Epidemiology and health burdens of antimicrobial resistant bacterial infection in Southeast Asia and impact of antibiotic use on patient survival



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Abstract

Antimicrobial-resistance (AMR) is a global health concern. Data to support estimates of the AMR health burden are important to guide policy makers in developing appropriate interventions. The objective of this thesis is to improve the understanding of the epidemiology of AMR in low and middle-income countries (LMICs), where data is scarce.

A prospective surveillance study on hospital-acquired infection (HAI) was conducted to estimate excess mortality attributable to AMR infection in a LMIC. Evaluating all types of infections and seven priority bacteria, I estimated that 16.0% of deaths involving bacterial infections were attributable to AMR. This is higher than is observed in high-income countries (HICs).

One of the barriers in sharing AMR data in LMICs is a lack of expertise, time, and resources to analyse the data and write reports. I developed an open-access, offline, and easy-to-use application to fill in this gap. The tool was tested in seven hospitals in seven countries. AMR surveillance reports from those hospitals were shared on an open-access data repository platform and each has a digital object identifier that can be appropriately cited when re-used.

It is known that low blood culture usage can bias the estimates of health burden of AMR. However, the magnitude of this effect is largely unknown. I performed a retrospective study with bootstrap sampling to illustrate the potential magnitude of biases in the

estimates of AMR prevalence and incidence rates due to low blood culture utilisation rates.

Finally, using a large retrospective data set, I applied a counterfactual framework to estimate the impact of delays in concordant antibiotic treatments. Attempts were made using observational data to emulate randomised controlled trials that are otherwise unethical to perform.

This thesis describes work which generated data, provided evidence, and created a new tool for quantifying the health burden of AMR infections in LMICs.

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Statement of contributions

My supervisors, Direk Limmathurotsakul, Ben S Cooper, Nicholas PJ Day, and Guy Thwaites provided methodological and scientific guidance throughout all stages of this work. Additional contributors for each chapter are listed below.

Chapter Two

Kanthiya Jirapornuwat and Rampaipan Wongwattanasatean were the research nurses who worked with me in collecting data in the prospective surveillance study. Gumphol Wongsuvan and his team collected and stored bacterial isolates from clinical samples of the enrolled patients. Prapit Teparrukkul, a medical doctor in Sunpasitthiprasong Hospital, reviewed the chest X-ray films and acted as the advisor for the clinical symptoms of hospital-acquired infections. Jitrapan Nilskul, Sompboon Nuntalohit and Somsamair Boonsong from Sunpasitthiprasong Hospital provided microbiology data.

Chapter Three

Miliya T, Chansamouth V, Aung MT, Karkey A, Teparrukkul P, Rahul B, Lan NPH, Turner P, Ashley E, van Doorn HR, Lin HN, Dunachie S, Yen LM, Tan LV, Hlaing HH, Mayxay M, Vongsouvath M, Basnyat B, Edgeworth J supported and tested the AMASS application in the local hospitals, and reviewed the AMR surveillance report before it was shared in FigShare.

Chapter Five

Prapit Teparrukkul and Maliwan Hongsuwan helped with data collection. Mo Yin reviewed the definition of concordant antibiotic treatments.

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Publications in relation to this thesis

Chapter One

Lim C, Ashley E, Hamers R, Turner P, Kesteman T, Akech S, Corso A, Mayxay M, Okeke I, Limmathurotsakul D, van Doorn RH. Surveillance strategies using routine microbiology for antimicrobial resistance in low and middle-income countries. Clin Microbiol Infect. 2021. doi: 10.1016/j.cmi.2021.05.037. Online ahead of print. PMID: 34111583

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Chapter Four

Lim C, Hantrakun V, Teerawattanasook N, Srisamang P, Teparrukkul P, Sumpradit N, Turner P, Day NP, Cooper BS, Peacock SJ, Limmathurotsakul D. Impact of low blood culture usage on rates of antimicrobial resistance. J Infect. 2021 Mar;82(3):355-362. doi: 10.1016/j.jinf.2020.10.040. Epub 2020 Dec 2. PMID: 33278401; PMCID: PMC7994019.

Chapter Five

Lim C, Mo Y, Teparrukkul P, Hongsuwan M, Day NPJ, Limmathurotsakul D, Cooper BS. Effect of delays in concordant antibiotic treatment on mortality in patients with hospital-acquired *Acinetobacter* spp. bacteremia: emulating a target randomised trial with a 13-year retrospective cohort. Am J Epidemiol. 2021 May;kwab158. doi: 10.1093/aje/kwab158. PMID: 34048554.

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List of abbreviations

AMASS	AutoMated tool for Antimicrobial resistance Surveillance System
AMR	antimicrobial-resistance
AMS	antimicrobial-susceptible
AMU	antimicrobial usage
ANSORP	Asian Network for Surveillance of Resistant Pathogens
AST	antimicrobial susceptibility tests
BLQS	Bureau of Laboratory Quality Standards
BSI	bloodstream infection
cAST	cumulative antimicrobial susceptibility test
CAB	community-acquired bacteraemia
CAP	community-acquired pneumonia
CCI	Charlson comorbidity index
CI	confidence interval
CLABSI	central line-associated BSI
CLSI	Clinical and Laboratory Standards Institute
DAG	direct acyclic graph
DALY	disability adjusted life years
DRI	drug-resistant infections
EARS-Net	European Antimicrobial Resistance Surveillance Network
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Prevention and Control
EQA	external quality assessment
EU/EEA	European Union and European Economic Area

EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
GLASS	Global Antimicrobial Resistance Surveillance System
HAB	hospital-acquired bacteraemia
HAP	hospital-acquired pneumonia
HCAB	healthcare-associated bacteraemia
HCAP	healthcare-associated pneumonia
HICs	high-income countries
HUS	Haemolytic uraemic syndrome
ICD	International Classification of Diseases
ICU	intensive care unit
JANIS	Japan Nosocomial Infections Surveillance
IPW	inverse-probability weight
LIS	laboratory information systems
LMICs	low and middle-income countries
LQAS	lot quality assurance sampling
LRTI	lower-respiratory tract infection
MDR	multidrug-resistant
MIC	minimal inhibitory concentration
MRSA	methicillin-resistant *S. aureus*
NARST	National Antimicrobial Resistance Surveillance Thailand
NEDSS	National Electronic Disease Surveillance System
OIE	Organisation for Animal Health
PAF	Population attributable fraction
PPS	point prevalence survey

SSI	surgical-site infections
SSTI	skin/soft tissue infections
STEC	Shiga-toxin-producing *Escherichia coli*
UTI	urinary-tract infections
VAP	ventilator-associated pneumonia
WHO	World Health Organization

1

Background and literature reviews

1.1 Antibiotics and resistant bacteria

Soon after the discovery of penicillin, a ß-lactam antibiotic, by Alexander Fleming in 1929,[1] an increasing number of antibiotics were discovered in the 1940s. Antibiotics have, since then, been the key treatment for many infectious diseases caused by pathogenic bacteria. In 1941, Selman Waksman used the term antibiotic for molecules with properties of antagonizing the growth of microbes.[2] Some antibiotics are bacteriostatic, which means they inhibit the multiplication of bacteria; when the antibiotics are removed, the bacteria can resume growth. Other antibiotics are bactericidal and can trigger pathways within bacterial cells that lead to bacterial death. Different antibiotic classes act against bacteria through different modes of action, which include interference with i) bacterial cell wall or cytoplasmic membrane synthesis; ii) bacterial protein synthesis; iii) bacterial nucleic acid synthesis by blocking DNA gyrase or DNA-dependent RNA polymerase; and iv) the metabolic pathway for folic acid (a precursor of nucleic acid) synthesis.[3]

There are several mechanisms bacterial cells have developed to increase the chance of survival in the presence of antibiotics. At the molecular level, individual bacterial cells can develop mechanisms to block, inactivate, or bypass the action of antibiotics and become resistant to the antibiotics. These mechanisms include i) production of enzymes that destroy or inactivate antibiotics; ii) increase of bacterial outer membrane

impermeability which prevents antibiotics from entering into the bacterial cell; iii) alteration of the targets that antibiotics bind to and act against; iv) utilisation of efflux pumps that export antibiotic as rapidly as it enters; and v) alteration of metabolic pathway to bypass the reactions that are inhibited by antibiotics.[4,5] Collectively, a community of mixed resistant and sensitive bacteria populations may develop cooperative resistance. For instance, antibiotic degradation by a sub-population of enzyme-producing (i.e., resistant) bacterial cells can indirectly protect the sensitive bacterial cells within the same community, which would otherwise be affected by the antibiotic at inhibitory drug concentrations.[6-8] Another example of cooperative resistance is the formation of dense surface-associated biofilms.[9] Biofilms are communities of bacteria that live in cellular clusters and are under the protection conferred by biofilm matrix polymers, which increase the difficulty for antibiotics to penetrate through and act on the bacterial cells. In the laboratory, it has been shown that an increase of 600-fold in the concentration of chlorine (one of the most effect antibacterial chemicals) is needed to kill biofilm cells of *Staphylococcus aureus* (a Gram-positive bacteria) compared to killing the same bacterium in a free-floating culture (without biofilm protection).[10]

The emergence of antimicrobial resistance results in increasing difficulties in treating infectious diseases.[11-13] We are falling behind in the race against antibiotic resistance, and the options for treating drug-resistant infections (DRI) are increasingly limited.[14] There is a significant decline in the discovery and development of new antibiotics and a dramatic increase in resistance to the currently available antibiotics.[15] An example is the use of colistin. Colistin is a polymyxin antibiotic discovered more than 50 years ago, and its therapeutic use was limited due to the drug's side effects.[16,17] In the early 2000s, the use of colistin resurged due to a lack of treatment options for multidrug resistant (MDR)

infections. In 2015, the mcr-1 colistin resistance gene was described in *Escherichia coli*, a Gram-negative bacterium, and the dissemination of the gene is increasingly reported globally.[18] Only two new classes of antibiotics have been developed and approved by international drug agencies in the past 20 years, and both provide coverage against only a sub-class of pathogenic bacteria, the Gram-positive bacteria.[19] The effect of antimicrobial resistance on our modern healthcare system is significant. A direct impact is the limited options for antibiotic treatment against resistant pathogens, which may lead to delays in appropriate antibiotic treatment and a decrease in the chance of survival in patients with DRI.[20-22] Indirectly, modern medicine, including surgery, care of premature infants, transplant medicine, and chemotherapy for cancer, is hampered without effective antibiotics. Moreover, the economic impact related to resistant infection is substantial. A report compiled by Jim O'Neill in 2015 projected that a total GDP loss of $100 trillion would be expected by 2050 if no effective action to combat resistant infections is taken.[13] DRI imposes additional costs on treatment and patient care for both hospitals and patients.

1.1.1 Evolution and transmission of antibiotic resistance

The emergence of antimicrobial resistance in bacteria occurs naturally.[23-26] Some bacteria possess mechanisms that overcome naturally-produced antibiotics in order to survive, and these bacteria are considered to be "intrinsically" resistant to one or more antibiotics. Nevertheless, in clinical settings, "acquired" resistance is used to describe a population of bacteria that was originally susceptible to an antibiotic but later developed resistance to the antibiotic.[4] Experimental studies have demonstrated the adaptation of pathogenic bacteria in response to survival pressure mainly through mutation and transfer of resistance genes between resistant bacteria and pathogenic bacteria through horizontal gene transfer.[27]

Gene mutation may occur randomly in a subpopulation of bacteria during the replication of the bacterial chromosomes and could lead to modifying cellular targets of antibiotics, that in turn result in the antimicrobial agents no longer being active against the bacteria. These successful strains would then further expand in population size under antibiotic selection pressure. Overuse and mis-use of antimicrobials may catalyse this process, and accelerate the spread of new antibiotic resistance mechanisms. Consumption of antibiotics for common viral infections is an example of mis-use of antimicrobials in many countries, especially in countries where antibiotics can be easily bought in pharmacy stores without a prescription. Use of antibiotics in livestock and aquatic farming as growth promoters are also often considered examples of misuse of antimicrobials. In hospital settings, intensive overuse of broad-spectrum antibiotics without effective antibiotic stewardship programs contributes to the emergence of resistance. DRI affects both humans and animals, and the resistant bacteria can be transmitted between humans and environment, humans and animals, and humans and humans.

Transfer of resistance genes between intrinsically resistant bacteria and pathogenic bacteria (i.e., via horizontal gene transfer) is promoted by the large variety of bacterial species sharing the same community. The human body is densely populated with micro-organisms, and the gastrointestinal tract, skin, and respiratory tract are home to both harmless commensals and pathogenic bacteria. The complex microbial community is a hub of various resistance genes, and the transfer of these resistance genes between different bacterial populations can occur through conjugation (transfer of gene via cell-to-cell contact) and/or transduction (transfer of gene via bacteriophages).[28]

1.1.2 Isolation and identification of bacteria from clinical samples

Microbiology culture is one of the most important methods of bacterial isolation, prior to bacterial identification and antimicrobial susceptibility testing, used in clinical settings. The process begins with the collection of clinical samples from patients suspected of having a bacterial infection. Here, I will use blood sampling as the example to discuss the process of bacteria isolation and identification in routine microbiology testing. A blood sample may be collected for bacterial culture from a patient who is suspected of bloodstream infection or systemic infection. Guidelines on sepsis management highlight the importance of blood sample collection for microbiological culture before administration of antibiotics, or immediately after when collection before antibiotic administration is not possible.[29,30] This is crucial because blood culture utilisation rate can influence the interpretation of the test results and surveillance data.[31,32] Moreover, improper sampling collection techniques and/or laboratory processing could lead to increased contamination rates, which could affect estimates of disease incidence. This could also affect the interpretation of the microbiology testing results and ultimately affect clinical decisions and patient outcome. Previous studies have shown that contaminants can lead to increased prescriptions of antibiotics and thus resistant infections.[33-35] Furthermore, contaminants can hamper the growth of pathogens, which could lead to delays in appropriate treatments, and result in extended hospital stays and increased mortality.[35]

The process of microbiological testing can influence the downstream data on AMR infections. It begins with inoculating clinical samples into an appropriate culture bottle, and then placing in an incubator in which bacteria will replicate to reach a detectable population size. This process determines the ability to detect the causative pathogen. A

manual method will require the blood culture bottle to be incubated for seven days at 35˚C. The growth of bacteria and fastidious organisms usually occurs within the first five days, and newly detectable growth often is limited on day six and seven.[36-38] There is no one optimal medium or commercial product to detect all microorganisms. Selection for the medium needs careful considerations. Moreover, the type of medium to use for specific organisms, additive solutions to inactivate antimicrobials in the blood sample, atmosphere of incubation, and bottle agitation all play important roles in growth of microorganisms, but descriptions of these are beyond the scope of this thesis.[36] Conventionally, Gram staining and blind subculturing (i.e., subculturing bottles that have no visual signs of bacterial growth) of blood culture media are performed after the first overnight incubation and, if cultures remain negative, then again at the end of incubation period.[36]

Automated blood culture systems, including BACTEC 9240 (Johnston Laboratories, Cockeysville, Md.) and BacT/ALERT (BioMerieux, Marcy d'Etoile, France) are gradually replacing manual blood culture systems especially in high income settings, and also in some low and middle-income settings. These systems rely on detection of carbon dioxide produced by microorganisms in the bottles using colorimetric or fluorescent detections. They perform better than manual systems in the yield of bacteria and speed of growth, and thus perform better in organism isolation.[39,40]

Conventionally, the manual procedures for identifying microorganisms involve a combination of multiple tests including Gram stain, observation of physical characteristics through colony morphological and odour examination on multiple agars, biochemical tests and antimicrobial susceptibility tests.[36]

More recently, systematic biochemical reactions and mass spectrometry techniques are increasingly used in automated instruments for bacterial identification. Examples of commercial instruments included VITEK 2 (bioMerieux, Marcy d'Etoile, France), MicroScan conventional panels (Siemens Healthcare Diagnostics, West Sacramento, CA, USA), and BD Phoenix system^{TM} (BD Biosciecnes, USA). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), which measures the mass molecules from a sample using a laser to ablate and desorb the molecules is increasingly used in both HICs and LMICs. After microorganisms are identified, the subculture of isolates is then extracted to an agar plate for further identification and for antibiotic susceptibility tests.

1.1.3 Antimicrobial susceptibility tests

Antimicrobial susceptibility testing (AST) is important for the detection of drug-resistant organisms and to guide antibiotic treatment in a clinical setting. The most commonly used methods for AST are the disk diffusion susceptibility test and one of several methods to detect the minimal inhibitory concentration (MIC). Disk diffusion is a semiquantitative method of manual testing that is performed using commercially-prepared, fixed concentration, paper antibiotic disks, which are placed on an agar containing the bacterial inoculum. The diameter of the diffusion zone around each antibiotic disk is measured after incubation. The diffusion zone reflects the growth inhibition around each antibiotic disk. The advantages of disk diffusion are that it is simple to perform, low cost, and flexible in the choice of disks for antibiotic testing. The limitation of disk diffusion is that it is a semi-quantitative method that only provides the classification of susceptible, intermediate, or resistant.

A quantitative AST method is the agar/broth dilution test, which involves preparing a gradient of dilutions for each antibiotic to be tested and then determining the lowest concentration of antibiotic that inhibits bacterial growth, which is measured in MIC. The advantage of this method is that it gives a quantitative result. The limitation is that manual preparation of antibiotic solutions can be tedious and labour intensive.

Alternatively, the MIC can also be determined using a method that combines the principles of both dilution methods and diffusion methods. Etest (BioMérieux) is a commercial antimicrobial gradient test method that involves strips with dried antibiotic concentration gradients marked on the strips. The MIC values can be read directly from the scales from the strip. The advantage is that it is easy to perform. The limitation is that the test is expensive, as each Etest strip costs $2-3. Thus, Etest is often used for one or two antibiotics for which MIC values are needed for reliable interpretation of susceptibility results.

Automated systems for AST are often incorporated into automated bacterial identification systems. For example, the VITEK 2 system performs both microbial identification and MIC testing automatically, but is expensive for resource-limited settings. Various automated systems differ in time to generate AST results, in maintenance time, in costs, and in the software programs utilised for data management.[41] AST methods other than those mentioned above, including detection of resistance genes, time-kill curves, the adenosine triphosphate bioluminescence assay, and flow cytofluorometric methods are not commonly used for clinical diagnosis due to high costs, and the expertise needed to perform the test and interpret the results.

Standardized guidelines on interpretation of the disk diffusion zone diameters and MIC values are available from the Clinical and Laboratory Standards Institute (CLSI, formally the National Committee for Clinical Laboratory Standards or NCCLS) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Interpretation involves the assignment of clinical breakpoints that categorize the test result into "susceptible (S)", "intermediate or susceptible, increased exposure (I)" or "resistant (R)". The CLSI guidelines are based on data from various sources including *in vitro* studies, pharmacokinetic-pharmacodynamic studies, and clinical studies.[42] The guideline is used in hospitals in the United States and many regions outside Europe. The EUCAST guidelines developed antimicrobial susceptibility breakpoints based on data from pharmacodynamic analysis, susceptibility distributions, drug resistance mechanisms, and clinical outcomes, and are predominately used in hospitals in Europe.[43] Several studies have been done to compare CLSI and EUCAST.[44-46]

A major difference between the CLSI and EUCAST guidelines is in the definition of the "I" category. CLSI defines "I" as an ambiguous AST result due to test variability and possible higher drug exposure, whereas EUCAST defines "I" as "susceptible, increased exposure".[47] The definition given in the EUCAST guideline implies that a drug for a microorganism under "I (susceptible, increased exposure)" category does not rule out the drug's therapeutic capability against the microorganism.[48,49] In other words, under such category a high chance of therapeutic success can be achieved with increasing exposure to the drug through adjusting the dosing regimen or its concentration at the site of infection.[48,49] Consequently, this would affect the re-grouping of "S", "I", and "R". For instance, under the CLSI classification grouping "I" and "R" to represent "non-susceptible" and "S" for "susceptible" is justified. However, under the EUCAST

classification grouping “S” and “I” is justified for “susceptible”, and “R” alone represents “resistance”.[48] Despite the differences, interpretations of AST results from both CLSI and EUCAST guidelines rely mainly on the *in vitro* activity of an antibiotic against a bacterial sample together with some pharmacological parameters, such as infection site concentration of the antimicrobial.[5,50]

Hence, regardless of which guideline is used for AST result interpretation, the treatment of antibiotic-resistant infections should also be based on clinical scenarios and the availability of treatment options. For example, a patient with lower urinary tract infection caused by an organism reported as gentamicin-resistant, defined by CLSI or EUCAST, could still be treatable using gentamicin. This is because gentamicin is excreted via urine and the concentration of the gentamicin in the urine could be sufficiently higher than its MIC. Similarly, different breakpoints for penicillin have been used for *Streptococcus pneumoniae* depending on whether the isolate is causing meningitis or other infections, since the levels of antibiotic that can pass the blood brain barrier and reach the cerebrospinal fluid have to be taken into consideration.[42,51]

1.1.4 Laboratory information systems

On a daily basis, a typical laboratory in a hospital processes multiple clinical samples for clinical diagnostic purposes, and a large amount of data is routinely generated. Such data may be recorded in paper forms, such as log books, electronically, or both. In very poorly resourced settings, paper forms remain the key data storage means and hamper the re-use of laboratory results. With the increasing use of computers, electronic storage of microbiology data is becoming common. Laboratory information systems (LIS) are tools that store and manage laboratory data electronically. The development and usage of a

LIS was noted as early as 1939 in Sweden.[52,53] The key features of a microbiology LIS include managing samples (and patients) with multiple results, archiving notes that are made routinely, conveying interpretable results for clinicians to support treatment, and facilitating result entry.[54]

Automated microbiology culture and AST machines are often accompanied by software to support a LIS. For instance, BD Phoenix has BDXpert and BD EpiCenter software installed within the machine, and VITEK 2 has Observa AES software installed. These machines are expensive and software maintenance incurs additional costs, which are unaffordable in laboratories in most LMICs. Independent LIS vendors are also available in different countries, and most incur purchasing and maintenance costs (i.e., not open-access), which impose extra economical burdens on resource-limited hospitals.

Alternatively, there are open-access software packages with functions to store data, such as WHONET. WHONET is designed for laboratories to manage and analyse AST results in both LMICs and HICs.[55,56] It allows data entry and data storage for settings without a LIS. In settings where a LIS is available, WHONET has the BacLink function that can translate data extracted from the local LIS to WHONET universal file format. The data analysis functions include outbreak detection, data quality checks, and descriptive data statistics for each organism of interest.[57-61]

1.1.5 Laboratory quality assurance in microbiology

Laboratory quality assurance is essential to ensure accurate identification of pathogenic organisms and their AST profile, which in turn is important for generating accurate routine microbiology data to inform treatment and support quality data generation. Both

international and national organizations have attempted to provide support to laboratories for participating in accreditation programs. In Thailand, the Bureau of Laboratory Quality Standards (BLQS) provides external quality assessment (EQA) programs and audits most of the national public health and private laboratories.[62] The Thailand national accreditation system was adapted from the ISO 17025 and ISO 15189, and CLSI guidelines that were translated into the national language.[62] In Africa, the WHO Regional Office for Africa established frameworks for improving the quality of laboratories to achieve ISO 15189 standards.[63] It can be labour-intensive to participate in an EQA scheme, and the cost can be as high as 24,558 US dollar per year [64]. These high costs hamper accessibility to the accreditation in many LMICs. In 2020, Fleming Fund grants have supported EQA for laboratory testing with a focus on bacteria identification and AST in twelve countries in Asia.[65] Training to ensure quality performance of bacterial identification and AST is important for laboratories in LMICs.[66]

Regardless of whether a laboratory uses automated machine or manual methods for microbiology culture, bacterial identification and AST, it is crucial to check and verify unusual AST results. For instance, *Enterococcus faecalis* isolates that are phenotypically resistant to ampicillin are uncommon and results should be confirmed.[67] Another example is an *Enterobacteriaceae* or *Pseudomonas aeruginosa* isolate that is more resistant to piperacillin-tazobactam than piperacillin, which would need re-testing as such a phenotype is rare.[68] Guidelines on identifying unusual results that require verification include the CLSI M100 document and EUCAST expert rules and intrinsic resistance guidelines (http://www.eucast.org/expert_rules_and_intrinsic_resistance/). Moreover, the laboratory quality at surveillance sites influences the ability to identify the correct organism and ultimately influences the overall estimates on incidence of infection. For

instance, a recent study in Thailand describes the misidentification of *Burkholderia pseudomallei* as *Acinetobacter* species in blood specimens.[69] This resulted in an overestimation of incidence of *Acinetobacter* spp. bacteraemia, an underestimation of the incidence of melioidosis (a disease caused by *Burkholderia pseudomallei*), and inappropriate treatment for melioidosis patients.

A substantial number of surveillance studies on antimicrobial resistance lack documentation on testing and quality control programs.[70] Performing data verification manually on a daily basis is a tedious and time-consuming job. There are very few commercial LISs that can perform data verification, and those that can are expensive and unaffordable for resource-limited settings. Open-access software such as WHONET has functions to highlight unusual AST results; however, these are not commonly utilised. There is a lack of easy-to-use, open-access tools that can support data verification and highlight unusual AST results.[71]

1.1.6 Routine microbiology data

On a routine basis the decision to collect clinical samples to send to the microbiology laboratory for clinical purposes is usually made by the treating physicians and motivated based on clinical suspicion of an infection. However, the general prescription behaviour varies among individual physicians in different hospitals and is based on the availability of laboratory resources. A study examining the utilisation of blood culture in Indonesia and Thailand showed that even when health expenditure per capita between the two countries was similar, the proportion of patients sampled for blood culture in similar size hospitals differed greatly.[72] There are several barriers that can hamper the physician's decision to collect clinical samples for microbiological testing. Technical barriers include

lack of resources to collect clinical specimens, to perform culture, and to store data for analysis. Social barriers include belief in microbiology testing results and the practice of empirical antibiotic treatment without collecting clinical specimens for microbiological tests. A mixed methods study in Germany showed only 23% of physicians would collect blood samples for culture even when the national guidelines recommend blood culture be performed for a given scenario.[73] The practices of microbiology culture for diagnosis could have an effect on the downstream data available for supporting patient management and disease burden estimation.

Microbiology results can be used for different purposes, ranging from guiding individual patient management to informing action plans for controlling the spread of antimicrobial resistance. First, in a typical clinical setting, an individual report on results of a sample type collected from a patient is sent, often in paper form, to the attending physician to guide antibiotic prescription. The individual report contains information such as patient demographics, the sample type that was sent to the laboratory for microbiology culture, date of sample collection, microorganism identified, and the resistance profile of the organism. Together with clinical observations, this information is essential to adjust or to determine the optimal antibiotic type, route of administration, dose, and frequency for the patient. Second, the utility of microbiology data is maximized in cases where hospitals store their data in electronic systems, such as a commercial LIS, Microsoft Excel, or Microsoft Access. The electronically stored data can be re-used and compiled to generate cumulative antimicrobial susceptibility test (cAST) reports, which is important for patient management.[74] For instance, while waiting for microbiology culture and AST to be available, a physician of a patient that is suspected to have a urinary tract infection may need to consider the most common pathogen, and the chance that an

antibiotic, such as ampicillin, for urinary tract infection will cover the causative pathogen. These considerations that eventually lead to treatment decisions need epidemiological evidence from cAST reports, which are aggregated from the individual AST results. Third, monitoring the trends of resistance, designing preventive interventions, and assessing the interventions at local, national and global levels to combat AMR needs evidence and support from accurate, valid AST data that are collected routinely in electronic databases, analysed systematically, and presented in a standardized format.[75,76]

1.1.7 Priority list of antibiotic-resistant bacteria

With recognition of the importance for understanding clinically-relevant bacteria, WHO published a priority list of antibiotic-resistant bacteria to direct research and development of new treatments and design of action plans to control the spread of the bacteria in 2017.[77,78] In brief, the list of bacteria includes *Acinetobacter baumannii, Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Enterococcus* spp., *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter*, *Salmonella* spp., *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Shigella* spp. (Table 1.1).[77,78] Among the pathogens in that list, *S. aureus*, *Enterococcus* spp., *Streptococcus pneumoniae*, *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp. are commonly isolated in human clinical samples in LMICs.[79-83] These pathogens will be the focus of discussion in this thesis. The section below briefly summarises the microbiology of the bacteria and their resistant strains.

Acinetobacter spp.

Acinetobacter spp. are Gram-negative coccobacilli that are increasingly recognised as a clinically relevant pathogen in hospitals worldwide.[77,84-86] The organism was described in 1911 as *Micrococcus calco-aceticus*[87] and became known as *Acinetobacter* in 1950s.[88] The bacterium is capable of acquiring diverse mechanisms of resistance. The emergence of strains that are resistant to all commercially available antibiotics is increasingly observed in many regions globally. Acinetobacter can survive weeks in the environment, and the transmission can be mediated by fomite contamination in the hospital.[89] In humans, Acinetobacter colonises skin, wounds, respiratory tracts, and gastrointestinal tracts. The most commonly reported clinical isolates of Acinetobacter are *A. baumannii*, *A. calcoaceticus*, and *A. lwoffii*, which together often are referred to as *A. calcoacetisu-A.baumannii* complex. Carbapenem-resistant *A. baumannii* is ranked as one of the critical antibiotic-resistant bacteria in the WHO priority list.[77]

Enterococcus spp.

These are Gram-positive bacteria that are commonly isolated from wounds and urine samples. *Enterococcus faecalis* and *Enterococcus faecium* are clinical pathogens that can cause infections. Vancomycin-resistant *E. faecalis* and *E. faecium* are clinically important, as they are difficult to treat, and are in the WHO priority list.[77]

Escherichia coli

E. coli, a Gram-negative bacterium, can be found in the environment and food. Typically, it colonises intestines of humans and animals as a common flora, but can cause infection in immunosuppressed hosts or when gastrointestinal barriers are compromised. The three main infections caused by pathogenic *E. coli* strains are urinary-tract infection, sepsis, and enteric disease. Moreover, Shiga-toxin-producing E. coli (STEC) can cause

diarrhoea-associated haemolytic uraemic syndromes (HUS), which is a thrombi disorder commonly encountered in children after gastrointestinal infection from the organism.[90] *E. coli* are serotyped based on their somatic (O), flagellar (H) and capsular (K) surface antigen profiles. The most common *E coli* clonal group among the extraintestinal pathogenic isolates worldwide is ST131. Isolates in this group produce extended-spectrum beta-lactamases and are resistant to almost all fluoroquinolones (an important class of antibiotics for treating *E. coli* infections).[91] The most frequent STEC found in humans is O157:H7, which has a strong aetiological association with HUS.[90] WHO ranked third-generation cephalosporin resistant *E. coli* and carbapenem-resistant *E. coli* as critical antibiotic-resistant bacteria.[77]

Klebsiella pneumoniae

K. pneumoniae, an encapsulated Gram-negative bacterium, was first isolated from lungs of a patient who died from pneumonia in 1882. Typically, the bacterium can be found in human mucosal surfaces of the oropharynx and gastrointestinal tract as common flora, but it causes infection when it enters the human body. Third-generation cephalosporin resistant *K. pneumoniae* and carbapenem-resistant *K. pneumoniae* are listed in the WHO priority list.[77]

Pseudomonas aeruginosa

P. aeruginosa, an aerobic, rod-shaped Gram-negative bacterium, is an opportunistic pathogen that causes infection in hospital patients. Carbapenem-resistant *P. aeruginosa* is in the WHO priority list.[77]

Salmonella spp.

Salmonella spp., anaerobic Gram-negative rod-shaped bacteria, can cause gastrointestinal infections when contaminated foods are consumed. The organism is primarily of animal origin, and is prevalent in food animals. Most of the *Salmonella* serotypes belong to *Salmonella enterica* subspecies *enterica*, which causes *Salmonella* infections in humans. Fluoroquinolone-resistant *Salmonella* spp. is rated as a high priority organism in the WHO priority list published in 2017,[77] and is prevalent in Asia.[92] Other clinically important isolates include third-generation cephalosporin resistant *Salmonella* spp.[77]

Staphylococcus aureus

S. aureus is a Gram-positive, round-shaped bacterium and is part of the microbiota of the human body. The bacterium commonly colonises the upper-respiratory tract and the human skin. However, when entering normally sterile sites such as the bloodstream or compromised sites such as wounds and even normal skin, clinical infections can occur. Methicillin-resistant *S. aureus* (MRSA) is rated as a high priority antibiotic-resistant bacteria by the WHO because BSIs caused by MRSA have high mortality.[77,78] Vancomycin-resistant *S. aureus* is also on the list as treatment options for infections related to this antibiotic-resistant bacteria are limited and development of new antibiotics is needed.

Streptococcus pneumoniae

S. pneumoniae, a lancet-shaped Gram-positive bacterium, was first isolated from saliva of a patient with rabies in 1881.[93,94] The bacterium is a facultative anaerobic organism that is typically seen in pairs or short chains. Over 90 serotypes of *S. pneumoniae* have been identified.[93] Encapsulated *S. pneumoniae* is pathogenic for humans and may cause infections such as pneumonia and meningitis that are particularly prevalent in

children.[95,96] Conjugate pneumococcal vaccines, since their introduction in 2000, have been shown to be protective against infections caused by the vaccine-type invasive pneumococcal infections.[97,98] Penicillin-non-susceptible *S. pneumoniae* is ranked as a medium priority organism in the WHO priority list published in 2017.[77] Other clinically relevant antibiotic-resistant strains are third-generation cephalosporin resistant, erythromycin-resistant, and clindamycin-resistant *S. pneumoniae*.

Table 1.1. List of pathogen-antibiotic combinations (ordered by alphabetical order) from the WHO priority list published in 2017. The commonly isolated pathogens from clinical samples in my study sites will be included as the focus of discussion in this thesis.

Pathogen	Resistant isolate	Priority rank	Note
Acinetobacter baumannii	• Carbapenem-resistant	Critical	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
Campylobacter	• Fluoroquinolones-resistant	High	Not included in my study
***Enterobacteriaceae* (including *Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp., *Morganella* spp.)**	• Third-generation cephalosporin-resistant • Carbapenem-resistant	Critical	*K. pneumoniae* and *E. coli* are commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
Enterococcus faecium	• Vancomycin-resistant	High	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
Haemophilus influenzae	• Ampicillin-resistant	Medium	Not included in my study
Helicobacter pylori	• Clarithromycin-resistant	High	Not included in my study
Neisseria gonorrhoeae	• Third-generation cephalosporin-resistant • Fluoroquinolones	High	Not included in my study

Pseudomonas aeruginosa	• Carbapenem-resistant	Critical	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
***Salmonella* spp.**	• Fluoroquinolone-resistant	High	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
***Shigella* spp.**	• Fluoroquinolone-resistant	Medium	Not included in my study
Staphylococcus aureus	• Methicillin-resistant • Vancomycin intermediate and resistant	High	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study
Streptococcus pneumoniae	• Penicillin-non-susceptible	Medium	Commonly isolated from clinical samples in Southeast Asia countries [79,80,83] and included in my study

1.2 Origin of infection and human organs affected by bacterial infections

Bacterial infections occur when pathogenic bacteria proliferate inside a human body and trigger a series of events that cause damage to cells. Every area of the human body can be a target of pathogenic bacteria, and infections may occur at one or more body sites including the bloodstream, lower-respiratory tract, surgical wounds, urinary tract and/or central nervous system.

The predominant pathogen varies across different body sites of infection as do risk factors related to the infection and the impact on patient mortality. For instance, *S. aureus* is commonly isolated from surgical wounds. A majority of urinary-tract infections are related to *E. coli*, which is commensal but can be pathogenic when the immune system of the host is weakened, the pathogen enters a site that is typically free of microorganisms,

or when the organism carries a toxin such as STEC. In general, urinary-tract infections without complications have a much lower mortality rate than bloodstream infections.

The complexity of defining infections varies from being as simple as recovering a pathogen from sets of clinical samples, as is the case for bloodstream infections (though the possibility of contamination cannot be neglected), to a combination of different diagnostic methods including chest imaging, clinical symptoms, microbiology test results, and laboratory chemistry tests, such as is the case for lower-respiratory tract infection. Moreover, pathogenic bacteria found in the community often differ in antimicrobial susceptibility patterns from those found in the hospital and thus further classification by the likely origin of infection can be important for treatment decisions, and action plans to control antimicrobial resistant infections.

1.2.1 Community-origin, healthcare-associated, and hospital-origin infections

There are different commonly used definitions that aim to classify bacterial infections by their likely place of acquisition. Traditionally, the term nosocomial, or hospital-acquired infection, was used to describe infections that occur in patients who are receiving care in the hospital, and any infection that is not nosocomial is considered to be community-acquired by default. The classifications by likely origin of infection can be useful for identifying patients with high risk of drug-resistant infection, in an effort to improve empirical antibiotic treatment, and ultimately reduce treatment failure and deaths.[99]

The World Health Organization Global Antimicrobial Resistance Surveillance System (WHO GLASS) defined patients with community origin infection as those being cared for at an outpatient clinic when the specimen was taken or those hospitalized for ≤2

calendar days when the specimen was taken.[100] Conversely, patients with hospital-origin infection are defined as those admitted to a healthcare facility for ≥2 calendar days when the specimen was taken or those admitted to a health care facility for <2 calendar days but transferred from another health care facility where they were admitted for ≥2 calendar days when the specimen was taken.[100] The definitions can be easily applied to classify the type of infection in cases when date of admission and date of specimen collection are available. This classification is important to ensure that the reporting on prevalence and incidence of antimicrobial resistant infections is epidemiologically pertinent,[100] as the resistance pattern often differs by the acquisition setting of infection.

Another classification on origin of acquisition for bloodstream infection was proposed to accommodate changes in healthcare seeking behaviours.[101] In this classification the origin of acquiring bacteraemia is classified into three categories: community-acquired bacteraemia (CAB), healthcare-associated bacteraemia (HCAB), or hospital-acquired bacteraemia (HAB).[101] CAB is defined in patients who had a pathogenic organism identified in a blood sample taken in the first two days of hospitalization and without a history of hospital stay within 30 days prior to the current hospitalization. HCAB is defined in patients who had a pathogenic organism identified in blood sample(s) taken within the first two days of hospitalization and with a history of hospital stay within 30 days prior to the current hospitalization. HAB is defined in patients who had a pathogenic organism identified in blood sample(s) when hospitalized for two or more days. The resistance patterns of the causative organisms typically vary by the origin of infection because of the differences in chances of exposure to the resistant bacterial population and antibiotic usage. For example, pathogenic bacteria that cause HAB are typically resistant to more antibiotics than those that cause CAB,[101] as patients who are hospitalized are

more likely to be have some exposure to antibiotics (including prophylaxis antibiotics), which is a driver of resistance (i.e., through natural selection for the resistant strains), and/or exposure to invasive devices (such as central line catheters), which are a common entry point for infections in general. Moreover, even without exposure to antibiotics prolonged length of hospitalisation increases the chance of exposure to the resistant strains of bacteria carried by other patients who may have taken antibiotics.

Similarly, for pneumonia the origin of infection can be classified into community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), or healthcare-associated pneumonia (HCAP).[102,103] In a retrospective study on hospitalized patients of hospitals in New York State, of all patients with pneumonia, 54% were CAP, 30% were HCAP, 14% were HAP, and the remaining 2% were ventilator-related.[104] In the Asia-Pacific region, *S. pneumoniae* was the predominant Gram-positive organism for CAP and *K. pneumoniae* was the predominant Gram-negative organism for CAP.[105]

1.2.2 Bloodstream infection

Bloodstream infection (BSI) is one of the most commonly studied site-specific infections because it has a large impact on patient mortality.[76,106] The isolation of a pathogenic bacteria from a blood sample of a suspected patient can typically be defined as a case of BSI. Based on the United States CDC definition, primary BSI is defined as laboratory-confirmed BSI that is not secondary to an infection at another body site.[107] Primary BSI can further be classified as central line-associated BSI (CLABSI) or non-CLABSI, based on whether or not an intravascular catheter line, that terminates at or close to the heart or in one of the large vessels which is used for infusion, withdrawal of blood, or

hemodynamic monitoring, was in place for more than two calendar days of infection onset.

The predominant causative pathogens identified from patients with BSI vary across different regions of the world, by origin of the infection, and by age. The report from a worldwide AMR survey showed that in the North America region the most common pathogens among the BSI isolates are *S. aureus* and *E. coli*, which together comprised 44% of BSIs, and followed by *K. pneumoniae*, *E. faecalis* and *P. aeruginosa*.[108] In the Asia-Pacific region, the most common pathogens among the BSI isolates were *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*.[108] The proportion of MRSA in the Asia-Pacific region is lower than that in the North America region (30% vs 40%).[108] *Acinetobacter* spp. is one of the top 10 pathogens related to BSIs in the Asia-Pacific, Latin America and European regions, but not in the North America region.[108] Moreover, studies in developing countries have reported higher catheter-related BSI compared to those in developed countries.[108] The reported incidence of catheter-related BSI ranged from 8.9 to 11.3 per 1000 device-days in developing countries, but ranged from 1.3-5.0 per 1000 device-days in developed countries.[109] In the South and Southeast Asia region, the common bacterial pathogens isolated from blood samples of adults with CAB were *Salmonella enterica* serotype Typhi, *S. aureus*, and *E. coli*.[110] The common bacterial pathogens isolated from blood samples of children with CAB were *Salmonella enterica* serotype Typhi, *S. pneumoniae*, and *Haemophilus influenzae*.[110]

Similarly, the antibiotic resistance pattern of the bacteria isolated from blood samples differed by geographical region and origin of infection. For instance, in high-income Asian countries including Japan [111,112] and Korea [112,113], the proportion of MRSA among

the *S. aureus* blood culture isolates is high. The proportion of MRSA is higher in the American continent (38.8-47.0%) than that in Asia (26.8-40.3%).[114] A recent report from a multinational study (the SENTRY Antimicrobial surveillance program) suggested that the proportion of MRSA is slightly higher in nosocomial infections (47.0%) compared to that in community-onset infection (36.8%).[114] The study was a report of surveillance data from 4 continents over 20 years. However, data from LMICs remains scarce and the existing data is likely to under-represent areas that lack surveillance systems for BSI.[111,115]

High mortality related to antibiotic-resistant BSI has been reported. The reported attributable mortality of hospital-acquired MDR *Acinetobacter* spp. BSI ranged from 18 to 41% in developing countries.[20,21,116-118] In a study conducted in Thailand, it was estimated that around 15,000 excess deaths per year were related to MDR *Acinetobacter* spp..[21] Other studies have suggested that the in-hospital mortality (including both mortality directly due to the infection and mortality unrelated to the infection) of BSI with carbapenem-susceptible *Acinetobacter* spp. is generally lower (ranging from 9% to 45%) compared to that with carbapenem-resistant *Acinetobacter* spp. (ranging from 27% to 70%).[20,116] Crude mortalities, even those from the same study cohort, are not directly comparable, as patient characteristics and appropriateness of treatment can influence the chance of survival and may be different between patients with carbapenem-resistant and carbapenem-susceptible *Acinetobacter* spp. BSI. However, this trend has been consistently seen in patients with *Acinetobacter* spp. BSI in different studies.[20,21,116-118] BSI caused by MRSA has been studied in many developed countries. For instance, a study conducted in Australia in 1998 estimated that the direct attributable mortality (defined based on clinical judgements) among patients with MRSA BSI and MSSA was

9% and 6%, respectively.[119] There are few studies in LMICs that estimate the attributable mortality of MRSA BSI. A retrospective cohort study conducted in Brazil showed the crude 30-day mortality for MRSA BSI was 54.9%, which is higher than that for MSSA BSI (24.7%).[120] There is an extensive literature highlighting the methodological issues of estimating mortality due to AMR infections.[121] A more in-depth discussion on the issues and measurements for describing health burdens (such mortality) of AMR infection is in Section 1.4 of this chapter.

1.2.3 Lower-respiratory tract infection

Lower-respiratory tract infection (LRTI) is commonly defined based on chest imaging, clinical presentations, and, when available, microbiology results. This reliance on clinical and radiological criteria complicates the surveillance and reporting of LRTI.[122,123] Nonetheless, LRTI is the most common type of hospital-acquired infection.[124] This infection can be further classified as to whether the infection is ventilator-associated pneumonia (VAP), which is defined in patients who were on mechanical ventilator for more than two calendar days prior to the day of onset.

The common pathogens associated with VAP are *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *E. coli*.[125] Colonization of pathogens at the oropharynx, at the ventilator circuit, and at the lower airways are risk factors for acquiring VAP, as the infection can be acquired through the aspiration of oropharyngeal secretions that pass the endotracheal tube or directly from inoculation into the respiratory tract.[126] In a prospective observational study conducted in 27 ICUs of nine European countries, 76% of pneumonia cases were HAP and of those around 56% were VAP.[127] The overall incidence of VAP exceeded 18 episodes per 1000 days of ventilator-days.[127] This means

that for every 50 days on a mechanical ventilator, on average one case of LRTI could be expected. The most commonly identified pathogens related to VAP varied by country.[127] For instance, *S. aureus* was commonly found in Spain, France, Belgium and Ireland, *P. aeruginosa* in Italy and Portugal, *Acinetobacter* spp. in Greece and Turkey, and *E. coli* in Germany. The incidence of VAP in the United States ranges from 1.9 to 3.8 per 1000 ventilator-days.[102] The most commonly identified pathogens from sputum samples of VAP in a long-term care hospital in the United States were *P. aeruginosa*, *Acinetobacter* spp., and *S. aureus*.[128] In hospitals in Thailand, *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* are commonly identified from clinical samples of patients with HAP.[129] In children with CAP, the most common bacteria isolated was *S. pneumoniae*.[130] In adults, the bacteria commonly isolated from patients with CAP vary by geographical region. For instance, in addition to *S. pneumoniae* which is commonly identified in western countries, Asian countries are also challenged with *Mycoplasma pneumoniae, K. pneumoniae,* and *Burkholderia pseudomallei*.[131]

Several studies have suggested high mortality among patients with hospital-acquired infection caused by Gram-negative bacteria, especially strains that are resistant to antibiotics.[132] The risk difference in case fatality of LRTI caused by resistant versus susceptible strains of bacteria varies by the causative organism and geographical region. For instance, a study in intensive care units in European countries showed that the case-fatality among patients with LRTI caused by ceftazidime resistant *A. baumannii* is similar compared to that in infections caused by the ceftazidime susceptible strains (38% vs 40%), whereas for patients with LRTI caused by third-generation cephalosporins resistant *E. coli* the case fatality is around 17% higher compared to infections caused by the susceptible strains.[132] This pattern is different from that in LMICs, where healthcare

quality, level of resources and the epidemiology of LRTI differ. Studies in LMICs, where LRTI caused by *A. baumannii* is common, suggest a large difference in case fatality between LRTIs caused to carbapenem-resistant *A. baumannii* and carbapenem-susceptible *A. baumannii.*[133-137]

1.2.4 Urinary-tract infection

Urinary-tract infections (UTIs) can be caused by bacteria which colonize the urethral opening and urine in the urethra but are often washed-out during urination. UTIs can be defined based on clinical symptoms and/or the microbiology culture results of urine samples. Based on the US CDC definition, UTI can be classified as symptomatic UTI, asymptomatic bacteraemic UTI, or urinary system infection. To define symptomatic UTI two criteria need to be met, which i) symptoms including fever, suprapubic tenderness, or costovertebral angle pain without other recognized causes; and ii) urine culture with no more than two species of organisms isolated. The quantity of uropathogen isolated from urine sample is an important factor of consideration in surveillance and reporting of UTI.[138-141] For patients less than one year old, the clinical symptoms to define symptomatic UTI are fever, hypothermia, apnoea, bradycardia, lethargy, vomiting and suprapubic tenderness without other recognized causes. Asymptomatic bacteraemia UTI is defined based on microbiology culture results and that is with at least one matching organism in blood and urine specimens. Urinary system infections are infections involving the kidney, ureter, bladder, urethra or tissue that surrounds the retroperitoneal or perinephric space. However, previous studies have criticized low specificity of clinical symptoms to define UTIs,[142,143] which may lead to overdiagnosis and overtreatment especially in the absence of urine cultures. The importance of positive urine culture without symptoms remains controversial, as asymptomatic bacteriuria results in no short-

term or long-term adverse outcomes in most studies but are risk factors for symptomatic UTIs, which may result in adverse outcomes.[144-147] UTI that occur in a normal female patient who has no structural or function abnormalities, is not pregnant, or did not have a catheter is considered as uncomplicated.[143] All other UTIs are classified as complicated.[143]

The most common bacteria identified from urine samples in UTIs is *E. coli*, followed by *Enterococcus* species.[148,149] In general, uncomplicated UTIs are self-limiting,[150] hence the primary goal of treatment for uncomplicated UTI is to ameliorate symptoms.[151] Randomized controlled studies suggest the rate of symptomatic relief is faster with antibiotic therapy compared to without,[152,153] however the treatments are not without adverse effects and resistance to antibiotics is increasingly reported.[154,155] Complicated UTI can lead to detrimental outcomes especially among elderly and immunocompromised populations when treatment is not given in time.[156]

1.2.5 Surgical-site infection

Surgical-site infections (SSI) are infections related to invasive procedures. They can be further categorized into three groups, which are superficial incisional SSI, deep incisional SSI, and organ/space SSI. Based on the CDC/NHSN surveillance definitions, an episode of superficial incisional SSI is defined as an infection occurring within 30 days after an operative procedure at body sites involving only skin and subcutaneous tissue of incision, with clinical symptoms and pathogen(s) identified from an aseptically-obtained specimen from the infected body site. An episode of deep incisional SSI is defined as an infection occurring within 30 or 90 days (based on operative procedure that the patient had) after the NHSN operative procedure involving deep soft tissues of incision (including fascial

and muscle layers), with clinical symptoms and pathogen(s) identified from the infected site. An episode of organ/space SSI is defined when an infection occurs within 30 or 90 days (based on the type of the NHSN operative procedure the patient had), involves body sites deeper than fascial/muscle layers that were opened or manipulated during the operative procedure, with clinical symptoms and pathogen(s) identified from aseptically-obtained fluid or tissue in the organ/space.

The incidence of SSI is high, even in HICs, and prevalent causative organisms vary by geographical regions. A nationwide study in US hospitals in 2005 showed that for every 100 surgical hospitalizations, around 1 case of SSI is identified.[157] Other studies suggested that SSI is the third most commonly reported hospital-acquired infection in the US and it is responsible for 14-16% of the inpatients with hospital-acquired infections.[158] In developing countries the prevalence of SSI ranged from 4-26%.[159-161] Large variation is observed across different studies, patient populations, and settings. Common pathogens associated with SSI are *S. aureus*, coagulase-negative staphylococci, and enterococci in the US hospitals.[158]

Mortality associated with SSI can be high. The relative risk of death among patients with SSIs was reported to be as high as 2.2 compared to patients without SSIs in a case-control study in the US in 1990s.[162] Studies of the impact of SSI on patient outcomes and healthcare costs suggested increased mortality, days of hospital stay, and costs are seen even in developed country settings.[163]

1.2.6 Skin/Soft tissue infection

Skin/soft tissue infections (SSTI) are infections involving with the skin, fascia or muscle.[164,165] The infection occurs when microorganisms invade the skin and cause damage at any anatomical site. SSTIs can be further classified into skin infection, soft tissue infection, decubitus ulcer infection, burn infection, and breast infection or mastitis based on the CDC/NHSN surveillance definitions. Each type has specific definition criteria, and all are based on clinical presentation and microbiology diagnosis.[166]

The most common causative organism of SSTIs is *S. aureus*.[167] The epidemiological and clinical determination of community-acquired or hospital-acquired SSTI is difficult, as the movement of patients and infections between hospitals and the community is dynamic.[168] A hospital outbreak of community-acquired MRSA after admission among colonized or infected patients has been reported.[169] At the molecular level, different strains of *S. aureus* are responsible for community-acquired and hospital-acquired SSTIs.[170,171] For instance, the prevalent lineage of community-acquired MRSA that cause SSTI are ST8, ST80, and ST30 in the USA, Europe and Australia, respectively. Management of bacterial SSTIs is based on antibiotic treatment guided by identification of the causative organism, control of infection source (sometimes requiring surgical intervention), and identifying the extent of infection (i.e., systemic or localized).

1.2.7 Infection at other body sites

Infections at other body sites include the digestive system, central neural system, upper respiratory tract, reproductive tract, among others. Each type of infection has its own diagnostic criteria that involves clinical symptoms and microbiology culture results. Some infections are more commonly acquired in hospitals, while others are more commonly acquired in the community. For instance, gastrointestinal infections caused by

Clostridium difficile are commonly seen in hospitalized patients in developed countries.[172] Pneumococcal meningitis caused by *Streptococcus pneumoniae* is a common reason of hospital admission among young children, older adults, and immunocompromised individuals, with high mortality and a high risk of neurological sequelae.[173,174]

1.3 Surveillance of antimicrobial resistant infections

Collecting, aggregating and interpreting microbiology results from individual patients is a collective act of performing surveillance, which is the foundation for combating the health crisis of increasing AMR infections.[12] Surveillance of AMR infections can be performed at the local hospital level, national level, regional level or global level, and may serve overlapping or different purposes.[175] For instance, at a local level, surveillance may tailor antibiotic use policies for the cohort of patients with specific characteristics and medical care-seeking behaviours. At a national level, surveillance data for AMR infections provides evidence for national action plans and helps to support resource allocation. At both local and national levels, surveillance data are important to understand the epidemiology of AMR patterns. There are different strategies for performing surveillance, including laboratory-based and case-based, and each is accompanied by various advantages, and limitations.[75,175] Furthermore, there are several different measurements to represent burdens of resistant infection from the surveillance data, and different estimation methods for those measurements are subject to various types of biases. Finally, reporting and interpreting surveillance data on resistant infections needs careful consideration concerning the context of the cohort under survey, and may have implications in supporting public health policy-makers in making decisions about population-level interventions.

1.3.1 Objectives of antimicrobial resistance surveillance

The main objectives of routine AMR surveillance include i) to provide local evidence for empiric treatment guidelines and clinical decision making; ii) to trace differences and changes in space and time; iii) to benchmark to assess the effect of interventions and iv) estimating disease burden for epidemiological purposes.[13,75] In 2017, Thailand published a national strategic plan on AMR, and surveillance data is essential to determine if the target of a 50% reduction in AMR morbidity is achieved.[176] Similarly, strengthening the national surveillance system was one of the goals in the national action plan on combating drug resistance in Vietnam in 2013.[177] Outbreak identification in healthcare facilities plays an important role in improving healthcare quality in hospitals, reducing workload of healthcare workers, and reducing morbidity and mortality. A successful outbreak identification system needs strong support from high quality surveillance data.[178-180] Empirical antibiotic treatment guidelines also rely on quality antimicrobial resistance data, and an in-depth understanding of the local AMR epidemiology.[181,182]

1.3.2 One Health approach to antimicrobial resistance surveillance

The One Health approach is increasingly recognized as an important approach to reduce the health threat due to AMR infections.[183,184] This approach is referred to as a collaborative effort involving different sectors to prevent the spread of AMR bacteria between humans, animals and the environment. The section below briefly summarises these three sectors that play roles in the One Health approach.

The human sector can refer to either infected patients or individuals colonized with AMR bacteria. Patients with suspected bacterial infection are one of the most common targets of interest. For instance, the ECDC surveillance system collects data on microbiology results of blood and cerebrospinal fluid samples from humans.[185] Often, the specificity of the sample in representing a specific infection is of great concern when using surveillance data from routine microbiology. For example, sputum samples without clinical data have rarely been used as a key sample type for surveillance due to the high contamination rate of sputum samples and the complex definition of pneumonia. On the other hand, blood and cerebrospinal fluid samples that are culture positive for pathogenic organisms are often used to estimate incidence of BSI and CNS bacterial infections, respectively, as isolation of a pathogen from either sample type is usually a specific indication of an infection. There is an extensive literature on bloodstream infections from high-income countries and also some from resource-limited settings that are equipped with microbiology laboratories. A systematic review in 2011 showed 14 studies from developing countries on healthcare associated bloodstream infections published between 1995 and 2008.[109] However, differentiating a true positive culture from contaminants may not always be straightforward, especially without clinical data. Blood samples can be contaminated with bacteria (e.g. *Staphylococcus epidermidis*) living on the skin when sampling is collected and protective equipment is not appropriately worn. Computerised laboratory-based algorithms have been proposed to identify potential contaminants but none is perfect.[186] Moreover, when surveillance sampling is performed predominantly in healthcare facilities, the representativeness of the data especially in regions where access to healthcare facilities varies between different groups of people (for example with different economic status, access to healthcare service, access to antibiotics, different practices of self-medication by the public, and physical distance from residential areas).

Individuals colonized with an AMR organism can also be the target of interest because the colonised individuals typically have an elevated risk of acquiring an AMR infection. Surveillance data on these high risk individuals is useful to define the baseline resistance burdens in this group and to identify modifiable risk factors that can be affected by interventions to reduce the risk of acquiring an AMR infection.[187] For instance, in a prospective surveillance study in Thailand swabs were collected from the nasal cavity, throat, inguinal area and rectum from patients within 48 hours of their hospitalization.[109] The study results showed a high colonization rate of ESBL-producing *Enterobacteriaceae*, and patients with cerebrovascular disease and previous hospitalization have high prevalence of carbapenem-resistant *A. baumannii*,[188] which translated to a high prevalence of infections related to *A. baumannii* among patients admitted to the hospital.[21,80] This provides evidence to support potential targets for interventions to prevent the spread of AMR within the hospital. Many studies in the current literature focus on decolonization of MRSA as an intervention to prevent spread of the disease and reduce morbidity, in recognition of the high prevalence of colonization in hospitals in many developed countries.[189] The US CDC recommends active surveillance testing for MRSA colonization and to consider decolonization for MRSA in neonatal ICU patients.[187,190-193]

Food-producing animals represent an important sector for the One Health approach. This sector plays a potentially major role in the spread of antimicrobial resistance, and represents an important target for surveillance. There are several surveillance programmes and studies reporting antimicrobial resistance in bacterial isolates from food-producing animals. A systematic review of antimicrobial resistance surveillance

programmes in livestock and meat in the European Union (EU) identified three types of surveillance systems: EU surveillance, industry-financed supranational surveillance systems, and national surveillance systems.[194] The sites from which samples were taken for surveillance varied from farms and slaughterhouses to retail and swab samples. Most of the surveillance systems included assessing antibiotic consumption among the animals. However, programmes and studies varied in sample types collected, sampling approaches and reporting methods, which lead to difficulties in making comparisons across time and surveillance sites. Moreover, there is a paucity of data on the potential links in the burden of antimicrobial resistance between humans and animals, and the impact of AMR in food-producing animals on human health remains unclear.

Finally, the environment also plays a role in the One Health approach of AMR surveillance. Environmental surveillance is an important approach to quantify the occurrence and abundance of resistance genes or resistant bacteria and to reduce environmental contamination with antimicrobials.[195] Examination of sewage treatment plants for polio surveillance has a long history of success in supplementing information to support the eradication of polio, especially in areas where persistent poliovirus circulation is suspected and clinical surveillance is absent or unreliable.[196,197] However, environmental surveillance of antimicrobial resistance is currently still in its infancy.[198] Only recently, emphasis on environmental surveillance to study the burden of AMR bacterial infection has been highlighted by the FAO of the UN.[199] The target samples included surface water, soil, animal and human waste streams, and food of plant origin. Sewage samples, in particular, have been a target of focus in environmental surveillance for AMR, as correlation between resistance data from sewage and from clinical surveillance has been suggested by some recent studies.[200,201] Common data collection

approaches include metagenomic sequencing that detects all resistance genes available in the database or quantitative polymerase chain reaction that targets selected resistance genes. One of the key advantages of environmental surveillance of AMR is that it adds complementary information about a wider population beyond the clinical setting. Importantly, environmental surveillance data can also provide evidence on overall human health impacts caused by exposure to environmental resistant bacteria and improve understanding of the contributions of different sources of antibiotics and AMR bacteria released into the environment. These are crucial for designing interventions to control the spread of AMR bacteria.

In the remaining sections of this thesis, I will, however, focus primarily on surveillance of bacterial infections in humans as environmental and agricultural surveillance is beyond the scope of my study objectives.

1.3.3 Types of antimicrobial resistance surveillance

There are several different types and definitions of AMR surveillance in humans. In 2002, a document from the WHO titled "Surveillance standards for AMR" suggested two general types of surveillance: comprehensive surveillance and sentinel surveillance.[202] Comprehensive surveillance considers a specific disease (or pathogen) in the entire population at risk.[202] This involves capturing data on all (or nearly all) cases of infection. In comparison, sentinel surveillance involves collecting data from a limited catchment area population that can be considered representative for the rest of the population.[202] The choice of performing comprehensive or sentinel surveillance depends on the practicality, type of infection, and surveillance capacity of a country or a setting. In general, sentinel surveillance generates limited data but reliability can be guaranteed if proper standardised

sample definitions are used and when such definitions are consistent between the sentinel sites. Another aspect of surveillance is the period of data collection. A surveillance system can be defined as continuous or episodic (undertaken over limited time period) based on the availability of resources and type of infection.[202] Episodic surveillance is suitable for predictably seasonal infections. Moreover, surveillance can be defined as either passive or active irrespective of whether it is continuous or episodic. A passive surveillance system relies on readily available data.[203,204] In other words, a passive surveillance system makes no attempt to seek voluntary reports or data (i.e., on infections) from the primary data collector in a surveillance system, and utilizes reports already readily available.[202] An active surveillance involves a planned collection of a targeted and representative study sample.[203-205] In other words, the primary data collector submits reports (on infections) to the surveillance system on a regular basis.[202] Both passive and active surveillance can be routine, which involves regular systematic collection of data, or enhanced, which involves collection of additional data about the reported cases under routine surveillance.[202]

1.3.4 Collection of antimicrobial resistance data in local sentinel sites

At sentinel sites, there are different ways to collect AMR data. The two commonly used strategies in identifying human cases of AMR infections are laboratory-based and case-based surveillance.[100,175] These two approaches used different methods and data sources to detect antimicrobial resistant infections. Laboratory-based surveillance relies heavily on routinely, passively reported laboratory data generated from samples collected for clinical diagnostic purposes. Case-based surveillance uses an approach of prospective data collection for a defined population or patient group for infections by particular clinical presentations or clinical syndromes. Neither of the methods is perfect, and each

has its advantages and disadvantages. The section below will dissect the two approaches in detail.

Laboratory-based surveillance is perhaps the most straightforward approach to collect data on AMR, as it can rely solely on routinely collected microbiology data. An alternative term that was used in the WHO GLASS manual is 'case-finding based on clinical specimens sent to laboratories for clinical purposes'. Here, for consistency, I will use the term laboratory-based surveillance. In general, there are two types of laboratory-based surveillance; pathogen-based and sample-based surveillance.[175] Pathogen-based surveillance focuses on samples collected from target patients testing positive for pathogens. The numerator is a count of those samples with a certain resistant pathogen (i.e., number of samples testing positive for *E. coli* that are resistant to third-generation cephalosporins), and the denominator is the total number of those samples testing positive for the pathogen (i.e., number of samples testing positive for *E. coli*). Sample-based surveillance focuses on samples collected from patients testing positive or negative for pathogens. The denominator is counts of samples collected for microbiology culture. Due to its simplicity in implementation, the laboratory-based surveillance approach is often used in large scale surveillance, such as surveillance at national, regional, or global levels. WHO GLASS and EARSS use this approach and collect microbiology data from surveillance laboratories to generate aggregated data reports on AMR stratified by the sites. Several national networks such as JANIS and the NARST are also using this approach to monitor national prevalence of AMR infections. The biggest advantage of this approach is that it requires minimal time, human resources, and efforts to perform. This increases the inclusiveness of the surveillance sites, especially for resource-limited

settings. As this approach is based on routinely collected microbiology data, the cost required to sustain the surveillance system in a resource-limited setting is relatively low.

There are, however, several caveats concerning laboratory-based surveillance. First, this approach has a high risk of sampling bias. Sampling patients for diagnostic purposes is influenced by local clinical practices, social factors, cost, and availability of resources, which can affect the estimates on prevalence and incidence of AMR infection. This would increase the difficulties in comparing statistics generated from a laboratory-based surveillance system across space and time.[206] Also, the representativeness of the sample for the population with infections could be low, which may lead to biased estimates of the true overall infection rates. For instance, in cases when clinical samples are only collected for bacterial culture from patients who failed antibiotic treatments, the estimated proportion of AMR infections could be overestimated while incidence rates of AMR infections could be underestimated. To understand the potential influences of blood culture utilisation rate on estimates of the proportions and incidence rates of AMR infection a literature search was done. I initially searched PubMed for articles using the following broad MeSH search terms: "microbial sensitivity tests" and "bias". In addition, I searched using the following additional terms: "recommendations", "guideline" or "reviews" together with the MeSH search term "microbial sensitivity tests". The references from the retrieved articles and recommendations on presentation of cAST data or AMR surveillance reports from international organizations such as the CLSI, EUCAST and the WHO were also reviewed. No articles on evaluating or describing the potential magnitude of impact or bias caused by low blood culture utilisation rate on proportions and incidence rates of AMR infections were found. Several studies have shown that failure to exclude duplicated specimens from the same patients could lead to

overestimation of the proportion of infections caused by AMR isolates.[31] A few studies have shown that BSI rates are underestimated if blood culture utilisation rate is low,[207,208] but its impact on estimates of proportions and incidence rates of AMR infections has not been evaluated.[208] The CLSI guidelines stated, "Susceptibility rates may be biased by more frequent sampling of patients with: a) treatment failure following prior antimicrobial therapy; and/or b) prolonged medical histories or hospitalizations.".[74] However, no references are provided.

Case-based surveillance begins with the detection of patients suspected of having an infection. The unit of surveillance is a patient with a specific condition. Example of this approach include point-prevalence survey studies performed by the ECDC on hospital-acquired infections.[139] The advantage of this approach is that the representativeness of the sample for the population with infection is high. In a well-resourced setting, data on treatment history, comorbidity of patients, and patient characteristics may be additionally collected. This could further enhance surveillance data and provide evidence to generate tailored guidelines to inform clinical decisions on antibiotic treatment. Different denominators can be derived from case-based surveillance data. For community-acquired infections, population coverage of a surveillance site has been a commonly used denominator. An example measure of population coverage is the number of tested patients over the total population.[100] Another example is the population size in the catchment area of sentinel sites, but it is often difficult to capture this measurement accurately in LMIC settings. For hospital-acquired infections, a commonly used denominator is the number of hospitalized patients who are at risk of infection. For instance, the burden of central line-associated bloodstream infection can be calculated from case-based surveillance data. The numerator is the number of hospitalized patients

with a central-line catheter for more than 48 hours and laboratory-confirmed bloodstream infection. The denominator is the total number of hospitalized patients with a central-line catheter for more than 48 hours.

However, one caveat is that the case-based surveillance can be labour-intensive and time consuming, especially for resource-limited settings. This is mainly because the ratio of patients with no infection to patients with either susceptible or resistant bacterial infections is usually large. For instance, in a general hospital, the majority of the hospitalized patients will not develop a bacterial infection during their hospital stay, and a labour-intensive screening period is usually required to identify one case of hospital-acquired bacterial infection. This is particularly the case in large hospitals in resource-limited settings where human resources needed to perform the screening may be lacking. Hence, the implementation of such an approach is low in LMICs.

1.3.5 Study designs for collecting antimicrobial resistant infection data

There are several different designs of surveillance studies including point prevalence survey (PPS) studies, observational cohort studies, and matched case-control studies. Each study design serves different objectives. Often, PPS studies for AMR use a case-based surveillance approach and cross sectionally identify patients who meet the study eligibility criteria from a population at a specific time period.[139] The main measurement derived is prevalence of AMR infections, which can be used to calculated incidence using the Rhame and Sudderth formula.[209] The majority of the current literature on PPS studies comes from the United States [124] and European countries [210-213] and study the morbidity of AMR infections and antibiotic usage.

A cohort study involves collection of data on a specific sample type from a defined population over a specific period. The purpose of this study design often involves

measuring incidence and, sometimes, performing analysis to test or generate a hypothesis. For instance, WHO-GLASS collects retrospective data on specific sample types from surveillance sites to estimate the global incidence of AMR. In 2020, WHO-GLASS published a protocol for measuring attributable mortality related to AMR among BSIs globally.[214] An important consideration when designing a cohort study to estimate detrimental outcomes of AMR is the definition of a comparator cohort.[214] A general recommendation is to define the comparator cohort based on the research goals and assumptions in the data analysis. For instance, in a setting where a new antibacterial (i.e. ceftarolin fosamil) targeting a specific pathogen, such as MRSA, was introduced, a relevant comparator cohort to measure burden could be patients with MSSA infections. On the other hand, when the goal is to prevent infection through general infectious disease control such as hand-hygiene and a cohort study is to identify risks of antimicrobial resistant infections among patients admitted to the hospital, an appropriate comparator cohort could be patients without an infection.

A matched case-control study is often used when the outcome is rare and the aim is to explore multiple exposures. An example is a study conducted in Brazil to identify predictors of mortality in cancer patients infected with Gram-negative bacteria, in which cases were patients who died after positive culture and controls were those who survived within the same time frame.[215]

1.3.5 Sampling methods in antimicrobial resistance surveillance

There are several methods to sample patients. A method of selecting patients with AMR infection is to screen all cases in the target population and to include all those who met pre-defined eligibility criteria. Examples of such surveillances include studies on

meningitis in South Africa,[216] on group B streptococcus in United States,[217] invasive pneumococcal disease in New Zealand,[218] and invasive pneumococcal disease in Brazil.[219] This approach could reduce selection bias, however may not be achievable in resources-limited settings and sampling approaches may need to be performed. When performed appropriately (i.e. with no systematic bias), a randomly selected sample could also generate unbiased estimates. For example, some studies sample for a certain number of consecutive isolates (in a laboratory-based surveillance) or suspected infections (in a case-based surveillance).[70] Alternatively, sampling patients using a Lot Quality Assurance Sampling (LQAS) survey has been done to study patients with UTIs in Indonesia.[206] The concept of LQAS is to determine whether the prevalence of resistance is "high" or "low" in a population through randomly selected samples from different "lots" and using a priori defined criteria.[220,221] The lots are similar to clusters sharing the same characteristics, for example, in the case to evaluate isolates ("unit of sample") of an organism for, say five antibiotics, then the number of lots would be five different lots. From each lot, a sample of pre-defined sample size is then drawn and pre-defined criteria would then be applied to the sample to determine whether the prevalence of resistance for each antibiotic is "high" or "low". This information is then used to reflect the population. The screening and patient selection procedure is identical to a conventional population-based survey. LQAS has now been used to guide designs of empirical antibiotic treatment policy in a setting.[220,221] This method has been shown to be more affordable than population-based surveys.[220]

1.3.6 Potential biases in measuring prevalence and incidence rate

There are several challenges in estimating the prevalence and incidence rate of AMR infections. Firstly, the measurements can be influenced by the availability of data and selection of samples for culture.[31,222] as discussed in the previous sections.

Second, quality of data varies across different settings and may influence prevalence and incidence estimates. In resource-limited settings, there is often a lack of trained personnel to monitor clinical specimen collection procedures, to perform laboratory quality checks, and to verify collected data. Quality of microbiology data varies among different settings.[222] Poor data quality may lead to either underestimates or overestimates of the prevalence and incidence rate. The quantity and direction of biases in estimating prevalence and incidence rates of AMR infections under the presence of poor data quality is understudied.

Third, approaches to the processing and analysis of data may affect the measurement estimates. Studies have demonstrated that failure to exclude duplicated specimens from the same patients can lead to overestimates of the prevalence in AMR infections.[31,223] A study in 2016 demonstrated that in a scenario where duplicated data are not removed, the proportions of antibiotic-resistant *S. aureus* and *P. aeruginosa* may rise by 5% and 10%, respectively.[223] Choosing the appropriate methods to estimate prevalence and incidence rate is often a challenge, especially in settings where there is a lack of data. The 2015 antimicrobial resistance report compiled by Jim O'Neill has been criticized for its estimation of the global number of infections using incidence based on European Antimicrobial Resistance Surveillance network data, which may not represent LMIC settings.[224] Moreover, the extrapolation in incidence from bacteraemia to infections at other body sites often needs parameter values. These parameter values, or so-called

correction or conversion factors for estimating overall morbidity of antibiotic resistant infections, were established based on the hospital data from the US and Spain,[225,226] which differed from LMIC settings in healthcare quality, epidemiology of antibiotic-resistant pathogens, and patient characteristics.[13,76,227] No study has yet been conducted in a hospital in a LMIC setting to establish conversion factors for incidence of antibiotic-resistant infections that can represent resource-limited settings.

Finally, the choice of reporting proportion or frequency for prevalence and incidence rate depends on the usage of the estimate. For clinical settings, in which a treating physician is interested in information that can guide empirical therapy and prevent delays in effective treatment, the proportion or prevalence of resistant isolates would be useful and is easy to understand. For policy makers, the proportion of resistant isolates could, sometimes, be misleading for decisions on effective interventions. Consider a scenario, where a prophylactic antibiotic is introduced in a particular year, infections caused by susceptible isolates would be reduced but those caused by resistant isolates may remain unchanged. However, the proportion of resistant infections would be increased as the denominator (total infected cases) is reduced. Incidence rates of resistant infections (for example, per 100,000 tested patients), as a measurement of morbidity, might be less vulnerable to biases in this type of scenario compared to the proportion. The choice of the optimal denominator remains a challenge, especially in settings where data are scarce.

1.4 Health impacts of antimicrobial resistance

Statistics that present the impact of AMR infection are important for policy-making and resource allocation, and thus efforts have been put into quantifying the key measures. Mortality, number of days hospitalised, and economic losses are some of the indicators

for measuring health impact of AMR infections. The focus of this section is on mortality. There are different ways to define mortality associated with AMR infections.[121,228] A clinical definition of death due to AMR infection may require autopsy, and careful clinical assessments that are subject to inter-observer variability, hence are time-consuming to obtain and impractical for routine surveillance.[229,230] Vital or civil registration, including International Classification of Diseases (ICD) code data and death registration, has been used as a source of data to estimate mortality related to an infection.[231] However, the process of ICD certification can be subjective, and may not capture cause of death accurately.[232] Moreover, the reliability of ICD10 coding in identifying comorbidities and infections is often questionable.[233] Hence, epidemiological definitions of mortality have been used as an alternative measurement for mortality associated with antibiotic-resistant infection.

1.4.1 All-cause mortality associated with antimicrobial resistance

All-cause mortality is a simple way of measuring deaths that can be used to represent the proportion of deaths among patients with AMR infections. The numerator of all-cause mortality is the total number of deaths in a defined cohort (i.e., a cohort of patients with AMR infections), which may consist of a mixture of patients who would not have died if they had not acquired AMR infection and patients who would inevitably have died even if the patient did not acquire AMR infection. The denominator is the total number of patients in that cohort of patients with AMR infections. The UK office for National Statistics has referred to the measurement as ‘deaths involving AMR infection’ to highlight the possible inclusion of deaths caused or contributed by other causes.[234,235] The advantage of reporting all-cause mortality associated with AMR is that it is easy to calculate. Examples of all-cause mortality measures that are reported are 30-day, 90-day,

or in-hospital mortality. As the measurements do not involve attributing death to a particular cause, all-cause mortality is therefore an objective measurement of the health burden of AMR. The limitations are that the number is vulnerable to the context of the study cohort, and lacks the ability to be compared between different settings and across time.

1.4.2 Mortality attributable to antimicrobial resistance

Mortality attributable to AMR can be briefly defined as the proportion of patients who would not have died if they had not acquired AMR infections. The measurement is important for policy-making as the statistics directly reflect the impact AMR has on clinical outcomes, and thus can assist in the design of preventive interventions and assessing the impact of the interventions. The caveat is that to estimate this measure, detailed patient data, several assumptions, and statistical modelling are often needed. Furthermore, this statistic is not straightforward to conceptualise.

In addition to using proportions to describe mortality attributable to AMR infections, frequency of attributable deaths has been used and is an informative estimate in global, regional and national reports. For instance, the 2015 antimicrobial-resistance Review compiled by Jim O'Neill reported 700,000 deaths due to antimicrobial-resistant infections per year globally.[13] A study by Cassini et al. reported 33,119 (28,480-38,430) deaths due to AMR infections per year in the European countries using a population-level modelling analysis.[20] A retrospective study in 2016, using routinely collected hospital data, reported 19,000 deaths due to hospital-acquired AMR infections per year in Thailand.[21] Here, the frequency of deaths represents the number of deaths attributable to resistant infections.

When estimating mortality attributable to AMR infections, several factors need careful consideration. The choice of comparator cohort is one of the important factors to consider.[236] The definition of the comparator cohort (collectively referred to either as the non-exposure group in a cohort study or the control group in a case-control study) is related to the assumptions made and infection control intervention that the study has in mind. For instance, when using susceptible isolates of the same bacteria as the comparator cohort to study the impact of AMR infection, an implicit assumption is that resistant and susceptible isolates may compete with each other for infecting patients and there is a control strategy that may reduce the number of infection with resistant bacteria while the number of infections with a sensitive bacteria may increase by the same amount.[236] An example of such strategy could be reductions in antimicrobial use that may lead to reduction in resistant infections assuming there is a fitness cost of resistant transmissions, and a corresponding increase in infections with competing sensitive bacteria.[237] On the other hand, when using patients with no bacterial infection as the comparator cohort to study the impact of AMR infection, the assumption is that the burden of infections caused by the resistant isolates adds to the burden of infections caused by the susceptible isolates of the same bacteria.[236] A control strategy could be a hand-hygiene compliance programme, which aims to reduce all bacterial infections (caused by both resistant and susceptible isolates).

The daily probability of being discharged or dying varies by the length of hospitalisation, even without an event of bacterial infection. At the same time, the cumulative probability of acquiring infection caused by AMR bacteria will increase with hospitalization time. This is because the longer time spent (time to exposure) in a hospital the higher chance of exposure to resistant strains of bacteria through invasive medical procedures, antibiotic

uses, contaminated surfaces, or other means. Hence, hospital-acquired AMR infection is often referred to as a time-dependent exposure, which would lead to time-dependent bias when not accounted for during study design or analysis. The period of time between admission and acquiring an infection can be referred to as exposure time. This is the time during which a patient is a risk of becoming infected. An approach to adjust for exposure time is to select a comparator cohort that has a matched exposure time with the cohort of AMR infection. This can be done randomly or using exposure density sampling.[238,239] In the former sampling method, participants of the comparator cohort are required to have stayed in the hospital at least as long as the cases have stayed at the time they acquired AMR infection. A caveat of this method is that often researchers using this approach restrict the comparator cohort to those who never acquired an infection, but this will exaggerate the risk differences of outcome (i.e., mortality) between the cases and controls.[238] An alternative method that avoids this source of bias is to use exposure density sampling to select comparator cohorts. In this method, the participants are those who survived at least as long as the cases before acquiring an infection and participants of the comparator cohort may change their status (i.e., acquire an infection) afterwards.[238]

Finally, estimating the mortality attributable to AMR can be complex. At the global level, the lack of denominator data or essential parameter values to estimate the overall burden of AMR may lead to the generation of under-representative estimates. For instance, conversion factors are essential parameters for estimating the total attributable mortality in cases when only BSI data are available.[13,21,76,227] They were used in Jim O'Neill's report[13] on antimicrobial resistance in 2015 to estimate the global burden of bacterial resistant infections, and have been used in the attributable mortality of antibiotic resistant infections study by Cassini A., et al.[20] The conversion factors were developed using data

from hospitals in developed countries,[240] and have never been established for LMICs where resources, quality of patient healthcare, and epidemiology of antimicrobial resistance differ from those in HICs.

1.4.3 Other measurements for burdens of antimicrobial resistance

Quantification of costs related to resistant infection is of interest for hospitals and governments, as interventions such as funding research in antibiotics, and incentivizing prudent antibiotic use at the hospital, national, and international levels need financial support. Several reviews in the literature have evaluated the costs related to antibiotic resistant infections.[241,242] At the global level, Jim O'Neill projected that by 2050 a total loss of $100 trillion GDP related to DRI would be expected, if no effective action is taken. The World Bank estimated 0.6-6.8 trillion dollars of costs per year would be related to resistant infections in 2030.[243] Alternatively, disease burdens expressed as Disability Adjusted Life Years (DALYs) have been used to quantify the premature mortality and disability of LRTI.[244] The measurement is the sum the years of life lost and years lived with disabilities due to the disease, and represents the health gaps between a current situation and an ideal situation where all members of a population live up to the age of the standard life expectancy in perfect health.

1.4.4 Networks and organisations supporting antimicrobial resistance surveillance

In recognizing the importance of surveillance data, networks within nations and between nations have been established. In general, the purposes of these networks are to harmonize the data collection approach and to enable data sharing between different participating organizations. The accumulated data can then be used as an evidence base for designing and assessing preventive interventions. Several surveillance networks

focusing on AMR data have been established globally. These networks vary in the number of participating countries, pathogen of focus, specimen type of focus, operational team, and other characteristics. Ashley EA, et al. compiled a report on surveillance networks that were active at a point of time between 2000 and 2016.[245] Overall, 105 supranational networks related to surveillance in bacterial infection, malaria, HIV or tuberculosis were identified. Of the 105 networks, 46 were related to AMR in bacteria. Here, I describe some of the most active and influential networks in producing AMR reports or publications on a regular basis as examples.

World Health Organization Global Antimicrobial Resistance and Use Surveillance System (GLASS)

In October 2015, the WHO launched the GLASS to support the global action plan on AMR. The aim is to strengthen the evidence base on AMR by standardizing the collection, analysis and sharing of AMR data at the global level. The objectives of GLASS are to 1) foster national surveillance systems; 2) estimate the global burden of AMR; 3) analyse and report global data on AMR on a regular basis; 4) monitor emergence and global spread of AMR; 5) inform preventive programmes to control AMR; and 6) assess the impact of the interventions. For instance, to harmonise the data analysis approach, WHO GLASS recommends that (a) duplicated results on bacterial isolates should be removed, and (b) data should be stratified by origin of infection (community-origin or hospital-origin) whenever possible. The minimal de-duplication process is to include only the first isolate of an organism per patient per specimen type per survey period. The WHO GLASS initially focused on AMR surveillance data from human clinical samples and priority organisms, including *E. coli*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *S. pneumoniae*, *Salmonella* spp., *Shigella* spp., and *Neisseria gonorrhoeae*. The priority

samples included blood, urine, faeces, and urethral and cervical swabs (Table 1.1). In 2017, 22 countries provided AMR data that were included in the GLASS report, and in 2018 that number increased to 49 countries. Recently, the WHO published a GLASS 2020 AMR report with an improved format, and the number of countries providing AMR data continued to rise and now stands at 66 countries, territories or areas. Of the 66 countries, territories or areas reporting AMR data to the WHO GLASS, 30 were high-income countries, territories or areas, and 36 were low and middle-income countries, territories or areas. A study in a tertiary hospital in Thailand (a middle-income country) shared their experience on implementing GLASS for data on patients with bacteraemia.[246] They concluded that case-based surveillance combined with patient clinical data was more beneficial and applicable for developing local antibiotic treatment guidelines; however, it consumed more time and resources compared to the conventional laboratory-based surveillance system. Progressively, the WHO GLASS is incorporating other information related to AMR in humans, such as use of antibiotics, foodborne AMR, and infections associated with health care. In 2019 GLASS-antimicrobial consumption (AMU) was launched, which aims to collect information on use of registered antimicrobial medicines, the quantities of medicines used per survey year, and other related contextual information. Recently, WHO GLASS published a protocol for patient-level data collection to assist global estimation of mortality attributable to AMR.[214] WHO GLASS is a laboratory-based surveillance system, in which the microbiology data is the main focus. The advantage is that it is easy to implement as data are routinely collected in the surveillance sites for diagnostic purposes.

European Antimicrobial Resistance Surveillance Network

The European Centre for Disease Prevention and Control (ECDC) established the European Antimicrobial Resistance Surveillance System (EARSS) in 1998, and later renamed the European Antimicrobial Resistance Surveillance Network (EARS-Net). The EARS-Net presents AMR data on invasive isolates from European Union (EU) and European Economic Area (EEA) countries annually, and data of 30 EU/EEA countries were included in the 2019 report (which contains data from 2018). The key organisms included in the report are *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter spp.*, *S. pneumoniae*, *S. aureus*, and *E. faecium*. The clinical specimens of focus are blood and cerebrospinal fluid. EARS-Net is based on a network of centres from the EU/EEA countries that collect routine AST data from national AMR surveillance initiatives. At the same time, EARS-Net also serves as a data hub in which ECDC retrieves data to submit to the WHO GLASS.[247] Many of the participating laboratories are engaged in the annual EARS-Net external quality assessment to ensure data quality and comparability of AMR data. In the annual report, EARS-Net includes data on self-assessed national coverage (i.e., estimated percent of national population coverage) by country and the blood culture sets/1000 patient days, as a measurement of blood culture utilisation[185]. In 2009, for the first time, a report, using the surveillance from EU/EEA countries, on AMR infections in EU countries was published. The report included a detailed map of prevalence statistics, and an estimation of 25,000 deaths due to AMR infections in EU countries. This number was cited by the WHO and had been used as evidence base to push AMR infection up in the policy agenda. The estimate of deaths due to AMR infections has been recently revised using updated data and a more systematic analytical approach. It is estimated that 35,000 deaths are due to AMR infections in EU/EEA countries yearly. EARS-Net utilises a laboratory-based surveillance approach for the bloodstream and cerebrospinal fluid samples data collection. At the same time, ECDC

also performs case-based PPS studies to estimate the burdens of hospital-acquired infections. The limitation is that the approach can be costly and requires a large amount of man-power and time. Hence, point-prevalence surveys sponsored by ECDC are performed only once in a year, and hospital participation rate is lower than the laboratory-based surveillance approach of EARS-Net.

Central Asia and Eastern European Surveillance of Antimicrobial resistance Network

The ECDC coordinates the Central Asian and European Surveillance of Antimicrobial Resistance Network (CAESAR), which consists of countries in the WHO European Region that were not part of the EARS-Net in 2019. The first annual AMR report from CAESAR was published in 2015. This first report mainly focused on the objective, methodology, process of routine data collection, protocols and AMR case definitions. The second CAESAR report published in 2016 focused on the AMR data collected. In the 2019 report, data from 11 countries were included. The seven organisms in EARS-Net were included, and with the addition of *E. faecalis* and *Salmonella* spp.. Similar to EARS-Net, the CAESAR network focuses on blood and cerebrospinal fluid.

Asian Network for Surveillance of Resistant Pathogens

The Asian Network for Surveillance of Resistant Pathogens (ANSORP) was established in 1996, and is an academically orientated network. The network consisted of 13 countries in the Asia region and one country in the Middle-East in 2019. Since 1996, research projects focusing on different themes have been conducted within the network. For instance, in 2000, a project focusing on *S. pneumoniae* was conducted in 11 countries within the network. The study described the AST pattern and molecular epidemiology of *S. pneumoniae* identified from patients with pneumococcal diseases.[248] A prospective

surveillance study on community-acquired pneumonia was conducted between 2002 and 2004 in eight Asian countries within the ANSORP network, and was published in 2008.[131]

Japan Nosocomial Infections Surveillance

The Ministry of Health, Labour and Welfare of Japan established the Japan Nosocomial Infections Surveillance (JANIS) in 2000, in response to the emergence and spread of multidrug-resistant *P. aeruginosa* and MRSA.[249] The aim of JANIS is to understand the prevalence and incidence of nosocomial infections and AMR bacteria in hospitals in Japan. It was reported that 1461 hospitals in Japan submitted data to JANIS in 2015. The data flow of JANIS begins with microbiology laboratories in surveillance sites, with LIS facilities that can convert microbiology results (of specimens with both culture negative and positive) into a standardized JANIS data format. After the data format is standardized, data are sent and stored in the JANIS data server. The JANIS Clinical Laboratory division collectively cleans (including de-duplicates), analyses and generates annual reports for all data submitted to the network. The annual reports on national AMR statistics are open-access and can be found on the JANIS website (https://janis.mhlw.go.jp/english/report/index.html, accessed on 20th June 2020).

Other organisations and efforts supporting antimicrobial resistance surveillance

In 2014, Jim O'Neill, who is a British economist, was asked to analyse the global problem of drug resistance and to propose actions to control it at a global level. The Review on AMR, chaired by Jim O'Neill, was established and funded by the UK Government and the Wellcome Trust to compile reports and propose solutions for resistant infections. The

key messages from the work included an estimate of 700,000 deaths due to resistant infections (including bacterial, malaria, and HIV) globally, a projection of 10 million deaths by 2050 if nothing effective is done to control the spread, a conclusion that the economic burden related to AMR infection is high, and an emphasis on the need for global solutions.[13] Nine classes of interventions were highlighted for addressing this global health crisis, which are 1) global public awareness campaigns; 2) improving sanitation and preventing the spread of the infection; 3) reducing unnecessary use of antimicrobials in agriculture and their dissemination into the environment; 4) improving global surveillance of drug resistance; 5) promoting new, rapid diagnostics to reduce unnecessary use of antimicrobials; 6) promoting development and use of vaccines and alternatives; 7) improving the number, pay and recognition of people working in infectious disease; 8) a global innovation fund for early stage and non-commercial research & development; and 9) improving incentives to promote investment for new drugs and improving existing ones. The list overlapped with many global and national action plans. For example, the WHO antibiotic awareness week, which happens annually in Thailand.

An integrated and holistic multisectoral One Health approach has been suggested as an approach to tackling the health crisis of AMR infections. Many of the therapeutic antibiotics are shared between humans and animals. Transmission of AMR infections happen not only between humans, but also between animals and humans, the environment and humans, and animals and the environment. Antibiotic use in animals, and quality of antibiotics, are all playing a role in the increasing prevalence of resistance. The WHO, the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (OIE) Tripartite Collaboration is an example of One

Health approach. Collectively, the aim to reduced incidence of AMR infections. The mobilized colistin resistance (*mcr-1*) gene responsible for plasmid-mediated resistance to colistin was first detected in *Enterobacteriaceae* from farm pigs and humans in 2015,[18] and is now increasingly reported globally.[250-255] In 2017, the Chinese government banned the use of colistin in animal feeds as a growth promoter, and subsequent reductions in prevalence of colistin resistance in both humans and animals have been observed.[256]

Consortia with panels of experts from various fields are playing important roles in motivating and leading the advances in action plans for controlling the spread of AMR. For instance, the Surveillance and Epidemiology of Drug Resistant Infections Consortium (SEDRIC) was established in recognition of the crucial role of surveillance and epidemiology in the control of AMR. The aims of SEDRIC are to advance and where needed, transform the ways to track, share, and analyse information on antimicrobial use, AMR rate and burden of disease. An additional aim is to support the understanding of contamination in the environment.[257] Several papers on recommendations for AMR data sources, methods to estimate burden of AMR, and priorities of surveillance priorities have been published by the SEDRIC committee.[121,258,259]

1.5 Factors impacting health burdens of antimicrobial resistant infections

The next relevant question to ask is through what mechanism AMR infection results in high mortality. A previous study in Thailand showed high attributable mortality in hospital-acquired MDR *Acinetobacter* spp. bloodstream infection suggesting the organism would be clinically important in the region. However, the mechanisms through which MDR *Acinetobacter* spp. causes high mortality remained to be elucidated. The commonly discussed mechanisms include high virulence of antibiotic-resistant isolates

of *Acinetobatcer* spp.,[260-263] and inappropriate empirical antibiotic treatment as a result of prevalent resistant infections. Both of these two important pathways may lead to low survival in patients with antibiotic-resistant *Acinetobacter* spp. infection. In this section I will focus on the role of empirical antibiotic treatment.

High prevalence of antibiotic-resistance may result in an increasing chance of selecting an inappropriate empirical antibiotic treatment, which in turn may result in reduced chance of treatment success. Therapeutic options for treating MDR *Acinetobacter* spp. infections are limited. Carbapenem, colistin, and tigecycline are currently the last resort antibiotics for drug-resistant *Acinetobacter* spp. bloodstream infection, and increasing resistance to these antibiotics has been reported in developing countries.[108,264] The spread of resistant pathogens can be fuelled by the overuse of broad-spectrum antibiotics in hospital settings,[265] and *Acinetobacter* spp. has an ability to assemble different mechanisms of resistance.[93] In general, it takes at least three days for microbiological testing (including bacterial isolation, identification and AST) results to be available.[266] While waiting for the results, physicians prescribe empirical antibiotic treatment to patients. The choice of antibiotic depends on the physician's experience, the local hospital antibiotic policy, and knowledge of the epidemiology of bacterial infections in the local setting. However, without the microbiological testing results there is always a chance of prescribing an antibiotic that would not be expected to work against the causative pathogen. It may not be wise to always use a last resort antibiotic immediately even though the chance of coverage is high, as overuse of the antibiotic would encourage the emergence of resistant strains of bacteria. In 2019, WHO published a guideline for evaluating and monitor usage of antibiotic classes.[267] The reserve group antibiotics, including carbapenems, polymyxins, and some tetracyclines, are to be reserved for suspected or confirmed infections caused by MDR bacteria. The choice of antibiotic

treatment needs to balance likely coverage and impact on patient outcomes with risk of population-level selection for resistance while also taking into account safety and cost, a major concern in LMIC settings. Quantifying the causal effect of delaying concordant empirical antibiotic treatment (i.e., antibiotics that the bacteria causing the infection are susceptible to) is, therefore, important to guide the design of antibiotic stewardship programmes.

1.5.1 Using a counterfactual framework to study the impact of delaying appropriate antibiotic treatments

Ideally, to know the causal effect of an exposure on an outcome for an individual, one needs to observe the outcome when the exposure is present as well as when the exposure is absent within the same individual.[268] Statistically, the causal effect of the exposure on the individual's outcome can be expressed as below:

$$Y^{a=1} \neq Y^{a=0}$$

where a is the value for the dichotomous exposure variable A (1: delays in appropriate antibiotic treatment, 0: no delays in appropriate antibiotic treatment), and Y is the outcome variable (1: death, 0: survival). $Y^{a=1}$ is outcome under treatment a=1, and $Y^{a=0}$ is outcome under treatment a=0. The terms $Y^{a=1}$ and $Y^{a=0}$ are refered to as potential outcomes or as counterfactual outcomes.[268]

In reality, it is not possible to observe both potential outcomes within the same individual, as being exposed and not being exposed to a condition are usually two mutually exclusive events (i.e., cannot both happen at the same time) for the individual. For instance, an individual cannot receive both appropriate and inappropriate antibiotic at the same time. Hence, we take an average causal effect in a population of individuals as the target to

estimate. The average of individual causal effects $Y^{a=1} - Y^{a=0}$ can be expressed as below:

$$E[Y^{a=1}] - E[Y^{a=0}]$$

This method of evaluating causal relationship is referred to as the counterfactual framework or the potential outcome approach.[268]

Randomised controlled trial: a 'gold standard' design to study causality

A randomized controlled trial when performed ideally in all aspects (i.e., adequate sample size, no loss to follow-up, full adherence to assigned treatment, a single version of treatment etc) is, sometimes, referred to as the gold standard design to estimate the average of individual causal effects. A key reason is that by randomly assigning patients into different treatment strategies, both the observed and unobserved confounders that influence both treatment and outcome can be equally distributed across different treatment groups. An alternative way of saying this is *exchangeability* can be achieved. This means that the risk of developing the outcome in the treatment group would have been the same as the risk of developing the outcome in the control group had individuals in the treatment group received the treatment given to the control group.[268] In other words, the probability of being assigned to the treatment group is equal to the probability of being assigned to the control group for any particular individual. Despite all the benefits of conducting a randomized controlled trial to answer a causal question, such an approach could not be applied to the question we are asking here as it would be unethical to intentionally delay the provision of appropriate antibiotic treatment to a patient.

Emulating a target trial using observational data

An alternative approach to answer a causal question when randomized trials are not feasible is to emulate a target trial using observational data.[268,269] When the emulation is successful, the analysis of the observation data can yield the same effect estimates (except for random variability) as the target trial would have yielded had the latter been conducted. The approach requires an in-depth understanding of the data and knowledge of the context of the causal question. Hernan and Robins established a framework to guide the emulation of target trials using observational data.[269] There are seven key components to be considered: 1) eligibility criteria of patients in this hypothetical target trial; 2) treatment strategies to emulate; 3) procedure of assigning the treatment strategy; 4) follow-up period; 5) outcome to be measured in this hypothetical target trial; 6) causal contrasts of interest (i.e., whether intention-to-treat effect or per-protocol effect or both are to be estimated); and 7) the analysis plan for the target trial.[269]

There are three essential assumptions that an observational study needs to meet to generate an unbiased estimate. The first assumption is the exchangeability of treated (A=1) and untreated (A=0) in a target trial. When exchangeability is achieved, the treated, had they remained untreated, would have experienced the same average outcome as the untreated did, and vice versa.[268] In observational data, often only conditional exchangeability can be achieved. This means that after appropriate adjustment for all known confounders, the treated (A=1) and untreated (A=0) are exchangeable. The second assumption is that the probability of being assigned to each treatment level must be more than zero in a target trial.[268] In observational data, equivalently this means that the probability of being in each exposure level must be positive. The third assumption is that the observed outcome under the observed treatment status needs to be consistent to the

counterfactual outcome under the observed treatment status in a target trial. Mathematically, it means that if A=a, then $Y=Y^a$. This assumption is to ensure that the exposure is well-defined. There were debates on the precise definition of a "well-defined" exposure (or treatment in a target trial). Nonetheless, the general principle is to avoid a broad definition that may result in composite exposure.[268] A commonly used example is the impact of obesity on mortality. There are multiple ways for an individual to get obese ranging from lack of exercise to imbalanced diet, and each of these ways may have different impact on mortality. Hence, a regimen of extreme physical inactivity with high caloric diet, for example, would be a better definition of exposure than just obesity.[268]

Causal diagrams: a tool to understand causal relationships between exposure and outcome

Graphical tools are useful in mapping causal relationship between exposure and outcome in counterfactual frameworks. A direct acyclic graph (DAG) is a type of causal diagram.[270-277] The tool is particularly useful for a causal question such as the one we are asking about the impact of delays in the provision of appropriate antibiotic use on patient mortality. This is because background empirical knowledge and assumptions about the causality between concordant antibiotic use (a treatment which the causative bacteria is susceptible to) and mortality can be mapped using DAGs.[271,272] It is important to identify variables that must be measured and controlled to obtain unconfounded effect estimates.[278,279] Equally critical, DAGs also help to identify variables that should not be controlled to obtain unbiased effect estimates.[278,280] A DAG consists of nodes representing variables and arrows representing the direction of causality. For instance, A causes Y can be represented by Figure 1.1, in which a unidirectional arrow is drawn from A to Y. A variable that is the common cause of A and Y is called a confounder. There

are different examples of confounding in observational data for clinical studies (Figure 1.1). A variable that lies in the middle of the causal pathway between A and Y is a mediator. A variable that is influenced by both A or a cause of A and Y or a cause of Y is a collider (Figure 1.2). If A is a binary variable representing concordant antibiotic treatment and Y is the survival status of the patient, a potential confounder could be resistant infection (Z) and a mediator could be severity of infection (M). A simplified version of a DAG to describe the possible causal relationship between concordant antibiotic use and patient outcome is shown in Figure 1.3. In reality, a DAG for this causal question can be much more complex (please see Chapter 5 for a more detailed example). Causal diagrams are useful tools to explicitly illustrate assumptions that have been made in a causal model, and to guide the analysis.

Figure 1.1. Simplified directed acyclic graphs (DAGs) to illustrate some of the examples of confounders that may influence the relationship between exposure of interest and outcome, when not adjusted appropriately,. A: exposure; Y: outcome; L: measured confounder; and U: unmeasured confounder.

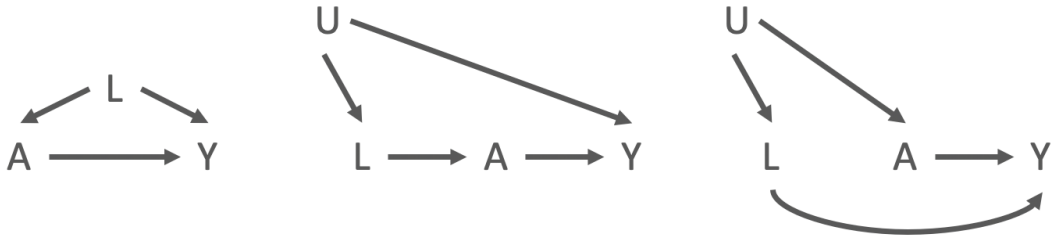


Figure 1.2. A simplified directed acyclic graph (DAG) to illustrate presence of a collider (C) in the causal relationship between exposure (A) and outcome (Y).

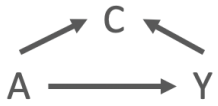
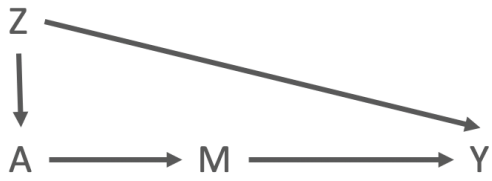


Figure 1.3. A simplified directed acyclic graph (DAG) to illustrate possible causal relationships relating antibiotic use to mortality. A: a binary exposure (i.e., exposure to inappropriate or appropriate antibiotic); Y: outcome (i.e., mortality); Z: confounder (i.e., underlying comorbidity); M: mediator (i.e., severity of the bacterial infection).



1.5.2 Potential biases in observation data

Time-varying exposure

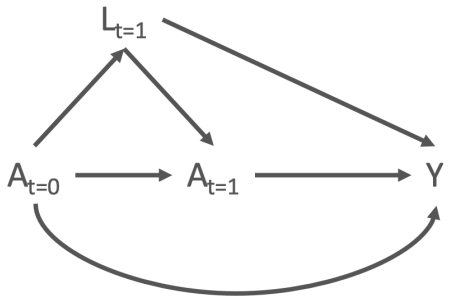
Empirical antibiotic treatment is often given over a period of time. During this treatment period, the type of antibiotic, dosage, frequency, and even route of administration may change. The changes may be based on the clinical presentation and severity of the infection, which in turn would have an impact on the chance of a patient surviving the bacterial infection. This is a common phenomenon in an observational data on antibiotic treatments, and needs to be accounted for during an analysis.[281]

Confounding bias

This is a well-recognised type of bias that arises from the failure to account (either by study design or analysis) for the common causes of treatment and outcome.[272] The assessment of whether a variable is a confounder (i.e., a common cause of treatment outcome) is typically determined using background knowledge of the causal question being addressed.[279] In cohort data, multiple approaches can be used to adjust for measured confounders during the analysis with the aim of achieving exchangeability between different levels of exposure. These approaches include inverse-probability weighting, standardization, and regression models. In the presence of a time-varying confounder, which acts as a confounder for the current exposure and a mediator for the future exposure (Figure 1.4), alternative analysis methods are needed. For instance, antibiotic treatment may change over the course of an infection, and these changes will

often be related to the time-varying clinical severity of the patient, which could be a confounder for the current antibiotic treatment and a mediator for the future antibiotic treatment and outcome (Figure 1.4). Here, controlling for severity of infection using the conventional approach (i.e., standard logistic regression models) is problematic. The reason is that severity of infection lies on the causal pathway between past antibiotic exposure and the outcome; hence, conditioning on severity of infection blocks some of the effect of the treatment and could introduce collider-stratification bias (since severity of infection is a consequence of antibiotic exposure). Methods such as g-estimation of structural nested models and inverse-probability weighted estimation of marginal structural models have been used to estimate the causal effect of a time-varying exposure in the presence of time-varying confounders that are affected by the exposure.[268,282-284]

Figure 1.4. A simplified directed acyclic graph (DAG) to illustrate the presence of a time-varying confounder in a causal relationship of antibiotic treatment and mortality. A_t: a binary exposure (i.e., exposure to inappropriate or appropriate antibiotic) that may change over the treatment period; Y: outcome (i.e., mortality); $L_{t=1}$: time-varying confounder (i.e., severity of the bacterial infection).



Selection bias

In epidemiology, the term "selection bias" encompasses different type of biases. This includes bias from inappropriate selection of controls in case-control studies, bias from differential loss-to-follow up, volunteer bias, healthy-worker bias, nonresponse bias and immortal-time bias.[280,285] The consequence of selection bias is that the association between exposure and outcome among those included in the analysis differs from the

association among those eligible to be included. When attempting to emulate a randomized trial using observational data to answer the causal question on the impact of delays in provision of concordant antibiotic treatment, one has to be aware of immortal time bias, especially in the cases when the antibiotic treatment is given over a period of time.

Immortal time bias, sometimes also known as survivorship bias among economists, is defined as bias arising when the analysis fails to take proper account of a period of time during which death or study outcome cannot occur by design (the period of "immortality").[286] A commonly discussed example is the analysis of Oscar winners and survival.[239,287,288] In 2001, an article reported that Oscar winners lived almost 4 years longer than Oscar nominees without winning the award.[287] In a survival analysis, when the analysis time starts from the nominees' date of birth (time 0), a sequence of events relating to the Oscar award would happen before an outcome, death, is observed. For instance, for each nominee there would be a period of time before being nominated (i.e., unlikely that one would be nominated since birth), and a period during which the individual was nominated for the award. The length of time during which a nominee is nominated for the award but without winning it can be different from person to person. In other words, some nominees may not win an award because they did not live long enough, while others may be nominated multiple times because they lived long enough and ultimately won the award. Hence, a direct comparison of the times to a death event between the nominees not winning and those winning the Oscar award, without appropriate analysis method, would exaggerate the difference, even in the absence of other types of bias.[239,288] There are examples of immortal time bias in epidemiological and clinical studies. A classical form is when there is a delay between eligibility criteria

being met and a treatment strategy being assigned in a randomized trial or a target trial that one tries to emulate using observational data.[289] This form of immortal time bias was described in heart transplant studies,[290] in cancer studies,[291] and in pharmaco-epidemiological studies.[292] In the case of evaluating the impact of delays in provision of concordant antibiotic treatment, comparing "no concordant treatment given within 48 hours of infection" against "at least one concordant antibiotic prescription within 48 hours of infection" in an ideal randomised controlled trial with an intention-to-treat analysis would give an unbiased estimate even if individuals in the "at least one concordant antibiotic prescription within 48 hours of infection" arm dropped out of the study within 24 hours of infection (Figure 6). This is because these individuals would be kept within the arm they were randomised to. However, in observational data when the emulation of intention-to-treat analysis cannot be achieved, the analysis will either exclude this same group of individuals (resulting in selection bias), or assign them to "no concordant treatment given within 48 hours of infection" (resulting in misclassification) (Figure 1.5).

1.5.3 Statistical models to evaluate the impact of delaying concordant antibiotic

In 1986, Robins proposed the g-computation formula for estimating causal effects of a time-varying treatment in the presence of time-varying covariates that are simultaneously both confounders and mediator variables.[293] Further methods, including marginal structural models (MSMs) using inverse-probability weighted (IPW) estimators and structural nested models (SNMs) using g-estimation,[282,294,295] were then suggested to be used for such settings. Several manuscripts and tutorials are available describing the three approaches[268,283,284] and in this thesis I will focus on MSMs and in particular estimating the parameters using IPW estimators.

Throughout this thesis, I will use *Y* to represent outcome, which is mortality in this case, A to present exposure, which is antibiotic treatment (a binary variable where 0: concordant treatment, and 1: discordant treatment), and *L*, which are confounders (such as severity of infection as described in the previous section). In brief, in the cases when the outcome is a binary variable (where 0: survived and 1: died), linear logistic MSM can be defined using the expression below. The expression is adapted from *Robins* (2000).[282]

$$logit\ \mathrm{P}[Y_{\bar{a}} = 1] = \beta_0 + \beta_1 cum(\bar{a})$$

where $cum(\bar{a})$ is the cumulative antibiotic exposure associated with the history $\bar{a}$ (past exposure of antibiotic treatment). The expression is the probability of observing a death event if all patients have antibiotic treatment history $\bar{a}$. In the presence of a time-varying confounder (i.e., severity of infection), represented by $\bar{L}_{k,}$ the unbiased causal parameter β_1 can be estimated by fitting a logistic model with inverse-probability weights, assuming no other types of biases. The IPWs can be presented as below (adapted from Robins J and colleague's paper on MSMs in 2000):[282]

$$IPW_i = \frac{1}{\prod_{k=0}^{K} \mathrm{P}(A_k = a_{ki} \mid \bar{A}_{k-1} = \bar{a}_{(k-1)i}, \bar{L}_k = \bar{l}_{ki})}$$

where *i* indicates individual patients and *k* indicates time.

Large IPWs may result from small values (close to zero) of $\mathrm{P}(A_k = a_{ki} \mid \bar{A}_{k-1} = \bar{a}_{(k-1)i}, \bar{L}_k = \bar{l}_{ki}$, and stabilized IPW can be used. The expression for stabilized IPW below is adapted from *Robins* (2000):[282]

$$stabilised\ IPW_i = \frac{\prod_{k=0}^{K} \mathrm{P}(A_k = a_{ki} \mid \bar{A}_{k-1} = \bar{a}_{(k-1)i})}{\prod_{k=0}^{K} \mathrm{P}(A_k = a_{ki} \mid \bar{A}_{k-1} = \bar{a}_{(k-1)i}, \bar{L}_k = \bar{l}_{ki})}$$

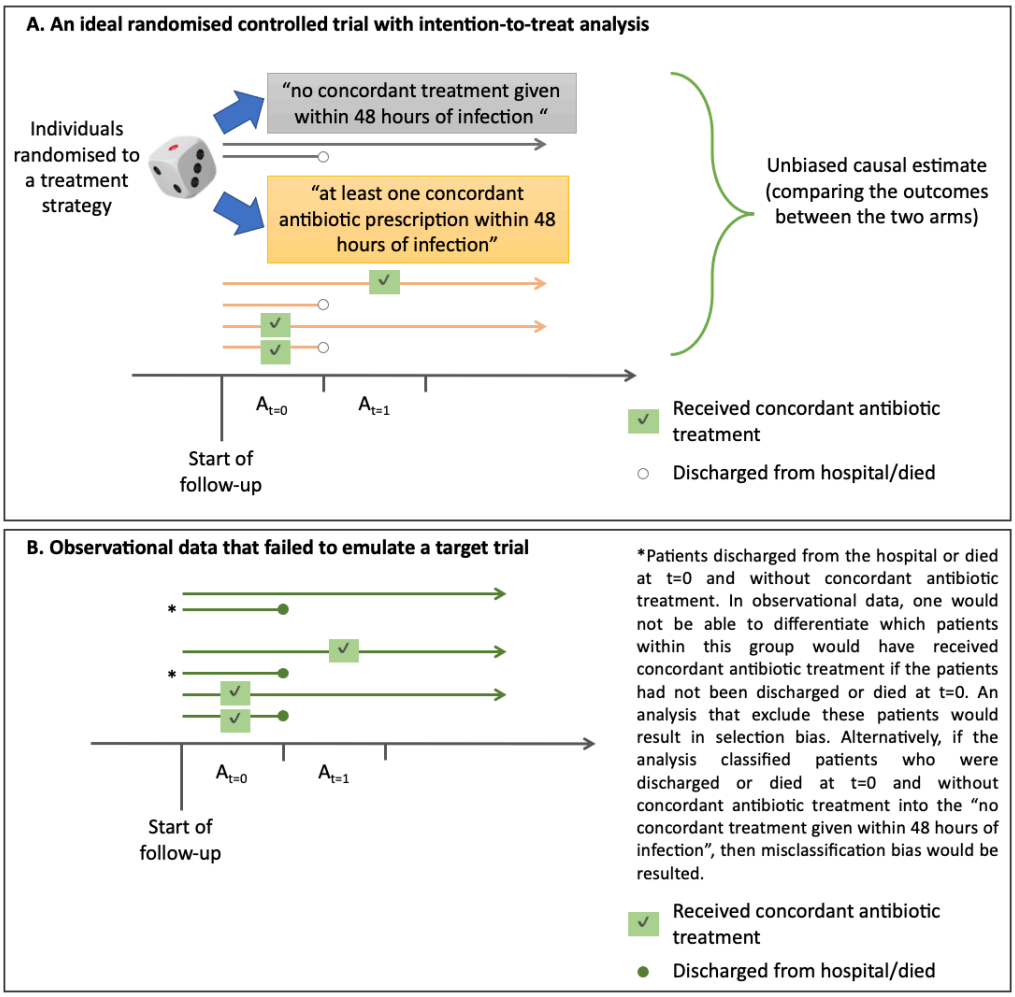
Essentially, IPWs (or stabilized IPWs) are calculated from the probability of exposure to *a* at time *k* for each individual *i*. Through assigning the weights (the inverse of the probabilities) to individuals in the cohort, a pseudopopulation that represents a randomised trial (in which conditional exchangeability is achieved) can be generated to estimate the parameters of the MSM applied to the cohort data.[268] In practice, unmeasured confounders, which are unobserved in a given data set but if present, can render the exchangeability assumption unrealistic. Diagnostic methods to assess the covariate balance after adjustment for measured confounders using plots of mean differences between variable values for exposed and unexposed groups can provide evidence about the remaining level of influence due to residual confounding.[296]

1.5.4 The counterfactual framework in the current literature

The counterfactual framework is not the only way to study causal effect estimands in clinical research. For instance, case-control studies have been used effectively to establish the causal effect of smoking on lung cancer. Negative control studies, which have been traditionally used in natural (laboratory) science experiments, have been adapted for epidemiological research questions. A decision-theoretical framework and causal Bayesian network are examples of approaches that have attempted to move away from the counterfactual framework by not using counterfactual outcomes (though in many ways the approaches carry assumptions that are entailed in approaches based on counterfactual outcomes, but this is beyond the scope of the discussion for this thesis).[297-299] There are three main reasons that I chose to use the counterfactual framework to evaluate the impact of delays in appropriate antibiotic treatment on mortality. First, the causal relationship between the exposure and outcome is complex and involves time-varying confounders (as described in the sections above), which cannot be adjusted

appropriately using standard analysis strategies. G-methods (such as MSM), which are based on the counterfactual framework, are well-developed and have been increasingly used to answer causal questions involving time-varying confounders.[284] Second, due to the complexity of the context in which this clinical question on impact of delays in appropriate antibiotic treatment is relevant, both empirical knowledge (on, for example, factors influencing antibiotic prescription behaviours, the antibiotic resistant pattern of *Acinetobacter* spp., and blood culture utilisation) and potential biases in observational data need special attention. An optimal and explicit approach to illustrate both aspects is by using a causal diagram. Third, the structure of the antibiotic use data-generating process is characterized by the way that it depends not only on the course of infection but also the survival status of the patients during the course of treatment. Causal estimates, when derived from observational data, are inevitably subject to different types of biases that need to be appropriately adjusted for. Hence, I chose to use the counterfactual framework to systematically identify the potential biases and to adjust for them in the routinely collected antibiotic use data.

Figure 1.5. An ideal randomised controlled trial with intention-to-treat analysis will give an unbiased causal estimate comparing outcomes of treatment arms (A), as individuals would remain in the randomised group despite dropping out of the study due to early death. In contrast, an observational study that failed to emulate a target trial would result in a biased estimate (B).



1.6 Thesis outline

The focus of this DPhil thesis is the epidemiology of AMR infection in Southeast Asia. In particular, the specific objectives are to 1) estimate the health burdens of hospital-acquired AMR infections; 2) develop a tool to support the utilisation of routinely collected data for AMR surveillance; and 3) evaluate the impact of delays in appropriate antibiotic use using causal inference framework.

Specific objective 1 (Chapter 2): To estimate mortality attributable to antibiotic-resistant infection in Thailand.

I have conducted a two-year prospective surveillance study on hospital-acquired infections in Sunpasitthiprasong Hospital, Thailand. Data on patient characteristics, microbiology culture results of isolated pathogens, and patient management were collected from the enrolled patients. Survival status on Day 28 post infection of the patients was recorded. Analyses were performed to estimate the incidence and mortality of the patients. The ratio (or conversion factors) of attributable mortality in patients with LRTI, SSI, UTI, and other infections to that in patients with BSI were calculated. It is the first time that conversion factors have been estimated using data from a LMIC setting. The conversion factors can be used to estimate the overall attributable mortality of hospital-acquired AMR infection when only BSI data are available, which is commonly seen in LMIC settings.

Specific objective 2 (Chapter 3): To develop and test an application that can support a local hospital to analyse routinely collected electronic data and generate AMR surveillance reports

I have developed an offline application named AutoMated tool for Antimicrobial resistance Surveillance System (AMASS), which is to be used in a resource-limited setting for processing AMR data locally. The tool is developed in the R environment. The features of AMASS include easy-to-use, open-access code, and does not require internet access to operate. AMASS cleans AMR data and generates AMR surveillance reports for users automatically. The aim is to fill the technological gap in processing microbiology and hospital admission data sets for resource-limited settings. AMASS was tested in

seven hospitals in seven countries. The AMR surveillance reports generated by each hospital were published in FigShare, which is an open-access platform.

Specific objective 3 (Chapter 4): To evaluate the impact of blood culture utilisation on proportion and incidence rates of antimicrobial resistance

Data from patients admitted to Sunpasitthiprasong Hospital between 2010 and 2015 was analysed. Microbiology data, antibiotic use data and patient basic demographic data were used in the analysis. Using bootstrap sampling, three scenarios were generated to represent various blood culture utilisation rates corresponding to 50%, 25%, and 10% of the current blood culture utilisation rates. The results showed that the proportion of AMR infections among hospital-acquired BSIs was overestimated as blood utilisation rate reduced. The incidence rate of patients with AMR BSIs per 100,000 population per year was underestimated when blood culture utilisation rate reduced. Moreover, the proportion of patients with AMR infections is higher when positive blood culture was taken after parenteral antibiotic treatment compared to when taken before parenteral antibiotic treatment. The findings suggest that blood culture utilisation rate is an important parameter to include in an AMR surveillance report. Furthermore, AMR surveillance reports should additionally include stratification by exposure to a parenteral antibiotic at the hospital.

Specific objective 4 (Chapter 5): To evaluate the impact of antibiotic use on mortality in a low and middle-income country using a causal inference framework

Firstly, a simulation study was performed to explore the magnitude of biases in the causal estimates under various scenarios that failed to adjust for time-varying and immortal time biases. Then, the target trials to address the causal relationship between delays in

concordant antibiotic treatment and mortality were defined. Protocols were described based on the framework developed by *Hernan & Robins* (2016). Finally, observational data of patients admitted to Sunpasitthiprasong Hospital between 2003 and 2015 was used to emulate the target trials. Patients with *Acinetobacter* spp. hospital-acquired BSIs were included in the analysis. Marginal structural models with inverse probability weighting were used to estimate the impact of delays in concordant antibiotic treatment on in-hospital mortality. The results suggested that delays in concordant antibiotic treatment increased mortality.

Conclusions (Chapter 6): Discussions, implications, and future research directions

I summarized the main findings and discussed how overall findings contribute to the growing literature on the epidemiology, burdens, and surveillance systems of AMR infections in the context of Southeast Asian countries. I also discussed the implication of the available evidence for health policy and the potential directions for future work.

2

Excess mortality attributable to hospital-acquired antimicrobial-resistant infections in a developing country: a two-year prospective surveillance study

2.1 Abstract

Background

Quantification of the mortality burden related to antimicrobial-resistant (AMR) bacterial infections is fundamental to understanding the extent of the health problem and to control the spread of these infections. The objective of this study is to estimate the excess mortality attributable to AMR infections using antimicrobial-susceptible (AMS) infections as a comparator in a hospital in Thailand.

Methods

A prospective surveillance study on patients with hospital-acquired infections (HAI) was conducted in Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand from January 2018 to February 2020. The types of infection under evaluation included bloodstream infection (BSI), lower respiratory tract infections (LRTI), surgical site infections (SSI), urinary tract infections (UTI) and other infections (OTH), which were defined based on the CDC/NHSN surveillance definitions. We focused on bacteria species listed in the WHO priority list of AMR bacteria. In particular, infections with methicillin-resistant *Staphylococcus aureus* (MRSA), 3rd generation cephalosporin resistant *Escherichia coli*

and *Klebsiella pneumoniae* (3GCREC and 3GCRKP), and carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (CRPA and CRACI) were defined as AMR infections. We also evaluated ampicillin-resistant *Enterococcus faecium* (AMPREfm) and *Enterococcus faecalis* (AMPREfc), as they are prevalent in this setting. Infections with one of those bacteria but susceptible to the antibiotics of interest were defined as AMS infections. The outcome measured was 28-day mortality, counting from the first date of culture-positive specimen collection of the HAI under evaluation. The 28-day outcome was confirmed via telephone call for patients who were no longer hospitalized and had been discharged alive. The average counterfactual outcomes of AMS and AMR infections were compared to estimate excess mortality attributable to AMR infections.

Results

Of 2,043 HAI patients enrolled and included in the analysis, 658 (32.2%) had AMS infections and 1,385 (67.8%) had AMR infections. Overall, the crude 28-day mortality of patients with AMS infections and AMR infections was 23.1% (152/658) and 35.5% (491/1,385), respectively. After adjustment for pre-defined confounders, we estimated that if all of the infections were AMS infections, the mortality in this cohort would be 26.4% (95% CI: 21.7, 31.2%), and if all of the infections were AMR infections, the mortality in this cohort would be 34.0% (95% CI: 31.3, 36.7%). The absolute risk difference was 7.7 percentage points (95% CI: 2.2, 13.2 percentage points). The population attributable fraction (PAF) of mortality attributable to AMR infections in this cohort was 16.3% (95% CI: 1.2, 29.1%). We estimated that 105 deaths (95% CI: 7, 187 deaths) out of the 643 observed deaths could be prevented if all of the infections in this study were AMS infections. Amongst the bacteria under evaluation, CRACI (42 deaths

[95% CI -5, 75]) was responsible for the highest number of excess deaths attributable to AMR infections. Of all types of infection under evaluation, UTI (39 deaths [95% CI -24, 84]) was responsible for the highest number of excess deaths attributable to AMR infections though with a wide 95% confidence interval. This was followed by LRTI (37 deaths [95% CI 1, 67]).

Interpretation

The mortality attributable to AMR bacterial infections in LMICs can be high. Our findings have implications for decisions about prioritising targets of interventions to control AMR infections in hospital with similar AMR prevalence, patient management, and diagnostic capacity as that in this study hospital.

2.2 Introduction

Quantification of health burden related to antimicrobial resistance (AMR) is essential to understand the extend of the health problem and to control the spread of drug resistant infections. In 2015, Jim O'Neill estimated that 700,000 deaths could be due to AMR bacterial infections, including multidrug-resistant and extensive drug-resistant tuberculosis, globally.[13,224] Due to a lack of data,[13,224] that 700,000 deaths were attributable to AMR infections was estimated based on the reports from European Centre for Disease Prevention and Control/European Medicines Agency (ECDC/EMEA) in 2009 and US Centers for Disease Control and Prevention (CDC) in 2013.[76,300] The ECDC/EMEA estimated 25,000 deaths attributable to AMR infections in European countries,[76] and this number was updated to 35,000 deaths by Cassini et al. in 2018.[20] The CDC estimated 23,000 deaths attributable to AMR infections in the US,[300] and this number was updated to 35,000 in 2019.[301] A study from Thailand in 2016 estimated that 19,000 deaths are attributable to multidrug-resistant hospital-acquired infections.[21]

Estimating excess deaths attributable to AMR infections for all types of infection is often difficult in both high-income countries (HICs) and low and middle-income countries (LMICs).[76,300] One of the reasons is that complete clinical data often are not readily accessible in electronic form. This leads to difficulties in defining whether patients with a clinical specimen culture positive for bacteria should be defined as having an infection or not. For instance, lower-respiratory tract infection (LRTI) is usually defined based on a combination of chest radiographs, blood chemistry, clinical symptoms, and microbiology culture.[166] As these data are not all accessible, it is difficult to estimate the overall excess deaths due to AMR for all types of infection in a target population. Attributable mortality (i.e., excess mortality attributable to AMR infections) of

bloodstream infections (BSI) is the most commonly reported[76,300] because BSI can be defined reasonably reliably based on a bacterial pathogen identified from blood culture specimens. When only attributable mortality of BSI is available, attributable mortality for each type of infection can be estimated by applying conversion factors corresponding to the relative mortality of HAIs from each type of infection compared to BSI.[76,240] For instance, in the ECDC/EMEA 2009 report, a set of conversion factors described by Martone *et al.* from the US hospital surveillance data in 1998 was used for a number of AMR bacteria when only attributable mortality of BSI was available.[76,240] This approached was also used in the paper that estimated 19,000 deaths due to AMR infections in Thailand in 2016.[21]

Alternatively, the estimate of 23,000 excess deaths attributable to AMR infections in the US[300] was based on the multiplication of an estimate of the total number of AMR infections for each bacteria species under evaluation for all types of infection by 6.5%, which is an overall estimate of mortality attributable to AMR hospital-onset infections for all types of infection of a public teaching hospital in Chicago in 2000.[302] The mortality attributable to AMR infections for all types of infection was estimated using a logistic regression adjusted for APACHE II score, ICU care and concurrent HAI. The comparator was antimicrobial susceptible (AMS) infections.[302]

Another method of estimating excess deaths attributable to AMR infections for all types of infection when complete clinical data are not available was presented by Cassini et al. in 2015, in which estimates were derived from a systematic review and a decision tree. The studies included in the systematic review varied in study design, type of infection, and analytical approach.[20] In the analysis, it is important to adjust for patient

characteristics including underlying illnesses, and for the bacteria isolated including bacterial loads and resistance patterns. These factors may influence the probability of acquiring hospital-acquired infections and chance of survival.

Recently, Global Antimicrobial Resistance and Use Surveillance System (GLASS) of the World Health Organization (WHO) published a guideline for estimating attributable mortality of AMR bloodstream infections.[214] A recommendation is to use two cohorts of patients as comparators. A cohort of patients without an infection as a comparator could be used to represent an additive scenario, which assumes the burdens of AMR infections adds to overall existing burden of bacterial infection. This could define the upper limit of the impact of AMR infections. Additionally, a cohort of patients with AMS infections could be used to represent a replacement scenario, which assumes every infection caused by AMR bacteria would be replaced by an infection caused by more susceptible bacteria if AMR bacteria could be prevented. Estimates of mortality attributable to AMR infections using this comparator could represent the lower limit of the impact of AMR infections.

Data on mortality attributable to AMR infections for each and all types of infection in LMICs are limited.[20] To address this knowledge gap, we prospectively enrolled patients with all types of hospital-acquired infection, and recorded the survival status on day 28. The objective of this study was to estimate excess mortality and deaths attributable to AMR infections compared to AMS infections for all types of hospital-acquired infections in a hospital in a LMIC setting. Our findings could be useful for the estimation of AMR burden in LMICs where only data of attributable mortality of BSI are available.

2.3 Methods

2.3.1 Study design

We prospectively enrolled patients who were admitted to Sunpasitthiprasong Hospital, a 1,500-bed tertiary hospital in Ubon Ratchathani, Thailand. The inclusion criteria were patients who developed BSI, LRTI, surgical-site infection (SSI), urinary tract infection (UTI) or other specific types of infections (OTH) after hospitalization for more than 48 hours, with clinical specimen culture-positive for *Staphylococcus aureus*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Acinetobacter* spp.. We focused on these six bacteria because they were in the WHO 2015 priority list[77] and are of clinically importance in hospitals in the Southeast Asia region.[80] Specifically, we analysed *A. baumannii* of *Acinetobacter* species and *E. faecium* and *E. faecalis* of *Enterococccus* species. We excluded patients who had an onset of signs and symptoms of the current infections within 48 hours of hospitalization. Only the first episode of the first hospital-acquired infection under evaluation of each patient were included. Protocol training and a dry-run were performed between January and February 2018, before the surveillance study was rolled out between 1st March 2018 and 29th February 2020.

Clinical and microbiology data were collected on a standardized case report form and then entered into an electronic database (Microsoft Access). The clinical data collected included patient demographics, history of invasive procedures, comorbidities, severity scores, antibiotic treatments (including type, route and frequency), and survival status within 28 days since the first date of culture-positive specimen collection of the HAI under evaluation. The culture-positive specimen included only the specimen culture positive for bacteria under evaluation. The microbiology data collected were: date of

clinical sample collection; bacterial culture results; date on which bacterial culture results were available; and antimicrobial susceptibility test results.

2.3.2 Definitions

The definitions of BSI, LRTI, SSI, UTI, and OTH were based on the US CDC surveillance guidelines,[107] the Infectious Diseases Society of America and American Thoracic Society guidelines for pneumonia,[103] and the ECDC point-prevalence survey study.[139] Detailed definitions used for each infection are given in Appendix A1. In brief, BSI was defined as growth of one or more pathogenic bacteria of interest from blood culture. We included patients with either pneumonia or LRTI that were not pneumonia, which together were classified as LRTI. LRTI were defined based on chest imaging, clinical symptoms and microbiology results. SSI included superficial incisional SSI, deep incisional SSI, and organ/space SSI. Patients with microbiologically confirmed symptomatic UTI were included in the study. OTH included skin/soft tissue, eye, mouth, genital, and other body site infections. More than one type of infection was defined as infections with more than one type including BSI, LRTI, SSI, UTI and OTH. This included, but was not limited to, secondary BSI with matching bacteria in blood specimen and in other clinical specimens (such as urine specimen, specimen collected from endotracheal aspirate, and specimens collected from tissues/organs).

Infections with methicillin-resistant *Staphylococcus aureus* (MRSA), ampicillin-resistant *Enterococcus faecium* (AMPREfm), *Enterococcus faecalis* (AMPREfc), 3rd generation cephalosporin resistant *Escherichia coli* and *Klebsiella pneumoniae* (3GCREC and 3GCRKP), and carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (CRPA and CRACI) were defined as AMR infections. Infections caused by

the same bacterial species but with strains that were susceptible to the corresponding antibiotics stated above were defined as AMS infections. Polymicrobial infections were defined as infections with more than one of the bacteria under evaluation.

Severity scores were collected. The Charlson comorbidity Index (CCI) was calculated based on the presence of the following comorbidities: congestive heart failure; myocardial infarction; peripheral vascular disease; chronic obstructive pulmonary disease; ulcer disease; liver disease; renal disease; connective tissue disease; cerebrovascular disease; hemiplegia or paraplegia; dementia; lymphoma; leukaemia; tumor; diabetes; recipient of organ transplant; and HIV infection.[303] Sequential organ failure assessment (SOFA) scores[304] on the day of hospitalisation and on the first date of culture-positive specimen collection of the HAI under evaluation were determined for each enrolled patient. Detailed definitions of the comorbidities and severity scores are in Appendix A2. The number of days between the date of sample collection and the date on which bacterial culture results were available has calculated and used as a proxy for bacterial load.

2.3.3 Laboratory procedures

Throughout the study, bacterial culture, species identification and antimicrobial susceptibility testing (AST) were performed by the microbiology laboratory of the study hospital as per routine practices using standard methodologies provided by Department of Medical Science, Ministry of Public Health (MoPH), Thailand.[305] In short, blood specimens (5-10 mL) were collected in BD BACTEC blood culture bottles and processed using the BD BACTEC automated system (Becton-Dickinson, Sparks, MD, USA). Other types of clinical specimen were collected in sterile containers and then transported to the

laboratory for bacterial culture. Identifications and AST of the bacteria isolated from blood and sterile-site specimens were performed using VITEK 2 system (bioMérieux). Identification and AST of the bacteria isolated from non-sterile site specimens were performed using biochemical testing and the disk diffusion method. The interpretation of the disk diffusion method was based on Clinical and Laboratory Standards Institute (CLSI) guidelines.[74] All microbiology test results were then input and stored in MLAB program every evening.[306] Then the microbiology data were exported in MS Excel format and sent to the hospital infection control nurse team and the study team on a daily basis throughout the study period.

2.3.4 Outcome

The primary outcome was mortality within 28 days from the first date of culture-positive specimen collection of the HAI under evaluation. The research team telephoned patients who were no longer hospitalized and had been discharged alive. Those who were lost to follow-up were assessed using the hospital admission data including the type of discharge and health status when discharged. Patients who were lost to follow-up and discharged against physician's advice without improved health condition were considered to have died within 28 days.

2.3.5 Statistical analysis

Estimation of excess mortality attributable to AMR infections

In this study, AMS infection was used as the comparator to estimate the impact of AMR infections on 28-day mortality. The population attributable fraction (PAF) of mortality attributable to AMR infection was estimated as described elsewhere.[268] In brief, the

observed mortality was compared with the counterfactual outcome of AMS infection as illustrated below:

$$PAF\ of\ mortality\ attributable\ to\ AMR = \frac{Pr[Y = 1] - Pr[Y^{AMS} = 1]}{Pr[Y = 1]} \quad [1]$$

where Pr[Y=1] was the observed mortality and Pr[Y^{AMS}=1] was the mortality that would have been observed if everyone with HAI infections in this cohort had AMS infections.

The attributable risks of mortality associated with AMR infection was defined as the absolute risk difference between the mortality that would be expected if all patients in the study cohort had AMR infections (Pr[Y^{AMR}=1]) and the mortality that would be expected if all patients in the study had AMS infections (Pr[Y^{AMS}=1]):

$$Attributable\ risks\ of\ mortality\ associated\ with\ AMR = Pr[Y^{AMR} = 1] - Pr[Y^{AMS} = 1] \quad [2]$$

As 28-day mortality was recorded regardless of whether or not patients were discharged from the hospital before observing the outcome, discharge was not a competing outcome of the 28-day mortality in this study. Hence, logistic regression models were used to estimate the impact of AMR infections on 28-day mortality. Firstly, a set logistic regression models were used to estimate the odds ratio for 28-day mortality of AMR infection compared to AMS infection, adjusted for the causative bacteria and types of infection. Secondly, an interaction term between the causative bacteria and AMR infection was added to take account of the varying effect of AMR infection of different causative bacteria on 28-day mortality.

Multivariable logistic regression models were built based on a direct acyclic graph (DAG). The pre-defined confounders were (a) the severity of underlying illness and patient characteristics on the hospitalisation day, and (b) risks of acquiring AMR infections during the hospitalisation (Figure 2.1; detailed descriptions for each potential confounder were in Appendix A2). Firstly, the severity of underlying illness and patient characteristics on the hospitalisation day could represent the potential exposure to various level of contacts with healthcare workers and to invasive procedures, which were factors that influenced the probability of the patient being infected by AMR or AMS bacteria. The proxy variables included the CCI score on hospital admission, SOFA score on the hospitalisation day, the ward to which patients were admitted on the hospitalisation day, whether or not the patient was referred from another hospital, and patient characteristics (including age and gender). Secondly, the set of events representing the risks of acquiring AMR infections during the hospitalisation included duration of hospital stay, duration of antibiotic treatment, and exposure to an invasive device prior to the first date of culture-positive specimen collection. These were potential drivers of acquiring AMR infections after the hospital admission. These drivers could involve within-host transmission dynamics of AMR and AMS bacteria which may determine the dominant strain of bacteria and the bacteria isolated from the clinical specimen.[237] Bacterial load, by definition, was not a confounder but was adjusted in the model to improve precision of the final estimate of attributable mortality. The bacterial load could determine development of infection and the probability that the diagnostic tests (i.e., bacterial culture) would be positive, the chance of treatment success, and consequently patient survival. The proxy variable to represent bacterial load was the number of days between the date of specimen collection and the date on which microbiology culture and AST results were reported.

We considered that SOFA score on the first date of culture-positive specimen collection and antibiotic treatment after the first date of culture-positive specimen collection were on the causal pathway to the 28-day outcome. Therefore, those two variables were not included in the models.

We also explored the estimated excess mortality attributable to AMR infections when models were adjusted for a subset of confounders, which could be retrievable from routinely collected electronic data in some resource-limited settings. The variables considered included ward of admission, direct admission to ICU, gender, age, CCI, and duration of hospital stay prior to the first date of culture-positive specimen collection (results are presented in Appendix A3). The CCI score could be defined by using data from International Classification of Disease (ICD)-9 or ICD-10 coding, and duration of hospital stay prior to the first date of culture-positive specimen collection could be calculated from hospital admission date and specimen collection date.

Sensitivity analyses

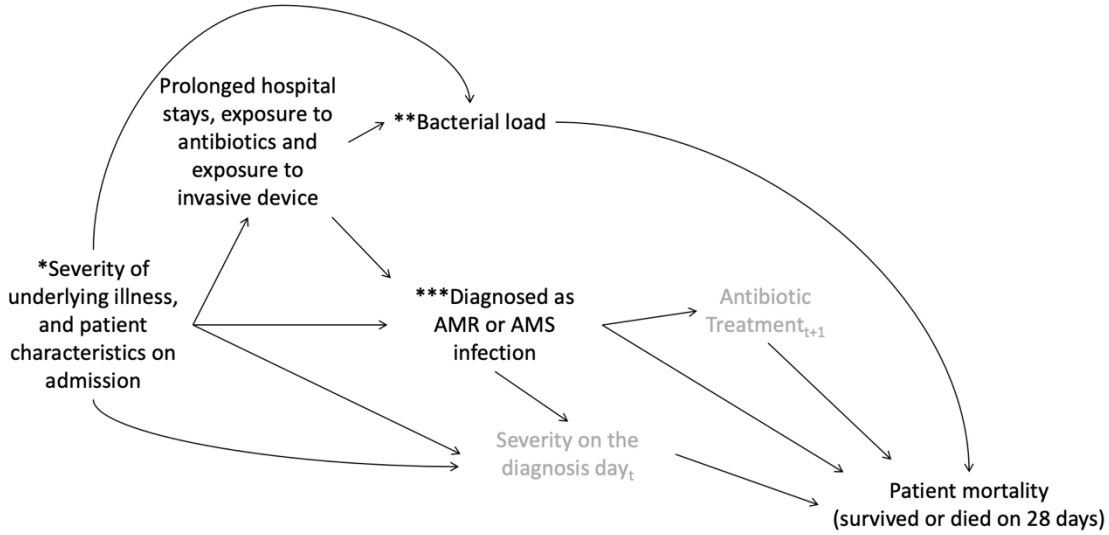
Finally, we assessed the strength of evidence for the causality between AMR infections and 28-day mortality using the 'E value'.[307] In brief, the E value was estimated for common outcomes (>15%) as follows:

$$E\ value = \sqrt{OR} + \sqrt{\sqrt{OR} \times \left(\sqrt{OR} - 1\right)} \quad [3]$$

The E value was developed to assess how strongly an unmeasured confounder would have to be related to the exposure and outcome to explain away the observed association.[307] This value can be calculated from the odds ratio (OR), which represents

the magnitude of the impact. When the outcome is >15%, the square root of OR is used instead of OR as illustrated in equation [2].

Figure 2.1. Directed acyclic graph to illustrate the relationship between hospital-acquired bacterial infections and 28-day mortality of patients.



Footnote: Arrows indicate the direction of a potential causal relationship; for instance, the relationship between severity of underlying illnesses and the probability of AMR infection is represented by an arrow from "Severity of underlying illness" to "AMR or AMS infection". *The proxy variables to represent the potential baseline confounders for the severity of underlying illnesses and patient characteristics included the Charlson Comorbidities Index score, department and ward type (ICU vs. non-ICU) to which patient was admitted on the day of hospitalisation, gender, age, whether or not patient was referred from another hospital, and SOFA score recorded when patients were hospitalised. After hospital admission the level of exposure to AMR bacteria in the hospital environment is a potential confounder, which was represented by proxy variables including the duration of hospitalisation, cumulative days of exposure to antibiotic treatment prior to specimen collection, and exposure to invasive device prior to the date the culture-positive specimen was collected. **The proxy variable used to represent bacterial load is the number of days it took to obtain the microbiology test results. ***Infections with the bacteria under evaluation. The events in grey colour are those that occur after the onset of or are caused by infections (i.e., mediators between the type of infection and 28-day mortality).

Sensitivity analyses were performed to assess the impact the patients lost to follow-up had on the estimated attributable mortality. Firstly, the final model was applied to the cohort of patients whose 28-day survival status was known (i.e., excluding those who were lost to follow-up). Secondly, the final model was applied to the full cohort and we assumed that all patients who were lost to follow-up had survived. Moreover, we include random effects to account for bacteria variation and allowed intercepts to vary for the

bacteria under evaluation. The results of the sensitivity analyses are presented in Appendix A3.

All statistical analyses were done using STATA, version 17.0 (StataCorp LP, College Station, Texas, USA).

2.3.6 Ethical approvals

The study was approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (Ref. TMEC 17-057) and by the ethics committee of Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand (Ref. 092/2560). Written informed consent was obtained from participants prior to enrollment. The study was registered at ClinicalTrials.gov (NCT03411538).

2.4 Results

2.4.1 Patient population

Over the entire study period, the team received bacterial culture results (both negative and positive culture results) from 282,247 clinical samples (with an average of 378 clinical samples per day) collected from 54,281 patients. On a daily basis, the study team identified clinical specimens that were culture positive for the bacteria under evaluation, and recorded specimen collection dates and hospital admission dates for screening. A total of 7,726 patients who had at least one of the bacteria under evaluation isolated from a clinical specimen collected more than 48 hours after admission were screened by the study team (Figure 2.2). Of the 7,726 patients screened, 4,117 were excluded because an onset of signs and symptoms of the current infections were documented within the first 48 hours of hospitalisation, and 1,552 were excluded because the diagnostic criteria of

LRTI, SSI, UTI or OTH were not fulfilled. Of the remaining 2,057 patients with HAI, we further excluded 14 patients because the total number of patients from whom a particular bacterial species was isolated was too small for the analysis (including *Enterococcus* spp. that were not *E. faecium* or *E. faecalis* [n=1]; and *Acinetobacter* spp. that were not *A. baumannii* [n=13]). In total, 2,043 patients were included in the analysis.

Of the 2,043 patients with HAI enrolled in this study, 1,385 (67.8%) had infections with at least one AMR bacteria (including MRSA, AMPREfm, AMPREfc, 3GCREC, 3GCRKP, CRPA and CRAS) (Table 2.1). 1,163 (56.9%) were males, and the median age was 61 years (interquartile range [IQR]: 45-72 years). There were 148 (7.2%) patients who were less than 15 years old (Table 2.1). The median length of hospital stays prior to the first date the culture-positive specimen collection was 11 days (IQR: 7-19), and was longer for the AMR infection group (median: 14 days [IQR: 8-22]) compared to the AMS infection group (median: 8 days [IQR: 6-14]). The median of CCI score on the hospital admission was 3 (IQR: 1-5) in both patients with AMR infections and those with AMS infections. The proportion of patients that were directly admitted to ICUs on hospital admission was slightly higher in the AMR infection group (25.3%; [351/1,385]) compared to AMS infection group (23.7% [156/658]). There were substantial differences in antibiotic use between the two groups of patients. The proportion of patients who had at least one dose of parenteral antibiotic prior to the first date of the culture-positive specimen collection was 90.6% (1,255/1,385) in the AMR infection group, and 84.5% (556/658) in the AMS infection group. The cumulative days of parenteral antibiotic exposure prior to the first date of the culture-positive specimen collection was longer in the AMR infection group compared to the AMS infection group (median: 9 vs 3 days) (Table 2.1). Furthermore, among the patients who were admitted to an ICU ward on the

day of hospitalisation, the cumulative days of parenteral antibiotic exposure prior to the first date of the culture-positive specimen collection was also longer in the AMR infection group compared to the AMS infection group (median: 9 vs 1 days). Similarly, the same pattern was observed in patients who were admitted to a non-ICU ward on the day of hospitalisation.

Of 2,043 enrolled patients, 39 patients (2%) could not be contacted on day 28 after the first date of culture-positive specimen collection of the HAI under evaluation. Of these 39 patients, 28 patients were discharged against advice and without improvement in health condition, hence we assumed they died soon after discharged from the hospital and within 28 days of the date of culture-positive clinical specimen collection of the HAI (it is a common practice in Thailand for moribund patients to be discharged and to die at home). Overall, crude mortality was higher in the AMR infection group (35.5%; n=491/1,385) than in the AMS infection group (23.1%; n=152/658). Of those who survived up to 28 days, the length of hospital stay after the first date of culture-positive specimen collection was higher in the patients with AMR infection (median: 17 days [IQR: 8-34]) compared to those with AMS infection (median: 12.5 days [IQR: 6-26]). Of those who died within 28 days, the outcome occurred slightly faster in patients with AMR infection (median 9 days [IQR: 3-17]) compared to in patients with AMS infection (median 10.5 days [IQR: 4-19]).

2.4.2 Causative bacteria

Overall, there were 1,662 patients (81.4%) who had monomicrobial infections (i.e., infections with a single bacterium of interest under this study). Among the patients with monomicrobial infections, *A. baumannii* (n=401), followed by *K. pneumoniae* (n=340)

and *E. coli* (n=289) were the most commonly identified bacteria. 381 (18.7%) patients had polymicrobial infection in this cohort (Figure 2.3), and close to half of these cases had LRTI (43.6%; n=166/381).

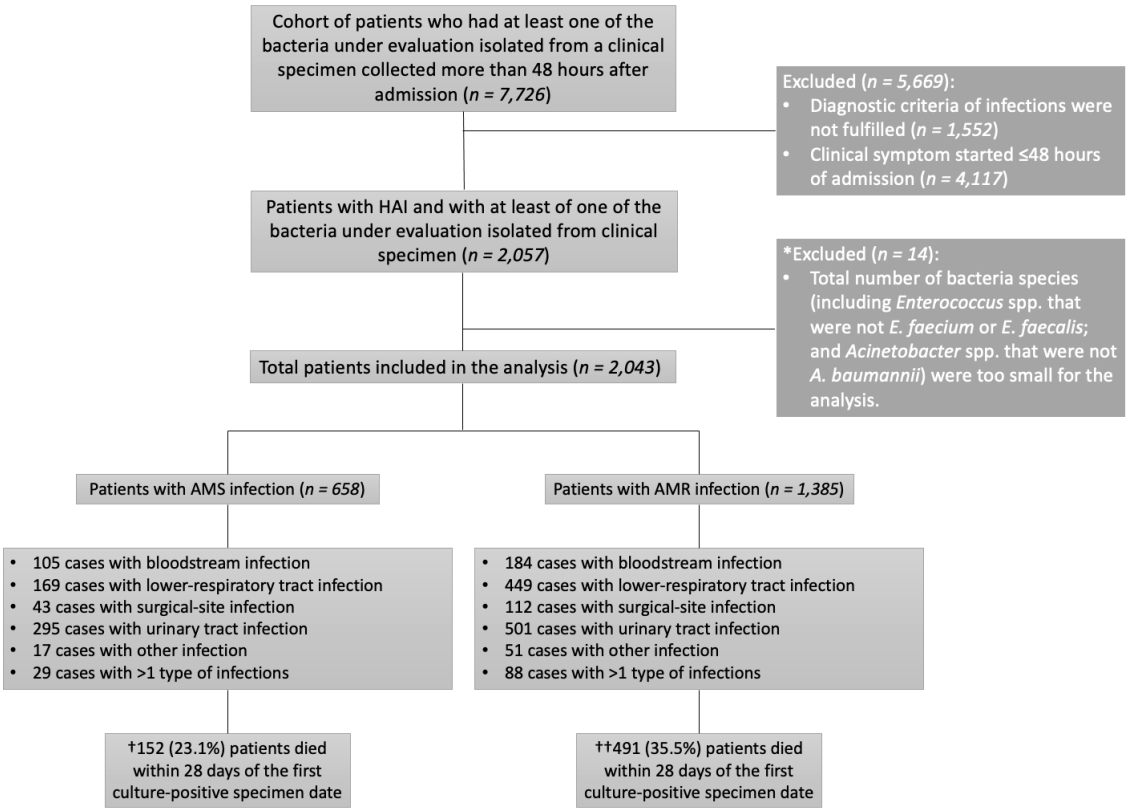
Of all monomicrobial infections, the proportion of MRSA, AMPREfm, AMPREfc, 3GCREC, 3GCRKP, CRPA, and CRACI among infections with *S. aureus*, *E. faecium*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumanii* were 7.3% (5/69), 96.7% (175/181), 18.6% (30/161), 65.1% (188/289), 78.5% (267/340), 36.7% (81/221) and 83.8% (336/401), respectively.

2.4.3 Types of infections

The majority of enrolled patients had infections involving a single body site (n=1,926/2,043; 94.3%). The most common type of infection was UTI (796 patients), followed by LRTI (618 patients), BSI (289 patients), SSI (155 patients), more than one type of infection (117 patients), and OTH (68 patients). Of 117 patients with more than one type of infection, 27 were secondary bacteraemia related to UTI (i.e., patients with matched bacteria in blood and urine samples), 48 were secondary bacteraemia related to LRTI, and 6 were secondary bacteraemia related to SSI. Details of patients' characteristics by type of infection and by causative bacteria are in Appendix A4-10.

Of 289 patients with BSI, 275 had infections with a single bacterium of interest (Figure 2.2). Among patients with monomicrobial BSI, the most commonly identified bacteria were *A. baumannii* (n=69), followed by *K. pneumoniae* (n=66) and *E. coli* (n=61). Amongst the patients with BSI, 35 (12.1%) had central-line associated BSI.

Figure 2.2. Study flow diagram



Footnote: †Of the 152 patients, 11 were lost to follow-up and amongst these patients 5 were discharged against medical advice with health condition not improving, and hence were assumed to have died within 28 days of the first date of culture-positive specimen collection. ††Of the 491 patients, 28 were lost to follow-up and amongst these patients 23 were discharged against medical advice with health condition not improving, and hence were assumed to have died within 28 days of the first date of culture-positive specimen collection. *Of these 14 patients, 2 had *Acinetobacter junii*, 2 had *Acinetobacter lwoffii*, 2 had *Acinetobacter pittii*, 7 had other *Acinetobacter* spp. (that were not *A. baumannii*), and 1 had other *Enterococcus* spp. (that were not *E. faecium* and *E. faecalis*) isolated from clinical sample, and the numbers were too small to compare the mortality between AMS and AMR for each bacterium.

Of 618 patients with LRTI, 452 (73.1%) had infections with a single bacterium of interest. Among patients with monomicrobial LRTI, the most commonly identified bacteria were *A. baumannii* (n=211), followed by *K. pneumoniae* (n=144) and *P. aeruginosa* (n=75). The majority of LRTI patients had ventilator-associated pneumonia (66.2%; n=409/618).

Of 796 patients with UTI, 713 (89.6%) patients had infections with a single bacterium of interest. Among patients with monomicrobial UTI, the most commonly identified

bacteria were *E. coli* (164 out of 796 patients), followed by *E. faecium* (n=159) and *E. faecalis* (n=135). The number of catheter-associated UTIs was 570 cases (out of 796 patients with UTI; 71.6%).

Of 155 patients with SSI, 122 (78.7%) had infections with a single bacterium of interest. Among patients with monomicrobial SSI, *E. coli* was the most commonly identified bacteria (n=33), followed by *A. baumannii* (n=31) and *K. pneumoniae* (n=25). Amongst the patients with SSI, 114 (73.6%), 11 (7.1%), and 30 (19.4%) had superficial incisional SSIs, deep incisional SSIs, and organ/space SSIs, respectively.

Patient characteristics on hospital admission varied by type of infection. Patients with more than one type of infection had, on average, the highest CCI score (Appendix A4). The median SOFA scores measured at hospital admission were highest among patients with BSI and OTH (median 3 [IQR: 1-5]), and lowest among SSI (median 1 [IQR: 0-2]). The proportion of patients directly admitted to an ICU on hospitalisation was highest among patients with LRTI (33.0%; 204 out of 618 patients) and lowest among patients with SSI (19.4%; 30 out of 155 patients). The age distribution in this cohort ranged from 55-80 years old. The proportion of patients less than 15 years of age was lowest among patients with UTI (3.6%; 29 out of 796 patients with UTI) and highest among those with OTH (19.1%; 13 out of 68 patients with OTH).

The exposure to antibiotic treatment and duration of hospitalisation prior to the first date of culture-positive specimen collection varied by type of infection. The median of the cumulative days on parenteral antibiotic treatment prior to the first date of culture-positive specimen collection was highest among patients with OTH (13 days; IQR: 4-

22.5 days) and shortest among patients with BSI (6 days; IQR: 1-14 days) and UTI (6 days; IQR: 1-14 days). The median hospitalisation days prior to the first date of culture-positive specimen collection was highest among those with OTH (median 18.5 days [IQR: 10.5-29 days]) and shortest among those with BSI (median 10 days [IQR: 6-18]).

The highest crude 28-day mortality was among patients with more than one type of infection (54 deaths out of 177 patients; 46.2%), followed by BSI (114 deaths out of 289 patients; 39.5%) and LRTI (231 deaths out of 618 patients; 37.4%) (Appendix A4). The lowest crude 28-day mortality was among patients with SSI infections (20 deaths out of 155 patients; 12.9%). The crude 28-day mortality slightly increased with the increasing number of days in hospital prior to the date the culture-positive specimen was collected (data not shown). The crude 28-day mortality also varied by causative bacteria (Table 2.2).

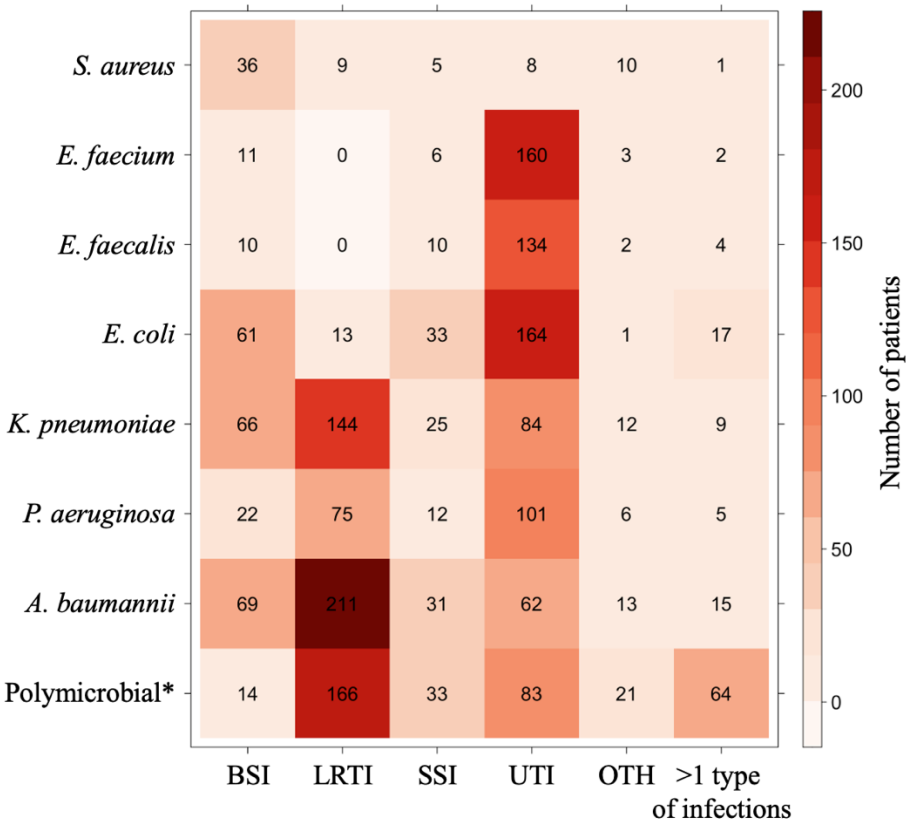
2.4.4 Estimated mortality attributable to AMR infections

We first used a logistic regression to model the association between AMR infections and mortality, adjusted for type of infection and causative bacteria. The odds ratio of 28-day mortality in AMR infections compared to AMS infections was 1.65 (95% CI: 1.29-2.13) for this cohort of HAI patients. This implies that an absolute increase of 10.0% (95% CI: 5.2-14.7%) in the estimated mortality from 24.6% (if all infections were AMS infection) to 34.6% (if all infections were AMR infections) without adjusting for pre-defined confounders. Additionally, when the model was further adjusted for ward of admission on the day of hospitalisation, sex, age, CCI and length of hospitalisation prior clinical sample collected for microbiology test, the estimated odds ratio was 1.60 (95% CI: 1.22-2.09). We further adjusted for all the pre-defined confounders (Figure 2.1; Appendix A3),

and the estimated odds ratio of 28-day mortality in AMR infections compared to AMS infections was 1.48 (95% CI: 1.12-1.96). This gave an estimate of excess mortality attributable to AMR infections of 16.1% (95% CI 4.4, 26.3%). Nonetheless, this model assumed that the impact of AMR infections was constant for different bacteria.

We then added an interaction term between bacteria and AMR infections to account for the varying effect on mortality. This set of models all gave similar estimates of overall excess mortality attributable to AMR (Appendix A3). As it is more biologically plausible to assume that the effect of AMR infections on 28-day mortality varied by the infecting AMR bacterium, we used the model with an interaction term between the bacteria and AMR infections adjusted for all pre-defined confounders as the final model to estimate PAF of mortality due to AMR infections. Random effects to account for species variation were then added and results are similar to the final model (Appendix A3). The estimated PAF of mortality due to AMR infections was 16.3% (95% CI: 1.6, 30.9%), resulting an estimate of 105 excess deaths. The bootstrap estimated standard error from the model with random effect is slightly larger, hence a wider 95% CI, than the estimates from the Delta method (from the STATA command: punaf). The AIC value of the model with the random effect is similar to that of the model without the random effect (2343.623 and 2341.623, respectively), hence the model without the random effect was used as the final model to estimate the attributable mortality for each bacterium and each type of infection under this surveillance study.

Figure 2.3. The number of patients by types of infection and by causative bacteria under the survey.



Footnote: BSI: bloodstream infection; LRTI: lower-respiratory tract infection; SSI: surgical-site infection; UTI: urinary-tract infection; OTH: infections at other body sites; ">1 types of infection" refers to more than one type of infection, which is defined as infections that occurred at two or more body sites, which include LRTI with secondary BSI, SSI with secondary BSI, and bacteriuria. *Polymicrobial refers to infections that caused by more than one of the six bacteria included in this survey.

Table 2.1. Characteristics of patients enrolled in the study

Characteristics	Hospital-acquired infections with AMS bacteria (n=658)	Hospital-acquired infections with AMR bacteria (n=1,385)
Sex		
Male	347 (52.7%)	816 (58.9%)
Age (year)		
≤0	19 (2.9%)	65 (4.7%)
1-4	7 (1.1%)	15 (1.1%)
5-14	17 (2.6%)	25 (1.8%)
15-24	37 (5.6%)	65 (4.7%)
25-34	26 (4.0%)	64 (4.6%)
35-44	52 (7.9%)	107 (7.7%)
45-54	102 (15.5%)	179 (12.9%)
55-64	126 (19.2%)	287 (20.7%)
65-80	200 (30.4%)	440 (31.8%)
≥81	72 (10.9%)	138 (10.0%)
Bacteria included in the study		
Staphylococcus aureus	64 (9.7%)	5 (0.4%)
Enterococcus faecium	6 (0.9)	176 (12.7)
Enterococcus faecalis	131 (19.9)	29 (2.1)
Escherichia coli	101 (15.4%)	188 (13.6%)
Klebsiella pneumoniae	73 (11.1%)	267 (19.3%)
Pseudomonas aeruginosa	140 (21.3%)	81 (5.9%)
Acinetobacter baumannii	65 (9.9%)	336 (24.3%)
Polymicrobial	78 (11.9%)	303 (21.9%)
Site-specific infections		
Bloodstream infections (BSI)	105 (16.0%)	184 (13.3%)
Lower-respiratory tract infections (LRTI)	169 (25.7%)	449 (32.4%)
Urinary-tract infections (UTI)	295 (44.8%)	501 (36.2%)
Surgical-site infections (SSI)	43 (6.5%)	112 (8.1%)
Other infections (OTH)	17 (2.6%)	51 (3.7%)
>1 types of infection	29 (4.4%)	88 (6.4%)
Admission wards		
Non-ICU Medical wards (adult/paediatrics/neonates)	221 (33.6%)	494 (35.7%)
Non-ICU Surgical wards (adult/paediatrics/neonates)	243 (36.9%)	497 (35.9%)

Haematology/oncology	28 (4.3%)	34 (2.5%)
Obstetrics/gynaecology	10 (1.5%)	9 (0.7%)
All ICU wards (adult/paediatrics/neonates)	156 (23.7)	351 (25.3%)
Health status on the hospital admission		
Charlson comorbidity index score*	3 (1-5)	3 (1-5)
Referred from other hospitals	445 (67.6%)	939 (67.8%)
SOFA score on the day of admission*	2 (0-4)	2 (1-4)
Parenteral antibiotic usage prior to the first date of culture-positive specimen collection		
Patients on parenteral antibiotic usage prior specimen collection	291 (44.2%)	989 (71.4%)
Cumulative days of exposure to parenteral antibiotic prior specimen collection*	3 (1-9)	9 (4-17)
Health status on the first date of first culture-positive specimen collection		
Admission in the ICU	267 (40.6%)	679 (49.0%)
SOFA score*	1 (0-4)	2 (0-4)
Bacterial load		
Proportion of reporting microbiology results within 3 days since specimen collection	82 (12.5%)	214 (15.5%)
Length of hospital stays		
Days in the hospital prior specimen collection	8 (6-14)	14 (8-22)
Outcome		
28-day mortality	152 (23.1%)	491 (35.5%)
Days since enrolment to death*^	10.5 (4-19)	9 (3-17)

Footnote: AMS: antimicrobial-susceptible; AMR: antimicrobial-resistant *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection among patients who had antibiotic treatment prior sample collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n (%). **Details on the classification is in Appendix A2. ^Statistics were estimated for those who died. ">1 types of infection" refers to patient who had infections that occurred at two or more body sites

Using the final model, adjusted for pre-define confounders and with an interaction term between bacteria and AMR infections, we estimated that if all of the infections were AMS infections, the mortality would be 26.4% (95% CI: 21.7, 31.0%), and if all of the infections were AMR infections, the mortality would be 34.0% (95% CI: 31.3, 36.7%). The absolute risk difference was 7.7 percentage points (95% CI: 2.2, 13.2 percentage

points). The PAF of mortality due to AMR infections accounted for 16.3% (95% CI: 1.2, 29.1%) of deaths involving hospital-acquired bacterial infections under evaluation. We estimated that 105 deaths (95% CI: 7, 187 deaths) out of the 643 observed deaths could be prevented if the HAI infections in this cohort were caused by AMS bacteria. Of all the causative bacteria evaluated in this survey, the number of excess deaths attributable to AMR was highest with CRACI (42 deaths [95% CI: -5, 75]), followed by AMPREfm and 3GCRKP (Table 2.3), adjusted for pre-defined confounders. Of all types of infections in this cohort of patients, UTI was responsible for the highest number of excess deaths attributable to AMR infections (39 deaths [95% CI: -24, 84]) but with a large uncertainty. This is followed by LRTI (37 [95% CI: 1, 67]), and BSI (17 [2, 29]). The estimated excess mortality attributable to AMR infection varied by type of infection (Table 2.4).

To assess the impact of residual confounders on the estimated impact, the E value was used.[307] In brief, the E value represents the minimum strength of association that an unmeasured confounder would need to have with both the treatment and outcome, conditional on the measured covariates, to explain away a treatment-outcome association. The E value was 1.73 for the estimated adjusted odds ratio for the model without an interaction term between resistance and bacteria, and the value was 1.31 for the lower confidence interval. The estimated odds ratio of 1.48 (95% CI: 1.12-1.96) could be explained away by an unmeasured confounder that was associated with both the treatment and the outcome by an odds ratio of 1.73-fold each, above and beyond the measured confounders, but weaker confounding could not do so. The evidence for causality from the calculated E value from the estimate was moderate, as unmeasured confounding would have to have a much larger effect (l.73-fold) than most observed risk factors to explain away the reported association.

Sensitivity analyses were done to assess the impact of AMR on mortality under different assumptions regarding the outcome of the 39 cases who were lost to follow-up. The results suggested that the estimated PAF of mortality due to AMR infections under the assumption that all 39 cases who were lost to follow-up survived would be 15.5%. Excluding the 39 cases who were lost to follow-up from the analysis, the estimated PAF of mortality due to AMR infection would be 15.3%. The results are presented in Appendix 3A.

Table 2.2. Crude 28-day mortality by type of infection and causative bacteria

	S. aureus		*E. faecium*		*E. faecalis*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*A. baumannii*		Polymicrobial*	
	MSSA	**MRSA**	**AMPS**	**AMPR**	**AMPS**	**AMPR**	**3GCS**	**3GCR**	**3GCS**	**3GCR**	**CS**	**CR**	**CS**	**CR**	**AMS**	**AMR**
BSI	18.8% (n=6/32)	25.0% (n=1/4)	0% (n=0/1)	40.0% (n=4/10)	22.2% (n=2/9)	100% (n=1/1)	17.6% (n=3/17)	47.7% (n=21/44)	16.7% (n=2/12)	44.4% (n=24/54)	29.4% (n=5/17)	40.0% (n=2/5)	20.0% (n=3/15)	63.0% (n=34/54)	50.0% (n=1/2)	41.7% (n=5/12)
LRTI	22.2% (n=2/9)	NA	NA	NA	NA	NA	50.0% (n=2/4)	66.7% (n=6/9)	37.8% (n=14/37)	36.4% (n=39/107)	21.6% (n=11/51)	25.0% (n=6/24)	25.7% (n=9/35)	41.5% (n=73/176)	39.4% (n=13/33)	42.1% (n=56/133)
SSI	0% (n=0/5)	NA	0% (n=0/1)	40.0% (n=2/5)	0% (n=0/7)	0% (n=0/3)	16.7% (n=1/6)	18.5% (n=5/27)	0% (n=0/5)	10.0% (n=2/20)	11.1% (n=1/9)	33.3% (n=1/3)	0.0% (n=0/5)	7.7% (n=2/26)	0% (n=0/5)	21.4% (n=6/28)
UTI	14.3% (n=1/7)	0% (n=0/1)	25.0% (n=1/4)	44.5% (n=49/155)	27.3% (n=30/110)	52.0% (n=13/25)	24.6% (n=17/69)	21.1% (n=20/95)	12.5% (n=2/16)	26.5% (n=18/68)	12.3% (n=7/57)	15.9% (n=7/44)	14.3% (n=1/7)	21.8% (n=12/55)	24.0% (n=6/25)	31.0% (n=18/58)
Other	0% (n=0/10)	NA	NA	0% (n=0/3)	100% (n=1/1)	0% (n=0/1)	NA	0% (n=0/1)	0% (n=0/1)	45.5% (n=5/11)	0% (n=0/2)	50.0% (n=2/4)	0.0% (n=0/1)	33.3% (n=4/12)	50.0% (n=1/2)	47.4% (n=9/19)
>1 body sites	0% (n=0/1)	NA	NA	50.0% (n=1/2)	25.0% (n=1/4)	NA	40.0% (n=2/5)	58.3% (n=7/12)	0% (n=0/2)	57.1% (n=4/7)	25.0% (n=1/4)	0% (n=0/1)	100% (n=2/2)	38.5% (n=5/13)	36.4% (n=4/11)	50.9% (n=27/53)

Footnote: *Polymicrobial refers to infections that were caused by more than one of the six bacteria included in this survey.

Table 2.3. Estimated population attributable fraction (PAF) of mortality due to AMR infections by causative bacterium adjusted for pre-defined confounders.

	Number of cases	Number of deaths	PAF of mortality due to AMR infection*	Excess deaths
Methicillin-susceptible *Staphylococcus aureus*	64	9	NA	NA
Methicillin-resistant *Staphylococcus aureus*	5	1	0.5% (-13.4, 12.7%)	0 (-1, 1)
Ampicillin-susceptible *Enterococcus faecium*	6	1	NA	NA
Ampicillin-resistant *Enterococcus. faecium*	175	56	34.5% (-204.9, 85.9%)	20 (-117, 49)
Ampicillin-susceptible *Enterococcus faecalis*	131	34	NA	NA
Ampicillin-resistant *Enterococcus faecalis*	30	14	11.5% (-2.0, 23.3%)	6 (-1, 11)
Third-generation cephalosporin-susceptible *Escherichia coli*	101	25	NA	NA
Third-generation cephalosporin-resistant *Escherichia coli*	188	59	11.6% (-15.8, 32.4%)	10 (-13, 27)
Third-generation cephalosporin-susceptible *Klebsiella pneumoniae*	73	18	NA	NA
Third-generation cephalosporin-resistant *Klebsiella pneumoniae*	267	92	15.1% (-18.9, 39.3%)	17 (-21, 43)
Carbapenem-susceptible *Pseudomonas aeruginosa*	140	25	NA	NA
Carbapenem-resistant *Pseudomonas aeruginosa*	81	18	10.9% (-11.4, 28.7%)	5 (-5, 12)
Carbapenem-susceptible *Acinetobacter baumannii*	65	15	NA	NA
Carbapenem-resistant *Acinetobacter baumannii*	336	130	29.1% (-3.5, 51.5%)	42 (-5, 75)
Antimicrobial-susceptible polymicrobial infection	78	25	NA	NA
Antimicrobial-resistant polymicrobial infection	303	121	4.1% (-22.6, 25.0%)	6 (-33, 36)
Total	**2043**	**643**	16.3% (1.2, 29.1%)	105 (7, 187)

Footnote: *Population Attributable Fraction (PAF) for mortality is defined as the mortality that could be prevented if the specific infections were caused by the AMS bacterium, adjusted for confounding factors.

Table 2.4. Estimated population attributable fraction (PAF) of mortality and deaths due to AMR infections by type of infection, after adjusting for pre-defined confounders in the final model.

	Observed 28-day mortality, % (n)	Predicted mortality if the infections were AMS infections (95% CI)	PAF of mortality due to AMR infections (95% CI)*	Excess deaths attributable to AMR infections (95% CI)
Bloodstream infections	39.5% (114/289)	33.6% (27.0, 40.2%)	14.8% (2.2, 25.7%)	17 (2, 29)
Lower-respiratory tract infections	37.4% (231/618)	31.4% (25.3, 37.6%)	15.9% (0.6, 28.8%)	37 (1, 67)
Surgical-site infections	12.9% (20/155)	10.3% (5.7, 14.9%)	20.4% (1.2, 35.8%)	4 (0, 7)
Urinary-tract infections	25.4% (202/796)	20.5% (13.3, 27.7%)	19.3% (-11.9, 41.8%)	39 (-24, 84)
Other infections	32.4% (22/68)	27.3% (16.6, 38.0%)	15.7% (-1.8, 30.1%)	3 (0, 7)
>1 type of infections	46.2% (54/117)	42.2% (31.9, 52.4%)	8.7% (-5.8, 21.1%)	5 (-3, 11)
Total	**31.5% (643/2043)**	**26.4% (21.7, 31.0%)**	**16.3% (1.2, 29.1%)**	**105 (7, 187)**

Footnote: *Population Attributable Fraction (PAF) for mortality is defined as mortality that could be prevented if each of the specific infections were caused by AMS bacteria, adjusted for confounding factors.

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2.5 Discussion

In this study I estimated the PAF of deaths caused by AMR bacteria for common infections. The study provides reliable data for population health indicators through a systematic approach to inform policy for controlling and preventing this global public health threat. The estimated mortality attributable to AMR infections is adjusted for underlying risk factors for death. We estimated that 16.3% of deaths involving hospital-acquired bacterial infections could be prevented if all of the AMR infections were AMS infections. Of the bacteria under evaluation, CRACI had the largest impact on excess deaths in our setting.

The estimated mortality attributable to AMR infections in this study cohort is relatively higher than typically observed in HICs. In 1992, Martone et al. reported 4.37% of deaths attributable to nosocomial BSI annually in the US hospitals.[240] A more recent study based in Chicago suggested the excess mortality attributable to AMR infections compared to AMS infections was 6.5%.[302] This estimate was used to calculate the 35,000 excess deaths in the US CDC report on AMR infections in 2019.[301] A recent study based on data from hospitals in European countries estimated the burden of antibiotic resistant infection using an estimate of 7.1-20.3% for the case fatality proportion among patients with BSI.[20] The differences in estimated excess mortality attributable to AMR infection between the current study and studies from HICs could be partly due to patient management, antibiotic resistance patterns, antibiotic usage, and patient characteristics. There are limited studies from LMICs reporting the number of excess deaths due to AMR infections. A previous study based in hospitals in Africa reported 33% crude in-patient mortality among patients with *E. coli* BSI.[308] The estimated hazard ratio in that study suggested the impact of 3GCREC versus the susceptible strain on mortality was above the value 1 but the 95%

confidence interval was wide.[308] However, differences in origin of infection, confounders adjusted for in the analysis, and clinical setting make direct comparison with the current study inappropriate.

There are differences in the epidemiology of AMR bacteria by type of infection between this study cohort and that in HICs. For instance, in European countries, a ratio of 1.38 for UTI to BSI among patients infected with CRPA has been reported in HICs,[20] whereas in this study cohort that ratio is as high as 8.80. A ratio of 4.67 for UTI to BSI caused by CREC was previously observed in hospitals of HICs,[20] but this ratio is much lower (ratio: 2.16) in the current study cohort. Moreover, *E. faecium* was a common causative bacterium of UTI in this cohort.

An important strength of this study is that we were able to collect detailed information about the studied patients and adjust for important confounders to estimate the excess mortality and PAF of mortality due to AMR infections. There are few studies from LMICs that attempted to systematically evaluate and analyse the excess fraction of deaths due to AMR infections for each type of infection. One reason is that clinical data about patients are often not recorded electronically and it is difficult to screen for site-specific infections using retrospective data. Hence, often systematic reviews on the relative risk from studies varying by design and by analysis methods had to be summarised to estimate excess mortality due to AMR infections for all types of infections other than BSI. Prospective studies that collected patient information and followed the cohort over a period of time to record outcome have been done, but most focused on a single type of infection, particularly BSI. An important advantage of examining the epidemiology of AMR infections for each and all types of infections in a complete cohort is that this allows

estimates of burdens of AMR to be adjusted for confounders systematically. Another strength of this study is that we recorded the 28-day outcome of almost all patients enrolled in the study, and this avoided the analysis issue of competing outcomes when patients are discharged from hospitals during the follow-up period.

There was substantial uncertainty about the PAF of mortality due to AMR (i.e., 95% confidence interval of the estimated PAF of mortality due to AMR infections covered the negative value) for the individual organisms evaluated in this survey. This is largely due to the study being underpowered to detect the difference for all types of infection and all AMR bacteria. Although the crude mortality of patients with HAI in this cohort is high, a larger sample size would still be needed especially if the difference in mortality of AMR infections and AMS infections for each type of infection and each AMR bacteria is small. However, the estimated 105 excess deaths that could be prevented if the HAI infections in this cohort were AMS infections is clinically important.

It is also possible that AMR infections did not cause more deaths compared to AMS infections. A reason could be empirical antibiotic treatments may influence the mortality, and when the treatments failed to cover the causative bacteria the differential mortality impact for AMR and AMS infections would be attenuated. Further studies on antibiotic treatment using mediation analysis on the same set of data would be useful to understand the mechanism of AMR infection influencing mortality through empirical antibiotic treatment. Additionally, molecular studies to compare virulent factors and body's immune responses between patients with AMR and AMS infections could help to explain the relationship between AMR infections and mortality.

2.6 Limitations

There are limitations in this study. First, additional analysis to estimate the excess deaths of AMR infections compared to that of no infection will be needed to inform the upper bound of burden of AMR infections. The use of AMS infection as a comparator in this study gave a lower bound of the impact of AMR infection on morality.[214] Second, there could be residual confounding and if present it may influence the estimated impact of AMR infections on excess mortality. Third, as the study was performed in a single-site, the results may not be generalisable to other settings where patient management and practices (including frequency and timing) of collecting clinical samples for microbiology culture may differ from the study hospital.

2.7 Implications

Health burden of AMR in LMICs could be underestimated because it was estimated by using the parameters, such as the excess mortality attributable to AMR for each and all types of infection, derived from HICs. Our study shows that the excess mortality and deaths due to AMR infections in LMICs could be much higher than that observed in HICs. We propose that regular estimating and monitoring of excess mortality and deaths attributable to AMR infections in LMICs should be implemented into national action plans on AMR in each LMIC. Understanding the extent of the health problem and capacity to monitor the excess deaths attributable to AMR infections in LMICs would allow policy makers at the local, national and global levels to implement and adjust actions against AMR infections effectively. Moreover, such data can help policy makers to identify targets of interventions.

2.8 Conclusions

Our findings have implications for decisions about prioritising targets of interventions to control AMR infections in hospitals with similar AMR prevalence, patient management, and diagnostic capacity as that in this study hospital.

3

Automating the generation of AMR surveillance reports

3.1 Abstract

Background

Many hospitals in low and middle-income countries (LMICs) lack well-trained personnel with adequate skills in the use of statistical software in order to analyse the routinely collected data and to generate reports on cumulative antimicrobial susceptibility test (cAST) results regularly and promptly. This chapter describes an application developed to support the generation of AMR surveillance reports automatically at the local level in LMICs.

Methods

The R programming language was used to developed an offline application to generate standardized AMR surveillance reports from routinely available microbiology and hospital data files. The data analysis procedure and report content were developed based on the recommendations of WHO GLASS. The application was tested in Microsoft Windows 10 and 7 using open-access example data sets. The application was then independently tested in seven hospitals in Cambodia, Lao People's Democratic Republic (PDR), Myanmar, Nepal, Thailand, the United Kingdom, and Vietnam.

Results

An AutoMated tool for Antimicrobial resistance Surveillance System (AMASS) was developed to support clinical microbiology laboratories to analyse their microbiology and hospital data files (in CSV or Excel format) onsite and to promptly generate AMR surveillance reports (in PDF and Excel formats). The data files could be those exported from WHONET and/or other similar laboratory information system (LIS). The automatically generated reports contain only summary data with no patient identifiers. The AMASS application is downloadable from www.amass.website, which was created as a resource hub for user and a communication channel. The participating hospitals tested the application and deposited their AMR surveillance reports in an open-access data repository. Overall, proportions of patients having *Escherichia coli* bacteraemia caused by 3rd generation cephalosporin resistant isolates (3GCREC) ranged from 19%-85%. The incidence rates of 3GCREC bacteraemia ranged from 283 to 2,737 per 100,000 tested patients among participating hospitals with available data on negative culture. In general, it took about 1 to 3 hours to input the data dictionary for the first time. This data dictionary files could then be re-used when the raw microbiology data file or hospital admission data file was updated. AMASS took about 1 to 3 minutes to run and automatically generate an AMR surveillance report using the local data and local hospital computers. The application was tested with data containing non-English (Latin and non-Latin characters) languages in Vietnam and in Thailand.

Interpretation

AMASS can be an add-on tool for clinical microbiology laboratories with routinely available and electronical data. The application can support the usage of local data to improve the understanding on the local epidemiology of AMR infections in resource-

limited settings through generation of AMR surveillance reports on a regular basis. Moreover, the tool can empower the sharing of AMR data, as no patient identifiable information is included in AMR surveillance report and summary data generated.

3.2 Introduction

Many hospitals in LMICs lack well-trained personnel with adequate skills in the use of statistical software in order to analyse routinely available data and to generate reports on cAST results regularly and promptly. There are open-access LISs,[309] and microbiology laboratory database software, including WHONET,[55] that are useful for recording and analysing the data, and generating figures to support the generation of AMR surveillance reports for hospitals in low and middle-income countries (LMICs). There are, however, no freely-available automated system that can generate summary reports for immediate use and be operated by non-technical staff.

In addition, to generate AMR surveillance reports stratified by the origin of infection, additional data on hospital admission are frequently needed.[21,155,310,311] This is because hospital admission dates are not generally collected in microbiology laboratory data. In many hospital settings, microbiology and hospital admission data are held in separate computers or systems with restricted access. Even in high-income countries, many hospitals lack well-trained clinical microbiologists, epidemiologists or data experts with adequate skills in statistical software (such as R, SAS, SPSS and STATA) to merge the separated databases, de-duplicate the data and generate reports stratified by the origin of infection.

Some high and middle-income countries have national surveillance systems that enable individual hospitals and healthcare settings to send data to a national organization or a centre to generate national level AMR surveillance reports. Examples include the National Electronic Disease Surveillance System (NEDSS) in the United States; JANIS in Japan;[249] National Antimicrobial Resistance Surveillance Thailand (NARST);

CEASAR network; and EARS-Net for European countries. WHO GLASS generates global level AMR surveillance reports. However, sending data to an external organization or centre to perform analysis risks breaching individual patient data confidentiality. Also, it takes time for local hospitals to prepare anonymous data, send the data, and wait for the report specific to their own hospitals, if generated at all. Commercial LISs are often expensive and would often require extra costs if users need additional functions in data analysis and report generation.

This chapter describes an application, termed the AutoMated tool for Antimicrobial resistance Surveillance System (AMASS), which was developed to support local hospitals to independently analyse routinely collected electronic data and rapidly generate AMR surveillance reports. AMASS was tested in seven hospitals in Cambodia, Lao PDR, Myanmar, Nepal, Thailand, the United Kingdom and Vietnam. AMR surveillance reports from the hospitals were deposited in Figshare, which is an open-access platform.

3.3 Methods

3.3.1 Design of the application

The application was built in R (version 3.6.2), which is a free software environment, and then placed within a user-friendly interface. This is to ensure the application can be fully open access and users can operate it without the need to understand the R program. In the downloadable package, both the R portable (version 3.4.3) and RStudio (version 1.1.423) were include. This is to ensure the application, once downloaded, can be operated without the need to install R or any other program.

3.3.2 Data processing and statistical analysis

A schematic overview of input data, data processing, statistical analyses and output is shown in Figure 3.1. Input data are microbiology data files (in CSV or Excel formats) with or without hospital data files, which can be exported from WHONET or a LIS with data export capacity. The data processing and analysis algorithms, including data-deduplication, were developed based on the WHO GLASS recommendations.[100,247] The reports were designed to have a similar format to the WHO GLASS 2018 report.[312]

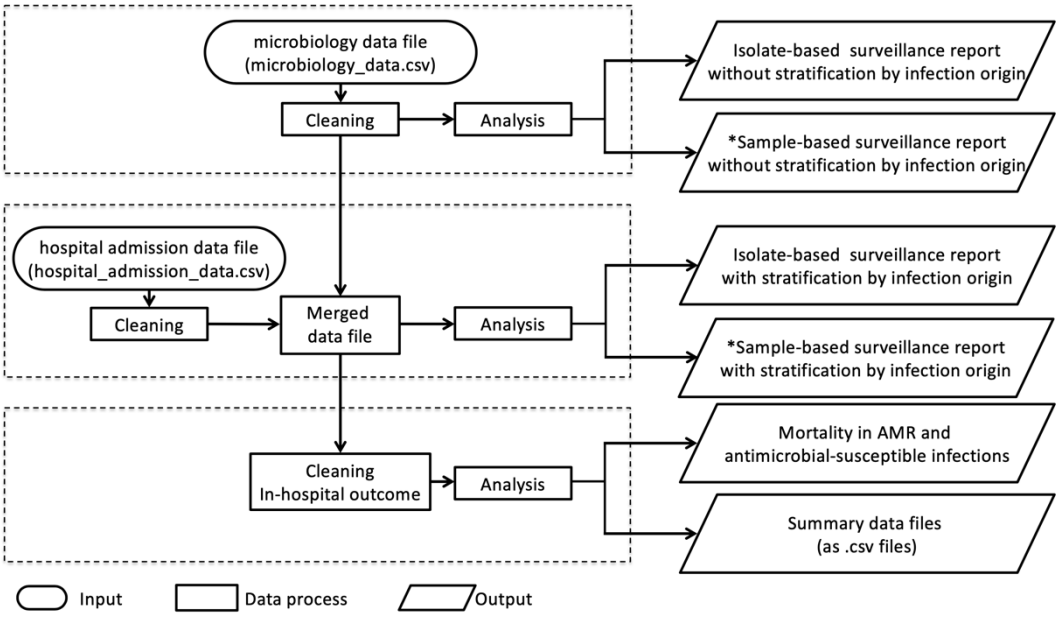
The application de-duplicates the data by including only the first isolate per sample type per pathogen per survey period for each patient.[100] The current version of the application includes only blood culture specimens, and eight pathogens (Table 3.1). The list of pathogen-antibiotic combinations was modified based on the WHO GLASS priority list.[77,247] Organisms that are common causes of bacteraemia[21,22] and in the 2015 global priority list of AMR bacteria were included.[77]

3.3.3 Definitions

Infections are stratified into community-origin or hospital-origin using hospital admission dates and specimen collection dates, when the data are available.[100,247] In cases where users have data on origin of infection assigned by the attending physicians or infection control team of the hospital, those categorizations to stratify the infection into community-origin or hospital-origin are used by the application. Alternatively, in cases where such a variable is absent from the data set, the definitions described by the WHO GLASS for community-origin and hospital-origin infection is used. Patients with community-origin infection are defined as those with the first specimen culture positive for the pathogen taken in the outpatient setting or on the first or second day of

hospitalization.[100,247] Patients with hospital-origin infection are defined as those with the first specimen culture positive for the pathogen taken on hospital day three or later.[100,247]

Figure 3.1. An overview on the flow of data processing and analyses performed by the AutoMated tool for antimicrobial resistance Surveillance system (AMASS).



Footnote: This figure was created by myself as the supplementary figure 1 for the published paper. The paper is an open-access article (doi:10.2196/19762).[313]

The data de-duplication and the calculations for prevalence and incidence rates are based on the recommendations of the WHO GLASS, and are as follows:[100,247]

Proportion of AMR pathogen:

$$\text{Proportion of patients with positive blood cultures who have resistant strains (\%, modified from WHO GLASS[100])} = \frac{\text{Number of patients with blood culture positive for resistant strains of bacterial species under surveillance (per pathogen per specimen per survey period)}}{\text{Total number of patients with blood culture positive for bacterial species under surveillance (per pathogen per specimen per survey period)}}$$

Proportion of AMR pathogen stratified by origin of infection:

$$\text{Proportion of patients with positive blood culture who have resistant strains per type of origin of infection (\%, modified from WHO GLASS[100])} = \frac{\text{Number of patients with blood culture positive for resistant strains of bacterial species under surveillance (per pathogen per specimen per survey period per origin of infection)}}{\text{Total number of patients with blood culture positive for bacterial species under surveillance (per pathogen per specimen per survey period per origin of infection)}}$$

Incidence of AMR pathogen:

$$\text{Frequency of patients with blood culture positive for pathogen (per 100,000 tested patients, modified from WHO GLASS}^{100}\text{)} = \frac{\text{Number of patients with blood culture positive for bacterial species in the population tested during reporting period * 100,000 (per pathogen per specimen per survey period)}}{\text{Total number of patients with blood culture performed during the reporting period}}$$

$$\text{Frequency of patients with blood culture positive for resistant strains (per 100,000 tested patients, modified from WHO GLASS}^{100}\text{)} = \frac{\text{Number of patients with blood culture positive for resistant strains of bacterial species under surveillance during reporting period * 100,000 (per pathogen per specimen per survey period)}}{\text{Total number of patients with blood culture performed during the reporting period}}$$

Incidence of AMR pathogen stratified by origin of infection:

$$\text{Frequency of patients with blood culture positive for pathogen (per 100,000 tested patients, modified from WHO GLASS}^{100}\text{)} = \frac{\text{Number of patients with blood culture positive for bacterial species in the population tested during reporting period * 100,000 (per pathogen per specimen per survey period per origin of infection)}}{\text{Total number of patients with the first blood culture performed } \underline{\textbf{within the first two calendar days of admissions}} \text{ during the reporting period}}$$

$$\text{Frequency of patients with blood culture positive for resistant strains (per 100,000 tested patients, modified from WHO GLASS}^{100}\text{)} = \frac{\text{Number of patients with blood culture positive for resistant strains of bacterial species under surveillance during reporting period * 100,000 (per pathogen per specimen per survey period per origin of infection)}}{\text{Total number of patients with the first blood culture performed } \underline{\textbf{after the first two calendar days of admission}} \text{ during the reporting period}}$$

In cases where data are available, an additional report on mortality involving AMR and non-AMR infections is generated. The measure of mortality includes deaths caused by or contributed to other underlying and intermediate causes.[1] Therefore, the term "involving AMR infections" is used, in accordance with that used by the UK Office for National Statistics.[234,235]

Table 3.1. The list of pathogen-antibiotic combinations modified from the WHO GLASS priority list and which are included in the AutoMated tool for Antimicrobial resistance Surveillance System (AMASS).

Type of Specimen	Pathogen	Antibacterial agents
Blood	*Staphylococcus aureus*	Methicillin* Vancomycin Clindamycin
Blood	*Enterococcus* spp.	Ampicillin Vancomycin Teicoplanin Linezolid Daptomycin
Blood	*Streptococcus pneumoniae*	Penicillin G Oxacillin Co-trimoxazole Ceftriaxone Cefotaxime Erythromycin Clindamycin Levofloxacin
Blood	*Salmonella* spp.	Ciprofloxacin Levofloxacin Ceftriaxone Cefotaxime Ceftazidime Imipenem Meropenem Ertapenem Doripenem
Blood	*Escherichia coli*	Gentamicin Amikacin

		Co-trimoxazole Ampicillin Ciprofloxacin Levofloxacin Cefpodoxime Ceftriaxone Cefotaxime Ceftazidime Cefepime Imipenem Meropenem Ertapenem Doripenem Colistin
Blood	*Klebsiella pneumoniae*	Gentamicin Amikacin Co-trimoxazole Ciprofloxacin Levofloxacin Cefpodoxime Ceftriaxone Cefotaxime Ceftazidime Cefepime Imipenem Meropenem Ertapenem Doripenem Colistin

Blood	*Pseudomonas aeruginosa*	Ceftazidime Ciprofloxacin Piperacillin/tazobactam Gentamicin Amikacin Imipenem Meropenem Doripenem Colistin
Blood	*Acinetobacter* spp.	Tigecycline Minocycline Gentamicin Amikacin Imipenem Meropenem Doripenem Colistin

Footnote: This table was in supplementary material in the published paper

3.3.4 Creating example data sets for users

Two example data sets are provided in the downloadable package of the AMASS application. The first example data set is the open-access demonstration file from WHONET.[309] The second example data set is a synthetic data set. The purpose of this hypothetical data set is for users to test the application and to visualize the data format required. Two hypothetical data files (microbiology_data.xlsx and hospital_admission_data.xlsx) were generated using the summary statistics in the AMR surveillance report from Sunpasitthiprasong Hospital in 2015.[314] The key variables generated were admission date, discharge date, gender, age (in year), in-hospital

discharge outcome, specimen date, specimen type, organism isolated, and antibiogram results. The admission date was sampled from a uniform distribution ranged between 1st January 2016 and 31st December 2016. For bloodstream infections of community-origin, we assumed that the first blood specimen culture positive for the pathogen was collected on the first calendar day of hospitalization. For bloodstream infections of hospital-origin, we assumed that the first blood specimen culture positive for the pathogen was collected on the fourth day of hospitalization. The data was generated using STATA, version 15.1 (StataCorp LP, College Station, Texas, USA).

3.3.5 Testing AMASS using hospital data

The application was tested in seven hospitals: Angkor Hospital for Children in Siem Reap, Cambodia; Mahosot Hospital in Vientiane, Lao PDR; North Okkalapa General and Teaching Hospital in Yangon, Myanmar; Patan Hospital in Kathmandu, Nepal; Sunpasitthiprasong Hospital in Ubon Ratchathani, Thailand; St Thomas' Hospital in London, United Kingdom; and the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. The hospitals were selected because routinely collected microbiology data are available. Moreover, the hospitals have prior experience in data quality control.

The study was approved by the Oxford Tropical Research Ethics Committee, University of Oxford, and local Ethics Committees in the participating institutions.

3.4 Results

3.4.1 Overview of AMASS

The AMASS application was developed to support clinical microbiology laboratories to automatically analyse hospital local data files (in CSV or Excel formats) and generate

AMR surveillance reports (in PDF and Excel formats) promptly (Figure 3.2). There are five key features of the AMASS. The application is:

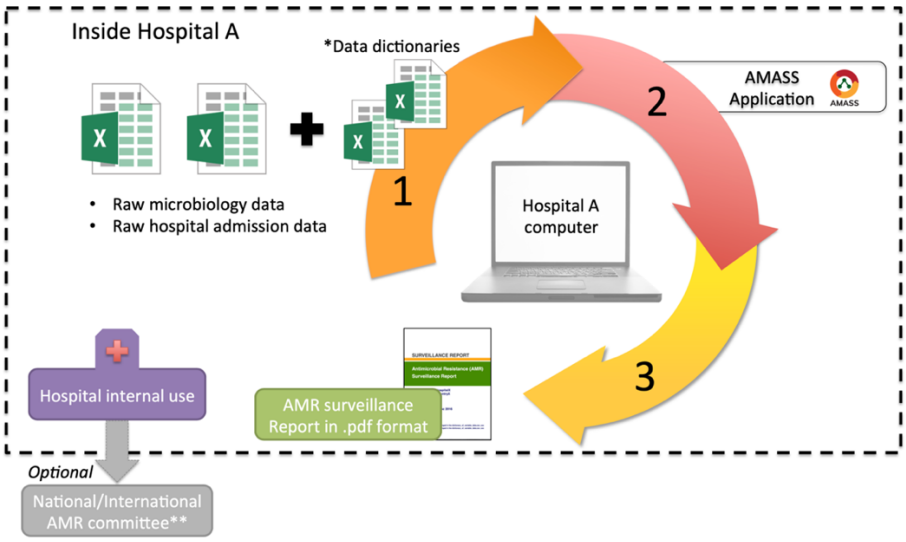
1. Open access (can be downloaded from www.amass.website and AMASS is under Creative Commons Attribution 4.0 International license (CC BY 4.0));
2. User-friendly (only requires users to double-click on the AMASS icon to perform data processing and analysis);
3. Highly compatible (AMASS reads CSV and EXCEL files that can be easily exported from WHONET, LIS, or electronic health record (eHR) systems);
4. High data security (Once downloaded AMASS can operate without Internet access. All data processing and report generation is done locally, no raw data needs to be shared, and the final reports and output summary data files contain no patient identifier);
5. Effective in generating sharable output (Summary reports are in PDF format, which can be readily printed and reviewed. Also, as the report contains no individual patient identifiers, it can be readily shared).

3.4.2 AMR surveillance report generated by AMASS

AMASS uses a tier-based approach to generate an AMR surveillance report (Table 3.2). There are in total six sections. In cases when only the microbiology data file containing the results of culture positive samples is available, only section one and two are automatically generated. In cases when data on admission dates are also available or a variable to classify infection into community-origin or hospital-origin infection is available, section three is automatically generated. In cases where the microbiology data file contains culture negative specimens (no microbial growth), section four is generated. Section five is section four stratified by origin of infection and is generated when data on admission dates are also available or a variable to classify infection into community-

origin or hospital-origin infection is available. Finally, in cases when mortality data is also available, section six is automatically generated.

Figure 3.2. An overview of how AMASS operates.



Footnote: *Users can configure the two data dictionaries files to match with the information in their microbiology data and hospital admission data files. **The outputs (including an AMR surveillance report and summary data) contain no patient identifiable information. As the files are locally saved into the user's computer, the decision to share the output with the national or international AMR organization is solely within the jurisdiction of the user (i.e., hospital, surveillance site).

3.4.3 Log files generated by AMASS

AMASS generates two log files. The first log file is automatically generated in PDF format. This file is for users to validate the input data used by AMASS to generate the AMR surveillance report. The information in this log file includes the total number of records analysed, age distribution, number of missing values and total number of isolates per organism in the raw microbiology data file.

A second log file is automatically generated in plain text format. This log file can be sent to programmers, R users, statisticians, or the AMASS development team when technical issue occurs.

Table 3.2. A summary on the sections generated by AMASS and the corresponding input requirements for each section.

Name of the section in AMR surveillance report	Input requirement	Description
Section [1]: Data overview **Section [2]: Isolate-based surveillance report**	1. microbiology_data*† 2. dictionary_for_microbiology_data.xls	This section is created by default (i.e., even in cases when only microbiology data file with results of culture positive samples is available)
Section [3]: Isolate-based surveillance report with stratification by infection origin	1. microbiology_data*† 2. dictionary_for_microbiology_data.xlsx 3. hospital_admission_data* (must contain include patient identifier that could be matched to those in microbiology_data file) 4. dictionary_for_hospital_admission_data.xlsx	This section is created only when admission date or a variable to classify infections by origin of infection is available
Section [4]: Sample-based surveillance report	1. microbiology_data*† (must contain both data of culture positive and negative specimens) 2. dictionary_for_microbiology_data.xlsx	This section is generated only when data of culture negative specimens (no microbial growth) are available in the microbiology data file.
Section [5]: Sample-based surveillance report with stratification by infection origin	1. microbiology_data*† (must contain both data of culture positive and negative specimens) 2. dictionary_for_microbiology_data.xlsx 3. hospital_admission_data* (must contain include patient identifier that could be matched to those in microbiology_data file) 4. dictionary_for_hospital_admission_data.xlsx	This section is generated only when data of culture negative specimens (no microbial growth) are available in the microbiology data file, and admission date or a variable to classify infections by origin of infection is available
Section [6]: Mortality involving AMR and antimicrobial-susceptible infections	1. microbiology_data*† (must contain both data of culture positive and negative specimens) 2. dictionary_for_microbiology_data.xlsx 3. hospital_admission_data* (must contain include patient identifier that could be matched to those in microbiology_data file) 4. dictionary_for_hospital_admission_data.xlsx	This section is generated only when mortality data are available
Methods	None	This section is created by default
Acknowledgements	None	This section is created by default

Footnote: *AMASS reads this data in both CSV and Excel formats; †The data files must be in wide format and contain variables including patient identifier, specimen collection date, specimen type, culture result, and antimicrobial susceptibility test interpretation per each antibiotic (i.e., each row contains data from a single clinical sample).

3.4.4 Support and resources for users of AMASS

A website (www.amass.website) was created for users. This website serves as the resource hub for user to access the application, tutorial documents, and reading materials related to AMR surveillance system. Short tutorial videos were created to guide users

through the process of operating the application. Currently, the videos are in English language and we are planning to translate the content into relevant local languages to ensure they can be used locally. The user can also access various versions of AMASS through the links provided in the website.

During the implementation phase, I conducted AMASS workshops in two hospitals. The first workshop was hosted by North Okkalapa General and Teaching hospital in Yangon, Myanmar, and co-organised by the Myanmar Oxford Clinical Research Unit (MOCRU). An introductory session and an intensive training session on the AMASS application were delivered. The hospital senior superintendent, deputy superintendent, and heads of different departments joined the introductory session, which sparked discussions on hospital data management systems during the session, and the heads of departments invited more than 20 more staff to join the intensive training session. The hospital used the AMASS application we developed and shared their antimicrobial resistance surveillance report on an open-access platform. The second workshop was hosted in Angkor Hospital for Children in Siem Reap, Cambodia, and organized by the Cambodia Oxford Medical Research Unit (COMRU). The hospital director, medical director and head of information technology (IT) of the hospital joined the workshop. We received positive feedbacks from the attendees and AMASS was well-received by the hospital.

3.4.5 Seven participating hospitals testing AMASS

Data storage systems in the seven hospitals

Data storage systems varied across the hospitals. Angkor Hospital for Children used ACORN LIS, a bespoke program written in Microsoft Access.[315] Mahosot Hospital used a local LIS which was also based on Microsoft Access.[316] Sunpasitthiprasong Hospital

used the MLAB programme.[306] St Thomas' used ICNET® (https://www.icnetsoftware.com), which is a commercial clinical surveillance software package. North Okkalapa General and Teaching Hospital used WHONET 2019 (a modernized version of WHONET 5.6).[309] Patan Hospital and the Hospital for Tropical Diseases both used a bespoke in-house LIS based on a MySQL system.

Data availability in the seven hospitals

All participating hospitals deposited their output files and their data dictionary files at Figshare.[314,317-322] Angkor Hospital for Children, North Okkalapa General and Teaching Hospital, and Sunpasitthiprasong Hospital had all six sections of the AMR surveillance reports, as microbiology and hospital admission data, data on negative cultures, and in-hospital discharge outcome data were available. Mahosot Hospital and Patan Hospital had sections one and two in the AMR surveillance report, as only microbiology data on positive cultures was available to test in AMASS. St Thomas' Hospital had sections one, two, three, and six in the AMR surveillance report because negative culture data were not available to test in AMASS. The Hospital for Tropical Diseases had sections one, two and four available, as hospital admission data was not available to test in AMASS.

Proportion and frequency of AMR infections in the seven hospitals

Table 3.3 is a summary of the proportions and incidence rates of the some of the key pathogen-antibiotic combinations. Overall, proportions of patients having *Escherichia coli* bacteraemia caused by 3rd generation cephalosporin resistant isolates (3GCREC) ranged from 19%-85%. The incidence rates of 3GCREC bacteraemia ranged from 283 to 2,737 per 100,000 tested patients among participating hospitals with available data on negative culture. The proportion of patients having *Acinetobacter* spp. bacteraemia

caused by carbapenem resistant (CRACI) isolates ranged from 0%-100%. The incidence rates of CRACI ranged from 0 to 818 per 100,000 tested patients. In one of the seven hospitals, no *Acinetobacter* spp. isolate identified was resistant to a carbapenem (out of six *Acinetobacter* spp. isolates identified). In another hospital, two isolates of *Acinetobacter* spp. were identified from their blood culture data and both were resistant to carbapenem.

3.4.6 Performance of AMASS

Inputting data dictionary files for the first time took about 1 to 3 hours. However, the data dictionary files could be stored and re-used when the raw microbiology data file or hospital admission data file was revised or updated. For instance, North Okkalapa General and Teaching Hospital used the data dictionary file from the example data set (in Example_Dataset_1_WHONET folder), which was generated to comply with WHONET exported data (in .XLSX format). This saved time and efforts needed to complete the data dictionaries for the hospital. The data dictionaries generated for each of the seven hospitals are uploaded in Figshare and can be re-used by the hospitals when additional raw data files or hospital admission data files are available in the future.

In general, AMASS took about 1 to 3 minutes to run and automatically generate an AMR surveillance report using the local data and local hospital computers. AMASS has been tested in Microsoft Windows 10, and tested with data containing a non-English (Latin and non-Latin characters) language. For example, “blood_specimen” was recorded as “Cấy máu định danh bằng máy tự động” in the raw microbiology Excel file at Hospital for Tropical Diseases, Vietnam. Another example is “Male” recorded as “ชาย” and

"Female" recorded as "หญิง" in the raw hospital admission Excel file at Sunpasitthiprasong Hospital, Thailand.

Table 3.3. A summary of proportions and incidence rates of bacteraemia caused by resistant isolates reported in the AMR surveillance reports generated by the seven hospitals independently using local data sets. The list of pathogen-antibiotic combinations is modified based on the WHO GLASS priority organism list.

Key pathogen-antibiotic combinations	Proportion of patients with bacteraemia caused by resistant isolates (minimum-maximum)	Incidence rates of bacteraemia caused by resistant isolates measuring in cases per 100,000 tested patients (minimum-maximum)
Carbapenem-resistant *Acinetobacter* spp. (CRACI)	0%-100%*	0-818*
Third-generation cephalosporin-resistant *E. coli* (3GCREC)	19%-85%	283-2737
Carbapenem-resistant *E. coli* (CREC)	0%-46%**	0-1474**
Vancomycin-resistant *Enterococcus* spp. (VRE)	0%-17%	0-16
Third-generation cephalosporin-resistant *K. pneumoniae* (3GCRKP)	4%-79%	26-2667
Carbapenem-resistant *K. pneumoniae* (CRKP)	0%-54%***	0-983***
Carbapenem-resistant *P. aeruginosa* (CRPA)	0%-67%****	0-281****
Methicillin-resistant *S. aureus* (MRSA)	10%-68%	43-818

Footnote: *One of the seven hospitals had no *Acinetobacter* spp. isolates resistant to carbapenems, and one hospital identified two isolates of *Acinetobacter* spp. from their blood culture data and both were resistant to carbapenems. **Three hospitals had no *E. coli* isolates resistant to carbapenems. ***Three hospitals had no *K. pneumoniae* isolates resistant to carbapenems. ****One hospital had no *P. aeruginosa* isolates resistant to carbapenems.

3.5 Discussion

AMASS is an open-access, offline and easy-to-use application that allows hospitals, especially in LMICs, to automatically generate AMR surveillance reports from routinely collected electronic microbiology data. AMASS was tested in seven hospitals. The hospitals shared and deposited their reports and summary data files in an open-access data repository website. Routine generation and sharing of AMR surveillance reports (i.e., cumulative antimicrobial susceptibility reports or antibiograms) in open-access data repositories from hospitals with a microbiology laboratory should be supported worldwide.[323]

AMASS empowers data sharing by reducing the time and effort needed to prepare the summary data and reports without the risks of breaching individual patient data confidentiality. This is because AMASS can analyse the data and generate the reports promptly without needing to transfer raw data files to any party outside of their hospital. The auto-generated AMR surveillance reports are in PDF format which can be reviewed and shared locally and immediately. This is important to improve understanding of the local AMR burden and to inform local patient management. The PDF report and summary Excel files contain no individual patient-level information and can be shared with national and international organizations to support action plans on AMR. Any attempt to compare AMR surveillance reports among different hospitals should be done cautiously, as factors including the type of hospital, blood culture utilisation rates and practices, and patient characteristics may influence the estimated prevalence and incidence rates of AMR infections.[74,312,324] An AMR surveillance report should clearly present denominators used to calculate incidence rates. Selecting an appropriate denominator that represents the local setting is important for estimating incidence rate of

AMR, but remains challenging.[32] AMASS calculates the total number of the tested population which is used as the denominator in Section four and Section Five of the output report based on the WHO GLASS recommendation; this denominator will not be available without data on negative cultures.

AMASS uses currently recommended analytical approaches to generate the summary data and reports. All of the code is open-access, verifiable and modifiable. AMASS can also be expanded and tailored based on the local requirements. For instance, notifiable bacterial infections that are important for a local setting can be included by revising the R code provided in the AMASS package. Analytical methods can also be constantly updated and improved over time.

AMASS is an add-on automatised report generating programme that can be easily used, even by hospital users with limited information technology skills. It does not replace WHONET, LIS, quality assurance programmes, or antimicrobial surveillance systems (including the WHO GLASS). AMASS differs from the 'Macros and Excel Reports' functions of the WHONET, which allow users to regularly generate reports on screen and export data as Excel files using macro language (i.e., a series of written study parameters generated automatically through the WHONET user interface grouped together).[309] In contrast AMASS is designed to automatically compile summary results of multiple organisms into a single report. The current version of AMASS does not support quality improvement or alert individuals to unexpected antibiogram results, which WHONET is capable of.[309] Thus, while WHONET is an appropriate choice for the main software package for microbiology laboratories in LMICs to capture and store microbiology data,

and to export data for the WHO GLASS,[309] AMASS provides significant additional functionality in merging microbiology and hospital admission data.

Verifying individual AST results and validating summary data in the reports (including those generated by AMASS) remain major barriers to the generation of accurate surveillance data on AMR. Only verified AST results on each patient's isolate should be used in the data analysis.[74,312] WHONET and many LISs include functions that automatically check the results to ensure that they appear reasonable or alert the users to confirm unusual results (e.g. amikacin resistance coexisting with gentamicin and tobramycin susceptibility in *E. coli* is unusual and data on such cases should be verified).[74,309,312] In addition, it is important to validate the calculations of any analytical software used to generate the summary data and reports. We recommended that users of AMASS perform manual validation (such as printing a line listing of all isolates of the species to cross-check with the reports), particularly when the program is used for the first time.[74,100,312] Users should also understand that the precision of the estimate is based on the sample size, and is illustrated by estimated confidence intervals. In addition, representativeness of the summary cAST data could be impacted by the sampling strategy used, particularly if blood cultures are mainly collected from patients with treatment failure or prolonged hospitalizations.[74,100,312] Nonetheless, generating AMR surveillance reports from an existing microbiology database could be a first step for hospitals in LMICs to understand and validate their own data, and support quality control programmes at their local level.

Local hospitals must not be discredited because of the statistics in any AMR surveillance report, as possible criticism could be a barrier to data sharing.[325] Negative criticism of the

data shared by the local hospitals needs to be avoided. It is inevitable for errors to occur in any data sets and reports especially in settings with limited resources and experience on quality assurance and data validation. Tools for data validation should be provided for local hospitals, and the statistics in an AMR report should act as a guidance on the direction of improving data quality control, data validation, and infection prevention and control. Credit should be given to the hospitals that share their local AMR data and reports in an open-access data repository platform. The data repository platform, such as Figshare, allows flexibility on updating the data and revising the AMR surveillance reports with appropriate version controls by the local hospitals. Publishing open-access AMR data on these platforms provides a digital object identifier that can be used for data citation.[326]

3.6 Limitations

AMASS has a number of limitations. Firstly, AMASS is not applicable for hospitals that only store data on paper forms. Secondly, AMASS cannot work with raw microbiology data files that are not in wide format or combine many files in multiple formats; for example, the raw microbiology data file where each row contains data of each antibiotic susceptibility result for one single specimen. Thirdly, the current version of AMASS can only analyse microbiology data that includes antimicrobial susceptibility test interpretive categories (susceptible, intermediate, resistant) based on guidelines that the local hospital uses. Fourthly, AMASS cannot automatically validate the reliability of data that are imported into the application and used for analysis. Data verification and quality checks will be included in future versions of the application. Fifthly, AMASS was tested with only a few data sets that included non-English languages, and further testing on data with other languages may be needed. The current version of AMASS has been tested in

Windows 10 and 7 and may not work under different operating systems. Finally, the current version of AMASS cannot provide options to export the AMR surveillance report in different formats (i.e., in document or text formats). However, users can re-use the summary statistics, which are saved in CSV format in the ResultData folder.

3.7 Implications

AMASS can be a useful tool to support the generation and sharing of AMR surveillance reports in resource-limited settings. AMASS can be an add-on tool of the existing local LIS (including WHONET and in-house LIS). The AMR surveillance report generated contains no identifier information, can be readily shared, and can support actions against AMR infections at local, national and international levels.

4

Blood culture utilisation rate and estimates of proportion and incidence rates of AMR

4.1 Abstract

Background

In resource-limited settings, blood cultures are often under-utilised in hospitals. Sampling patients for blood culture is more frequently performed when empirical antibiotic treatment has failed. This may bias the estimates of the proportion and frequencies of infections caused by antimicrobial resistant (AMR) organisms. The study described in this chapter aims to examine the magnitude of the biases.

Methods

Routinely collected electronic microbiology data, hospital admission data, and antibiotic prescription data from Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand, were used in the analysis. The potential effects of low blood culture utilisation rate were quantified using a simulation study. We created three scenarios that represents 50%, 25%, and 10% of first blood culture specimens collected, respectively. A blood culture episode was defined as all blood samples collected within two calendar days beginning when a blood sample was collected from the same patient. For example, a blood sample collected on 1 January 2010 from patient A and a blood sample collected on 2 January 2010 from the same patient A were considered as the same blood culture episode. The proportion of

infections caused by an AMR organism, incidence rates of an AMR infection, and blood culture utilisation rates were then estimated under each simulated scenario. The results were then compared against with the observations from the complete data set (i.e., 100% of first blood culture specimen collected). The analyses were performed using data from blood cultures with growth of *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa, and Acinetobacter* spp.. The pathogen-antibiotic combinations investigated in this study are methicillin-resistant *S. aureus* (MRSA), third-generation cephalosporin resistant *E. coli* (3GCREC) and *K. pneumoniae* (3GCRKP), carbapenem-resistant *P. aeruginosa* and (CRPA) and *Acinetobacter* spp. (CRACI).

Results

The proportion of *S. aureus* bloodstream infections (BSI) caused by MRSA was 23% (252/1092); those of *E. coli* and *K. pneumoniae* BSI caused by third generation cephalosporin resistant isolates were 44% (868/1959) and 51% (534/1045), respectively; and that of *P. aeruginosa* and *Acinetobacter* spp. caused by carbapenem resistant isolates was 28% (125/450) and 65% (647/1000), respectively. The estimates of the proportions increased from 23% to 43%, from 44% to 57%, from 51% to 75%, from 28% to 43%, and from 65% to 81%, respectively, when only 10% of first blood culture specimens were collected. Decreases in the incidence rates of patients with blood cultures positive for AMR isolates were also observed. The observed magnitudes of those decreases were 0.9 (from 2.7 to 1.8), 7.1 (from 9.5 to 2.4), 2.7 (from 5.8 to 3.1), 0.4 (from 1.4 to 1.0), and 1.6 (from 7.0 to 5.4) patients per 100,000 population per year, for MRSA, 3GCREC, 3GGRKP, CRPA, and CRACI, respectively. The proportion of MRSA, 3GCREC, 3GCRKP, CRPA, and CRACI identified from the blood samples that were collected within ±1 calendar day when a parenteral antibiotic was prescribed during hospitalisation

was 9%, 30%, 37%, 12%, 32%, respectively. These estimated were lower than those identified from the blood samples that were collected outside ±1 calendar day (74%, 79%, 86%, 42%, and 81%, respectively) of parenteral antibiotic prescription during hospitalisation.

Interpretation

The impact on estimates of the proportions and incidence rates of AMR infections due to low blood culture utilisation rate can be large and vary by organism. This could occur even in the absence of changes in the true susceptibility profiles of organisms and in the true infection rates in the setting. AMR Surveillance reports should include blood culture utilisation rates. Moreover, stratification by exposure to a parenteral antibiotic as proposed by the current study could help to interpret prevalence and incidence rates of AMR in the setting in which the surveillance is performed.

4.2 Introduction

Blood culture is the most important clinical diagnostic test for bloodstream infections. Identification of causative pathogen(s) and antimicrobial susceptibility profile allow a targeted effective antibiotic treatment, which is associated with an increased chance of survival. The utilisation rate of blood culture may influence the estimation of proportion and incidence of AMR infections in a hospital. For instance, in cases where there is a delay in blood sample collection from patients, especially after administration of antibiotics, overestimation on the proportion of antimicrobial resistant bloodstream infection is expected. Hence, the blood culture utilisation rate is an important indicator to support the interpretation of antimicrobial resistant infection burdens.

Previous studies have shown that BSI rates are underestimated if blood culture utilisation rate is low,[207,327] but the impact on estimates of proportions and incidence rates of AMR infections is largely unknown.[208] Recommendations for target blood culture utilisation rates have been published in microbiological guidelines in the United States and Germany, but how such recommendations could apply to low and middle-income countries (LMICs) is unclear. In the United States, the Cumitech guideline recommends 103 to 188 blood culture sets per 1,000 patients-days.[328] The German Mikrobiologish-Infektiologische Qualitätsstandards (MIQ) guideline recommends a range of 100 to 200 blood culture sets per 1,000 patient-days, referring directly to the U.S. guideline.[207,329] However, the target blood culture utilisation rates in LMICs is unknown, and is likely to be dependent on the type of hospital, patient case mix, physician training, number of ICU beds (as patients in ICUs have a higher rate of hospital-acquired infection[330]), and the true hospital-acquired infection rate in each hospital.[72,207]

This chapter explores the potential magnitude of effects on estimates of proportions and incidence rates of AMR infection due to low blood culture utilisation rates in Thailand using bootstrap simulations on data collected retrospectively.

4.3 Methods

4.3.1 Study population and Study design

We conducted a simulation study using retrospective data from Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand. Ubon Ratchathani is the second largest province in northeast Thailand. Sunpasitthiprasong Hospital is a referral centre for the district hospitals in the province and provincial hospitals in the adjacent provinces in the region. The hospital is well-equipped with a microbiology laboratory that provides bacterial culture services. Routine electronic databases of patients admitted in the hospital between 01 January 2011 and 31 December 2015 were collected. We focused on blood culture samples. Five pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp., were included in the study. These pathogens were selected because they are the top five pathogens related to increased mortality among bloodstream infections in Thailand.[79,80]

The three key databases collected held microbiology data, hospital admission data, and drug prescription data. The microbiology data obtained included patient hospital number, specimen collection date, specimen type, pathogen(s) identified, and antimicrobial susceptibility testing results. The hospital admission data included patient hospital number, admission number, sex, age, admission date, discharge date, and in-hospital discharge outcome. The drug prescription data collected included patient hospital number,

admission number, drug names, administration routes, date of start, and date of stop. All data obtained were in electronic files with a readily accessible format.

Ethics approvals were obtained from the Oxford Tropical Research Ethics Committee (Ref. 557-17), and the ethical committee of Sunpasitthiprasong Hospital (Ref. 005/2560). Individual informed consent was waived by the ethics committees due to the study design and minimal risk to the subjects.

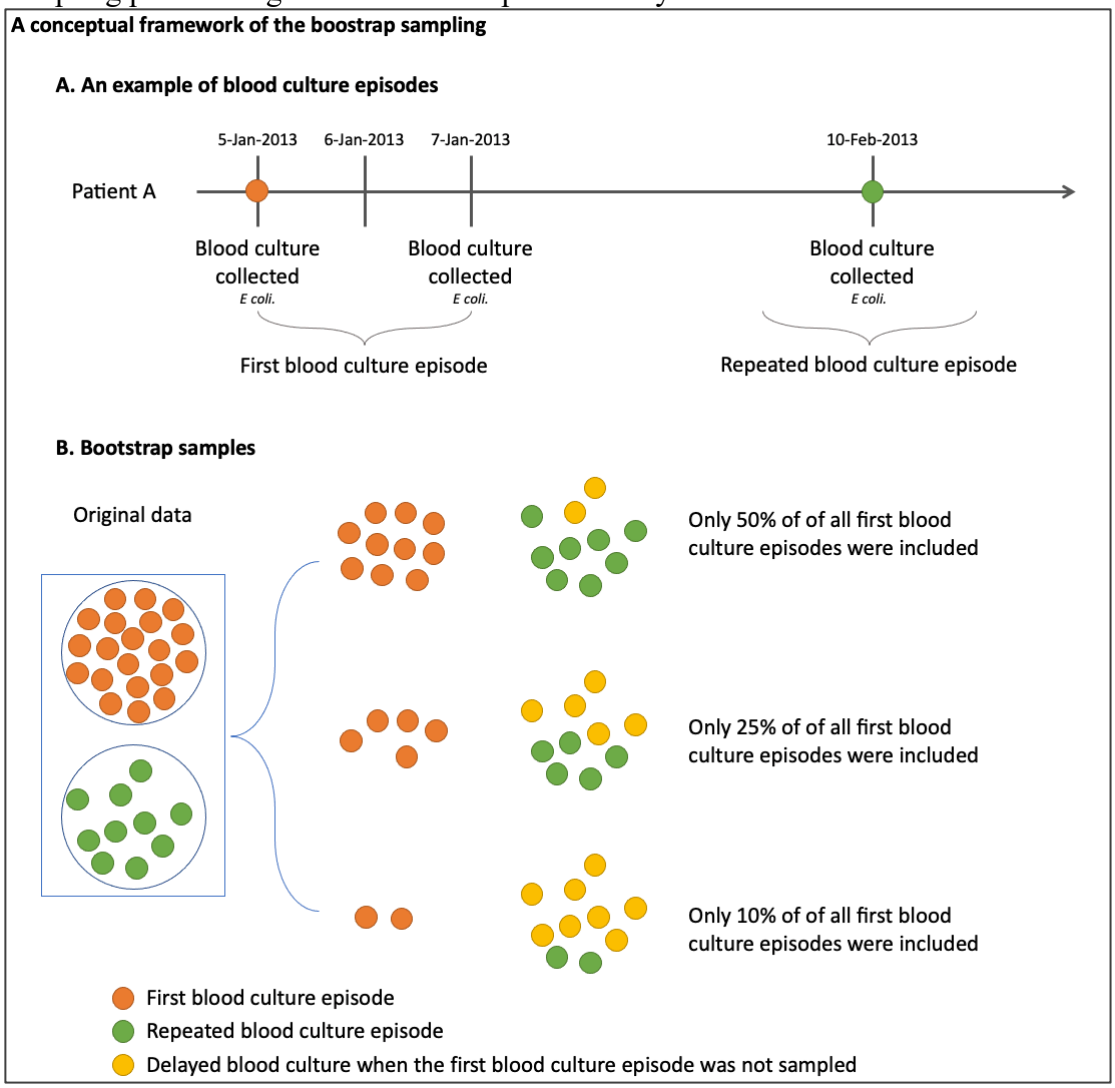
4.3.2 Simulation

A single blood culture episode is defined as all blood cultures collected within two calendar days of the first specimen (Figure 4.1A). To quantify the potential magnitude of biases related to blood culture utilisation patterns, we used data from patients with blood cultures to generate bootstrap samples in which only 10%, 25%, and 50% of all first blood culture episodes were included (Figure 4.1B). All repeated blood culture episodes were included to represent delayed blood culture when the first blood culture episodes were not sampled from the bootstrap sampling. Bootstrap resampling with 200 iterations for each simulation scenario were performed. The median and the 2.5^{th} and 97.5^{th} percentiles were calculated.

4.3.3 Definitions

We used the definitions of infection origin as proposed by WHO GLASS.[331] In brief, community-origin (or hospital-origin) BSI was defined for patients in the hospital within (or longer than) the first two calendar days of admission when blood culture positive specimens were taken.[331]

Figure 4.1. An illustration for (A) definition of blood culture episodes; and (b) bootstrap sampling process to generate data to represent delays in blood culture collection.



Proportions of methicillin-resistant *for S. aureus* (MRSA), 3rd generation cephalosporin resistance in *E. coli* (3GCREC) and *K. pneumoniae* (3GCRKP), and carbapenem resistance in *P aeruginosa* (CRPA), and *Acinetobacter* spp. were defined as the ratio of the number of patients having a blood culture positive for MRSA, 3GCREC, 3GCRKP, CRPA, and CRACI, and the total number of patients with at least one positive blood culture with the bacterial species, respectively. Only the first isolate per patient, per pathogen, per study period was included in the analyses.

The number of blood culture specimens per 1,000 patient-days was estimated to represent blood culture utilisation rate in different simulations. We also developed a new measurement 'proportion of patients having a blood culture taken within ±1 calendar day (i.e., either the day before or the day after) of the day when a parenteral antibiotic was started and continued for at least four consecutive days' to represent blood culture utilisation rate. This measurement captures severity of illness at the time of blood sampling and the practice of blood sampling (relative to antibiotic prescription). A key reason that a new parameter is needed is that "blood culture specimens per 1,000 patient-days" cannot be stratified by origin of infection. We used four consecutive days of parenteral antibiotics as a proxy for severe infection,[332] a conditional under which blood culture is generally recommended.[29,30] Patients who died, were discharged to a hospice or transferred to another hospital before completing four consecutive days of antibiotics and had antibiotics continuously until the day prior to death were considered to have experienced an event of death, a hospice discharge or transfer, respectively, and were included in the analysis.[332] Consecutive calendar days with any parenteral antibiotics after a parenteral antibiotic was initiated were included in the analysis.

4.3.4 Statistical analysis

Five parameters were estimated to present the prevalence and incidence of AMR infections from each of the simulated data set. These parameters are i) 'proportion of patients with blood culture positive for resistant isolates'; ii) 'incidence rates of new cases of antimicrobial resistant bloodstream infections per 100,000 tested patients'; iii) 'incidence rates of new cases with blood culture positive for resistant isolates per 100,000 population per year; iv) 'incidence rates of patients with community-origin BSI and blood

culture positive for resistant isolates'; and v) 'incidence rates of patients with hospital-origin BSI and blood culture positive for resistant isolates'.

The parameters to present proportion and incidence rates of AMR infections were calculated as follow:

$$\text{Proportion of patients with blood culture positive for resistant strains (\%, modified from WHO, 2019)} = \frac{\text{Number of patients with blood culture positive for resistant strains of bacterial species under surveillance (per pathogen, antibiotic and origin of infection)}}{\text{Total number of patients with blood culture positive for bacterial species under surveillance (per pathogen and origin of infection)}}$$

$$\text{Incidence rates of patients with blood culture positive for resistant strains (per 100,000 population per year, modified from PHE, 2019)} = \frac{\text{Number of new patients with blood culture positive for resistant strains of bacterial species during reporting period * 100,000 (per pathogen, antibiotic and origin of infection)}}{\text{Total financial year population during reporting period}}$$

$$\text{Incidence rates of patients with blood culture positive for resistant strains (per 100,000 tested patients; modified from WHO, 2019)} = \frac{\text{Number of new patients with blood culture positive for resistant strains of bacterial species in the population tested during reporting period * 100,000 (per pathogen and antibiotic)}}{\text{Total number of patients with blood culture performed during the reporting period}}$$

Incidence rates of patients with community-origin bacteraemia and blood culture positive for resistant strains (per 100,000 patients tested; modified from WHO, 2019; Bonita, Beaglehole, Kjellstrom, & WHO., 2006) = Number of new patients admitted within the first two calendar days when the first blood specimen culture positive for resistant strains * 100,000 (per pathogen and antibiotic) / Total number of patients with the first blood culture performed **within the first two calendar days of admissions** during the reporting period

Incidence rates of patients with hospital-origin bacteraemia and blood culture positive for resistant strains (per 100,000 patients; modified from WHO, 2019; Bonita, Beaglehole, Kjellstrom, & WHO., 2006) = Number of new patients admitted longer than the first two calendar days when the first blood specimen culture positive for resistant strains * 100,000 (per pathogen and antibiotic) / Total number of patients with the first blood culture performed **after the first two calendar days of admissions** during the reporting period

The parameters to represent blood culture utilisation rate were estimated as follow:

Blood culture utilisation rate (per 1,000 patient-days; modified from ECDC, 2016) = Number of blood cultures used during reporting period * 1,000 / Total patient-days occupancy during reporting period

Proportion of patients having blood culture sampled within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days (new parameter generated for this study)	=	Total number of patients having blood culture sampled within ±1 calendar day when parenteral antibiotics was first prescribed and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, were discharged to a hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer --- Total number of patients who had parenteral antibiotics first prescribed and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, was discharged to hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer
Proportion of patients **<u>at risk of BSI of community-origin</u>** and having blood culture sampled within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days (new parameter generated for this study)	=	Total number of patients having blood culture sampled within ±1 calendar day of the day when a parenteral antibiotic was started **<u>within the first two calendar days of admission</u>** and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, were discharged to a hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer --- Total number of patients who had a parenteral antibiotic first started **<u>within the first two calendar days of admission</u>** and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, were discharged to a hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer

Proportion of patients **at risk of BSI of hospital-origin** and having blood culture sampled within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days

(new parameter generated for this study)

=

$$\frac{\text{Total number of patients having blood culture sampled within ±1 calendar day of the day when a parenteral antibiotic was started } \textbf{after the first two calendar days of admission} \text{ and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, were discharged to a hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer}}{\text{Total number of patients who had a parenteral antibiotic started } \textbf{after the first two calendar days of admission} \text{ and (a) continued for at least four consecutive days or (b) continued for less than four calendar days if the patients died, were discharged to a hospice or transferred to another hospital less than four days after antibiotics were started, and antibiotics were continued until the day prior to death, hospice discharge or transfer}}$$

The rate of blood culture utilisation per 1,000 patient-days was calculated from each simulated data set. We then estimated three new measurements to represent the blood culture utilisation rates accounting for the antibiotic prescription behaviour (a key factor that influences the estimates of prevalence and incidence rates of AMR infections). These newly generated parameters are i) 'overall proportion of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days'; ii) 'proportion of patients at risk of community-origin BSI and having a blood sample cultured within ±1 calendar day of the day at which a parenteral antibiotic started and continued for at least four consecutive days'; and iii) 'proportion of patients at risk of hospital-origin BSI and had blood sample cultured within ±1 calendar day on the day at which a parenteral antibiotic started'.

The simulation, data cleaning and data analyses were performed using STATA 17.0 (StataCorp LP, College Station, Texas).

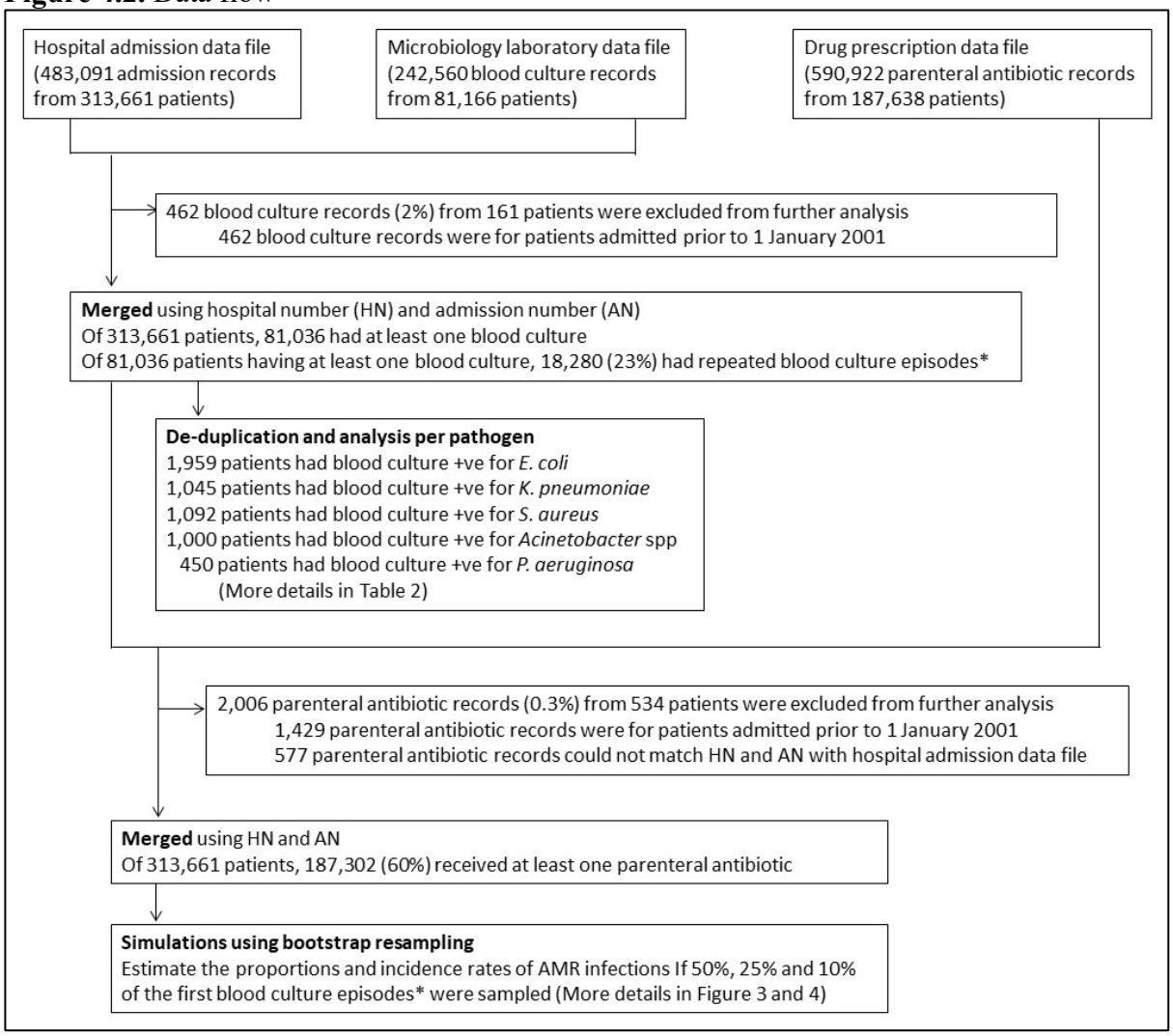
4.4 Results

4.4.1 Baseline characteristics

Between 2011 and 2015, there were 313,661 patients admitted to Sunpasitthiprasong hospital, and out of those patients 81,036 had at least one blood culture taken (Figure 4.2). The hospital bed capacity in 2015 was 1,183 beds. The total patient days were 2,956,643 and the total number of blood cultures were 242,098, which together gave a blood culture utilisation rate of 82 blood cultures per 1,000 patient days. 62,756 (out of 81,036 patients; 77%) patients had a single blood culture episode during their hospitalization. A single blood culture episode is defined as all blood cultures collected within two calendar days of the first specimen. 11,410 (14%), 3,751 (5%), 1,538 (2%), and 1,581 (2%) patients had two, three, four and at least five blood culture episodes during their admissions, respectively. For the patients who had multiple blood culture episodes, the median time between the first and second blood culture episode was 5 calendar days (IQR, 3 to 9 days). The number of parenteral antibiotic records in the drug prescription data set were 590,922 records. This represented 1,456,027 patient-days of antibiotic prescription and 187,302 patients with a parenteral antibiotic over the entire study period.

The crude fatality rate of in-hospital patients during the study period is 5% (15,589 out of 313,661 patients had a discharge record of ‘in-hospital mortality’). Of 484,225 admissions, 13,171 admissions (3%) had a discharge record of ‘against advice and not improved’ and was assumed to be discharged to a hospice, and 22,506 admissions (5%) had a discharge record of ‘transferred to another hospital’.

Figure 4.2. Data flow



Footnote: *A blood culture episode was defined as all blood culture specimens collected within two calendar days beginning when a blood culture specimen was collected. This figure is taken from the published paper (of which I am the first author) and with the permission of the corresponding author of the paper.

4.4.2 Proportion and incidence rates of AMR BSI

There were 1092, 1959, 1045, 450, and 1000 patients, who had blood cultures with growth of *S. aureus, E. coli, K. pneumoniae, P. aeruginosa*, and *Acinetobacter* spp., respectively. The overall proportions of MRSA, 3GCREC, 3GCRKP, CRPA and CRACI were 23%, 44%, 51%, 28%, and 65% respectively (Table 4.1). The proportions of patients with MRSA, 3GCREC, 3GCRKP, CRPA, and CRACI isolates were higher in hospital-origin BSI (44%, 53%, 72, 36%, and 75%, respectively) compared to that in community-origin BSI (14%, 42%, 34%, 14%, and 33%, respectively). The estimated

incidence rates of MRSA, 3GCREC, 3GCRKP, CRPA, and CRACI BSI per 100,000 population per year were 2.7, 9.5, 5.8, 1.4, and 7.0, respectively (Table 4.2). The estimated incidence rates of MRSA, 3GCREC, 3GCRKP, CRPA, and CRACI BSI per 100,000 tested patients were 311, 1,071, 659, 154, and 798, respectively (Table 4.3).

Table 4.1. Proportion of patients with bloodstream infection and blood culture positive for the pathogen-antibiotic combinations of interest in this study.

Species	**Resistance of interest**	**Proportion with specified resistance**		
		Total	BSI of community-origin*	BSI of hospital-origin*
S. aureus	MRSA	23% (252/1,092 patients)	14% (103/750 patients)	44% (149/342 patients)
E. coli	3GCREC	44% (868/1,959 patients)	42% (631/1,514 patients)	53% (237/445 patients)
K. pneumoniae	3GCRKP	51% (534/1,045 patients)	34% (193/569 patients)	72% (341/476 patients)
P. aeruginosa	CRPA	28% (125/450 patients)	14% (23/170 patients)	36% (102/280 patients)
***Acinetobacter* spp.**	CRACI	65% (647/1,000 patients)	33% (80/242 patients)	75% (567/758 patients)

Footnote: BSI: bloodstream infection. MRSA: methicillin-resistant S. aureus; 3GCREC: third-generation cephalosporin resistant E. coli; 3GCRKP: third-generation cephalosporin-resistant K. pneumoniae; CRPA: carbapenem-resistant P. aeruginosa; CRACI: carbapenem-resistant Acinetobacter spp..*BSI of community-origin was defined as patients in hospital for ≤2 calendar days when the first blood-culture positive for the pathogen was taken, and BSI of hospital-origin was defined as patients admitted for >2 calendar days when the first blood-culture positive for pathogen was taken.[331] Only the first isolate per patient, per pathogen, per study period was included in the analysis.

4.4.3 Antibiotic prescriptions

In total, 187,302 patients (60%) received at least one parenteral antibiotic at the study hospital (Figure 4.3). The most commonly used antibiotic was ceftriaxone (346,167 patient-days), followed by carbapenem drugs (253,466 patient-days) and ceftazidime (251,548 patient-days).

The 'proportion of patients who had a blood culture taken within ±1 calendar day of the start of parenteral antibiotic treatment that was then continued for at least four consecutive days' was 44% (47,132/106,341). This proportion was higher in patients who began a parenteral antibiotic within the first two calendar days of hospital admission (46%; 41,489/90,685) than those who began a parenteral antibiotic after two calendar days of hospital admission (36%; 5,643/15,656; p<0·001).

Table 4.2. Incidence rates measured in cases per 100,000 population per year of bloodstream infection and blood culture positive for the pathogen-antibiotic combinations of interest in this study.

Pathogen-antibiotic combinations	**Incidence rate (per 100,000 population per year)**		
	Total	BSI of community-origin*	BSI of hospital-origin*
MRSA	2.7 ($252*10^5$ /9181598)	1.1 ($103*10^5$ /9181598)	1.6 ($149*10^5$ /9181598)
3GCREC	9.5 ($868*10^5$ /9181598)	6.9 ($631*10^5$ /9181598)	2.6 ($237*10^5$ /9181598)
3GCRKP	5.8 ($534*10^5$ /9181598)	2.1 ($193*10^5$ /9181598)	3.7 ($341*10^5$ /9181598)
CRPA	1.4 ($125*10^5$ /9181598)	0.3 ($23*10^5$ /9181598)	1.1 ($102*10^5$ /9181598)
CRACI	7.0 ($647*10^5$ /9181598)	0.9 ($80*10^5$ /9181598)	6.2 ($567*10^5$ /9181598)

Footnote: BSI: bloodstream infection. MRSA: methicillin-resistant *S. aureus*; 3GCREC: third-generation cephalosporin resistant *E. coli*; 3GCRKP: third-generation cephalosporin-resistant *K. pneumoniae*; CRPA: carbapenem-resistant *P. aeruginosa*; CRACI: carbapenem-resistant *Acinetobacter* spp..*BSI of community-origin was defined as patients in hospital for ≤2 calendar days when the first blood-culture positive for the pathogen was taken, and BSI of hospital-origin was defined as patients admitted for >2 calendar days when the first blood-culture positive for pathogen was taken.[331] Only the first isolate per patient, per pathogen, per study period was included in the analysis.

4.4.4 Impact of low blood culture utilisation rate on estimated proportions of AMR infections

The results from bootstrap simulations showed that the estimate of the proportions of resistant isolates can be overestimated when blood culture utilisation rate is reduced. For instance, when only 10% of the first blood culture episodes were sampled, the proportions of MRSA would increase by 20 percentage points (from 23% to 43%) (Figure 4.4). The proportion of MRSA in patients with community-origin BSI changed by a 1 percentage

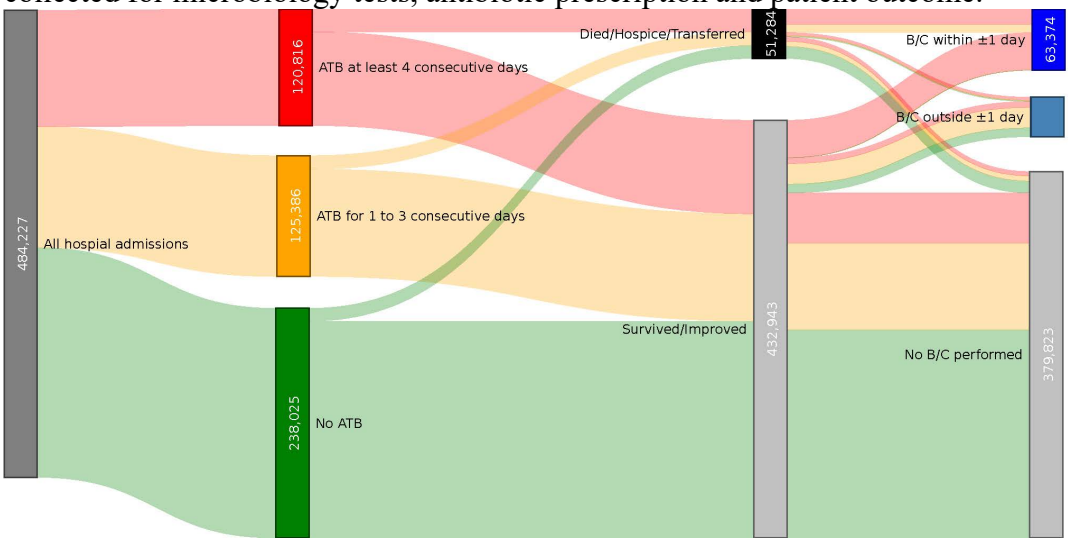
point of absolute increase but the 95% confidence intervals from the bootstrap sampling widen due to the reduced sample size. The proportion of MRSA in patients with hospital-origin BSI would increase by 10 percentage point (from 44% to 53%), when only 10% of the first blood culture episode were sampled. Similarly, the proportion of 3GCREC would increase from 44% to 57%, when only 10% of the first blood culture episodes were sampled (Figure 4.4). The impact of low blood culture utilisation on estimates of proportion of AMR infection is large in patients with hospital-origin BSI compared to that in patients with community-origin BSI. Similar patterns were also observed for infections caused by 3GCRKP, CRPA, and CRACI (Figure 4.4). The changes were the largest in patients with 3GCRKP BSI.

Table 4.3. Incidence rates measured in cases per 100,000 tested population per year of bloodstream infections and blood culture positive for the pathogen-antibiotic combinations of interest in this study.

Pathogen-antibiotic combinations	**Incidence rate (per 100,000 population per year)**		
	Total	BSI of community-origin*	BSI of hospital-origin*
MRSA	311 ($252*10^5$ /81036)	152 ($103*10^5$ /67641)	829 ($149*10^5$ /17983)
3GCREC	1,071 ($868*10^5$ /81036)	933 ($631*10^5$ /67641)	1,318 ($237*10^5$ /17983)
3GCRKP	659 ($534*10^5$ /81036)	285 ($193*10^5$ /67641)	1,896 ($341*10^5$ /17983)
CRPA	154 ($125*10^5$ /81036)	34 ($23*10^5$ /67641)	567 ($102*10^5$ /17983)
CRACI	798 ($647*10^5$ /81036)	118 ($80*10^5$ /67641)	3153 ($567*10^5$ /17983)

Footnote: BSI: bloodstream infection. MRSA: methicillin-resistant *S. aureus*; 3GCREC: third-generation cephalosporin resistant *E. coli*; 3GCRKP: third-generation cephalosporin-resistant *K. pneumoniae*; CRPA: carbapenem-resistant *P. aeruginosa*; CRACI: carbapenem-resistant *Acinetobacter* spp..*BSI of community-origin was defined as patients in hospital for ≤2 calendar days when the first blood-culture positive for the pathogen was taken, and BSI of hospital-origin was defined as patients admitted for >2 calendar days when the first blood-culture positive for pathogen was taken.[331] Only the first isolate per patient, per pathogen, per study period was included in the analysis.

Figure 4.3. Sankey diagram showing number of hospital admissions, blood sample collected for microbiology tests, antibiotic prescription and patient outcome.



Footnotes: ATB: parenteral antibiotics; B/C: blood culture. B/C within ±1 calendar day is defined as having a blood culture specimen taken within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days. This group also includes patients who died, were discharged to a hospice or transferred to another hospital within four days after a parenteral antibiotic was started, and a parenteral antibiotic was continued until the day prior to death, hospice discharge or transfer. B/C outside ±1 calendar day is defined as having a blood culture specimen taken outside ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days. This group also includes patients who had no parenteral antibiotic prescribed. This figure is taken from the supplementary material of the published manuscript (of which I am the first author) and with the permission of the corresponding author of the paper.

4.4.5 Impact of low blood culture utilisation rate on estimated incidence rates

If only 10% of the first blood culture episodes were sampled, the incidence rate of patients with MRSA BSI per 100,000 population per year would decrease by 33.3% (from 2.6 to 1.8; Figure 4.5). This could be explained by the changes in the numerator of the incidence rate. The number of patients with MRSA BSI identified (numerator) would decrease from 252 to 163 cases, while the total population coverage (denominator for the incidence rate) remained constant. This pattern is also observed in the estimates of incidence rate, measured in cases per 100,000 population per year, of 3GCREC, 3GCRKP, CRPA, and CRACI BSI. The decrease in the incidence rate of community-origin antimicrobial-resistant organisms per 100,000 population was higher than that for hospital-origin antimicrobial-resistant organisms for all organisms under evaluation except *S. aureus*.

For instance, the incidence rate of community-origin 3GCREC BSI decreased from 6.9 to 0.8 cases per 100,000 population per year and that of hospital-origin 3GCREC BSI decreased from 2.6 to 1.7 cases per 100,000 population per year.

When only 10% of the first blood culture episodes were sampled, the incidence rates (measured in cases per 100,000 tested patients) of 3GCREC decreased from 1,071 to 890 per 100,000 tested patients (Figure 4.5). On the other hand, under the same scenario, the incidence rates of MRSA, 3GCRKP, CRPA and CRACI BSI per 100,000 tested patients would increase by 108% (from 311 to 648 per 100,000 tested patients), 42% (from 659 to 1,128 per 100,000 tested patients), 132% (154 to 358 per 100,000 tested patients), and 147% (from 798 to 1975 per 100,000 tested patients), respectively (Figure 4.5).

4.4.6 Impact of blood culture practice on estimates of blood culture utilisation rate

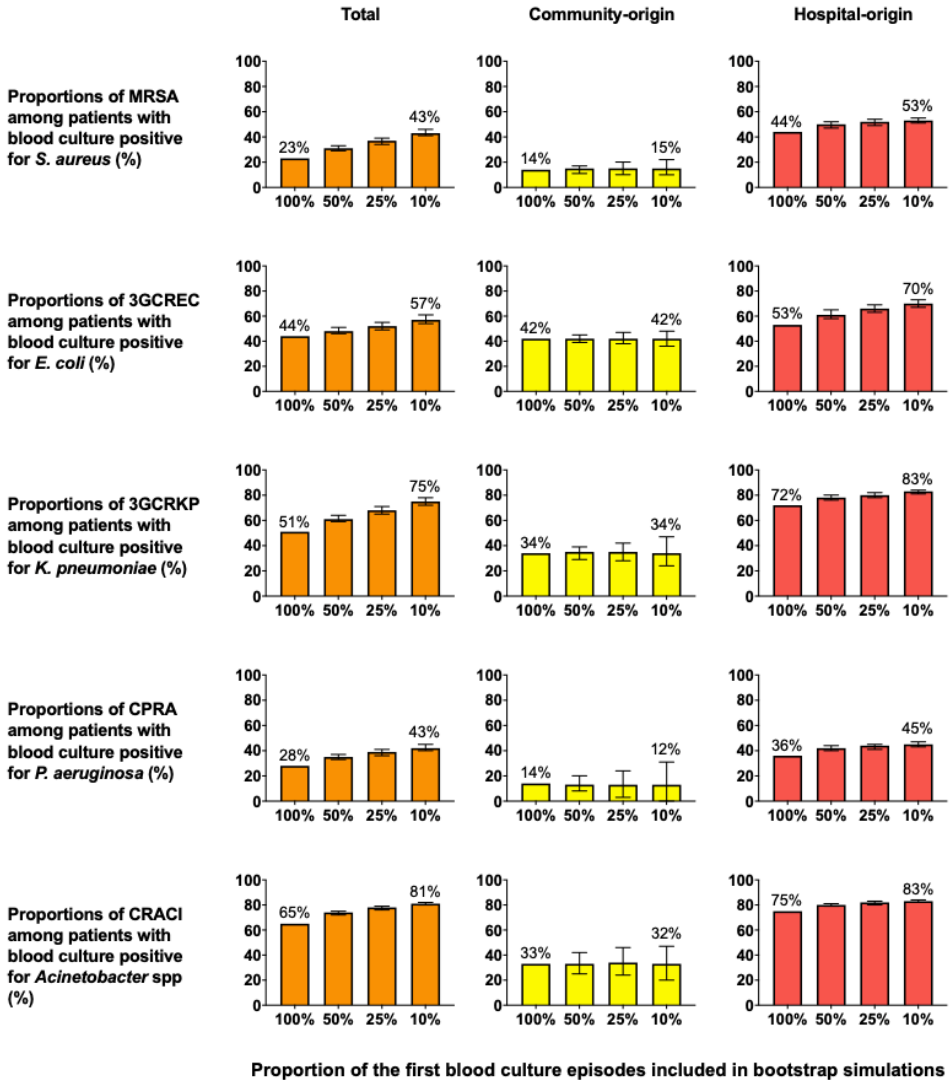
Unsurprisingly, when only 10% of the first blood culture episodes were sampled, the parameter representing blood culture utilisation rate decreased. Results from the simulation study showed such a decrease could be close to 3-fold (from 82 to 25 per 1,000 patient days). A caveat when using this parameter to represent blood culture practice is that it cannot be stratified by origins of infection. It is useful to stratify the blood culture utilisation rate by origins of infection because the proportion of AMR in community-acquired infections often differed from that in hospital-acquired infections due to differences in the level of antibiotic exposure and other risk factors of AMR infections. A blood culture utilisation rate that failed to account for such differences could not provide a fair comparison between different sentinel sites that varied by patient management, epidemiology of bacterial infections, or over surveillance time.

Alternatively, a parameter indicated as 'the proportion of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days' can be useful to represent blood culture utilisation rate. Results from the simulation study showed that the estimate of this parameter reduced as the number of the first blood culture episodes sampled reduced. For example, when only 10% of the first blood culture episodes were sampled, a reduction from 44% to 6% was observed (Table 4.4). Furthermore, the parameter can be stratified by origin of infection and represent the blood culture utilisation rate relative to antibiotic usage, which an important driver of AMR infections. Overall, in this study cohort, the proportion of patients with community-origin BSI having a blood culture taken within ±1 calendar days of the day when a parental antibiotic was started and continued for at least four consecutive days was 46% and that of patients with hospital-origin BSI was 36%. When only 10% of the first blood culture episodes were sampled, the proportions decreased from 46% to 5%, and from 36% to 11% for patients with community-origin BSI and hospital-origin BSI, respectively.

Table 4.4. Estimated blood culture utilisation rates, when 50%, 25% and 10% of the first blood culture episodes were sampled

Proportion of the first blood culture episodes sampled	**Blood culture per 1,000 patient-days**	**Proportions of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days.**		
		Total	**Community-origin BSI**	**Hospital-origin BSI**
100% (based on all data)	82 (242098*1000/2956643)	44% (47132/106341)	46% (41,489/90,685)	36% (5,643/15,656)
50%	51 (150769*1000/2942386)	19% (19523/102422)	16% (16803/90581)	23% (2714/11841)
25%	36 (105093*1000/2942386)	11% (11668/102422)	11% (9790/90581)	16% (1880/11841)
10%	26 (77690*1000/2942386)	6% (5931/102422)	5% (4663/90581)	11% (1265/11841)

Figure 4.4. Estimated proportions of antimicrobial-resistant isolates if 100%, 50%, 25% and 10% of the first blood culture episodes were sampled from community-origin and hospital-origin bloodstream infections.



Footnote: A blood culture episode was defined as all blood culture specimens collected within two calendar days beginning when a blood culture specimen was collected.

Figure 4.5. Estimated incidence rates of antimicrobial resistance per 100,000 poulation per year if 100%, 50%, 25% and 10% of the first blood culture episodes were sampled from community-origin and hospital-origin bloodstream infections.

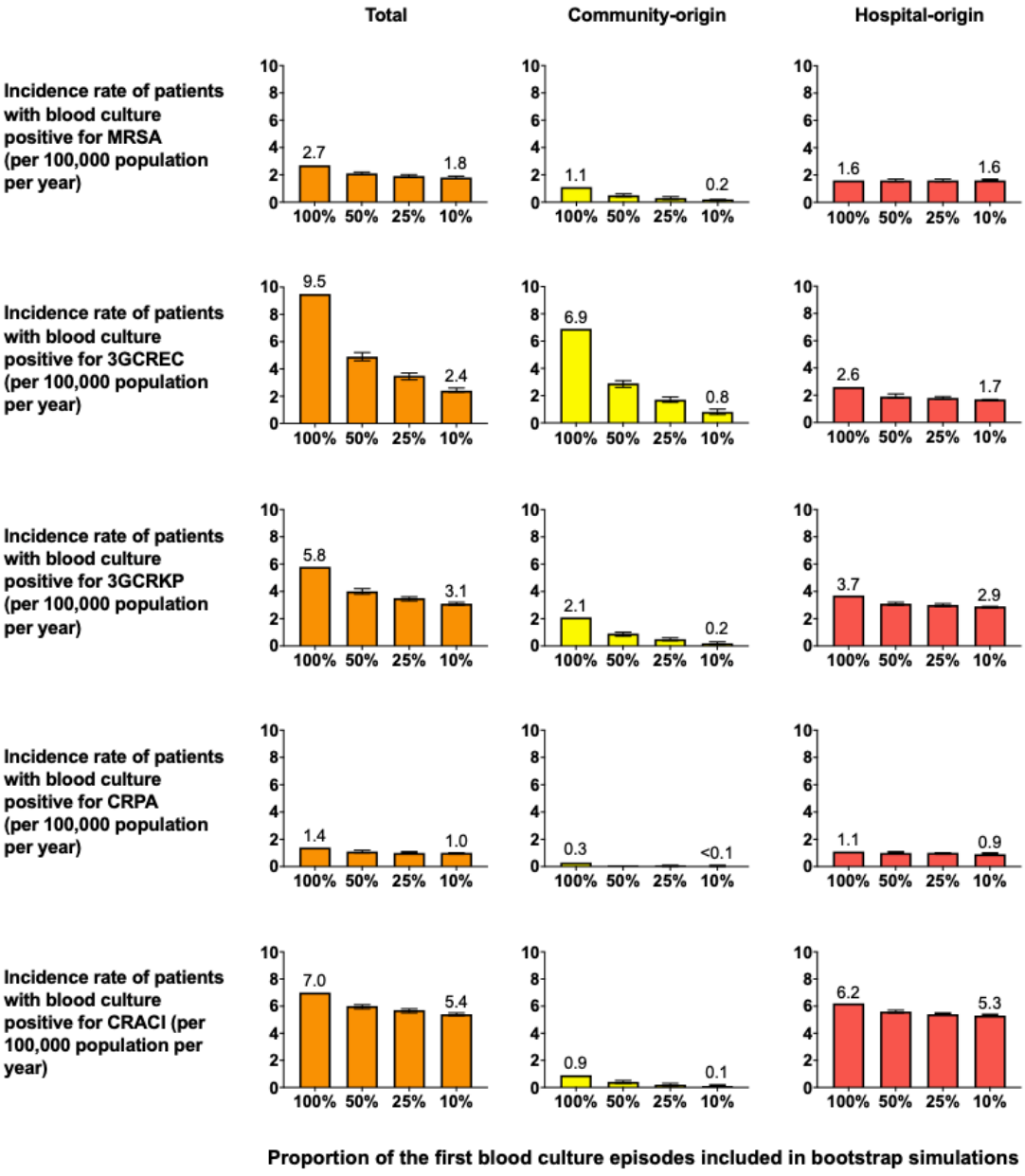
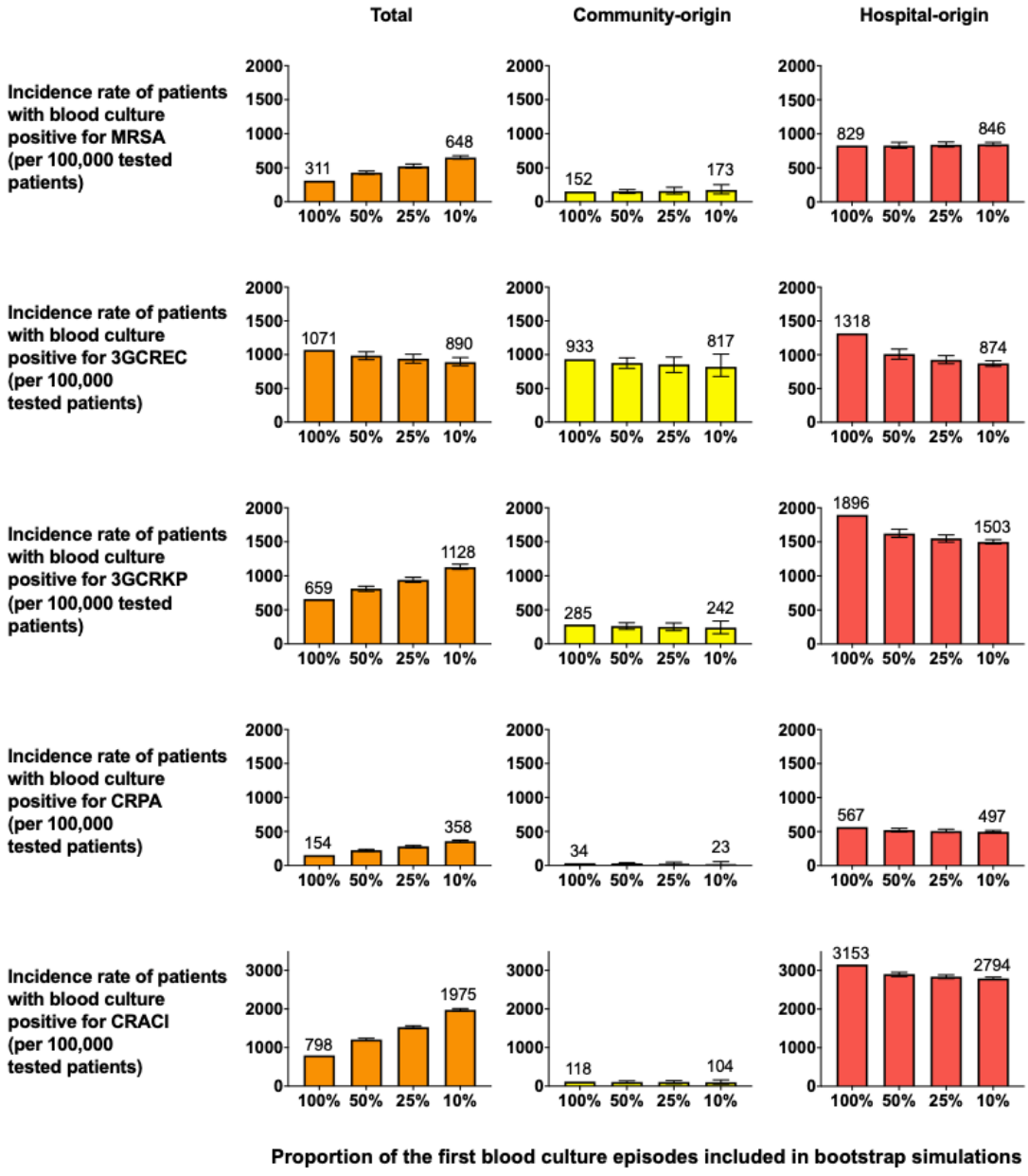


Figure 4.6. Estimated incidence rates of antimicrobial resistance per 100,000 tested patients if 100%, 50%, 25% and 10% of the first blood culture episodes were sampled from community-origin and hospital-origin bloodstream infections.



4.4.7 Proportions of AMR infections stratified by exposure to a parenteral antibiotic

After observing the increase in the proportion of AMR infection in patients with hospital-origin BSI when blood culture was delayed, a further investigation was made to determine whether antibiotic exposure could lead to the change in the proportion of AMR infections observed. Results from the simulation study showed that the proportion of MRSA in patients whose first positive blood culture was taken within ±1 calendar day of the start of a parenteral antibiotic was substantially lower compared with patients whose first positive blood culture was taken later into parenteral antibiotic treatment (9% versus 74%; Figure 4.6). The median duration of hospitalization prior to blood specimen collected for culture in the former group was around half that of the latter group (5 vs. 14 days, Appendix B1). Moreover, the median duration of exposure to a parenteral antibiotic in the former group was significantly lower than that of the latter group (0 vs. 12 days, Appendix B1).

The changes in the proportion of AMR infection stratified by antibiotic exposure in cases when culture sampling was delayed were further investigated. For *S. aureus* BSI, the proportion of MRSA identified from blood culture taken ±1 calendar day of the start of a parenteral antibiotic increased from 9% to 14% (Figure 4.7).

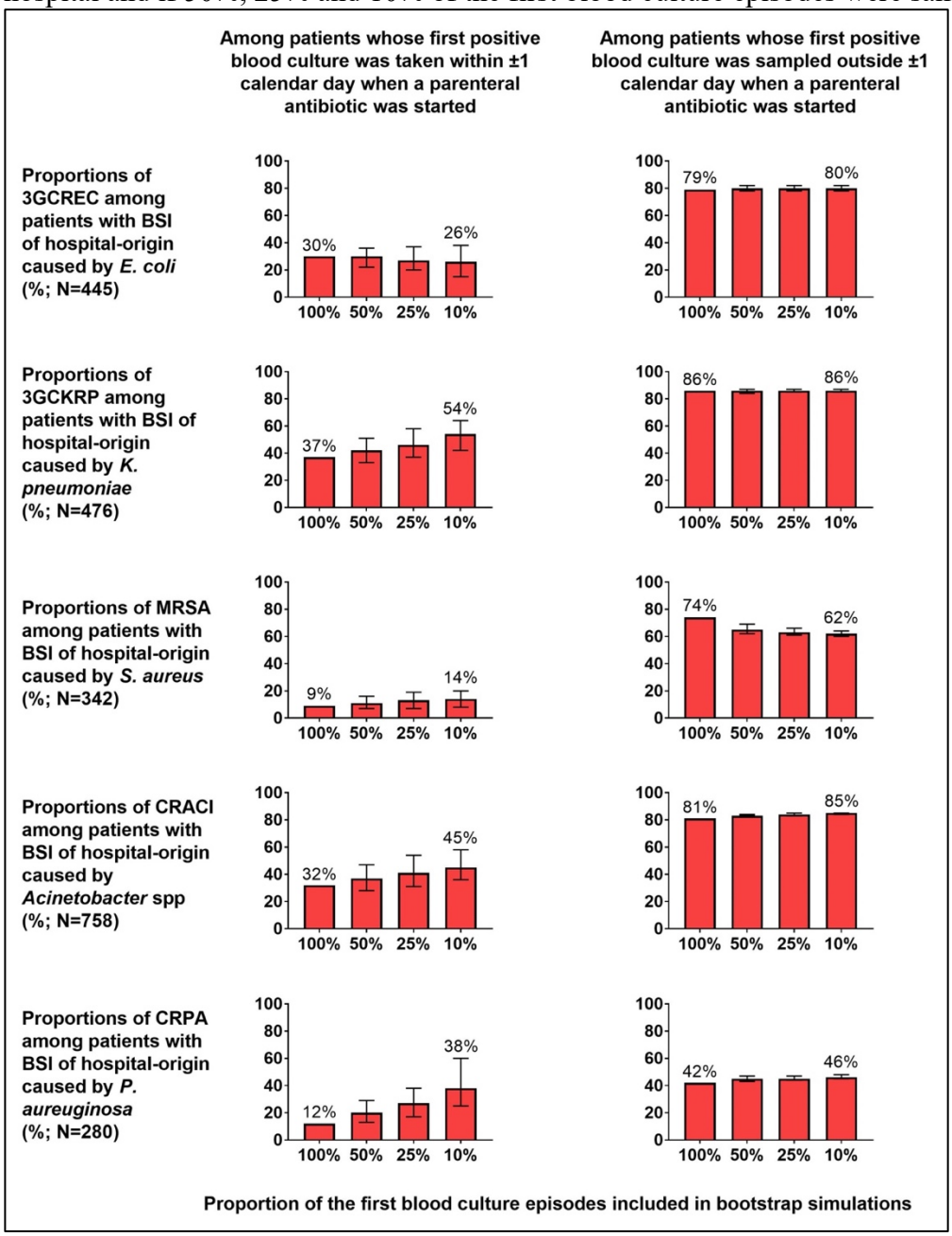
The large difference between proportions of AMR isolates from blood cultures taken within ±1 calendar day of the start of a parenteral antibiotic and the corresponding proportions from blood cultures taken later were observed in all other organisms in the study, except for *P. aeruginosa*, in the scenario when only 10% of the first blood culture episodes were sampled (Figure 4.7).

4.5 Discussion

The findings showed that the low blood culture utilisation rates could impact the estimates of the proportions and incidence rates of AMR infections. The proportions of 3GCREC, 3GCRKP, MRSA, CRACI, and CRPA would increase by 13, 24, 20, 16, and 15 percentage points, respectively, if blood culture utilisation rate was reduced from 82 to 26 per 1,000 patient days. On the other hand, the estimates of the incidence rates of AMR infections (measured as number of cases per 100,000 population per year) would decrease if blood culture utilisation rate was reduced. However, the changes in incidence rates of AMR infections per 100,000 tested patients varied by organism, and such changes ranged from a 17% decrease for 3GCREC to a 148% increase for CRACI. The proportions of AMR isolates from blood taken within ±1 calendar day of the day when a parenteral antibiotic is started are considerably lower compared to those from blood culture taken later into parenteral antibiotic treatment. This observation was seen for most of the organisms (3GREC, 3GCRKP, MRSA, and CRACI) even when blood culture utilisation was low.

Multiple reasons are responsible for the observed increase in overall proportions of AMR infections under the scenario when blood culture is delayed. First, the reduction in total number of patients with community-origin BSI is larger than the reduction in that of patients with hospital-origin BSI. Since the proportion of AMR infections among patients with community-origin infections is lower compared to that among patients with hospital-origin infections; hence, the overall (without stratification by origin of infection) proportion of AMR infection increased. Second, delays in blood culture would mean that the overall length of hospitalization prior to blood culture sampling increased as would the proportion of patients with prior exposure to parenteral antibiotics.

Figure 4.7. Proportions of 3GCEC, 3GCRKP, MRSA, CRACI and CRPA in patients with BSI of hospital-origin stratified by exposure to a parenteral antibiotic at the study hospital and if 50%, 25% and 10% of the first blood culture episodes were sampled.



Footnote: A blood culture episode was defined as all blood culture specimens collected within two calendar days beginning when a blood culture specimen was collected. This figure is taken from the published manuscript (of which I am the first author) and with the permission of the corresponding author of the paper.

Both factors may increase the risk of AMR infections.[333,334] Moreover, patients with hospital-origin BSI whose clinical condition improved after empirical treatment and without blood specimen sample would be missing from the calculation. Also, the

exposure to parenteral antibiotics may reduce the chance of detecting non-AMR bacteria and hence a reduced count of patients with antimicrobial-susceptible infection.[335]

The findings from the simulation study suggested that the new parameter (the proportion of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started at the study hospital and continued for at least four consecutive days) for blood culture rate performed well in representing the blood culture utilisation rate. This new parameter correlated well with the reduction in blood culture sampling, and hence could be useful for understanding blood culture practices in different settings. Moreover, the parameter can be stratified by origin of infection.

4.6 Limitations

There are limitations in this study. First, the magnitude of impact shown in the study cohort may not be generalizable to other cohort of patients. Second, potential misclassification of patients into the two origins of infection is possible, as the definition of origin of infection is only a proxy for community-acquired and hospital-acquired infection.[331] Furthermore, a proportion of patients with community-origin BSI may be patients with nosocomial infections acquired at transferring hospitals. Third, data to differentiate whether two or more blood cultures obtained on the same day were drawn from a single phlebotomy (in which those specimens could be defined as a single blood culture set) was not available.[336,337] Our reported blood culture specimens per 1,000 patient-days could be higher than the true value of blood culture sets per 1,000 patient days.[336] Fourth, data on parenteral antibiotics prior to hospital admission was not available, hence the impact of that on estimates of proportions and incidence rate of AMR infection could not be determined.

4.7 Implications

Results of this study showed that the impact on estimates of the proportions and incidence rates of AMR infections due to low blood culture utilisation rate could be high. This could occur even when there are no changes in true susceptibility profiles of organisms and in the true infection rates in the setting. The proportion of AMR infections from blood cultures taken within ±1 calendar day of the start of a parenteral antibiotic were much lower than the proportion of AMR infections from blood cultures taken later. The evidence supported the previous recommendation on that the cAST or AMR surveillance reports could be strongly biased by low blood culture utilisation rate. Overall, the work could improve the understanding on the burden and methods to monitor AMR infections in settings where blood culture utilisation rate is low.

Based on the results from this study, a set of recommendations on how to improve the AMR surveillance reports in the future was made. The text below is adapted from the published article of this study and the recommendations were collectively drafted by the authors of the paper.

4.8 Five recommendations to improve AMR surveillance report

1. The estimates of parameters included in AMR surveillance reports should be stratified by origin of infection.[331] Moreover, each parameter should be accompanied by clear terminology, methodology, and numerator values and denominator values used in the calculation. This would increase comparability of the proportions and incidence rates of AMR infections across different settings.[222,338]

2. AMR surveillance reports should include parameters to represent blood culture practices (such as blood culture utilisation rates[339] and the proportion of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started at the study hospital and continued for at least four consecutive days). The reason is that the estimates of proportion and incidence rates of AMR infection are influenced by the utilisation of microbiology testing.
3. Incidence rates per 100,000 tested patients stratified by origin of infection should be estimated and reported, especially in LMICs where the blood-culture utilisation rate is low or unknown.[72,340,341] This is because the magnitude of change in the incidence rates per 100,000 tested patients is likely to be lower than that on the incidence rate per 100,000 population per year if the blood culture utilisation rate is low (Figure 4.6). The incidence rate per 100,000 population per year is still a good parameter for monitoring total AMR burden in high-income countries where blood culture utilisation rates are considered high.[339,342]
4. The interpretation of AMR trends needs to take account of the change of blood culture utilisation. Our results suggest that if a hospital improves their blood culture utilisation rate over time (e.g., doubling their utilisation rate), the observed proportions of AMR infections could decline considerably while observed incidence rates of AMR infections (per 100,000 population per year) could increase considerably, even if there are no changes in true susceptibility profiles of pathogenic organisms and in true infection rates in that environment.
5. Hospitals in LMICs with a low blood culture utilisation rate should use AMR surveillance reports stratified by origin of infection and by exposure to an empirical antibiotic at the study hospital to guide choice of empiric antimicrobial therapy rather than the total antibiogram. This is because the cumulative

antibiogram reports mainly based on blood culture after failure of the first empiric treatment should not be used to guide choice of the first empiric therapy. This is similar to the recommendations for tuberculosis, where proportions of multi-drug resistant tuberculosis are stratified for new (never treated) tuberculosis cases and previously treated tuberculosis cases, and the choice of first-line anti-tuberculosis drugs is based on the proportion of drug-resistant tuberculosis found in new cases.[343-345]

5

Impacts of delays in concordant antibiotic treatment on mortality

5.1 Abstract

Background

Therapeutic options for multidrug-resistant *Acinetobacter* spp. are limited, and resistance to last resort antibiotics in hospitals is increasing globally. Quantifying the impact of delays in concordant antibiotic treatment on patient mortality is important for designing hospital antibiotic policies. The gold standard for studying the causal relationship is a randomized controlled trial. However, it is unethical to intentionally delay concordant antibiotic treatment. Alternatively, observational cohort data would be useful to study the causal relationship when potentially important biases can be addressed appropriately. The aims of this chapter are to perform a simulation study to explore analysis methods to address biases, and then apply an appropriate analysis to quantify impact of delays in concordant antibiotic treatment on patient mortality using observational data.

Methods

Firstly, a simulation study to generate individuals' trajectories since hospitalization was done. Data were simulated under two scenarios. These were: no causal relationship between delays in concordant antibiotic treatment and mortality and a dose-response causal relationship between delays in concordant antibiotic treatment and mortality. For

each scenario, two data sets were created. The first data set was simulated under the assumption that the number of cases of AMR infection adds to non-AMR infections. This corresponds to an assumption that there is no competition in an ecological niche between resistant and susceptible strains of bacteria. Under this assumption, the daily increase in probability of acquiring infection caused by resistant strains is independent of that caused by susceptible strains. The second data set was simulated under the assumption of AMR infections replacing non-AMR infections. This corresponds to an assumption that the resistant and susceptible strains of bacteria compete for the same niche in colonising patients and causing infections (i.e., a daily decrease in the probability of acquiring infection caused by a susceptible strain of bacteria is accompanied by a daily increase in the probability of acquiring infection caused by resistant strains for each patient). Estimated impacts of delays in concordant antibiotic treatment on mortality using different analysis methods were compared. Secondly, observational cohort data were collected. Patients with *Acinetobacter* spp. hospital-acquired bacteraemia (HAB) in a hospital in Thailand over a 13-year period were included in the study. For each day of stay following the first positive blood culture antibiotic treatment was considered as concordant to AST results if the isolated organism was susceptible to at least one antibiotic given. Data analysis was done using marginal structural models with inverse-probability weightings.

Results

The simulation study shows that standard logistic regression gives biased estimates in the presence of time-varying confounders and immortal time bias. The magnitude of the bias, measured as the absolute difference between the true value and the estimated value, can be as large as 11 percentage points (when the true mortality was 31% and estimated 30-

day mortality was 20%). Marginal structural models resolved the biases and gave unbiased estimates. The observational cohort data analysis included 1,203 patients who had HAB with *Acinetobacter* spp., of which 682 patients (56·7%) had one or more days of delay in concordant treatment. These delays were associated with an absolute increase in 30-day mortality of 6.6 percentage points (95% CI 0.2, 13.0 percentage points), from 33.8% to 40.4%. Crude 30-day mortality was substantially lower in patients with three or more days of delays in concordant treatment compared to those with one to two days of delays. Accounting for confounders and immortal time bias resolved this paradox, and showed similar 30-day mortality for one, two and three or more days of delays.

Interpretation

Marginal structural models with inverse probability weightings can be used to adjust for confounding and immortal time biases in observational data. Delays in concordant antibiotic treatment were associated with a 6.6 percentage points increase in mortality among patients with hospital-acquired *Acinetobacter* spp. bacteraemia. If this association is causal, switching fifteen patients from discordant to concordant initial treatment would be expected to prevent one death in this cohort of patients.

5.2 Introduction

Empirical antibiotic treatment, which is given before the causative organism or its susceptibility to antibiotics is known, is often designed to have broad coverage to ensure the causative organism is susceptible to one of the prescribed antibiotics (i.e., treatment is said to be "concordant"). Considerations about concordance must be balanced against concerns about selection for resistance.[265,346] In settings where antimicrobial resistance is common, empirical treatment may often be "non-concordant", i.e., the causative organism is not susceptible to any of the antibiotics given. The resulting delay in the time until the patient receives concordant antibiotics may lead to worse patient outcomes[347] and is a key mechanism by which antimicrobial resistance is thought to add to the burden of disease (i.e., health impact due to antibiotic-resistant infections).[265,348] Changes in empirical antibiotic treatment are also possible in the absence of antibiotic susceptibility results if the patient shows no clinical improvement.[281] Many hospitals in LMICs lack access to a microbiology testing service, and even in the cases where access is available the utilisation of microbiology testing is often low compared to hospitals in HICs. Both high prevalence of resistance isolates and low utilisation of microbiology testing can lead to delays to concordant antibiotic treatment. Therefore, understanding the magnitude of the impact of delays in concordant antibiotic treatment on patient outcomes is important for estimating the potential benefits of investments in microbiological testing capacity, changes in microbiology testing practice, and improvements in empirical antibiotic use policies. It is also important for estimating the potential benefits of innovative technologies that can shorten the time taken to identify the causative organism and to know antimicrobial susceptibility profiles.

Quantifying the causal effect of delays in concordant treatment on patient outcomes using observational data poses a number of challenges. Firstly, key baseline confounders need to be considered when studying the causal relationship between antibiotic treatment and patient in-hospital mortality, and should be adjusted for in the analysis. Secondly, antibiotic treatment may change over the course of an infection (time-varying exposure), and these changes may be related to the time-varying clinical severity of the patient, which could be a confounder for the current treatment and a mediator for the future treatment. Finally, observational studies are vulnerable to bias when patients are classified into exposure groups after time zero.[239,269,289,349] As explained in detail in Hernan et al (2016) these biases can be avoided by specifying a target trial.[269]

The aim of this work is to explore the methods that are commonly used in the current literature, and then apply the optimal method to quantify the impact of delays in concordant antibiotics for treating *Acinetobacter* spp. bloodstream infections in Northeast Thailand. *Acinetobacter* spp. are amongst the leading causes of bloodstream infections in Thailand, and multidrug-resistant (MDR) *Acinetobacter* spp. have been estimated to cause 15,000 excess death per year.[21,78,93]

5.3 Methods

5.3.1 Literature search

PubMed was searched using the search term: (((((Acinetobacter [TITLE]) AND ((("antibiotic" or "antibiotics" or "antimicrobial therapy" or "empiric therapy") and ("mortality" or "death" or "failure" or "survival") and ("bloodstream" or "bacteraemia" or "bacteremia" or “septicaemia” or "septicemia" or "sepsis") and ("inappropriate" or "appropriate" or "discordant" or "non concordant" or "concordant" or "delayed" or "covering" or "noncovering")))))))), with no language restriction.

5.3.2 Simulation study

Two hypothetical scenarios based on the causal diagram in Figure 5.1 were simulated. A cohort of 5,000 patients who were admitted to the hospital without any infection and stayed in the hospital for at least 3 days were generated for each hypothetical scenario. The maximum number of hospitalization days was 200 days. In the first hypothetical scenario, there was no causal relationship between delays in concordant empirical antibiotic treatment and patient mortality (i.e., delay in the time until the patient receives concordant empirical antibiotics does not lead to worse patient outcomes). The second hypothetical scenario assumed that there was a dose-response relationship between delays in concordant empirical antibiotic treatment and patient mortality (i.e., delay in the time until the patient receives concordant empirical antibiotics leads to worse patient outcomes).

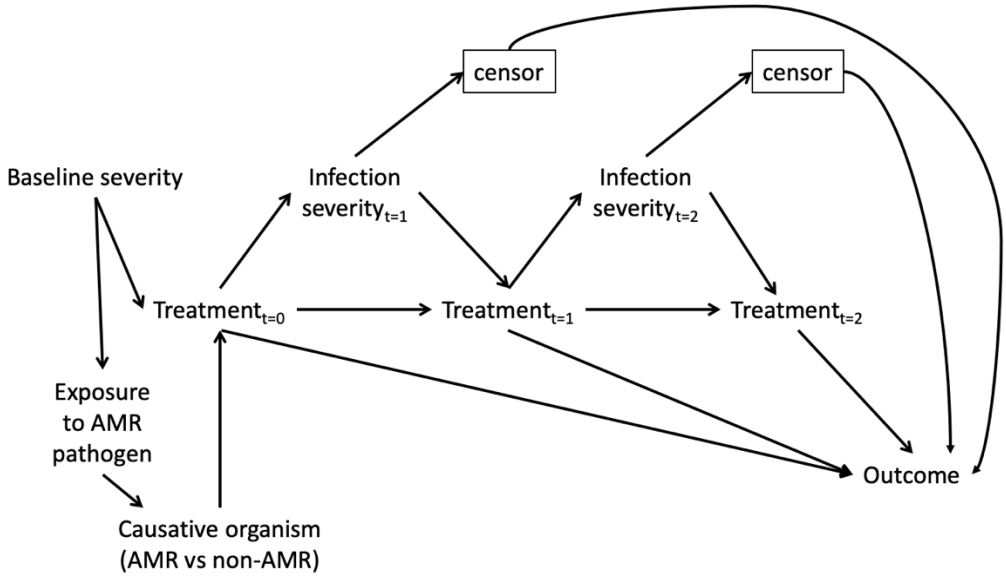
In each of the two hypothetical scenarios, two risk factors that influenced the probability of acquiring hospital-acquired infections are the severity of underlying illness and length of hospitalisation (Figure 5.1). Starting from day 3 of hospitalization, patients are at risk

of acquiring a hospital-acquired infection, and this risk (measured as a probability) increases with length of hospitalisation. The daily infection status of each patient was simulated based on a Markov process (i.e., the transition probability is only determined by the infection status on the previous day and risk factors). The daily transition events of each patient are summarised in Figure 5.2. The parameter values used in the simulations are in Appendix C1.

In addition, two data sets were then simulated for each hypothetical scenario. The first data set was simulated based on the assumption that AMR infections add to non-AMR infections. This corresponds to an assumption that there is no competition in an ecological niche between resistant and susceptible strains of bacteria (i.e., the daily increase in probability of acquiring infection caused by resistant strains is independent of that caused by susceptible strains). Evidence in support of the assumption that the burden of AMR infections adds to that of non-AMR infections comes from European surveillance data and US hospital data depicting secular trends of infections caused by MRSA and MSSA.[350-352] The second data set was simulated based on the assumption that AMR infections replace non-AMR infections. In this case I assumed that the resistant and susceptible strains of bacteria compete for the same niche in colonising patients, which in turn cause infections (i.e., a daily decrease in probability of acquiring infection caused by a susceptible strain of bacteria is accompanied by a daily increase in probability of acquiring infection caused by a resistant strain for each patient). There is evidence suggesting such competition occurs between different lineages of *S. pneumoniae*.[353] The two synthetic data sets are simulated in order to explore the magnitude of biases under different assumptions about transmission dynamics of the resistant and susceptible strains of pathogen.

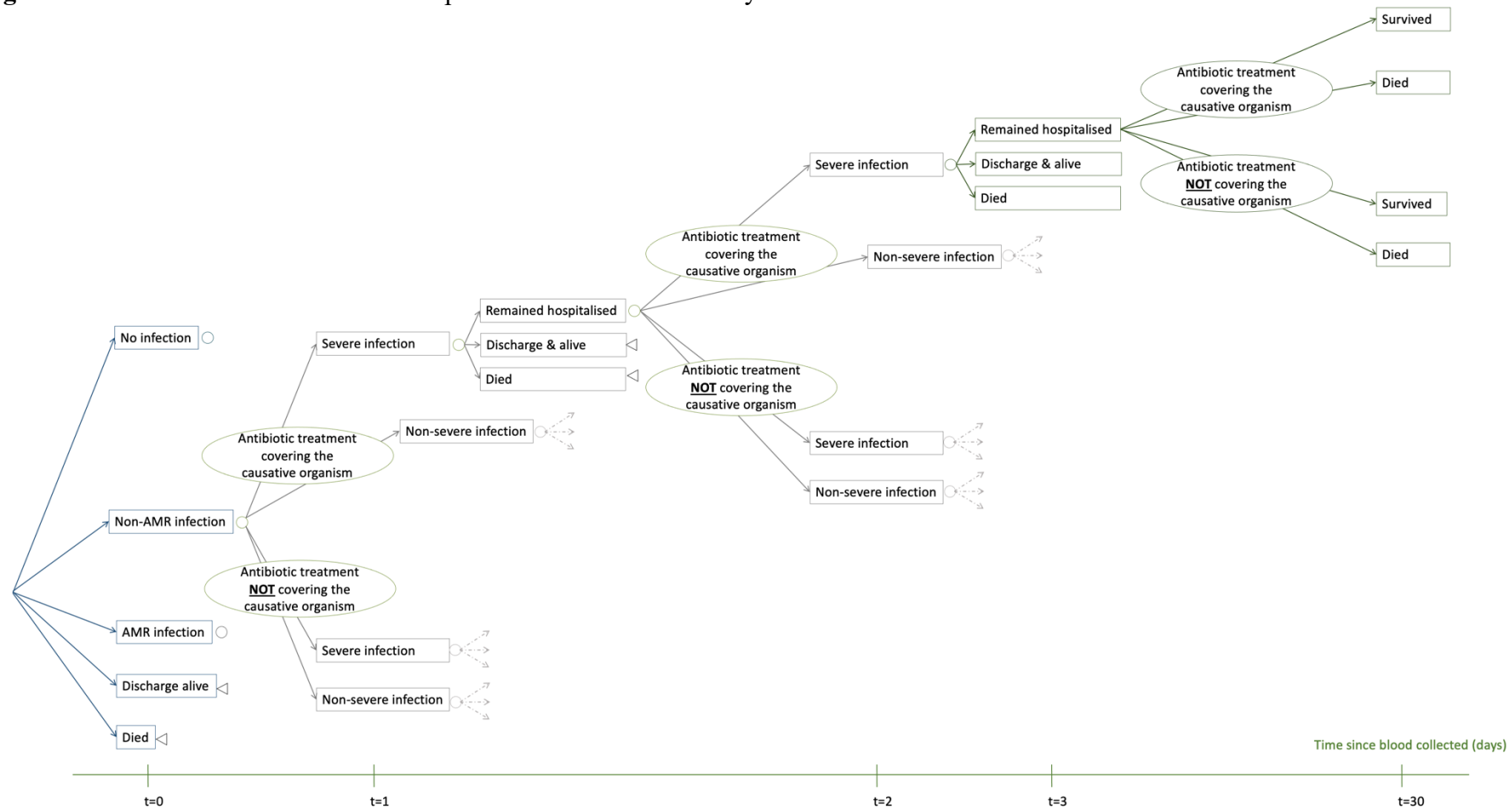
The simulated data under each hypothetical scenario and assumption were then analysed using four statistical approaches: 1) a simple logistic regression model based on the per-protocol assumption; 2) a simple logistic regression model on the whole cohort; 3) a marginal structural model with IPW for time-varying confounders; and 4) a marginal structural model with IPWs for time-varying confounders and immortal-time bias. The estimated 30-day mortalities from the statistical models were compared. The simulation study was performed in R (version 3.6.2), and analysis for the simulated data was performed in STATA, version 15.1 (StataCorp LP, College Station, Texas, USA).

Figure 5.1. A simplified directed acyclic graph (DAG) to represent the possible causal relationship between concordant empirical treatment and patient mortality, and used to guide the simulation study.



Footnote: Arrows indicate the direction of a causal relationship; for instance, the relationship "baseline severity increases the probability of antibiotic-resistant infection" is represented by an arrow from "Baseline severity" to "exposure to AMR pathogen". A time-varying confounder that could affect changes in empirical antibiotic treatment post blood sample collection is severity of infection, which could be affected by the history of treatment and itself may influence the decision on future treatment. "censor" refers to patients who discharged from the hospital or died before completing the treatment.

Figure 5.2. Patient health status transition paths in the simulation study.



Footnote: The rectangles represent the infection and survival status of patients and the ovals represent factors that affect state transitions. The triangles represent the absorbing states under which patients are no longer observed after the event (i.e., death or discharged from the hospital) occurred. The state transition patterns following the grey dashed arrows are the same as those following the grey solid arrows.

5.3.3 Emulating two target trials

Trying to make causal inferences from observational data can be thought of as attempting to emulate a target randomized trial.[269] Two hypothetical target trials are described using the framework outlined by Hernan and Robins.[269] Summaries of these protocols are in Table 1 and 2. In summary, inclusion criteria are the same in both trials: patients of any age and gender hospitalised for ≥2 days when a blood sample was collected and *Acinetobacter* spp. identified from the blood sample. In the first target trial, two treatment strategies are compared. These strategies are i) at least one-day of delay in concordant antibiotic treatment and ii) concordant treatment on the same day as blood sample collection (i.e., no delays in the time patient received concordant empirical antibiotic treatment). In the second target trial, patients are randomly assigned to one of four treatment strategies: i) no delay, ii) one-day of delay, iii) two days of delay, and iv) at least three days delay in receiving concordant antibiotic treatment. For these two target trials, patients are randomly assigned to the treatment group on the date of blood sample collection (assuming causative organism identification and AST can be obtained on the day of blood sample collection using for example molecular techniques). The follow-up period starts on the day of randomization and ends at the day of discharge from the hospital, day of death within the hospital, or 30 days post-randomization, whichever occurs first. The primary outcome of both trials is survival status at the end of follow-up. The contrasts of interest in both target trials are the per-protocol effect. The empirical antibiotic prescribed within the first three days after a blood sample is taken for microbiology culture is the main focus on the hypothetical trials. The dosing and frequency are not accounted for in the trials.

Table 5.1. A summary of target trial 1 to compare two treatment strategies

Protocol component	Description
Eligibility criteria	Patients who had been hospitalized for at least 2 calendar days on the date of collection of a blood sample from which *Acinetobacter* spp. was identified.
Treatment strategies	Days of delay in receiving concordant antibiotic treatment. The two treatment strategies were i) initiate an antibiotic regimen that is concordant to the *Acinetobacter* spp. isolated from blood sample without delay (i.e., on the same day as the blood sample is taken); ii) initiate a treatment that does not cover the *Acinetobacter* spp. isolated from blood sample at baseline.
Assignment procedures	Patients will be randomly assigned to one of two the antibiotic treatment strategies at baseline (on the day of blood sample collection).
Follow-up period	Starts at randomization and ends at day of discharge from the hospital, day of death within the hospital, or 30 days post randomization, whichever occurs first.
Outcome	Survival status within 30 days post randomization.
Causal contrasts of interest	Per-protocol effect.
Analysis plan	Per-protocol effect estimate requires adjustments for pre-defined confounders and immortal-time bias using marginal structural models with inverse-probability weights.

Table 5.2. A summary of target trial 2 to compare four treatment strategies.

Protocol component	Description
Eligibility criteria	Patients who had been hospitalized for at least 2 calendar days when a blood sample was collected and *Acinetobacter* spp. was identified.
Treatment strategies	Days of delay in receiving concordant antibiotic treatment. The four treatment strategies are: i) initiate an antibiotic treatment that is concordant to the *Acinetobacter* spp. isolated from blood sample without delay (i.e., on the same day as the blood sample is taken); ii) initiate a concordant antibiotic treatment one calendar day after the blood sample was taken; iii) initiate a concordant antibiotic treatment two calendar days after the blood sample was taken; and iv) no concordant antibiotic treatment for at least 3 days after blood sample collected. Patients are to keep the treatment strategies for at least 3 days post blood sample collection unless severity of infection increases during the treatment.
Assignment procedures	Patients will be randomly assigned to one of the four antibiotic treatment strategies at baseline (on the day of blood sample collection).
Follow-up period	Starts at randomization and ends at day of discharge from the hospital, day of death within the hospital, or 30 days post randomization, whichever occurs first.
Outcome	Survival status within 30 days post randomization.
Causal contrasts of interest	Per-protocol effect.
Analysis plan	Per-protocol effect estimate requires adjustments for pre-defined confounders and immortal-time bias using marginal structural models with inverse-probability weights.

5.3.4 Retrospective cohort data to emulate the target trials

A 13-year retrospective cohort study was performed. Patients admitted to Sunpasitthiprasong Hospital, Thailand between 2003 and 2015, and with hospital-acquired *Acinetobacter* spp. bacteraemia were included in the study. Sunpasitthiprasong Hospital is a provincial hospital with 1,500 beds. The hospital has a microbiology laboratory that performs microbial culture for isolate identification and antimicrobial susceptibility tests (AST) on a daily basis.

Patients were included in this study if they had stayed in the hospital >2 calendar days when a blood sample was collected and the culture results showed growth of *Acinetobacter* spp.. During the study period, Sunpasitthiprasong Hospital performed bacterial culture using standard methodologies for bacterial identification and the interpretation of AST results were based on guidelines of the Bureau of Laboratory Quality and Standards, Ministry of Public Health, Thailand.[354] In brief, Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret the results of AST from the disk diffusion method.[74] The first episode of *Acinetobacter* spp. bloodstream infection (BSI) per patient was included in the analysis. For the cases that have more than one isolate of *Acinetobacter* spp. with different susceptibility profiles identified on the same day, the isolate resistant to the largest number of antibiotics tested was included in the analysis. Additional data on antibiotic prescription, ICD-10 diagnosis, and patient basic demographics were collected and used for analysis.

The study was approved by the Institutional Review Board of Sunpasitthiprasong Hospital (Ref. 005/2560).

5.3.5 Covariate selection for the analysis in the observational study

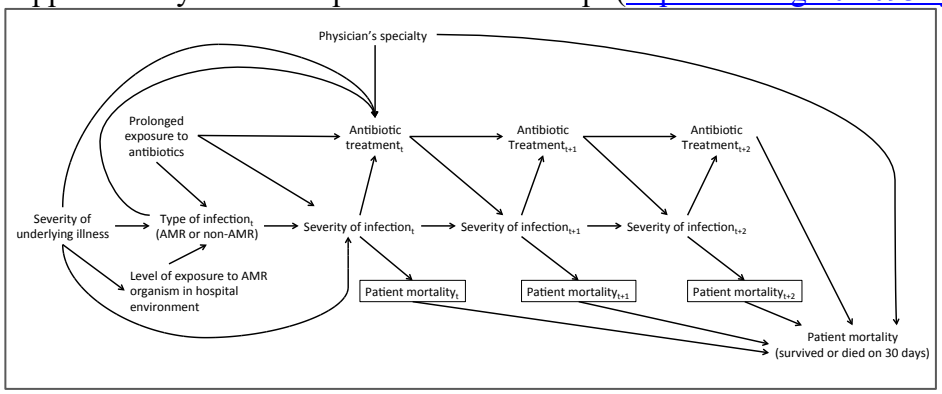
A directed acyclic graph to represent the presumed causal relationships between antibiotic treatment and patient mortality was drawn to identify the potential confounders (Figure 5.3).[268] The baseline (time-fixed) confounders identified were severity of underlying illness,[121] AST results of the *Acinetobacter* spp. isolates, year in which the blood samples were collected, and the attending physician. The time between date of admission and date of blood sample collection, whether or not admitted to intensive care unit (ICU) on the day of hospital admission, the cumulative days on antibiotic treatment before blood sample was collected, and age-stratified Charlson comorbidity index (CCI) scores as surrogates of severity of underlying illness. The ICD 10 code was used to calculate CCI scores.[355] MDR *Acinetobacter* spp. was defined as previously described.[356] The department in which the patient was treated on the day of blood collection was used as a proxy variable for the attending physician. A time-varying confounder that could affect changes in empirical antibiotic treatment post blood sample collection is severity of infection, and this itself could be affected by the history of treatment and may influence the decision on future antibiotic treatment. As data on daily severity of infection was not available, the prescription of a vasopressor and transfer to ICU during the infection within the analysis time period were used as proxy variables. Patient demographic information, age and gender, were also included as time-fixed covariates.

5.3.6 Exposure groups of the observational study

An antibiotic regimen was defined as concordant if AST results indicated that the *Acinetobacter* spp. isolates were susceptible to at least one of the antibiotics given. Otherwise, the regimen was defined as discordant treatment. For each eligible patient, concordance of the antibiotic treatment was determined on the day of blood sample

collection (which is defined as t=0), two days after blood sample collection (t=1), and three days after blood sample collection (t=2). The four exposure groups are: 1) patients with no delays in concordant antibiotic treatment (i.e., patients who had concordant treatment on the day of blood sample collection); 2) patients with one-day delay in concordant treatment (i.e., patients who did not have concordant antibiotic treatment at the least on t=1, which included those who died or were discharged at t=1); 3) patients with two days of delay in concordant treatment (i.e., patients who did not have concordant antibiotic treatment at the least on t=1 and t=2, which included those who died or were discharged at t=2); and 4) patients with at least three days of delay in concordant treatment.

Figure 5.3. A directed acyclic graph (DAG) to represent the causal relationship between antibiotic treatment and treatment outcome. This figure is modified from the supplementary file of the published manuscript (https://doi.org/10.1093/aje/kwab158).



Footnote: Arrows indicate the direction of a causal relationship; for instance, the relationship "severity of underlying illnesses may cause increases in the probability of antibiotic-resistant infection" is represented by an arrow from "Severity of underlying illness" to "Type of infection (AMR or non-AMR)". The key potential baseline confounders identified were severity of underlying illness, antibiotic resistance pattern of the *Acinetobacter* spp. isolated from the blood sample, year in which the blood samples were collected, and specialty of the attending physician. We used the time between date of admission and date of blood sample collection, admission to intensive care unit (ICU) on the day of hospital admission, the number of days on antibiotic treatment prior to blood sample collection, and age-stratified Charlson comorbidity index (CCI). score as surrogates of severity of underlying illness. The CCI scores were calculated from the ICD 10 codes given to each patient by the attending physicians. MDR *Acinetobacter* spp. was defined as previously described. As data on specialty of the attending physician is not routinely collected in the electronic record, we used the department in which the patient was treated on the day of blood collection as a proxy variable. A time-varying confounder that could affect changes in empirical antibiotic treatment post blood sample collection is severity of infection, which could be affected by the history of treatment and itself may influence the decision on future treatment. The prescription of a vasopressor and transfer to ICU during the infection within the analysis time period were used to represent severity of the infection and both coded as binary time varying variables. Patient demographic information, age and gender, were also included as covariates.

5.3.7 Outcomes of the observational study

The primary outcome was in-hospital all-cause mortality within 30 days of the date of collecting the first blood sample from which *Acinetobacter* spp. was isolated (t=30). A patient was considered to have survived if the patient was discharged alive from the hospital before t=30 or remained in the hospital on t=30.

5.3.8 Statistical analysis for the observational cohort data

Having observed the results from the simulation study, marginal structural models were used to estimate the effects of delays in concordant empirical antibiotic treatment on 30-day mortality.[282] Two analyses were performed. The first analysis was to evaluate the impact of one or more days of delays in concordant antibiotic treatment. The analysis is to emulate the first target trial described in the previous section. The second analysis was to evaluate the effect of one-day, two-day, and three or more days of delays in concordant antibiotic treatment (i.e., emulating the second target trial), which is to emulate the second target trial described in the section above. Stabilized inverse-probability weights (IPWs) were calculated for the two analyses independently based on methods described elsewhere.[349] Baseline variables, including age, gender, and CCI, were used for stabilising the weights. In brief, two sets of IPWs were generated. The first set was calculated from propensity scores representing the probability of being prescribed with a concordant antibiotic treatment on the day of blood sample collection, one day after, and two days after. Stabilized IPWs were then calculated from these propensity scores. The second set of IPWs, which is to emulate the second target trial with treatment regimen assigned on enrolment, was calculated using the three-step procedure described by Hernan.[349] A marginal structural logistic regression model with the stabilized IPWs to

estimate the marginal probability of 30-day mortality under each treatment regimen was then used to analyse the observational data.

Statistical analyses were performed in STATA, version 15.1 (StataCorp LP, College Station, Texas, USA).

5.4 Results

5.4.1 Literature review

The search identified 77 articles that were published before December 2019. No studies were found that aimed to evaluate the impact of delays in concordant empirical antibiotic treatment among patients with *Acinetobacter* spp. bacteraemia in low and middle-income countries (LMICs). There were 4 studies that aimed specifically to evaluate the relationship between inappropriate empirical antibiotics and mortality in high-income countries. Three of these four studies reported that appropriate empirical antibiotic therapy was associated with a reduction (ranging from 42-85%) in the relative risk of mortality. One study reported no association. The proportion of isolates that were resistant to carbapenem was 18% in one study, 58% in another, and not reported in the other two. The largest study was performed in the United States on a cohort of 1,423 patients with community-acquired *A. baumannii* pneumonia and sepsis. The remaining three studies had sample sizes of fewer than 270 patients. The four studies suffered from one or more of the following methodological limitations. First, the exposure was not well-defined. For example, one of the studies did not specify the time period over which the exposure was assessed (i.e., the period of time at which antibiotic treatment was considered as empiric). Second, the findings were vulnerable to time-varying and immortal time biases as treatment was given over an interval of days and appropriate analytical methods to adjust for these biases were lacking. Detailed assessments on the

risk of bias for the four studies using the ROBINS-I tool for assessing risk of bias in observational research are in Table 5.3. Of the remaining 73 studies, 39 articles written in English were relevant and sought to identify "risk factors" for mortality among patients with *Acinetobacter* spp. bacteraemia, but did not explicitly set out to answer causal questions. These studies tended to be small (sample sizes ranged from 26 to 399, IQR 54-177) and, while many reported associations of mortality with inappropriate empirical antibiotic treatment, these studies used methods that would not be expected to yield causally interpretable results.

The assessment results of the four studies and the current retrospective study using the Risk of Bias in Nonrandomized Studies of Interventions tools (ROBINS-I) are in Table 5.3.

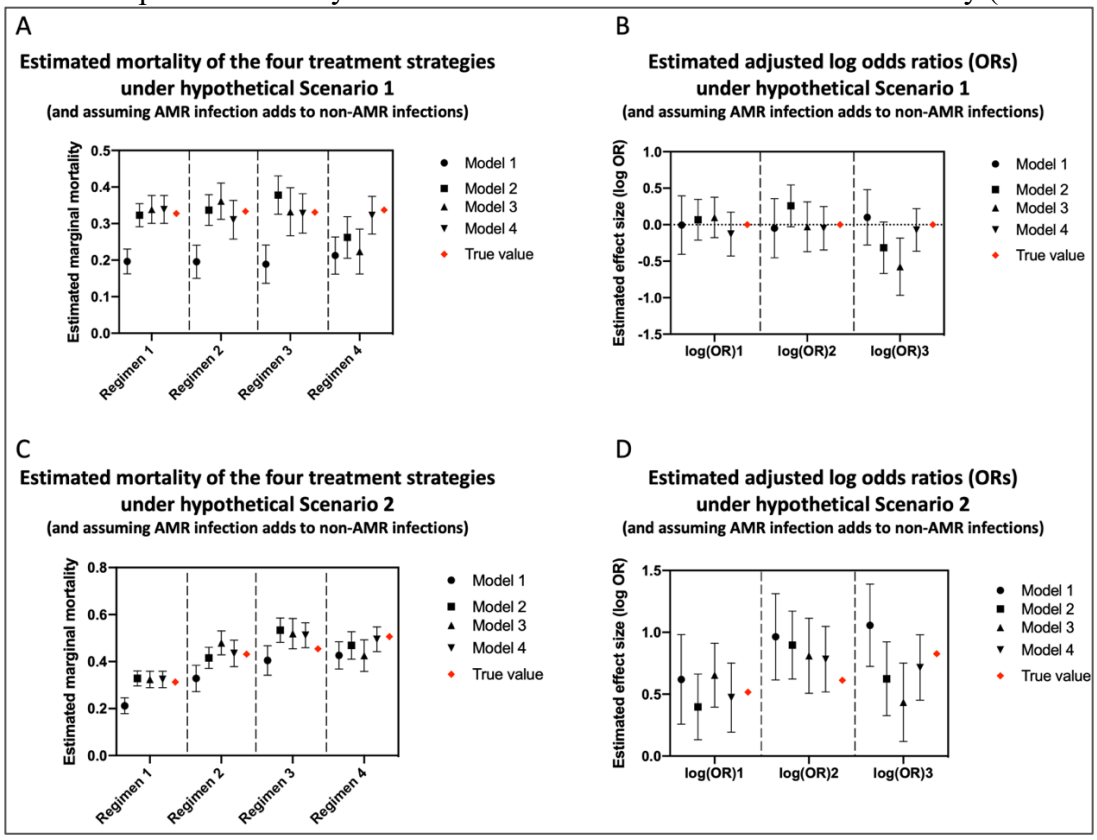
Table 5.3. Assessment results of the four articles identified with a similar study objective and the current retrospective cohort study performed in Sunpasitthiprasong Hospital using the Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) tool.

Study*	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of reported result	Overall risk of bias
The current study	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias
Zilberberg MD, et al. 2016[357]	Serious risk of bias	Moderate risk of bias	Serious risk of bias	Low risk of bias	No information	Low risk of bias	Low risk of bias	Serious risk of bias
Al-Dorzi HM, et al. 2015[358]	No information	No information	Serious risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Serious risk of bias	Serious risk of bias
Shorr AF, et al. 2014[359]	Serious risk of bias	No information	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Serious risk of bias
Kuo SC, et al. 2013[360]	Serious risk of bias	Moderate risk of bias	Serious risk of bias	Low risk of bias	No information	Low risk of bias	Serious risk of bias	Serious risk of bias

5.4.2 Simulation study

Figure 5.4 and Table 5.4 show the results of the four statistical models for estimating the expected 30-day mortality under scenario 1 (no causal relationship between delay in the time until patient receives concordant empirical antibiotic treatment and mortality) and under scenario 2 (increased delay in the time until patient receives concordant empirical antibiotic treatment would increase mortality). In the first set of simulations, the assumption that antimicrobial resistant infections add to antimicrobial susceptible infections was applied (Figure 5.4). Then in the second set of simulation, the assumption that antimicrobial resistant infections replace antimicrobial susceptible infections were applied (Figure 5.5). Simple multivariable logistic regression models (model 1 and 2) gave biased estimates in both scenarios. More precisely, both models underestimated the 30-day mortality for the exposure groups that represent long delays in time until a patient receives concordant antibiotic treatment (Table 5.4 and 5.5). The absolute difference between the true value and estimated mortality could be as big as 11 percentage points (from a true mortality of 31% to an estimated mortality of 20%) in scenario 1 under the assumption that AMR infection adds to non-AMR infection. Marginal structural models with IPW adjusted for time-varying confounders alone are not sufficient to provide an unbiased estimate, as the approach does not account for immortal-time bias. Finally, marginal structural models with IPWs adjusted for both time-varying confounders and immortal-time bias provided an unbiased estimate. Having seen the results of the simulation study, marginal structural models were used to analyse the observational data collected from Sunpasitthiprasong Hospital.

Figure 5.4. Comparisons of the estimated mortalities and effect sizes from four statistical models. The assumption that antimicrobial resistant infection added to antimicrobial susceptible infection is applied when simulating both scenario 1 and scenario 2. The magnitude of bias in the estimated 30-day mortality is the largest when using (A) a standard multivariable logistic regression model (Model 1) on the cohort that completed the treatment strategy (i.e., survived and remained in the study within the first 3 days of empirical antibiotic treatment) and minimal when using a marginal structural model accounted for both time-varying confounder and immortal time bias (Model 4). The adjusted log odds ratio (B) comparing the odds of 30-day mortality under regimen 3 and regimen 1 deviated from the true effect size when using a standard multivariable logistic regression model (Model 2) on the entire eligible cohort (i.e., including both patients who remained in the study and who dropped out from the study [due to a death or a discharged event] within the first 3 days of empirical antibiotic treatment), and when using a marginal structural model accounted for both time-varying confounder alone (Model 3). A similar pattern was observed under scenario 2, which assumed there is a dose-response relationship between delays in concordant antibiotic treatment and mortality (C and D).



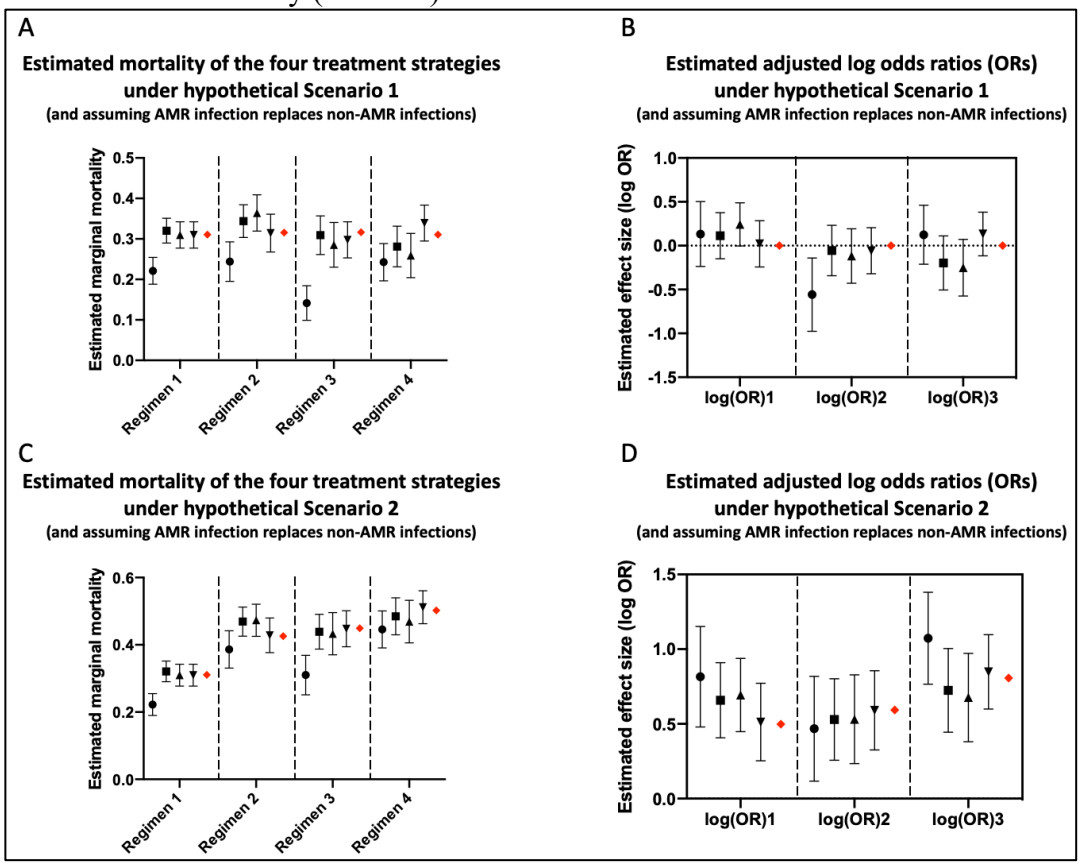
Footnote: Scenario 1 assumes no causal relationship between delays in concordant antibiotic treatment and 30-day mortality. Scenario 2 assumes a dose-response relationship between delays in concordant antibiotic treatment and 30-day mortality.

Table 5.4. The absolute differences between the true mortality and estimated mortality for the four treatment strategies under the assumption that AMR infection add to non-AMR infections (i.e., no competition between the AMR and non-AMR strains of the same bacteria over the same ecological niche area).

	Scenario 1 under the addition assumption				**Scenario 2 under the addition assumption**			
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Regimen 1	0.11	-0.01	-0.01	-0.01	0.10	-0.01	-0.01	-0.01
Regimen 2	0.11	0.02	-0.03	0.02	0.10	0.02	-0.05	0.00
Regimen 3	0.09	-0.08	-0.07	-0.05	0.05	-0.08	-0.06	-0.06
Regimen 4	0.08	0.04	0.08	-0.02	0.08	0.04	0.08	0.01

Footnote: Regimen 1, 2, 3, and 4 represent the treatment strategy of no delays in concordant empirical antibiotic treatment, one-day delay, two-day delay, and at least three-day delays, respectively. Models 1, 2, 3, and 4 are respectively: a standard multivariable logistic regression model on the cohort of patients who completed the 3 days of empirical antibiotic treatment; a standard multivariable logistic regression model on the entire cohort of patients who met the eligible criteria of the study (i.e., including both of those who completed and not completed the 3 days of empirical antibiotic treatment); a marginal structural model with inverse-probability weighting adjusting for time-varying confounder; and a marginal structural model with inverse-probability weightings adjusting for time-varying confounder and immortal time bias, respectively. In scenario 1, no causal relationship between number of days of delays in concordant treatment and patient mortality is assumed. In scenario 2, a dose-response causal relationship between number of days of delays in concordant treatment and patient mortality is assumed.

Figure 5.5. Comparisons of the estimated mortalities and effect sizes from four statistical models. The assumption that antimicrobial resistant infection replaces antimicrobial susceptible infection is applied. The magnitude of bias in the estimated 30-day mortality is largest when using (A) a standard multivariable logistic regression model (Model 1) on the cohort that completed the treatment strategy (i.e., survived and remained in the study within the first 3 days of empirical antibiotic treatment) and minimal when using a marginal structural model accounting for both time-varying confounders and immortal time bias (Model 4). The adjusted log odds ratio (B) comparing the odds of 30-day mortality under regimen 3 and regimen 1 deviated from the true effect size when using a standard multivariable logistic regression model (Model 2) on the entire eligible cohort (i.e., including both patients who remained in the study and who dropped out from the study [due to a death or a discharge event] within the first 3 days of empirical antibiotic treatment), and when using a marginal structural model accounting for both time-varying confounders alone (Model 3). A similar pattern was observed under scenario 2, which assumed there is a dose-response relationship between delays in concordant antibiotic treatment and mortality (C and D).



Footnote: Scenario 1 assumes no causal relationship between delays in concordant antibiotic treatment and 30-day mortality. Scenario 2 assumes a dose-response relationship between delays in concordant antibiotic treatment and 30-day mortality.

Table 5.5. The absolute differences between the true mortality and estimated mortality of the four treatment strategies under the assumption that AMR infections replace non-AMR infections (i.e., there is competition between AMR and non-AMR strains of the same bacteria over the same ecological niche, and the AMR strain causes decreases of the non-AMR strains).

	Scenario 1 under the replacement assumption				**Scenario 2 under the replacement assumption**			
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Regimen 1	0.09	-0.01	0.00	0.00	0.09	-0.01	0.00	0.00
Regimen 2	0.07	-0.03	-0.05	0.00	0.04	-0.04	-0.05	0.00
Regimen 3	0.17	0.01	0.03	0.02	0.14	0.01	0.02	0.00
Regimen 4	0.07	0.03	0.05	-0.03	0.06	0.02	0.03	-0.01

Footnote: Regimens 1, 2, 3, and 4 represent the treatment strategies of no delays in concordant empirical antibiotic treatment, one-day delay, two-day delay, and at least three-day delays, respectively. Models 1, 2, 3, and 4 are: a standard multivariable logistic regression model on the cohort of patients who completed the 3 days of empirical antibiotic treatment; a standard multivariable logistic regression model on the entire cohort of patients who met the eligible criteria of the study (i.e., including both of those who completed and not completed the 3 days of empirical antibiotic treatment); a marginal structural model with inverse-probability weighting adjusted for time-varying confounder; and marginal structural model with inverse-probability weightings adjusted for time-varying confounder and immortal time bias, respectively. In scenario 1, no causal relationship between the number of days of delays in concordant treatment and patient mortality is assumed. In scenario 2, a dose-response causal relationship between number of days of delays in concordant treatment and patient mortality is assumed.

5.4.3 Characteristic of patients in the retrospective observational study

There were 1,203 inpatients who had a blood sample collected two or more days after hospital admission yielding *Acinetobacter* spp. between 2003 and 2015 (Figure 5.6). Amongst the eligible cohort, 521 patients had concordant treatment on the day of blood sample collection (no delays in concordant antibiotic treatment); 224 patients had a one-day delay; 119 patients had a two-day delay; and 339 patients had three or more days of delays in concordant antibiotic treatment. Patient characteristics varied across the four groups of exposures (Table 5.6). The proportion of patients admitted to ICU wards on the day of hospitalization was highest among those having a one-day delay in concordant antibiotic treatment (159 of 224 patients; 71.0%), followed by those having a two-day

delay (72 of 119 patients, 60.5%). The proportion of patients with carbapenem-resistant *Acinetobacter* spp. isolates was highest among those who had a one-day delay in concordant antibiotic treatment (195 of 224; 87.1%), followed by those who had two-day delay (103 of 339 patients; 86.6%). The number of days on antibiotic treatment prior to blood sample collection was highest among those with no delays in concordant antibiotic treatment (9 days; interquartile range [IQR]: 5-15) and lowest among those with one-day delay (5 days; IQR: 0-10).

Figure 5.6. Flow chart of patients identified in the hospital microbiology database and included in the analysis.

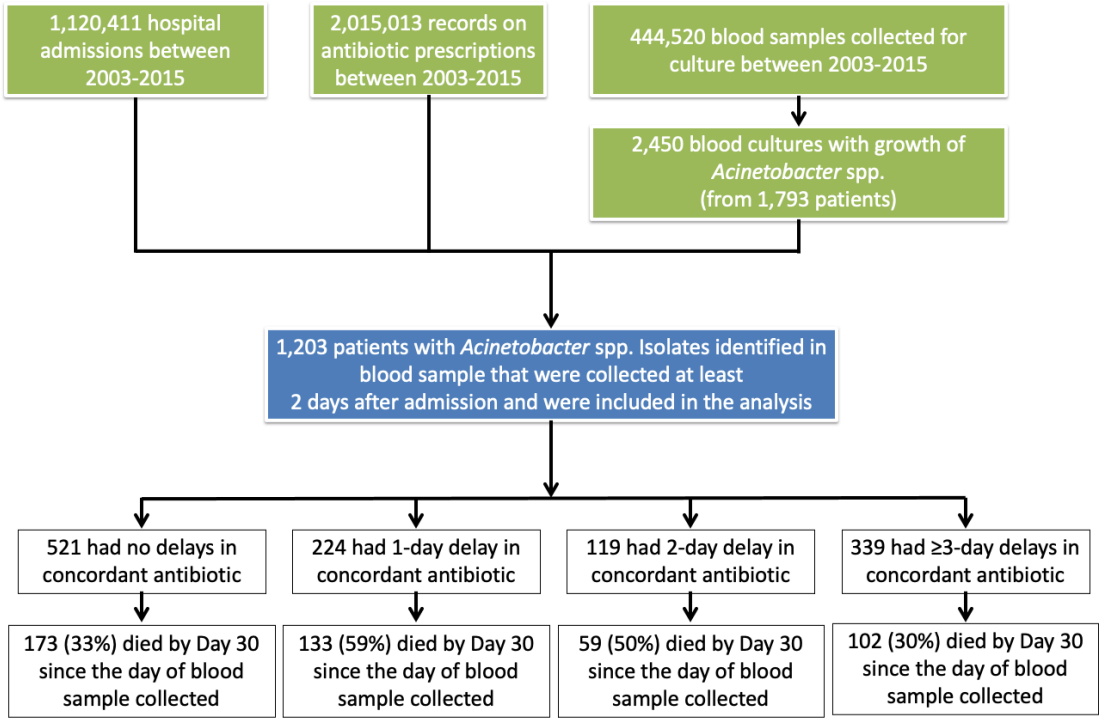


Table 5.6. Characteristics of patients with hospital-acquired bloodstream infection related to *Acinetobacter* spp.. This table is modified from Table 1 in the article published at American Journal of Epidemiology (https://doi.org/10.1093/aje/kwab158).

	No delays in concordant antibiotic treatment (n=521)	**One-day of delay in concordant antibiotic treatment (n=224)**	**Two-day of delay in concordant antibiotic treatment (n=119)**	**Three or more days of delay in concordant antibiotic treatment (n=339)**
Age (years)	54 (26-69)	57 (12-70)	51 (14-69)	54 (6-70)
Female gender	212 (40.7%)	103 (46.0%)	56 (47.1%)	143 (42.2%)
Fluoroquinolone-resistance	309 (59.3%)	191 (85.3%)	93 (78.2%)	266 (78.5%)
Carbapenem-resistance	312 (59.9%)	195 (87.1%)	103 (86.6%)	286 (84.4%)
Multi-drug resistance	364 (69.9%)	206 (92.0%)	103 (86.6%)	302 (89.1%)
Age-adjusted CCI score on admission	2 (0-4)	2 (0-5)	2 (0-4)	2 (0-4)
Patients with vasopressor prescription on the day of blood sample collected for culture	211 (40.5%)	129 (57.6%)	50 (42.0%)	103 (30.4%)
Patients with vasopressor prescription on the second day of blood sample collected for culture	176 (38.7%)[a]	22 (40.7%)[c]	43 (36.1%)	117 (34.5%)
Patients with vasopressor prescription on the third day of blood sample collected for culture	133 (34.1%)[b]	17 (34.7%)[d]	15 (38.5%)[e]	97 (28.6%)
Patients admitted to ICU on the day of hospitalisation	285 (54.7%)	159 (71.0%)	72 (60.5%)	162 (47.8%)
Patients in the ICU on the day of blood sample collected for culture	304 (58.3%)	156 (69.6%)	70 (58.8%)	191 (56.3%)
Overall in-hospital mortality	194 (37.2%)	137 (61.2%)	59 (49.6%)	120 (35.4%)
30-day in-hospital mortality since blood collection	173 (33.2%)	133 (59.4%)	59 (49.6%)	102 (30.1%)
Length of hospital stay since admission to blood sample collected for culture (days)	9 (6-19)	8 (6-15)	9 (6-15)	10 (6-17)

Days on antibiotic prior blood sample collection	8 (5-15)	5 (0-10)	7 (3-12)	7 (4-14)

Footnote: [a]Denominator is 455 as 66 patients with no delays in concordant antibiotic treatment were discharged from the hospital or died after t=0. [b]Denominator is 390 as 65 more patients with no delays in concordant antibiotic treatment were discharged from the hospital or died after t=1. [c]Denominator is 54 as 170 patients with a one-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=0. [d]Denominator is 49 as 5 more patients with a one-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=1. [e]Denominator is 39 as 55 patients with a two-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=0. Age, CCI score, length of hospital stays since admission to blood collection, and days on antibiotic prior blood collection are median (IQR), and other data is n (%). ICU=intensive care unit. CCI=Charlson Comorbidity Index Score; defined using ICD10 scores.[116] Multidrug resistance is defined as non-susceptible to ≥1 agent in ≥3 antimicrobial categories.[117]

5.4.4 Antibiotic treatment concordance on the day of blood sample collection and 30-day in-hospital mortality

The analysis to emulate the first target trial showed that receiving concordant antibiotic treatment on the day of blood collection was associated with reduced 30-day mortality, after adjusting for the pre-specified time-fixed confounders. The expected 30-day mortality was 33.8% (95% CI: 29.1%, 38.5%), if all patients were given concordant antibiotic treatment on the day of blood collection, compared with the expected 30-day mortality of 40.4% (95% CI: 36.1%, 44.7%) if all patients were not treated with concordant antibiotics on the day of blood collection. 6.6 percentage points (95% CI: 0.2, 13.0 percentage points) was the absolute difference.

5.4.5 Days of delays in concordant antibiotic treatment and 30-day in-hospital mortality

The analysis to emulate the second target trial showed that the crude 30-day in-hospital all-cause mortality was highest among those with a one-day delay in concordant antibiotic treatment (133 of 224 patients; 59.4%), and lowest among those with three or more days of delays in concordant antibiotic treatment (102 of 339 patients; 30.1%) (Table 5.7). The discharge pattern of patients under different treatment groups varied

over the three days of the initial treatment period (Figure 5.7). Amongst the 1,203 eligible patients, 236 (19.6%) either died or were discharged from the hospital one day after blood was collected for culture and, of those patients, 150 patients died within the hospital (Appendix C2).

The marginal structural model adjusting for baseline confounders, time-varying confounders, and immortal time bias resolved paradoxical observations in the crude data (Table 5.7). While the crude analysis showed that patients with three or more days of delays in concordant antibiotic treatment had the lowest mortality, the adjusted analysis showed that the expected mortality was lowest if the patients had no delays. However, no evidence of increasing mortality with increasing delays was observed, and uncertainty in the estimates was large.

Figure 5.7. Discharge patterns over 3 days after blood sample collected among the analysed cohort.

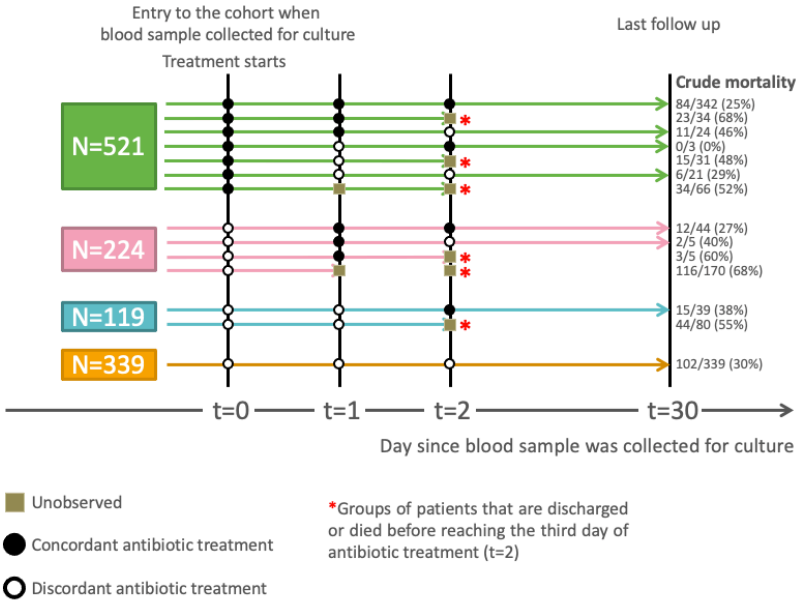


Table 5.7. Estimated expected 30-day mortality for each exposure group.

	Crude 30-days in-hospital all-cause mortality (n)	Expected 30-days mortality (95% CI) under each treatment regimen
No delays in concordant antibiotic treatment (n=521)	33.2% (173/521)	39.8% (32.3%-47.2%)
A one day of delay in concordant antibiotic treatment (n=224)	59.4% (133/224)	42.8% (29.8%-55.7%)
A two day of delay in concordant antibiotic treatment (n=119)	49.6% (59/119)	51.0% (38.9%-63.1%)
Three or more days of delays in concordant antibiotic treatment (n=339)	30.1% (102/339)	40.9% (36.0%-45.8%)

5.5 Discussion

The simulation study suggested that marginal structural models are useful to analyse observational data when time-varying confounder and immortal time bias are present in the data. In the first analysis attempting to emulate a target trial with two treatment arms and using marginal structural models, results showed that delays in concordant antibiotic treatment of one or more days were associated with an absolute increase of 6.6 percentage points in 30-day mortality from 33.8% to 40.4%. No evidence of a dose response relationship between days of delays in concordant antibiotics and 30-day mortality was observed in the second analysis.

HAB related to MDR *Acinetobacter* spp. is associated with increased patient mortality, especially in developing countries.[93] The proportion of hospital-acquired *Acinetobacter* spp. bacteraemias that are MDR has been reported as high as 75%, and the range of attributable was from 18-41% in developing countries (10-12, 20-23).[20,21,78,93,116-118] The magnitude of impact of delays to concordant antibiotic treatment is important when estimating the potential benefits of diagnostic stewardship and of the efforts to reduce the time taken to obtain antibiotic susceptibility testing results. Our estimates of the impact

of delays in concordant empirical antibiotic treatment on patient mortality among those with hospital-acquired *Acinetobacter* spp. bloodstream infection will be of particular relevance in settings with a high incidence of drug-resistant *Acinetobacter* spp. infection.

Immortal time bias is a common phenomenon and needs to be considered in studies comparing treatment regimens where the observed treatment durations vary.[239,289] The research question asked in this chapter involved empirical antibiotic treatments that were given to patients over a period of three days after blood sample collection was considered and only patients who survived up to three days after blood collection could be classified into the group of "≥3 days of delays in concordant treatment". Hence, by definition, these patients cannot have died within the first 3 days and during this time period they are effectively assumed "immortal". Consequently, the bias will tend to make this group of patients appear to survive longer compared to the group who had no delays in concordant antibiotic treatment. This is reflected in the paradoxical observation that the crude all-cause 30-day mortality was lowest among patients with ≥3 days of delay in concordant antibiotic treatment. This type of bias can be avoided by adopting the target trial emulation methodology as described by *Hernan M* (2018)[349] and was employed in the analysis described in this chapter.

Previous analyses have evaluated the impact of delayed antibiotic treatment on outcomes for patients with bacteraemia related to *Acinetobacter* spp.,[360-366] but there is a lack of appropriate adjustment for biases. A study of 252 patients with ICU-acquired bloodstream infections caused by *A. baumannii* suggested appropriate antibiotic therapy, which was defined as administration of at least one antibiotic treatment that is appropriate in type, route and dosage within 48 hours of bacteraemia onset, reduced 14-day mortality.

The adjusted odds ratio was 0.22 (95% CI: 0.10, 0.50).[365] The analysis used a multivariable logistic regression model adjusted for APACHE II score measured two days prior to bacteraemia onset and for malignancy. In that study, more than 70% of patients died within 24 hours in the inappropriate antibiotic group while no patients died in the appropriate treatment group within the initial 48 hours treatment period among those with APACHE II score >35.[365] It is likely that some of the reported differences in survival probability due to immortal time bias.

Most of the previous studies considered antibiotic use as a binary variable and switching of antibiotic regimens due to changes in clinical symptoms has been neglected.[281] However, in practice antibiotics are sometimes prescribed even before a clinical sample is taken for culture, especially in hospitals in resource-limited settings. Moreover, switching regimen in response to severity of infection is not uncommon.[281] Biased estimates may result when the changes in regimen determined by clinical signs were not incorporated using appropriate analysis designed for time-varying confounders.[269,282] A previous study has used marginal structural models to adjust for time-varying confounders to evaluate the association between appropriate antibiotic treatment for bacteraemia on the day the blood culture was taken and treatment outcome.[367] There was no evidence for a protective effect of appropriate empirical antibiotic treatment on treatment outcome reported in the study, but confidence intervals for the estimates were wide.[367] A direct comparison of results from that study and the findings in this chapter is not appropriate as the two differed in bacterial species considered, patient characteristics and patient management.

5.6 Limitations

There are four key limitations in the retrospective study. Firstly, data on the severity of infections were not routinely collected, and this is typically the case in LMIC settings. We used admission to ICU and prescription of vasopressor as proxy variables for the severity of infection. These proxy variables will only imperfectly represent the severity of infection and residual confounding is to be expected; however, both are specific in representing severe clinical conditions. Secondly, despite the relatively large sample size, the power to detect a dose-response relationship in the four regimens under evaluation might be low. This is reflected in the wide confidence intervals in our expected mortality under each regimen. Thirdly, our emulation included only ACI-HAB. Initiating empirical antibiotic treatment for patients with hospital-acquired sepsis would include both ACI-HAB and non-ACI-HAB. Nonetheless, point-of-care rapid diagnostic tests for ACI-HAB with AST results are critically needed to differentiate from non-ACI-HAB to avoid overuse of antibiotics that target non-MDR and MDR AS-HAB. Fourthly, residual confounding factors may be present.

5.7 Implication

The evidence of a causal link between delays in concordant antibiotic treatment of hospital-acquired *Acinetobacter* spp. bacteraemia and increases in mortality has four main implications: first, it highlights the pressing need for substantial investment in effective infection control measures in LMIC hospital settings where multidrug-resistant strains of *Acinetobacter* spp. are endemic; second it provides key quantitative information to inform rational design of hospital antibiotic prescribing guidelines which has hitherto been lacking; such guidelines must balance the twin aims of minimizing mortality in patients needing antibiotic treatment and preserving therapeutic options for future

patients; third, it provides further data to support needed investment in clinical microbiology in LMIC settings and for research to enable rapid and low cost susceptibility testing for *Acinetobacter* spp.; and fourth, it provides further evidence of the critical need for the development of new antibiotics to reduce deaths from carbapenem-resistant *Acinetobacter* spp. bacteraemia, especially in settings where it is endemic.

5.8 Conclusions

A 6.6 percentage points (95% CI: 0.2, 13.0 percentage points) increase in mortality among patients with hospital-acquired *Acinetobacter* spp. bacteraemia when concordant antibiotic treatment was delayed for one or more days was observed. Accounting for confounding and immortal time biases is necessary when attempting to estimate causal effects of delayed concordant treatment and, in this case, helped resolve paradoxical results in the initial crude data analysis.

6

Conclusions

This chapter starts with an overview of the main findings and a summary of how the findings contribute to the growing literature on the epidemiology, burdens, and surveillance systems of antimicrobial resistance (AMR) infections in the context of Southeast Asian countries. This is followed by a discussion of the implication of the available evidence for health policy and the potential directions for future work.

6.1 An overview of the key findings

The overall aim of the research performed in this thesis is to improve the understanding of the epidemiology and health impact of AMR infections in LMICs. Specifically, I have: i) estimated excess mortality attributable to AMR infections in a LMIC; ii) developed an open-access application that can be used in LMIC settings for analysing routinely collected data and generating AMR surveillance reports automatically; iii) quantified the impact of low blood culture utilisation rates on estimates of proportions and incidence rates of AMR infection in LMICs; and iv) evaluated the impact of delays in concordant antibiotic treatments on mortality of patients with hospital-acquired *Acinetobacter* spp. bacteraemia.

6.1.1 Excess mortality attributable to AMR infections

The results from the prospective surveillance study on hospital-acquired infections suggested that the PAF of mortality attributable to AMR infections can be as high as 16.3% (95% CI: 1.2, 29.1%) after adjusting for comorbidities and other risk factors of deaths (Chapter 2). It implies that 16.3% (105 deaths) of the observed 643 deaths could be prevented if all of the patients with hospital-acquired infections in the study were infected by AMS organisms rather than the observed pathogens. The excess mortality and deaths attributable to AMR infections varied by bacteria and type of infection. The findings suggest that if the proportion of HAI caused by AMR bacteria increased (for instance due to misuse or overuse of antibiotics in hospitals), the total number of people dying could also increase even if the incidence rate of HAI remains constant over time. Interventions that decrease the proportion of HAI due to resistant pathogens could decrease patient mortality even if they failed to reduce the incidence of HAI.

To my knowledge, this is the first prospective study in a LMIC that systematically estimates the mortalities attributable to AMR infections among lower-respiratory tract infection (LRTI), surgical-site infection (SSI), urinary-tract infection (UTI), other site-specific infection (OTH), and more than one types of infection relative to the mortality attributable to BSI. Using these estimates and local data on incidence rates of AMR infections, one may produce an estimate of the overall number of excess deaths attributable to hospital-acquired AMR infections from BSI data alone, which is more accessible than data for the other types of infections in LMICs. However, the excess mortality attributable to AMR infections estimated from this study should be used with caution. The parameter values estimated here may not be generalisable to all resource-limited hospitals. Nevertheless, the estimates help to provide a better understanding of potential impact of AMR infections in tertiary hospitals in Southeast Asia, where

hospital-acquired AMR infections are common. The findings suggested that excess mortality and deaths due to AMR infections in LMICs could be much higher than that observed in HICs. Hence, underestimation of AMR burdens in LMICs would be expected if the estimates were derived using parameters established in HICs.

6.1.2 Tool to support AMR data sharing

The second aim of the thesis is to narrow the gaps in data analysis and data sharing in LMICs (Chapter 3). There is a lack of high-quality data to inform estimates of the prevalence and incidence of AMR infections globally. A recent study estimated that more than 2 billion people are living in countries without data on the WHO priority list of pathogens, and most countries are LMICs.[368] Efforts to fill in this data gap are essential in producing evidence to inform and assess strategies in controlling the spread of AMR infections. In Chapter 3, I described an open-access and easy-to-use application that can support resource-limited settings in analysing AMR surveillance data while preserving data security. AutoMated tool for Antimicrobial resistance Surveillance System (AMASS) is an application that was developed to meet the local needs in analysing routinely collected electronic data and in generating summary reports on cAST reports automatically (Chapter 3). In many developing countries, even when microbiology and hospital data are collected on a regular basis there is a lack of expertise, time, and skills to process, analyse and report the data at the local setting. National surveillance systems exist in some countries to collect individual data sets and to analyse them collectively; however, results specific to the local settings (i.e., hospital or surveillance site) are often not readily available. AMASS is a tool to demonstrate the concept of locally and automatically generated AMR surveillance reports to increase understanding of the local epidemiology of AMR infections and to fill the data gap in resource-limited settings.

AMASS was tested in six hospitals in six countries in South and Southeast Asia, and one hospital in the United Kingdom. The users shared their AMR surveillance reports generated from the local data using AMASS on an open-access data repository platform. The tool could increase the utilisation of the existing data as well as increase the confidence in sharing AMR surveillance reports locally, regionally and globally. The concept of AMASS can be easily adapted further to provide support for users to validate microbiology data in order to increase the quality of AMR data from resource-limited settings.

6.1.3 Magnitude of bias in estimating AMR infections when blood culture utilisation rate is low

The third angle that this thesis looked into is factors that influence the estimates of proportion and incidence rates of AMR infections. In particular, the magnitude of biases caused by low blood culture utilisation rate was examined (Chapter 4). It is known that low blood culture utilisation rate can bias the estimates on incidence rates of BSI. However, to my knowledge, the magnitude of such biases to proportions and incidence rates of AMR BSI has never been quantified. Using retrospective data and bootstrap sampling, I simulated different levels of blood culture utilisation rate to explore the possible magnitudes of biases. The findings aligned with previous expectations on the direction of biases. The proportion of AMR infections was overestimated in the scenarios when blood culture utilisation was low, and this bias could be as large as an absolute increase of 13 to 24 percentage points. Conversely, the incidence rate of AMR BSI (per 100,000 population per year) was underestimated when blood culture utilisation rate was low. Our findings suggest that AMR surveillance reports should consider reporting prevalence and incidence rates of AMR infections together with the blood culture

utilisation rate in the setting. This is particularly important when researchers, healthcare providers or policy makers are to compare proportions and/or incidence rates between different hospitals with a similar setting or within the same hospitals but between different time points.

Currently, a commonly used measurement of blood cultures utilisation rate is the number of blood culture specimens per 1,000 patient-days. A limitation of such measurement is that it cannot be stratified by origin of infection and it does not account for the incidence rates of bacterial infections in different hospital settings. The simulation study (Chapter 4) showed an alternative measurement, which is to report based on antibiotic prescription behaviour. The alternative measurement is the proportion of patients having a blood culture taken within ±1 calendar day of the day when a parenteral antibiotic was started and continued for at least four consecutive days. The antibiotic prescription for at least four consecutive days is a good marker for the incidence rates of bacterial infections and the requirement of blood culture utilisation. This is because it is strongly recommended that a blood culture should be taken prior to the start of parenteral antibiotics for treating infectious diseases and that antibiotic prophylaxis prior to clean and clean-contaminated surgical procedures should be limited to a single dose. Moreover, this measurement of blood culture utilisation rate based on antibiotic prescriptive behaviour can be stratified by origin infection.

The findings from this study add evidence to the growing literature on common pitfalls in estimating and interpreting statistics on AMR infections. They also addressed an important knowledge gap on the extent of biases as a result of low blood culture utilisation, and offered an alternative parameter to better represent blood culture

utilisation rate in order to ensure comparability of AMR infections across different settings and between different time points.

6.1.4 Delays in concordant antibiotic treatment and mortality

Finally, this thesis includes an investigation of the burden of AMR infections through discordant empirical antibiotic treatment. An absolute increase in 30-day mortality of 6.6 percentage points (95% CI: 0.2, 13.0 percentage points), from 33.8% to 40.4% for patients with delays in concordant antibiotic treatments for *Acinetobacter* spp. bacteraemia was estimated (Chapter 5). There are many publications evaluating the impacts of inappropriate antibiotic use on mortality, but the analytical methods used have been vulnerable to potentially important biases, in particular time-varying confounding and immortal time biases. This is because the causal relationship between antibiotic use, AMR infections, and mortality are complex. Chapter 5 illustrates the application of a counterfactual framework to observational data to systematically identify potential biases and then use appropriate methods to account for those biases.

In settings where AMR infections are common, empirical antibiotic treatments can often be discordant to the antimicrobial susceptibility profile (i.e., the causative organisms are not susceptible to the antibiotics given). A delay in the time until the patient receives appropriate antibiotics may in turn decrease chance of survival. This is a potential mechanism through which AMR infections add to the health burden of infectious diseases. In LMIC settings, often it may take at least 3 days for the microbiology culture and AST results to be available. Moreover, when the diagnostic microbiology service is limited and the threshold of clinical suspicion for a blood culture is high, longer delays to concordant antibiotic treatment would often result. Hence, the findings of Chapter 5 are

critical to take into account when assessing the potential benefits of investing in building the microbiological diagnostic capacity, in changing blood culture practice, and designing empirical antibiotic prescribing guidelines. Further, the estimates generated are important to measure the additional benefits of developing novel techniques that can shorten the time it takes to obtain microbiology culture and AST results.

6.1.5 Generalisability of the findings in this thesis

Throughout the thesis, I have used LMIC to refer to countries that are considered as either low- or middle- income settings. This is to differentiate from the high-income setting, such as the United Kingdom, in Chapter 3. However, not all the findings from this thesis can be generalised to hospitals in the low-income, lower-middle-income, or upper-middle-income settings, as patient characteristics, diagnostic capacity, patient management and healthcare seeking behaviour, which can impact the epidemiology of AMR, can be different from that in the study sites in this thesis. The works presented in Chapter 2, 4 and 5 are only starting points to illustrate the importance of surveillance data and to demonstrate the richness of routinely collected microbiology data. For instance, the estimated mortality attributable to AMR infections from the prospective surveillance study in Chapter 2 may not be generalisable to other settings, but the methodology used could be adapted to better implement in other hospitals. Moreover, transposing a causal effect from a study to a specific target population has been demonstrated previously using inverse odds of sampling weights[369] and do-calculus[370] under specific assumptions, however this is beyond the scope of this thesis. Similarly, the analysis approach used in Chapter 5 has been previously applied to evaluate the impact of inappropriate empirical antibiotic treatment in a cohort of patients with bacteraemia in a high-income setting[367], and could also be applied to other settings where microbiology data, clinical data, and

antibiotic use data are available. Also, the magnitude of bias in measuring prevalence and incidence of AMR due to low blood culture utilisation rate illustrated in Chapter 4 could vary by settings with different diagnostic capacity, and further work to understand the drivers of low blood culture utilisation rate would be useful for designing diagnostic stewardship and for comparing AMR burden across time and settings. AMASS (Chapter 3) can be used in settings in which electronic data are available regardless of the socioeconomic status of the setting. For instance, AMASS was tested in hospitals in high-income country (the United Kingdom), upper-middle-income country (Thailand), and lower-middle-income countries (Cambodia, Lao PDR, Nepal, Myanmar and Vietnam).

6.2 Implications to healthcare policy

Overall, the research reported in this thesis highlights the threat of AMR infections, and gaps in data and information to support the design of strategies to mitigate the impact of AMR infections in LMICs. The high health burden of AMR infection is worrying. Local capacity to share AMR surveillance reports, even when microbiology data is collected on a routine basis, is still limited. This can be improved using tools that can automatise data processing and report generating steps. Chapter 3 illustrates the potential benefits and feasibility of such a tool in various settings. Strategies to enhance the local capacity in managing, storing and processing microbiology data could help fill in the data gap in resource-limited settings. Estimates of proportions and incidence rates of AMR infections should be reported to local, regional, national, and global AMR committees to assist the design and assessment of interventions to control the spread of AMR infections. Moreover, the new parameter for blood culture utilisation rate developed in Chapter 4 can reflect the local culture practice, and is useful to report to enable fair comparisons. The reporting of blood culture utilisation rate would improve comparisons in estimates

of AMR burdens between different hospitals within similar settings and between different time points in a hospital or a surveillance site. Moreover, innovative strategies that can shorten the time to obtain microbiology culture and AST results would avoid delays in appropriate antibiotic treatment, which in turn could improve patient outcomes.

6.3 Future research areas

A crucial extension of the prospective surveillance study is to estimate the excess mortality and PAF of mortality attributable to AMR infections using patients without infections as a comparator. This can be done by collecting retrospective hospital admission data, using the prospective data of HAIs evaluated in this thesis, and applying exposure density sampling to select comparator cohorts matched by the length of hospitalisation prior to clinical sample collection for microbiology culture. Another dimension to expand is to implement the same protocol to a wide range of hospitals in LMICs. Data on AMR for each and all types of infection as well as data on AMR for the WHO GLASS priority pathogens remain scarce in LMICs. This limits the ability to accurately estimate and monitor the burdens of AMR infections in LMICs, where AMR infections have the largest impact. Multi-national and multi-centre prospective surveillance studies to estimate excess mortality attributable to AMR infections could help fill the data gap. In parallel, trials on interventions that aim to reduce health burden of AMR infections would be useful to define the burden of AMR infections.[371] For instance, vaccine probe studies have been done on *Haemophilus influenzae* type b[372] to quantify the difference in disease burden between the vaccinated and unvaccinated individuals, which in turn was used to define the vaccine-preventable burden of the infection. Vaccines for AMR infections are still under development, an example being passive vaccination with monoclonal antibodies developed for preventing the spread of

infectious diseases and AMR pathogens.[373] Future studies on efficacy and effectiveness of such vaccines may consider reporting the vaccine-preventable burden of AMR infection.

The accuracy of microbiology results is crucial for estimating the burden of AMR infections and also for guiding treatment of patients on a daily basis. Future development to expand AMASS to provide microbiology data quality control reports has been considered. Indeed, we were recently awarded with a grant from the institutional translational partnership of Mahidol-Oxford Tropical Medicine Research Unit (MORU) to expand the functions of AMASS and to test in hospitals in developing countries. A mixed-methods study will be performed to evaluate the utilisation of the application and to provide detailed understanding on the local operational challenges of AMR surveillance systems and of sharing AMR surveillance reports in resource-limited settings.

Future studies on approaches to tackle the methodological challenges in measuring burdens of AMR infection are needed to provide an accurate and precise understanding on the extent of this global health crisis. In particular, it is important to untangle the transmission dynamics of AMR and AMS infections. The degree to which resistant strains could replace susceptible strains of the same organism may vary by the type of bacteria and the class of antibiotics under evaluation. This may vary by the mechanisms through which resistant and susceptible strains co-exist. Such knowledge would inform the appropriate analysis approach and support a more precise estimate of excess deaths attributable to AMR infections. More importantly, it will be crucial for designing national action plans aimed at reducing AMR infections.

6.4 Conclusions

The thesis investigated data, tools, and methods of estimating burdens of AMR infections in LMICs. Data and evidence generated from this thesis can inform the design and assessment of preventive interventions for AMR infections in LMICs.

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Appendices

A. Appendices to Chapter Two

Appendix A1. Definitions of the types of infection included in this prospective surveillance study.

Type of infection	Definition
Bloodstream infection (BSI)	*At least one of the six organisms under this survey culture from blood sample <u>Central line-associated BSI</u> Patient enrolled as a case of BSI where central line or umbilical catheter was in place for >2 calendar days on the date of blood collection and the line was also in place on the date of blood collection or the day before. <u>Primary BSI</u> Patient enrolled as a case of BSI that are not secondary to an infection at another body site. <u>Secondary BSI</u> Patient enrolled as a case of BSI and enrolled as a case of a site-specific infection and with at least one matching organism to the site-specific specimen collected on the same day.
Lower-respiratory tract infection (LRTI)	All of the following 4 items must be met: 1. At least one of the below: • Fever (>38°C) • Leukopenia (≤4,000 WBC/mm^3) or leukocytosis (≥12,000 WBC/mm^3) • Altered mental status with no other recognized cause (if ≥70 years old) 2. At least one of the below clinical symptoms: • New onset of purulent sputum or change in character of sputum, or

	increased respiratory secretions, or increased suctioning requirements • New onset or worsening cough, or dyspnea or tachypnea • Rales/Crepitation or bronchial breath sounds, or wheezing or rhonchi • Worsening gas exchange (i.e., O_2 desaturation [i.e., $PaO_2/FiO_2 \leq 240$], increased oxygen requirements, or increased ventilator demand) 3. Imaging result suggested one of the followings • Consolidation • Cavitation • New or progressive and persistent infiltration • Pneumatoceles, in infants ≤1 year old 4. *At least one of the six organisms under this survey isolated from blood or lower-respiratory tract specimen.
Surgical site infection (SSI)	<u>Superficial incisional SSI (all must meet)</u> • Infection occurs within 30 days after operative procedure • Involves only skin and subcutaneous tissue of incision • *At least one of the six organisms cultured from an aseptically-obtained specimen from the superficial incision or subcutaneous tissue <u>Deep incisional SSI (all must meet)</u> • Infection occurs within 30 or 90 days after operative† • Involves deep soft tissue of the incision (i.e., fascial and muscle layer) • *At least one of the six organisms cultured from an aseptically-obtained specimen from the deep incision **<u>and</u>** fever (38˚C) or/and localized pain or tenderness <u>Organ/Space SSI (all must meet)</u> • Infection occurs within 30 or 90 days after operative procedure† • Infection involves any part of the body deeper than the fascial/muscle layers, that is opened or manipulated during the operative procedure • *At least one of the six organisms cultured from an aseptically-obtained fluid or tissue in the organ/space
Urinary tract infection (UTI)	All the following two items must be met: 1. At least one of the following is presented • ≥1 of these symptpms: fever (>38˚C), Suprapubic tenderness**, Costovertebral angle pain or tenderness**, urinary

	frequency***, urinary urgency***, dysuria*** • If patient **≤1yo**, at least **<u>ONE</u>** of these symptoms: fever (>38˚C), hypothermia (<36˚C), apnea**, bradycardia**, lethargy**, vomiting**, suprapubic tenderness** • There is a matching organism from blood specimen to the urine specimen 2. *At least one of the six organisms cultured from urine specimen and the organism(s) is of ≥10^5CFU/ml.
Other infections (OTH)	<u>Skin/Soft tissue infection</u> All of the following two items must be met 1. At least one of the symptoms below: • Purulent drainage, pustules, vesicles, or boils • At least two of the symptoms: localized pain or tenderness, swelling, warmth, or redness 2. *At least one of the six organisms cultured from aspirate or drainage from affected site <u>Other infections:</u> *At least one of the six organisms cultured from other sterile sites and affected sites including: • CSF • Purulent drainage/material (from eye, ear canal, oral cavity, reproductive tract, etc) • Fluid (joint fluid, peritoneal fluid, pericardial fluid, etc) • Abscess • Tissues (lung tissue, brain tissue, spinal epidural, disc space, heart valve, bone, etc)

* The bacteria under evaluation are *Staphylococcus aureus*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp..

Appendix A2. Descriptions of confounders defined based on the directed acyclic graph in the thesis (Figure 2.1).

Pre-defined confounder	Definition	Description
Ward admitted on the day of hospitalisation	Responsible medical specialty at time of hospitalisation (Based on WHO GLASS guideline)	Categorical variable 1= Medical wards (Adult/Paediatrics/Neonate) 2= Surgical wards (Adult/Paediatrics/Neonate) 3= Haematology/oncology 4= Obstetrics/gynaecology 5= All ICUs
SOFA score measured on the day of hospitalisation	Health condition at time of hospitalisation	Continuous variable
Referred from other hospitals	Whether or not patient was referred from another hospital. The study hospital is a tertiary hospital where patients with severe condition would sometimes be referred to. This variable is a proxy for the severity of underlying illness.	Binary variable 0= Did not refer from another hospital 1= Referred from another hospital
Charlson Comorbidity Index score	Based on congestive heart failure, myocardial infarction, peripheral vascular disease, chronic obstructive pulmonary disease, ulcer disease, liver diseases, renal disease, connective tissue disease, cerebrovascular disease, hemiplegia or paraplegia, dementia, lymphoma, leukemia, tumor, diabetes, recipient of organ transplant, and HIV infection	Categorical (9 groups) 0= score is 0 1= score is 1 . . . 8= score is 8 or above
Exposure to invasive device	Invasive device referred to either peripheral-line catheter, central-line catheter, urinary-catheter,	Binary variable 1= on invasive device for at least 48 hours

	ventilator, or umbilical catheter	0= if otherwise
Gender	Female or male	Binary variable
Age	Age in year	Continuous variable
Cumulative days on antibiotics prior the day of sample collection for microbiology culture	Counts of the number of days continuously on antibiotic one day prior clinical specimen collected for microbiology culture	Spline with 3 knots at 1, 8 and 14 days
Length of hospitalisation prior the day of sample collection for microbiology culture	Counts of the number of days being hospitalised before clinical specimen collected for microbiology culture	Spline with 5 knots at 4, 7, 11, 19, and 31 days
Number of days took to report microbiology testing results **(not a confounder but adjusted for in the model to increase precision)**	Counts of the number of days it took for the microbiology culture results (including AST) to be available and reported	Binary variable 0= 3 days 1= more than 3 days

Appendix A3. Estimated parameter values from different models

Models*	Confounders adjusted in the model	Estimated causal risk difference between AMR infections and AMS infections (95% CI)	PAF of mortality due to AMR infections (95% CI)ˆ	Estimated deaths due to AMR infections (95% CI)
Final model on the full analysis cohort**	Pre-defined confounders†	7.7% (2.2, 13.2%)	16.3% (1.2, 29.1%)	105 (7, 187)
Logistic regression model with interaction term on the full analysis cohort and random effects to account for the bacterial variation**	Pre-defined confounders†	7.7% (6.2, 9.1%)	16.3% (1.6, 30.9%)***	105 (10, 199)
Logistic regression model with interaction term on the full analysis cohort**	Selected variables††	9.3% (3.9, 14.7%)	19.7% (5.3, 32.0%)	127 (34, 206)
Logistic regression model with interaction term on the full analysis cohort**	Unadjusted	10.9% (5.6, 16.2%)	23.8% (9.5, 35.9%)	153 (61, 231)
Logistic regression model with no interaction term on the full analysis cohort	Pre-defined confounders†	7.3% (2.3, 12.2%)	16.1% (4.4, 26.3%)	104 (29, 170)
Logistic regression model with no interaction term on the full analysis cohort	Selected variables††	8.7% (3.9, 13.6%)	19.2% (8.0, 29.0%)	123 (51, 187)
Logistic regression model with no interaction term on the full analysis cohort	Unadjusted	10.0% (5.2, 14.7%)	21.8% (10.8-31.5%)	140 (70, 202)
Final model on the cohort excluding 39 cases who were lost to follow-up**	Pre-defined confounders†	6.7% (1.7, 11.7%)	15.3% (3.1, 25.8%)	94 (19, 159)
Final model on the full analysis cohort and assuming those who were lost to follow-up survived within 28 days of infection**	Pre-defined confounders†	6.7% (1.7, 11.6%)	15.5% (3.3, 26.1%)	95 (20, 161)

CI: confidence interval; *All models were adjusted for site-infection infection and causative organism. **An interaction term between causative organism and AMR infection was included in the model. ***95% confidence interval was estimated using bootstrap with 1000 samples. †Pre-defined confounders adjusted in the model are ward of admission on the day of hospitalisation, gender, age, CCI, length of hospitalisation

prior clinical sample collected for microbiology test, SOFA score of patients on time of hospitalisation, whether or not patient was referred from other hospital, whether or not the number of days it took to report microbiology test result within 3 days after sample collection, and cumulative days on parenteral antibiotics prior clinical sample collected for microbiology test. ††Selected variables adjusted in the model are confounders that could be captured in hospital admission records in a retrospective study design, and these are ward of admission on the day of hospitalisation, gender, age, CCI, and length of hospitalisation prior clinical sample collected for microbiology test. ^Excess mortality attributable to AMR infections is estimated by comparing the observed risk with the counterfactual outcome of AMR infections as illustrated in equation [1] in Chapter 2

Appendix A4. Characteristics of patients included in the analysis stratified by specific type of infections.

Characteristics	All enrolled patients (N=2,043)	BSI (N=289)	LRTI (N=618)	SSI (N=155)	UTI (N=796)	OTH (N=68)	More than one type of infections (N=117)
Sex							
Male	1,163 (56.9%)	161 (55.7%)	388 (62.8%)	93 (60.0%)	408 (51.3%)	43 (63.2%)	70 (59.8%)
Age							
≤0	84 (4.1%)	20 (6.9%)	31 (5.0%)	12 (7.7%)	9 (1.1%)	8 (11.8%)	4 (3.4%)
1-4	22 (1.1%)	3 (1.0%)	7 (1.1%)	2 (1.3%)	7 (0.9%)	1 (1.5%)	2 (1.7%)
5-14	42 (2.1%)	6 (2.1%)	11 (1.8%)	7 (4.5%)	13 (1.6%)	4 (5.9%)	1 (0.9%)
15-24	102 (5.0%)	17 (5.9%)	28 (4.5%)	18 (11.6%)	33 (4.1%)	3 (4.4%)	3 (2.6%)
25-34	90 (4.4%)	18 (6.2%)	25 (4.0%)	9 (5.8%)	32 (4.0%)	1 (1.5%)	5 (4.3%)
35-44	159 (7.8%)	30 (10.4%)	39 (6.3%)	13 (8.4%)	62 (7.8%)	3 (4.4%)	12 (10.3%)
45-54	281 (13.8%)	42 (14.5%)	88 (14.2%)	20 (12.9%)	108 (13.6%)	8 (11.8%)	15 (12.8%)
55-64	413 (20.2%)	68 (23.5%)	109 (17.6%)	25 (16.1%)	170 (21.4%)	12 (17.6%)	29 (24.8%)
65-80	640 (31.3%)	66 (22.8%)	209 (33.8%)	38 (24.5%)	264 (33.2%)	24 (35.3%)	39 (33.3%)
≥81	210 (10.3%)	19 (6.6%)	71 (11.5%)	11 (7.1%)	98 (12.3%)	4 (5.9%)	7 (6.0%)
Severity of underlying illness							
Charlson comorbidity index score*	3 (1-5)	3 (1-5)	3 (1-5)	2 (0-4)	3 (2-5)	3 (1-5)	4 (2-6)
Transferred from other hospitals	1,384 (67.7%)	171 (59.2%)	449 (72.7%)	94 (60.6%)	548 (68.8%)	41 (60.3%)	81 (69.2%)
Admission to ICU on hospitalisation day	505 (24.7%)	68 (23.5%)	204 (33.0%)	30 (19.4%)	161 (20.2%)	14 (20.6%)	28 (23.9%)
SOFA score on the day of admission*	2 (0-4)	3 (1-5)	2 (1-4)	1 (0-2)	2 (0-4)	3 (1-5)	2 (1-5)
Health status on day of specimen collection							
SOFA score on the day of specimen collection*	1 (0-4)	3 (0-6)	2 (1-4)	0 (0-2)	1 (0-3)	1 (0-3)	4 (1-7)
Admission to ICU on enrolment day	946 (46.3%)	120 (41.5%)	413 (66.8%)	56 (36.1%)	267 (33.5%)	20 (29.4%)	70 (59.8%)
Proportion of reporting microbiology results within 3 days since specimen collection	1,747 (85.5%)	93 (32.2%)	589 (95.3%)	138 (89.0%)	757 (95.1%)	64 (94.1%)	106 (90.6%)
Parenteral antibiotic usage							

Patients on parenteral antibiotic usage prior specimen collection	1,807 (88.4%)	269 (93.1%)	581 (94.0%)	141 (91.0%)	653 (82.0%)	56 (82.4%)	107 (91.5%)
Cumulative days of exposure to parenteral antibiotic prior specimen collection*	8 (1-14)	6 (1-13)	9 (5-15)	8 (5-16)	6 (1-14)	13 (4-22.5)	9 (4-15)
Length of hospital stay							
Days in the hospital prior specimen collection*	11 (7-19)	10 (6-18)	12 (7-18)	11 (7-19)	11 (7-20)	19 (11-29)	14 (7-21)
Outcome							
28-day mortality	643 (31.5%)	114 (39.5%)	231 (37.4%)	20 (12.9%)	202 (25.4%)	22 (32.4%)	54 (46.2%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

Appendix A5. Characteristics of patients who enrolled in the survey and had hospital-acquired bloodstream infection stratified by causative bacteria.

	Bloodstream infection (n=289)																	
	***Staphylococcus aureus* (n=36)**		***Enterococcus faecium* (n=11)**		***Enterococcus faecalis* (n=10)**		***Escherichia coli* (n=61)**			***Klebsiella pneumoniae* (n=66)**			***Pseudomonas aeruginosa* (n=22)**		***Acinetobacter baumannii* (n=69)**		**Polymicrobial (n=14)**	
Characteristics	**MRSA (n=4)**	**MSSA (n=32)**	**AMPR (n=10)**	**AMPS (n=1)**	**AMPR (n=1)**	**AMPS (n=9)**	**3GCR-CR (n=9)**	**3GCR-CS (n=35)**	**3GCS (n=17)**	**3GCR-CR (n=11)**	**3GCR-CS (n=43)**	**3GCS (n=12)**	**CR (n=5)**	**CS (n=17)**	**CR (n=55)**	**CS (n=19)**	**AMR (n=12)**	**AMS (n=2)**
Sex																		
Male	3 (75.0%)	15 (46.9%)	6 (60.0%)	0 (0%)	1 (100%)	7 (77.8%)	4 (44.4%)	13 (37.1%)	9 (52.9%)	9 (81.8%)	27 (62.8%)	8 (66.7%)	3 (60.0%)	11 (64.7%)	31 (57.4%)	5 (33.3%)	9 (75.0%)	0 (0.0%)
Age																		
≤0	0 (0.0%)	2 (6.3%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0.0%)	1 (2.9%)	1 (5.9%)	0 (0%)	6 (14.0%)	0 (0%)	1 (20.0%)	0 (0%)	6 (11.1%)	1 (6.7%)	1 (8.3%)	0 (0%)
1-4	0 (0.0%)	0 (0.0%)	0 (0%)	1 (100%)	0 (0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (3.7%)	0 (0%)	0 (0%)	0 (0%)
5-14	0 (0.0%)	1 (3.1%)	1 (10.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0.0%)	1 (2.9%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.9%)	2 (13.3%)	0 (0%)	0 (0%)
15-24	0 (0.0%)	1 (3.1%)	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)	1 (11.1%)	2 (5.7%)	0 (0.0%)	1 (9.1%)	4 (9.3%)	0 (0%)	0 (0%)	2 (11.8%)	5 (9.3%)	0 (0%)	0 (0%)	0 (0%)
25-34	2 (50.0%)	1 (3.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0.0%)	1 (2.9%)	1 (5.9%)	0 (0%)	4 (9.3%)	0 (0%)	0 (0%)	1 (5.9%)	3 (5.6%)	3 (20.0%)	2 (16.7%)	0 (0%)
35-44	0 (0.0%)	3 (9.4%)	1 (10.0%)	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)	4 (11.4%)	2 (11.8%)	3 (27.3%)	3 (7.0%)	4 (33.3%)	0 (0%)	3 (17.6%)	2 (3.7%)	2 (13.3%)	1 (8.3%)	0 (0%)
45-54	1 (25.0%)	7 (21.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)	8 (22.9%)	6 (35.3%)	0 (0%)	3 (7.0%)	1 (8.3%)	0 (0%)	3 (17.6%)	7 (13.0%)	1 (6.7%)	2 (16.7%)	1 (50.0%)
55-64	1 (25.0%)	6 (18.8%)	4 (40.0%)	0 (0%)	0 (0%)	3 (33.3%)	1 (11.1%)	8 (22.9%)	3 (17.6%)	4 (36.4%)	11 (25.6%)	3 (25.0%)	2 (40.0%)	1 (5.9%)	18 (33.3%)	2 (13.3%)	1 (8.3%)	0 (0%)
65-80	0 (0.0%)	6 (18.8%)	3 (30.0%)	0 (0%)	1 (100%)	3 (33.3%)	3 (33.3%)	9 (25.7%)	3 (17.6%)	3 (27.3%)	11 (25.6%)	2 (16.7%)	1 (20.0%)	5 (29.4%)	9 (16.7%)	4 (26.7%)	2 (16.7%)	1 (50.0%)
≥81	0 (0.0%)	5 (15.6%)	1 (10.0%)	0 (0%)	0 (0%)	1 (11.1%)	0 (0.0%)	1 (2.9%)	1 (5.9%)	0 (0%)	1 (2.3%)	2 (16.7%)	1 (20.0%)	2 (11.8%)	1 (1.9%)	0 (0%)	3 (25.0%)	0 (0%)
Device-related																		
Central-line associated	0 (0.0%)	2 (6.3%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	3 (8.6%)	0 (0.0%)	3 (27.3%)	6 (14.0%)	0 (0.0%)	0 (0.0%)	2 (11.8%)	13 (24.1%)	2 (13.3%)	2 (16.7%)	0 (0.0%)

Severity of underlying illness																		
Charlson comorbidity index score*	2 (2-4)	4 (1-5)	3 (2-4)	0 (0-0)	5 (5-5)	4 (2-4)	5 (2-6)	3 (1-4)	3 (2-3)	3 (1-9)	2 (0-4)	3 (0-5)	2 (2-8)	3 (2-5)	3 (1-5)	3 (2-6)	3 (1-4)	2 (0-3)
Transferred from other hospitals	2 (50.0%)	15 (46.9%)	8 (80.0%)	0 (0.0%)	0 (0%)	7 (77.8%)	4 (44.4%)	19 (54.3%)	5 (29.4%)	6 (54.5%)	26 (60.5%)	11 (91.7%)	3 (60.0%)	8 (47.1%)	36 (66.7%)	11 (73.3%)	8 (66.7%)	2 (100.0%)
Admission to ICU on hospitalisation day	1 (25.0%)	6 (18.8%)	4 (40.0%)	1 (100%)	1 (100%)	2 (22.2%)	0 (0.0%)	5 (14.3%)	2 (11.8%)	3 (27.3%)	12 (27.9%)	1 (8.3%)	1 (20.0%)	3 (17.6%)	19 (35.2%)	3 (20.0%)	3 (25.0%)	1 (50.0%)
SOFA score on the day of admission*	2 (1-10)	4 (2-5)	2 (0-7)	7 (7-7)	11 (11-11)	2 (2-5)	4 (2-4)	3 (0-4)	1 (0-2)	4 (0-6)	2 (0-4)	1 (0-5)	3 (2-4)	1 (0-4)	4 (2-7)	3 (1-4)	2 (1-5)	3 (1-4)
Health status on day of specimen collection																		
SOFA score on the day of specimen collection*	3 (0-10)	3 (0-4)	2 (0-5)	5 (5-5)	5 (5-5)	5 (0-6)	2 (1-7)	3 (0-7)	2 (0-4)	1 (0-6)	3 (1-7)	2 (0-6)	2 (1-4)	4 (3-4)	3 (1-8)	3 (0-6)	3 (1-7)	3 (0-5)
Admission to ICU on enrolment day	1 (25.0%)	5 (15.6%)	5 (50.0%)	1 (100%)	1 (100%)	7 (77.8%)	1 (11.1%)	11 (31.4%)	4 (23.5%)	4 (36.4%)	24 (55.8%)	2 (16.7%)	3 (60.0%)	6 (35.3%)	37 (68.5%)	3 (20.0%)	4 (33.3%)	1 (50.0%)
Proportion of reporting microbiology results within 3 days since specimen collection	1 (25.0%)	27 (84.4%)	4 (40.0%)	0 (0%)	1 (100%)	2 (22.2%)	2 (22.2%)	12 (34.3%)	8 (47.1%)	1 (9.1%)	13 (30.2%)	5 (41.7%)	2 (11.8%)	0 (0.0%)	9 (16.7%)	3 (20.0%)	2 (16.7%)	1 (50.0%)
Parenteral antibiotic usage																		
Patients on parenteral antibiotic usage	4 (100%)	29 (90.6%)	10 (100%)	1 (100%)	1 (100%)	9 (100%)	9 (100%)	31 (88.6%)	17 (100.0%)	10 (90.9%)	40 (93.0%)	11 (91.7%)	3 (60.0%)	14 (82.4%)	15 (100%)	53 (98.1%)	2 (100.0%)	10 (83.3%)

prior specimen collection																		
Cumulative Days of exposure to systematic antibiotic prior specimen collection*	1.5 (1-9)	1 (1-1)	11.5 (6-19)	18 (18-18)	6 (6-6)	14 (9-23)	6 (5-11)	4 (1-8)	1 (1-1)	17 (6-24)	8 (5-16)	1 (1-1)	5 (0-14)	5 (1-9)	10 (6-14)	7 (1-9)	11 (2.5-28)	3 (1-5)
Length of hospital stay																		
Days in the hospital prior specimen collection*	8 (5-14)	7 (5-10)	13 (6-26)	28 (28-28)	6 (6-6)	22 (11-27)	9 (5-14)	7 (6-11)	7 (5-12)	18 (12-24)	14 (8-21)	5 (4-8)	15 (14-24)	18 (11-22)	12 (8-17)	8 (5-24)	21 (15-30)	8 (5-10)
Outcome																		
28-day mortality	1 (25.0%)	6 (18.8%)	4 (40.0%)	0 (0%)	1 (100%)	2 (22.2%)	4 (44.4%)	17 (48.6%)	3 (17.6%)	9 (81.8%)	15 (34.9%)	2 (16.7%)	2 (40.0%)	5 (29.4%)	34 (63.0%)	3 (20.0%)	5 (41.7%)	1 (50.0%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

Appendix A6. Characteristics of patients who enrolled in the survey and had hospital-acquired lower-respiratory tract infection stratified by causative bacteria.

	Lower-respiratory tract infection (n=618)													
	Staphylococcus aureus (n=9)		*Escherichia coli* (n=13)			*Klebsiella pneumoniae* (n=144)			*Pseudomonas aeruginosa* (n=75)		*Acinetobacter baumannii* (n=211)		Polymicrobial (n=166)	
Characteristics	**MRSA (n=0)**	**MSSA (n=9)**	**3GCR-CR (n=2)**	**3GCR-CS (n=7)**	**3GCS (n=4)**	**3GCR-CR (n=9)**	**3GCR-CS (n=98)**	**3GCS (n=37)**	**CR (n=24)**	**CS (n=51)**	**CR (n=176)**	**CS (n=35)**	**AMR (n=133)**	**AMS (n=33)**
Sex														
Male	NA	3 (33.3%)	2 (100.0%)	6 (85.7%)	2 (50.0%)	6 (66.7%)	61 (62.2%)	23 (62.2%)	17 (70.8%)	30 (58.8%)	117 (66.5%)	17 (48.6%)	83 (62.4%)	21 (63.6%)
Age														
≤0	NA	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0%)	3 (3.1%)	1 (2.7%)	0 (0%)	1 (2.0%)	16 (9.1%)	1 (2.9%)	6 (4.5%)	2 (6.1%)
1-4	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)	1 (2.0%)	2 (1.1%)	0 (0%)	3 (2.3%)	0 (0%)
5-14	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	0 (0%)	2 (1.1%)	4 (11.4%)	3 (2.3%)	1 (3.0%)
15-24	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0%)	6 (6.1%)	1 (2.7%)	1 (4.2%)	2 (3.9%)	4 (2.3%)	4 (11.4%)	7 (5.3%)	3 (9.1%)
25-34	NA	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0%)	2 (2.0%)	2 (5.4%)	4 (16.7%)	2 (3.9%)	7 (4.0%)	2 (5.7%)	4 (3.0%)	1 (3.0%)
35-44	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (1.0%)	2 (5.4%)	0 (0%)	5 (9.8%)	16 (9.1%)	3 (8.6%)	8 (6.0%)	3 (9.1%)
45-54	NA	1 (11.1%)	1 (50.0%)	0 (0.0%)	1 (25.0%)	4 (44.4%)	9 (9.2%)	4 (10.8%)	4 (16.7%)	6 (11.8%)	25 (14.2%)	5 (14.3%)	22 (16.5%)	6 (18.2%)
55-64	NA	2 (22.2%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	1 (11.1%)	22 (22.4%)	7 (18.9%)	2 (8.3%)	7 (13.7%)	23 (13.1%)	5 (14.3%)	31 (23.3%)	7 (21.2%)
65-80	NA	5 (55.6%)	0 (0.0%)	4 (57.1%)	1 (25.0%)	3 (33.3%)	37 (37.8%)	16 (43.2%)	9 (37.5%)	21 (41.2%)	60 (34.1%)	7 (20.0%)	37 (27.8%)	9 (27.3%)
≥81	NA	1 (11.1%)	1 (50.0%)	1 (14.3%)	0 (0.0%)	0 (0%)	17 (17.3%)	4 (10.8%)	3 (12.5%)	6 (11.8%)	21 (11.9%)	4 (11.4%)	12 (9.0%)	1 (3.0%)
Device-related														
ventilator-associated	NA	3 (33.3%)	1 (50.0%)	0 (0.0%)	4 (100.0%)	6 (66.7%)	42 (42.9%)	21 (56.8%)	17 (70.8%)	36 (70.6%)	139 (79.0%)	25 (71.4%)	89 (66.9%)	26 (78.8%)
Severity of underlying illness														

Charlson comorbidity index score	NA	3 (2-6)	4 (3-5)	6 (3-8)	3 (2-4)	3 (2-5)	4 (3-6)	4 (2-6)	4 (1-5)	4 (2-6)	3 (1-5)	3 (0-5)	3 (1-5)	2 (0-4)
Transferred from other hospitals	NA	5 (55.6%)	2 (100.0%)	3 (42.9%)	3 (75.0%)	7 (77.8%)	72 (73.5%)	25 (67.6%)	17 (70.8%)	37 (72.5%)	129 (73.3%)	28 (80.0%)	98 (73.7%)	23 (69.7%)
Admission to ICU on hospitalisation day	NA	0 (0.0%)	0 (0.0%)	1 (14.3%)	2 (50.0%)	2 (22.2%)	25 (25.5%)	14 (37.8%)	10 (41.7%)	18 (35.3%)	57 (32.4%)	10 (28.6%)	43 (32.3%)	22 (66.7%)
SOFA score on the day of admission	NA	2 (0-3)	3 (1-5)	2 (1-4)	2 (0-6)	2 (2-3)	2 (1-4)	1 (0-3)	2 (1-4)	3 (0-5)	3 (1-5)	2 (1-3)	2 (1-4)	3 (2-4)
Health status on day of specimen collection														
SOFA score on the day of specimen collection	NA	3 (1-4)	4 (2-6)	3 (1-6)	1 (0-2)	4 (2-8)	2 (0-4)	1 (0-3)	1 (0-4)	1 (1-4)	2 (1-5)	2 (1-5)	2 (1-4)	2 (1-3)
Admission to ICU on enrolment day	NA	6 (66.7%)	2 (100.0%)	3 (42.9%)	3 (75.0%)	6 (66.7%)	59 (60.2%)	21 (56.8%)	14 (58.3%)	36 (70.6%)	131 (74.4%)	27 (77.1%)	77 (57.9%)	28 (84.8%)
Proportion of reporting microbiology results within 3 days since specimen collection	NA	9 (100.0%)	2 (100.0%)	7 (100.0%)	4 (100.0%)	8 (88.9%)	94 (95.9%)	36 (97.3%)	23 (95.8%)	48 (94.1%)	169 (96.0%)	31 (88.6%)	128 (96.2%)	32 (97.0%)
Parenteral antibiotic usage														
Patients on parenteral antibiotic usage prior specimen collection	NA	8 (88.9%)	2 (100.0%)	7 (100.0%)	4 (100.0%)	8 (88.9%)	94 (95.9%)	29 (78.4%)	22 (91.7%)	47 (92.2%)	165 (93.8%)	33 (94.3%)	128 (96.2%)	32 (97.0%)

Cumulative Days of exposure to systematic antibiotic prior specimen collection	NA	1 (1-9)	7.5 (2-13)	10 (2-40)	5 (4-5.5)	11 (7-16)	9 (5-16)	1 (1-5)	12.5 (6.5-19.5)	9 (6-14)	10 (7-16.5)	6 (2-8)	9 (6-16)	6 (1-11)
Length of hospital stay														
Days in the hospital prior specimen collection	NA	11 (6-13)	12 (10-13)	11 (6-41)	6 (6-8)	15 (12-20)	14 (9-20)	7 (4-12)	19 (8-28)	15 (9-21)	12 (8-18)	9 (6-13)	12 (7-20)	8 (5-14)
Outcome														
28-day mortality	NA	2 (22.2%)	2 (100%)	4 (57.1%)	2 (50.0%)	5 (55.6%)	34 (34.7%)	14 (37.8%)	6 (25.0%)	11 (21.6%)	73 (41.5%)	9 (25.7%)	56 (42.1%)	13 (39.4%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

Appendix A7. Characteristics of patients who enrolled in the survey and had hospital-acquired surgical-site infection stratified by causative bacteria.

	Surgical-site infection (n=155)																	
	***Staphylococcus aureus* (n=5)**		***Enterococcus faecium* (n=6)**		***Enterococcus faecalis* (n=10)**		***Escherichia coli* (n=33)**			***Klebsiella pneumoniae* (n=25)**			***Pseudomonas aeruginosa* (n=12)**		***Acinetobacter baumannii* (n=31)**		**Polymicrobial (n=33)**	
Characteristics	**MRSA (n=0)**	**MSSA (n=5)**	**AMPR (n=5)**	**AMPS (n=1)**	**AMPR (n=1)**	**AMPS (n=9)**	**3GCR-CR (n=3)**	**3GCR-CS (n=24)**	**3GCS (n=6)**	**3GCR-CR (n=5)**	**3GCR-CS (n=15)**	**3GCS (n=5)**	**CR (n=3)**	**CS (n=9)**	**CR (n=26)**	**CS (n=5)**	**AMR (n=28)**	**AMS (n=5)**
Sex																		
Male	NA	2 (40.0%)	3 (60.0%)	1 (100%)	1 (33.3%)	6 (85.7%)	2 (66.7%)	13 (54.2%)	3 (50.0%)	3 (60.0%)	11 (73.3%)	3 (60.0%)	3 (100%)	5 (55.6%)	19 (73.1%)	3 (60.0%)	14 (50.0%)	1 (20.0%)
Age																		
≤0	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	3 (50.0%)	0 (0%)	2 (13.3%)	0 (0%)	1 (33.3%)	1 (11.1%)	2 (7.7%)	0 (0%)	2 (7.1%)	0 (0%)
"1-4"	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	1 (20.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
"5-14"	NA	1 (20.0%)	1 (20.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8.3%)	1 (16.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.8%)	0 (0%)	1 (3.6%)	0 (0%)
15-24	NA	3 (60.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8.3%)	0 (0%)	1 (20.0%)	1 (6.7%)	0 (0%)	0 (0%)	2 (22.2%)	4 (15.4%)	0 (0%)	2 (7.1%)	3 (60.0%)
25-34	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	1 (11.1%)	2 (7.7%)	1 (20.0%)	2 (7.1%)	1 (20.0%)
35-44	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	0 (0%)	0 (0%)	3 (20.0%)	0 (0%)	0 (0%)	1 (11.1%)	4 (15.4%)	1 (20.0%)	3 (10.7%)	0 (0%)
45-54	NA	1 (20.0%)	1 (20.0%)	1 (100%)	1 (33.3%)	0 (0%)	2 (66.7%)	1 (4.2%)	0 (0%)	0 (0%)	3 (20.0%)	0 (0%)	1 (33.3%)	2 (22.2%)	5 (19.2%)	1 (20.0%)	1 (3.6%)	0 (0%)
55-64	NA	0 (0%)	2 (40.0%)	0 (0%)	0 (0%)	2 (28.6%)	0 (0%)	7 (29.2%)	1 (16.7%)	0 (0%)	2 (13.3%)	3 (60.0%)	0 (0%)	1 (11.1%)	2 (7.7%)	2 (40.0%)	3 (10.7%)	0 (0%)
65-80	NA	0 (0%)	1 (20.0%)	0 (0%)	2 (66.7%)	5 (71.4%)	1 (33.3%)	7 (29.2%)	1 (16.7%)	4 (80.0%)	3 (20.0%)	0 (0%)	0 (0%)	1 (11.1%)	4 (15.4%)	0 (0%)	8 (28.6%)	1 (20.0%)
≥81	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	1 (20.0%)	1 (33.3%)	0 (0%)	2 (7.7%)	0 (0%)	6 (21.4%)	0 (0%)

Type of SSI																		
Superficial SSI	NA	3 (60.0%)	4 (80.0%)	0 (0%)	2 (66.7%)	6 (85.7%)	3 (100%)	18 (75.0%)	5 (83.3%)	4 (80.0%)	9 (60.0%)	3 (60.0%)	3 (100%)	8 (88.9%)	19 (73.1%)	5 (100%)	18 (64.3%)	4 (80.0%)
Deep SSI	NA	1 (20.0%)	1 (20.0%)	0 (0%)	0 (0%)	1 (14.3%)	0 (0%)	3 (12.5%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	1 (11.1%)	2 (7.7%)	0 (0%)	1 (3.6%)	0 (0%)
Organ/Space SSI	NA	1 (20.0%)	0 (0%)	1 (100%)	1 (33.3%)	0 (0%)	0 (0%)	3 (12.5%)	1 (16.7%)	1 (20.0%)	5 (33.3%)	2 (40.0%)	0 (0%)	0 (0%)	5 (19.2%)	0 (0%)	9 (32.1%)	1 (20.0%)
Severity of underlying illness																		
Charlson comorbidity index score*	NA	2 (0-2)	3 (1-4)	3 (3-3)	3 (2-3)	3 (2-4)	3 (1-3)	2 (1-5)	0 (0-5)	5 (3-6)	0 (0-5)	1 (1-3)	4 (0-5)	0 (0-2)	2 (0-3)	1 (0-6)	6 (4-28)	0 (0-5)
Transferred from other hospitals	NA	1 (20.0%)	2 (40.0%)	0 (0%)	1 (33.3%)	5 (71.4%)	5 (83.3%)	13 (54.2%)	1 (33.3%)	4 (80.0%)	9 (60.0%)	3 (60.0%)	3 (100%)	8 (88.9%)	15 (57.7%)	4 (80.0%)	19 (67.9%)	1 (20.0%)
Admission to ICU on hospitalisation day	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (28.6%)	0 (0%)	3 (12.5%)	0 (0%)	2 (40.0%)	1 (6.7%)	0 (0.0%)	3 (100%)	4 (44.4%)	8 (30.8%)	0 (0%)	7 (25.0%)	0 (0.0%)
SOFA score on the day of admission*	NA	0 (0-0)	1 (1-1)	1 (1-1)	0 (0-0)	1 (0-2)	0 (0-1)	1 (0-2)	1 (0-3)	1 (1-4)	1 (0-3)	0 (0-0)	4 (2-4)	1 (0-1)	1 (0-2)	1 (0-4)	2 (1-3)	0 (0-0)
Health status on day of specimen collection																		
SOFA score on the day of specimen collection*	NA	0 (0-0)	1 (0-4)	2 (2-2)	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-2)	0 (0-0)	0 (0-4)	0 (0-0)	0 (0-0)	4 (2-7)	1 (0-1)	1 (0-2)	0 (0-3)	1 (0-3)	0 (0-0)
Admission to ICU on enrolment day	NA	1 (20.0%)	2 (40.0%)	0 (0%)	0 (0.0%)	2 (28.6%)	1 (16.7%)	7 (29.2%)	1 (33.3%)	3 (60.0%)	3 (20.0%)	2 (40.0%)	3 (100%)	4 (44.4%)	13 (50.0%)	1 (20.0%)	13 (46.4%)	0 (0.0%)

Proportion of reporting microbiology results within 3 days since specimen collection	NA	4 (80.0%)	3 (60.0%)	1 (100%)	2 (66.7%)	7 (100%)	5 (83.3%)	22 (91.7%)	3 (100%)	5 (100.0%)	14 (93.3%)	2 (40.0%)	3 (100.0%)	9 (100%)	24 (92.3%)	5 (100%)	25 (89.3%)	4 (80.0%)
Parenteral antibiotic usage																		
Patients on parenteral antibiotic usage prior specimen collection	NA	3 (60.0%)	4 (80.0%)	1 (100%)	7 (100%)	3 (100%)	6 (100%)	22 (91.7%)	3 (100%)	5 (100%)	12 (80.0%)	5 (100%)	3 (100%)	7 (77.8%)	25 (96.2%)	4 (80.0%)	28 (100%)	3 (60.0%)
Cumulative Days of exposure to systematic antibiotic prior specimen collection*	NA	1 (0-5)	14 (10-25)	1 (1-1)	7 (4-13)	7 (5-9)	6 (5-27)	8 (5-13)	10 (8-11)	14 (11-14)	13 (6-18)	6 (6-6)	31 (11-33)	14 (7-18)	10 (6-13)	10 (7-16)	7 (5-21)	1 (1-1)
Length of hospital stay																		
Days in the hospital prior specimen collection*	NA	9 (8-11)	19 (11-25)	7 (7-7)	7 (5-13)	7 (6-10)	8 (6-27)	10 (8-12)	10 (8-11)	18 (11-22)	16 (8-20)	7 (7-8)	32 (11-33)	15 (8-19)	13 (7-20)	15 (13-21)	12 (7-24)	4 (4-4)
Outcome																		
28-day mortality	NA	0 (0%)	2 (40.0%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	4 (16.7%)	1 (33.3%)	2 (40.0%)	0 (0%)	0 (0%)	1 (33.3%)	1 (11.1%)	1 (3.8%)	0 (0%)	6 (21.4%)	0 (0%)

Appendix A8. Characteristics of patients who enrolled in the survey and had hospital-acquired urinary-tract infection stratified by causative bacteria.

	Urinary-tract infection (n=796)																	
	***Staphylococcus aureus* (n=8)**		***Enterococcus faecium* (n=159)**		***Enterococcus faecalis* (n=135)**		***Escherichia coli* (n=164)**			***Klebsiella pneumoniae* (n=84)**			***Pseudomonas aeruginosa* (n=101)**		***Acinetobacter baumannii* (n=62)**		**Polymicrobial (n=83)**	
Characteristics	**MRSA (n=1)**	**MSSA (n=7)**	**AMPR (n=155)**	**AMPS (n=4)**	**AMPR (n=25)**	**AMPS (n=110)**	**3GCR-CR (n=25)**	**3GCR-CS (n=70)**	**3GCS (n=69)**	**3GCR-CR (n=28)**	**3GCR-CS (n=40)**	**3GCS (n=16)**	**CR (n=44)**	**CS (n=57)**	**CR (n=55)**	**CS (n=7)**	**AMR (n=58)**	**AMS (n=25)**
Sex																		
Male	0 (0%)	6 (85.7%)	67 (43.2%)	3 (75.0%)	9 (36.0%)	60 (54.5%)	11 (44.0%)	31 (44.3%)	21 (30.4%)	16 (57.1%)	23 (57.5%)	5 (31.3%)	39 (88.6%)	33 (57.9%)	38 (69.1%)	6 (85.7%)	29 (50.0%)	11 (44.0%)
Age																		
≤0	0 (0%)	0 (0%)	6 (3.9%)	0 (0%)	0 (0%)	1 (0.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)	0 (0%)	1 (1.7%)	0 (0%)
1-4	0 (0%)	0 (0%)	2 (1.3%)	0 (0%)	0 (0%)	1 (0.9%)	0 (0%)	0 (0%)	1 (1.4%)	1 (3.6%)	1 (2.5%)	0 (0%)	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
5-14	0 (0%)	0 (0%)	2 (1.3%)	0 (0%)	0 (0%)	4 (3.6%)	1 (4.0%)	1 (1.4%)	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)	2 (3.6%)	0 (0%)	0 (0%)	1 (4.0%)
15-24	0 (0%)	0 (0%)	5 (3.2%)	0 (0%)	0 (0%)	3 (2.7%)	1 (4.0%)	3 (4.3%)	2 (2.9%)	0 (0%)	3 (7.5%)	1 (6.3%)	0 (0%)	6 (10.5%)	6 (10.9%)	1 (14.3%)	2 (3.4%)	0 (0%)
25-34	0 (0%)	0 (0%)	6 (3.9%)	0 (0%)	1 (4.0%)	4 (3.6%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)	3 (7.5%)	1 (6.3%)	1 (2.3%)	4 (7.0%)	5 (9.1%)	1 (14.3%)	5 (8.6%)	0 (0%)
35-44	1 (100.0 %)	1 (14.3%)	14 (9.0%)	0 (0%)	0 (0%)	9 (8.2%)	1 (4.0%)	4 (5.7%)	5 (7.2%)	3 (10.7%)	3 (7.5%)	0 (0%)	3 (6.8%)	3 (5.3%)	3 (5.5%)	2 (28.6%)	8 (13.8%)	2 (8.0%)
45-54	0 (0%)	2 (28.6%)	20 (12.9%)	1 (25.0%)	0 (0%)	17 (15.5%)	5 (20.0%)	5 (7.1%)	10 (14.5%)	6 (21.4%)	5 (12.5%)	2 (12.5%)	9 (20.5%)	6 (10.5%)	7 (12.7%)	0 (0%)	6 (10.3%)	7 (28.0%)
55-64	0 (0%)	0 (0%)	30 (19.4%)	0 (0%)	8 (32.0%)	24 (21.8%)	3 (12.0%)	20 (28.6%)	16 (23.2%)	8 (28.6%)	7 (17.5%)	6 (37.5%)	11 (25.0%)	10 (17.5%)	11 (20.0%)	2 (28.6%)	12 (20.7%)	2 (8.0%)
65-80	0 (0%)	3 (42.9%)	47 (30.3%)	2 (50.0%)	12 (48.0%)	37 (33.6%)	14 (56.0%)	26 (37.1%)	21 (30.4%)	7 (25.0%)	13 (32.5%)	4 (25.0%)	14 (31.8%)	17 (29.8%)	19 (34.5%)	1 (14.3%)	18 (31.0%)	9 (36.0%)
≥81	0 (0%)	1 (14.3%)	23 (14.8%)	1 (25.0%)	4 (16.0%)	10 (9.1%)	0 (0%)	10 (14.3%)	13 (18.8%)	3 (10.7%)	5 (12.5%)	2 (12.5%)	5 (11.4%)	10 (17.5%)	1 (1.8%)	0 (0%)	6 (10.3%)	4 (16.0%)
Device-related																		

Urinary catheter-associated	1 (100.0%)	5 (71.4%)	109 (70.3%)	3 (75.0%)	18 (72.0%)	84 (76.4%)	18 (72.0%)	43 (61.4%)	45 (65.2%)	17 (60.7%)	25 (62.5%)	14 (87.5%)	34 (77.3%)	46 (80.7%)	45 (81.8%)	6 (85.7%)	43 (74.1%)	14 (56.0%)
Severity of underlying illness																		
Charlson comorbidity index score	0 (0-0)	5 (1-6)	3 (2-5)	5 (3-6)	4 (3-5)	4 (2-5)	4 (2-5)	3 (2-5)	4 (2-5)	3 (2-5)	3 (1-6)	3 (2-5)	3 (1-4)	3 (1-5)	3 (0-4)	0 (0-4)	4 (1-6)	4 (3-6)
Transferred from other hospitals	0 (0.0%)	7 (100.0%)	107 (69.0%)	3 (75.0%)	20 (80.0%)	76 (69.1%)	15 (60.0%)	42 (60.0%)	50 (72.5%)	17 (60.7%)	24 (60.0%)	9 (56.3%)	32 (72.7%)	42 (73.7%)	44 (80.0%)	5 (71.4%)	37 (63.8%)	18 (72.0%)
Admission to ICU on hospitalisation day	1 (100.0%)	1 (14.3%)	39 (25.2%)	2 (50.0%)	6 (24.0%)	18 (16.4%)	1 (4.0%)	6 (8.6%)	9 (13.0%)	2 (7.1%)	6 (15.0%)	3 (18.8%)	13 (29.5%)	19 (33.3%)	17 (30.9%)	3 (42.9%)	14 (24.1%)	1 (4.0%)
SOFA score on the day of admission	2 (2-2)	3 (0-4)	2 (0-4)	2 (1-4)	2 (1-4)	2 (0-4)	2 (0-4)	1 (0-3)	2 (0-3)	1 (0-2)	2 (0-5)	1 (0-3)	2 (0-4)	2 (1-3)	2 (1-4)	2 (1-4)	3 (1-4)	1 (0-3)
Health status on day of specimen collection																		
SOFA score on the day of specimen collection	2 (2-2)	3 (0-5)	1 (0-4)	2 (1-4)	2 (1-3)	1 (0-3)	1 (0-3)	1 (0-2)	0 (0-2)	0 (0-2)	1 (0-3)	2 (0-3)	1 (0-2)	1 (0-2)	1 (0-2)	0 (0-1)	1 (0-3)	2 (1-3)
Admission to ICU on enrolment day	0 (0%)	1 (14.3%)	65 (41.9%)	1 (25.0%)	12 (48.0%)	35 (31.8%)	6 (24.0%)	14 (20.0%)	12 (17.4%)	7 (25.0%)	10 (25.0%)	2 (12.5%)	17 (38.6%)	29 (50.9%)	27 (49.1%)	2 (28.6%)	22 (37.9%)	5 (20.0%)
Proportion of reporting microbiology results within 3 days since specimen collection	1 (100.0%)	7 (100.0%)	136 (87.7%)	3 (75.0%)	25 (100.0%)	108 (98.2%)	25 (100.0%)	70 (100.0%)	65 (94.2%)	27 (96.4%)	39 (97.5%)	16 (100.0%)	41 (93.2%)	56 (98.2%)	52 (94.5%)	7 (100.0%)	54 (93.1%)	25 (100%)
Parenteral antibiotic usage																		
Patients on parenteral antibiotic usage prior specimen collection	1 (100%)	5 (71.4%)	145 (93.5%)	4 (100.0%)	22 (88.0%)	91 (82.7%)	22 (88.0%)	48 (68.6%)	49 (71.0%)	22 (78.6%)	32 (80.0%)	12 (75.0%)	39 (88.6%)	47 (82.5%)	44 (80.0%)	6 (85.7%)	46 (79.3%)	18 (72.0%)

Cumulative Days of exposure to systematic antibiotic prior specimen collection	1 (1-1)	1 (0-1)	12 (6-20)	4.5 (2.5-7)	12 (6-20)	3 (1-9)	8 (1-14)	1 (0-6)	1 (0-1)	8 (2-21)	2 (1-11)	1 (0.5-1)	10 (4.5-21.5)	7 (1-13)	10 (3-17)	2 (1-8)	9 (1-19)	1 (1-4)
Length of hospital stay																		
Days in the hospital prior specimen collection	6 (6-6)	5 (4-9)	16 (9-24)	8 (6-23)	10 (8-18)	8 (6-12)	14 (8-21)	11 (7-20)	7 (5-9)	17 (10-26)	12 (6-18)	7 (5-12)	16 (11-28)	12 (7-17)	15 (9-23)	6 (4-9)	17 (11-30)	8 (6-13)
Outcome																		
28-day mortality	0 (0%)	1 (14.3%)	49 (31.6%)	1 (25.0%)	13 (52.0%)	30 (27.3%)	6 (24.0%)	14 (20.0%)	17 (24.6%)	7 (25.0%)	11 (27.5%)	2 (12.5%)	7 (15.9%)	7 (12.3%)	12 (21.8%)	1 (14.3%)	18 (31.0%)	6 (24.0%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

Appendix A9. Characteristics of patients who enrolled in the survey and had other hospital-acquired infection stratified by causative bacteria.

	Other infection (n=68)																	
	Staphylococcus aureus (n=10)		*Enterococcus faecium* (n=3)		*Enterococcus faecalis* (n=2)		*Escherichia coli* (n=1)			*Klebsiella pneumoniae* (n=12)			*Pseudomonas aeruginosa* (n=6)		*Acinetobacter baumannii* (n=13)		Polymicrobial (n=21)	
Characteristics	**MRSA (n=0)**	**MSSA (n=10)**	**AMPR (n=3)**	**AMPS (n=0)**	**AMPR (n=1)**	**AMPS (n=1)**	**3GCR-CR (n=0)**	**3GCR-CS (n=1)**	**3GCS (n=0)**	**3GCR-CR (n=3)**	**3GCR-CS (n=8)**	**3GCS (n=1)**	**CR (n=4)**	**CS (n=2)**	**CR (n=12)**	**CS (n=1)**	**AMR (n=19)**	**AMS (n=2)**
Sex																		
Male	NA	7 (70.0%)	1 (25.0%)	NA	0 (0.0%)	1 (100%)	NA	1 (100%)	NA	2 (66.7%)	7 (87.5%)	1 (100%)	4 (100%)	1 (50.0%)	6 (50.0%)	1 (100%)	11 (57.9%)	0 (0%)
Age																		
≤0	NA	4 (40.0%)	1 (33.3%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	0 (0%)	1 (5.3%)	0 (0%)
1-4	NA	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)
5-14	NA	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	0 (0.0%)	0 (0%)	1 (25.0%)	0 (0%)	1 (8.3%)	0 (0%)	2 (10.5%)	0 (0%)
15-24	NA	1 (10.0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	1 (33.3%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)
25-34	NA	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)
35-44	NA	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	2 (25.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%))	1 (5.3%)	0 (0%)
45-54	NA	2 (20.0%)	0 (0%)	NA	1 (100%)	0 (0%)	NA	0 (0%)	NA	1 (33.3%)	0 (0.0%)	1 (100%)	0 (0%)	1 (50.0%)	1 (8.3%)	0 (0%)	1 (5.3%)	0 (0%)
55-64	NA	1 (10.0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	1 (100%)	NA	0 (0.0%)	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	1 (100%)	7 (36.8%)	0 (0%)
65-80	NA	1 (10.0%)	2 (66.7%)	NA	0 (0%)	1 (100%)	NA	0 (0%)	NA	1 (33.3%)	4 (50.0%)	0 (0.0%)	3 (75.0%)	0 (0%)	5 (41.7%)	0 (0%)	5 (26.3%)	2 (100%)
≥81	NA	1 (10.0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0%)	2 (10.5%)	0 (0%)
Severity of underlying illness																		
Charlson comorbidity index score	NA	1 (0-6)	3 (0-5)	NA	2 (2-2)	7 (7-7)	NA	2 (2-2)	NA	2 (2-6)	4 (1-6)	2 (2-2)	4 (2-5)	3 (0-6)	4 (0-4)	2 (2-2)	2 (2-5)	4 (3-5)

Transferred from other hospitals	NA	3 (30.0%)	2 (66.7%)	NA	1 (100%)	1 (100%)	NA	0 (0%)	NA	2 (66.7%)	4 (50.0%)	1 (100.0%)	2 (50.0%)	0 (0.0%)	8 (66.7%)	0 (0.0%)	16 (84.2%)	1 (50.0%)
Admission to ICU on hospitalisation day	NA	1 (10.0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	5 (41.7%)	0 (0.0%)	6 (31.6%)	0 (0.0%)
SOFA score on the day of admission	NA	1 (0-2)	4 (3-5)	NA	7 (7-7)	5 (5-5)	NA	1 (1-1)	NA	5 (3-11)	3 (1-4)	4 (4-4)	2 (1-3)	2 (0-4)	5 (3-9)	0 (0-0)	3 (2-5)	3 (2-3)
Health status on day of specimen collection																		
SOFA score on the day of specimen collection	NA	0 (0-1)	0 (0-3)	NA	0 (0-0)	3 (3-3)	NA	0 (0-0)	NA	4 (2-5)	2 (0-5)	0 (0-0)	2 (1-5)	1 (0-2)	1 (0-6)	0 (0-0)	1 (0-4)	0 (0-0)
Admission to ICU on enrolment day	NA	2 (20.0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	NA	2 (66.7%)	2 (25.0%)	0 (0.0%)	1 (25.0%)	1 (50.0%)	5 (41.7%)	1 (100.0%)	6 (31.6%)	0 (0.0%)
Proportion of reporting microbiology results within 3 days since specimen collection	NA	10 (100.0%)	3 (100.0%)	NA	1 (100%)	1 (100%)	NA	1 (100%)	NA	3 (100.0%)	7 (87.5%)	1 (100.0%)	3 (75.0%)	2 (100.0%)	11 (91.7%)	1 (100.0%)	18 (94.7%)	2 (100.0%)
Parenteral antibiotic usage																		
Patients on parenteral antibiotic usage prior specimen collection	NA	6 (60.0%)	3 (50.0%)	NA	1 (100%)	1 (100%)	NA	1 (100%)	NA	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (25.0%)	2 (100.0%)	1 (8.3%)	1 (100.0%)	18 (94.7%)	1 (50.0%)
Cumulative Days of exposure to systematic antibiotic prior	NA	1.5 (0-5)	15 (13-23)	NA	30 (30-30)	23 (23-23)	NA	4 (4-4)	NA	17 (13-28)	11.5 (4.5-15.5)	7 (7-7)	18 (9-27)	0 (0-0)	11.5 (8-17)	0 (0-0))	18 (9-28)	15.5 (0-31)

specimen collection																	
Length of hospital stay																	
Days in the hospital prior specimen collection	NA	7 (5-14)	15 (13-26)	NA	34 (34-34)	23 (23-23)	NA	10 (10-10)	NA	19 (13-28)	17 (11-22)	19 (19-19)	30 (21-37)	23 (8-37)	16 (10-23)	80 (80-80)	23 (16-47)	23 (6-39)
Outcome																	
28-day mortality	NA	0 (0%)	0 (0%)	NA	0 (0%)	1 (100%)	NA	0 (0%)	NA	1 (33.3%)	4 (50.0%)	0 (0%)	2 (50.0%)	0 (0%)	4 (33.3%)	0 (0%)	9 (47.4%)	1 (50.0%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

Appendix A10. Characteristics of patients who enrolled in the survey and had more than one type of infection by causative organism.

	More than one types of infections (n=117)																	
	Staphylococcus aureus (n=2)		*Enterococcus faecium* (n=2)		*Enterococcus faecalis* (n=4)		*Escherichia coli* (n=17)			*Klebsiella pneumoniae* (n=9)			*Pseudomonas aeruginosa* (n=5)		*Acinetobacter baumannii* (n=15)		Polymicrobial (n=64)	
Characteristics	**MRSA (n=0)**	**MSSA (n=1)**	**AMPR (n=2)**	**AMPS (n=0)**	**AMPR (n=0)**	**AMPS (n=4)**	**3GCR-CR (n=2)**	**3GCR-CS (n=10)**	**3GCS (n=5)**	**3GCR-CR (n=0)**	**3GCR-CS (n=7)**	**3GCS (n=2)**	**CR (n=1)**	**CS (n=4)**	**CR (n=13)**	**CS (n=2)**	**AMR (n=53)**	**AMS (n=11)**
Sex																		
Male	NA	1 (50.0%)	2 (100%)	NA	NA	3 (75.0%)	2 (100%)	5 (50.0%)	3 (60.0%)	NA	5 (71.4%)	1 (50.0%)	1 (100%)	3 (75.0%)	8 (61.5%)	1 (50.0%)	31 (58.5%)	4 (36.4%)
Age																		
≤0	NA	0 (0%)	0 (0%)	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	1 (14.3%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	0 (0%)	2 (3.8%)	0 (0%)
1-4	NA	0 (0%)	0 (0%)	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)
5-14	NA	0 (0%)	0 (0%)	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)
15-24	NA	1 (100%)	0 (0%)	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (3.8%)	0 (0%)
25-34	NA	0 (0%)	0 (0%)	NA	NA	0 (0%)	1 (50.0%)	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	0 (0%)	3 (5.7%)	0 (0%)
35-44	NA	0 (0%)	0 (0%)	NA	NA	0 (0%)	1 (50.0%)	1 (10.0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	1 (25.0%)	1 (7.7%)	0 (0%)	8 (15.1%)	0 (0%)
45-54	NA	0 (0%)	0 (0%)	NA	NA	1 (25.0%)	0 (0%)	1 (10.0%)	2 (40.0%)	NA	0 (0%)	0 (0%)	1 (100%)	0 (0%)	4 (30.8%)	2 (100%)	3 (5.7%)	1 (9.1%)
55-64	NA	0 (0%)	1 (50.0%)	NA	NA	1 (25.0%)	0 (0%)	2 (20.0%)	1 (20.0%)	NA	2 (28.6%)	0 (0%)	0 (0%)	1 (25.0%)	2 (15.4%)	0 (0%)	15 (28.3%)	4 (36.4%)
65-80	NA	0 (0%)	1 (50.0%)	NA	NA	2 (50.0%)	0 (0%)	4 (40.0%)	2 (40.0%)	NA	4 (57.1%)	2 (100%)	0 (0%)	2 (50.0%)	3 (23.1%)	0 (0%)	16 (30.2%)	3 (27.3%)
≥81	NA	0 (0%)	0 (0%)	NA	NA		0 (0%)	2 (20.0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	0 (0%)	3 (5.7%)	1 (9.1%)
Severity of underlying illness																		
Charlson comorbidity index score	NA	2 (2-2)	7 (5-9)	NA	NA	6 (4-7)	0 (0-0)	5 (3-6)	4 (2-5)	NA	3 (2-5)	4 (2-5)	1 (1-1)	3 (1-4)	1 (1-5)	5 (3-7)	4 (2-6)	4 (2-5)
Transferred from other hospitals	NA	0 (0%)	2 (100%)	NA	NA	3 (75.0%)	1 (50.0%)	6 (60.0%)	3 (60.0%)	NA	3 (42.9%)	2 (100.0%)	1 (100%)	3 (75.0%)	11 (84.6%)	2 (100%)	35 (66.0%)	9 (81.8%)

Admission to ICU on hospitalisation day	NA	0 (0%)	0 (0%)	NA	NA	1 (25.0%)	0 (0%)	1 (10.0%)	1 (20.0%)	NA	0 (0%)	0 (0%)	0 (0%)	2 (50.0%)	8 (61.5%)	0 (0.0%)	11 (20.8%)	4 (36.4%)
SOFA score on the day of admission	NA	2 (2-2)	3 (2-4)	NA	NA	4 (3-6)	7 (2-11)	2 (2-2)	1 (1-3)	NA	3 (1-4)	3 (1-4)	1 (1-1)	4 (1-7)	4 (2-8)	5 (4-5)	2 (0-4)	3 (1-6)
Health status on day of specimen collection																		
SOFA score on the day of specimen collection	NA	1 (1-1)	4 (1-6)	NA	NA	3 (2-7)	12 (10-13)	5 (2-8)	5 (1-5)	NA	5 (2-7)	3 (2-4)	1 (1-1)	1 (0-2)	4 (2-10)	7 (7-7)	4 (1-6)	5 (1-7)
Admission to ICU on enrolment day	NA	0 (0%)	0 (0%)	NA	NA	2 (50.0%)	0 (0%)	3 (30.0%)	2 (40.0%)	NA	4 (57.1%)	1 (50.0%)	0 (0%)	3 (75.0%)	10 (76.9%)	1 (50.0%)	38 (71.7%)	6 (54.5%)
Proportion of reporting microbiology results within 3 days since specimen collection	NA	2 (100.0%)	2 (100%)	NA	NA	3 (75.0%)	2 (100.0%)	9 (90.0%)	4 (80.0%)	NA	5 (71.4%)	1 (50.0%)	1 (100%)	4 (100%)	12 (92.3%)	2 (100%)	50 (94.3%)	10 (90.9%)
Parenteral antibiotic usage																		
Patients on parenteral antibiotic usage prior specimen collection	NA	1 (100%)	2 (100%)	NA	NA	4 (100%)	2 (100%)	8 (80.0%)	4 (80.0%)	NA	5 (71.4%)	2 (100.0%)	4 (100%)	1 (100%)	2 (100%)	13 (100%)	49 (100%)	10 (100%)
Cumulative Days of exposure to systematic antibiotic prior specimen collection	NA	1 (1-1)	10 (10-10)	NA	NA	4 (1-9.5)	1 (1-11)	4 (1-8)	7.5 (1-14)	NA	3 (0-24)	1 (1-1)	30 (30-30)	11 (7.5-22.5)	14 (9-15)	2.5 (1-4)	12 (6-17)	7 (1-9)
Length of hospital stay																		
Days in the hospital prior specimen collection	NA	25 (25-25)	11 (10-11)	NA	NA	8 (6-10)	20 (18-21)	7 (6-36)	6 (6-11)	NA	14 (6-33)	6 (4-7)	31 (31-31)	17 (11-26)	14 (10-16)	8 (4-11)	15 (12-25)	9 (6-12)

Outcome																		
28-day mortality	NA	0 (0%)	1 (50.0%)	NA	NA	1 (25.0%)	2 (100%)	5 (50.0%)	2 (40.0%)	NA	4 (57.1%)	0 (0%)	0 (0%)	1 (25.0%)	5 (38.5%)	2 (100%)	27 (50.9%)	4 (36.4%)

Footnote: *Charlson comorbidity index score, SOFA scores, cumulative days of exposure to parenteral antibiotic prior specimen collection, and days in the hospital prior specimen collected are in median (interquartile range), and other data is in n(%). MSSA=methicillin-susceptible *Staphylococcus aureus*; MRSA= methicillin-resistant *Staphylococcus aureus*; AMPS=ampicillin-susceptible; AMPR= ampicillin-resistant; 3GCS=third-generation cephalosporin susceptible; 3GCR=third-generation cephalosporin resistant; CS=carbapenem-susceptible; CR=carbapenem-resistant; AMR=antimicrobial-resistant.

B Appendices to Chapter Three

Appendix B1. Characteristics of BSI patients stratified by causative organism and by exposure to a parenteral antibiotic during hospitalisation.

	First positive blood culture was sampled within ±1 calendar day when a parenteral antibiotic was started and continued for at least four consecutive days†	First positive blood culture was sampled outside ±1 calendar day when a parenteral antibiotic was started and continued for at least four consecutive days†	First positive blood culture was sampled at any time point when a parenteral antibiotic was given less than four consecutive days††
Stapylococcus aureus (N=342)			
Time from hospital admission to the collection of the first blood culture specimen positive for *S. aureus* (calendar days; median, IQR, N)	5 (3-9, N=130)	14 (8-27; N=183)	4 (3-6, N=29)
Time from the start of parenteral antibiotic to the collection of the first blood culture specimen positive for *S. aureus* (calendar days; median, IQR, N)	0 (0-0, N=130)	12 (6-25, N=183)	0 (0-0, N=18)†††
In-hospital mortality (%)	22% (28/130)	34% (62/183)	7% (2/29)
Escherichia coli (N=445)			
Time from hospital admission to the collection of the first blood culture specimen positive for *E. coli* (calendar days; median, IQR, N)	5 (3-12, N=207)	10.5 (5-23, N=210)	4 (3-5, N=28)
Time from the start of parenteral antibiotic to the collection of the first blood culture specimen positive for *E. coli* (calendar days; median, IQR, N)	0 (0-1, N=207)	7.5 (4-21, N=210)	0 (0-1, N=24)
In-hospital mortality (%)	18% (37/207)	26% (55/210)	4% (1/28)
Klebsiella pneumoniae (N=476)			
Time from hospital admission to the collection of the first blood culture specimen positive for *K.*	6.5 (4-12; N=122)	14 (8-27; N=341)	6 (4-7; N=13)

pneumoniae (calendar days; median, IQR, N)			
Time from the start of parenteral antibiotic to the collection of the first blood culture specimen positive for *K. pneumoniae* (calendar days; median, IQR, N)	0 (0-1; N=122)	11 (6-23; N=341)	0 (0-0; N=9)†††
In-hospital mortality (%)	28% (34/122)	35% (118/341)	0% (0/13)
Pseudomonas aeruginosa (N=280)			
Time from hospital admission to the collection of the first blood culture specimen positive for *P. aeruginosa* (calendar days; median, IQR, N)	8.5 (5-13, N=42)	17 (9-30, N=231)	5 (3-19, N=7)
Time from the start of parenteral antibiotic to the collection of the first blood culture specimen positive for *P. aeruginosa* (calendar days; median, IQR, N)	0 (0-0, N=42)	14 (6-28, N=231)	0.5 (0-1.5, N=4)†††
In-hospital mortality (%)	29% (12/42)	35% (82/231)	14% (1/7)
Acinetobacter spp. (N=758)			
Time from hospital admission to the collection of the first blood culture specimen positive for *Acinetobacter* spp. (calendar days; median, IQR, N)	6 (4-8, N=78)	11 (7-19, N=663)	4 (3-5, N=17)
Time from the start of parenteral antibiotic to the collection of the first blood culture specimen positive for *Acinetobacter* spp. (calendar days; median, IQR, N)	0 (0-1, N=78)	9 (5-16, N=663)	0 (0-0, N=6)†††
In-hospital mortality (%)	23% (18/78)	44% (294/663)	0% (0/17)

†Included patients who died, were discharged to a hospice or transferred to another hospital before completing four consecutive days of antibiotics and had antibiotics continuously until the day prior to death, hospice discharge or transfer, respectively.

††Excluded patients who died, were discharged to a hospice or transferred to another hospital before completing four consecutive days of antibiotics and had antibiotics continuously until the day prior to death, hospice discharge or transfer, respectively.

C Appendices to Chapter Five

Appendix C.1. Parameter values used in the simulation study.

Events	Parameter	Measurement	Value	Description
Event 1	p_severe_adm	%	0.56	Probability of patients admitted to hospital with sever underlying illness; defined based on Sunpasit hospital data
Event 1-2	pdaily_amr	%	((1-exp(-((abs(expo_amr)/20)^0.5))))/20	The level of exposure to AMR organism (compared to non-AMR organisms); under the "addition" assumption this value in taken away from the daily probability of remaining uninfected; under the replacement assumption this value is taken away from the probability of infection caused by non-AMR infection
Event 1-2	expo_amr	NA	0.1 + b1_expo_amr*t[j] + b2_expo_amr*bl_severe[i,1] + expo_amr_error[j]	Rate at which patients expose to AMR organisms during hospitalization
Event 1-2	a0_expo_amr	CFU/ml	0.005	Baseline value
Event 1-2	b1_expo_amr	CFU/ml	0.8	Unite of increase in level of exposure to AMR organism per 1 additional day in the hospital
Event 1-2	b2_expo_amr	CFU/ml	1.5	Difference in level of exposure to AMR organism between patients with severe underlying illness and those with non-severe underlying illness
Event 1-2	expo_amr_error	NA	rnorm(t,0,expo_amr_error_sigma)	Random error sampled from a normal distribution
Event 1-2	expo_amr_error_sigma	NA	0.5	Standard deviation of the error term
Event 2	Inf0_00	%	0.8	Probability of remaining in no infection state among patients with non-severe underlying illness
Event 2	Inf0_01	%	(0.1/3)*1.5	Probability of transiting from no infection state to non-AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_02	%	(0.1/3)*1.5	Probability of transiting from no infection state to AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_03	%	0.09	Probability of transiting from no infection state to discharged state among patients with non-severe underlying illness
Event 2	Inf0_04	%	0.01	Probability of transiting from no infection state to died state among patients with non-severe underlying illness
Event 2	Inf0_10	%	0	Probability of transiting from non-AMR infection state to no infection state among patients with non-severe underlying illness
Event 2	Inf0_11	%	0.9	Probability of transiting from non-AMR infection state to non-AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_12	%	0	Probability of transiting from non-AMR infection state to AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_13	%	0.07	Probability of transiting from non-AMR infection state to discharged state among patients with non-severe underlying illness
Event 2	Inf0_14	%	0.03	Probability of transiting from non-AMR infection state to died state among patients with non-severe underlying illness

Event 2	Inf0_20	%	0	Probability of transiting from AMR infection state to no infection state among patients with non-severe underlying illness
Event 2	Inf0_21	%	0	Probability of transiting from AMR infection state to non-AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_22	%	0.9	Probability of transiting from AMR infection state to AMR infection state among patients with non-severe underlying illness
Event 2	Inf0_23	%	0.07	Probability of transiting from AMR infection state to discharged state among patients with non-severe underlying illness
Event 2	Inf0_24	%	0.03	Probability of transiting from AMR infection state to died state among patients with non-severe underlying illness
Event 2	Inf1_00	%	0.8	Probability of remaining in no infection state among patients with severe underlying illness
Event 2	Inf1_01	%	(0.1/3)*1.0	Probability of transiting from no infection state to non-AMR infection state among patients with severe underlying illness
Event 2	Inf1_02	%	(0.1/3)*2.0	Probability of transiting from no infection state to AMR infection state among patients with severe underlying illness
Event 2	Inf1_03	%	0.01	Probability of transiting from no infection state to discharged state among patients with severe underlying illness
Event 2	Inf1_04	%	0.09	Probability of transiting from no infection state to died state among patients with severe underlying illness
Event 2	Inf1_10	%	0	Probability of transiting from non-AMR infection state to no infection state among patients with severe underlying illness
Event 2	Inf1_11	%	0.95	Probability of transiting from non-AMR infection state to non-AMR infection state among patients with severe underlying illness
Event 2	Inf1_12	%	0	Probability of transiting from non-AMR infection state to AMR infection state among patients with severe underlying illness
Event 2	Inf1_13	%	0.02	Probability of transiting from non-AMR infection state to discharged state among patients with severe underlying illness
Event 2	Inf1_14	%	0.03	Probability of transiting from non-AMR infection state to died state among patients with severe underlying illness
Event 2	Inf1_20	%	0	Probability of transiting from AMR infection state to no infection state among patients with severe underlying illness
Event 2	Inf1_21	%	0	Probability of transiting from AMR infection state to non-AMR infection state among patients with severe underlying illness
Event 2	Inf1_22	%	0.95	Probability of transiting from AMR infection state to AMR infection state among patients with severe underlying illness
Event 2	Inf1_23	%	0.02	Probability of transiting from AMR infection state to discharged state among patients with severe underlying illness
Event 2	Inf1_24	%	0.03	Probability of transiting from AMR infection state to died state among patients with severe underlying illness

Event 4	p_concordant_t[, 1]	%	1-(1/(1+exp(a0_abx_concordant_t + b1_abx_concordant_t*sim_data[i,"Inf_status"]+b2_abx_concordant_t*sim_data[i,"bl_severe"])))	The probability of having concordant treatment (trt_d0=1) depends on type of infection (non-AMR or AMR); severity of underlying illness
Event 4	a0_abx_concordant_t	log(odds)	log(2.099553)	log odds of appropriate treatment for patients who admitted to hospital with non-severe underlying illness and had an infection caused by non-AMR strain of bacteria
Event 4	b1_abx_concordant_t	log(odds ratio)	-1.382404	log odds ratio of receiving inappropriate antibiotic treatment comparing patients with AMR infection to those with non-AMR infections
Event 4	b2_abx_concordant_t	log(odds ratio)	0.1927966	log odds ratio of receiving inappropriate antibiotic treatment comparing patients with severe underlying illness to those with non-severe underlying illness
Event 5	p_severe1_d1_trt0	%	0.6	Probability of a patient developed severe infection given if at t=0, the patient had discordant treatment
Event 5	p_severe1_d1_trt1	%	0.1	Probability of a patient developed severe infection given if at t=0, the patient had concordant treatment
Event 6	p_concordant_t[, 2]	%	1-(1/(1+exp(a0_abx_concordant_t1 + b1_abx_concordant_t1*sim_data[i,"trt_d0"]+ b2_abx_concordant_t1*sim_data[i,"severe_d1"])))	The probability of having concordant treatment (trt_d1=1) depends on the treatment on the previous day (trt_d0); severity of infection on the same day t=1 (severe_d1)
Event 5-2	p_discharged_d1_s0	%	0.10	Probability of discharged and alive given severe_d1=0 (not severe)
Event 5-2	p_discharged_d1_s1	%	0.05	Probability of discharged and alive given severe_d1=1 (severe)
Event 5-2	p_died_d1_s0	%	0.05	Probability of died given severe_d1=0
Event 5-2	p_died_d1_s1	%	0.10	Probability of died given severe_d1=1
Event 6	a0_abx_concordant_t1	odds	log(0.2/0.8)	log odds of appropriate treatment for patients who admitted to hospital with non-severe underlying illness and had appropriate antibiotic treatment on the previous day
Event 6	b1_abx_concordant_t1	odds ratio	log(0.6/0.4)	log odds ratio for the effect of previous days treatment on the current day's treatment
Event 6	b2_abx_concordant_t1	odds ratio	log(0.8/0.2)	log odds ratio for the effect of severity of underlying illnesses on the current day's treatment
Event 7	p_severe1_d2_trt0	%	0.6	Probability of a patient developed severe infection given if at t=1, the patient had discordant treatment
Event 7	p_severe1_d2_trt1	%	0.1	Probability of a patient developed severe infection given if at t=1, the patient had concordant treatment
Event 7-2	p_discharged_d2_s0	%	0.12	Probability of discharged and alive given severe_d1=0 (not severe)
Event 7-2	p_discharged_d2_s1	%	0.03	Probability of discharged and alive given severe_d1=1 (severe)
Event 7-2	p_died_d2_s0	%	0.05	Probability of died given severe_d1=0
Event 7-2	p_died_d2_s1	%	0.15	Probability of died given severe_d1=1
Event 8	b0_abx_concordant_t2	log(odds)	log(0.2/0.8)	log odds of appropriate treatment for patients who admitted to hospital with non-severe underlying illness and had appropriate antibiotic treatment on the previous day
Event 8	b1_abx_concordant_t2	log(odds ratio)	log(0.6/0.4)-a0_abx_concordant_t	log odds ratio for the effect of previous days treatment on the current day's treatment

Event 8	b2_abx_concordant_t2	log(odds ratio)	log(0.8/0.2)-b1_abx_concordant_t-a0_abx_concordant_t	log odds ratio for the effect of severity of underlying illnesses on the current day's treatment
Event 9	a0_outcome	log(odds)	log(0.1/0.9)	log odds of developing a death event (y=1) for when the infection was caused by non-AMR bacteria; no severe underlying illness; and no delays in appropriate antibiotic treatment
Event 9	b1_outcome	log(odds ratio)	log(0.2/0.8)-a0_outcome	coefficient for the effect of AMR infection on outcome
Event 9	b2_outcome	log(odds ratio)	log(0.25/0.75)-a0_outcome-b1_outcome	coefficient for the effect of severity of underlying illness on outcome
Event 9	b3_outcome_dum1	log(odds ratio)	0 (in scenario 1); log(0.4/0.6)-a0_outcome-b1_outcome-b2_outcome (in scenario 3)	coefficent for the effect of 1-day delay in appropriate antibiotic treatment
Event 9	b3_outcome_dum3	log(odds ratio)	0 (in scenario 1); log(0.45/0.6)-a0_outcome-b1_outcome-b2_outcome (in scenario 3)	coefficent for the effect of 2-day delay in appropriate antibiotic treatment
Event 9	b3_outcome_dum3	log(odds ratio)	0 (in scenario 1); log(0.5/0.5)-a0_outcome-b1_outcome-b2_outcome (in scenario 3)	coefficent for the effect of 3-day delay in appropriate antibiotic treatment