline	1
cefoperazone & beta-lactamase inhibitor	4	panipenem & betamipron	2
ceforanide	4	pazufloxacin	1
cefotaxime	4	pefloxacin	0.8
cefotaxime & beta-lactamase inhibitor	4	penicillin g	3.6
cefotetan	4	penicillin v	2
cefotiam	4	pheneticillin	1

cefoxitin	6	piperacillin	14
cefazopran	4	piperacillin & beta-lactamase inhibitor	14
cefpiramide	2	pivmecillinam	0.6
cefprome	4	pristinamycin	2
cefpodoxime	0.4	prulifloxacin	0.6
cefprozil	1	ribostamycin	1
cefradine	2	rifabutin	0.15
cefroxadine	2.1	rifampicin	0.6
ceftaroline fosamil	1.2	rifamycin	0.6
ceftazidime & beta-lactamase inhibitor	6	roxithromycin	0.3
ceftazidime	4	rufloxacin	0.2
cefteram pivoxil	0.4	sitafloxacin	0.1
ceftezole	3	sparfloxacin	0.2
ceftibuten	0.4	spectinomycin	3
ceftizoxime	4	streptomycin	1
ceftolozane & beta-lactamase inhibitor	3	sulbactam	1
ceftriaxone	2	sulbenicillin	15
ceftriaxone & beta-lactamase inhibitor	2	sulfadiazine	0.6
cefuroxime	0.5	sulfadiazine & trimethoprim	1
chloramfenicol	3	sulfamethoxazole	2
chlortetracycline	1	sulfamethoxazole & trimethoprim	1.92
ciprofloxacin	1	sulfametrole & trimethoprim	1.92
clarithromycin	0.5	sultamicillin	1.5
clindamycin	1.2	tebipenem pivoxil	0.56
clofoctol	1.5	tedizolid	0.2
cloxacillin	2	teicoplanin	0.4

combination penicillins	2	telavancin	0.7
daptomycin	0.27	telithromycin	0.8
demeclocycline	0.6	temocillin	2
dibekacin	0.14	tetracycline	1
dicloxacillin	2	thiamphenicol	1.5
dirithromycin	0.5	ticarcillin & beta-lactamase inhibitor	15
doripenem	1.5	tigecycline	0.1
doxycycline	0.1	tinidazole	1.5
enoxacin	0.8	tobramycin	0.24
ertapenem	1	tosufloxacin	0.45
erythromycin	1	trimethoprim	0.4
faropenem	0.75	vancomycin	2
fleroxacin	0.4		

Table C.1: DDD values for each included J01 antibiotic

Antimicrobial	Oral DDD (g)	Parenteral DDD (g)
Amoxicillin	1.5	3
Ampicillin	2	6
Azithromycin	0.3	0.5
Cefotiam	1.2	4
Cefuroxime	0.5	3
Ciprofloxacin	1	0.8
Clarithromycin	0.5	1
Clindamycin	1.2	1.8
Erythromycin	1	2
Fosfomycin	3	8
Linezolid	1.5	1.2
Tobramycin	0.3	0.24

Table C.2: DDD values for differing oral and parenteral preparations. The oral and parenteral DDD values for each included J01 antibiotic where they differ. Expert opinion was used to select the most suitable values, highlighted in bold in the table

AWaRe	Antibiotic Categorisation
Access	Amikacin, Amoxicillin, Amoxicillin/clavulanic Acid, Ampicillin, Ampicillin/sulbactam, Bacampicillin, Benzathine benzylpenicillin, Benzylpenicillin, Cefacetrile, Cefadroxil, Cefalexin, Cefalotin, Cefapirin, Cefatrizine, Cefazedone, Cefazolin, Cefradine, Cefroxadine, Ceftezole, Chloramphenicol, Clindamycin, Clometocillin, Cloxacillin, Dicloxacillin, Doxycycline, Flucloxacillin, Gentamicin, Mecillinam, Metronidazole, Nafcillin, Nitrofurantoin, Oxacillin, Penamecillin, Phenoxymethylpenicillin, Pivampicillin, Pivmecillinam, Procaine benzylpenicillin, Spectinomycin, Sulfadiazine/trimethoprim, Sulfamethizole/trimethoprim, Sulfamethoxazole/trimethoprim, Sulfametrole/trimethoprim, Sulfamoxole/trimethoprim, Sultamicillin, Tetracycline, Thiamphenicol
Watch	Arbekacin, Azithromycin, Azlocillin, Biapenem, Carbenicillin, Cefaclor, Cefamandole, Cefbuperazone, Cefcapene pivoxil, Cefdinir, Cefditoren pivoxil, Cefepime, Cefetamet pivoxil, Cefixime, Cefmenoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperazone, Ceforanide, Cefoselis, Cefotaxime, Cefotetan, Cefotiam, Cefotiam hexetil, Cefoxitin, Cefozopran, Cefpiramide, Cefpirome, Cefpodoxime proxetil, Cefprozil, Ceftazidime, Cefteram pivoxil, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefuroxime, Chlortetracycline, Ciprofloxacin, Clarithromycin, Clofoctol, Delafloxacin, Dibekacin, Dirithromycin, Doripenem, Enoxacin, Ertapenem, Erythromycin, Fleroxacin, Flomoxef, Flumequine, Fosfomycin, Fusidic Acid, Garenoxacin, Gatifloxacin, Gemifloxacin, Imipenem/cilastatin, Isepamicin, Josamycin, Kanamycin, Latamoxef, Levofloxacin, Lincomycin, Lomefloxacin, Lymecline, Meropenem, Metacycline, Mezlocillin, Micronomicin, Midecamycin, Minocycline, Moxifloxacin, Neomycin, Netilmicin, Norfloxacin, Ofloxacin, Oleandomycin, Oxytetracycline, Panipenem, Pazufloxacin, Pefloxacin, Pheneticillin, Piperacillin, Piperacillin/tazobactam, Pristinamycin, Prulifloxacin, Ribostamycin, Rifabutin, Rifampicin, Rifamycin, Rifaximin, Roxithromycin, Rufloxacin, Sisomicin, Sitafoxacin, Sparfloxacin, Spiramycin, Spiramycin/metronidazole, Streptomycin, Sulbenicillin, Tebipenem, Teicoplanin, Telithromycin, Temocillin, Ticarcillin, Tobramycin, Tosufloxacin, Vancomycin
Reserve	Aztreonam, Ceftaroline fosamil, Ceftazidime-avibactam, Ceftobiprole medocartil, Ceftolozane-tazobactam, Colistin, Dalbavancin, Dalfopristin-quinupristin, Daptomycin, Eravacycline, Faropenem, Linezolid, Meropenem-vaborbactam, Omadacycline, Oritavancin, Plazomicin, Polymyxin B, Tedizolid, Telavancin, Tigecycline

Table C.3: AWaRe categorisation of J01 antibiotics¹

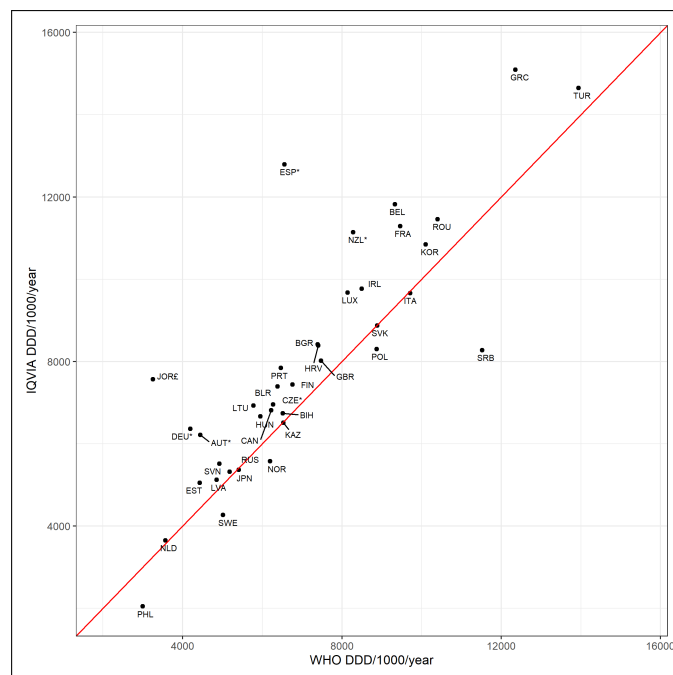


Figure C.1: Comparison of IQVIA and WHO antibiotic consumption data. DDDs/1,000 population/year from the IQVIA Analytics database on the y-axis and from the WHO report on surveillance of antibiotic consumption on the x-axis. This includes data from all country-years from both sources. The red line indicates a 1:1 relationship between the data (intercept = 0, slope = 1). Countries followed by * indicated that the WHO estimates are for community antibiotic consumption only. Countries followed by £ indicate that the WHO estimate is for <70% of the population and has not been population adjusted.

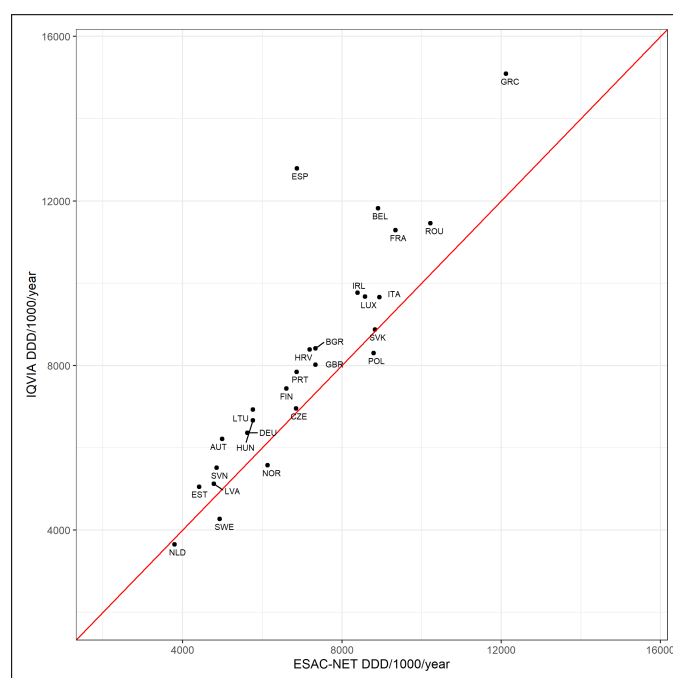


Figure C.2: Comparison of IQVIA and ESAC-Net antibiotic consumption data. All data are for 2015 and are presented in DDD/1,000 population /year. The red line indicates a 1:1 relationship between the data (intercept = 0, slope = 1).

Stacked ensemble models were investigated to use spatially and temporally refined covariates to estimate the consumption of antibiotics in HICs and in all countries by ATC level three class. For the ATC level three classes, each class of antibiotic were modelled, with the aim of combining the estimates after modelling. For both of these models no selection of covariates available were able to produce satisfactory predictions of antibiotic consumption out-of-sample. A variety of covariates were trialled and input into each of the child models specified. The correlation and RMSE between the input data and the out-of-sample estimates were calculated and are shown in the following tables for the final selection of covariates. This could not be improved on and therefore the spatial modelling strategy was deemed unsuitable.

Child model	RMSE	R^2
xgboost	2346	0.14
gam	3009	0.01
ridge	2527	0.13
nnet	2410	0.13
cubist	2570	0.12
Stacked results	2268	0.14

Table C.4: Stacked ensemble model statistics for the model of antibiotic consumption in HICs.

Child model	RMSE	R^2
xgboost	707	0.01
gam	531	0.01
ridge	500	0.08
lasso	504	0.04
nnet	779	0.06
rf	541	0.00
cubist	2194	0.00
Stackers	470	0.07

Table C.5: Stacked ensemble model statistics for the model of antibiotic consumption in ATC level three J01M antibiotics. Example of model performance for J01M - Quinolones. Similar results were seen for other ATC level three classes trialled.

Level 0	Level 1 - Super-region	Level 2 - Region	Level 3 - Country	Income level
Global				
	Central Europe, Eastern Europe, and Central Asia			
		Central Asia		
			Armenia	Lower middle
			Azerbaijan	Upper middle
			Georgia	Lower middle
			Kazakhstan	Upper middle
			Kyrgyzstan	Lower middle
			Mongolia	Lower middle
			Tajikistan	Lower middle
			Turkmenistan	Upper middle
			Uzbekistan	Lower middle
		Central Europe		
			Albania	Upper middle
			Bosnia and Herzegovina	Upper middle
			Bulgaria	Upper middle
			Croatia	Upper middle
			Czech Republic	High
			Hungary	High
			Macedonia	Upper middle

High-income	Eastern Europe	Montenegro	Upper middle
		Poland	High
		Romania	Upper middle
		Serbia	Upper middle
		Slovakia	High
		Slovenia	High
	Australasia		
		Belarus	Upper middle
		Estonia	High
		Latvia	High
		Lithuania	High
		Moldova	Lower middle
		Russian Federation	High
		Ukraine	Lower middle
	High-income Asia Pacific		
		Australia	High
		New Zealand	High
		Brunei	High
		Japan	High
		South Korea	High
		Singapore	High

High-income North America	Canada	High
	Greenland	High
	United States	High
Southern Latin America	Argentina	High
	Chile	High
	Uruguay	High
Western Europe	Andorra	High
	Austria	High
	Belgium	High
	Cyprus	High
	Denmark	High
	Finland	High
	France	High
	Germany	High
	Greece	High
	Iceland	High
	Ireland	High
	Israel	High
	Italy	High
	Luxembourg	High

		Malta	High
		Monaco	High
		Netherlands	High
		Norway	High
		Portugal	High
		San Marino	High
		Spain	High
		Sweden	High
		Switzerland	High
		United Kingdom	High
Latin America and Caribbean			
	Andean Latin America		
		Bolivia	Lower middle
		Ecuador	Upper middle
		Peru	Upper middle
	Caribbean		
		Antigua and Barbuda	High
		The Bahamas	High
		Barbados	High
		Belize	Upper middle
		Bermuda	High
		Cuba	Upper middle
		Dominica	Upper middle

	Dominican Republic	Upper middle
	Grenada	Upper middle
	Guyana	Upper middle
	Haiti	Low
	Jamaica	Upper middle
	Puerto Rico	High
	Saint Kitts and Nevis	High
	Saint Lucia	Upper middle
	Saint Vincent and the Grenadines	Upper middle
	Suriname	Upper middle
	Trinidad and Tobago	High
	Virgin Islands, U.S.	High
Central Latin America		
	Colombia	Upper middle
	Costa Rica	Upper middle
	El Salvador	Lower middle
	Guatemala	Lower middle
	Honduras	Lower middle
	Mexico	Upper middle
	Nicaragua	Lower middle
	Panama	Upper middle
	Venezuela	Upper middle

	Tropical Latin America		Brazil	Upper middle
			Paraguay	Upper middle
North Africa and Middle East	North Africa and Middle East		Afghanistan	Low
			Algeria	Upper middle
			Bahrain	High
			Egypt	Lower middle
			Iran	Upper middle
			Iraq	Upper middle
			Jordan	Lower middle
			Kuwait	High
			Lebanon	Upper middle
			Libya	Upper middle
			Morocco	Lower middle
			Palestine	Lower middle
			Oman	High
			Qatar	High
			Saudi Arabia	High
			Sudan	Lower middle

		Syria	Lower middle
		Tunisia	Lower middle
		Turkey	Upper middle
		United Arab Emirates	High
		Yemen	Lower middle
	South Asia		
	South Asia		
		Bangladesh	Lower middle
		Bhutan	Lower middle
		India	Lower middle
		Nepal	Low
		Pakistan	Lower middle
	Southeast Asia, East Asia, and Oceania		
	East Asia		
		China	Upper middle
		North Korea	Low
		Taiwan	High
	Oceania		
		American Samoa	Upper middle
		Cook Islands	High
		Federated States of Micronesia	Lower middle
		Fiji	Upper middle
		Guam	High
		Kiribati	Lower middle

	Marshall Islands	Upper middle
	Nauru	UPPER
		MIDDLE
	Niue	High
	Northern	High
	Mariana Islands	
	Palau	High
	Papua New Guinea	Lower middle
	Samoa	Upper middle
	Solomon Islands	Lower middle
	Tokelau	High
	Tonga	Upper middle
	Tuvalu	Upper middle
	Vanuatu	Lower middle
	Southeast Asia	
	Cambodia	Lower middle
	Indonesia	Lower middle
	Laos	Lower middle
	Malaysia	Upper middle
	Maldives	Upper middle
	Mauritius	Upper middle
	Myanmar	Lower middle
	Philippines	Lower middle
	Sri Lanka	Lower middle
	Seychelles	High
	Thailand	Upper middle
	Timor-Leste	Lower middle

		Vietnam	Lower middle
	Sub-Saharan Africa		
	Central Sub- Saharan Africa		
		Angola	Lower middle
		Central African Republic	Low
		Congo Democratic Republic of the Congo	Lower middle Low
		Equatorial Guinea	Upper middle
		Gabon	Upper middle
	Eastern Sub- Saharan Africa		
		Burundi	Low
		Comoros	Low
		Djibouti	Lower middle
		Eritrea	Low
		Ethiopia	Low
		Kenya	Lower middle
		Madagascar	Low
		Malawi	Low

	Mozambique	Low
	Rwanda	Low
	Somalia	Low
	South Sudan	Low
	Tanzania	Low
	Uganda	Low
	Zambia	Lower middle
Southern Sub- Saharan Africa	Botswana	Upper middle
	Lesotho	Lower middle
	Namibia	Upper middle
	South Africa	Upper middle
	Swaziland	Lower middle
	Zimbabwe	Low
Western Sub- Saharan Africa	Benin	Low
	Burkina Faso	Low
	Cameroon	Lower middle
	Cape Verde	Lower middle
	Chad	Low
	Côte d'Ivoire	Lower middle
	The Gambia	Low
	Ghana	Lower middle

Guinea	Low
Guinea-Bissau	Low
Liberia	Low
Mali	Low
Mauritania	Lower middle
Niger	Low
Nigeria	Lower middle
Sao Tome and Principe	Lower middle
Senegal	Low
Sierra Leone	Low
Togo	Low

Table C.6: Global Burden of Disease location hierarchy. Each country/territory is assigned to one of 21 regions and 7 super-regions in a hierarchical structure, based on their geographic locations and sociodemographic and epidemiological characteristics.

Year	Using the 2019 DDD values				Using the historic (pre-2019) DDD values			
	LMIC		HIC		LMIC		HIC	
	DDD _s	DDD/1000/DAY	DDD _s	DDD/1000/DAY	DDD _s	DDD/1000/DAY	DDD _s	DDD/1000/DAY
2000	7,941,273,121	5.3	8,180,210,981	20.5	8,782,752,352	5.8	9,317,706,888	23.4
2001	8,135,122,711	5.3	8,423,974,386	21	9,049,989,370	5.9	9,590,958,821	23.9
2002	8,632,942,591	5.6	8,267,659,921	20.5	9,625,730,124	6.2	9,432,438,766	23.4
2003	8,957,001,860	5.7	8,455,591,732	20.8	9,974,750,377	6.4	9,680,632,834	23.9
2004	9,355,688,655	5.9	8,177,810,129	20	10,423,804,696	6.6	9,367,309,339	22.9
2005	10,706,204,184	6.7	8,763,631,276	21.3	11,851,729,781	7.4	10,038,898,121	24.4
2006	11,035,958,469	6.8	8,729,105,068	21.1	12,228,980,551	7.5	10,025,765,573	24.2
2007	11,307,828,042	6.9	8,857,180,354	21.2	12,559,914,143	7.6	10,203,135,664	24.4
2008	12,509,263,703	7.5	8,961,369,504	21.3	13,823,260,463	8.3	10,369,383,530	24.6
2009	13,295,998,894	7.9	9,206,162,654	21.7	14,721,185,039	8.8	10,659,559,946	25.1
2010	14,421,006,630	8.5	9,283,336,460	21.7	15,967,073,294	9.4	10,736,314,874	25.1
2011	15,774,694,530	9.2	9,537,837,951	22.2	17,558,520,419	10.2	11,048,105,112	25.7
2012	18,258,322,653	10.5	9,056,231,163	21	20,358,120,918	11.7	10,527,788,978	24.4
2013	18,288,340,383	10.4	9,136,754,953	21	20,482,247,939	11.7	10,675,488,193	24.6
2014	19,754,398,040	16	9,382,566,526	21.6	17,705,278,173	14.3	11,102,186,075	25.5
2015	21,014,630,910	16.8	9,575,548,330	21.9	18,823,195,548	15	11,365,282,682	26
2016	22,006,487,057	17.3	9,502,828,938	21.6	19,859,085,212	15.6	11,298,381,362	25.7
2017	21,492,408,297	16.7	9,360,217,484	21.2	19,559,778,903	15.2	11,143,202,033	25.2
2018	22,133,931,886	17	9,061,093,060	20.5	20,455,854,131	15.7	10,805,800,040	24.4

Table C.7: Comparison of new and historic DDD values. Total DDDs and DDD/1000/day in HICs and LMICs for 2000-2018 based on calculations using the updated 2019 DDD values compared to the historic (pre-2019) DDD values

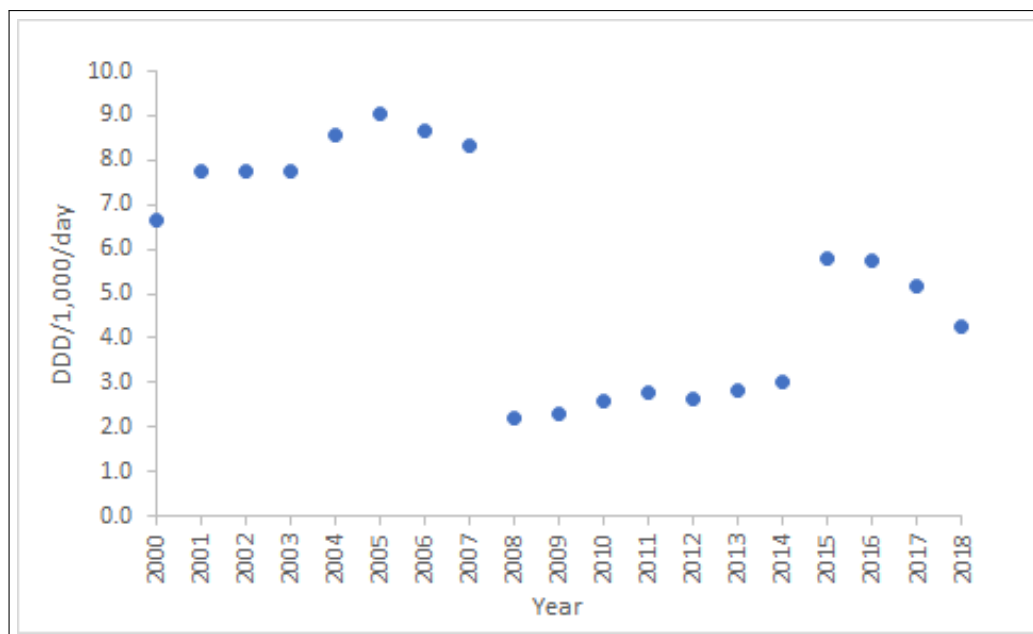


Figure C.3: Indonesia antibiotic consumption data from the IQVIA dataset. The reported rate of antibiotic consumption is shown in DDD/1,000 population/day for each year of the study, 2000 to 2018. DDD/1,000/day were calculated from the IQVIA dataset using the methodology specified in Chapter Four.

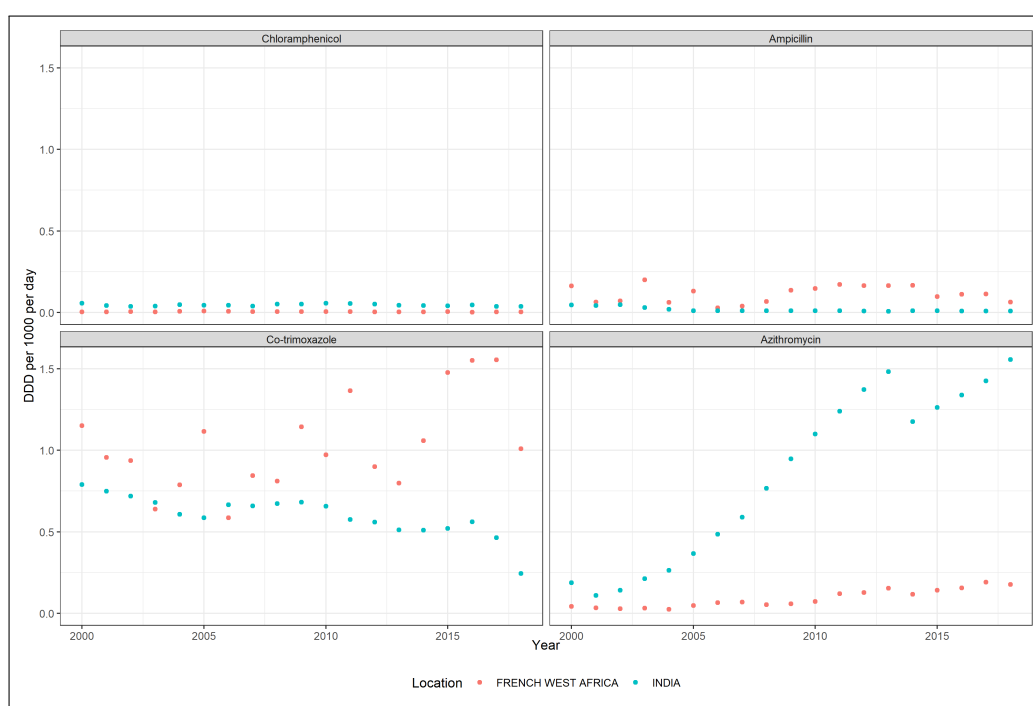


Figure C.4: Consumption rates of selected antibiotics for India and French West Africa from the IQVIA dataset. The reported rates of antibiotic consumption for ampicillin, chloramphenicol, co-trimoxazole and azithromycin are shown in DDD/1,000 population/day for each year of the study, 2000 to 2018. DDD/1,000/day were calculated from the IQVIA dataset using the methodology specified in Chapter Four.

Appendix to Chapter Six

This section contains additional tables and figures relating to Chapter Six: The prevalence of antimicrobial resistance in *Salmonella enterica* serovars Typhi and Paratyphi A infections, in endemic countries, 1990 to 2018. This includes additional information on the model, model uncertainty and model validation statistics.

Various modelling strategies were compared to estimate the prevalence of MDR in *S. Typhi* by location and year. The following models were fit with five-fold cross-validation and the RMSE and R^2 between the out-of-sample estimates and the input data were calculated to quantify the predictive validity of the models. The models fit were:

- MBG models as specified in Chapter Six
- Stacked ensemble plus ST-GPR model as specified in Chapter 6
- Stacked ensemble model as specified in Chapter 6, with no second stage model
- ST-GPR as specified in Chapter Six but with a generalised linear mixed effects model (GLMM) with nested random effects for country, region and super-region as the first stage model (instead of the stacked ensemble)
- Meta-regression - Bayesian, regularised, trimmed (MR-BRT) model from IHME^{2,3}
- MR-BRT as the first stage model, followed by an ST-GPR
- GLMM with nested random effects for country, region and super-region

All models were fit with the same selection of covariates as detailed in Chapter Six, except for the MBG model. The MBG model estimated the prevalence of MDR at a

5x5km spatial resolution, therefore a similar selection of covariates available at this spatial resolution were selected for inclusion. The model performance is shown in Figure D.1 and Table D.1.

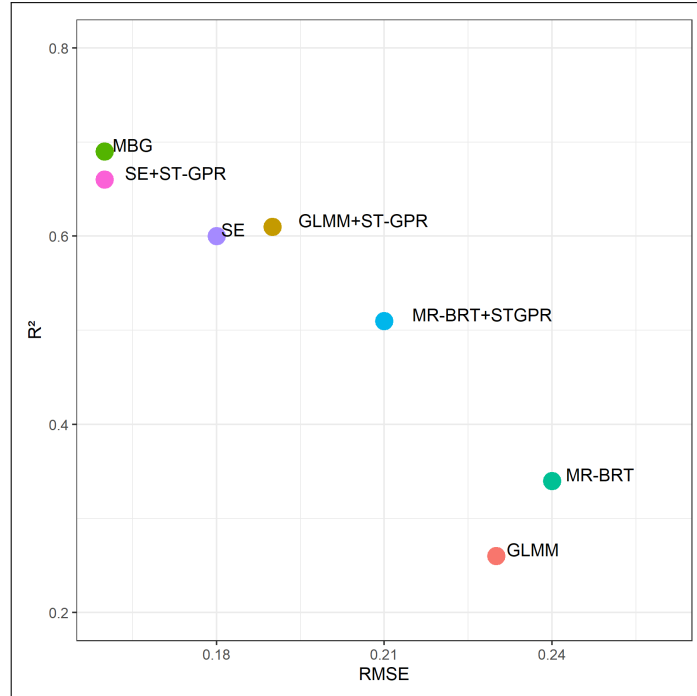


Figure D.1: Model comparison. The R^2 and RMSE between the input data and the out-of-sample model estimates for selected models of the prevalence of MDR in *S. Typhi*. R^2 is shown on the y-axis and RMSE on the x-axis

Model	RMSE	R^2
MBG	0.16	0.69
SE+ST-GPR	0.16	0.66
SE	0.18	0.6
GLMM+ST-GPR	0.19	0.61
MR-BRT+STGPR	0.21	0.51
MR-BRT	0.24	0.34
GLMM	0.23	0.26

Table D.1: Model comparison. The R^2 and RMSE between the input data and the out-of-sample model estimates for selected models of the prevalence of MDR in *S. Typhi*.

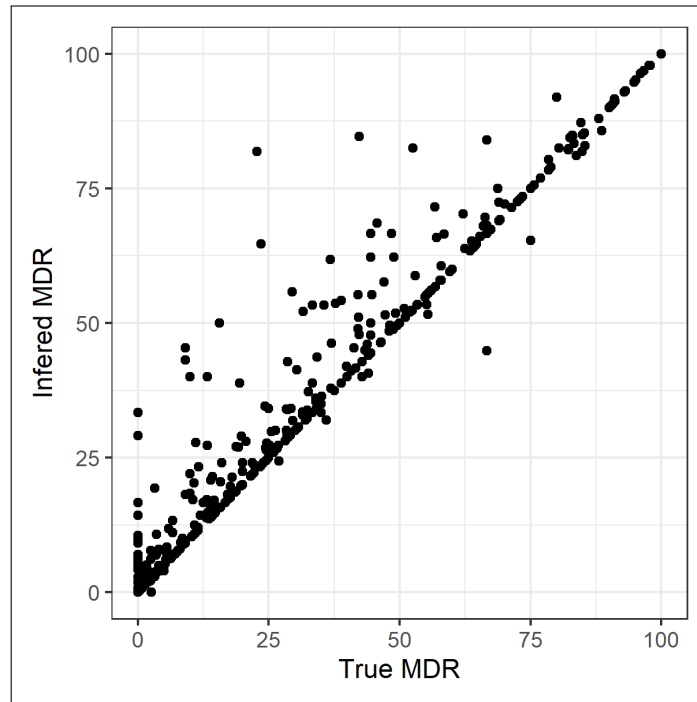


Figure D.2: Actual compared to inferred MDR. A comparison between the prevalence of MDR in *S. Typhi* based on concurrent resistance to ampicillin, chloramphenicol and co-trimoxazole, and inferred MDR defined as the minimum aggregated prevalence of resistance from the three individual antimicrobials. The r^2 between MDR and inferred MDR is 0.95.

Model (data density)	Spatial smoothing (ζ)	Temporal smoothing (λ)	GPR scale	GPR amplitude
MDR <i>S. Typhi</i>				
0-2 datapoints	0.1	0.1	25	2
3-9 datapoints	0.01	0.1	25	2
10+ datapoints	0.005	0.25	25	2
FQNS <i>S. Typhi</i>				
0-2 datapoints	0.1	0.05	25	5
3+ datapoints	0.1	0.1	25	5
MDR <i>S. Paratyphi</i>				
All data densities	0.5	0.01	25	20
FQNS <i>S. Paratyphi</i>				
All data densities	0.0001	0.1	25	3

Table D.2: ST-GPR model parameters for MDR and FQNS *S. Typhi* and *S. Paratyphi* A. The parameters controlling the spatial and temporal smoothing, and the GPR are displayed for each indicator by the density of datapoints within a country, where applicable. A higher ζ indicates more spatial smoothing; a higher λ indicates lower temporal smoothing, the GPR scale indicates the number of years over which the data are correlated and the GPR amplitude indicates much the GPR can vary from the spatial-temporal model estimates.

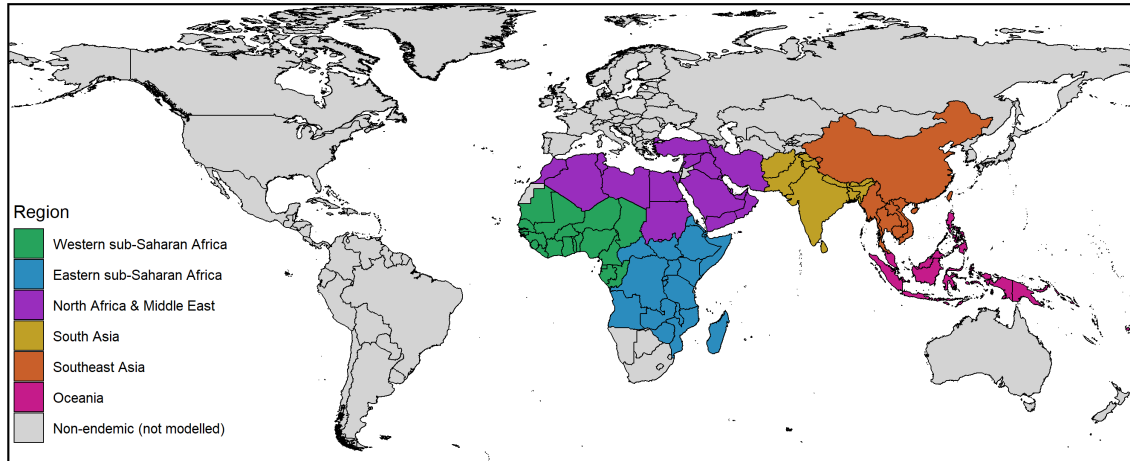


Figure D.3: Epidemiological regions for the subnational MDR *S. Typhi* model.

Parameter	Prior specification
ϵ_C	$\text{loggamma}(\alpha = 2, \gamma = 0.05)$
ϵ_i	$\text{loggamma}(\alpha = 2, \gamma = 0.05)$
ϵ_s	$\text{loggamma}(\alpha = 2, \gamma = 0.5)$
$\theta_2 t$	$N(\mu = 3, \sigma^2 = 1/1.1)$

Table D.3: MBG MDR *S. Typhi* model priors. Priors for each of the parameters in the MBG model of the prevalence of MDR in *S. Typhi*.

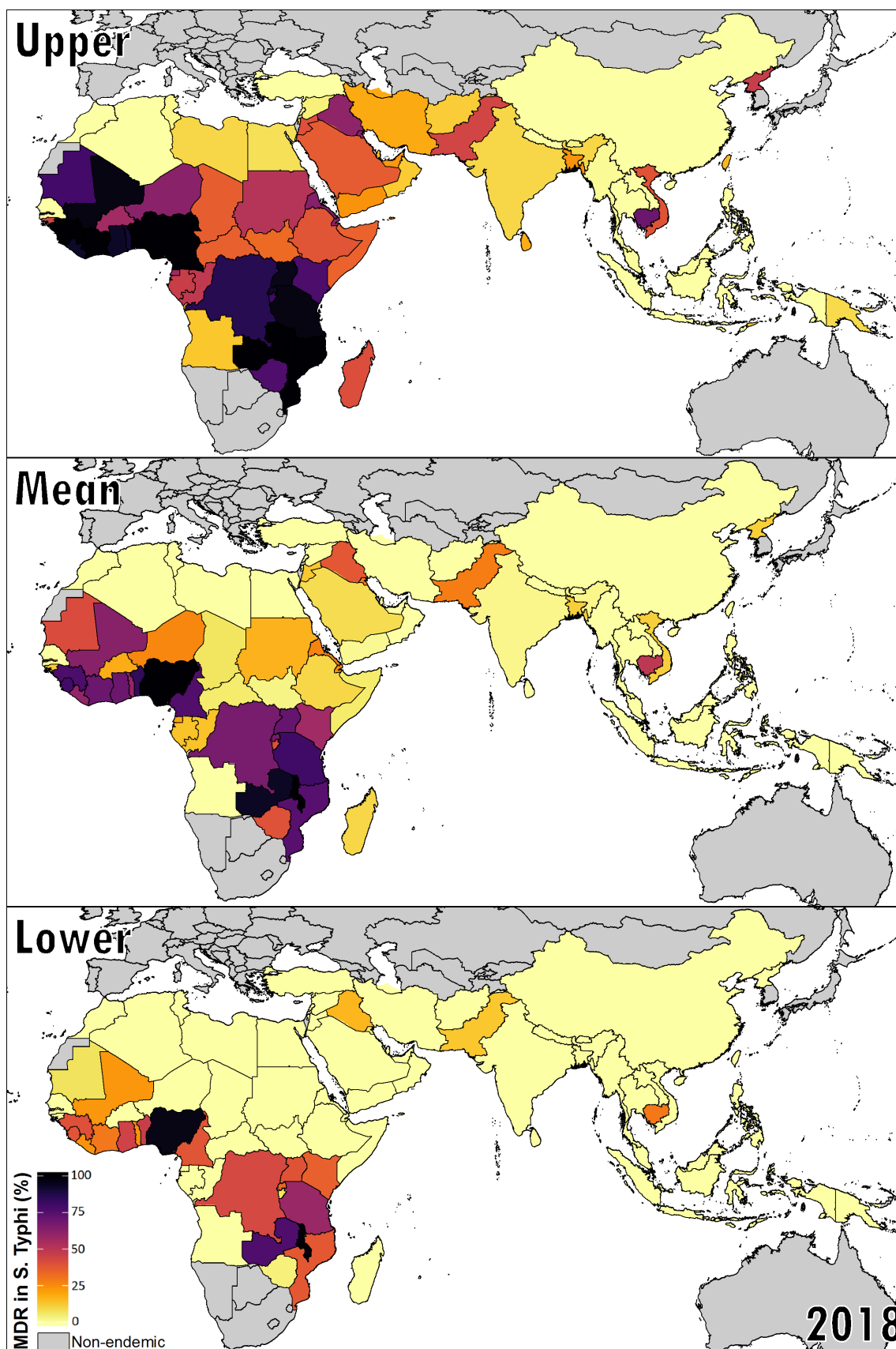


Figure D.4: Prevalence of MDR in *S. Typhi* in 2018, model uncertainty. Modelled estimates of the prevalence of MDR in *S. Typhi* from the stacked ensemble plus ST-GPR model. The mean, upper and lower 95% uncertainty interval values are shown for each country for 2018. Non-endemic countries are shown in grey.

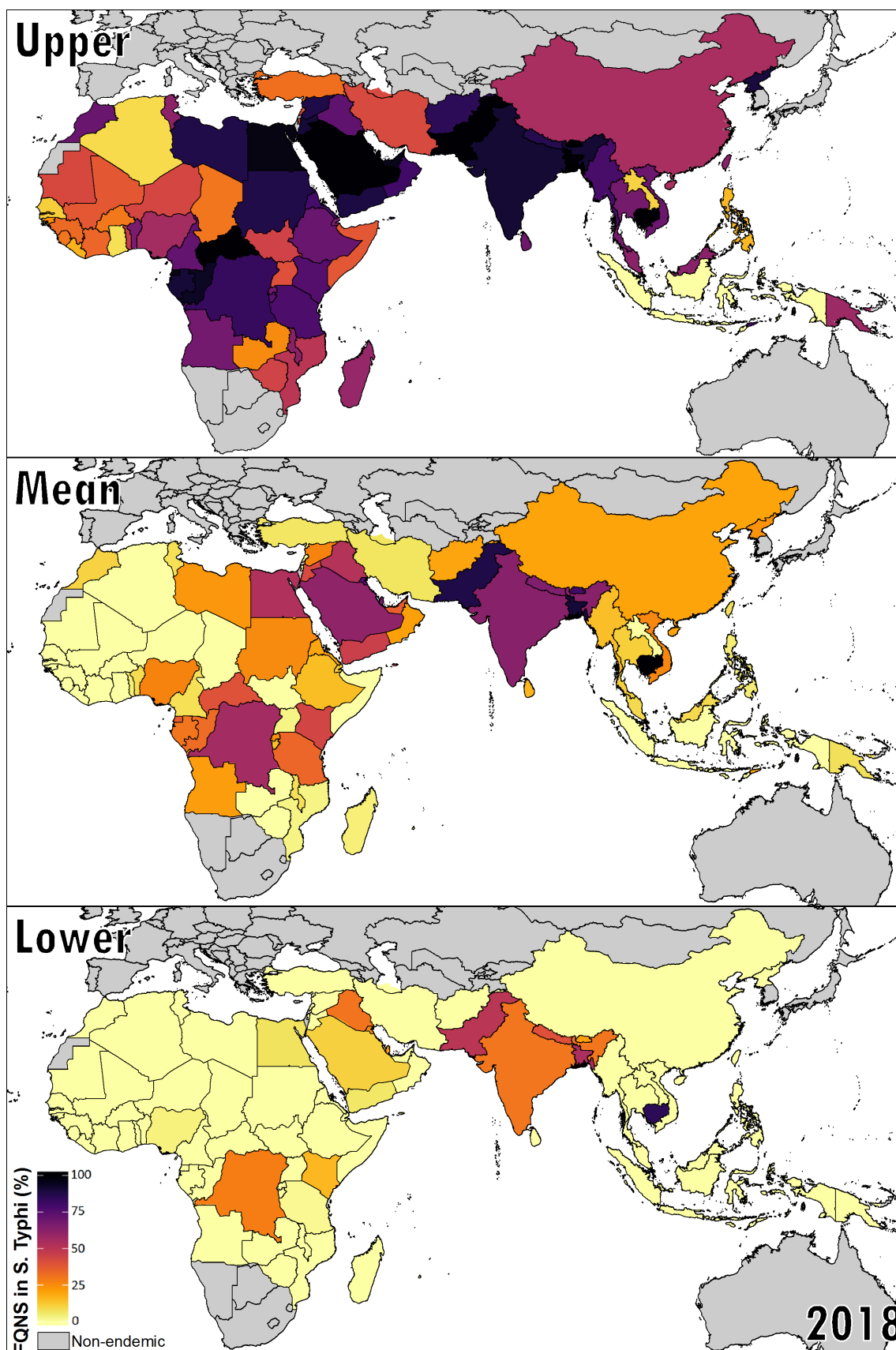


Figure D.5: Prevalence of FQNS in *S. Typhi* in 2018, model uncertainty. Modelled estimates of the prevalence of MDR in *S. Typhi* from the stacked ensemble plus ST-GPR model. The mean, upper and lower 95% uncertainty interval values are shown for each country for 2018. Non-endemic countries are shown in grey.

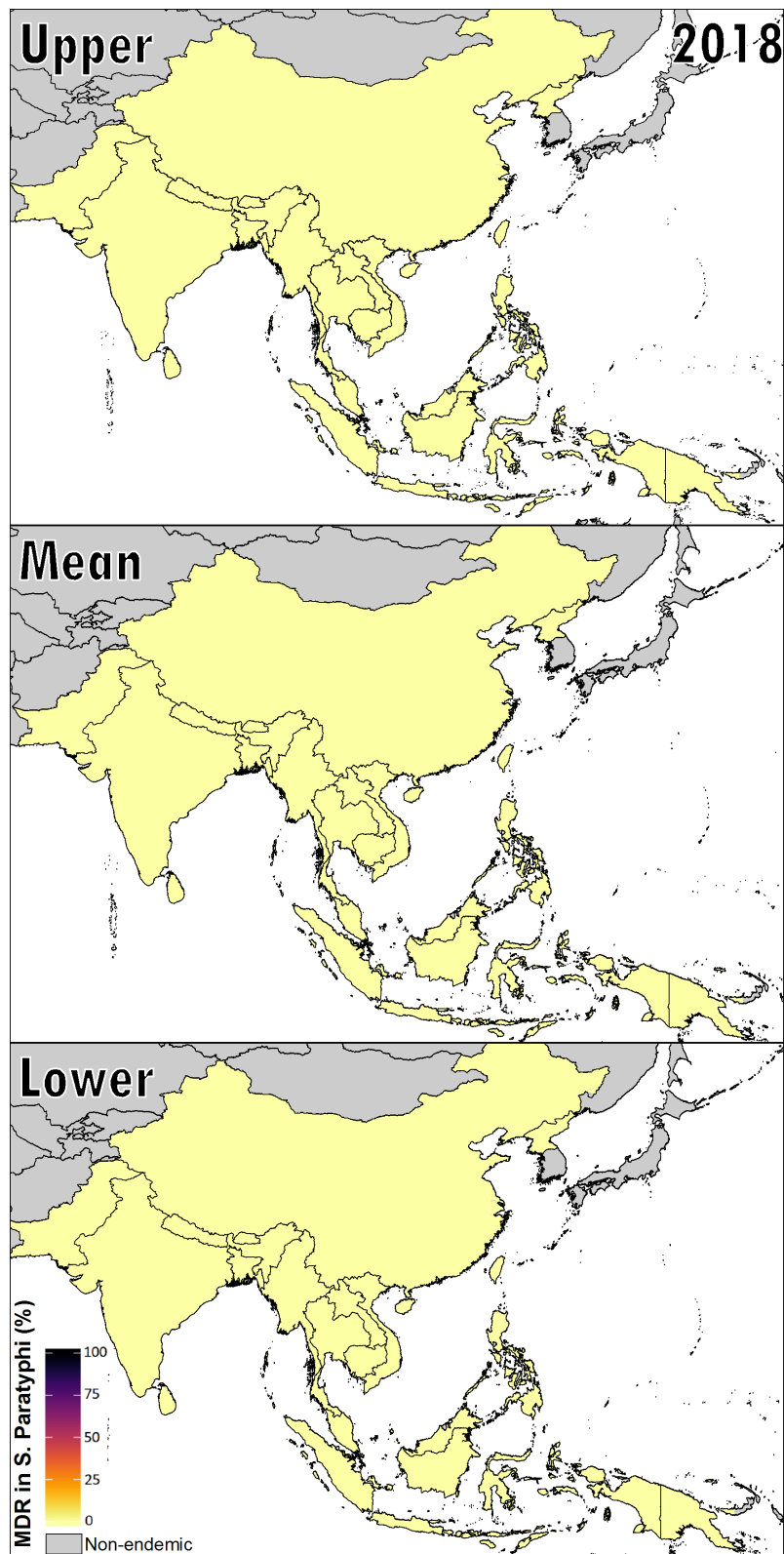


Figure D.6: Prevalence of MDR in *S. Paratyphi* in 2018, model uncertainty. Modelled estimates of the prevalence of MDR in *S. Paratyphi* from the stacked ensemble plus ST-GPR model. The mean, upper and lower 95% uncertainty interval values are shown for each country for 2018. Non-endemic countries are shown in grey.

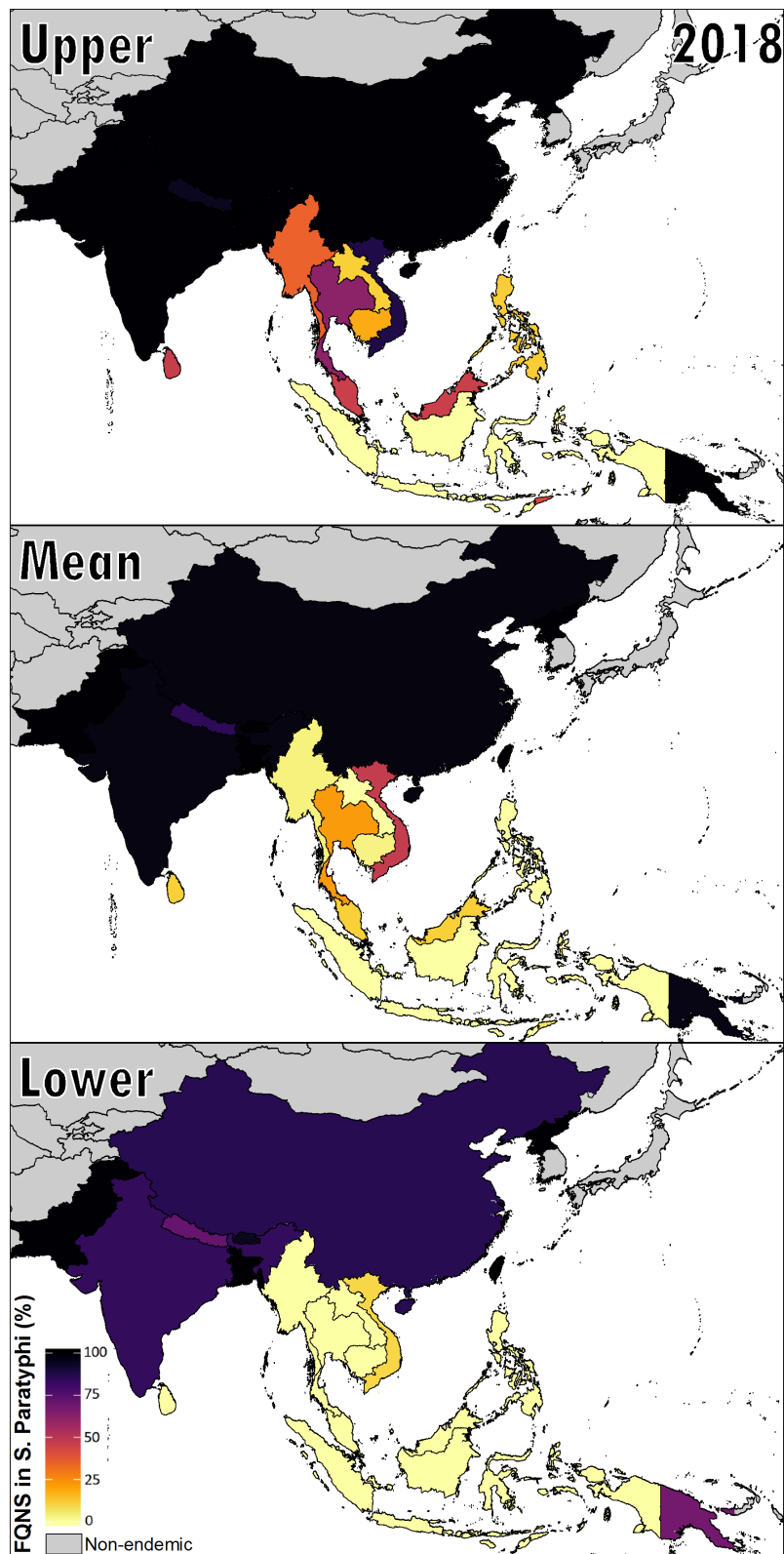


Figure D.7: Prevalence of FQNS in *S. Paratyphi* in 2018, model uncertainty. Modelled estimates of the prevalence of FQNS in *S. Paratyphi* from the stacked ensemble plus ST-GPR model. The mean, upper and lower 95% uncertainty interval values are shown for each country for 2018. Non-endemic countries are shown in grey.

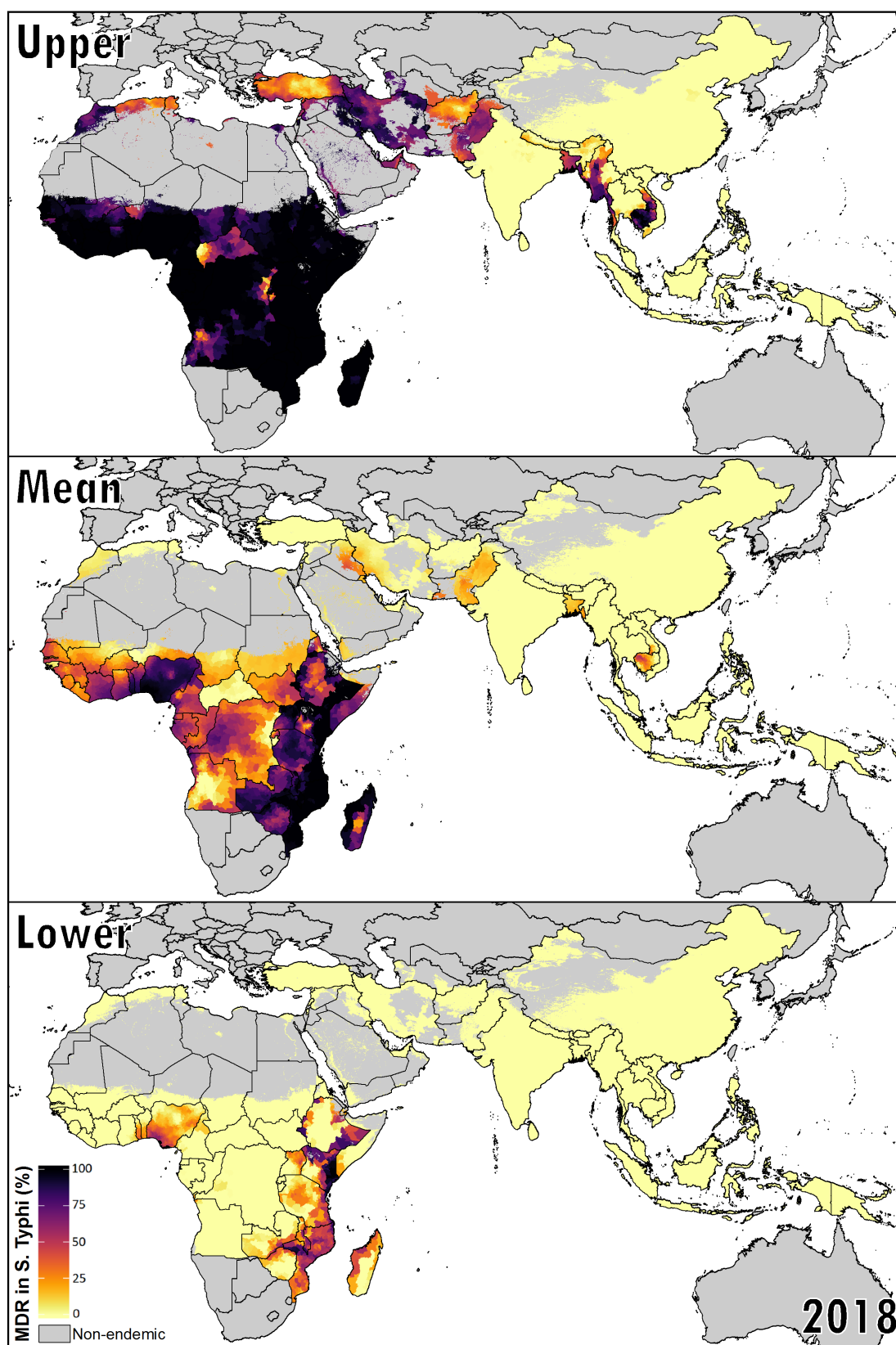


Figure D.8: Subnational prevalence of MDR in *S. Typhi* in 2018, model uncertainty. Modelled estimates of the prevalence of MDR in *S. Typhi* from the MBG model. The mean, upper and lower 95% uncertainty intervals are shown for each country for 2018. Non-endemic countries are shown in grey and pixels (1x1km) with populations of less than 10 are masked in grey.



Publications and conference proceedings

Publications related to this thesis

Browne AJ, Kashef Hamadani BH, Kumaran EAP, et al. Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. BMC Med. 2020 Jan 3;18(1).

Browne AJ, Chipeta MG, Haines-Woodhouse G et al. Global antibiotic consumption in humans, 2000 to 2018: a spatial modelling study [In review: Lancet Planetary Health].

Publications alongside this thesis

Burstein R, Henry NJ, ...**Browne AJ**, ...Murray CJL, Hay SI. Mapping 123 million neonatal, infant and child deaths between 2000 and 2017. Nature. 2019 Oct;574(7778):353-358.

Stanaway JD, Reiner RC, ...**Browne AJ**, ...Murray CJL, Hay SI. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis. 2019 Apr;19(4):369-381.

Graetz N, Friedman J, ... **Browne AJ**, ... Gakidou E, Hay SI. Mapping local variation in educational attainment across Africa. Nature. 2018 Feb 28;555(7694):48-53.

Osgood-Zimmerman A, Millear AI, ... **Browne AJ**, ... Kassebaum NJ, Hay SI. Mapping child growth failure in Africa between 2000 and 2015. *Nature*. 2018 Feb 28;555(7694):41-47.

Golding N, Burstein R, Longbottom J, **Browne AJ**, Fullman N, et al. Mapping under-5 and neonatal mortality in Africa, 2000-15: a baseline analysis for the Sustainable Development Goals. *Lancet*. 2017 Nov 11;390(10108):2171-2182.

Conference presentations

‘Global antibiotic consumption: A modelling study’ - Wellcome Trust Antimicrobial Resistance - Genomes, Big Data and Emerging Technologies conference; Online. Oct 2020 (Oral).

‘Geospatial mapping of the global prevalence of antimicrobial resistant *Salmonella* Typhi and Paratyphi A isolates’ – International conference on infectious diseases; Sept 2020 (Cancelled due to Covid-19) (Oral).

‘Geospatial mapping of antimicrobial resistant *Salmonella* Typhi’ - Oxford Tropical Network Conference; Oxford, UK. Sept 2019 (Short talk).

‘Geospatial mapping of the global prevalence of antimicrobial resistant *Salmonella* Typhi’ - 11th International conference on Typhoid and other invasive salmonellosis; Hanoi, Vietnam. Mar 2019 (Poster).

‘Developing a geospatial modelling framework to estimate the burden of antimicrobial resistance’ Wellcome Trust Antimicrobial Resistance - Genomes, Big Data and Emerging Technologies conference; Cambridge, UK. Nov 2018 (Oral).

‘Antimicrobial drug resistance in Salmonella Typhi and Paratyphi isolates worldwide, 1990 to 2017: A systematic review of the literature’ International conference on infectious diseases; Buenos Aires, Argentina. March 2018 (Poster).



Code repositories

All code used for the data cleaning, modelling and production of figures is available on GitHub in the following repositories:

Chapter Two:

`https://github.com/The-Oxford-GBD-group/typhi\_paratyphi\_systematic\_review\_code`

Chapter Three:

`https://github.com/The-Oxford-GBD-group/antibiotic\_modelling\_code/tree/master/antibiotic\_usage`

Chapter Four:

`https://github.com/The-Oxford-GBD-group/antibiotic\_modelling\_code/tree/master/antibiotic\_usage`

Chapter Six:

`https://github.com/The-Oxford-GBD-group/typhi\_paratyphi\_modelling\_code`