Antibiotic Resistance Profiles and Population Structure of Healthcare-associated and Community-associated *Staphylococcus aureus* isolated in Fort Portal, Western Uganda

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Author:

Gavin Ackers Johnson

Supervisors:

Dr Chloe James & Prof. Richard Birtles

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Declaration

Some of the data analysed as part of this thesis has been used in the following publications:

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Ackers, L., Ackers-Johnson, G., Seekles, M., Odur, J. & Opio, S. (2020). Opportunities and Challenges for Improving Antimicrobial Stewardship in Low- and Middle-Income Countries; Lessons Learnt from the Maternal Sepsis Intervention in Western Uganda. *Antibiotics*, 9.

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Abbreviations

Access, Watch and Reserve (AWaRe)

Aminoglycoside-Modifying Enzymes (AMEs)

Ampicillin (AMP)

Antimicrobial Resistance (AMR)

Antimicrobial Resistance Gene (ARG)

Antimicrobial Susceptibility Testing (AST)

Base Pairs (BP)

Brain Heart Infusion (BHI)

Carbapenem-resistant *Enterobacteriaceae* (CRE)

Cefoxitin (FOX)

Ceftriaxone (CRO)

Chemotaxis Inhibitory Protein (Chp)

Chemotaxis Inhibitory Protein of *S. aureus* (CHIPS)

Chloramphenicol (C)

Chloramphenicol Acetyltransferase (*cat*)

Ciprofloxacin (CIP)

Clindamycin (DA)

Clonal Complex (CC)

Community-Associated (CA)

Dihydrofolate Reductase (DHFR)

Erythromycin (E)

Erythromycin-Resistance Methylase (Erm)

ESAT-6 Secretion System (ESS)

European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Extended-Spectrum β-lactamase (ESBL)

Extracellular Adherence Protein (Eap)

Fort Portal Regional Referral Hospital (FPRRH)

Gross Domestic Product (GDP)

Gentamicin (CN)

Global Action Plan (GAP)

Global Antimicrobial Resistance Surveillance System (GLASS)

Government of Uganda (GoU)

Healthcare Worker (HCW)

Infection, Prevention and Control (IPC)

Knowledge for Change (K4C)

Low-Middle Income Countrie (LMIC)

Luria Bertani (LB)

MacConkey Agar (MAC)

Macrolides, Lincosamides and Streptogramin Type B (MLSB)

Mannitol Salt Agar (MSA)

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin-Susceptible Staphylococcus aureus (MSSA)

MHC Analogous Protein (Map)

Millennium Development Goals (MDG)

Multi-Drug Resistant (MDR)

Multilocus Sequence typing (MLST)

N-acetyltransferase (AAC)

National Action Plan (NAP)

National Antimicrobial Resistance Sub-Committee (NAMRSC)

O-nucleotidyltransferase (ANT)

O-phosphotransferase (APH)

Oxacillin (OX)

Panton-Valentine Leukocidin (PVL)

Penicillin Binding Protein (PBP)

Peptidyltransferase (PTase)

Phosphate Buffered Saline (PBS)

Polymerase Chain Reaction (PCR)

Staphylococcal Cassette Chromosome (SCCmec)

Staphylococcal Protein A (spa)

Staphylococci Enterotoxin (SE)

Staphylococcus intermedius chloramphenicol acetyltransferase (CARD)

Sequence Type (ST)

sub-Saharan Africa (SSA)

Surgical Site Infection (SSI)

Tetracycline (TE)

Toxic Shock Syndrome Toxin-1 (TSST-1)

Type VII Secretion System (T7SS)

Ugandan National Action Plan (UNAP)

United Nations (UN)

Urinary Tract Infection (UTI)

von Willebrand Factor-Binding Protein (vWbp)

Whole Genome Sequencing (WGS)

World Health Organisation (WHO)

Abstract

Antimicrobials currently hold infectious pathogens at bay, but with cases of resistance ever increasing, those same pathogens have the potential to reverse decades of medical progress, creating one of the biggest threats to global health, food security and development in the world today. The impact of antimicrobial resistance (AMR) is of particular concern for low-income countries which already suffer from a high burden of infectious bacterial diseases in both humans and animals, with cost constraints preventing the widespread application of newer, more expensive agents. Tackling AMR is particularly challenging in settings such as Fort Portal Regional Referral Hospital (FPRRH), Western Uganda, where specific knowledge of local AMR epidemiology is required to inform evidence-based improvement of antibiotic stewardship measures in the local area.

This study focused on the evaluation of *Staphylococcus aureus*, a commensal bacterium carried by roughly 30% of the human population commonly in the nasopharynx and/or on skin. *S. aureus* also acts as a major human pathogen frequently associated with nosocomial infections where the skin has been broken, for example from a wound or surgical procedure. The three core aims of this study involved the evaluation of clinical and community associated *S. aureus* resistance profiles; evaluation of the population diversity of clinically associated *S. aureus*; and the elucidation of key resistance associated genes through the whole genome sequencing (WGS) of 41 clinical isolates.

A population structure for the 41 sequenced isolates was inferred by comparing their core genomes. Twenty isolates formed a tight cluster corresponding to multilocus sequence typing (MLST) clonal complex (CC) 152, a CC found to be particularly prevalent in northern Africa. In agreement with other studies, the occurrence of Panton-Valentine leukocidin

toxin-encoding genes was significantly higher among CC152 strains than non-CC152 strains. However, it was also observed that the coagulase gene was over-represented in this CC, further defining the virulence strategy of this important pathogen.

Initial analysis of antimicrobial susceptibility testing (AST) data of *S. aureus* isolated from both clinical infections at FPRRH and the hand swabs of people in the local community revealed 64% (45/70) and 83% (104/125) of isolates were resistant to one or more antibiotic and 26% (18/70) and 49% (61/125) were multidrug resistant (MDR) respectively. Methicillin-resistant *S. aureus* (MRSA) was isolated at rates of 38% (8/21) and 22% (27/125) for the clinical and community associated isolates respectively.

WGS of the 41 clinical *S. aureus* isolates revealed resistance phenotypes were largely explained by the presence of antibiotic resistance genes. Although all isolates were susceptible to clindamycin, a 24% carriage of *erm* genes suggests potential for rapid development of resistance. The frequency of genes associated with methicillin, chloramphenicol and ciprofloxacin resistance were significantly lower amongst CC152 strains than non-CC152 strains; thus, in keeping with previous work, it was found that CC152 was almost exclusively methicillin-sensitive *S. aureus* (MSSA).

By generating detailed information about the epidemiology of circulating *S. aureus* and their antibiotic susceptibility, this study has provided, for the first time, data on which evidence-based infection and AMR interventions at FPRRH can be based, including the procurement and prescription of antibiotics. Furthermore, this study was able to build and promote international collaborations between Salford University, FPRRH, Ugandan Infectious Diseases Institute and Makerere University for the effective transfer of knowledge and completion of advanced research.

Chapter 1 - Introduction

1.1 Thesis Overview

This thesis explores not only my research objectives and their greater context, but also aims to describe and evaluate my experiences facilitating research at a transnational level and what it means to be part of a global health project.

Chapter 1 describes the global issue of antimicrobial resistance (AMR), its prevalence, impact and associated risk factors; followed by a closer look at Uganda as an example of a developing country dealing with the implications of AMR. The World Health Organisation (WHO) has generated a Global Action Plan (GAP) to help with deal with multidisciplinary issues, based upon which Uganda has recently implemented their National Action Plan (NAP) to combat AMR.

Chapter 2 provides a detailed description of the methods used to identify and characterise *Staphylococcus aureus*, as well as the antimicrobial susceptibility testing and molecular methods used to explore population structure and investigate key virulence and AMR determinants.

Chapter 3 focuses on a range of molecular typing methods used to characterise the *S. aureus* isolates collected, including spa typing, multilocus sequence typing (MLST) and whole genome sequencing (WGS). Phylogenetic relationships are investigated as well as key virulence determinants.

Chapter 4 delves deeper into the resistance mechanisms identified in *Staphylococcus aureus* and contributes to evidence-based policy development at a local level. The data provides a

comparison of antibiotic resistance profiles of *S. aureus* from patients, healthcare workers and members of the local community. The clinical phenotypic AMR profiles observed were assessed against WGS data to determine whether the observed susceptibilities and resistance profiles matched up with the presence of AMR genes, thus evaluating the relative value of each approach to surveillance.

Chapter 5 addresses the implementation of AMR related healthcare policies and antimicrobial stewardship at Fort Portal Regional Referral Hospital (FPRRH), including an overview of antibiotic prescribing in Uganda, surveillance systems and one health. It also highlights specific prescribing practices and antibiotic procurement at FPRRH. Final reflections examine the challenges of research in low resource settings including the transportation of infectious samples and the ethical implications of doing so, in addition to highlighting the benefits of forming collaborations in low resource settings.

Chapter 6 forms the final discussion of the thesis as a whole, bringing together all the findings of the research in a regional and global context as well as describing the impact of the study and how it can evolve in the future.

1.2 The evolving dynamic of investing in human health

With reductions in mortality rates accounting for roughly 11% of recent economic growth in low and middle-income countries, there is a clear benefit towards investing in public health and healthcare provision (Jamison et al., 2013). Having a solid understanding of how such health improvements correlate with economic value reinforces the foundation for appropriate resource allocation across multiple sectors. When the economies of countries strengthen a gradual shift in paradigm from communicable to non-communicable

diseases and injuries can be witnessed. As infectious diseases are successfully targeted, an ageing population comes with its own health complications. Mortality as a result of non-communicable diseases is now dominant in lower-middle, upper-middle- and high-income countries. Whilst a rise in income correlates with a reduction in deaths attributed to AIDS, tuberculosis, malaria, diarrhoea and lower respiratory tract infections; it also correlates with an increased proportion of heart disease and cancer related deaths due to risk factors including smoking, diet, exercise and air pollution (Jamison et al., 2013). However, whilst investments are understandably redistributed towards non-communicable diseases and injuries, it is important not to forget the serious threat infectious diseases possess.

1.2.1 Transmission of Disease on a Global Scale

With ever improving technological advancements, the ease through which global trade, travel and labour can be facilitated is always increasing. As a result, one of the of the first things that comes to mind in relation to health is the capacity for communicable diseases to spread on both a national and global level. Not only does this have an impact with respect to healthcare but given the economical interdependency of countries there can be huge financial implications (Koplan et al., 2009). This has been seen with the ongoing coronavirus pandemic originating in China, which over the course of a year has since spread to countries across the globe. In addition to the direct implications on human health, the knock-on effect of people unable to attend work has had a massive economic impact – especially when factoring in the global dependency on Chinese manufacturing exports. This has led to global economic markets falling in value (Prescott, 2020). That being said, international outbreaks of disease are nothing new and pre-date the ease of modern-day

travel, one example being how the plague was able to spread across Europe and Asia in the Middle Ages. Factors affecting non-communicable diseases are also at play, for example the historic tobacco trade, which along with other drugs have led to an increase in premature disease and death (Koplan et al., 2009). Nevertheless, as our exposure to transnational threats increases, it only accentuates the need for a global strategy to address them.

1.3 Antimicrobials and Antimicrobial Resistance

Antimicrobials currently hold infectious pathogens at bay, but with cases of resistance ever increasing, those same pathogens have the potential to reverse decades of medical progress, creating one of the biggest threats to global health, food security and development in the world today. The impact of antimicrobial resistance (AMR) is of particular concern for low-income countries which already suffer from a high burden of infectious bacterial diseases in both humans and animals, with cost constraints preventing the widespread application of newer, more expensive agents (Okeke et al., 2005). One example of such a developing country under threat from AMR is Uganda, where bacterial infections are responsible for >37% of hospitalised patients; and of the top ten leading causes of death among hospitalised patients, bacterial infections account for roughly 20% in adults and 25% in children under 5 (Ugandan National Academy of Sciences, 2015). *Staphylococcus aureus* is a bacterium of particular importance due to its strong association with nosocomial infections correlating with recurrent hospitalisations and increased mortality rates (Almeida et al., 2014). Furthermore, its ability to colonise both humans and animals alike provides an ample reservoir for the generation and transmission of AMR (Smith, 2015).

1.3.1 How do Antibiotics Work?

The first antibiotic, penicillin, was discovered by Alexander Fleming in 1928, having observed its production by *Penicillium notatum* and antibacterial properties on *Staphylococcus* cultures. However, it wasn't until the early 1940's that penicillin was available for therapeutic use. Following the production of penicillin the next ten years saw the development of several other antimicrobial agents; streptomycin, chloramphenicol and tetracycline all isolated from soil bacteria, ushering in a new age of medical therapies with reliable and amazingly quick treatment for previously life-threatening bacterial infections including those of pneumonia, syphilis and tuberculosis (Clardy et al., 2009). In addition to the direct treatment of infections, antibiotics given prophylactically in specific medical and surgical procedures also show greatly improved clinical outcomes. However, with the development of new antimicrobial agents dwindling over time, major problems are on the horizon when taking into account the rising number of bacteria becoming resistant to the antimicrobial agents available (Ramalingam, 2015).

Antibacterial agents can be classified along three primary selection criteria revolving around their target site; chemical structure; or whether they are bactericidal (kill bacteria) or bacteriostatic (prevent further growth). Given that bacteriostatic agents only inhibit growth, as opposed to killing the bacteria, their effect is typically viewed as reversible. Preventing further growth allows time for the host immune system to generate an adequate response. Whilst their success is evident in the treatment of some infections, certain situations deem them less efficacious, for example immunocompromised patients (Goering et al., 2012).

Classification based upon the target site of the antibacterial agent includes five key features of synthetic processes within bacteria that are susceptible to attack. These are i) cell wall synthesis (inhibited by all classes of β-lactam antibiotics including penicillins, cephalosporins, carbapenems and monobactams, as well as others such as vancomycin), ii) protein synthesis (commonly inhibited by binding to 30S or 50S subunits of the bacterial ribosomes, e.g. aminoglycosides, tetracyclines, and macrolides), iii) nucleic acid synthesis (inhibited by preventing the actions of DNAgyrase or topoisomerase II e.g. fluoroquinolones, or RNA polymerase, e.g. rifampicin), iv) metabolic pathways such as folic acid synthesis (e.g. inhibited by trimethoprim and sulfamethoxazole) and v) cell membrane function disruption e.g. by polymyxins (Amyes et al., 1996). With each of these processes providing a core focus of antibiotic action, inhibition can be achieved through the targeting of any number of enzymes and/or substrates involved; where more complex therapies are able to target multiple factors at once. However, the generation of such agents must take into account host cell mechanisms to ensure there is no inhibition of the equivalent mammalian cell targets (Goering et al., 2012).

For the purposes of this research a panel of eight antibiotics were utilised. The mechanism of action each of these has been described in detail below:

I. Chloramphenicol

Chloramphenicol is a broad-spectrum bacteriostatic agent that inhibits bacterial protein synthesis. The antibiotic is lipid soluble allowing it to naturally diffuse through the cytoplasmic membranes of bacterial cells. Once inside it binds to the 50S ribosomal subunit blocking basic functions including peptidyltransferase (PTase) activity and the binding and

movement of ribosomal substrates through the PTase centre - both required for protein chain elongation (Xaplanteri et al., 2003).

II. Gentamicin

Gentamicin is a broad-spectrum bactericidal antibiotic belonging to the aminoglycoside family, aptly named due to the presence of an amino-modified glycoside group within the molecule (Mingeot-Leclercq et al., 1999). Traditionally aminoglycosides have been used in the treatment of Gram-negative infections, but effectiveness of gentamycin against both Gram-negative and positive organisms in addition to its' low cost and wide availability have led it to become one of the most frequently prescribed aminoglycosides (Kushner et al., 2016). Gentamicin is also recommended in combination with other antibiotics for the empirical treatment of problematic infections creating a further increase in demand as the number of multidrug resistant pathogens increases (Krause et al., 2016).

The primary target of aminoglycoside action, gentamicin included, is the bacterial 30S ribosome. Once bound the aminoglycoside inhibits effective protein translation, with mistranslated proteins putatively causing further damage to the cytoplasmic membrane allowing even more antibiotic entry into the cell. The final stage is also energy-dependent, serving as the culmination of the positive feedback loop whereby more aminoglycoside activity inside the cell increases aminoglycoside entry into the cell leading to the eventual concentration-dependent killing of the bacteria. It is the feedback loop that allows this antibiotic to be bactericidal, where others which also target the bacterial ribosome (e.g. chloramphenicol) are bacteriostatic. However, due to the energy-dependent nature of these steps, aminoglycosides have been shown to be less effective against anaerobic bacteria (Serio et al., 2018).

III. Cefoxitin

Cefoxitin is a broad-spectrum bacteriostatic agent originally synthesised from cephamycin. Incorporating a β-lactam ring it is a second generation cephalosporin and is of particular importance as it acts as an indicator for methicillin resistant *S. aureus* (MRSA) (Gootz, 1990). β-lactam antibiotics can be classified into four basic groups: penicillins, cephalosporins, carbapenems and monobactams. All these feature a central β-lactam ring fused with a secondary ring consisting of 5-6 atoms forming a bicyclic ring structure as shown in Figure 1.1. Further sub-classifications can be made based on the nature of the side chain. In penicillins the β-lactam ring is fused with thiazolidine, with carbapenems being effectively the same except for substituting out the sulphur atom for a carbon. Cephalosporins possess a dihydrothiazine ring fused to the Beta- lactam ring, whilst monobactams have no fused secondary ring (Ramalingam, 2015).

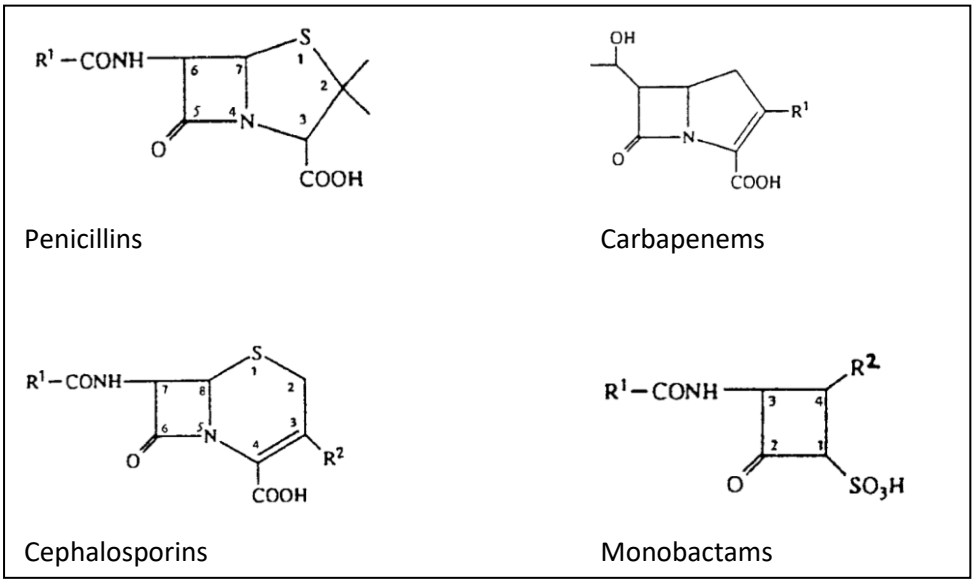


Figure 1.1: The chemical structure of the core groups of β-lactam antibiotics; penicillins, carbapenems, cephalosporins and monobactams. Adapted from Essack (2001)**.**

β-lactam antibiotics act through the disruption of cell wall synthesis, as such they prevent the successful formation of new cells. The cell wall consists of long polysaccharide chains cross-linked between one another by peptides, hence the peptides are largely responsible for its overall stability. The generation of peptide cross-links involves the addition of a new molecule - consisting of a monosaccharide attached to a peptide containing two D-alanine groups - into the growing polysaccharide chain of the cell wall. At this point, with the D-alanine-D-alanine peptide having a structural analogy to the β-lactam ring, β-lactam antibiotics are able to bind to penicillin-binding proteins (PBPs) inhibiting the transpeptidation reaction, resulting in the inhibition of the cross-linking of cell wall formation. As such the newly formed cell wall is unstable making the cell susceptible to bacteriolysis (Sköld, 2011).

IV. Trimethoprim/sulfamethoxazole

Trimethoprim/sulfamethoxazole, also known as co-trimoxazole, consists of one part trimethoprim to five parts sulfamethoxazole (Hamilton, 2015). Trimethoprim and sulfamethoxazole are frequently used as a combination therapy as they inhibit two consecutive steps on the same pathway of bacterial DNA and protein synthesis. Inhibiting two separate elements of the pathway simultaneously also reduces the rate at which resistance can develop. Trimethoprim acts through reversibly inhibiting the enzyme dihydrofolate reductase (DHFR), responsible for the conversion of dihydrofolic acid into tetrahydrofolic acid. Being an essential precursor in the thymidine synthesis pathway, Tetrahydrofolic acid is essential for the biosynthesis of nucleic acids. In its absence the bacteria will penultimately die (Brogden et al., 1982). Sulfamethoxazole further inhibits this pathway through preventing the initial synthesis of dihydrofolic acid. For the majority of

bacteria, the enzyme dihydropteroate synthase is responsible for the production of folic acid, as opposed to mammals where it is a dietary requirement. Sulfamethoxazole acts as a competitive inhibitor of this enzyme, hence preventing the same pathway as noted above for trimethoprim (Yun et al., 2012). When administered individually this drug combination is bacteriostatic. However, in combination the effects can be bactericidal (Kemnic & Coleman, 2021).

V. Ciprofloxacin

Nalidixic acid was the first member of the quinolone antibacterial group to be developed, with a fluorine substitution in the basic molecule leading to the development of fluoroquinolones (Andersson & MacGowan, 2003). Ciprofloxacin is a bactericidal second-generation fluoroquinolone, targeting bacterial type II DNA topoisomerases in both Gram-negative and positive bacteria. The two key enzymes targeted are DNA gyrase and topoisomerase IV, which are made up of 2 sub-units each. In *S. aureus,* GyrA and GyrB constitute DNA gyrase and GrlA and GrlB make up topoisomerase IV. DNA gyrase catalyses the energy-dependent supercoiling of relaxed covalently closed circular DNA, whilst topoisomerase IV catalyses the energy-dependent decatenation of catenated DNA. Ciprofloxacin and other quinolones act to stabilise an intermediary component of the enzymatic reaction resulting in a quinolone-DNA-enzyme complex halting the further replication of DNA. This ultimately creates a chain of events leading to the death of the cell (Tanaka et al., 2000).

VI. Erythromycin

Erythromycin was the first of its kind in the macrolide family, being the parent molecule of other later derived macrolides, for example azithromycin. The class is characterised by a

lactone ring with two sugar groups attached which target specifically the 50S ribosomal subunit of the bacterial ribosome (Novak & Kovač, 2015). Erythromycin itself is bacteriostatic, but in high enough concentrations possesses bactericidal activity, and since its discovery it has remained one of the safest broad-spectrum antibiotics available. Through reversibly binding to the 50S ribosomal subunit, erythromycin functions in a similar fashion to that of chloramphenicol (Ahmed & Sennik, 2011). Binding to the PTase centre and preventing the new addition of ribosomal substrates inhibits RNA-dependent protein synthesis, hence preventing cell replication (Dancer, 2014).

VII. Clindamycin

Despite multiple antibiotic derivatives within the lincosamide class, only clindamycin and lincomycin are utilised in clinical practice, where clindamycin is generally more effective especially against anaerobic bacteria and aerobic Gram-positive cocci including *S. aureus*. Both agents, similar to erythromycin, are bacteriostatic through their inhibition of protein synthesis, yet are capable of having bactericidal effects in high enough concentrations (Spízek & Rezanka, 2004).

VIII. Tetracycline

Tetracyclines have an extensive broad spectrum of activity including Gram-negative and positive aerobic and anaerobic microorganisms. Combined with their relative safety and cheap production costs they rose to prominence across the world being second in usage only to penicillin. However, their use has been in decline as the burden of resistance has increased. This effect has been further compounded by their use in agriculture and animal husbandry, with genetic resistance markers first reported in animals or humans subsequently found later in the other (Roberts, 1996). Tetracycline is able to bind reversibly

to the 30S subunit of ribosomes, as opposed to MLSB antibiotics which bind to the larger 50S subunit. Whilst the precise binding sites and mechanisms of action are still disputed, it is understood that tetracycline prevents the binding of aminoacyl-tRNA to the mRNA-ribosome complex, hence preventing translation and protein synthesis, without which the bacteria is unable to replicate (Chukwudi, 2016).

1.3.2 What is Antimicrobial Resistance?

According to the WHO, antimicrobial resistance (AMR) happens when microorganisms (bacteria, fungi, viruses, and parasites) change to resist the effects of an antimicrobial drug (antibiotic, antifungal, antiviral, antimalarial, or anthelmintic) to which it has been exposed. Microorganisms that develop AMR are sometimes referred to as “superbugs”. As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others (World Health Organisation, 2020). Similarly, Jindal et al. (2015) define AMR as the resistance of microorganisms to an antimicrobial agent to which they were at first susceptible. Such resistance can arise in a number of ways; for example, random spontaneous chromosomal mutations can result in the generation of resistance to an antimicrobial through either a single or series of alterations. Typically, this will confer resistance to a single or single class of antibiotics. Once given a selective advantage over the susceptible population, these mutations allow resistant bacteria to become increasingly prevalent. AMR genes (ARGs) can also be carried on plasmids. Transmissible plasmids carrying multiple ARGs are capable of crossing species barriers giving a cell the opportunity to gain multiple drug resistance to several unrelated antibiotic families in a single action. Transposons can also incorporate ARGs and are capable of generating copies of genes which can integrate into either the

chromosome or plasmids. Chromosomal genes are generally more stable, whilst incorporation onto a mobile element (plasmid, integron, transposon, or bacteriophage) allows for much greater dissemination (Goering et al., 2012). These concepts are discussed further in Chapter 4.

1.3.3 The 'Cat and Mouse' Evolution of Antibiotic Drug Development

The nature of evolution dictates that bacteria will always adapt under the selective pressure of antibiotics to develop resistance. Indeed, since antibiotics are naturally produced by bacteria and fungi in the natural environment, AMR existed long before the discovery of antibiotics. Mass-production and intensive use of clinically and agriculturally relevant antibiotics has promoted the generation of resistance at ever increasing rates. Effective control of bacterial diseases therefore requires continual development of new therapeutics, either by adaptation of existing antibiotics or novel drug discovery. A classic example of this is the development of several generations of cephalosporins, capable of permeating the outer membrane of Gram-negative bacteria more readily than penicillins. Clinical use of first-generation cephalosporins began in the 1960s, but diminished permeability was soon reported in clinical strains. This prompted the development of second-generation cephalosporins, which exhibited increased activity against Gram-negative bacteria. However, resistance developed in a number of bacterial species including *Klebsiella*, *Enterobacteriaceae* and *Pseudomonas* spp. through the hyperproduction of β-lactamases. Whilst less effective against Gram-positive cocci, third-generation cephalosporins are much more active against *Enterobacteriaceae.* However, hyperproduction of species-specific β-lactamases were soon able to convey clinically significant levels of resistance to a wide spectrum of β-lactams including cephamycins and

monobactams, leaving only carbapenems as an effective substitute (Essack, 2001). Fourth generation cephalosporins contain a positively charged nitrogen, with the charge helping to better orient the molecule in relation to the entrance of the porin channel. As such they exhibit enhanced permeation across the outer membrane in comparison to their third generation counterparts. Their improved effectiveness can also be attributed to an increased resistance to hydrolysis from β-lactamases and cephalosporinases. Whilst generally taking longer to develop, resistance against fourth generation cephalosporins is possible, most often seen through a combination of hyperproduction of β-lactamases, plasmid-mediated extended spectrum β-lactamases and porin modification in Gram-negative bacteria (Fung-Tomc, 1997).

With the levels of AMR increasing faster than the development of new drugs, the WHO developed the AWaRe antibiotic classification system, short for Access, Watch and Reserve groups. Access antibiotics are first or second choice offering the best therapeutic value whilst minimising potential resistance. The watch group consists of first and second choice antibiotics with usage limited to specific infective syndromes. Finally the reserve group is comprised of antibiotics of last resort, with usage closely monitored and only recommended in life-threatening situations due to multi-drug resistant (MDR) bacteria (World Health Organisation, 2021). That being said, antibiotics of last resort are being increasingly prescribed. Carbapenems, for example, host a broad antimicrobial spectrum of activity. Being effective against Gram-positive, Gram-negative and anaerobic bacteria they are often prescribed as a last resort for nosocomial infections and sepsis caused by MDR microorganisms (Ramalingam, 2015). However, resistance even to these has been increasingly documented, resulting in bleak public health warnings. One example of this is the rise in carbapenem-resistant *Enterobacteriaceae* (CRE), known to cause bladder, lung

and blood infections which can lead to life-threatening septic shock. Whilst it's not too late to intervene in the growth of such cases, it consolidates the need for improved antimicrobial surveillance (McKenna, 2013).

1.3.4 Why is Antimicrobial Resistance a Concern?

With the potential to reverse decades of medical progress, AMR is one of the biggest threats to global health, food security and development in the world today. As such, a large number of resources are being directed towards AMR research and evaluation. A review in 2015 commissioned by the UK government estimated that between 2015 and 2050, annual deaths related to AMR could reach as high as 10 million globally (O'Niel, 2015); with the concluding report the following year indicating a current global rate of 700,000 deaths per year (O'Niel, 2016). Whilst the numbers quoted are loose estimates due to the sporadic nature of available data, they do still give major cause for concern.

Whilst human health is a priority, AMR will also have an inevitable impact on the global economy. The same study commissioned by the UK government estimated that between 2015 and 2050 AMR could cost globally US$100 trillion (O'Niel, 2015); with a study published by the World Bank estimating a reduction on global GDP of between 1.1 and 3.8 percentage points between 2017 and 2050 due to AMR (Jonas et al., 2017). It should be pointed out that the impact of AMR does not only effect human wellbeing. The livestock industry is also heavily reliant on antimicrobials as "growth factors" as well as for infection control. Here, antibiotic usage encompasses ethical, economic and environmental concerns. The risk of antibiotics in the environment involves increased selectivity for resistance genes in soil bacteria, with manure being the primary vehicle for antibiotic delivery when livestock

are present. Such genes have the potential to transfer to other infectious bacterial species, hence the necessity for reduced antibiotic usage. On the other hand, ethically speaking, minimising pain and treating disease are one of the "five freedoms" afforded to livestock by contemporary animal husbandry for which antibiotics are essential. Equally, from an economic perspective the use of antibiotics allows for increased efficiency meaning fewer animals needed for the same volume of produce, less cropland required for the growth of animal feed, decreased manure production and the associated economic benefits for both consumers and producers (Durso & Cook, 2014). Given its wide implications, it is essential that research is facilitated to both reduce the growth rate of AMR in addition to the development of new, effective antibiotics.

1.3.5 Mechanisms through which Antimicrobial Resistance Occurs

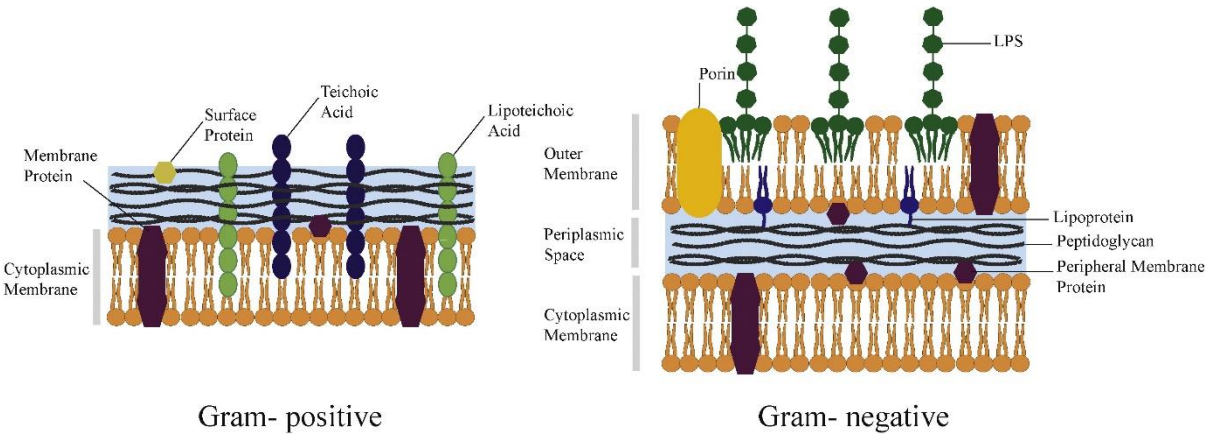


Figure 1.2: Schematic Diagram comparing Gram-positive (left) and Gram-negative (right) bacterial cell membranes (Epand et al., 2016).

When assessing the efficacy of antimicrobials, the cell membrane compositional differences between Gram-positive and Gram-negative bacteria must first be taken into account. As shown in Figure 1.2, Gram negative bacteria have an additional outer

membrane which, as a general rule, gives an extra layer of protection against antimicrobial agents. That being said, both cell types possess a cell wall enclosing the cytoplasmic membrane, which is considerably greater in thickness in Gram positive bacteria (Epand et al., 2016).

Resistance mechanisms can vary depending on the nature of the microorganism and the antimicrobials that it has been exposed to, these concepts are discussed further in Chapter 4. In general, there are three common pathways through which resistance is facilitated:

i) Alteration of target site: the target of the antibacterial agent may be altered to lower the affinity of binding or be removed entirely. Many antibiotics belonging to classes such as the aminoglycosides, tetracyclines, macrolides and chloramphenicol target the bacterial ribosomal subunits to inhibit protein synthesis. However, multiple alterations of drug binding sites within the ribosomal subunits and other components of the bacterial protein synthesis machinery can confer resistance. In some cases, single nucleotide changes have been shown to confer multidrug resistance affecting protein synthesis inhibitors that have overlapping binding sites within the ribosome (Dunkle et al., 2010).

ii) Altered permeability: For an antibiotic to be effective, a threshold concentration needs to reach the target site in the bacterial cell. This can be altered by either pumping the drug out of the cell or inhibiting its initial entry. Porins are passive transport proteins which enable diffusion of hydrophilic solutes across the lipid bilayer of Gram-negative bacteria, hence providing a route of access for antimicrobial agents into the cell. As such, a loss or reduced function of one or more porins can block or slow the accumulation of antibiotic at the target site and lead to further

resistance development. Resistance arising in this manner is typically reported to develop during prolonged use of antibiotics (Pagès et al., 2008). Efflux pumps can also provide an efficient form of resistance through facilitating the active transport of antimicrobial agents out of the cell (Nishino & Yamaguchi, 2001). Efflux pumps and reduced cell permeability are collectively responsibly for conveying a general resistance to trimethoprim and sulphonamides simultaneously (Eliopoulos & Huovinen, 2001).

iii) Antibiotic inactivation: Enzymatic inhibition forms the dominant resistance strategy in bacteria, with multiple mechanisms through which this can occur. Typically, the antibacterial structure can be altered through either hydrolysis (e.g. β-lactamases), the transfer of functional groups that interfere with activity (e.g. acetyl-transferases) or other chemical modifications (e.g. redox) (Bhullar et al., 2012). The primary route of aminoglycoside resistance is enzymatic inactivation via aminoglycoside-modifying enzymes (AMEs) (Garneau-Tsodikova & Labby, 2016).

1.3.6 Use of Antimicrobials and Promotion of Resistance

Improvements to clean water supplies, infrastructure, public health services and vaccination have all contributed to reducing the incidence of infection; but antibiotics have been crucial in the reduction of morbidity and mortality associated with bacterial infections (Adedeji, 2016). In the USA between the years 1936 and 1952 the death rate due to bacterial illness (typhoid and paratyphoid fever, typhus fever, scarlet fever, whooping cough, diphtheria, tuberculosis, pneumonia, diarrhoea and enteritis, appendicitis and puerperal septicaemia) fell from 216.3 to 59.7 deaths per 100,000 population. This time

period encompassed the introduction of antibiotics to treat the major types of bacteria: gram-positive, gram-negative and mycobacterium (Gottfried, 2005). The high efficacy, low toxicity and relatively low cost of antibiotics has led to overuse and mis-use in both clinical and agricultural settings for many decades and it is well known that any use of antimicrobials contributes to the development of resistance (Ayukekbong et al., 2017).

Low-Middle Income Countries (LMICs) already suffer from a higher burden of infectious bacterial diseases in both humans and animals, with cost constraints preventing the widespread application of newer, more expensive antibiotics (Okeke et al., 2005). Thus, the indiscriminate use of antibiotics in developing countries as empirical treatments and variable drug efficacy associated with the import or manufacture of unregulated cheap antibiotics, available on demand over the counter, provides an ideal environment for the generation of resistance. This effect is further compounded given the wide prophylactic use of antibiotics in animal husbandry and human health. Even where antibiotics are solely intended for use within the confines of veterinary medicine the risk of cross-resistance developing with those used for humans are still present (Tiong et al., 2016). Welsh's research found very high levels of prophylactic antibiotic use amongst pregnant women attending lower-level health facilities in Uganda (Welsh, 2019). Additionally, the poor access to appropriate antibiotics in Uganda promotes misuse, where full courses cannot be completed or inappropriate doses or alternatives are used in replacement.

Strategic action plans to tackle AMR recognise the importance of evidence-based policies. However, despite the present risks of AMR, little data are available for countries across sub-Saharan Africa (Omulo et al., 2015); a recent systematic review including 144 articles found national data on rates of resistance were not available for 42.6% of the countries on the African continent, with a limited number of published studies focused on

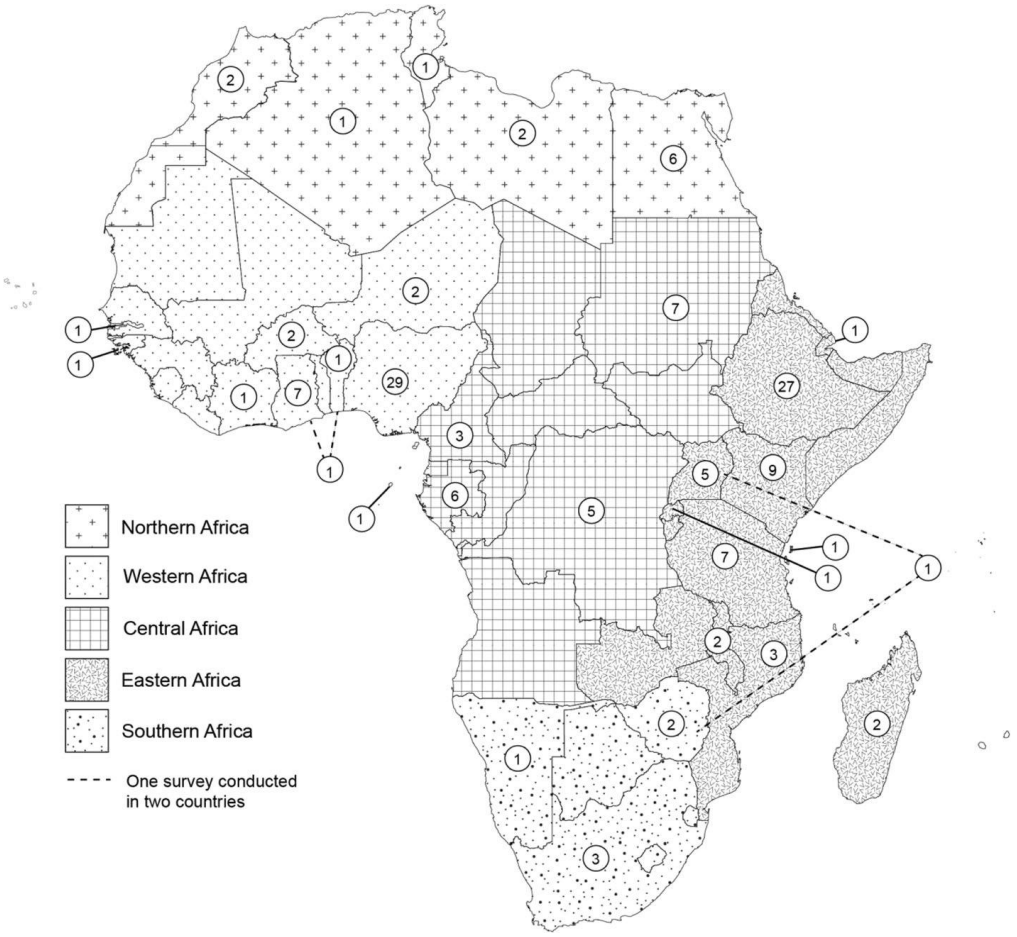


Figure 1.3: Geographical distribution and number of selected studies between January 2013 and January 2016 in the different African countries. Countries were grouped based on the United Nations Statistics Division classification into Eastern Africa, Southern Africa, Central Africa, Northern Africa and Western Africa (Tadesse et al., 2017).

AMR as shown in Figure 1.3 (Tadesse et al., 2017). These gaps in surveillance data need to be addressed as a matter of urgency. That being said, whilst the review provided an interesting oversight of the region, there were a few outliers which may have arisen as a result of the inclusion and exclusion criteria. Nigeria and Ethiopia had far more studies than all other countries assessed with a total of 29 and 27 respectively. Being the two most populated countries in Africa (United Nations, 2019) and both ranking amongst the top six in terms of GDP (International Monetary Fund, 2021) it could be somewhat expected to find more healthcare related studies, but not to the extent witnessed relative to other countries;

a case in point being South Africa which has a better functioning healthcare system and AMR surveillance than many of its neighbours despite only three studies being revealed through this review. As such it is plausible that whilst data is being collected it is not being reported in an adequate fashion for further downstream analysis and comparisons. Notable exclusions within the review included AMR reported by antibiotic groups (n=25), genetic studies without AMR data (n=124) and AMR not recorded for specific organisms (n=31). These highlight the need for a uniform approach in not only data collection, but also reporting for the benefit of large-scale data evaluation.

1.3.7 The 'One Health' Agenda

Whilst coordinating the multisectoral governance of AMR prevention it is important not to overlook the wide variety of targets for action. Without an understanding of how interventions can affect people in their respective communities the preventative strategies can ultimately fail in their desired impact. One facet of this is how AMR can affect men and women differently; for example, it is not uncommon for women who have delivered via caesarean section to receive prophylactic antibiotics to combat the development of sepsis (Sharma et al., 2013). Alternatively, outside of patient care, do certain professions present increased risk of AMR exposure? Employees in the farming and agriculture industry are more likely to be exposed to resistant bacteria being carried by animals. The specific bacterial species can vary depending on the type of animal and the production setting, but resistant strains can equally be carried by the workers and spread to others in the community (World Health Organisation, 2020). Additionally, resistance can be selected for

in food animals, where possible contamination of animal-derived food with such resistant bacteria then posing a threat to human health (Phillips et al., 2004).

Such interplaying factors have led to the growth and adoption of the 'One Health Agenda'. With the same microorganisms capable of infecting humans and animals alike, drug resistant strains can equally be transmitted between them and their environments either through direct contact or contaminated intermediates. In order to address the overall problem, solutions must be fashioned through effective collaboration between all affected sectors - be it human health, animal health, plant health or environmental organisations. This involves cooperation on the front line as well as research facilitation through the sharing of epidemiological data and laboratory information (World Health Organisation, 2017).

1.4 Addressing Antimicrobial Resistance on a Global Platform

Alongside surveillance, there is an emerging consensus that efforts to contain the threat of AMR should focus on four key objectives (Holmes et al., 2016). The first is to address the overuse of antibiotics in both human and animal populations, with high usage being the predominant factor in the development of AMR. Secondly, the effective treatment of liquid waste and sewage to reduce the volumes of antibiotics being dispersed into the environment. Thirdly, through utilising effective infection prevention and control (IPC) strategies in conjunction with water and sanitation programmes, the need for antibiotic usage can be reduced. Finally, it is essential that effective, affordable and regulated antibiotics are available to those that require them to avoid the self-prescription of uncertified alternatives. These objectives are most effective in combination with new

diagnostic and treatment technologies (Rochford et al., 2018). Mirroring these ideas, in order to help tackle the threat of antibiotic resistance on a global scale, the World Health Assembly endorsed a global action plan in May 2015 to act as a foundation through which enrolled countries can better address the issues at hand. The central aim of the plan was to ensure the continuity of successful treatment and prevention of infectious diseases with safe and effective medicines. To facilitate this, five key strategic objectives were generated (World Health Organisation, 2015a):

1. Improve awareness and understanding of antimicrobial resistance through effective communication, education and training

To promote positive behaviour change, awareness must be raised across all disciplines implicated in antibiotic use; including human health, animal health and other agricultural practices. In addition to this, education at multiple stages is vital to promote better understanding of antimicrobials and the repercussions of resistance, ranging from early age school curricula through to professional education, training and certification in health, veterinary and agricultural sectors.

2. Strengthen the knowledge and evidence base through surveillance and research

To understand how and why antimicrobial resistance arises it is essential to have information on the incidence, prevalence and patterns of resistance present. Such data can not only inform public health policies on how best to address the situation, but also contribute to the research and development of medical and agricultural alternatives to antimicrobials.

3. Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures

A clear pathway to reducing antibiotic usage is reducing the incidence of infection through effective infection prevention and control. Whilst this is largely improved through better sanitation and hygiene, increasing the availability and administration of appropriate vaccinations helps reduce the number of patients with viral infections who can then be mistreated with antimicrobial medicines. Such factors are also applicable to animal husbandry where indiscriminate use of antibiotics can lead to resistance spreading to humans.

4. Optimise the use of antimicrobial medicines in human and animal health

To reduce the rate at which resistance is generated to specific antibiotics, the means for their indiscriminate usage must be decreased. This comes from responsible antimicrobial stewardship and reduced unnecessary dispensing on all fronts. Prescriptions for such drugs should be evidence based through effective, cheap and fast diagnostic tests to allow for the optimal effectiveness of treatments. Following on from prescription, stronger compliance to treatment schedules is essential using quality assured medication.

5. Develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions

With the majority of pharmaceutical companies ceasing investments in novel antibiotics, it is vital that the global community encourages the research and development of new medicines, vaccines and diagnostic tools with economic incentives. Not only are new

treatments required in the fight against severe multi-drug resistant infections, but more stable, affordable and effective tools and medicines are also hugely beneficial across multiple different settings. Increasing the amount of funding available also allows for subsidised medical costs thus improving accessibility to essential treatments.

1.4.1 Global Antimicrobial Resistance Surveillance System (GLASS)

With less published data available and a lack of national action plans across Africa, trends of resistance in developing countries are generally on the rise (Ayukekbong et al., 2017). As of 2017 only seven countries in Africa have had their AMR surveillance National Action Plan approved by national authorities (World Health Organisation, 2017). Uganda itself, however, has been enrolled in GLASS since July 2016 and implemented its National Action Plan soon after in 2018 (World Health Organisation, 2018b).

The WHO developed GLASS in an attempt to improve the coordination between countries and effectively address the issues posed (Jee et al., 2018). The first phase of GLASS covered the early implementation period between 2015 and 2019. During this period GLASS provided assistance and tools in surveillance and laboratory guidance as well as support to countries in developing effective AMR surveillance systems. The initial focus revolved around bacterial pathogens specific to humans, with other factors added in progressively. With that in mind the following objectives for GLASS were derived (World Health Organisation, 2015b):

- Foster national surveillance systems and harmonized global standards
- Estimate the extent and burden of AMR globally by selected indicators

- Analyse and report global data on AMR on a regular basis
- Detect emerging resistance and its international spread
- Inform implementation of targeted prevention and control programmes
- Assess the impact of intervention

Table 1.1: WHO member countries per region enrolled in GLASS and reporting information on the implementation of national surveillance systems in the second data call. Adapted from World Health Organisation (2018b)**.**

Region	Number of Countries in the Region	Number of Countries Enrolled to GLASS	Number of Countries Reporting Implementation to GLASS
Africa	47	15	14
Americas/Pan American	35	3	3
Eastern Mediterranean	21	14	14
Europe	53	24	22
South-East Asia	11	9	8
Western Pacific	27	6	6

A total of 69 countries were enrolled in GLASS by the end of the second data call on 31 July 2018, a 64% increase in relation to the first call of July 2017. Additionally, the depth of information is also improving from the initial status reports of national AMR surveillance systems through to the presentation of AMR data. Of the 68 countries providing data, there were 10 low-income, 16 lower middle-income, 15 upper middle-income and 27 high-income countries. In total 3097 hospitals and 2358 outpatient's clinics reported AMR data to GLASS, with the most prominent resistant pathogens being *E. coli, K. pneumoniae, Salmonella spp.,*

Acinetobacter spp., S. aureus, S. pneumoniae, N. gonorrhoea and Shigella spp. respectively. These were collected from blood, urine, stool, cervical and urethral specimens (World Health Organisation, 2018b).

1.5 Uganda

Uganda is an East African country located on the equator. Being 800km inland from the Indian Ocean the country is landlocked, bordered by Kenya to east, South Sudan to the north, Democratic Republic of Congo to the west, Tanzania to the south and Rwanda to the south-west. Uganda itself is divided into 116 districts including the capital city, Kampala, and has an estimated population of 34.6 million, with a population growth rate of 3.03% and estimated total fertility rate of 6.7 children per woman. 47.9% of the population were aged 0-14 making Uganda one of the youngest and fastest growing populations in the world (World Health Organisation, 2016b). Life expectancy at birth in 2014 was 62.2 for males and 64.2 for females (Uganda Bureau of Statistics, 2016), compared to 79.5 and 83.2 for men and women in the UK respectively (World Health Organisation, 2016a).

1.5.1 Ugandan Healthcare System

The Ugandan healthcare system is comprised of both public and private sector institutions. That being said, due to the sizeable inequalities observed between the two, patients typically seek private care as high as they can afford (Ackers & Ackers-Johnson, 2017). The provision of public care is divided into two core levels, national and district based. The national level includes both national (n=2) and regional (n=8) referral hospitals,

whilst at the district level exists a tiered system of healthcare provision provided at healthcare centres I – IV (Welsh, 2019):

- Health Centre I's act as more of link between the community and the nearest healthcare facility with village health technicians promoting health and engagement with local healthcare services.
- Health Centre II's are staffed by nurses and run basic outpatient and community outreach services. They are qualified to prescribe certain drugs including antibiotics and malaria treatment.
- Health Centre III's have an outpatient department managed by a clinical officer and provide basic obstetric, antenatal and labour care. Antibiotics can be prescribed for maternity patients by on site midwives.
- Health Centre IV's have an operating theatre and inpatient wards for adults and children in addition to the services provided by lower-level healthcare centres.
- National and regional referral hospitals offer specialist clinical services with increased capacity and higher levels of medical, surgical and laboratory support.

Theoretically speaking patients should seek care at the lowest level healthcare facilities and be referred upwards where services are inadequate, however, in practice patients often travel to the facility which is most convenient for them or to where they believe they will receive the best care.

1.5.2 AMR prevalence in Uganda

From a review of currently available data, key indicators of resistance, MRSA and extended-spectrum β-lactamase (ESBL)-producing bacteria display a highly variable prevalence between hospitals in Uganda, ranging from 2 - 90% and 10 - 75% bacterial isolates respectively. Whilst resistance to carbapenems, last resort antibiotics (see Chapter 4), is growing with a reported prevalence between 4 and 30% (Uganda National Academy of Sciences, 2015).

Judging from this research it is clear that levels of antibiotic resistance are edging closer to critical levels, at least in some regions. However, it is also clear that data collection is highly sporadic with variable results observed (Reinhart et al., 2017). Published studies based on AMR in Uganda have mainly focused on the Capital city (Kampala) where a recent study indicated 23.7% of clinical isolates from surgical site infections were *Escherichia coli* and 21.1% were *S. aureus*. Of those isolated more than 75% of the *E. coli* were determined to be ESBL producers, whilst 37.5% of *S. aureus* isolates were methicillin resistant (Jeremiah Seni et al., 2013). The levels of MRSA are relatively consistent with other nearby countries with reported rates of 53.4% in Kenya (Wangai et al., 2019), 32.5% in Ethiopia (Eshetie et al., 2016) and 44% Tanzania (Manyahi et al., 2014). Levels of ESBL producing *E. coli* were far more variable with rates of 92.3% in Tanzania (Manyahi et al., 2014), 41.2% in Ethiopia (Abayneh & Worku, 2020) and 37.4% Kenya (Storberg, 2014). Far fewer studies, however, focus on more rural/isolated settings outside of the major cities. As such it is not well known whether the same incidence and resistance patterns occur in more isolated settings. One study aiming to shed light on this area assessed two rural districts, 74km and 29.7km

outside of Kampala, in relation to samples acquired in Kampala and essentially found that the rates of antibiotic resistance were higher in urban areas (Najjuka et al., 2016).

1.5.3 The impact of AMR on agriculture in Uganda

Whilst often under-reported in relation to human cases of infection, agriculture and animal husbandry are an important component of the UNAP, with specific objectives noted to encourage support for One Health networks at local and regional levels (Government of Uganda, 2018). Agriculture forms a core economic sector of Uganda employing upwards of 80% of the labour force. Producing 40% of the goods exported from the country it's accountable for 22% of the GDP. However, subsistence farming is also practised by 69% of the population resulting in low productivity and earnings for the sector (National Population Council, 2017). In the drive to boost production various supplements and drugs (including antibiotics) are given to livestock on a regular basis. Whilst veterinary surgeons are the only cadre of workers with legal authority to prescribe antibiotics, they constitute a small proportion of the people who actually do, which include para-veterinarians, farm managers and farmers. Given the large number of people participating in farming it can be difficult for the Ministry of Agriculture, Animal Industry and Fisheries to fulfil their objectives of managing crop and animal diseases in addition to regulating the use of veterinary drugs (Uganda National Academy of Sciences, 2015). Whilst no data from Uganda was available, a study in neighbouring Rwanda which interviewed 229 farmers of various animals found 95.6% were able to name at least one specific antibiotic, 97.4% had used antibiotics on their farm animals and 55.6% of farmers had used non-prescribed antibiotics (Manishimwe et al., 2017).

In addition to the wide distribution of antibiotics to livestock increasing the risk of resistance developing, the frequency of small-scale subsistence farming within the population in and around the home provide ample opportunity for transmission between humans, animals and the local environment. Further compounding this are the lack of access to clean water, effective sanitation and proper waste management seen in multiple communities (Afema et al., 2016). Despite the implications research and documentation of animal health is severely deficient in Uganda, with only 3 out of 16 veterinary diagnostic laboratories able to carry out antimicrobial susceptibility tests. The most commonly reported condition, bovine mastitis, indicates an alarming >50% pathogens are resistant to common antibiotics (Uganda National Academy of Sciences, 2015).

1.5.4 Uganda's Antimicrobial Resistance National Action Plan (UNAP)

Given the multi-contextual nature of AMR, the UNAP takes a "whole-of-society engagement" approach embracing the *One Health* Agenda. This ensures that AMR can be targeted at every level be it related to humans, animals or the environment. To help provide structure and enhance collaboration a multidisciplinary National Antimicrobial Resistance Sub-Committee (NAMRSC) was formed which comprises representatives from multiple industries including fisheries and agriculture, water and waste management, human health, wildlife, science and research and professional societies. Through the utilisation of such sub-committees it is hoped that the UNAP will have a national, regional and local impact. Mirroring the GAP, Uganda's National Action Plan contains five key strategic objectives (Government of Uganda, 2018):

1. Awareness

2. Infection Prevention and Control
3. Stewardship
4. Surveillance
5. Research and Innovation

1.5.5 Aligning my Research with the National Action Plan in Uganda

Through monitoring patterns of antibiotic resistance at Fort Portal Regional Referral Hospital (FPRRH) and completing the genetic sequencing elements in partnership with Makerere University, my core research revolved around strategic objectives 1, 2 and 4 of the World Health Authority Global Action Plan and strategic objectives 3, 4 and 5 of the UNAP, contributing towards AMR surveillance as well as education at a local level (raising awareness when collecting samples and reporting back results) and wider research and innovation (including working with colleagues at Makerere University on the genome sequencing aspect). It is also worth noting that improving surveillance is central to the achievement of the other pillars creating the evidence-base for more credible awareness-raising processes amongst health workers and the public. Collecting effective microbiology data on resistance patterns is an essential prerequisite for effective stewardship, creating opportunities for a shift from empirical to rational prescribing. Through collaborating with the microbiology laboratory and pharmacy at FPRRH we were able to provide the evidence required for shifts in antibiotic procurement and the generation of an antibiogram, both having a direct impact on stewardship and prescription practices.

1.5.6 Strategic Objective 3: Promote Optimal Access and Use of Antimicrobials

The overuse of antimicrobial agents has been identified as a major modifiable driver of antimicrobial resistance (AMR). As such, there are recommendations to only use antibiotics when the situation requires. This is critical to the success of both prolonging the efficacy of specific antibiotics as well as reducing the development of AMR. The strategic objective has a number of facets spanning across human, animal and agricultural sectors and revolves around ensuring the availability of a range of effective antibiotics in addition to educating prescribers and consumers about their circumstantial usage. Many aspects of strategic objective 3 go hand in hand with those of strategic objective 4 (AMR surveillance), where the acquisition of data through culture and sensitivity testing is essential for policy developments and procurement plans relating to the availability and appropriate use of effective treatments (Government of Uganda, 2018). This can be observed through empirical use of antibiograms as well as through personally tailored treatment regimens. That being said, knowing the appropriate treatment of choice is futile if it cannot be accessed, hence why strategic objective 3.1 focuses on optimising access to effective antimicrobial medicines and diagnostics (Government of Uganda, 2018).

In Uganda, funds for the procurement of drugs and supplies in the public sector are highly centralised and inadequate. National Medical Stores procures and distributes supplies to health facilities based on a centrally allocated annual supplies budget, with deliveries scheduled once every two months. Each hospital is required to produce an annual procurement plan which, once agreed upon, is fixed and cannot be varied over the year. This rigid structure reduces the opportunity for flexibility and responsiveness to local hospital laboratory results. Additionally, with the exception of private wards, it is not

possible for a regional referral hospital to source supplies from elsewhere. During the annual procurement process, the hospital may only order those items authorised by the 'Essential Medicines and Health Supplies List for Uganda'. However, not all essential drugs feature in the National Medical Stores catalogue (L. Ackers et al., 2020).

1.5.7 Challenges of Effective Antibiotic Use in Clinical and Community Settings

In Ugandan healthcare settings, infection control measures are very limited and access to antibiotics is intermittent in terms of both availability and type (World Health Organisation, 2018a). These issues are compounded by task-shifting, where junior staff end up with prescribing responsibilities with insufficient training. Inappropriate prescribing, dispensing and administration of antibiotics compromise their utility and increase the risk of adverse drug reactions. Lack of appropriate supplies in the healthcare setting means that patients often purchase their own antibiotics to supplement their prescription, many of which are un-regulated. Moreover, people will often self-medicate without accessing any healthcare advice at all, particularly in rural community settings, and there is considerable evidence of counterfeit drugs on the market (Omulo et al., 2015; Ugandan National Academy of Sciences, 2015, 2018; Vialle-Valentin et al., 2012; World Health Organisation, 2019).

1.5.8 Uganda NAP Strategic Objective 4 - AMR Surveillance

Strategic objective 4 of Uganda's NAP further diverges into multiple smaller interlinking objectives, with those most directly linked to this research being objectives:

4.1.1 – Support the implementation of a national AMR surveillance programme to generate actionable data

4.1.3 - Strengthen and support improvement of laboratory infrastructure, human resources, access to laboratory supplies and equipment for microbiological testing and quality data reporting platforms

4.4.4 - Support the routine generation and use of microbiological culture and sensitivity tests on prioritized microorganisms and antimicrobials in health facilities

At the conception of this research laboratory facilities at FPRRH were relatively poor. Whilst culture and sensitivity testing were available in a limited capacity, the services were largely under-used. However, as the effects of the UNAP came into play alongside Ebola-readiness-associated factors the laboratory was selected for large scale refurbishments. As such the hospital became a prime location for increased data collection and AMR surveillance. This aligned with strategic objective 4.1.3 of the UNAP to strengthen and support improvement of laboratory infrastructure, human resources, access to laboratory supplies and equipment for microbiological testing and quality data reporting platforms (Government of Uganda, 2018). Improving laboratory capacity is essential for the evaluation of AMR prevalence at both a local and national level.

1.5.9 Uganda NAP Strategic Objective 5 – Research and Innovation

The NAP aims to promote research on the resistance mechanisms and transmission of high-risk and high-burden strains, as well as supporting more localised research on

resistance and transmission pathways involving the environment, humans, animals and food supply chains.

For this objective my PhD has supported the emphasis on research and research capacity-building in 2 key respects. In the first instance through the development of strong relationships with the Infectious Disease Laboratory Team based at FPRRH. The IDI laboratory was developed at FPRRH in 2018 with the objective of building laboratory capacity in a hospital close to the Congo border, an area identified as the high-risk entry point for Ebola virus disease. The IDI team shared a need to collect samples from the hospital and its environment as the basis for the generation of a significant evidence base on antimicrobial resistance. However, it was struggling to access the volume of samples required in the face of poor awareness and commitment to sample-taking in the hospital. Secondly, this project has played a key role in the validation of laboratory testing at the IDI facility. These concepts align well with the strategic sub-objectives outlined below and shall be explored further in Chapter 4.

5.3 - Collaborate with International Partners in Basic Intervention Research

5.3.1 - Promote research to identify high-risk and high-burden resistant strains, their resistance mechanisms and their transmission

5.3.3 - Invest and support collaboration in high-throughput genomics and sequencing technologies that have the potential to enhance product development

5.3.4 - Support research on the burden of AMR and its interventions to inform policy for investment in interventions

5.4 - Enhance Operational and Health Systems Research at the Local Level

5.4.2 - Promote local research on antimicrobial use patterns with the goal of producing more context specific stewardship approaches

1.6 The Burden of Staphylococcus aureus on Human Health and how Antibiotic Resistance Affects its Treatment

Carried by roughly 30% of the human population, *Staphylococcus aureus* is a Gram-positive coccus found frequently in the nasopharynx, respiratory tract and on skin. Whilst commonly found as a commensal organism, *S. aureus* is also a major human pathogen with the ability to cause a wide range of clinical infections resulting in skin (where the skin has been broken, for example from a wound or surgery) and respiratory diseases and is the most prevalent pathogen isolated from hospitalised patients (Tong et al., 2015). The variable levels of pathogenic activity can often be described through the presence or absence of virulence factors, many of which contribute directly to immune evasion (Mrochen et al., 2021). Serious infections occur most regularly in hospitalised patients where the consequences can be severe. Though often easily treated with antibiotics, if left neglected the infection can worsen and spread leading to the possible development of sepsis. Being one of several organisms commonly linked to bloodstream infections, *S. aureus* is one of the most predominant and fatal, with an estimated mortality of 20% and upwards of 50% of patients with *S. aureus* bacteraemia going on to develop complicated bacteraemia (Gu et al., 2020). A large meta-analysis of community acquired bloodstream infections in Africa examined 5578 patients with non-malaria bloodstream infections, where 531 (9.5%) cases were due to *S. aureus* (Reddy et al., 2010). Furthermore, *S aureus* is recognised as one of the most prevalent causes of skin and soft tissue infections, with the potential to cause

infections as a result of surgery, pressure ulcers, diabetic ulcers and hospital or community-acquired injuries. It has a strong association with nosocomial infections leading not only to recurrent hospitalisations but also increased mortality rates, increased duration of hospital stays and increased healthcare costs (Almeida et al., 2014). Also of note, whilst not being one of the more common causes of urinary tract infections (UTI), the incidence of *S. aureus* causing such has been increasing. This has a strong nosocomial link where a higher frequency of patients fitted with various urinary catheters with UTIs and other related health complications are increasingly susceptible to *S aureus* infection (Mitiku et al., 2021).

Treatment has become increasingly complicated due to the rise of methicillin resistant *S. aureus* (MRSA), which is now often multi-drug resistant (MDR). Figure 1.4 highlights how MRSA has become highly prevalent amongst hospitals on a global level, where the highest rates (>50%) are reported in North and South America, Asia and Malta. Intermediate rates (25–50%) are reported in China and Australia; whilst countries across Africa and Europe show variable prevalence ranging from low to intermediate where data is available (Stefani et al., 2012). As MRSA treatment becomes more complicated, methods of infection prevention have become valuable (Kluytmans et al., 1997). That being said, whilst initial outbreaks of MRSA were typically confined to hospitals, there has been a large increase in the number of cases reported with no exposure to healthcare settings. These new strains are frequently referred to as community-associated (CA-) MRSA (David & Daum, 2010), with mathematical models predicting they will displace the traditional health care-associated strains (Mediavilla et al., 2012). As the lines between community and health care-associated strains become blurred, the value of genotypic screening to describe the differences between strains has become increasingly valuable (David & Daum, 2010).

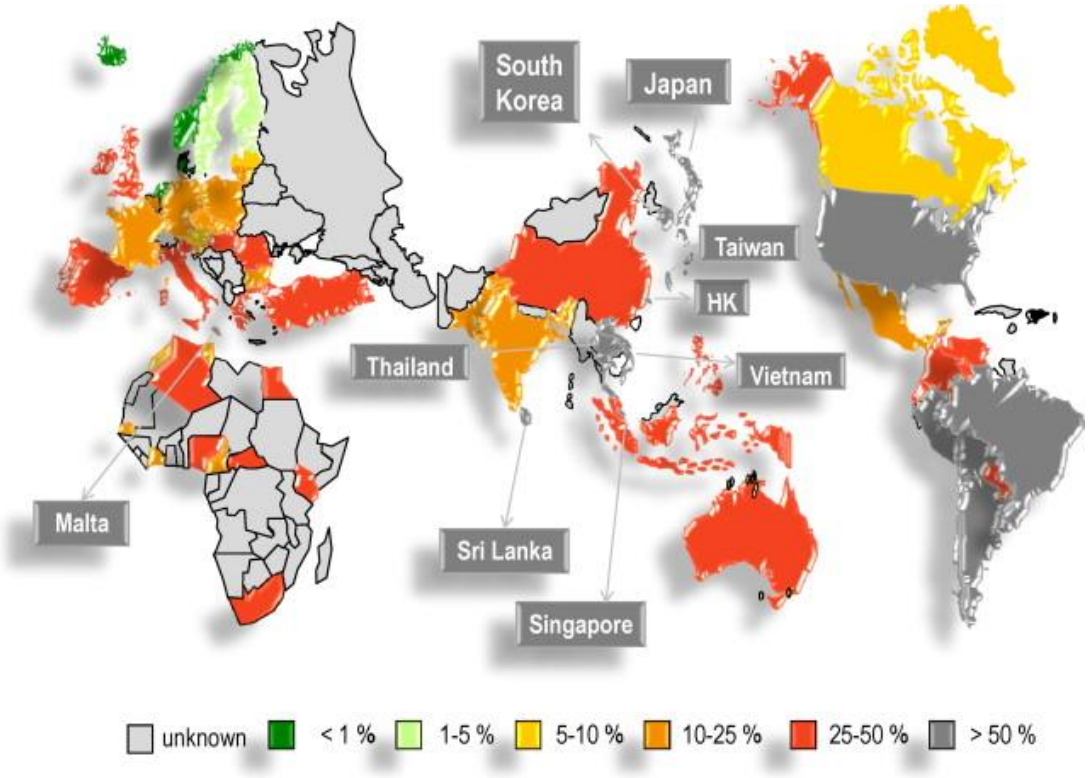


Figure 1.4: Worldwide prevalence of hospital-acquired methicillin-resistant *Staphylococcus aureus* (Stefani et al., 2012). Smaller countries/regions included in the study with very high rates of MRSA (>50%) are highlighted for clarity.

Whilst MRSA infections are an issue on a global scale, there is no single pandemic strain, rather a successive emergence of regionally predominant strain types with infections occurring in waves. Examples of such include livestock-associated ST93 in Australia, HA-MRSA clonal complex 30 (CC30) in North America and Europe and CA-MRSA USA300 in North America. On the other hand, a more diverse range of strain types can be seen across Asia and the Middle East (Turner et al., 2019). The prevalence of MRSA infections in lower developed regions has been harder to ascertain, however judging from the available information *S. aureus* has been identified as a serious pathogen. Populations in Europe and the USA where rates of MRSA infection increased rapidly between the 1990s and early 2000s have seen signs of a decrease and/or stagnation of new HA- and CAMRSA infections since 2005, especially among bloodstream and soft tissue infections. Despite this decrease 7

of the 29 European Union countries still report 25% or more of invasive *S. aureus* isolates as MRSA (Hassoun et al., 2017). The reasons for the serial rise and fall of specific strain types remains poorly understood, with evidence in the UK suggesting reduced levels of MRSA pre-dates the implementation of increased infection prevention and control measures (Wyllie et al., 2011). Further research in molecular epidemiology is required to improve the increasingly complex understanding of *S. aureus* population dynamics.

S. aureus also has the ability to colonise a variety of animal species including cattle, poultry, pigs and dogs. The most commonly reported infections are mastitis (inflammation of breast tissue) in dairy producing animals and 'bumblefoot' (bacterial infection and inflammatory reaction that occurs on the feet of birds) in chickens. Whilst livestock associated strains are typically different to those found on humans, many classes of antibiotics used for the treatment of livestock overlap with those used for humans. As such, resistance generated amongst animals has the potential to spread to humans through direct contact with animals and/or meat products in addition to transmission via the environment (Smith, 2015). Whilst colonisation alone comes without any harm, livestock-associated MRSA has been reported to cause severe infections in humans. A study of 1800 poultry in sub-Saharan Africa revealed a 13.7% prevalence of *S. aureus*, with 6.1% of isolates being MRSA (Nworie et al., 2017).

This is of particular importance in countries like Uganda, where agriculture forms a core economic sector employing upwards of 80% of the labour force. Producing 40% of the goods exported from the country, it is accountable for 22% of the GDP. Furthermore, local subsistence farming is also practised by 69% of the population (National Population Council, 2017). The frequency of this small-scale farming within the population in and around the

home provide ample opportunity for transmission between humans, animals and the local environment. Further compounding this are the lack of access to clean water, effective sanitation and proper waste management seen in multiple communities (Afema et al., 2016). Despite the implications, research and documentation of animal health is severely deficient in Uganda, with only 3 out of 16 veterinary diagnostic laboratories able to carry out antimicrobial susceptibility tests. The most commonly reported condition, bovine mastitis, indicates an alarming >50% pathogens are resistant to common antibiotics (Uganda National Academy of Sciences, 2015).

S aureus has a highly clonal population structure (Price et al., 2013); and whilst it has been shown that any genotype has the potential to become a life-threatening pathogen (Donker et al., 2009), the majority of disease-causing isolates belong to a small number of lineages or clonal complexes (Price et al., 2013). Most of the strains identified colonising humans belong to one of ten dominant lineages (Clonal complex 1, 5, 8, 15, 12, 25, 51, 22, 45 and 30) (Lindsay et al., 2006).

1.7 Utilising genomics to trace S. aureus populations

Molecular typing methods are vital for the surveillance and control of *S. aureus* populations, especially those with resistance in and around healthcare settings. With methicillin resistance arising due to the modified penicillin binding protein (PBP) products of the *mecA* gene found within the staphylococcal cassette chromosome mec (SCCmec), the first typing technique (SCCmec typing) classified SCCmec elements based on their structural differences. Advancing through several techniques including phage typing and pulsed-field gel electrophoresis, most current typing is in the form of multi-locus sequence typing (MLST)

and staphylococcal protein A (spa) typing. Spa typing, often chosen due to its ease of performance and cheaper procedure, analyses the number of and sequence variation in tandem repeats at the polymorphic x region of the spa gene. As such, each 24 bp sequence motif is assigned a unique code, with the repeat succession and precise sequences of the repeats determining the spa type (Asadollahi et al., 2018).

1.7.1 Staphylococcal Protein A (Spa) Typing

The primary binding site of Staphylococcal protein A (Spa) is the Fc region of mammalian immunoglobulins, where binding helps protect the bacteria from phagocytosis by host immune cells (Asadollahi et al., 2018). The *spa* gene is present in all strains of *S. aureus* and spa typing schemes have exploited the repeat structure of its X region, enabling both micro- and macro-variations to be tracked through single-locus based typing. As such, spa typing has been widely utilised in both local and global epidemiological studies (Hallin et al., 2009). Using a single-locus marker is also inherently less expensive, time-consuming, and error prone than multi-locus techniques (Koreen et al., 2004), with previous studies demonstrating a good correlation between the clonal groupings obtained by spa-typing and those obtained by other typing techniques (Satta et al., 2013).

The utilisation of typing techniques allows for the geographical analysis of strain variation and domination in populations from different regions. A recent global meta study by (Asadollahi et al., 2018) included 843 articles from 5 continents (Europe, Asia, America, Africa and Australia) and assessed the most prevalent spa types among clinical isolates of MRSA and susceptible *S. aureus* (MSSA). Spa type t032 was the most prevalent in Europe with its presence mostly found around the UK and Germany, it was also among the top 5

dominant spa types in Austria. However, it wasn't reported in any other European countries as one of the most frequent. The second most prominent spa type was t008 which was more widely distributed across 11 of the 22 European countries and being the most frequent in Italy and France. Similarly in Asia, whilst t030 was the most predominant spa type in China and Asia overall, t037 was the second most common, yet was recorded across a much wider distribution of countries (5/10). Equally, t037 was the most prevalent spa type in Africa. America exhibited common spa types closer to those of Europe with t008 and t002 being the most predominant respectively.

1.7.2 Multi Locus Sequence Typing (MLST)

Where spa typing addresses variations at the x region of the *spa* gene (Asadollahi et al., 2018), MLST scrutinises variations in typically 6-8 housekeeping genes. Roughly 500 bp fragments are sequenced from each locus, with each unique sequence designated a specific allele marker (Jolley et al., 2004). Through utilising alleles as a means of comparison, MLST is able to accommodate conflicting signals from both vertical and horizontal gene transfer. As each allelic change is counted as a single event, the data is not skewed by horizontal transfer events which are attributed to a wide range of polymorphisms in relation to the less common single point mutations (Maiden et al., 2013). The combination of these allele markers from across the different loci are collectively assigned a sequence type (ST), where each individual ST has a unique array of alleles attributed to it. STs can be further grouped in terms of clonal complexes (CC) where, in the case of *S aureus*, STs which share at least five of seven identical alleles are assigned to the same CC (Feil et al., 2003). This typing method was designed as a useful tool for global epidemiology and surveillance with the primary

advantage of creating highly reproducible data with robust portability. Since its' conception multiple databases have been generated which act as central repositories providing reference data sets through which evolutionary relationships can be examined (Jolley et al., 2004).

The variability of housekeeping genes amongst different bacteria means there is no single standard MLST technique that can be uniformly used as a comparison tool, rather different MLST schemes must be derived to compare those that are closely related. In some cases, more than one targeting scheme may be required for a single genus of bacteria. Additionally, due to the aforementioned use of alleles as markers, MLST can be weaker in discriminating amongst single-clone, low diversity, pathogens as well as more diverse pathogens of the same lineage. In such circumstances MLST is best utilised in conjunction with other specific typing techniques (Maiden et al., 2013).

In relation to *S. aureus*, the frequency of point mutations far outweighs the occurrence of recombination events, somewhat dampening the advantages of MLST (Harmsen et al., 2003). In such cases single locus typing techniques, for example spa typing, can provide a reliable and accurate means of discriminating between strain variations. This becomes more relevant in hospital settings where advanced sequencing facilities are less readily available, further combined with the increased necessity of tracing MRSA. With only a small number of clonal groupings of MRSA circulating worldwide, spa typing helps to offer a reliable means of rapid, high resolution and low-cost typing. With that in mind, various studies have examined the relationship between MLST sequence types and spa types. Whilst there is a good correlation between clonal groupings and the respective spa types, there is not always a direct association. Whilst isolates of the same spa type have been

found belonging to related MLST sequence types differing at only a single locus, there are also cases where a considerable difference in spa gene repeat number variation have all been assigned to the same sequence type, implying in some cases spa typing can provide higher resolution (Harmsen et al., 2003). That being said, MLST remains an effective tool for defining population structures, where at the time of writing 7059 unique STs have been described, with 4496 of them being attributed to a total of 97 CCs (Jolley et al., 2018). With *S aureus* able to colonise different environments, be it community, healthcare or livestock associated, being able to unambiguously track strains of interest is critical in identifying their origins and spread (Enright et al., 2000). For example, whilst in the past certain MRSA lineages have been limited almost exclusively to healthcare or community settings, MLST has been utilised to describe the transition of strains across different niches (Chen et al., 2018). Equally, strains local to specific geographical areas can be assessed as they expand into previously uninhabited regions (Frieder Schaumburg et al., 2011).

1.7.3 Whole genome sequencing

Due to advances in technology whole genome sequencing (WGS) has become a more accessible and affordable tool for bacterial typing (Chen et al., 2018). This is evidenced by the PubMLST database which indicates that at the time of writing over 26,000 *S aureus* genomes have been characterised utilising the technique (Jolley et al., 2018). WGS surpasses all previous typing techniques in relation to detail and resolution, allowing for the comparison of isolates across time and space down to a single nucleotide. The high precision of the technique means different lineages can be accurately described at a local level providing insight into their evolutionary history, estimated time scales of delineation

and highly localised outbreak recognition. Furthermore, interpretation in the context of databases such as PubMLST allows for the identification of newly emerging or expanding strains (Price et al., 2013). MLSTs can also be derived from WGS data providing a backwards compatible tool for the interpretation of such (Chen et al., 2018).

The characterisation of strains is essential for understanding the sources and spread of infections and AMR. *S. aureus* is generally viewed as an opportunistic pathogen and is known to have highly clonal populations. As such, it is possible that certain clones are more commonly associated with invasive disease than others due to the carriage of certain virulence factors, for example increasing the chance of successfully invading host tissues. Other factors contribute more directly to cell survival (Feil et al., 2003). Of growing interest are the phage-encoded Panton-Valentine Leukocidin (PVL) genes which, when associated with *S. aureus*, provide enhanced protection against the immune system through the lysis of leukocyte cells (Boyle-Vavra & Daum, 2007). Genomic differences can also be observed amongst resistance factors. In the case of *S. aureus*, SCCmec (which hosts the *mecA* gene) has seven main variations described ranging in sizes from 20.9 to 66.9 kb. For example, I, IV, V, VI and VII cause only β-lactam antibiotic resistance, whilst II and III cause resistance to multiple classes of antibiotics (Deurenberg & Stobberingh, 2008). Initial exploration into the genomics of *S. aureus* revealed the core and accessory components of the genome. However, with the advent of relatively rapid, inexpensive, high throughput sequencing large numbers of strains have been assessed providing insights into the emergence, global spread, epidemic clones, the source and transmission routes of *S. aureus*. Such data can be used to compare strains from a variety of niches including hospital outbreaks, community-associated and livestock associated strains (Fitzgerald & Holden, 2016).

1.8 Virulence Factors

Virulence factors encompass cellular components and regulatory systems which enable a pathogen to replicate and disseminate within a host more effectively. These factors utilise mechanisms including both evasion and inhibition of the host's immune response, promotion of movement towards and attachment to host cells, and the acquisition of nutrients from the host (Casadevall & Pirofski, 2009). Whilst virulence factors are attributed to the presence of particular genes, the overall virulence of a pathogen is determined by both the virulence factors present as well as their respective gene expression (Chua et al., 2014). In light of this it has been observed that virulence can both be attenuated or enhanced for a specific bacteria under different environmental / host conditions (Casadevall & Pirofski, 2009). Virulence factors in relation to *S. aureus* are of particular interest due to both its prevalence of infection and its dual nature of being a commensal and pathogenic organism. It possesses a wide repertoire of associated factors including both structural and secreted products which participate in the pathogenesis of infection (Plata et al., 2009).

S. aureus genomes have been studied in depth and are known to possess a highly conserved core genome alongside a variable accessory genome, with unique features affecting the phenotypic behaviour of the strain often found in the latter. The accessory genome houses multiple genetic elements that are or have once been mobile, transferring horizontally. These include insertion sequences, plasmids, transposons, integrated bacteriophages, and genomic or pathogenicity islands. Conversely, whilst being larger the core genome predominantly encompasses general housekeeping genes required for cell growth and maintenance. That being said, virulence factors have been isolated from both subsections of the genome (Lindsay & Holden, 2004). Whilst *S. aureus* houses significant

barriers to horizontal gene transfer through the action of restriction modification systems, multiple strains have been able to acquire mobile genetic elements. Evidence of these transfer events are typically found within the accessory genome where the potentially large-scale recombination of genetic material can lead not only to multiple sources of virulence but also resistance to a range of antibiotics (Cameron et al., 2011). These horizontal transfer events are more easily described through sequencing different strains; however, it is important not to overlook the impact of smaller mutations which can take place within the core genome. The increased *in vitro* expression of multiple molecules has been shown to have a significant impact on a strains' virulence (Li et al., 2009).

1.9 Structuring my Research

Initial research at FPRRH began on the maternity ward as part of a public health intervention focussing specifically with a view to reduce maternal mortality. The Ugandan healthcare system faces many complex challenges, with maternal healthcare being high on the list. Despite this it has shown resistance to change despite major international investments (Lozano et al., 2011). The Millennium Development Goals (MDG) report for Uganda (2015) stated that MDG 5 'improving maternal health' was 'not achieved' (United Nations, 2015). Maternal mortality can arise as the result of multiple issues, whilst the most direct causes were reported to be haemorrhage and sepsis (Mbonye et al., 2007). Despite sporadic data collection across lower income regions, a recent study based at Mbarara Regional Referral Hospital, Uganda, reached the same conclusion with puerperal sepsis contributing to 30.9% of maternal deaths followed by obstetric haemorrhage at 21.6% (Ngonzi et al., 2016). Whilst the figure for haemorrhage falls largely in line with other Sub-

Saharan African countries (Say et al., 2014), sepsis appears to be contributing to a much larger degree. Possible causes noted include the sporadic availability / use of antibiotics in addition to poor infection prevention control practice.

Sepsis is a condition which arises through a disproportionate immune response to an infection causing damage to the hosts' own tissues and organs, the result of which if left untreated can lead to multiple organ failure, shock and death. On a molecular level, this is the convergence of both host-derived and foreign molecules acting upon pathogen recognition receptors. The activation of these receptors results in the release of immune mediators that produce the clinical signs and symptoms of sepsis (Czura, 2011). One area susceptible to the onset of sepsis are surgical site infections (SSI), which also encompass a large number of maternal sepsis cases as a result of caesarean sections. As shown in Mulago hospital, where 314 patients with clinical SSI's were examined, 145 (46%) were from caesarean sections. Of the 304 isolates obtained from wound swabs, 64 (21%) were identified as *S. aureus*, second only to *Escherichia coli* with 72 (24%) isolates (Jeremiah Seni et al., 2013). Other similar studies recovered *S. aureus* at an even higher rate of 29% at Bugando Medical Centre Mwanza (BMC), Tanzania (Mawalla et al., 2011) and 45% at Jinja Regional Referral Hospital, Uganda (Anguzu & Olila, 2007).

After commencing the initial research, the microbiology laboratory at FPRRH underwent a large-scale re-development greatly improving their bacterial identification and antimicrobial susceptibility testing capabilities. Based on these updates I was able to expand my initial study to factor in all wards at the hospital, focusing specifically on *Staphylococcus aureus* found both clinically at the hospital and locally in the community (further described in results Chapters). Another collaboration developed with Makerere University in Kampala

allowed for the transport and sequencing of *S. aureus* isolates (results described in Chapters 3 & 4). Additionally, through links made at Makerere and IDI I was able to attend the national antimicrobial resistance task force meetings as they finalised and published the Ugandan NAP, allowing me to fine tune my research for maximum value.

1.9.1 Research Objectives

My research focused on the patterns of antibiotic resistance found within a collection of *Staphylococcus aureus* isolates from Fort Portal, a city located in the western Kabarole district of Uganda, and revolved around the following objectives:

1. *Evaluate the antimicrobial resistance profiles of S. aureus isolated from clinical and community sources in Fort Portal, Uganda*
2. *Assess the population diversity of S. aureus isolated from clinical cases of infection at Fort Portal Regional Referral Hospital*
3. *Investigate the key resistance-associated genes and mechanisms present through which the clinical S. aureus isolates convey antibiotic resistance.*

Chapter 2 - Methods

2.1 Study site

Fort Portal, roughly 300km west of Kampala, is home to Fort Portal Regional Referral Hospital (FPRRH), one of 8 regional referral hospitals in the country. According to the most recent estimates by the Ugandan Bureau of Statistics (UBOS, 2019a), Kabarole district (Fort Portal included) has a total population of around 344,500, where 75% of the population are aged 30 and below. Only 46.5% of households have access to running water with 76.3% of households living in semi-permanent dwellings (UBOS, 2019b).

FPRRH formed the focal point for this research where *S. aureus* strains were clinically isolated by hospital staff. Further isolates were obtained through swabbing healthcare workers at various health centres and local people within the community.

Ethical clearance was obtained from Makerere University College of Health Sciences, School of Biomedical Sciences Higher Degrees Research and Ethics Committee; File: SBS 584 (appendix 1) and the University of Salford Research, Innovation and Academic Engagement Ethical Approval Panel; application STR1617-104 (appendix 2).

2.2 Bacterial Isolates:

2.2.1 Isolation of clinical blood isolates during pilot study

This aspect of the study centred around FPRRH. Following a clinical diagnoses of sepsis, blood (2ml) was collected from the patient as part of routine practices, and added to (8ml) brain heart infusion (BHI) broth (LabM), at which point the sample was immediately

transported to the hospital laboratory for the lab technician to process. The pilot study was performed before installation of the BACTEC blood culture machine at FPRRH). Technicians detected blood infection by incubating the sample (37°C, 5% CO_2) for up to 5 days, visually checking for the presence of bacteria every 24 hours through assessing the turbidity of the media. Should a sample exhibit bacterial growth, 100µl of culture media was spread onto blood agar plates (Columbia agar base supplemented with 5% blood (TCS), LabM) and incubated for up to 48 hours. Isolates were further sub-cultured onto MacConkey Agar and Mannitol Salt Agar plates and incubated for up to 48 hours (37°C, 5% CO_2) to aid in bacterial identification.

For wound samples a sterile cotton swab was used to gently swab the area of the wound. The swab was transported to the laboratory where it was streaked on to blood agar plates. The plates were incubated (37°C, 5% CO_2) for up to 48 hours and assessed for any growth. Colonies were further sub-cultured onto MacConkey and Mannitol Salt Agar plates and incubated for up to 48 hours (37°C, 5% CO_2) to aid in bacterial identification.

2.2.2 Bacterial isolation from hand swabs

Cotton swabs were moistened with PBS solution and swabbed across the candidates' hands and between their fingers. Swabs were used to inoculate three different agar plates; nutrient agar, Mannitol Salt Agar and MacConkey agar. These plates were incubated for up to 48 hours (37°C, 5% CO_2). Colony colour and morphologies were recorded and the isolates were further assessed by Gram's staining.

2.2.3 Isolates from healthcare workers

S. aureus isolates were obtained from the hand swabbing of healthcare workers at FPRRH and two local healthcare centres (Kagote and Bukuuku) in the surrounding Kabarole district area to determine the carriage level of *S. aureus* by healthcare workers and the level of AMR in circulation.

2.2.4 Community Isolates

To gain an insight into the level of *S. aureus* carriage and AMR in the community, swab samples were taken from the hands of participants from villages in the Kabarole district. Participants were primarily approached as part of the mobile blood collection activities for the Ugandan Blood Transfusion Service, who sourced participants primarily through local schools and universities. Swab samples were collected during 9 blood donation drives with UBTS and 2 local community events.

2.2.5 Bacterial isolation and identification (main study)

S. aureus isolates were routinely collected and phenotypically characterised by the FPRRH microbiology laboratory between 2017 and 2019, utilising the BACTEC blood culture machine where appropriate. Samples were obtained from patients with signs of infection across all major wards: obstetrics and gynaecology, surgical, general male and female wards. These samples were primarily derived from patients with infected skin wounds or suspected urinary tract or bloodstream infections.

When swabs were taken, they were transported to the laboratory and used to streak on blood agar plates. The plates were incubated (37°C, 5% CO_2) for up to 48 hours and assessed for any growth. Colonies were further sub-cultured onto MacConkey and chocolate agar (Oxoid) plates and incubated for up to 48 hours (37°C, 5% CO_2) to aid in bacterial identification.

When blood samples were taken, blood (2ml) was added to a ready-made BD BACTEC standard aerobic media bottle and incubated (37°C, 5% CO_2) for up to 48 hours. Detection of bacteria growth was automated through monitoring the turbidity of the solution. Where growth was detected within 48 hours, bacteria was cultured on blood agar plates (37°C, 5% CO_2) for up to 48 hours and further sub-cultured onto MacConkey and chocolate agar (Oxoid) plates and incubated for up to 48 hours (37°C, 5% CO_2) to aid in bacterial identification.

Putative *S. aureus* isolates were confirmed by Gram staining followed by a positive catalase and Staphaurex latex agglutination test (Thermo Fisher Scientific). All isolates were stored at -80°C.

2.3 Gram staining

A bacterial colony was homogenised with a drop of water on a standard microscope slide, which was allowed to dry and heat fixed over a Bunsen flame. The heat-fixed smear was flooded with crystal violet for 1 minute, followed by a gentle rinse with tap water, flooded with Gram's iodine for 1 minute, followed by a gentle rinse with tap water, washed with 95% ethyl alcohol immediately followed by a gentle rinse with tap water, flooded with

safranin and left to stand for 1 minute, followed by a gentle rinse with tap water. The slide was allowed to dry completely and viewed using a light-microscope (x100 objective) under oil-immersion.

2.4 Catalase test

A drop of 3% H_2O_2 and a fresh bacterial colony were mixed on a glass slide. A positive result was indicated by the production of bubbles of oxygen.

2.5 Staphaurex agglutination test

The test reagent was added onto the reaction card followed by the addition of bacterial culture. The mixture was emulsified and the reaction card gently rotated for up to 20 seconds. Positive samples showed signs of agglutination.

2.6 Antibiotic Susceptibility Testing

For each isolate, a single colony was isolated and sub-cultured, prior to screening by a disk diffusion assay according to EUCAST guidelines (EUCAST, 2021). Susceptibility to eight different antibiotics belonging to different classes were tested to ensure a wide range of resistance and putative resistance traits could be screened for. These were: cefoxitin (30µg), a second-generation cephalosporin used to indicate MRSA; ciprofloxacin (5µg), a fluoroquinolone; cotrimoxazole (sulfamethoxazole - trimethoprim (1.2µg and 23.75µg

respectively)); tetracycline (30µg); erythromycin (15µg), a macrolide, clindamycin (2µg), a lincosamide; gentamycin (10µg), an aminoglycoside; and chloramphenicol (30µg) (Oxoid).

Briefly, fresh bacterial material was thoroughly suspended in 5 ml sterile phosphate buffered saline (PBS, Oxoid) to a density of McFarlands Standard 0.5. A sterile cotton swab was dipped into the suspension, and excess liquid removed by pressing against the inside of the rim. A Mueller Hinton Agar plate (Oxoid) was then inoculated by thoroughly spreading the swab across the entire area of the surface in three directions to achieve confluence. Antibiotic disks were then placed aseptically on the inoculated plate using sterile forceps. Plates were inverted and incubated at 37 ^{o}C for 18-20 h. Zones of inhibition were measured in mm and compared to EUCAST clinical breakpoint tables to determine whether the isolate was susceptible, resistant or intermediate to each antibiotic.

2.7 Crude DNA extraction during pilot study

Crude extraction of DNA for PCR screening was carried out using basic boil preps. In each case, a bacterial colony was suspended in distilled water (50 µl) and incubated at 95 °C for 15 min, followed by a cooling period of 15 minutes at 10°C.

2.8 DNA Extraction and Quality Control

Bacterial cultures were grown in Luria Bertani (LB) broth for 18-24h at 37 °C and DNA was extracted using the Isolate II Genomic DNA Kit (Bioline, UK) according to the manufacturer's instructions, including the extra lysozyme pre-treatment step for efficient lysis of Gram-positive cells. DNA concentration and purity were measured using the Qubit

3.0 Fluorometer (Thermo Fisher Scientific) with Quant-iT dsDNA Assay Kit, High Sensitivity (Invitrogen). DNA library quality control was performed using a Tapestation 2200 with a High Sensitivity D1000 kit (Agilent Technologies) according to the manufacturer's instructions.

2.9 Spa typing

Polymerase Chain Reaction (PCR) was facilitated utilising the primers 1095F and 1517R detailed below (Votintseva et al., 2014) with an expected product size of 422bp. Reactions totalled 20 µl and consisted of (6 µl) ddH2O, (10 µl) 1xPCR MyTaq master mix (Bioline), (1 µl) 0.25 µM of each primer (Eurofins genomics) and 2 µl boil prep template. PCR cycling began with a melting step at 94°C for 2 min; followed by 35 amplification cycles each involving 94°C melting for 30s, 50°C annealing for 30s, and 72°C extension for 60s; and a final extension at 72°C for 5 min. *S. aureus* strain ATCC 25923 was used as a positive control.

1095F 5'-AGACGATCCTTCGGTGAGC-3'

1517R 5'-GCTTTTGCAATGTCATTTACTG-3'

2.10 16S rRNA gene PCR

PCR reactions totalled 20 µl and consisted of (6 µl) ddH2O, (10 µl) 1xPCR MyTaq master mix (Bioline), (1 µl) 10 pmol of each primer 806R and 496F (Caporaso et al., 2011) and 2 µl boil prep template. PCR conditions were 94°C for 2 min; 35 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 60s; and a final extension at 72°C for 5 min.

515F 5’-GTGCCAGCMGCCGCGGTAA-3’

806R 5’-GGACTACHVGGGTWTCTAAT-3’

2.11 Gel electrophoresis

5µl of product was run on a 1% agarose gel (Bioline) for 60 minutes at 110V with a molecular weight marker HyperLadderTM 1kbp (Bioline). The bands were visualised on a transilluminator to assess the size of each amplicon.

2.12 Whole Genome Sequencing, Draft Genome Assembly and Analysis

Isolates were selected for sequencing based on DNA quality: All pure, confirmed *S. aureus* isolates collected within the timeframe with appropriate DNA quality are included in this analysis. Whole genome DNA libraries were prepared using the Nextera XT library preparation kit according to the manufacturer’s instructions. Samples were multiplexed on a single MiSeq flowcell using Nextera XT barcodes and normalised using the bead-based normalisation kit (Illumina). Sequencing of 41 pooled genomes was performed simultaneously at Makerere University, Uganda and the University of Salford, UK, as part of a training activity to support UNAP AMR initiatives. In both cases, DNA sequencing was performed using a MiSeq v2 500 cycle kit (2x250bp reads; Illumina). Raw reads were trimmed using fastq-mcf and assembled using SPADES v3.9.1 (Bankevich et al., 2012). The resulting draft assemblies were annotated using PROKKA v 1.13.3 (Seemann, 2014).

The online webtool Pathogenwatch (https://pathogen.watch) was used to cluster the annotated genomes of each isolate to predict antibiotic resistance profiles. Pathogenwatch uses PAARSNP, bespoke software that uses BLAST against a custom database (combined from public AMR databases such as CARD, ResFinder and NCBI) to highlight the presence or absence of genes and SNPs implicated in antimicrobial resistance and infers their combined effect. Chloramphenicol resistance was predicted by CARD (*Staphylococcus intermedius* chloramphenicol acetyltransferase) analysis (Alcock et al., 2019) implemented in ABRICATE (https://github.com/tseemann/abricate). Virulence gene presence was also predicted using ABRICATE, against the VFDB database (Liu et al., 2019). Multi-locus sequence types (MLST) were confirmed against the pubMLST database (Jolley et al., 2018) using the software "MLST" (https://github.com/tseemann/mlst). Core genome alignment was ascertained by SNIPPY (Seemann, 2015), and a phylogenetic tree generated by FastTree using a GTR+CAT model of nucleotide evolution (Price et al., 2009). Additional genomic analyses were performed using ABRICATE and ROARY (Page et al., 2015).

Univariant statistical analysis of antimicrobial resistance and virulence gene presence/absence was performed in Minitab.

Chapter 3 - Population structure of *Staphylococcus aureus* isolates from FPRRH and distribution of virulence factor-encoding genes among them

3.1 Introduction

S. aureus is not only found as a commensal organism, but is also responsible for a diverse range of human infections from basic soft skin and tissue infections to life-threatening invasive diseases. As such it has become one of the most prevalent pathogens in both hospital- and community-associated disease (Thomer et al., 2016). A variety of molecular typing techniques form the basis of epidemiological studies investigating the clonal relatedness, genetic diversity, evolutionary pathways and spread of *S. aureus* infections, for example the use of multi-locus sequence typing (MLST). Despite being such a prevalent pathogen, different strains are often restricted to a specific geographical area, whilst others have more of a pandemic spread (Goudarzi et al., 2017).

In addition to a range of antimicrobial capabilities, treatment of *S. aureus* infection is further inhibited through its ability to produce a wide variety of virulence factors including specific enterotoxins, proteases, leukocidins and proteins associated with immune-evasion or adhesion (Ilczyszyn et al., 2016). Fortunately, with advances in next generation sequencing technologies many novel virulence genes have been elucidated providing clues about how these factors influence disease thus enhancing our understanding of disease mechanisms.

3.1.1 *S. aureus* MLST types and Clonal Complexes

MLST remains a useful tool for studying large scale population structures (Huygens et al., 2006). In respect to *S. aureus*, sequence types (STs) are assigned according to seven loci, with STs sharing at least five of the seven loci grouped into the same clonal complex (CC) (Feil et al., 2003). Currently, 7059 unique STs have been identified, with 4496 of them being attributed to a total of 97 CCs (Jolley et al., 2018). That being said, the majority of strains identified colonising humans belong to one of ten dominant lineages (Clonal complex 1, 5, 8, 15, 12, 25, 51, 22, 45 and 30) (Lindsay et al., 2006); these complexes are found at varying levels depending on the geographical area of interest. For example, in Europe, strains analysed tended to fall under six major complexes (CC5, CC22, CC8, CC30, CC45 and CC15) (Aanensen et al., 2016), whilst in Africa CC5, CC15 and CC30 are observed more frequently – albeit from a much smaller number of available studies. More recent research has shown CC152 and CC121 to be much more prevalent in Africa, these differences can be observed in Figure 3.1 (Ruffing et al., 2017). Figure 3.1 also highlights at best weak associations between complexes associated with invasive disease and those found as a commensal organism. Previous studies have also observed some contradictions (Rasmussen et al., 2013). Conversely, correlations have been observed between clones which are typically MRSA (CC22, CC5, CC8) and those which are MSSA (CC45 and CC30), although this can vary by geographical region where MRSA variants tend to show geographic clustering whilst MSSA isolates do not (Aanensen et al., 2016).

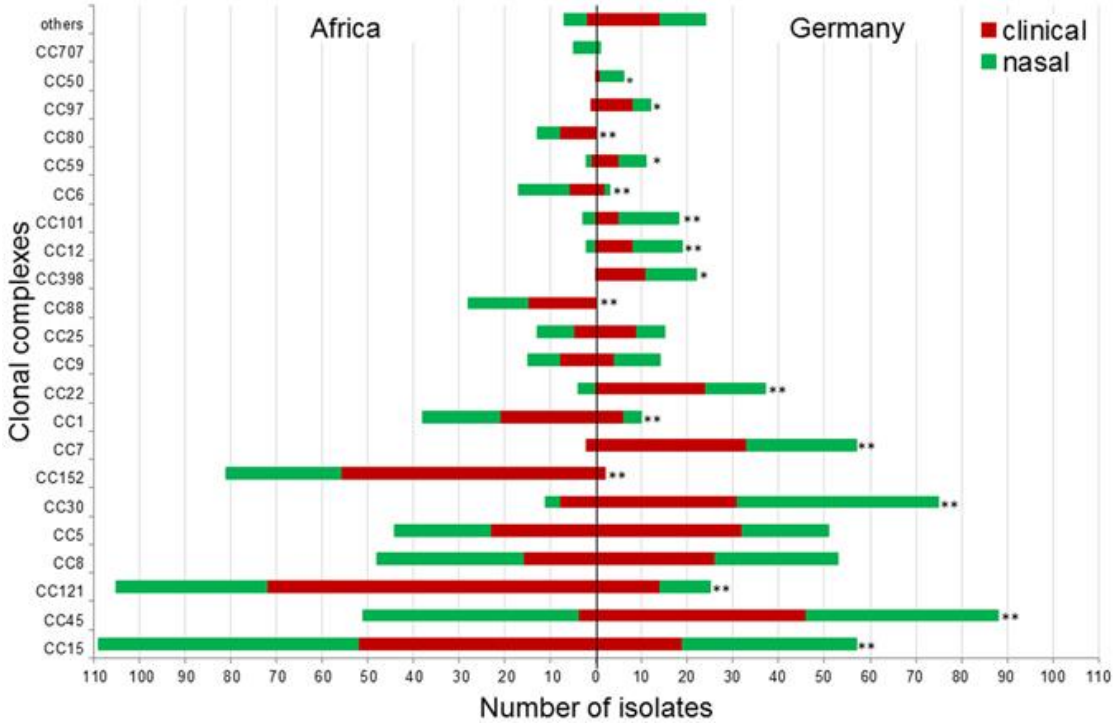


Figure 3.1: Distribution of the 22 most prevalent clonal complexes (CC) in Africa and Germany among isolates from colonization and infection. CCs of low prevalence (<6 isolates) where grouped together (others). The CCs were sorted in ascending order according to the total number of isolates in the respective CC. The proportions of clinical (red) and nasal (green) isolates in the African and German group are shown.

3.1.2 *S. aureus* associated virulence factors

A wide variety of virulence factors have been uncovered in *S. aureus* including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), toxic shock syndrome toxin-1 (TST-1), panton-valentine leukocidin (PVL), staphylococcal enterotoxins (SEs), staphylokinase, hemolysin (alpha, beta, gamma and delta), capsular polysaccharides, lipase and exfoliative toxins (*eta, etb*) in addition to several types of cell wall-associated adhesive molecules (*fnb*, encoding fibronectin binding protein; *cna*, encoding collagen binding protein; *clf*, encoding clumping factor; *ebp*, elastin-binding protein) (Goudarzi et al., 2017). As part of this research thirteen virulence factors were

observed to positively or negatively associate with the prevalent CC152 isolates, these are described in detail below.

3.2.2.1 Host Cell Adhesion

The adherence of *S. aureus* to host tissue is an important stage in the initiation of pathogenesis. A number of different adhesins have been described which help facilitate this process in different ways. Extracellular adherence protein (Eap), also known as MHC analogous protein (Map), has a broad binding specificity with an affinity for extracellular matrix and plasma proteins including fibronectin and fibrinogen. Additionally, Eap has been shown to bind ICAM-1 on epithelial and endothelial cells. These interactions inhibit leukocyte adhesion as well as neutrophil recruitment as a means of immunosuppression (Harraghy et al., 2003).

Another adhesion related molecule, SdrC, promotes bacterial adherence to surfaces as well as biofilm formation. Biofilms provide a useful protective structure for bacteria, capable of forming on biotic and abiotic surfaces. *S. aureus* biofilm formation is of particular concern as it has been prevalent on medical equipment leading to nosocomial infections. Biofilms themselves allow the free flow of nutrients, water and metabolites allowing the microbes within to exist as reservoirs in chronic infections and to better tolerate stresses such as antibiotics (Barbu et al., 2014).

3.2.2.2 Evasion of host immune system

Bloodstream dissemination forms a crucial part of *S. aureus* related systemic diseases such as sepsis. Facilitation of this requires defence against bloodstream elements

of the immune system, achieved in one approach through the formation and dispersion of blood clots. *S. aureus* utilises two key coagulases in this manner, staphylocoagulase (Coa) and von Willebrand factor-binding protein (vWbp). These coagulases are able to bind to host prothrombin to produce and enzymatically active staphylothrombin, capable of proteolytically converting host fibrinogen into a fibrin meshwork. Not only does the fibrin network protect *S. aureus* from phagocytosis, it also contributes to abscess formation. Furthermore, staphylothrombin is immune to the action of normal host thrombin feedback inhibition and can avoid the activation of inflammatory factors (Thammavongsa et al., 2015).

Chemotaxis Inhibitory Protein of *S. aureus* (CHIPS) further aids the bacteria in evading the hosts immune response. Once a host has detected signs of a bacterial infection the immediate reaction is to divert leukocytes in circulation around the body towards the sites of infection. Numerous chemo-attractants are utilised to facilitate this process. When secreted by *S. aureus*, CHIPS is able to inhibit host protein C5a and formyl peptide 1 receptors, hence blocking C5a-mediated neutrophil activation and N-formyl-peptide chemo-attractant associated chemotaxis (de Haas et al., 2004).

3.2.2.3 Host Cell Damage

S. aureus is known to produce a number of toxins providing a means for evading a hosts' innate immune response. One facet of these are cytotoxins, capable of lysing mammalian cells; those which target leukocytes specifically are known as leukotoxins, the most notorious being Panton-Valentine leukocidin (PVL). PVL became a prominent feature of investigations due to its role in the pathogenesis of community-associated methicillin

resistant *S. aureus* (CA-MRSA) and its clinical association with severe disease. Whilst its virulence acts independently of methicillin susceptibility, a high association with CA-MRSA despite its low presence in isolates overall indicates a close genetic relationship with SCCmec. PVL itself is a bicomponent cytotoxin that belongs to the staphylococcal synergohymenotropic toxin family. It is encoded by two contiguous and cotranscribed genes, *lukS-PV* and *lukF-PV*, located on the staphylococcus genome and carried on lysogenised bacteriophages (Chua et al., 2014). Whilst its role in virulence was initially subject to numerous contradictory results, Löffler *et al.* (2010) later clarified its potential for rapid *in vitro* activation and apoptosis of human and rabbit neutrophils.

Production of PVL toxins requires the presence and expression of both *lukS-PV* and *lukF-PV*, which are considered the most frequently transferred mobile toxin genes amongst CA-MRSA. The respective protein subunits are secreted as water soluble monomers and act synergistically at the host cells' membrane to formulate transmembrane pore-forming proteins in a multistep process (Nawrotek et al., 2018). Whilst the PVL toxins are the most frequently referenced, other leukotoxins can act in a similar manner and share a high sequence similarity, for example LukD and LukE (Morinaga et al., 2003).

Another family of secreted toxins are pyrogenic toxin superantigens which include toxic shock syndrome toxin-1 (TSST-1) and staphylococci enterotoxin (SE) serotypes. Similar to PVL, most genes associated with SEs are located on mobile genetic elements such as plasmids, bacteriophages and pathogenicity islands. Over 20 different SEs have been identified, most commonly being associated with food poisoning. SEC on the other hand has been identified from both humans and animal isolates, being implicated in a variety of diseases in domestic animals. SEs are able to bind non-specifically to host T-cells inducing

proliferation and the mass release of host cytokines associated with acute inflammatory responses causing severe symptoms including hypotension, shock and multi-organ failure (Pinchuk et al., 2010).

3.2.2.4 *Staphylococcus aureus* Type VII Secretion System

The *S. aureus* type VII secretion system (T7SS), also known as the ESAT-6 secretion system (ESS), is responsible for exporting multiple proteins associated with bacterial virulence. Whilst different strains express T7SS heterogeneity, those most extensively studied exhibit four core membrane proteins (EsaA, EssA, EssB and EssC), two cytosolic proteins (EsaB and EsaG) and five secreted substrates (EsxA, EsxB, EsxC, EsxD and EsaD). Collectively these proteins are crucial in determining virulence, where *S. aureus* mutants lacking either all or select components are consistently seen to be less virulent (Kengmo Tchoupa et al., 2020). Whilst the precise mechanisms underlying this action are poorly described, two of the more researched components EsaD (EssD) and EsaG (EssI) have a speculative relationship. The secreted substrate EsaD is a nuclease toxin capable of cleaving DNA but not RNA. Essential to its biogenesis, EsaG acts as an antitoxin to nullify EsaD nuclease activity inside the staphylococcal cytoplasm (Ohr et al., 2017). Furthermore, the presence of multiple homologues of EsaG in strain variants which do not possess EsaD implies a protective role against intraspecies competition (Cao et al., 2016).

3.7 Aims and Objectives

This chapter explores a range of molecular typing techniques to investigate the strains of *S. aureus* circulating in Fort Portal, Uganda. Key objectives were to determine whether there are any dominant clones, and whether they reflect the same trends as observed in other parts of Uganda.

The initial objective was to investigate bacterial causes of maternal sepsis, for which a pilot study was facilitated to investigate the most prevalent bacteria and to compare the resistance profiles of isolates from clinically acquired blood samples and the hands of healthcare workers. Considerable challenges in reliably obtaining blood samples from the maternity ward at FPRRH drove a shift in focus to any clinical *S. aureus* isolates from across the hospital.

3.8 Pilot Study: Highlighting the challenges of reliable surveillance in low resource settings

The core aims of the initial pilot study were to first assess the bacteria associated with causing maternal sepsis at Fort Portal Regional Referral Hospital (FPRRH), Uganda; and secondly assess any relationships between the clinical *S. aureus* isolates and those isolated from the hands of healthcare workers. This involved assessing their antimicrobial activity through sensitivity testing (see Chapter 4) and population structure through spa typing. Plans to include community associated *S. aureus* were later developed as part of the main study, whilst plans for livestock associated strains had to be abandoned due to time constraints.

3.8.1: Isolates obtained at FPRRH

Samples of blood, urine or wound swabs were collected as part of routine procedures by ward staff from a total of 13 patients diagnosed with sepsis at FPRRH. A total of 17 bacterial isolates were obtained from the samples by culture on selective media (Table 3.2). It is worth noting this work was conducted prior to the installation of the automated BACTEC blood culture machine and hence involved the use of manually assembled culture medium/bottles incubated at 37°C for up to 5 days before isolating on solid media.

The large majority of samples collected were in the form of a blood sample (n = 11), with urine samples (n= 1) and wound swabs (n=2) rarely being collected. Each bacterial isolate was given a presumptive identity through the use of selective media (MacConkey and mannitol salt agar) in addition to Gram staining (Table 3.1).

Table 3.1: The presumptive bacterial species isolated and their respective identifiable characteristics

Presumptive Bacteria	Identifiable Characteristics
E. coli	Red colony on MAC, gram –ve, rod shaped
S. aureus	Pink-orange colony on MAC, Yellow colony on MSA, gram +ve, round clusters
S. epidermidis	White colony on MSA, gram +ve, round clusters
Enterococci	Pink-deep red colony on MAC, gram +ve, round short chains
Proteus spp.	Yellow colony on MAC, gram –ve, rod shaped

The most prevalent bacterial species identified was *Staphylococcus aureus* (7 isolates) followed by *E. coli* (4 isolates). Three patients had suspected co-infections as two species of bacteria were isolated from their samples (Table 3.2).

Table 3.2: Clinical sample acquired from each patient and the respective presumptive bacteria isolated. Collectively there were seven *S. aureus*, four *E. coli*, three *S. epidermidis*, two *Proteus spp*. and one *Enterococci* presumptively identified from a total of eleven blood samples, one urine sample and two puss samples.

Patient	Sample	Presumptive Bacteria isolated
C1	Abscess (puss)	*E. coli*
C2	Blood	*S. aureus*
C3	Blood	*S. epidermidis* *Proteus spp.*
C4	Blood	*S. aureus*
C5	Blood Urine	*E. coli* *E. coli*
C6	Blood	*S. epidermidis*
C7	Blood	*S. aureus* *Enterococci*
C8	Blood	*S. aureus*
C9	Blood	*Proteus spp.* *S. aureus*
C10	Blood	*S. aureus*
C11	Blood	*E. coli*
C12	Blood	*S. epidermidis*
C13	Wound (puss)	*S. aureus*

Poor infection prevention control (IPC) presents a major challenge in healthcare settings, particularly in poor-resource settings, presenting an increased threat of healthcare acquired transmission of infection. Poor handwashing facilities and rapid turnover of staff means that infection can spread via healthcare workers (Louise Ackers et al., 2020). As such, the pilot study included an investigation of *S. aureus* from healthcare workers hands since this was the most commonly isolated species from blood samples and is a common inhabitant of healthy skin. Healthcare workers hands were swabbed as part of a hand-hygiene training workshop. A total of 21 healthcare worker-associated *S. aureus* isolates were collected (see Methods) and their spa types compared to those of clinical isolates.

3.8.2: Spa typing trials

Molecular typing was facilitated for all isolates gathered in the pilot study utilising boil preps that were prepared in Fort Portal and transported to Salford, UK (see Methods). With a presumptive identification of *S. aureus*, spa typing was first performed as a means of secondary identification. The PCR amplified the *spa* target from the positive control (390 bp) and only three boil preps (450 bp) from the pilot study (Figure 3.2).

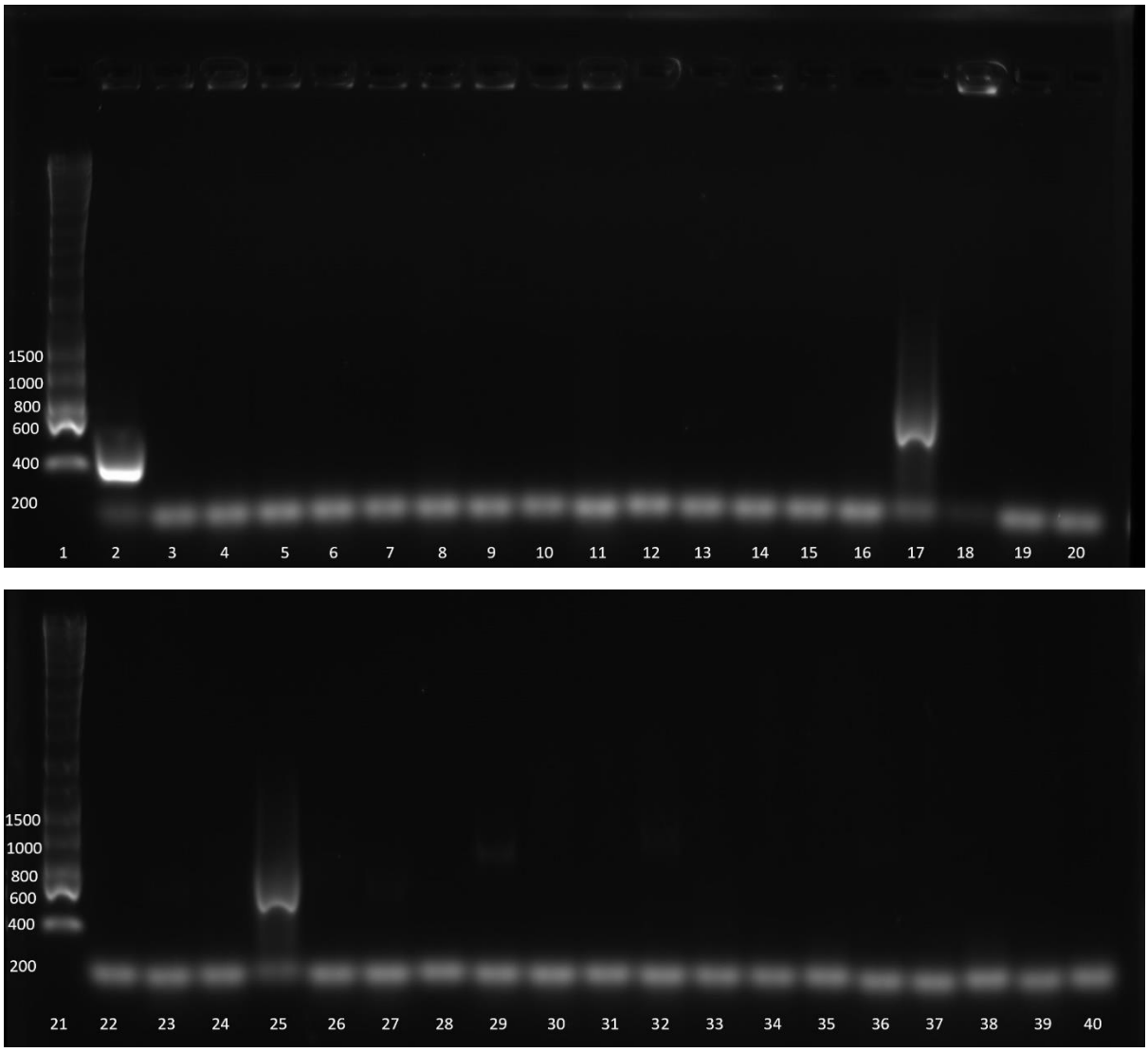


Figure 3.2: Spa typing PCR products of *S. aureus* isolates from clinical samples (lanes 3-9) and Healthcare workers hands (lanes 10-20 and 22-40). Products were separated on a 1% agarose gel at 100 v. Molecular weight marker hyper-ladder 1kb was used to estimate size (lanes 1 and 21) and a boil prep from a fresh colony of *S. aureus* strain ATCC 25923 was used

as a positive control (lane 2). Lanes 17 and 25 provided a strong positive result, with a very faint band observed at the expected position on lane 27.

3.8.3: Identification by 16S rRNA gene sequencing

An alternative explanation for the low success rate of positive *spa* amplicons was the possibility of incorrect species identification. If the isolates were not *S. aureus,* this would explain the negative result. To investigate this, amplification and sequencing of the 16S rRNA gene was performed in attempt to more accurately identify the original bacterial isolates used to prepare the boil prep templates. The same boil prep samples were used as DNA templates for 16S rRNA gene PCR as were used for spa typing. The outcome of 16S PCR was much more successful than with the spa typing primers (Figure 3.3). An observable product was amplified from all but two of the samples. This implied that viable DNA was present in the samples. As such it may be that some of the bacteria were given an incorrect identification as *S. aureus*, or the spa typing primers used are not effective enough for the quality of DNA present.

In order to determine species identity, the 16S PCR products from the 17 clinical isolates obtained during the pilot study were sequenced on a 272 base pair alignment of all the unambiguous sequences. Whilst the delineation of species was not possible from such a short stretch of 16S rDNA, a clear variance from the preliminary identification was observed (Table 3.3). Of the samples sent for sequencing, 76% (13/17) were identified to genus level. Of these, only 38% (5/13) were identified as belonging to the genus *Staphylococci.* Others were identified as belonging to the *Enterococcus* (n=1)*, Micrococcus* (n=1), *Bacillus* (n=1), *Brevibacillus* (n=2) *Leucobacter* (n=1), *Kocuria* (n=1) and *Arthobacter* (n=1) genus. For the healthcare worker isolates (Table 3.4), all of which were given a preliminary identification of

S. aureus, identities were more consistent with 95% of samples that were successfully amplified (n=16) identifying as *Staphylococcus* species (15/16). Just one sample was identified as a *Brevibacillus* species indicating possible contamination. 5 isolates out of the total 21 collected from healthcare workers hands were not identifiable. Of note the three *spa* positive isolates were identified as *Staphylococcus spp.* through 16S sequencing.

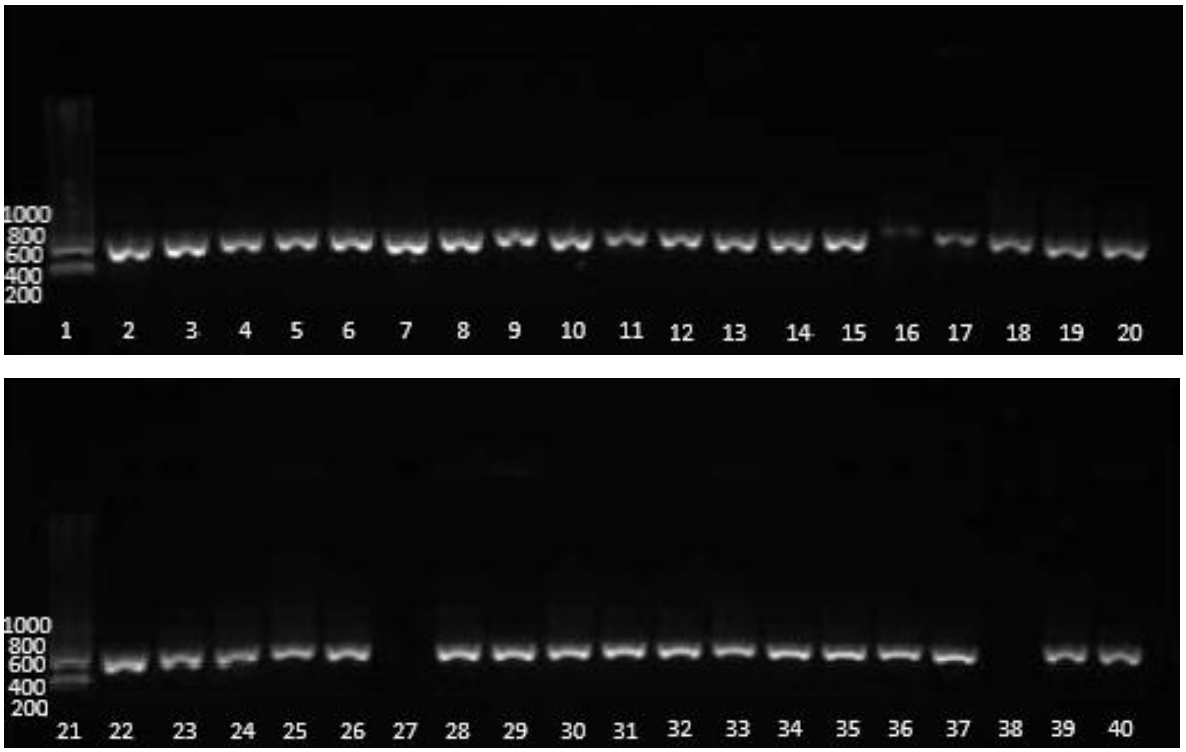


Figure 3.3: The 16S PCR products of *S. aureus* isolates from clinical samples (lanes 3-9) and Healthcare workers hands (lanes 10-20 and 22-40). Products were separated on a 1% agarose gel at 100 v. Molecular weight marker hyperladder 1kb was used to estimate size (lanes 1 and 21) and a boil prep from a fresh colony of *S. aureus* strain ATCC 25923 was used as a positive control (lane 2). Positive results can be seen in all lanes except 27 and 38.

Table 3.3: 16S rDNA gene Identification of isolates from cases of maternal sepsis in the pilot study. A total of eight different species were identified (*Staphylococcus*, *Leucobacter*, *Brevibacillus*, *Micrococcus*, *Enterococcus*, *Kocuria*, *Bacillus* and *Arthobacter*) with all *S. aureus* isolates correctly identified where data was available.

Isolate	Presumptive Identification	16S rDNA sequence identity
C1	*E. coli*	-
C2	*S. aureus*	*Staphylococcus spp.*
C3a	*S. epidermidis*	*Leucobacter spp.*
C3b	*Proteus spp.*	*Brevibacillus spp.*
C4	*S. aureus*	-
C5a	*E. coli*	*Brevibacillus spp.*
C5b	*E. coli*	-
C6	*S. epidermidis*	*Micrococcus spp.*
C7a	*S. aureus*	-
C7b	*Enterococci*	*Enterococcus spp.*
C8	*S. aureus*	*Staphylococcus spp.*
C9a	*Proteus spp.*	*Kocuria spp.*
C9b	*S. aureus*	*Staphylococcus spp.*
C10	*S. aureus*	*Staphylococcus spp.*
C11	*E. coli*	*Bacillus spp.*
C12	*S. epidermidis*	*Arthobacter spp.*
C13	*S. aureus*	*Staphylococcus spp.*

Table 3.4: 16S rDNA gene identification of isolates from healthcare workers hands in the pilot study. Where data was available all *S. aureus* isolates were correctly identified except for a single isolate representing a *Brevibacillus spp*. (HCW3).

Isolate	Presumptive Identification	16S rDNA sequence identity
HCW1	*S. aureus*	-
HCW2	*S. aureus*	*Staphylococcus spp.*
HCW3	*S. aureus*	*Brevibacillus spp.*
HCW4	*S. aureus*	*Staphylococcus spp.*
HCW5	*S. aureus*	*Staphylococcus spp.*
HCW6	*S. aureus*	*Staphylococcus spp.*
HCW7	*S. aureus*	*Staphylococcus spp.*
HCW8	*S. aureus*	-
HCW9	*S. aureus*	*Staphylococcus spp.*
HCW10	*S. aureus*	-
HCW11	*S. aureus*	*Staphylococcus spp.*
HCW12	*S. aureus*	*Staphylococcus spp.*
HCW13	*S. aureus*	*Staphylococcus spp.*
HCW14	*S. aureus*	*Staphylococcus spp.*
HCW15	*S. aureus*	*Staphylococcus spp.*
HCW16	*S. aureus*	-
HCW17	*S. aureus*	*Staphylococcus spp.*
HCW18	*S. aureus*	*Staphylococcus spp.*
HCW19	*S. aureus*	-
HCW20	*S. aureus*	*Staphylococcus spp.*
HCW21	*S. aureus*	*Staphylococcus spp.*

3.9: Dominance of Common Sequence Types in Clinical isolates of *S. aureus* from FPRRH

Following the initial pilot study that was limited to the maternity ward and a makeshift lab with poor resources, a decision was made to focus on *S. aureus* isolates that had been collected as part of routine practice from patients with suspected infection from several wards throughout Fort Portal Regional Referral Hospital. This follow-on study was based on isolates collected between August 2017 and April 2019 and benefitted from new microbiology lab facilities and staff funded by the infectious diseases institute. Isolation,

identification and storage were much more reliable [a Memorandum of Understanding and Material Transfer Agreement with Makerere University enabled shipment and storage of the isolates at the strain bank in Makerere and Salford]. DNA was extracted from 73 isolates and 47 were chosen for whole genome sequencing based on DNA quality (Table 3.5). This provided an opportunity to assess the *S. aureus* population structure and determine any patterns with associated virulence genes (this chapter) or antibiotic resistance genes (Chapter 4).

Table 3.5: *S. aureus* isolates selected for whole genome sequencing. The isolates were selected based upon their Nanodrop concentration and 260/280 ratio. Following selection, concentrations were assessed utilising Qubit prior to commencing WGS.

Sample	Nanodrop Concentration (ng/ul)	Qubit Concentration (ng/ul)	260/280 ratio
128	130.3	3.64	2.29
145	96.9	27.3	1.8
150	92.7	13.2	1.82
161	91.5	3.14	1.76
171	90.9	0.485	1.83
155	86.4	2.97	1.67
158	84.2	1.47	1.76
127	84.1	0.258	1.98
162	80.5	1.5	1.76
137	77.2	2.47	1.62
160	76.3	4.63	1.75
159	75.1	2.21	1.76
136	74.7	1.25	1.59
129	73.5	0.783	1.74
174	71.2	8.02	1.87
133	71.2	4.16	1.67
134	70.6	6.83	1.74
149	68.3	16.4	1.72
152	68	2.81	1.71
169	67.7	2.47	2.07
163	66.2	0.838	1.78
164	62.5	0.856	1.78
147	62.4	5.04	1.7
146	61.7	2.17	1.65
142	60.6	1.35	1.63
165	59	3.07	1.69

166	58.1	0.397	1.65
131	57.3	2.69	1.65
135	56.5	6.64	1.62
157	55.1	2.23	1.77
156	55	0.997	1.72
154	54.1	4.61	1.73
130	53.7	1.6	1.62
168	53.5	3.11	1.72
151	53.2	3.8	1.66
132	53	3.1	1.65
138	50.9	2	1.6
148	47	3.21	1.62
167	46.1	4.88	1.67
139	44.9	1.09	1.54
170	44.7	1.88	1.93
153	44.4	4.91	1.7
143	42	2.17	1.56
140	37.1	1.15	1.52
141	36.2	1.64	1.5
144	34.1	0.937	1.49
172	17.6	0.642	2.04
173	17.6	0.269	1.93

3.9.1 WGS read quality

Draft whole genomes were generated for 47 *S. aureus* isolates using Illumina MiSeq data acquired from two sequencing runs (yield = 8.83Gbp and 3.79Gbp, average %Q30 = 90.91% and 84.07%, % reads identified (PF) per index = 90.13% and 85.14% respectively). All sequence read data used for genome assembly and annotation are available on the European Nucleotide Archive under project accession: PRJEB40863. According to Pathogenwatch (https://pathogen.watch/collection/ddhx5rrchbht-antibiotic-resistance-profiles-and-population-structure-of-disea), between 86% to 100 % of the core gene families were identified for all isolates (mean 95% per isolate). N50 ranged from 3,045 bp to 145,228 bp (mean 26.8 Kbp).

Of the 46 sequenced isolates, 41 were *S. aureus,* and the remaining 5 were identified as other closely related species *S. haemolyticus* (n=3)*, S. xyloxus* (n=1) and *S. saprophyticus* (n=1), all of which are coagulase negative.

Of the 41 *S. aureus* isolates 6 were from blood, 10 from wounds, 11 from puss, 3 from urine, one from an ear swab and one from synovial fluid as well as 9 with no source information. Core genome alignment was ascertained by SNIPPY (Seemann, 2015), and a phylogenetic tree generated by FastTree using a GTR+CAT model of nucleotide evolution (Price et al., 2009) (Figure 3.4). MLSTs were confirmed against the pubMLST database (Jolley et al., 2018) using the software "MLST" (https://github.com/tseemann/mlst). Twenty five of the sequenced isolates contained the complete MLST data required for this comparison.

A total of nine different sequence types were translated from the data (ST1, ST5, ST8, ST25, ST152, ST508, ST1633, ST2019, ST2178), 37% (15/41) of which belonged to ST152 and its single locus variant ST1633. Both of these fall into the clonal complex CC152 classification. Furthermore, three isolates with which only 6 out of 7 loci were confirmed through sequencing were identified as CC152, along with another two isolates which had more incomplete MLST data, but core genome analysis indicated they clustered tightly with other CC152 isolates. Hence collectively it was assumed that 20/41 (49%) isolates tested were CC152. The second most prevalent ST with 4 isolates was ST8 (CC8), whilst ST1 (CC1), ST5 (CC5), ST2178 (CC30), ST508 (CC45), ST25 and ST2019 (no associated CC) were all observed once.

Table 3.6: Whole Genome Sequencing data for the 41 successfully sequenced *S. aureus* isolates. Core matches refers to the number of core genes identified that matched those found in the reference genome core library. Percentage core families corresponds to the percentage of core gene families identified relative to the reference genome core library. Percentage non-core corresponds to the percentage of the genome assembly that has not been assigned to a core gene. Total data length represents the length of the genome in nucleotide pairs.

Sample	CORE MATCHES	% CORE FAMILIES	% NON-CORE	TOTAL DATA LENGTH (bp)
132	1434	88.1	44	2735195
139	1583	97.2	38.9	2761861
140	1460	88.6	58.7	3634745
141	1420	87.3	44	2749787
142	1551	95.3	40.4	2767123
143	1600	98.3	36.9	2718946
145	1601	98.3	38.4	2789950
146	1516	93.1	41.9	2813893
149	1564	95.1	51.4	3339664
155	1618	99.4	39.5	2860026
157	1618	99.4	37.8	2784224
158	1417	87.1	42.8	2656604
159	1514	93	40.6	2727273
160	1494	91.8	44.4	2869474
161	1508	92.7	39.6	2654798
162	1522	93.5	38.9	2679537
163	1489	91.4	46.9	2970567
167	1406	86.3	42.5	2616280
168	1489	91.4	40.7	2677133
169	1552	95.3	40.2	2789398
170	1487	91.3	39.4	2637112
171	1626	99.9	39.1	2861353
172	1880	98.8	59.4	4916569
174	1601	98.3	45	3126018
175	1610	98.9	37.9	2776700
176	1553	95.4	38.6	2692943
177	1593	97.8	39.1	2800277
178	1628	100	43.3	3072352
181	1619	99.4	39.9	2881335
182	1472	86.7	58.9	3820827
183	1571	96.6	39.3	2747269
185	1592	97.8	36.6	2688914
186	1750	96	62.6	4945722
187	1628	100	38.8	2848091
188	1544	94.6	55.1	3548364

190	1456	89.4	43.2	2738487
191	1967	98.6	66.1	6077323
192	1873	96	62	5244925
193	1895	99.3	63.3	5467494
195	1862	97.1	61.1	5087142
196	1425	86.3	62.7	3816584

Table 3.7: Whole Genome Sequencing data for the 41 successfully sequenced *S. aureus* isolates. N50 is a measure of how many contigs are required to cover more than half of the genome, relative to the size of the genome. No. contigs refers to the total number of contigs used in the assembly of the genome. Non-ATCG refers to the number of non-ATCG characters, i.e., 'N' may have been used where an uncertain nucleotide was present. Percentage GC content corresponds to the percentage of nucleotides that are either guanine or cytosine.

Sample	N50	NO. CONTIGS	NON-ATCG	% GC CONTENT
132	5524	1364	0	33.5
139	21512	746	118	33.2
140	3782	2849	11694	34.1
141	5288	1243	20	33.8
142	11786	973	0	33.3
143	21389	307	10	32.9
145	38591	327	307	32.9
146	9446	1495	306	33.7
149	10749	1301	11312	33.7
155	93655	600	511	33.1
157	100865	146	58	32.8
158	5611	1479	20	33.8
159	10742	1023	153	33.3
160	7317	2558	80	34.3
161	8291	667	10	33.1
162	10381	683	0	33.1
163	7247	1620	8717	33.7
167	6035	1439	600	33.8
168	7884	1004	20	33.4
169	18045	471	20	33.1
170	9328	629	50	33.2
171	128231	229	614	32.8
172	9558	1880	127	33.3
174	20683	2045	47	33.4
175	52284	208	10	32.8
176	9356	1226	0	33.4
177	30430	601	141	33

178	62076	613	2753	32.9
181	71183	830	0	33.1
182	3045	2517	1942	34.2
183	14907	509	49	33
185	36620	193	10	32.8
186	5586	2641	14093	33.5
187	145228	126	0	32.7
188	18123	4570	18759	34.7
190	7244	1352	387	33.7
191	34251	2032	70	33.2
192	8238	1686	30	32.9
193	10630	2274	3864	33.4
195	7032	2021	3749	33.2
196	11487	4827	51530	34.2

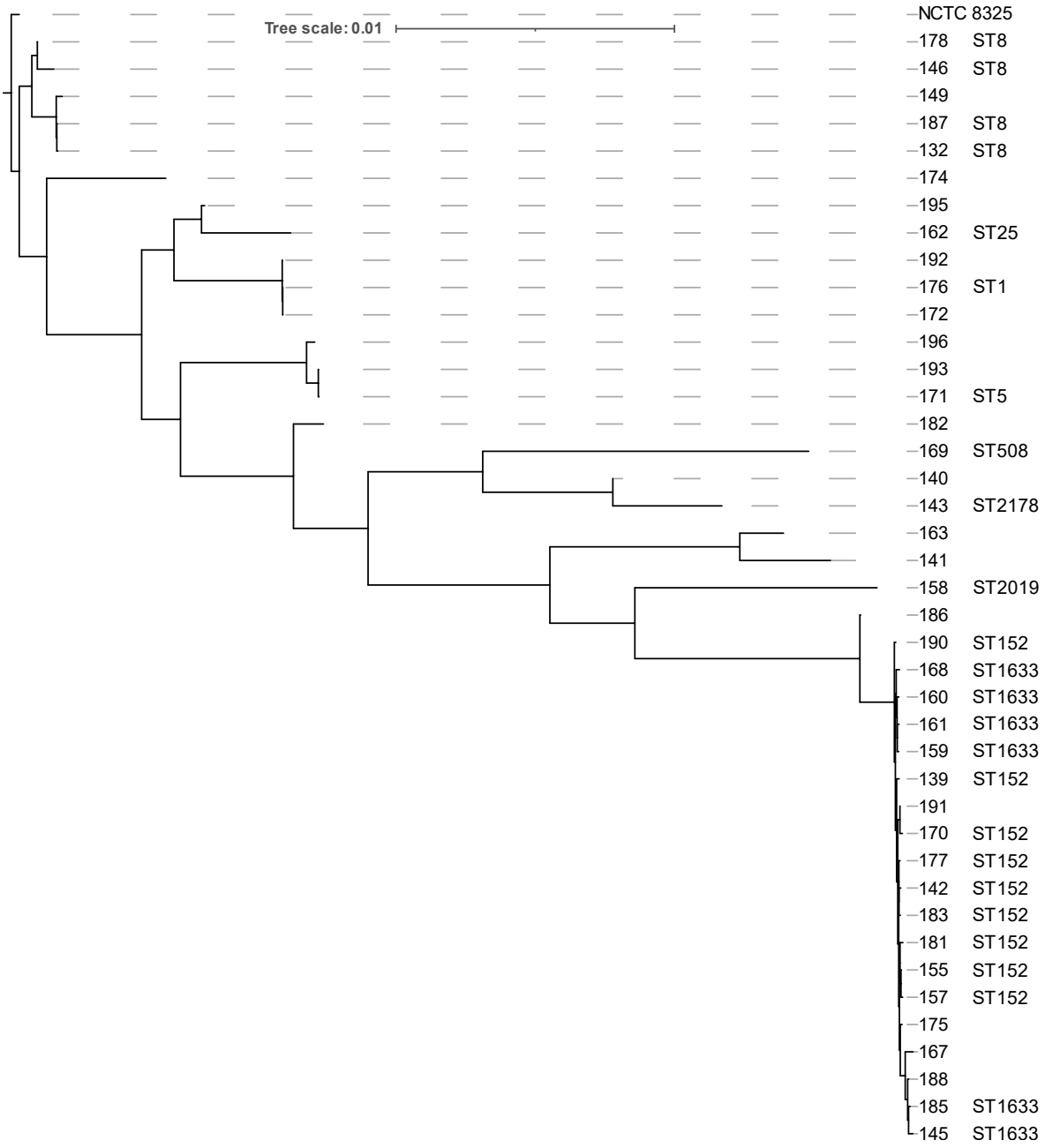


Figure 3.4: Core genome phylogeny of 41 *Staphylococcus aureus* isolates from Fort Portal Regional Referral Hospital isolated between 2017 and 2019. Aligned against the NCTC 8325 reference genome. Isolates 190 through 145 were collectively identified as CC152 isolates.

3.10 Association of Virulence Factors with CC152

The presence of virulence genes in the 41 sequenced *S. aureus* genomes was predicted using ABRICATE, against the VFDB database (Liu et al., 2019). Given the

dominance of CC152 strains, it was pertinent to explore variation in virulence-associated gene content between isolates belonging to CC152 (n = 20) and all the others (n = 21). The majority of virulence-associated genes were neither over- nor under-represented among CC152 stains. However, thirteen virulence-related genes were found to have a stronger correlation with the CC152 isolates (Figure 3.5).

3.10.1 *lukF-PV*, *lukR-PV* and *coa* significantly more prevalent amongst CC152 isolates

The most striking observation was that *lukF-PV* and *lukR-PV* genes were significantly more common among CC152 strains compared to the others (χ^2 =20.74, P <0.001 and χ^2 =18.09, P<0.001 respectively). *lukD, luKF-PV, lukS-PV* and *sec* are all virulence factors associated with toxins which damage host cells. The most prevalent of these were the leukotoxins found collectively in 38/41 isolates. Individually *lukD* was the most prevalent virulence factor isolated (30/41), with PVL associated genes lukF-PV and lukS-PV identified in 22/41 and 17/41 isolates respectively. The least prevalent virulence factor across all data assessed was sec (5/41).

Blood clot associated virulence factor gene *coa* was the second most prevalent factor observed (25/41) and was more closely associated with the CC152 group (χ^2 = 13.82, P<0.001). The von Willebrand factor binding protein, encoded by *vWbp*, exhibits multiple similarities in terms of coagulation induction function to Coa (Thomas et al., 2019) and was present in just under 20% of isolates (8/41). Interestingly, the gene was found to be absent from CC152 isolates, but present in 37% of non-CC152 isolates (χ^2 =9.47, P=0.002).

3.10.2 *chp, sec, map,* and *sdrC* significantly under-represented amongst CC152 isolates

Several other virulence factor-encoding genes were encountered significantly less frequently among CC152 isolates than non-CC152 isolates. These included genes encoding the chemotaxis inhibitory protein Chp (χ^2 =7.96, P=0.005) present in 10/41 isolates, and the Sec enterotoxin (χ^2= 5.42, P=0.020) present in 5/41 isolates. Adhesion associated virulence factors Eap (map) and SdrC were identified at 15/41 and 10/41 isolates respectively. Both were more associated with non-CC152 strains (χ^2=12.60, P=0.003 and χ^2=16.79, P<0.001) respectively.

3.10.3 Multiple genes encoding EsaG show variable representation amongst CC152 and non-CC152 isolates

Multiple genes encoding EsaG have been encountered in *S. aureus* genomes. The most predominant homologue observed in the 41 clinical *S. aureus* isolates was *esa*G5 identified in 21/41 isolates, with other EasG1, EsaG3, EsaG4 being identified at largely similar rates of 12/41, 11/41 and 10/41 samples respectively. Significant variation was observed in the presence/absence of these genes among CC152 members and other isolates, such that some copies (*esaG1, esaG3*, and *esaG4*) were under-represented (χ^2= 11.11, 9.48, 12.60, P <0.001, 0.002, <0.001 respectively) among CC152 strains whereas other *esaG5* was over-represented (χ^2= 8.84, P<0.01).

In summary, the CC152 isolates more commonly harboured *coa, esaG5, lukD, lukF-PV* and *lukS-PV*, genes whilst other common virulence genes *chp, esaG1, esaG3, esaG4, map, sdrC*, sec and *vWbp* were not associated with this group at all.

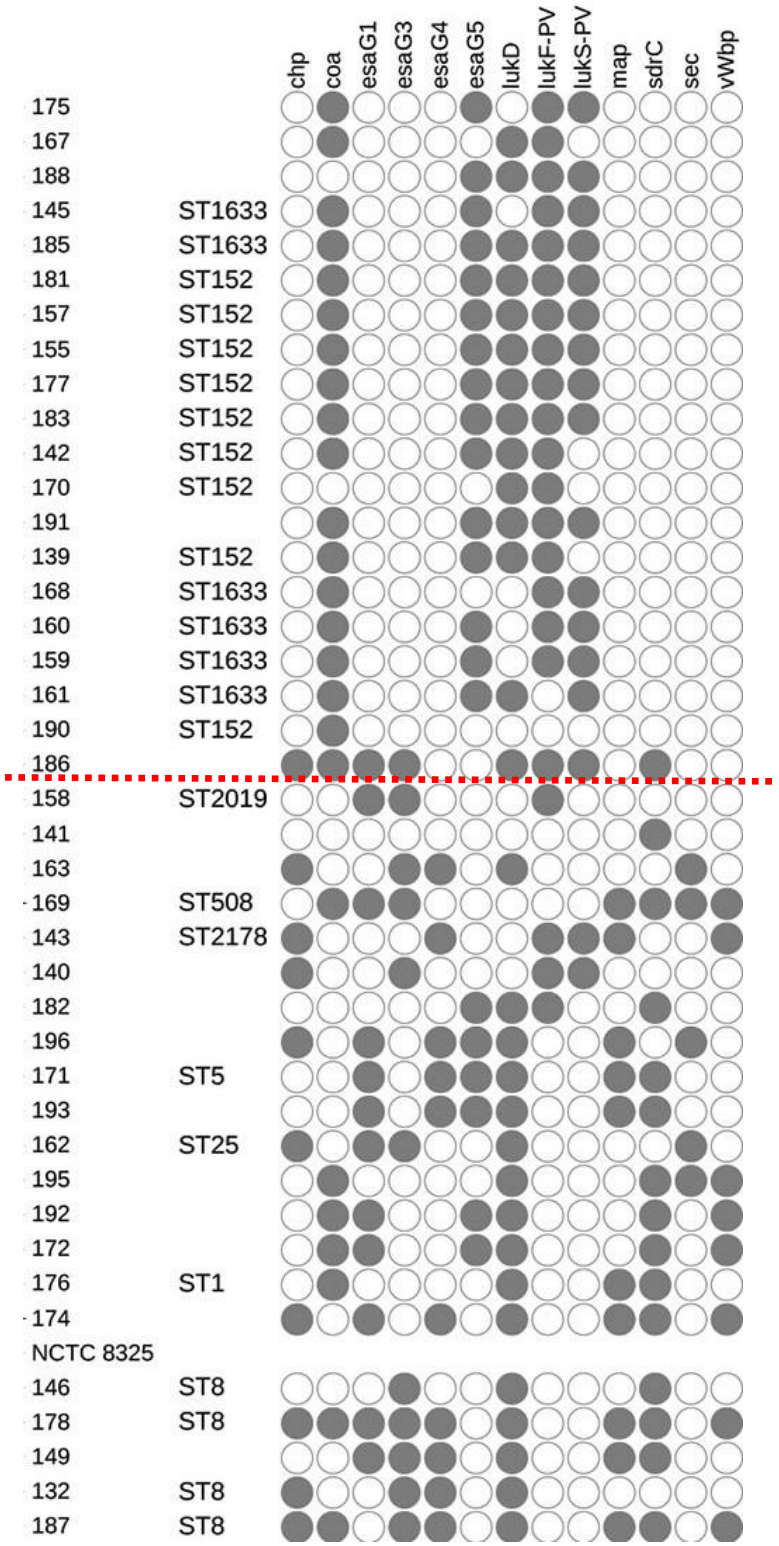


Figure 3.5: Virulence gene presence of 41 *Staphylococcus aureus* isolates from Fort Portal Regional Referral Hospital isolated between 2017 and 2019. Presence of a virulence gene is indicated as a filled-in grey circle (absence indicated by empty grey circle). A red dotted line indicates the cut-off between CC152 isolates (above) and others (below).

3.11 Discussion

3.11.1: The Pilot Study Highlighted a Number of Complications

A large number of discrepancies were observed in relation to the identity of the bacteria isolated from patients at FPRRH during the pilot study, and whether they were in fact causes of sepsis. However, assessment of healthcare worker associated isolates was largely accurate. These differences may be attributed to the methods of sample collection and bacterial isolation. Where taken, hand swabs were used to directly inoculate the respective selective agar medium, with only the presumptive *S. aureus* isolates based on colony morphology being utilised further. As such, the species identification of other bacteria was scrutinised to a far lesser extent in relation to the clinical samples. Additionally, clinically acquired blood cultures were incubated for up to 5 days in non-selective broth medium, where potential contaminants have more capacity to grow.

Interestingly, all clinical samples identified as *S. aureus* were successfully classified as *Staphylococcus* species through sequencing, with the exception of one isolate which was not successfully sequenced. This higher level of success may be due to *S. aureus* being the primary target of this research, hence influencing the basis of the identification techniques used. Together, these findings serve to highlight the challenges of working in poor-resource settings. However, the process enabled the decision to focus on *S. aureus* since it was the most frequently isolated species as well as being frequently isolated from health care workers hands and known to be a major health concern in Uganda.

Despite *S. aureus* being identified phenotypically and through 16S rRNA gene sequencce profiling, spa typing was largely unsuccessful across all the samples. No product was amplified from the large majority of samples, with only primer-dimers visible on the gel.

The same protocols were used for a selection of *S. aureus* samples freshly prepared in the laboratory including the positive control prepared from a fresh colony of *S. aureus* strain ATCC 25923, where strong positive results were obtained. As such, the most likely explanation for the failure was degradation of DNA in transit from Uganda and were kept under less consistent environmental conditions. This may also account for the unsuccessful amplification of the 16SrRNA gene target from some samples. Variations in size of the amplified *spa* products could be attributed to the presence of different spa types, however given the low success rate and likely degradation of the DNA in the sample preps there was little evidence to justify further attempts to type these samples in this way at the time.

Based on the preliminary identification of bacteria from the clinical samples, there was an exceedingly high isolation rate of bacteria from blood samples (100%), where rates in other sub-Saharan African countries have been shown to range from 7-28% (Habyarimana et al., 2021). With samples only taken from 13 patients it is hard to deduce reliable conclusions, however with bacteria present in all samples it becomes more likely some are due to contamination. That being said, typical hospital practices must also be taken into account. The laboratory at FPRRH was largely under-used at the time, with healthcare workers often misguided as to the availability and scope of tests on offer. On the rare occasions where tests were facilitated it was more likely the patient was suffering from a serious condition where empirical treatments had been ineffective, hence increasing the likelihood of isolating bacteria from the sample.

16S rRNA gene sequencing identified *Staphylococcus spp.* as the most prevalent bacteria isolated, in line with Habyarimana *et al*. (2021). The data suggested the presence of other less common causes of bactaeremia including *Micrococcus* (previously reported in

immunocompromised patients) and *Enterococcus spp*., commensal organisms found in the normal flora of skin (Smith et al., 1999) and the human gastro-intestinal tract respectively (Ike, 2017). Other identified genus included *Kocuria spp.*, also found amongst the normal flora of skin but largely considered non-pathogenic, although they have been associated with catheter-related bacteraemia (Ma et al., 2005). It is therefore plausible that these organisms were the underlying cause of infection, especially considering the prevalence of HIV in Uganda where oportunistic bacterial infections are more likely to arise in immunocompromised patients. Alternatively, *Leucobacter* (Clark & Hodgkin, 2015), *Bacillus*, *Brevibacillus* (Panda et al., 2014) and *Arthobacter spp* (Busse, 2016) are all found in the environment, (predominantly in soil) and are not associated with human bacteraemia. As such, these identified isolates are likely to be the result of contamination. These findings do however still raise questions as to whether *S. aureus* was successfully identified as a direct causal factor of infection or if it was in fact also the result of contamination.

3.11.2 CC152 and ST8 dominance aligns with a growing continental trend of isolation rates

When evaluating MLST data with respect to global *S. aureus* population structures there are certain observable biases caused by increased sample collection of isolates causing disease and also increased isolates collected in generally wealthier countries, for example Western Europe, United States, Australia and Japan. Working to address this issue a study by Ruimy and colleagues in Mali (Ruimy et al., 2008) assessed a cohort of isolates recovered from the nasopharynx of asymptomatic members of the general population and were inadvertently the first to document the dominance of CC152 (24% of isolates gathered). Multiple subsequent studies across Africa have since reported similar results with

CC152 being one of the most predominant classifications (Schaumburg et al., 2014), the most recent studies being in Kenya (Kyany'a et al., 2019) and Nigeria (Obasuyi et al., 2020) where CC152 was reported as the most common clonal complex at rates of 28% and 24.7% respectively. CC152 is a community associated complex and has been observed across the globe (Figure 3.6), with methicillin resistant strains largely limited to Europe (discussed further in Chapter 4).

Figure 3.6: Global geographical distribution of *S. aureus* CC152 isolates. Samples collected from 1999 to 2015 in 28 different countries (Baig et al., 2020). Black and red dots represent MSSA (n=93) and MRSA (n=56) isolates respectively.

The second most prevalent sequence type was ST8, with its clonal cluster (CC8) being one of the very first clinically isolated MRSA strains circulating in the UK and Europe. More recently it has since become one of the most prevalent, hypervirulent strains in the

USA known as community associated MRSA clone USA300 (Chambers & Deleo, 2009). Similar to ST152, ST8 is known to be widespread in sub-Saharan Africa (SSA), with specific USA300-like clones found in Cameroon, Gabon and Ghana. It is speculated that whilst ST8 was likely introduced to SSA from single introduction events, it has since proven to spread rapidly within the region leading to other locally evolved African ST8 clones (Strauß et al., 2017). Similar to ST8, whilst the presence of ST1 and ST5 were only confirmed once in this study, they are both prevalent strains in the US (Chambers & Deleo, 2009; Vignaroli, 2009) that have been isolated in multiple other studies in SSA. (Kyany'a et al., 2019; Obasuyi et al., 2020; Schaumburg et al., 2014). CC30 and CC45 are two further clonal complexes that have been isolated globally at rates typically higher than found within this study (Enright et al., 2002). Collectively the aforementioned clonal complexes (CC1, CC5, CC8, CC30 and CC45), with the exception of CC22, form all the core subset of *S. aureus* complexes which convey methicillin resistant strains (Chambers & Deleo, 2009) – this is further discussed in Chapter 4.

Bearing in mind that the majority of isolates identified were from wound infections and classified as community-associated complexes, it is plausible that infections were arising as a result of poor patient personal hygiene. Anecdotal evidence described by Welsh (2019) within the local region with respect to maternity agrees with this, where women who have recently delivered go home to an unsanitary environment and use dirty cloths as maternity pads. It can be inferred that patients from other wards in the hospital also return home to such settings creating a high risk of wound infection. However, Welsh is also clear that the hygiene of healthcare environments is also far from ideal, with midwives rarely seen washing hands between patients. These ideas are discussed further in Chapter 5.

3.11.3 Assessing virulence trends in sub-Saharan Africa

Whilst WGS is still a relatively new technology available in Uganda, multiple studies have been conducted in countries across sub-Saharan Africa evaluating the presence of virulence genes found in *S. aureus* isolates. As previously noted, one of the first studies in Africa highlighting the high prevalence of CC152 (23.9% of isolates) was conducted in Mali. The study also observed a strong association between CC152 and the presence of PVL encoding genes, where 100% of the respective isolates recovered from nasal carriage samples were PVL positive (Ruimy et al., 2008). Further studies compounded this finding: Okon et al. (2009) obtained a similar value of 25% (24/96) of isolates collected across 6 hospitals in Nigeria attributed to CC152, all but one of which were PVL positive; F. Schaumburg et al. (2011) investigated isolates from a province in Gabon and identified 6% (10/163) of carriage associated and 11% (6/54) disease associated isolates as CC152, with a single carriage associated isolate being PVL negative; finally, Kyany'a et al. (2019) identified 28% (9/32) of isolates from four hospitals across Kenya as CC152, all of which were PVL positive. Interestingly, despite PVL frequently being found in community-acquired MRSA strains, only a single MRSA isolate (1/10) was PVL positive in this study. Equally, MRSA strains frequently produce superantigen toxins (Souza et al., 2009), yet again in this study only a single MRSA isolate was found to house *sec*. That being said, CC152 is predominantly methicillin resistant in Europe (Baig et al., 2020), highlighting the need for the clonal complex to be closely monitored in future research.

With Kyany'a providing a more in-depth characterisation of isolates further associations could be observed. As with this study, no CC152 isolates possessed any of the

virulence markers *chp, map, sec* or *vWbp*. With *Coa* exhibiting a strong association with CC152 isolates within this study and fulfilling a similar role to *vWbp*, it may point to the two proteins fulfilling similar roles in different *S. aureus* lineages. Further similarities can be drawn with CC8 which showed a strong association with the absence of *lukS*, *lukF* and *sec*, and the presence of *vWbp*, *chp* and *map* (Kyany'a et al., 2019). That being said, with only four ST8 isolates identified as part of this study a larger sample size is required to draw stronger correlations.

With this study going some way to provide a solid foundation of data for the prevalence of virulence factors identified at FPRRH, further in-depth research is required including the genomes of environmental, community and healthcare worker associated *S. aureus* to aid in elucidating a source of possible infection and virulence characteristics. This would provide more insight into the local relationships between PVL, CC152 and community associated infections. Also of use would be statistics regarding the severity of infection; at the time of data collection, with new laboratory systems being implemented, the presence of detailed sample information was often sporadic (for example which ward the sample had been sent from and patient outcomes). Without such data it can be hard to attribute the presence or absence of specific virulence factors with increased *S. aureus* virulence. These associations have been highlighted in other studies which investigated 28/30-day mortality rates. MRSA is capable of producing various virulence factors including staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), leukocidins, hemolysins, exfoliative toxins, and immune-modulatory factors all of which have been shown to increase the mortality rate of patients with MRSA bacteraemia. An independent association was also reported between superantigenic toxins and staphylococcal superantigen genes (*sec*, *sel*, and *tst*) in relation to patient mortality (Hyun Jin et al., 2020). Similar observations have

been made with different types of coagulase, where coagulase gene size and the presence of *fib* associated with 28-day mortality rates in patients with *S. aureus* bacteraemia (Karer et al., 2020).

Chapter 4 – Phenotypic and Genotypic Indicators of Antimicrobial Resistance in *Staphylococcus aureus*

4.1 Introduction

S. aureus is capable of causing life-threatening diseases and is a major cause of nosocomial infections. Furthermore, it has a strong capacity for the development of resistance to multiple antimicrobials (Dancer, 2014). Commonly found as a commensal organism on skin and in the nasopharynx as well as on livestock and certain environments (Tong et al., 2015), *S. aureus* strains are frequently referred to as community- or healthcare-associated, both capable of causing serious infection (Cohen, 2007). These attributes are particularly harmful in resource-constrained settings where basic infection, prevention and control measures are lacking (Louise Ackers et al., 2020). In such situations antibiotics can often be over-prescribed as a fail-safe measure to compensate for poor hygiene practices, itself becoming a driver for increased resistance (Welsh, 2019). Given the widespread use of antibiotics both as a treatment and prophylactic it is important to keep track of AMR prevalence in addition to the respective resistance mechanisms employed to enable future effective antibiotic use.

4.1.1 Key Antimicrobial Resistance Mechanisms Observed in *S. aureus*

As described in Chapter 1, Fort Portal Regional Referral Hospital (FPRRH) routinely screened for phenotypic susceptibility to a panel of eight antibiotics commonly

recommended for treating *S. aureus* infections. The mechanisms of resistance identified in *S. aureus* to these antibiotics are described in detail below.

4.1.1.1 Chloramphenicol

There are two primary ways through which chloramphenicol resistance occurs: i) a low-level resistance conferred by an observable reduction in the permeability of the cell membrane as a result of a series of spontaneous mutations, thus reducing the rate at which the antibiotic can enter the cell; ii) a high-level resistance facilitated by the enzymatic inactivation of chloramphenicol by acetylation. Genes encoding these acetylation enzymes can be located chromosomally or on plasmids allowing for both horizontal and vertical gene transfer (Sköld, 2011). A third method of resistance only recently described involves altering the structure of the ribosomal subunit such that the antibiotic can no longer effectively bind to it (Wu et al., 2021).

4.1.1.2 Gentamicin

There is a diverse range of mechanisms through which bacteria have become resistant to aminoglycosides, these include the modification and/or mutation of the ribosomal target, decreased permeability of the cell wall and active transport of aminoglycosides out of the cell. The most prevalent, however, is the enzymatic inactivation of aminoglycosides by aminoglycoside-modifying enzymes (AMEs) (Garneau-Tsodikova & Labby, 2016). Three primary types of AME have been identified in *S. aureus*: N-acetyltransferase (AAC), O-nucleotidyltransferase (ANT) and O-phosphotransferase (APH), where resistance to gentamicin (as well as tobramycin, amikacin and kanamycin) is mediated by AAC(6') and APH(2") (Dancer, 2014).

4.1.1.3 Cefoxitin

S. aureus resistance to β-lactams is typically due to the presence of penicillinases (encoded by *bla* genes) capable of directly inactivating the antibiotic via hydrolysis of the amide bond of the β-lactam ring, resulting in an inactive product. The effectiveness of this mechanism is influenced by the quantity of β-lactamase produced, the affinity of the enzyme for the antibiotic and the rate of hydrolysis. There are slight differences between Gram-positive bacteria, which excrete enzymes into their immediate environment, and Gram-negative bacteria, which maintain the enzyme in the periplasmic space (Essack, 2001), or package them in outer-membrane vesicles. β-lactamase genes are commonly mobilized on plasmids and are widely distributed in several key pathogenic bacteria that have been identified as major threats to human and animal health. To combat the initial resistance observed, newly modified antibiotics were generated, including methicillin, to help in the treatment of penicillin-resistant *S. aureus* infections (Dancer, 2014). Despite being a β-lactamase inducer, cefoxitin has high resistance to hydrolysis by β-lactamases due to presence of a methoxy group in the 7-alpha position and a thienyl acetyl group at the R^1 position of the base cephalosporin shown in Figure 1.1 (Gootz, 1990). Extended-spectrum β-lactamases have been widely reported, with the ability to hydrolyse penicillins and all cephalosporins (Paterson et al., 2014). Furthermore, it did not take long for the more nefarious methicillin-resistant *S. aureus* (MRSA) to develop utilising a secondary resistance mechanism. Production of a fifth PBP, PBP2a, enabled the continuation of transpeptidation in cell wall synthesis due to its low affinity to β-lactams. Not only does this convey resistance to methicillin, but nearly all β-lactams and their derivatives including cephalosporins and carbapenems (Dancer, 2014).

4.1.1.4 Trimethoprim/sulfamethoxazole

Several different mechanisms have been reported to confer resistance to cotrimoxazole. With resistance to both individual components being readily transferable, for example through transposons, resistance to both parts are frequently carried together. Such mechanisms can involve reducing the permeability of the cell wall, efflux pumps which remove the antibiotic from the cell, modifications to the target enzyme which can naturally exhibit insensitivity, mutational/recombinational changes, or acquired resistance by drug-resistant target enzymes. With changes to cell permeability and efflux pumps conveying general resistance to trimethoprim and sulfonamides simultaneously, other modifications are more specific (Eliopoulos & Huovinen, 2001). In *S. aureus*, a single amino acid substitution in DHFR (encoded by *dfr* genes) has been found to convey resistance to trimethoprim (Dale et al., 1997). In clinical cases of human infections, a single mutation to *dfrB* conveys intermediate resistance, whilst acquired *dfrA* and *dfrG* gene variants are typically responsible for high level resistance (Nurjadi et al., 2014). Cotrimoxazole resistance has been observed at various levels, being relatively low across North America, Europe and China with the highest rates observed across Africa (up to 55% of colonising and 72% of clinical *S. aureus* isolates). A recent genetic study across three African countries highlighted the dominant presence of *dfrG* (78%) relative to *dfrA* (19%) where 122 resistant isolates from nasal swabs were assessed (Coelho et al., 2017).

4.1.1.5 Ciprofloxacin

Fluoroquinolones are clinically significant as they confer no cross-resistance with other antibiotic classes (Varshney et al., 2014). Resistance is conferred through a series of mutations in target genes resulting in reduced antibiotic binding, where each mutation

individually contributes to a slight increase in tolerance up to a point where the bacteria is no longer susceptible (Pietsch et al., 2017). Resistance observed occurs through two core pathways - primarily through mutations in the target enzymes and to a lesser extent active efflux of the antibiotic. The fluoroquinolone efflux membrane protein in *S. aureus* is known as NorA (Neyfakh et al., 1993). Alternatively, resistance derived from mutations in DNA gyrase and topoisomerase IV is conferred primarily by the genes *gyrA* and *grlA* respectively, with *gyrB* and *grlB* involved to a lesser extent. These mutations typically involve the substitution of an amino acid (Tanaka et al., 2000).

4.1.1.6 Erythromycin

Three key mechanisms of macrolide resistance have been reportedly observed in *S. aureus*: enzymatic inactivation, active efflux and most importantly target-site modification. The *msrA* gene encodes an efflux pump, whilst *ereA* and *ereB* genes encode macrolide-inactivating enzymes. However, it is rare for individual isolates to carry both sets of resistance genes. Modifications to the ribosomal target result from methylation via erythromycin-resistance methylase (Erm) enzymes, encoded by *erm* genes, the most common being *ermA* and *ermC*. The expression of *erm* genes can be inducible or constitutive, with erythromycin itself being a potent inducer. The importance of this particular ribosomal modification stems from the fact that several classes of antibiotics share the same 50S target region; macrolides, lincosamides and streptogramin type B (MLSB). As such, resistance attributed to Erm enzymes conveys resistance to all MLSB antibiotics (Dancer, 2014).

4.1.1.7 Clindamycin

Lincosamides are included in the MLSB antibiotic group and intrinsically have overlapping target sites on the 50S subunit of bacterial ribosomes. They largely function in the same way to inhibit protein synthesis and as such where resistance occurs to one particular type it can also convey resistance to all other MLSBs. This is the case for ribosomal modifications via *erm* genes as described above. Alternatively, enzymatic inactivation and efflux of lincosamides is attributed to the *lnu* and *lsa* genes respectively (Dancer, 2014), with *lsa* also conferring cross resistance to streptogramin A and pleuromutilins (Wendlandt et al., 2015).

4.1.1.8 Tetracycline

Resistance to tetracyclines occurs through active efflux and ribosomal protection proteins. Tetracycline efflux proteins have been observed in both Gram-negative and positive bacteria, they also exhibit amino acid and protein structural similarities with efflux proteins conveying resistance to other antibiotics including chloramphenicol and quinolones. A number of genes are responsible for such proteins across different bacterial species, with *tet(K)* and *tet(L)* being the primary factors in *S. aureus*. Ribosomal protection proteins express a wider spectrum of tetracycline resistance relative to efflux proteins and have been observed to allow the binding of amino-acyl tRNA to the ribosome through the release of tetracycline, thus allowing the continuation of protein synthesis. Protection proteins are thought to act in a GTPase dependent manner, with the genes *tet(M)* and *tet(O)* being largely responsible for their expression in *S. aureus* (Chopra & Roberts, 2001).

4.1.2 Genomic correlation with phenotypic AMR profiles

Whilst phenotypic antimicrobial susceptibility testing (AST) is the most reliable technique to determine the resistance of a particular isolate, there is a limit to the physical number of antibiotics that can be feasibly tested in a practical laboratory setting. This effect is compounded in resource-limited facilities, for example FPRRH, where antibiotic availability can be sporadic. Furthermore, time delays associated with culture and sensitivity testing are often impractical where the empirical use of broad-spectrum antibiotics for the treatment of severe infection is required. In such situations genomic analysis can prove useful not only for detecting resistance to a variety of antibiotics both inducible and non-inducible, but also to assess new resistance genes, mutations or synergistic relationships even before they are functionally expressed (Kyany'a et al., 2019). A recent study of 778 MRSA isolates from the UK, comparing Illumina whole genome sequencing (WGS) data – based analysis with disc diffusion AST data showed 99.7% accuracy (Kumar et al., 2020). That being said, using genomic screening alone poses its own risks when purely assessing gene presence/absence, where mutations, accessory genes, gene expression modifiers and redundant resistance mechanisms can all have an impact on the phenotypic resistance observed. As such, genomic testing is most effective when used in conjunction with AST data (Kyany'a et al., 2019). To that end, this study has compared AST results from FPRRH with antibiotic resistance gene (ARG) presence/absence data drawn from WGS.

4.2 Aims and Objectives

Working in line with the UNAP, this study aimed to assess the prevalence of antimicrobial resistance amongst *S. aureus* isolates from clinical cases of infection at FPRRH,

in addition to those isolated from the hand swabs of healthcare workers and members of the local community. It also investigated the molecular mechanisms of AMR in clinical *S. aureus* isolates and compared phenotypic and genotypic resistance profiles to evaluate the usefulness of genomics for improving AMR surveillance. This work not only contributed to the development of the 1st antibiogram at FPRRH, but also provided a wider view of resistance circulation in the community and touched upon the importance of a one-health approach.

4.3 Pilot Studies

Pilot studies were conducted to assess logistics and optimise protocols for surveillance of clinical and non-clinical isolates including the best ways to collect and process samples from clinical sources as well as healthcare workers hands and the community around Fort Portal with limited resources.

4.3.1 Pilot Study of Clinical Isolates from Suspected Sepsis Patients Suggested Either High Frequency of MRSA or Problems with Sampling Procedures

Initial research began as a pilot study to assess the range of bacteria associated with causing maternal sepsis at FPRRH (see Chapter 3). All bacteria were isolated from 5-day blood cultures from patients that had been diagnosed with sepsis by the on-duty doctor. Bacterial isolates were preliminarily identified through colony morphology on selective media and Gram staining. Antibiotic susceptibility testing was performed using Kirby Bauer

disk diffusion assays, with resistance determined using EUCAST breakpoint tables (see Methods).

Supplies of consumables in Fort Portal were in limited supply during the pilot stages of the project. Given that the most frequently isolated species was *S. aureus,* the core focus of the study revolved around this species and the antibiotic disks selected for use were prioritised to fully assess the resistance profiles of *S. aureus.* There were some overlaps with relevant antibiotics for assessing other species, however, some of the antibiotic disks used weren't reliable for assessing all resistance profiles (Table 4.1). In general, resistance was very common in the *Staphylococcal* isolates (7 *S. aureus* and 3 *S. epidermidis*). Of note was the relatively high frequency of resistance to oxacillin, with 5/7 *S. aureus* and 3/3 *S. epidermidis* exhibiting resistance. Oxacillin resistance acts as an indicator for methicillin resistance and can be used for reporting MRSA. Thus, 71% of *S. aureus* isolates were considered MRSA. A considerable diversity of *S. aureus* resistance profiles were observed. Over half of these isolates (4/7) were resistant to ciprofloxacin and/or clindamycin. Tetracycline resistance was exhibited by 43% (3) *S. aureus* and 29% (2) *S. epidermidis* isolates. Lower rates of erythromycin and ceftriaxone resistance were observed, whilst only gentamicin proved effective against all strains. 57% (4/7) of *S. aureus* and 66% (2/3) *S. epidermidis* isolates were MDR, with the highest level of resistance observed by a single isolate being 7/9 antibiotics tested (Table 4.2).

Of the other species, only one of the three isolates of *E. coli* exhibited resistance (to ceftriaxone) and one isolate of *Proteus* was resistant to ampicillin and chloramphenicol (Table 4.1).

Table 4.1: Antibiotic resistance profiles of bacteria isolated from patients clinically diagnosed with sepsis on the postnatal and gynaecology wards at FPRRH

	Number of isolates exhibiting resistance								
Bacterial species	AMP	CIP	OX	DA	C	E	CRO	CN	TE
E. coli (4)	0	0	-	-	0	-	1	0	-
S. aureus (7)	1	4	5	4	2	2	2	0	3
S. epidermidis (3)	0	0	3	0	1	1	1	0	2
Enterococci (1)	0	0	-	-	-	-	-	-	-
Proteus spp. (2)	1	0	-	-	1	-	0	0	-

*AMP – Ampicillin, CIP – Ciprofloxacin, OX – Oxacillin, DA – Clindamycin, C – Chloramphenicol, E – Erythromycin, CRO – Ceftriaxone, CN – Gentamicin, TE – Tetracycline. Values are not present where EUCAST breakpoint tables determined disk diffusion assays to be unsuitable for sensitivity testing.

Table 4.2: Antibiotic Resistance Profiles of bacteria resistant to two or more antibiotics isolated from patients clinically diagnosed with sepsis on the postnatal and gynaecology wards at FPRRH

	Polyresistant Isolates Exhibiting Resistance (R) or Susceptibility (S) to the Respective Antibiotics									
Bacterial Isolate	AMP	CIP	OX	DA	C	E	CRO	CN	TE	Total number of antibiotics resistant to (9 maximum)
S. aureus	S	R	R	S	R	R	S	S	S	4
S. epidermidis	S	S	R	S	S	R	R	S	R	4
S. aureus	S	R	R	R	S	S	S	S	R	4
S. epidermidis	S	S	R	S	R	S	S	S	R	3
S. aureus	S	R	R	R	R	R	R	S	R	7
S. aureus	S	S	R	R	S	S	S	S	S	2
S. aureus	S	R	R	S	S	S	R	S	S	3
S. aureus	R	S	S	S	S	S	S	S	R	2
Total Resistant Isolates (8 maximum)	1	4	7	3	3	3	3	0	5	

*AMP – Ampicillin, CIP – Ciprofloxacin, OX – Oxacillin, DA – Clindamycin, C – Chloramphenicol, E – Erythromycin, CRO – Ceftriaxone, CN – Gentamicin, TE – Tetracycline

This pilot study served to i) identify *S. aureus* as the most commonly isolated bacterial species from sepsis patients and ii) a need for more robust systems and facilities to reliably isolate and store bacterial species from clinical samples at the hospital.

4.3.2 Pilot Study of *S. aureus* Isolates from Healthcare Workers Hands Suggested High Frequency of MRSA

To investigate the potential role of healthcare workers (HCW) in spreading infection, a second pilot study was performed as part of a series of hand hygiene workshops. *S. aureus* isolates were collected by swabbing HCW hands at local healthcare centres within the Fort Portal region.

Out of the 21 *S. aureus* isolates obtained all of them were resistant to at least one antibiotic, with 38% (8/21) displaying multidrug resistance. As shown in Table 4.3, the most reliable antibiotic was gentamicin (effective against 95% (20/21) of isolates), followed by ciprofloxacin (effective against 86% (18/21) of isolates). Closely following were ampicillin, and clindamycin, effective against 71% and 67% of isolates respectively, as well as tetracycline and chloramphenicol each effective against 62% of isolates. However, a relatively high number of isolates showed resistance or intermediate resistance to clindamycin (33% - 7/21) and tetracycline (38% - 8/21). The most unreliable antibiotics were erythromycin and oxacillin, only effective against 38% and 33% of isolates respectively. Since oxacillin is a preliminary indicator of methicillin resistance, this suggests that 67% (14/21) of isolates were MRSA.

Table 4.3: Antibiotic resistance profiles of *S. aureus* isolates from HCW hands at two healthcare centres within the FPRRH catchment area (Bukuuku and Kagote)

	Susceptible	Intermediate	Resistant	% Resistant Isolates
DA	14	3	4	19
E	8	0	13	62
TE	13	5	3	14
CN	20	0	1	5
C	13	0	8	38
CIP	18	1	3	14
AMP	15	0	6	29
OX	7	0	14	67

* DA – Clindamycin, E – Erythromycin, TE – Tetracycline, CN – Gentamicin, C – Chloramphenicol, CIP – Ciprofloxacin, AMP – Ampicillin, OX – Oxacillin. 5 *S. aureus* isolates were obtained from Kagote and 16 were obtained from Bukuuku

There are several key similarities and differences observed when comparing the resistance patterns of the clinical isolates to those from the HCW hands. Whilst the clinical isolates were significantly more commonly resistant to clindamycin (57% (4/7) vs 19% (4/21); χ^2 =3.73, P=0.05), it was one of the stronger performing antibiotics against the HCW isolates at a susceptibility rate of 67%. Performing equally well across the board were gentamicin and ciprofloxacin. At a first glance there appears to be a large discrepancy amongst the tetracycline resistance observed (14% for HCW and 45% for clinical isolates); however, a much greater number of isolates with intermediate resistance were found within the HCW isolates which could potentially lower the perceived effectiveness of tetracycline, bringing the levels more in line with rates of non-susceptibility of 38% (8/21). Further differences were observed in resistance rates to chloramphenicol and erythromycin which were much higher in the HCW isolates (38% and 62% respectively) compared to 28%

in each case in the clinical isolates, although these differences were not significant. However, it should be noted that later analysis using 16S rRNA gene sequencing suggested that some of the clinical isolates may not in fact have been *S. aureus* (see Chapter 3).

As well as informing surveillance (strategic objective 4), the hand-swabbing activity during health-centre workshops, and the associated data were useful as a visual aid to convey the importance of hand hygiene to healthcare staff as part of their IPC training and understanding of AMR towards improved stewardship (strategic objective 3) (Government of Uganda, 2018).

4.3.3 Pilot Study of *S. aureus* Skin Isolates from Healthy Members of the Community Suggested High Prevalence of Multi-Drug Resistance

In addition to the patient and HCW isolates being collected, *S. aureus* isolates were also obtained through swabbing the hands of members of the public from around the Fort portal region. This was performed by travelling with the mobile blood transfusion team, who visit educational centres in Kabarole to collect heathy blood for the transfusion service in Fort Portal. In total, *S. aureus* was isolated from 67 participants. The resistance profiles were higher than those obtained from patient samples, and for the majority of cases higher than those isolated from healthcare workers hands. A single isolate (1/67) was susceptible to all antibiotics tested, with 66% (44/67) of isolates being MDR – statistically higher than the HCW isolates (χ^2 =5.03, P=0.02).

The prevalence of community-isolated *S. aureus* resistance to chloramphenicol was the highest at 81%, significantly higher than observed in clinical (29%) or HCW (38%) isolates

(χ^2 = 9.31 and 13.88, P<0.01 and 0.01 respectively). Whilst chloramphenicol was one of the most effective antibiotics against the patient isolates, resistance was consistently more common in bacteria isolated from hand swabs. Erythromycin resistance was also highly prevalent in the general community (69% - 46/67). With similar levels observed to HCW (62%) it was significantly higher in comparison to the patient isolates (29%) (χ^2 =4.47, P=0.03). Prevalence of resistance to tetracycline (64%) was also higher than observed in the other pilot studies. Although oxacillin disks were not available at the time of performing this 3rd pilot study, they were replaced by cefoxitin, which is also commonly used as an indicator of methicillin resistance, hence MRSA. The prevalence of cefoxitin resistance in community isolates was 69%, which is similar to prevalence of oxacillin resistance observed in isolates from HCW (67%) and clinical isolates (71%). At the opposite end of the spectrum, relatively low levels of resistance were again observed to gentamicin (13%) and ciprofloxacin (24%). In contrast to the HCW study, no intermediate resistance was observed to any of the antibiotics.

Table 4.4: Antibiotic resistance patterns observed when assessing 67 *S. aureus* samples isolated from community hand swabs around the Fort Portal region

	Susceptible	Intermediate	Resistant	% of Resistant Isolates
FOX	21	0	46	69
E	21	0	46	69
TE	24	0	43	64
CN	58	0	9	13
C	13	0	54	81
CIP	51	0	16	24

* FOX – Cefoxitin, E – Erythromycin, TE – Tetracycline, CN – Gentamicin, C – Chloramphenicol, CIP – Ciprofloxacin

4.4 Main Study

4.4.1 Clinical *S. aureus* isolates from FPRRH

With increased laboratory service provision, research was expanded to encapsulate all *S. aureus* isolated at FPRRH, with the majority of samples attributed to maternity, surgical and general wards. The samples collected were predominantly wound (24%) and puss (25%) swabs, but also included blood (11%) and urine (7%) cultures as well as single isolates from ear swab and synovial fluid. It is worth noting that sample records were not always complete when arriving from the ward. The newly developed laboratory followed a regulated set of standard operating procedures for culture and sensitivity testing, further described in Methods. Alongside the clinical isolates, a total of 125 additional *S. aureus* isolates were collected through more swabbing the hands of people in the local community. It is worth noting that the hand swab samples were processed in a different laboratory to those of the clinical isolates as noted in Methods.

4.4.2 Clinical Isolates from FPRRH Demonstrate Diverse and Prevalent Multi-Drug Resistance

The results of disk diffusion assays performed in the FPRRH diagnostics lab (Table 4.5) indicated the strongest performing antibiotic was clindamycin with no resistance observed. This was followed by chloramphenicol, gentamicin and ciprofloxacin with clinical isolates collectively exhibiting similar rates of resistance at 10%, 18%, and 20% respectively. By contrast, the largest frequencies of resistance were observed to tetracycline (55%), followed by cotrimoxazole (46%). With cefoxitin being utilised as a marker for MRSA it was

unfortunate that laboratory stock-outs during the upscaling of practices resulted in a low number of isolates being tested. That being said, 38% of isolates tested (8/21) were found to be resistant, indicating a high presence of MRSA amongst the samples. 36% (25/70) of isolates were susceptible to all antibiotics tested, 39% (27/70) were resistant to one or two antibiotics, whilst 26% (18/70) were MDR.

Table 4.5: Antibiotic resistance patterns of *S. aureus* clinically isolated from all wards at FPRRH

Staphylococcus aureus (n=70)				
Antibiotic agent	**Susceptible**	**Intermediate**	**Resistant**	**Resistant (%)**
Chloramphenicol (n=59)	53	0	6	10
Gentamicin (n=68)	54	2	12	18
Cefoxitin (n=21)	13	0	8	38
Trimethoprim/sulfame (n=57)	25	6	26	46
Ciprofloxacin (n=69)	51	4	14	20
Clindamycin (n=62)	62	0	0	0
Erythromycin (n=69)	47	1	21	30
Tetracycline (n=53)	24	0	29	55

Given the resistance patterns recorded in the pilot study with resistance to clindamycin observed in 4/7 (57%) *S. aureus* isolates, it was surprising to observe no resistance in the clinically acquired isolates as part of the main study ($\chi^2 = 42.2$, $P<0.01$). However, the sample sizes being compared are relatively small. Gentamicin being the second most effective antibiotic falls in line with previous expectations, although the resistance shown in patient cases (16%) was significantly higher than that of isolates from the pilot where 100% of isolates were susceptible ($\chi^2 = 22.0$, $P<0.01$). Tetracycline

effectiveness also showed more consistency with relatively high resistance recorded in 43% of cases in the pilot study.

4.4.3 *S. aureus* Isolates from The Local Community Demonstrate Prevalence of Clindamycin Resistance not Observed in Clinical Isolates.

Observing the resistance profiles of *S. aureus* found on the hands of healthy members of the public within the catchment area of FPRRH highlights how AMR is not a problem reserved exclusively to hospitalised patients.

Table 4.6: Antibiotic resistance patterns of *Staphylococcus aureus* isolated from hand swabs of the general public

Staphylococcus aureus (n=125)				
Antibiotic agent	**Susceptible**	**Intermediate**	**Resistant**	**Resistant (%)**
Chloramphenicol (n=124)	87	0	37	30
Gentamicin (n=123)	116	0	7	6
Cefoxitin	98	0	27	22
Trimethoprim/sulfame	54	4	67	54
Ciprofloxacin	81	0	44	35
Clindamycin (n=124)	24	53	47	38
Erythromycin (n=124)	39	28	57	46
Tetracycline	57	31	37	30

18% (22/125) of isolates were susceptible to all antibiotics tested, 34% (42/125) were resistant to one or two antibiotics, whilst 49% (61/125) were MDR – a value significantly higher than that observed from the clinical isolates ($\chi^2 = 9.9$, $P<0.01$). In line with the pilot study, Table 4.6 shows high levels of resistance to all antibiotics tested with

the exception of gentamicin at 6%. Moreover, resistance to clindamycin, which was shown to be 100% effective against isolates obtained clinically at FPRRH, was significantly higher within the local community at 38% (χ^2 = 69.5, P<0.01). That being said, cefoxitin resistance in the community, whilst still being high (22% isolates), was lower than observed amongst the clinical isolates (38%), although not significantly so (P=0.1). Similarly, reduced prevalence of ciprofloxacin and tetracycline resistance, whilst not statistically significant, were observed within the community isolates (35% and 30% respectively).

Table 4.7: A comparison of the antimicrobial resistance observed (%) in *S. aureus* clinical isolates from FPRRH and community isolates from around the Fort Portal region

Antibiotic agent	Resistance observed in clinical isolates (%)	Resistance observed in community isolates (%)
Chloramphenicol	10	30
Gentamicin	18	6
Cefoxitin	38	22
Trimethoprim/sulfame	46	54
Ciprofloxacin	20	35
Clindamycin	0	38
Erythromycin	30	46
Tetracycline	55	30

4.4.4 Assessing the genotypic resistance profiles of 41 clinical isolates from FPRRH

Whole genome sequencing data of 41 clinical isolates from FPRRH was utilised to assess the presence of genetic markers of resistance. Genotypic and phenotypic predictions of susceptibility to each antibiotic are shown in Figure 4.1 below.

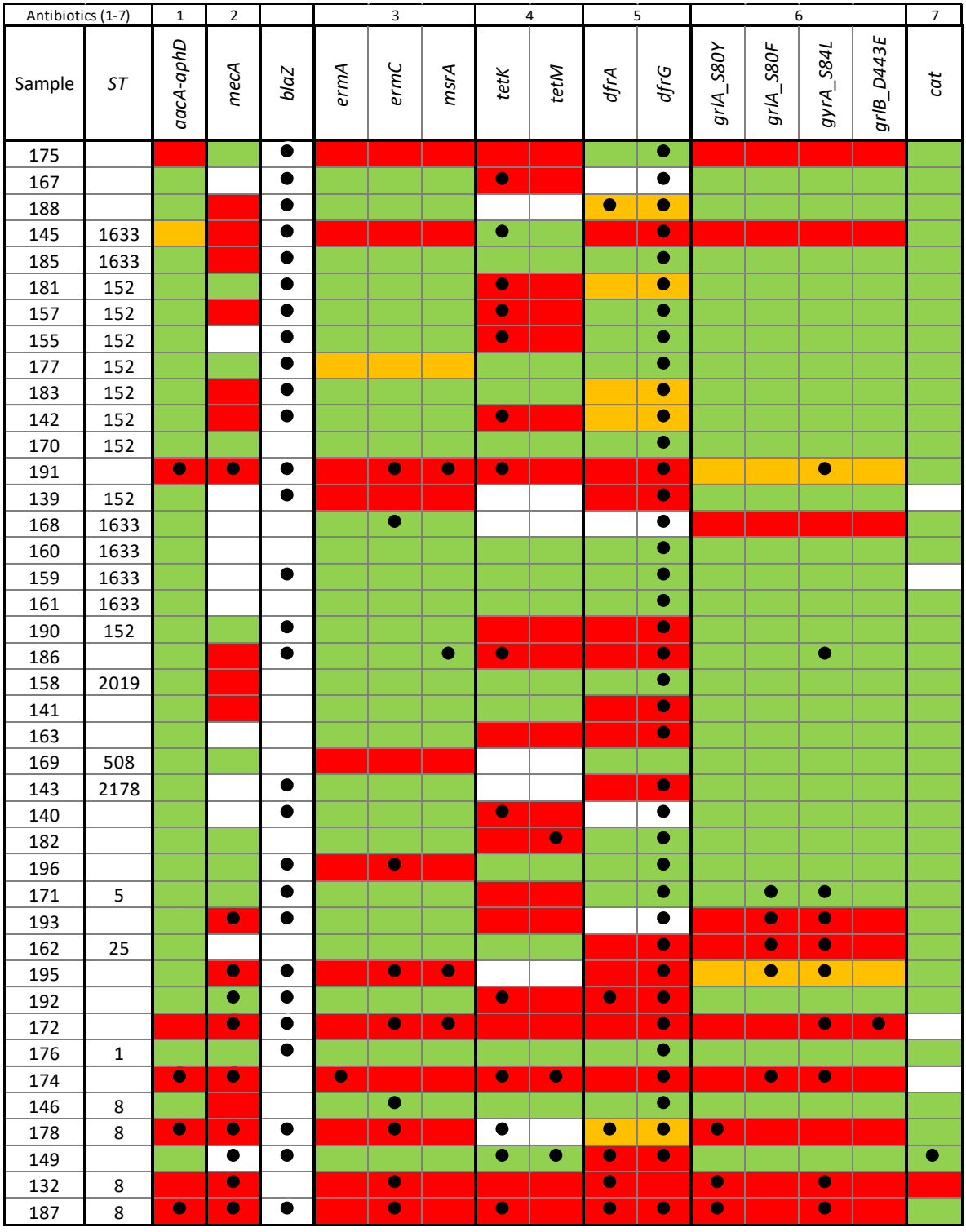


Figure 4.1: Comparing phenotypic antimicrobial susceptibility testing data with predicted genotypic resistance. Cells highlighted green, orange and red correspond to susceptible, intermediate and resistant isolates respectively, with resistance gene presence signified by a black dot. Cells highlighted white have no associated phenotypic susceptibility data available. Antibiotics utilised for susceptibility testing: gentamicin (1), cefoxitin (2), oxacillin (2), erythromycin (3), tetracycline (4), co-trimoxazole (5), ciprofloxacin (6) and chloramphenicol (7). Samples are listed in relation to their core genome phylogeny as described in Chapter 3.

4.5 Discussion

4.5.1 The Pilot Study highlighted challenges working with laboratory and equipment constraints

Together, the pilot studies highlighted multiple issues that needed to be addressed moving forwards, the majority of which were rectified through the refurbishment and development of laboratory facilities. Prior to this, access to the laboratory was limited with support staff frequently unavailable. Additionally, there was an extremely low uptake of samples from the respective maternity wards. Where samples were taken, they were often not transported to the laboratory in a timely manner, despite being just a short distance away. Furthermore, reagents were frequently unavailable and had to be acquired privately. Once the laboratory renovations had been finalised these processes were streamlined and became much more efficient. Not only were facilities improved for enhanced testing with their respective uniform reagents, but additional staff were hired to cope with the increased service demand. These factors were taken into account when developing the main study.

It is worth noting that the assessment of community isolates was done in a different lab to those of the patient isolates, with two antibiotics included in the clinical panel not included in the panel used to screen the community isolates. Hence it is possible that some of the differences observed were due to alternative brands/batches of antibiotics in addition to less equipped storage facilities at the laboratory where the community isolates were assessed, leading to a possible reduced effectiveness of the antibiotic disks over time.

4.5.2 The presence of *tetK* and *tetM* genes accounted for 84% of tetracycline resistance phenotype

Tetracycline was the least effective antibiotic observed in this study against the clinical isolates tested where 56% (19/34) displayed resistance. This figure is however similar to multiple studies at Mulago Hospital where resistance rates of 36%, 42%, 45% and 44.1% have been reported when investigating *S. aureus* isolated from surgical site infections and blood cultures (Kajumbula et al., 2018; Jeremiah Seni et al., 2013; Tumuhamye et al., 2020; Wekesa et al., 2020). A recent review of two hospitals in Eastern Uganda (Mbale and Soroti) observed a much higher level of tetracycline resistance at 85.6% (Obakiro et al., 2021). Whilst the study had a much larger sample size (376 isolates between January 2016 and December 2018) it was the only study in Uganda reporting such a high level of clinical resistance, the second highest being 68% among environmental/carriage isolates (Kateete et al., 2011), with a third matching this study at 55% resistance evaluating *S. aureus* isolated from the eye swabs of patients at Mulago Hospital (Mshangila et al., 2013).

Genomic analysis through Pathogenwatch successfully predicted 63% of resistant isolates (12/19) and 87% of susceptible isolates (13/15). Tetracyclines act through targeting of the 30S bacterial ribosome subunit, with resistance occurring through two core pathways; active efflux of the antibiotic and ribosomal protection proteins. Translation of all these proteins are controlled by a number of *tet* genes, with Pathogenwatch utilising specific genes *tetK* (active efflux) and *tetM* (ribosomal protection protein) as markers of resistance. Roary analysis was able to detect the presence of *tetK* in a further four isolates (18 total), three of which were phenotypically resistant with the remaining isolate having no AST data. Similarly, it was able to detect an additional isolate harbouring *tetM* (4 total)

which was also phenotypically resistant – this increased the percentage of successfully predicted resistant isolates to 84% (16/19). It is worth noting that the presence of tetM in CC152 isolates was significantly lower than that of non-CC152 isolates (χ^2 =4.2, P=0.04). Whilst these are the primary factors in *S. aureus*, it is not uncommon for others to be present (Chopra & Roberts, 2001) which may account for some discrepancy between phenotypic and genotypic analysis. Specifically, *tetL* and tetO act in the same manner as *tetK* and *tetM* respectively in *S. aureus*. Resistance correlation between WGS and AST data has been reported as high as 100% (Kumar et al., 2020), with others reporting 83% correlation of resistance phenotypes by the presence of *tetK* and *tetM* genes (Khoramrooz et al., 2017). Further Roary analysis was unable to detect the presence of *tetL* or *tetO*, it did however identify 38 isolates harbouring *tet(A)* and 40 isolates harbouring *tet(38)* – both tetracycline efflux proteins present in *S aureus*. That being said, their presence in such a high number of isolates highlights a weak association with phenotypic resistance.

Whilst *tetM* has the potential to convey a wider spectrum of resistance to tetracyclines (Chopra & Roberts, 2001), it was only found in 10% (4/41) isolates compared to tetK which was present in 44% (18/41). A recent study in Kenya also found *tetK* to be the principal mediator of resistance (Kyany'a et al., 2019).

4.5.3 Trimethoprim/Sulfamethoxazole (Co-trimoxazole) resistance was only partially explained by the presence of *dfr* resistance markers

The second highest level of resistance witnessed within this study was cotrimoxazole at 46%, a figure lower than all other recent clinical studies observed across Uganda with rates of 100%, 74%, 88%, 89%, 97.1% and 88% (Kityamuwesi et al., 2015; Obakiro et al.,

2021; Odongo et al., 2013; Ojulong et al., 2009; Jeremiah Seni et al., 2013; Wekesa et al., 2020). These high figures are thought to be linked to the use of cotrimoxazole as a prophylactic to prevent secondary bacterial infections in HIV patients, the rate being very high in Uganda (Marwa et al., 2015; William et al., 2012) and highlights the need for wider study and control of other antibiotics showing decreased efficacy. Why the rate is much lower in Fort Portal however is not clear seeing as the region is one the most severely affected by HIV in Uganda with prevalence rates as high as 16% (Schumann et al., 2020).

Co-trimoxazole is interesting as it consists of two antibiotics functioning independently to inhibit thymidine synthesis. Of the 37 sequenced isolates tested, 16 (43%) were resistant, 5 (14%) showed an intermediate result and the remaining 16 (43%) were susceptible. That being said, Pathogenwatch predicted trimethoprim resistance in 98% of isolates (40/41) through the presence of dfrG (39/41) and dfrA (6/41).

Dihydrofolate reductase (DHFR) is the enzyme targeted by trimethoprim and has multiple variations spanning across different bacterial species, with a select few able to confer trimethoprim resistance (Charpentier & Courvalin, 1997). In human clinical infections *dfrA* and *dfrG* gene variants have been observed causing high level resistance (Nurjadi et al., 2014). In this study, however, *dfrG* appeared to be a weak indicator of resistance due to its presence in nearly all isolates. *dfrA* on the other hand, whilst present in only 6 isolates, did always convey at least an intermediate level of resistance. Again, the presence of *dfrA* in ST8 isolates was significantly higher than in non-ST8 isolates (χ^2 =12.9, P<0.01). Others have reported trimethoprim resistant phenotypes where isolates were genotypically predicted to be susceptible, in one case despite the presence of a *dfrA* gene, suggesting that in silico prediction of trimethoprim resistance may require further work (Fowler et al., 2018; Kumar

et al., 2020). It must be highlighted, however, that for this study phenotypic resistance was assessed utilising co-trimoxazole, whilst sulfamethoxazole isn't genotypically scrutinised in Pathogenwatch. Sulfamethoxazole targets dihydropteroate synthase with resistance tied to mutations/variations of the *dhps* gene (Eliopoulos & Huovinen, 2001). As such, *dfrG* may have played a more important role had trimethoprim been individually phenotypically assessed.

Co-trimoxazole has been widely successful as a second antibiotic of choice in treating MRSA infections in Europe and North America, with rising levels of resistance in Africa being a cause for concern (Nurjadi et al., 2014). Its overuse in lower-income countries stems from its cheap cost, oral administration and recommendation by the WHO for its prophylactic use in HIV-infected/exposed patients against secondary infections (Coelho et al., 2017). As such increasing levels of *dfrG*-related trimethoprim resistance are of clinical significance due to their presence on transmissible mobile genetic elements and potential global export out of Africa, highlighting the need for wider study and control of other antibiotics showing decreased efficacy (Nurjadi et al., 2014).

4.5.4 Inadequate Supplies of Cefoxitin and Oxacillin Disks Resulted in Missed Reports of MRSA

Empirical treatment (ceftriaxone + metronidazole) is prescribed for suspected infections at FPRRH. Whilst metronidazole covers anaerobic bacteria, ceftriaxone is a broad-spectrum cephalosporin that is well tolerated and commonly used to effectively treat infections caused by most aerobes including methicillin susceptible *S. aureus* (MSSA). Cefoxitin plays an important role in AMR surveillance as it acts as a proxy for determining

susceptibility to all penicillins and the majority of cephalosporins and carbapenems, thus reporting incidence of MRSA (EUCAST, 2021). Unfortunately, due to the nature of working in a resource-constrained facility, stocks of cefoxitin disks were poorly maintained at FPRRH during the study period and 28 isolates were not able to be tested. 16 of these missing isolates were tested against oxacillin, another marker for MRSA. The observed frequency of MRSA at FPRRH (38%) suggests a potentially high level of failure of the empirical treatment, and the need for informed decisions about procurement of alternative antibiotics. MRSA prevalence was similar or higher than those reported elsewhere (Kampala: 37.5% (J. Seni et al., 2013), 29% (Ojulong et al., 2009) and 28% (Mshangila et al., 2013); Mbale: 27% (George et al., 2018) and Gulu: 22% (Odongo et al., 2013)). That being said, more recent studies have observed similar and much higher rates relative to FPRRH of 33%, 44.4% and 91.2% (Johnson et al., 2021; Obakiro et al., 2021; Wekesa et al., 2020). This indicates an alarming trend of increased resistance. Whilst the Ugandan national action plan (UNAP) reports prevalence of MRSA in healthcare settings at 2-50% (L. Ackers et al., 2020), the WHO AMR global report on surveillance (2014) found overall MRSA prevalence to range from 12-80% across Africa and to exceed 20% in all six WHO regions (World Health Organisation, 2014). There have been reports of more than 80% MRSA prevalence in African, American and Western Pacific regions (World Health Organisation, 2014).

Present in 71% of isolates (30/41), the *blaZ* gene is responsible for the production of penicillinase, a β-lactamase (Takayama et al., 2018) conferring resistance to a range of penicillins, including ampicillin and amoxycillin. Whilst the majority of *S. aureus* show penicillinase activity (EUCAST, 2021), these results are of particular importance as antibiotic prescription practices at FPRRH have until very recently involved the widespread use of

amoxycillin and ampicillin. Further compounding the issue is their use in the public community, where such antibiotics are available over-the-counter at local pharmacies and used preferentially for a wide range of self-diagnosed ailments (L. Ackers et al., 2020).

β-lactamases initially rose to prominence through their ability to inactivate penicillins through hydrolysis of the β-lactam ring. Later derivatives of the antibiotic, such as oxacillin and cefoxitin, are far more resistant to hydrolysis resulting in the rise of genes like *mecA*. *mecA* facilitates the translation of an alternate PBP, PBP2a, which has a much lower binding affinity to β-lactams, so much so that they confer resistance to all β-lactam antibiotics (Takayama et al., 2018). 24% of the genomes assessed by Pathogenwatch (10/41) carried the *mecA* gene, with one additional isolate identified through Roary analysis. Based on this genetic evidence, the prevalence of MRSA appears slightly lower than reported elsewhere in Uganda (Kampala: 37.5% (J. Seni et al., 2013), 29% (Ojulong et al., 2009) and 28% (Mshangila et al., 2013); Mbale: 27% (George et al., 2018) and slightly higher than in Gulu: 22% (Odongo et al., 2013)). However, AST suggested much higher prevalence of MRSA: 38% of tested isolates were resistant to cefoxitin (5/13) and 81% (13/16) were resistant to oxacillin, the collective total being 62% (18/29). Of the isolates tested for phenotypic sensitivity, all five testing positive for cefoxitin resistance possessed the *mecA* gene, with one susceptible isolate genetically predicted to be resistant. However, whilst three isolates possessing *mecA* also showed phenotypic resistance to oxacillin, 77% of isolates found phenotypically resistant (10/13) had no genetic marker for methicillin resistance. From the isolates not screened for susceptibility to cefoxitin or oxacillin, 8% harboured *mecA* (1/12). Indicating that some incidents of MRSA are going un-detected and un-reported. Population-wise the predominant CC152 isolates harboured *mecA* at a

significantly lower level than non-CC152 isolates (χ^2 =7.96, P=0.05). Conversely, ST8 isolates carried *mecA* at significantly higher rates (χ^2 =6.16, P=0.01), with two further closely related isolates also harbouring the gene.

Without data directly comparing the effectiveness of cefoxitin against oxacillin, drawing conclusions can be difficult when questioning the efficacy of each antibiotic, especially considering the low number of samples. Given that all the aforementioned studies also used oxacillin as a predictor of MRSA, the levels of resistance at FPRRH seem exceptionally high. Additionally, since the presence of *mecA* successfully predicted resistance in all cefoxitin resistant isolates, it seems unlikely it could be so unreliable relative to oxacillin. Oxacillin and cefoxitin are both resistant to hydrolysis by β-lactamases. As such, any resistance present is likely to be conferred by alternate PBPs. Other mechanisms of resistance to oxacillin are rarely reported. The most obvious explanation for these discrepancies would be to question the validity of the antibiotic disks or the results, as oxacillin disk diffusion tests are often not reliable in determining resistance (Center for Disease Control and Prevention, 2019). Molecular analysis of *mecA* is considered the 'gold standard' in identifying MRSA, where *mecA* is highly conserved in the staphylococcal cassette chromosome (SCCmec). That being said, it is feasible that another homologue of the gene, *mecC*, can convey resistance in the same manor, but lacks the DNA sequence similarity to be identified when *mecA* is being targeted. However, *mecC*-encoded PBP2a binds to oxacillin with a stronger affinity than cefoxitin. Hence if *mecC*-encoded resistance was present it is more likely a reduced oxacillin resistance relative to cefoxitin would be observed (Paterson et al., 2014).

In relation to the single isolate that tested phenotypically susceptible to cefoxitin, yet was shown genetically to harbour *mecA*, it is possible that the gene simply wasn't expressed in *vitro*. Known as heteroresistance, two subpopulations of *S. aureus* can coexist within a culture, one resistant and the other susceptible. This has been known to occur in staphylococci resistant to β-lactamases and can cause problems with the accurate detection of methicillin resistance (Center for Disease Control and Prevention, 2019).

4.5.5 Clindamycin and Erythromycin Resistance Genes were Identified in Susceptible Isolates

Clindamycin is another WHO access antibiotic recommended as a second choice for bone/joint infections. It was the only antibiotic in this study to which no isolates showed any phenotypic resistance despite the detection of resistance genes in 12 isolates. Whilst clindamycin has not been used clinically at FPRRH, it has been used elsewhere in Uganda with various levels of resistance reported. A 2014 study based at Mbarara Regional Referral Hospital in South-western Uganda reported 36% of isolates as clindamycin-resistant and also showed discrepancies between phenotypes and genotypic prediction (Mwambi et al., 2014). Clindamycin resistance rates of 0-59% have been reported elsewhere in Uganda between 2009-2018 (Ojulong et al., 2009) and was reported as early as 2008 in Tanzania (Mshana et al., 2009). Interestingly, 30% of isolates were resistant to erythromycin which has overlapping genetic markers of resistance. It is possible that the genes that confer clindamycin resistance are under erythromycin-inducible regulation, and that exposure to clindamycin *in vivo* could select for constitutive expression mutants that would confer resistance to both (Drinkovic et al., 2001). In all cases where data is available erythromycin

resistance is observably then higher clindamycin resistance: 70.6% and 58.8% (Wekesa et al., 2020), 47% and 41% (Jeremiah Seni et al., 2013), 88% and 0% (Ojulong et al., 2009), 73% and 22% (George et al., 2018), 88% and 50% (Kityamuwesi et al., 2015) and 48% and 36% (Mwambi et al., 2014) respectively. Taking these figures into account both levels of clindamycin and erythromycin resistance were lower at FPRRH than elsewhere in Uganda with two clinical studies reporting only clindamycin resistance at 21.5% (Obakiro et al., 2021) and 59% (Kajumbula et al., 2018); and two studies reporting only erythromycin resistance at 45% (Johnson et al., 2021) and 72% (Tumuhamye et al., 2020).

Within this study erythromycin AST data suggested that 71% of sequenced isolates (29/41) were susceptible, with genotypes matching phenotypes in the majority of cases (26/29 susceptible and 8/12 resistant isolates). Three isolates observed to be susceptible did harbour a single resistance gene each (two *ermC* and one *msrA*) Thirteen isolates (32%) were found to be resistant to erythromycin, which is considerably lower than reported by several other Ugandan studies (George et al., 2018; Mwambi et al., 2014; Ojulong et al., 2009; Tumuhamye et al., 2020). There were no significant correlations between different isolate clonal populations amongst any of these three genes, except for *ermC* which was harboured at significantly higher levels in ST8 isolates (4/4) than other populations (χ^2 =18.0, P<0.01). Interestingly ST8 isolates harboured *ermC* exclusively out of the three tested, whilst closely related isolates 149 and 174 harboured no genes and *ermA* respectively.

The relationship between clindamycin and erythromycin is clinically significant as they are part of the MLSB (macrolides, lincosamides and streptogramin type B) group of antibiotics all targeting a similar region of the 50S bacterial ribosome. As such, resistance mechanisms can overlap as a result of structural ribosomal modifications (Dancer, 2014).

The *erm* genes (erythromycin-resistance methylase) are capable of facilitating this process, with *ermA* and *ermC* being the primary feature identified by Pathogenwatch as genetic markers of both clindamycin and erythromycin resistance. Given that 24% of isolates harboured *ermA* or *ermC* (1/41 and 9/41 respectively) it is surprising that no clindamycin resistance was phenotypically observed, especially where erythromycin resistance was present. This is likely explained by the inducible nature of the *erm* genes. Erythromycin has been shown to be a strong inducer of *erm* genes, with clindamycin being so to a much lesser degree. Even though the gene itself is present, in the presence of clindamycin it fails to be expressed at a sufficient level to promote the structural ribosome modifications required for resistance. That being said, care must be taken where the bacteria has been previously exposed to erythromycin as induction of the respective genes can lead to clindamycin resistance. Additionally, regular exposure to clindamycin *in vivo* could lead to bacterial mutants with constitutive expression (Drinkovic et al., 2001). This could be investigated further using the D test (Tankeshwar, 2021) which would determine whether clindamycin resistance was inducible in apparently susceptible isolates. If this were the case, it would not be advisable to begin prescribing clindamycin.

Despite being the most prevalent mechanism of resistance against these antibiotics, there are other factors to consider in addition to *ermA* and *ermC* when evaluating the phenotypic and genotypic discrepancies observed. Firstly, there are other *erm* genes that may have gone undetected, for example *ermB* and *ermF*, which although uncommon function in a similar fashion (Ghanbari et al., 2016). Secondly, other mechanisms involving enzymatic inactivation through *ereA* and *ereB* and antibiotic efflux through genes including *msrA* may be involved (Dancer, 2014). Of these, only *msrA* was highlighted by Pathogenwatch (the others were also absent when utilising Roary analysis) and was present

in 10% of isolates (4/41). The impact of this efflux protein is questionable however, as its presence in three resistant isolates coincided with the presence of *ermC*, with the single isolate remaining being phenotypically susceptible where no other genetic resistance indicators were present. It is therefore clear that erythromycin/clindamycin resistance is a complex issue that requires ongoing monitoring given the potential to expanded resistance.

4.5.6 Gentamicin Resistance was not Fully Explained by Genome Data

Gentamicin is on the WHO list of access antibiotics, recommended for use as an empirical treatment when others have failed due to its wide range of activity and reduced resistance potential (Ojulong et al., 2009). Gentamicin is one of the more commonly used aminoglycosides at FPRRH (L. Ackers et al., 2020), where 17% (7/41) of the sequenced isolates tested were resistant, and although resistance rates were observed to be low relative other antibiotics tested it still poses cause for concern. Whilst several studies report similar levels of resistance ranging from 6%-24% (Kityamuwesi et al., 2015; Odongo et al., 2013; Jeremiah Seni et al., 2013; Tumuhamye et al., 2020), an equal number of generally more recent studies report much higher figures ranging from 30%-83.2% (George et al., 2018; Johnson et al., 2021; Kajumbula et al., 2018; Obakiro et al., 2021; Ojulong et al., 2009; Turyasiima et al., 2020; Wekesa et al., 2020) Despite these observations, the specific gentamicin resistance gene *aacA-aphD* was only found by Pathogenwatch in four of the *S. aureus* genomes. These four isolates all showed phenotypic resistance implying the gene was being functionally expressed. Alternatively, all isolates which tested phenotypically susceptible had no observed *aacA-aphD* gene presence. That being said, Roary analysis identified a fifth isolate (192) carrying the gene which was phenotypically susceptible.

Aminoglycoside acetyltransferase (AAC(6')) and aminoglycoside phosphotransferase (APH(2")) enzymes both confer resistance to multiple aminoglycoside antibiotics, with AAC responsible for tobramycin and kanamycin and APH for gentamicin resistance (Rouch et al., 1987). These enzymes form the primary means of glycoside resistance through antibiotic inactivation, with the gene *aacA-aphD* first located on a transferable plasmid in a sequence now designated as transposon Tn*4001* (Udou, 2004). However, this does not explain the phenotypes of the other 3 resistant isolates, and a 4th exhibiting an intermediate response, none of which harboured any of the expected genetic associations. Further Roary analysis did identify the presence of *aphA* (aminoglycoside-3-phosphotransferase) in isolate 174 where *aacA-aphD* was already present, in addition to *aadK* (aminoglycoside adenyltransferase) in five isolates (149, 172, 176, 192 and 195). Out of these five only isolate 176 was phenotypically resistant showing the gene to be a weak indicator. As previously mentioned, there is a diverse range of less prevalent resistance mechanisms which may be responsible, these include the modification and/or mutation of the ribosomal target, decreased permeability of the cell wall and active transport of aminoglycosides out of the cell; although such mechanisms are predominantly observed in non-*S. aureus* species (Garneau-Tsodikova & Labby, 2016). When assessing the presence of *aacA-aphD* as described by Pathogenwatch there was no clear correlation amongst CC152 and non-CC152 isolates. However, the gene was significantly more prevalent amongst ST8 isolates relative to the others (χ^2 =8.15, P=0.004). In addition, whilst isolate 174 had no designated sequence type, it was still closely related to ST8 phylogenetically and also harboured *aacA-aphD*. Conflicts between genotype and phenotype have been reported previously (Davis et al., 2011), but may also be representative of the short read sequencing used in this study,

analysis of which could have missed other genetic markers of resistance and which could be clarified using long-read technologies or complementary techniques.

4.5.7 Isolates Harboured the Least Genetic Markers of Resistance to Chloramphenicol

Similar to gentamicin, chloramphenicol is another access antibiotic with a low level of resistance observed at FPRRH. With 10% of isolates exhibiting resistance, it was the lowest rate recorded amongst recent clinical studies where lower values lay around 14%-24% (Kityamuwesi et al., 2015; Jeremiah Seni et al., 2013; Tumuhamye et al., 2020; Wekesa et al., 2020) and higher values ranged from 48.5%-88% (Johnson et al., 2021; Kajumbula et al., 2018; Obakiro et al., 2021; Ojulong et al., 2009). From the 41 isolates sequenced as part of this study, only a single isolate displayed phenotypic resistance. Additionally, utilising CARD analysis only a single isolate predicted to carry the chloramphenicol acetyltransferase (*cat*) gene, with Roary analysis picking up an extra isolate for a total of two.

Whilst overall phenotypic and genotypic analysis of chloramphenicol susceptibility were largely in agreement, the sole phenotypically resistant isolates did not match either of the two genetically predicted ones. The *cat* gene encodes chloramphenicol acetyltransferase which transfers an acetyl group to directly inactivate chloramphenicol (Sköld, 2011), is used as the genotypic resistance predictor through CARD analysis. In relation to the falsely positive isolates, it is possible that the gene was present but simply was not being expressed in vitro. A study in the DRC had a similar concordance of 97% when observing *cat*-related chloramphenicol resistance (Phaku et al., 2016). Isolate 132 was phenotypically resistant despite lacking the *cat* gene. Here resistance could be due to the

action of relatively novel genes *cfr* and *fexA*, an rRNA methyltransferase and florfenicol efflux pump respectively (Wu et al., 2021). *cfr* is of particular importance as it has frequently been detected in MRSA from both animals and humans (Li et al., 2018). Additionally, through its action of modifying the 23S rRNA it can convey resistance to four other classes of antimicrobial agents due to overlapping binding sites including phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A compounds (Shore et al., 2016). This is a cause for concern as isolate 132 is not only the sole isolate resistant to chloramphenicol, but also shows phenotypic resistance to all other antibiotics tested except clindamycin (which may be inducible as it is resistant to erythromycin) and harbours *mecA* and *ermC*. Hence it is clear that chloramphenicol resistance should be closely monitored both phenotypically and genotypically.

4.5.8 Ciprofloxacin Resistance Strongly Correlates with MRSA

Ciprofloxacin (a fluoroquinolone) appears on the WHO watch list of essential antibiotics that are considered at high risk of selecting for further bacterial resistance, and should be prioritised for stewardship programmes (World Health Organisation, 2021). Rates of resistance vary across other Ugandan studies, most of which report higher rates of ciprofloxacin resistance than at FPRRH ranging from 28% - 71% (Johnson et al., 2021; Kajumbula et al., 2018; Mshangila et al., 2013; Obakiro et al., 2021; Odongo et al., 2013; Ojulong et al., 2009; Jeremiah Seni et al., 2013; Wekesa et al., 2020). Only one study investigating oral infections observed a lower rate ciprofloxacin resistance at 12% (Kityamuwesi et al., 2015).

A total of 24% (10/41) of sequenced *S. aureus* isolates were phenotypically resistant to ciprofloxacin and two more exhibited intermediate resistance. The presence of resistance was correctly predicted genotypically for 70% (7/10) of isolates, with susceptibility correctly predicted for 93% (27/29) of isolates. Both intermediate isolates harboured resistance genes.

Resistance to ciprofloxacin occurs in a more linear fashion in relation to other antibiotics, with the accumulation of multiple single mutations gradually improving bacterial survivability at each point (Pietsch et al., 2017). However, resistance is still capable of rapidly developing through this pathway (Blumberg et al., 1991). Resistance was genetically predicted by Pathogenwatch through analysis of *grlA*, *gyrA* and *grlB* genetic variants, where the *grlB_D443E* variant was found in only a single isolate as opposed to eight *grlA* and ten *gyrA* variants. This is in agreement with Tanaka *et al*. (2000) who stated that *gyrB* and *grlB* were involved in resistance to a much lesser extent. Another study in the Democratic Republic of the Congo found only a loose concordance of 78% when observing levofloxacin, a value similar to these results (Phaku et al., 2016).

It is also worth noting that ciprofloxacin resistance can be attributed to the efflux protein NorA (Neyfakh et al., 1993), which is not described by Pathogenwatch. Whilst this mechanism is far less prevalent it could have a small impact the phenotypic resistance observed. Roary analysis also failed to identify the presence of *norA*; it did however reveal all isolates possessed *norB*, responsible for the production of MDR efflux protein NorB. NorB has roughly 30% structural similarity to NorA and its overexpression is associated with increased resistance to NorB substrates including fluoroquinolones and tetracycline (Santos Costa et al., 2013). Given that only 24% of isolates were phenotypically resistant it is evident

that *norB* either was not being expressed in high amounts or is a very weak genetic indicator of resistance.

Also of importance is how ciprofloxacin resistance correlates with MRSA, with previous studies having observed a substantially lower rate of ciprofloxacin resistance amongst MSSA isolates relative to MRSA (Kwak et al., 2013). This study observed similar results with 80% of *mecA* positive isolates also harbouring ciprofloxacin resistance genes, a significantly higher value than the 10% of *mecA* negative isolates (χ^2 =19.05, P<0.01). The relationship between these factors also plays out in regards to population dynamics, where CC152 isolates harbour significantly less and ST8 isolates harbour significantly more ciprofloxacin resistance genes in relation to other isolates (χ^2 =5.63 and 5.24, P=0.02 and 0.02 respectively).

4.5.9 *S. aureus* multi-drug resistance in the context of clinical settings across Uganda and SSA

The observed levels of resistance and MDR in this study are not unexpected; for example, Seni and colleagues (J. Seni et al., 2013) reported 21% of Gram-positive isolates from surgical site wounds of patients in Mulago Hospital, Kampala (Uganda) were MDR, whereas Kateete and colleagues (Kateete et al., 2020) observed 31% of *S. aureus* isolates recovered from the nasopharyx of children in rural Eastern Uganda were MDR. A study of post-operative sepsis cases in rural Eastern Uganda (George et al., 2018) reported 14% *S. aureus* isolates to be resistant to five different classes of antibiotics and 3% resistant to six different classes (George et al., 2018). All eight MRSA isolates were MDR; Kateete and

colleagues (Kateete et al., 2011) also observed markedly higher rates of MDR among MRSA strains compared to MSSA strains.

4.5.10 Isolates Acquired from Community Hand Swabs Show Different Resistance Profiles than Clinical Isolates.

Whilst the study of asymptomatic *S. aureus* carriage is less well documented than clinically obtained isolates, several recent studies have been facilitated through which to draw comparisons. Testing against the same panel of antibiotics minus clindamycin Kateete et al. (2020) observed much lower levels of resistance in *S. aureus* carried by children in Eastern Uganda ranging from 2%-29%. Only resistance rates of 29% and 16% for cefoxitin and gentamicin respectively were higher than observed in this study. Another study of healthcare workers in Kampala tested only three comparable antibiotics; ciprofloxacin, cotrimoxazole and clindamycin with resistance rates of 55%, 41% and 8% respectively (Abimana et al., 2019). Of note is the comparable level of cotrimoxazole resistance to this study, whereas previously noted multiple clinical studies found much higher rates. An investigation facilitated at a Nigerian University (Adeoye-Isijola et al., 2020) bares the most resemblance to the community cohort investigated as part of this study with generally lower but largely similar levels of resistance ranging from 7.3%-43.4%. Again, cotrimoxazole resistance was much lower than observed clinically (39.5%) with the most effective antibiotics being chloramphenicol and gentamicin (7.3% and 12.5% respectively).

With high levels of resistance present in the community relative to those clinically isolated, the requirement for effective IPC is further emphasised to reduce the incidence of infection from harmful resistant bacteria already present on skin. This raises questions about

the role that attendants (hospital visitors) may play as vectors of infection on the wards especially when they are involved in clinical roles (wound and canula management) and the impact of stock-outs of iodine on surgical safety practices in theatre (Ackers *et al* 2020). Hsieh *et al* (2014) generated a theoretical model to predict the impact of hospital visitors on nosocomial transmission and spread to the community concluding that, 'transmission rates of infective residents in the community and of infective visitors at the healthcare facility have a decisive impact on disease eradication/persistence'. These concerns are emerging in current research on COVID-19 (Halbfinger, 2020; Hsu *et al*, 2020; Wee *et al*, 2020).

4.5.11 ST8 Isolates Harbour Significantly More Resistance Genes than CC152 Isolates

With 20/41 isolates classified as CC152 they formed the predominant clonal grouping within this data set. As highlighted in Figure 4.1, with the exception of *dfrG* and *blaZ* found in high levels across all populations, genetic markers of resistance observed were far less prevalent amongst CC152 isolates, the most prevalent being *tetK* which bore no significant difference to non-CC152 isolates. Whilst this result is largely positive in terms of hospital treatment regimes, a single isolate (191) belonging to this cluster did harbour eight genetic markers of resistance conveying resistance to gentamicin, cefoxitin, erythromycin, tetracycline, cotrimoxazole and an intermediate response to ciprofloxacin. Given the dominance of this clonal complex it is vital that these genetic markers are monitored to avoid further transmission. Of equal importance is the highly resistant nature of ST8, which possesses significantly higher levels of nearly all genetic markers assessed in relation to other isolates. Initially isolated and observed widely across USA and Europe, populations of ST8 and other closely related sequence types have been on the rise across sub-Saharan Africa (Chambers & Deleo, 2009). That being said, with only four ST8 isolates identified and

even fewer of the remaining sequence types it can be hard to draw reliable conclusions, stressing the need for continued data collection and evaluation.

4.5.12 The clinical value of phenotypic and genotypic AMR data

When evaluating both phenotypic and genotypic data it is important to consider its practical applications at the hospital. One facet of this is the presentation of information on bacteria exhibiting an intermediate level of resistance to an antibiotic, which can have one of several clinical implications. An intermediate response correlates to an uncertain therapeutic effect of the drug at a given concentration. *In vitro* antibiotic efficacy can be easily assessed, *in vivo* on the other hand requires more factors to be considered. With antibiotics being utilised for a variety of conditions, sometimes the target bacteria may be easily accessed (e.g. the urinary tract), other times they are more difficult to reach (e.g. the meninges). Equally, antibiotic concentrations can be different in different tissues and organs (Rodloff et al., 2008). In these situations, the half-life of the drug and the type of infection must be taken into account to ensure the appropriate dosage is prescribed.

An intermediate response can also signify the generation of resistance via two different pathways. Firstly, a series of stepwise mutations can occur gradually improving bacterial survivability at each point until an intermediate and subsequent resistant response is achieved (Pietsch et al., 2017). Alternatively, the bacterial population may be heterogenous, where different subpopulations of bacteria are resistant or susceptible. Treatment strategies for these can vary in a clinical setting. An increased dosage may be able to successfully treat an individual isolate that has only built up partial resistance; conversely, where heterogenous populations are involved continued use of the antibiotic at

any dosage will only enhance selection for resistant subpopulations causing increased problems further down the line (Liu & Chambers, 2003).

The ability to evaluate genotypic data provides essential information to enhance our understanding of phenotypic patterns, case in point being the ability to detect whether resistance is a result of the accumulation of a series of mutations or the consequence of a single gene – each requiring different treatment strategies. It can be equally useful to visualise the prevalence of resistance genes where isolates are phenotypically susceptible, potentially foreshadowing a future rise in phenotypically resistant isolates. As such different antibiotics can be pre-emptively prescribed. Genetic analysis also has the power to reveal co-resistances not assayed on standard drug panels including disinfectants and heavy metal resistance (Hendriksen et al., 2019) leading to more expansive policy changes.

4.6 Conclusions

4.6.1 Whole Genome Sequencing was an Effective Tool for Evaluating Hospital AST Data

Overall, the WGS data provided a more detailed view of the AMR mechanisms circulating in *S. aureus*-associated infections at FPRRH, although susceptibility was more accurately predicted than resistance. Given the non-contiguous nature of the draft genomes this could be due to a lack of complete gene coverage enabling confident prediction of gene presence or absence. Complete genomes, such as using PacBio or Oxford Nanopore sequencing would increase confidence in prediction, but the additional effort may not be cost-effective in low-middle income resource settings. These data are nevertheless useful for in-filling missing information from AST data due to the poor supply of appropriate

materials, towards informing more-effective treatment of *S. aureus* infections at FPRRH. In particular, the data identified *mecA* in isolates that were not screened for cefoxitin resistance. This highlights the need for improved surveillance at a local level. Clear knowledge of circulating resistance mechanisms could promote evidence-based decisions for procurement of antibiotic disk supplies as well as antibiotics for treatment.

4.6.2 Pathogenwatch and Roary Analysis Largely in Agreement

For the purposes of this study Pathogenwatch was utilised as the core means of evaluating any genetic markers of resistance present, with Roary analysis providing supplementary information. Regarding the genes tracked by Pathogenwatch, Roary analysis frequently identified the same isolates, occasionally picking up one extra which Pathogenwatch had missed. Whilst Roary analysis provided a far more comprehensive repertoire of genes, these often did not translate to meaningful correlations with phenotypic AST data. For example, several multidrug efflux pumps/proteins were identified linked to resistance including *bmrA*, *mdtG*, *mdtH*, *mdtD*, *ykkC* and *emrY* (putative). *bmrA*, *mdtG*, *mdtH*, *mdtD* and *emrY* were present in upwards of 90% of all isolates resulting in a poor correlation with phenotypic data; on the other hand, *ykkC* was present in five isolates although only alongside other resistance gene markers notified by Pathogenwatch. Whilst it is possible that such genes contribute towards resistance and can provide further insight into the data observed, it can be difficult to directly attribute a cause and effect.

Chapter 5 – Instigating Healthcare Policy Change and Antimicrobial Stewardship at Fort Portal Regional Referral Hospital as part of a Global Health Project

5.1 Introduction

5.1.1 Defining Global Health

Global health is widely seen as an amalgamation of public health and international health, which itself evolved from hygiene and tropical medicine. The term has since proved popular in stimulating a great deal of interest across media, student and faculty institutions alike (Koplan et al., 2009). A more formal definition from the Institute of Medicine's Expert Committee on the U.S Commitment to Global Health (Fineberg & Hunter, 2013) states:

"*Global health is the goal of improving health for all people in all nations by promoting wellness and eliminating avoidable diseases, disabilities, and deaths. It can be attained by combining clinical care at the level of the individual person with population-based measures to promote health and prevent disease. This ambitious endeavour calls for an understanding of health determinants, practices, and solutions, as well as basic and applied research concerning risk factors, disease, and disability*."

As part of its evolution from international health, global health has typically seen a core focus on population-based preventative strategies with an emphasis on poorer, vulnerable and underserved populations. As such the majority of global attention and interest has centred around infectious diseases and maternal and child health. That being said, health issues related to other populations including smoking, lack of physical activity, stress and mental health can't be neglected (Koplan et al., 2009). It is also worth noting that

combining reduced mortality rates with increased life expectancy will lead to an ageing world population, bringing with it its' own implications for disease and global health in the future (Fineberg & Hunter, 2013).

5.1.2 Healthcare Systems as Mixed Economies

Healthcare systems are an essential component through which improvements to global health can be enabled and can broadly be viewed as facilitating one of two primary objectives; providing primary care in the form of quality, safe, accessible, affordable and equitable health services, for example treatment of injury and disease; and secondly, improving health and health-related services, for example education and clean water / sanitation (Nicholson et al., 2015).

When it comes to the provision of healthcare, services are typically delivered through facilities with one of three generic types of ownership: public, private for profit (PFP) and private not for profit (PNFP). Public health systems are owned and administrated by the government, with funding coming primarily from public sources. Conversely, PFP institutions are owned privately by investors/ shareholders who seek to generate profits for the aforementioned parties involved. Finally, PNFP organisations are typically run by a committee of members, be it for religious, charitable, educational, scientific or benevolent purposes. With the goal of economic sustainability all profits are invested back into the institution for the improved provision of care (Gray & McNerney, 1986).

In low- and middle-income countries healthcare is more typically delivered via a mixed health system, which Nishtar (2007) defines as a health system in which out-of-

pocket payments and market provision of services predominate as a means of financing and providing services in an environment where publicly financed government health delivery coexists with privately financed market delivery. In such cases the local healthcare systems can suffer because of three interplaying factors which undermine the quality and equity of public healthcare provision (Nishtar, 2010):

1. Insufficient state funding – low levels of public funding result in reduced investment and maintenance of infrastructure and equipment. Without the necessary facilities available patients are driven to purchase their own care, which sets a dangerous precedent when considering the most vulnerable in society. Additionally, with reduced funding comes reduced staff salaries. Workers without a viable living wage often turn to holding a second job in the private sector with the benefits of higher pay and added incentives. Where supervision cannot be guaranteed, absenteeism and the "ghost worker" phenomenon is prevalent. As public services struggle to meet demand, private services are quick to collect what they can.
2. An environment which lacks the enforceable regulatory framework for private sector establishments to adhere to – without an effective framework the private sector can charge extortionate fees for poor quality and unnecessary service
3. A lack of transparency in governance – the availability of resources can be subject to corruption as a result of collusion in procurement and/or theft from the supply chain.

Given the varying wealth, quality, ownership and incentives different healthcare settings hold, coordination across different systems can prove difficult to achieve. This is

especially important when it comes to implementing guidelines and interventions throughout multiple regions to combat epidemics (Herrera et al., 2014).

5.1.3 Investing in Global Health Partnerships

There can be a huge health and financial benefit associated with investments in global health, with many organisations capitalising on this to facilitate the implementation of their own strategies. In 2018 the UK alone provided £14.6 billion official development assistance, behind that of USA (£25.7bn) and Germany (£18.7bn) (Department for International Development, 2019). Efficient utilisation of such funds to promote the facilitation of aid relies upon global connectivity. A study by Manzi et al. (2017) spanning five sub-Saharan countries concluded that mentorship and coaching was an essential factor in health system strengthening across all locations. Arriving at the same conclusion was Walker et al. (2014), who added that long term partnerships and institutional commitment are essential for success and sustainability. In addition to their training and clinical aspects, such findings are also applicable to research facilitation, where disparities between different healthcare settings deem collaborations essential. Collective research can provide many resolutions to global health problems through the development of new technologies. These can have both direct implications on health, for example safer, cheaper and more effective treatments and vaccines; as well as more indirect implications revolving around better designed health care and governance systems, a well-trained workforce and the efficient attribution of funds and resources. Regarding parasitic and infectious diseases, the end goal could be the total eradication of the disease (Fineberg & Hunter, 2013).

It is worth noting that the 'developed world' doesn't hold a monopoly on policies and concepts to address the issues of disease treatment and prevention, healthy environments, food production and distribution. Consequently, the idea and success of global health relies upon the amalgamation of experience, knowledge and resources from a diverse range of cultures to solve health challenges throughout the world (Koplan et al., 2009).

5.1.4 Multi-Disciplinary Challenges

As the burden of infectious diseases increases, any hindrance to viable treatments available can have dire consequences. These aren't just limited to medical health, but to the environment, food production, poverty and health security – all key elements of the Sustainable Development Goals as set by the United Nations (Jee et al., 2018). When combined with the widely targeted objectives previously mentioned there is an essential need for multi-disciplinary cooperation at both local and national levels. As such, a flexible framework was produced in a collaborative effort between the World Health Organization (WHO), Food and Agriculture Organisation of the United Nations and World Organisation for Animal Health. The guidance features advice intended for country policy makers to formulate their own National Action Plans (NAP) in line with that of the global action plan set out by the World Health Assembly. The framework is adaptable to suit the needs of individual countries where specific needs, circumstances and available resources can be variable (World Health Organization et al., 2016).

Unifying public and private sectors across multiple industries under one general plan is no small feat, with multiple challenges to overcome across different periods of

implementation. However, based on past experiences a number of tools and strategies are available to enhance cooperation, with the driving factors being condensed into four key categories: political commitment, resources, governance mechanisms and practical management (World Health Organisation, 2018c).

Political leadership and commitment are essential components in the facilitation of AMR projects at a local and international level, providing the means for resource allocation and mobilisation. It has been demonstrated that countries with AMR related officials in senior enough positions to enforce action are the most progressive and sustainable projects over time. That being said, stimulation of political commitment requires action on several fronts, including increased AMR awareness, the production of new data highlighting both complimentary and critical aspects of AMR and providing a legal means to enforce commitment. Political governance aside, combating AMR requires a huge investment in terms of time, finance and human resources. Connecting different industries across varying levels requires trust and respect between participants such that everyone can contribute on an equal platform. These qualities take time to build and require the involvement of key stakeholders from as early as possible. However, there is a balance to be made between garnering the involvement of multiple industries and actually taking steps forward, ensuring that where differences in capacity and resources are present, they don't inhibit the potential progress of individual sectors. Last but not least, as a branch of human resource comes quality communication and organisation. To ensure effective horizontal (cross-industry) and vertical (national to local) collaboration administrations often require dedicated personnel to coordinate people and meetings as well as the facilitation of knowledge transfer through the cascades of governing bodies (World Health Organisation, 2018c).

5.2 Aims and Objectives

Antimicrobial stewardship represents a core objective of the WHO global action plan (strategic objective 4) and strategic objective 3 of Uganda's National Action Plan (UNAP), (Government of Uganda, 2018) and serves to promote the optimal access and usage of antimicrobials. Potential issues can arise where national advice on control strategy based on national clinical guidelines, or data that may be focused upon major urban centres, are not always aligned with antibiotic susceptibility observed at the local level, emphasising the requirement for local knowledge (Odongo et al., 2013).

The main goal of this study, in keeping with the UNAP, was to create a local knowledge base of circulating AMR traits in community- and clinically-associated *S. aureus* strains. This would help provide the evidence required for future policies on the optimal use of antibiotics to preserve their efficacy for use in treatment at FPRRH and its surrounding catchment area.

5.3 Creating an Evidence Base for the Hospital Antibiogram

A significant pillar of AMR surveillance involves strategic objective 4.1.6: to analyse, disseminate and share surveillance data and information to facilitate decision making on diagnoses and treatments in clinical public health (Government of Uganda, 2018). A key research outcome through culture and sensitivity testing at FPRRH has been the generation of the first antibiogram – a collection of data, based on laboratory testing of pathogens isolated in a specific facility, that summarises patterns of resistance to different antimicrobial agents (or antibiotics). Whilst initial culture and sensitivity testing provides

direct feedback for the benefit of the respective patient, facilitation of results from start to finish can take several days. In situations where severe infection is present, for example sepsis, immediate empirical treatment is often given based on the doctors' own perception of what works best and what is available to use from their own experience. This may or may not be relevant to the setting in which they are currently practicing. An antibiogram, however, negates these personal biases and provides a means of empirical treatment tailored to the local healthcare setting providing a more accurate means of prescription (L. Ackers et al., 2020).

5.4 Antibiotic usage and procurement

A second key outcome of this research revolved around antibiotic procurement at FPRRH. The process of procurement is generally inflexible and adheres to rigid guidelines reducing adaptability to locally based results (L. Ackers et al., 2020). That being said, slow changes can be observed when analysing the antibiotic procurement plan (Figure 5.1). Most notably from 2019/2020 to 2020/2021 an increase from 0 to 5 x 1000 units of 250mg chloramphenicol tablets and 0 to 5 x 50 units of chloramphenicol 1g injections can be seen. This is a strong reflection of the low levels of resistance to chloramphenicol witnessed amongst clinical isolates, second only to clindamycin in this study. Similarly, increased orders of ciprofloxacin are comparable to the low levels of resistance observed. In addition to the increased procurement of suitable antibiotics, a reduction in the amount of amoxicillin ordered (a popular antibiotic of choice requested by the general public) is promising given the high rates of resistance observed.

Antibiotic(s)		Unit	Price (UGX)	Past Average Consumption	2019/2020 Plan	2020/2021 Plan	Bi-monthly cost (UGX)
Amoxicillin	250g Capsule	1000	54,100	134	101	90	4,869,000
Ampicillin/cloxacillin	Capsule	100	14,400	42	30	50	720,000
Ampicillin/cloxacillin	250mg Injection	100	71,700	5	2	2	143,400
Cefotaxime	Injection	1	13,400	767	1	0	0
Ceftriaxone	1g Vial	1	1,700	6000	6000	5000	8,500,000
Chloramphenicol	250mg Tablet	1000	100,200	0	0	5	501,000
Chloramphenicol	1g Injection	50	80,200	0	0	5	401,000
Ciprofloxacin	50mg Tablet	100	10,500	118	70	70	735,000
Ciprofloxacin	200mg Tablet	50	33,000	217	100	150	4,950,000
Cloxacillin	500mg Injections	50	33,000	2	0	1	33,000
Gentamicin	80mg Injection	100	32,800	0	40	0	0
Meropenem	500mg Injection	1	16,000	92	0	150	2,400,000
Metronidazole	Tablet	1000	17,100	70	50	70	1,197,000
Metronidazole	500mg Injection	1	800	5833	7000	7000	5,600,000
Penicillin Benzathine	2.4MU Vial	10	11,400	4	5	5	57,000

Figure 5.1: Extract from the 2020/2021 Procurement Plan focusing on key antibiotics used on the postnatal and gynaecology wards. The units indicate how many individual units of medication are contained within each individual order (i.e. 1000 units = 1000 tablets), with the cost of orders presented in Ugandan shillings (approximate exchange rate at time of writing £1 = $4850 UGX). The Plans indicate the number of orders placed for the pre-specified units of medication available, which will be delivered on a bi-monthly basis throughout the year. Blue and red coloured text indicate a reduction or increase in the number of units ordered respectively.

5.5 Discussion - Facilitating Research as Part of a Global Health Project

5.5.1 Multi-Disciplinary Challenges

Whilst my initial research at Fort Portal aimed to capture all aspects of the One Health agenda, time constraints led me to exclude animal and environmental sampling in favour of human commensal and clinically associated isolates. Whilst this seemed like a relatively narrow spectrum compared to the multi-faceted original plan, there was still a large amount multi-disciplinarity involved, particularly in relation to obtaining the relevant

ethical approval. Whilst front line staff on the ground are generally very positive, the strong hierarchical nature of different cadres often meant to obtain the appropriate permissions you had to acquire approval from those at the top of the ladder, of which I had variable experiences with. The first step of obtaining ethical approval required approval from FPRRH, for which I had to speak directly with the hospital director. Throughout the course of my research there were three different directors, each of which I had to speak to personally to ensure my research could continue; and this required a meeting in-person which couldn't be scheduled ahead of time. Instead, it was customary to wait outside their office and they would see people on a first come first served basis until they had to move on to other business. Thankfully as I was working under the umbrella of Knowledge for Change (K4C), a non-government organisation based in Fort Portal and at Salford University, there was already a track record of successful partnerships. Whilst one of the hospital directors was more abrasive than the others, overall I was successfully able to continue my research. As such I feel that working with a locally based reputable organisation was critical to completing my research in a timely manner.

Further complications arose when seeking ethical approval. I acquired initial approval from a university local to Fort Portal, only to later be told they weren't fully accredited, hence I was unable to transport any isolates. Instead, I had to apply via Makerere University in Kampala and subsequently create another application to the Ugandan National Council of Science and Technology. Key problems encountered at this stage involved the initial requirement for a fully qualified supervisor based at Makerere University – a point later conceded; and further issues of friction with the hospital director at the time who wanted to see my ethical approval, but to acquire ethical approval I needed permission from the hospital. Whilst at the time ethical approval was the cause of major

time delays, I am overall grateful as I was able to develop strong connections with researchers based at Makerere University who were essential to the completion of my studies. Whilst I fully understand the need for a comprehensive ethical review system, I feel that the process could have been better streamlined with more application information disseminated to the relevant organisational bodies. A lot of individuals at different organisations were giving different interpretations of the correct sequence of events which added complications to the application procedure. Conducting any research in future I will be sure to comprehensively review all the relevant organisations capable of providing ethical approval to ensure a streamlined timeline of events.

5.5.2 Advantages of Linking Healthcare Systems

As previously touched upon, the collaborations developed with multiple organisations including Makerere University, the Infectious Diseases Institute and FPRRH were paramount to the successful completion of my research. That being said, through these partnerships the local organisations were also able to benefit beyond the direct research findings. Whilst the laboratory at Makerere housed the appropriate facilities for WGS, they lacked the funds and high throughput of samples to make it cost efficient, hence its usage was rare. As a result of my research a laboratory technician was able to travel to the UK for WGS training to further compound his knowledge of the process. On his return to Uganda, he was able to utilise the knowledge he had gained to sequence the same isolates in Uganda that he had in UK as a means of quality control. Having such expertise locally is crucial for future research at Makerere and Uganda as a whole when utilising advanced techniques. Similarly, WGS of the 41 clinical isolates was a useful means of quality control

for laboratory practices at FPRRH managed by the Infectious Diseases Institute. On reporting back the sequencing data the technicians were able to assess the accuracy of their *S. aureus* identification techniques in addition to the in depth genetic resistance data highlighting the need for comprehensive AST data. Furthermore, effective AST allowed for improvements in antibiotic procurement at FPRRH, slowly improving the policy driven governance systems and the efficient attribution of funds.

5.5.3 Health Systems as Mixed Economies

Whilst extending beyond the boundaries of what my study was able to attain, future AMR research needs to include the involvement of local partners and businesses specifically in relation to effective antimicrobial stewardship. Although this study was able to influence the procurement and prescription practices at FPRRH, these antibiotics account for a small proportion of what is available to the public. Fort Portal houses multiple independent pharmacies which offer antibiotics over the counter without prescription and as such distribute an unknown quantity of antibiotics which circulate in the community. Not only does this provide a platform for the unnecessary consumption of antibiotics, but also provides a means for patients to unknowingly consume cheaper, less effective antibiotics than what is required for their condition. Future AMR interventions need to take this into account when developing effective treatment strategies.

Chapter 6 – Final Thoughts and Conclusions

6.1 Introduction

Antimicrobial resistance is a growing problem of global concern and as such requires a concerted effort to decelerate its expansion. With numerous initiatives derived to help facilitate this process, it is essential that aid is provided to developing countries to help address these issues. With no 'one size fits all' approach due to the varying prevalence of different resistance characteristics and population diversity amongst different strains of bacteria, it is essential that data is collected at a local and national level and tailor-made solutions developed. To this end, FPRRH where no data has been previously published, in combination with a newly developed microbiology laboratory, provided the optimum research environment for improved AMR surveillance and the implementation of policies to counter the spread of resistance. In this study, *S aureus* was isolated from both community and clinical sources in and around Fort Portal. The phenotypic antimicrobial resistance traits of all isolates were assessed, with the resistance genotypes of 41 clinical isolates also evaluated. Furthermore, whole genome sequencing allowed for the identification of sequence types and known virulence genes providing an additional means to investigate any associations.

6.2 Overview of the Research Findings

Phenotypic antimicrobial susceptibility testing indicated 36% (25/70) of clinical isolates were susceptible to all antibiotics tested, 39% (27/70) were resistant to one or two

antibiotics, whilst 26% (18/70) were MDR. Regarding the community isolates, 18% (22/125) were susceptible to all antibiotics tested, 34% (42/125) were resistant to one or two antibiotics, whilst 49% (61/125) were MDR. The resistance profiles observed were largely in accordance with other Ugandan studies (George et al., 2018; Mshangila et al., 2013; Odongo et al., 2013; Ojulong et al., 2009; Jeremiah Seni et al., 2013) with notable outliers being clindamycin (no clinical resistance), chloramphenicol (10% clinical resistance) and cotrimoxazole (46% clinical resistance).

Overall, the WGS data provided a more detailed view of the AMR mechanisms circulating in *S. aureus*-associated infections at FPRRH, although susceptibility was more commonly predicted than resistance. Given the non-contiguous nature of the draft genomes, this could be due to a lack of complete gene coverage enabling confident prediction of gene presence or absence. Regarding MLST sequence types, CC152 was observed to be the dominant complex encompassing nearly half of the isolates assessed (20/41), followed by CC8 (4/41) and ST 1, 5, 25, 508, 2019 and 2178 all identified once. The data also revealed genetic markers of resistance across all isolates were more prevalent amongst non-CC152 isolates (with the exception of *dfrG* and *blaZ* found in high levels across all populations). Whilst 100% of isolates tested (35/35) appeared susceptible to clindamycin, *ermA* and/or *ermC* were detected in 12 isolates. This suggested that the genes are under erythromycin-inducible regulation, but that exposure to clindamycin *in vivo* could select for constitutive expression mutants, that would confer resistance to both.

Given the high prevalence of CC152, the presence of virulence factors (also derived from WGS data) were assessed for any associations between the different populations. Of particular note was the presence of *lukF* and *lukS* PVL genes being strongly associated with

CC152 isolates relative to non-CC152 isolates. This was also the case for *coa*, whilst *vWbp, chp, map, sdrC* and *sec* showed the reverse with their presence more associated with non-CC152 isolates.

6.3 Contextualising the Research Findings

As noted earlier, no data had been previously published from in and around Fort Portal relating to the prevalence of antimicrobial resistance from either a phenotypic or genotypic perspective, or in terms of community and clinical isolates. As such, one of the core outcomes of this research was the generation of data which created a solid foundation for future initiatives. With an established data set of circulating AMR genes and their respective phenotypes in place, future research can be accumulated to form a comprehensive view of the changing face of antimicrobial resistance over time and utilised to dictate future changes in healthcare policy. The data collected as part of this research aided in the production of the hospitals' first antibiogram and has since led to changes in pharmacy procurement practices.

Not only was the study a useful source of data in its own right, but also served to formulise and further enhance the process of data acquisition in terms of both infrastructure and personnel. Following the laboratory modernisation at FPRRH one of the key hurdles to research was sample acquisition. Having adapted to life with a semi-functional laboratory many medical staff had either lost faith in the laboratory staff or were simply unaware of the available services. As a result, laboratory facilities were heavily under-utilised. As part of this study, we helped to revitalise communication pathways and rebuild trust where required allowing not just the production of a solid foundation of data

to evaluate, but more importantly improved patient care through increased access to laboratory services. Furthermore, through performing more advanced secondary analysis of select clinical isolates we were able to provide welcome feedback to FPRRH laboratory on the (strong) reliability of their microbiology procedures. However, there was cause for concern given the low availability of cefoxitin disks affecting the reliable detection of MRSA. These situations are not uncommon when working in a low resource setting, with unreliable supply chains leading to stock outs of various reagents. Such circumstances highlighted an additional benefit of performing WGS in being able to provide an insight where AST was not facilitated, for example due to a lack of cefoxitin disks, as the data did indeed identify the *mecA* resistance gene in isolates that were not screened for cefoxitin resistance.

Building relationships formed a key part of the study, one example already mentioned being the communication pathways between FPRRH medical and laboratory staff. A second example of great importance was developing a link with Makerere University to facilitate the preliminary requirements of WGS. A Memorandum of Understanding and Material Transfer Agreement between Makerere University and the University of Salford enabled shipment and storage of the required isolates. Whilst this was immediately essential for my research to continue, it also paved the way for future collaborations allowing for data collection and analysis to be streamlined.

Whilst antimicrobial resistance forms a unified global threat, geographically it exhibits considerable variation (Mitchell et al., 2021). Socio-economic factors related to AMR including the presence of hospitals, industries, urbanisation, animal-based agriculture and wastewater systems can all impact AMR prevalence at a local and regional level (Medina-Pizzali et al., 2021). Hence, performing novel research in areas like Fort Portal

provide high value data to aid in understanding resistance transmission. Equally, *S. aureus* sequence types tend to vary depending on geographic location (Aanensen et al., 2016; Ruffing et al., 2017). Whilst *S. aureus* sequence types do not always associate with invasive disease, they have been observed to correlate with respect to MRSA (Aanensen et al., 2016). The results discussed in Chapter 4 corroborated with this where CC152 was reported as the dominant strain, mirrored in other African studies (Kyany'a et al., 2019; Obasuyi et al., 2020). Also, a high proportion of CC152 isolates had either a no resistance phenotype or were resistant to just one antibiotic, with comparisons to non-CC152 isolates highlighting significantly less prevalent methicillin, chloramphenicol and ciprofloxacin resistance. Whilst this is largely positive due to the majority of isolates being identified as CC152, it does provide cause for concern regarding the high level of resistance witnessed amongst CC8 isolates. Although only four CC8 isolates were identified, it is one of the most hypervirulent community-associated strains in the USA (Chambers & Deleo, 2009) and has proven to spread rapidly in Africa (Strauß et al., 2017). Of equal concern is CC152's ability to acquire methicillin resistance. Whilst MSSA CC152 is dominant in Africa (also evidenced by this research), much higher rates of MRSA CC152 are observed in Europe (Baig et al., 2020). Furthermore, CC152 isolated as part of this study strongly correlated with the presence of PVL, which itself has a strong association with community-acquired MRSA strains (Souza et al., 2009). These results indicated the need for persistent surveillance of sequence types and related AMR profiles at FPRRH to monitor the spread and acquisition of resistance genes.

6.4 Future research

As previously noted, a key outcome of this research was the generation of a solid foundation of data of which to build future research projects upon. Future initiatives have the option of expanding either horizontally through broadening the research to encompass unexplored bacteria and agricultural environments, or vertically through investigating the clinical impact and origin of *S. aureus* infections and any relative AMR mechanisms at a greater depth. Such research could be facilitated as a series of smaller work packages over time, or be compiled into a larger overarching project.

6.4.1 Investigating the clinical burden of bacterial infections and AMR at FPRRH

Moving forwards, one of the most important interventions at FPRRH would be to improve record keeping in terms of both patient information and laboratory results. To adequately assess the impact of bacteria and their respective virulence and AMR genes, information including the severity, duration and location of the infection in addition to patient outcomes are essential. Given the basic framework for this package is already in place in line with research facilitated as part of this thesis, it provides an ideal opportunity to examine other clinically relevant bacteria, for example *E. coli*. Comprehensive susceptibility testing must also be planned for to ensure there are no shortages of reagents. Where appropriate slightly more advanced testing should be facilitated to identify any heterogenous colonies or induced antibiotic resistances, for example a disk approximation test to check for induced clindamycin resistance. Without these phenotypic and genotypic discrepancies may be observed. Having detailed information on patient outcomes post-treatment will also shed light on this.

When working in a low resource setting sustainability is vital. For research purposes it may be useful to examine other whole genome sequencing tools, for example PacBio or Oxford Nanopore, to assess the confidence in AMR and virulence gene prediction. However, despite the cost of WGS gradually reducing over time, in terms of longevity it would be useful to examine cheaper alternatives. To that end it would be useful to assess PCR based strategies to target specific resistance and virulence genes.

6.4.2 Investigating Antibiotic Consumption in Fort Portal

Increased antibiotic consumption is the core driver for the generation of resistance, yet little is known of the quantities of antibiotics in circulation around Fort Portal or their potential use. This package can be broken down into two subsections: procurement and usage. Whilst simple as a concept, in practice it may prove difficult to achieve. Working at FPRRH anecdotal evidence suggested collecting data from public healthcare settings is much easier than private settings who, understandably, have their own political and economic motivations. As such, much of this package would revolve around settings where collaborations have already been established.

The package would function almost exclusively through surveys and data collection with respect to the following groups:

1. Patients and people in the community: Patients and community members would be surveyed with the assistance of healthcare staff and village health technicians acting as a link. Questions would revolve around: what drives them to acquire antibiotics (e.g. what symptoms of illness), where do they acquire antibiotics from (e.g.

purchase from local pharmacy), what influences their choice of antibiotic (e.g. familiar name or recommendation) and how long they take antibiotics for (e.g. a set time or until they feel better).

2. Farmers: Farmers would be approached through links with veterinary staff and agricultural schools. Questions would revolve around: what drives them to acquire antibiotics (e.g. animal welfare/prophylaxis), where do they acquire antibiotics from (e.g. purchase from local partners), what influences their choice of antibiotic (e.g. familiar name or recommendation) and how often do they treat their animals with antibiotics (e.g. 'x' amount added to feed 'y' times a year).
3. Pharmacies: Predominantly government associated healthcare pharmacies would be assessed but also privately owned pharmacies where feasible. Questions would revolve around: what informs antibiotic procurement (e.g. local resistance trends), how flexible is the procurement process (e.g. how easy is it to stock new antibiotics), what informs antibiotic prescriptions (e.g. whatever is in stock) and what volume of antibiotics are being dispensed (e.g. per month/year).

6.4.3 Investigating the Origins of *S. aureus* infection and AMR in Fort Portal

Similar to the first work package, investigating the origin of *S. aureus* infections requires comprehensive patient notes including where the patient has arrived from (e.g. self-admitted or referred from another healthcare centre), duration of stay at the hospital and reason for admittance / which ward is the patient staying in (e.g. maternity with infected caesarean wound). Such data can help infer whether the infection is community or hospital acquired allowing for the appropriate downstream investigations. To draw reliable

associations a wide range of samples on top of the clinical isolates would need to be taken to isolate *S. aureus* strains from different settings including: the local hospital environment, for example beds, medical equipment and surfaces in theatre; nasal and/or hand swabs from patients, attendants, healthcare workers and members of the community; and animals linked to local subsistence farming.

Ideally, as with this thesis, whole genome sequencing would be used in conjunction with phenotypic antimicrobial susceptibility testing to identify different strain types and the presence or absence of resistance genes. Alternatively, where costs deem it necessary, PCR based assays could be used to target specific genes of interest. Narrowing the costs of sequencing would allow for a much greater proportion of the isolates to be sequenced thus inferring more reliable conclusions.

6.5 Conclusions

The main goal of this study was to create local knowledge of circulating AMR in disease-associated S. aureus. It is clear that the procurement of sufficient materials to perform AST tests for resistances identified in the antibiogram, supported by molecular data, will be important for robust future surveillance to inform effective treatment and to ensure a rapid, appropriately planned response to treatment failures. Given the frequent circulation of *S. aureus* isolates between community and hospital settings, combined with the variability in resistance rates across Ugandan studies, this further highlights the need for continued, robust epidemiology at the local level to inform policy on control strategies that can minimise treatment failures and the spread of antimicrobial resistance.

Chapter 7 References

Aanensen, D. M., Feil, E. J., Holden, M. T. G., Dordel, J., Yeats, C. A., Fedosejev, A., Goater, R., Castillo-Ramírez, S., Corander, J., Colijn, C., Chlebowicz, M. A., Schouls, L., Heck, M., Pluister, G., Ruimy, R., Kahlmeter, G., Åhman, J., Matuschek, E., Friedrich, A. W., . . . European, S. R. L. W. G. (2016). Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive Staphylococcus aureus in Europe. *mBio*, *7*(3), e00444-00416. https://doi.org/10.1128/mBio.00444-16

Abayneh, M., & Worku, T. (2020). Prevalence of multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacilli: A meta-analysis report in Ethiopia. *Drug Target Insights*, *14*(1), 16-25. https://doi.org/10.33393/dti.2020.2170

Abimana, J. B., Kato, C. D., & Bazira, J. (2019). Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization among Healthcare Workers at Kampala International University Teaching Hospital, Southwestern Uganda. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2019*, Article 4157869. https://doi.org/10.1155/2019/4157869

Ackers-Johnson, G., Kibombo, D., Kusiima, B., Nsubuga, M. L., Kigozi, E., Kajumbula, H. M., Kateete, D. P., Walwema, R., Ackers, H. L., Goodhead, I. B., Birtles, R. J., & James, C. E. (2021). Antibiotic resistance profiles and population structure of disease-associated *Staphylococcus aureus* infecting patients in Fort Portal Regional Referral Hospital, Western Uganda. *Microbiology*, *167*(5). https://doi.org/https://doi.org/10.1099/mic.0.001000

Ackers, L., Ackers-Johnson, G., Seekles, M., Odur, J., & Opio, S. (2020). Opportunities and Challenges for Improving Antimicrobial Stewardship in Low- and Middle-Income Countries; Lessons Learnt from the Maternal Sepsis Intervention in Western Uganda. *Antibiotics*, *9*(6). https://doi.org/10.3390/antibiotics9060315

Ackers, L., Ackers-Johnson, G., Welsh, J., Kibombo, D., & Opio, S. (2020). *Antimicrobial Resistance in Global Perspective*. Palgrave Macmillan. https://doi.org/10.1007/978-3-030-62662-4

Ackers, L., & Ackers-Johnson, J. (2017). *Mobile Professional Voluntarism and International Development*. Palgrave Pivot. https://doi.org/https://doi.org/10.1057/978-1-137-55833-6

Adedeji, W. A. (2016). The Treasure Called Antibiotics. *Annals of Ibadan postgraduate medicine*, *14*(2), 56-57. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5354621/

Adeoye-Isijola, M., Olajuyigbe, O., Adebola, K., Coopoosamy, R., & Afolayan, A. (2020). Vancomycin intermediate resistant *Staphylococcus aureus* in the nasal cavity of asymptomatic individuals: a potential public health challenge. *African Health Sciences*, *20*(3), 1109-1117. https://doi.org/10.4314/ahs.v20i3.12

Afema, J. A., Byarugaba, D. K., Shah, D. H., Atukwase, E., Nambi, M., & Sischo, W. M. (2016). Potential Sources and Transmission of Salmonella and Antimicrobial Resistance in Kampala, Uganda. *PLoS One*, *11*(3), e0152130-e0152130. https://doi.org/10.1371/journal.pone.0152130

Ahmed, F. W., & Sennik, D. (2011). Erythromycin. *Practical Diabetes*, *28*, 364-365.

Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H.-K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., . . . McArthur, A. G. (2019). CARD 2020: Antibiotic Resistome Surveillance with the Comprehensive Antibiotic Resistance Database. *Nucleic acids research*, *48*(D1), D517-D525. https://doi.org/10.1093/nar/gkz935

Almeida, G. C. M., dos Santos, M. M., Lima, N. G. M., Cidral, T. A., Melo, M. C. N., & Lima, K. C. (2014). Prevalence and factors associated with wound colonization by *Staphylococcus spp*. and *Staphylococcus aureus* in hospitalized patients in inland northeastern Brazil: a cross-sectional study. *BMC Infectious Diseases*, *14*, 328-328. https://doi.org/10.1186/1471-2334-14-328

Amyes, S., Miles, R. S., Thomson, C. J., & Tillotson, G. (1996). *Antimicrobial Chemotherapy, Theory, Practice and Problems* (1st ed.). Martin Dunitz.

Andersson, M. I., & MacGowan, A. P. (2003). Development of the quinolones. *Journal of Antimicrobial Chemotherapy*, *51*(suppl_1), 1-11. https://doi.org/10.1093/jac/dkg212

Anguzu, J. R., & Olila, D. (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *African Health Sciences*, *7*(3), 148-154. https://doi.org/10.5555/afhs.2007.7.3.148

Asadollahi, P., Farahani, N. N., Mirzaii, M., Khoramrooz, S. S., van Belkum, A., Asadollahi, K., Dadashi, M., & Darban-Sarokhalil, D. (2018). Distribution of the Most Prevalent Spa Types among Clinical Isolates of Methicillin-Resistant and -Susceptible *Staphylococcus aureus* around the World: A Review. *Frontiers in microbiology*, *9*, 163. https://doi.org/10.3389/fmicb.2018.00163

Ayukekbong, J. A., Ntemgwa, M., & Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance & Infection Control*, *6*(1), 47. https://doi.org/10.1186/s13756-017-0208-x

Baig, S., Rhod Larsen, A., Martins Simões, P., Laurent, F., Johannesen Thor, B., Lilje, B., Tristan, A., Schaumburg, F., Egyir, B., Cirkovic, I., Nimmo, G. R., Spiliopoulou, I., Blanc, D. S., Mernelius, S., Moen Aina Elisabeth, F., David, M. Z., Andersen, P. S., & Stegger, M. (2020). Evolution and Population Dynamics of Clonal Complex 152 Community-Associated Methicillin-Resistant *Staphylococcus aureus*. *mSphere*, *5*(4). https://doi.org/http://dx.doi.org/10.1128/mSphere.00226-20

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and its Applications to Single-Cell Sequencing. *Journal of Computational Biology*, *19*(5), 455-477. https://doi.org/10.1089/cmb.2012.0021

Barbu, E. M., Mackenzie, C., Foster, T. J., & Höök, M. (2014). SdrC Induces *Staphylococcal* Biofilm Formation Through a Homophilic Interaction. *Molecular microbiology*, *94*(1), 172-185. https://doi.org/10.1111/mmi.12750

Bhullar, K., Waglechner, N., Pawlowski, A., Koteva, K., Banks, E. D., Johnston, M. D., Barton, H. A., & Wright, G. D. (2012). Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome. *PLoS One*, *7*(4), e34953. https://doi.org/10.1371/journal.pone.0034953

Blumberg, H. M., Rimland, D., Carroll, D. J., Terry, P., & Wachsmuth, I. K. (1991). Rapid development of ciprofloxacin resistance in methicillin-susceptible and -resistant *Staphylococcus aureus*. *Journal of Infectious Diseases*, *163*(6), 1279-1285. https://doi.org/10.1093/infdis/163.6.1279

Boyle-Vavra, S., & Daum, R. S. (2007). Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton-Valentine leukocidin. *Laboratory Investigation*, *87*(1), 3-9. https://doi.org/http://dx.doi.org/10.1038/labinvest.3700501

Brogden, R. N., Carmine, A. A., Heel, R. C., Speight, T. M., & Avery, G. S. (1982). Trimethoprim: a review of its antibacterial activity, pharmacokinetics and therapeutic use in urinary tract infections. *Drugs*, *23*(6), 405-430. https://doi.org/10.2165/00003495-198223060-00001

Busse, H.-J. (2016). Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter sensu lato*, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. *International Journal of Systematic and Evolutionary Microbiology*, *66*(1), 9-37. https://doi.org/https://doi.org/10.1099/ijsem.0.000702

Cameron, D. R., Howden, B. P., & Peleg, A. Y. (2011). The Interface Between Antibiotic Resistance and Virulence in Staphylococcus aureus and Its Impact Upon Clinical Outcomes. *Clinical Infectious Diseases*, *53*(6), 576-582. https://doi.org/10.1093/cid/cir473

Cao, Z., Casabona, M. G., Kneuper, H., Chalmers, J. D., & Palmer, T. (2016). The type VII secretion system of Staphylococcus aureus secretes a nuclease toxin that targets competitor bacteria. *Nature microbiology*, *2*, 16183-16183. https://doi.org/10.1038/nmicrobiol.2016.183

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(Supplement 1), 4516-4522.

Casadevall, A., & Pirofski, L.-a. (2009). Virulence factors and their mechanisms of action: the view from a damage–response framework. *Journal of Water and Health*, *7*(S1), S2-S18. https://doi.org/10.2166/wh.2009.036

Center for Disease Control and Prevention. (2019). *Methicillin-resistant Staphylococcus aureus (MRSA) – Laboratory Testing*. Retrieved 08/06/21 from https://www.cdc.gov/mrsa/lab/

Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews. Microbiology*, *7*(9), 629-641. https://doi.org/10.1038/nrmicro2200

Charpentier, E., & Courvalin, P. (1997). Emergence of the trimethoprim resistance gene dfrD in Listeria monocytogenes BM4293. *Antimicrobial Agents and Chemotherapy*, *41*(5), 1134-1136. https://doi.org/10.1128/aac.41.5.1134

Chen, Y., Sun, L., Wu, D., Wang, H., Ji, S., & Yu, Y. (2018). Using Core-genome Multilocus Sequence Typing to Monitor the Changing Epidemiology of Methicillin-resistant *Staphylococcus aureus* in a Teaching Hospital. *Clinical Infectious Diseases*, *67*(suppl_2), S241-S248. https://doi.org/10.1093/cid/ciy644

Chopra, I., & Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232-260. https://doi.org/doi:10.1128/MMBR.65.2.232-260.2001

Chua, K. Y., Howden, B. P., Jiang, J. H., Stinear, T., & Peleg, A. Y. (2014). Population genetics and the evolution of virulence in *Staphylococcus aureus*. *Infection, Genetics and Evolution*, *21*, 554-562. https://doi.org/10.1016/j.meegid.2013.04.026

Chukwudi, C. U. (2016). rRNA Binding Sites and the Molecular Mechanism of Action of the Tetracyclines. *Antimicrobial Agents and Chemotherapy*, *60*(8), 4433-4441. https://doi.org/doi:10.1128/AAC.00594-16

Clardy, J., Fischbach, M. A., & Currie, C. R. (2009). The natural history of antibiotics. *Current Biology*, *19*(11), R437-R441. https://doi.org/10.1016/j.cub.2009.04.001

Clark, L. C., & Hodgkin, J. (2015). *Leucobacter musarum* subsp. *musarum* sp. nov., subsp. nov., *Leucobacter musarum* subsp. *japonicus* subsp. nov., and *Leucobacter celer* subsp. *astrifaciens* subsp. nov., three nematopathogenic bacteria isolated from *Caenorhabditis,* with an emended description of *Leucobacter* celer. *International Journal of Systematic and Evolutionary Microbiology*, *65*(Pt_11), 3977-3984. https://doi.org/https://doi.org/10.1099/ijsem.0.000523

Coelho, C., de Lencastre, H., & Aires-de-Sousa, M. (2017). Frequent occurrence of trimethoprim-sulfamethoxazole hetero-resistant *Staphylococcus aureus* isolates in different African countries. *European journal of clinical microbiology & infectious diseases*, *36*(7), 1243-1252. https://doi.org/10.1007/s10096-017-2915-x

Cohen, P. R. (2007). Community-acquired methicillin-resistant Staphylococcus aureus skin infections: implications for patients and practitioners [Article]. *American Journal of Clinical Dermatology*, *8*, 259+. https://link.gale.com/apps/doc/A199397564/AONE?u=salcal2&sid=bookmark-AONE&xid=88f23125

Czura, C. J. (2011). "Merinoff symposium 2010: sepsis"-speaking with one voice. *Molecular Medicine*, *17*(1-2), 2-3. https://doi.org/10.2119/molmed.2010.00001.commentary

Dale, G. E., Broger, C., D'Arcy, A., Hartman, P. G., DeHoogt, R., Jolidon, S., Kompis, I., Labhardt, A. M., Langen, H., Locher, H., Page, M. G., Stüber, D., Then, R. L., Wipf, B., & Oefner, C. (1997). A

single amino acid substitution in *Staphylococcus aureus* dihydrofolate reductase determines trimethoprim resistance. *Journal of Molecular Biology*, *266*(1), 23-30. https://doi.org/10.1006/jmbi.1996.0770

Dancer, S. J. (2014). *Staphylococcus aureus* antibiotic resistance. In *Clinical Insights: Staphylococcus aureus Antibiotic Resistance* (pp. 2-5). Future Medicine Ltd. https://doi.org/doi:10.2217/ebo.13.716

10.2217/ebo.13.716

David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*, *23*(3), 616-687. https://doi.org/10.1128/CMR.00081-09

Davis, M. A., Besser, T. E., Orfe, L. H., Baker, K. N. K., Lanier, A. S., Broschat, S. L., New, D., & Call, D. R. (2011). Genotypic-Phenotypic Discrepancies between Antibiotic Resistance Characteristics of *Escherichia coli* Isolates from Calves in Management Settings with High and Low Antibiotic Use. *Applied and Environmental Microbiology*, *77*(10), 3293-3299. https://doi.org/doi:10.1128/AEM.02588-10

de Haas , C. J. C., Veldkamp , K. E., Peschel , A., Weerkamp , F., Van Wamel , W. J. B., Heezius , E. C. J. M., Poppelier , M. J. J. G., Van Kessel , K. P. M., & van Strijp , J. A. G. (2004). Chemotaxis Inhibitory Protein of *Staphylococcus aureus*, a Bacterial Anti-inflammatory Agent. *Journal of Experimental Medicine*, *199*(5), 687-695. https://doi.org/10.1084/jem.20031636

Department for International Development. (2019). *Statistics on International Development: Final UK Aid spend 2018*. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/857904/Statistics-on-International-Development-final-aid-spend-2018d.pdf

Deurenberg, R. H., & Stobberingh, E. E. (2008). The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution*, *8*(6), 747-763. https://doi.org/10.1016/j.meegid.2008.07.007

Donker, G. A., Deurenberg, R. H., Driessen, C., Sebastian, S., Nys, S., & Stobberingh, E. E. (2009). The population structure of *Staphylococcus aureus* among general practice patients from The Netherlands. *Clinical Microbiology and Infection*, *15*(2), 137-143. https://doi.org/10.1111/j.1469-0691.2008.02662.x

Drinkovic, D., Fuller, E. R., Shore, K. P., Holland, D. J., & Ellis-Pegler, R. (2001). Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *Journal of Antimicrobial Chemotherapy*, *48*(2), 315-316. https://doi.org/10.1093/jac/48.2.315

Dunkle, J. A., Xiong, L., Mankin, A. S., & Cate, J. H. D. (2010). Structures of the *Escherichia coli* ribosome with antibiotics bound near the peptidyl transferase center explain spectra of drug action. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(40), 17152. https://doi.org/10.1073/pnas.1007988107

Durso, L. M., & Cook, K. L. (2014). Impacts of antibiotic use in agriculture: what are the benefits and risks? *Current Opinion in Microbiology*, *19*, 37-44. https://doi.org/https://doi.org/10.1016/j.mib.2014.05.019

Eliopoulos, G. M., & Huovinen, P. (2001). Resistance to Trimethoprim-Sulfamethoxazole. *Clinical Infectious Diseases*, *32*(11), 1608-1614. https://doi.org/10.1086/320532

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J., & Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of clinical microbiology*, *38*(3), 1008-1015. https://doi.org/10.1128/JCM.38.3.1008-1015.2000

Enright, M. C., Robinson, D. A., Randle, G., Feil, E. J., Grundmann, H., & Spratt, B. G. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of the National Academy of Sciences of the United States of America*, *99*(11), 7687-7692. https://doi.org/10.1073/pnas.122108599

Epand, R. M., Walker, C., Epand, R. F., & Magarvey, N. A. (2016). Molecular mechanisms of membrane targeting antibiotics. *Biochimica et Biophysica Acta*, *1858*(5), 980-987. https://doi.org/10.1016/j.bbamem.2015.10.018

Eshetie, S., Tarekegn, F., Moges, F., Amsalu, A., Birhan, W., & Huruy, K. (2016). Methicillin resistant Staphylococcus aureus in Ethiopia: a meta-analysis. *BMC Infectious Diseases*, *16*(1), 689-689. https://doi.org/10.1186/s12879-016-2014-0

Essack, S. Y. (2001). The development of beta-lactam antibiotics in response to the evolution of beta-lactamases. *Pharmaceutical Research*, *18*(10), 1391-1399. https://doi.org/10.1023/a:1012272403776

EUCAST. (2021). *Clinical Breakpoints - breakpoints and guidance* https://www.eucast.org/clinical_breakpoints/

Feil, E. J., Cooper, J. E., Grundmann, H., Robinson, D. A., Enright, M. C., Berendt, T., Peacock, S. J., Smith, J. M., Murphy, M., Spratt, B. G., Moore, C. E., & Day, N. P. (2003). How clonal is *Staphylococcus aureus*? *Journal of Bacteriology*, *185*(11), 3307-3316. https://doi.org/10.1128/jb.185.11.3307-3316.2003

Fineberg, H. V., & Hunter, D. J. (2013). A global view of health - an unfolding series. *The New England Journal of Medicine*, *368*(1), 78-79. https://doi.org/10.1056/NEJMe1208801

Fitzgerald, J. R., & Holden, M. T. (2016). Genomics of Natural Populations of *Staphylococcus aureus*. *Annual Review of Microbiology*, *70*, 459-478. https://doi.org/10.1146/annurev-micro-102215-095547

Fowler, P. W., Cole, K., Gordon, N. C., Kearns, A. M., Llewelyn, M. J., Peto, T. E. A., Crook, D. W., & Walker, A. S. (2018). Robust Prediction of Resistance to Trimethoprim in *Staphylococcus aureus*. *Cell Chemical Biology*, *25*(3), 339-349.e334. https://doi.org/10.1016/j.chembiol.2017.12.009

Fung-Tomc, J. C. (1997). Fourth-generation cephalosporins. *Clinical Microbiology Newsletter*, *19*(17), 129-136. https://doi.org/https://doi.org/10.1016/S0196-4399(97)82485-3

Garneau-Tsodikova, S., & Labby, K. J. (2016). Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives [10.1039/C5MD00344J]. *MedChemComm*, *7*(1), 11-27. https://doi.org/10.1039/C5MD00344J

George, M., Iramiot, J. S., Muhindo, R., Olupot-Olupot, P., & Nanteza, A. (2018). Bacterial Aetiology and Antibiotic Susceptibility Profile of Post-Operative Sepsis among Surgical Patients in a Tertiary Hospital in Rural Eastern Uganda. *Microbiology Research Journal International*, *24*(2). https://doi.org/10.9734/mrji/2018/41690

Ghanbari, F., Ghajavand, H., Havaei, R., Jami, M.-S., Khademi, F., Heydari, L., Shahin, M., & Havaei, S. (2016). Distribution of *erm* genes among *Staphylococcus aureus* isolates with inducible resistance to clindamycin in Isfahan, Iran [Original Article]. *Advanced Biomedical Research*, *5*(1), 62-62. https://doi.org/10.4103/2277-9175.179184

Goering, R., Dockrell, H., Zuckerman, M., Roitt, I., & Chiodini, P. L. (2012). *Mims' Medical Microbiology, 5th Edition*. Elsevier Saunders.

Gootz, T. D. (1990). Discovery and development of new antimicrobial agents. *Clinical Microbiology Reviews*, *3*(1), 13-31. https://doi.org/10.1128/CMR.3.1.13

Gottfried, J. (2005). *History Repeating? Avoiding a Return to the Pre-Antibiotic Age*. Digital Access to Scholarship at Harvard. Retrieved 27/10/2021 from http://nrs.harvard.edu/urn-3:HUL.InstRepos:8889467

Goudarzi, M., Seyedjavadi, S. S., Nasiri, M. J., Goudarzi, H., Sajadi Nia, R., & Dabiri, H. (2017). Molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis. *Microbial Pathogenesis*, *104*, 328-335. https://doi.org/https://doi.org/10.1016/j.micpath.2017.01.055

Government of Uganda. (2018). *Antimicrobial Resistance National Action Plan*. E. P. Center for Disease Dynamics. https://cddep.org/wp-content/uploads/2019/03/Final-Signed-NAP-AMR-2018-2023-Final.pdf

Gray, B. H., & McNerney, W. J. (1986). For-profit enterprise in health care. The Institute of Medicine Study. *The New England Journal of Medicine*, *314*(23), 1523-1528. https://doi.org/10.1056/nejm198606053142335

Gu, F., He, W., Xiao, S., Wang, S., Li, X., Zeng, Q., Ni, Y., & Han, L. (2020). Antimicrobial Resistance and Molecular Epidemiology of *Staphylococcus aureus* Causing Bloodstream Infections at Ruijin Hospital in Shanghai from 2013 to 2018. *Scientific Reports*, *10*(1), 6019. https://doi.org/10.1038/s41598-020-63248-5

Habyarimana, T., Murenzi, D., Musoni, E., Yadufashije, C., & N Niyonzima, F. (2021). Bacteriological Profile and Antimicrobial Susceptibility Patterns of Bloodstream Infection at Kigali University Teaching Hospital. *Infection and drug resistance*, *14*, 699-707. https://doi.org/10.2147/IDR.S299520

Hallin, M., Friedrich, A. W., & Struelens, M. J. (2009). spa typing for epidemiological surveillance of *Staphylococcus aureus*. *Methods in Molecular Biology*, *551*, 189-202. https://doi.org/10.1007/978-1-60327-999-4_15

Hamilton, R. (2015). *Tarascon Pocket Pharmacopoeia*. Jones & Bartlett Learning.

Harmsen, D., Claus, H., Witte, W., Rothgänger, J., Claus, H., Turnwald, D., & Vogel, U. (2003). Typing of Methicillin-Resistant *Staphylococcus aureus* in a University Hospital Setting by Using Novel Software for *spa* Repeat Determination and Database Management. *Journal of clinical microbiology*, *41*(12), 5442-5448. https://doi.org/doi:10.1128/JCM.41.12.5442-5448.2003

Harraghy, N., Hussain, M., Haggar, A., Chavakis, T., Sinha, B., Herrmann, M., & Flock, J.-I. (2003). The adhesive and immunomodulating properties of the multifunctional *Staphylococcus aureus* protein Eap. *Microbiology*, *149*(10), 2701-2707. https://doi.org/https://doi.org/10.1099/mic.0.26465-0

Hassoun, A., Linden, P. K., & Friedman, B. (2017). Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. *Critical care (London, England)*, *21*(1), 211-211. https://doi.org/10.1186/s13054-017-1801-3

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using Genomics to Track Global Antimicrobial Resistance [Review]. *Frontiers in Public Health*, *7*. https://doi.org/10.3389/fpubh.2019.00242

Herrera, C. A., Rada, G., Kuhn-Barrientos, L., & Barrios, X. (2014). Does ownership matter? An overview of systematic reviews of the performance of private for-profit, private not-for-profit and public healthcare providers. *PLoS One*, *9*(12), e93456. https://doi.org/10.1371/journal.pone.0093456

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176-187. https://doi.org/http://dx.doi.org/10.1016/S0140-6736(15)00473-0

Huygens, F., Inman-Bamber, J., Nimmo, G. R., Munckhof, W., Schooneveldt, J., Harrison, B., McMahon, J. A., & Giffard, P. M. (2006). Staphylococcus aureus genotyping using novel real-time PCR formats. *Journal of clinical microbiology*, *44*(10), 3712-3719. https://doi.org/10.1128/JCM.00843-06

Hyun Jin, K., Choi, Q., Kwon, G. C., & Koo, S. H. (2020). Molecular epidemiology and virulence factors of methicillin-resistant Staphylococcus aureus isolated from patients with bacteremia. *Journal of Clinical Laboratory Analysis*, *34*(3). https://doi.org/http://dx.doi.org/10.1002/jcla.23077

Ike, Y. (2017). Pathogenicity of *Enterococci*. *Nihon Saikingaku Zasshi*, *72*(2), 189-211. https://doi.org/10.3412/jsb.72.189

Ilczyszyn, W. M., Sabat, A. J., Akkerboom, V., Szkarlat, A., Klepacka, J., Sowa-Sierant, I., Wasik, B., Kosecka-Strojek, M., Buda, A., Miedzobrodzki, J., & Friedrich, A. W. (2016). Clonal Structure and Characterization of Staphylococcus aureus Strains from Invasive Infections in Paediatric Patients from South Poland: Association between Age, spa Types, Clonal Complexes, and Genetic Markers. *PLoS One*, *11*(3), e0151937-e0151937. https://doi.org/10.1371/journal.pone.0151937

International Monetary Fund. (2021). *World Economic Outlook (October 2021), GDP, current prices*. Retrieved 11/01/2022 from https://www.imf.org/external/datamapper/NGDPD@WEO/WEOWORLD/MYS/SGP/IDN/AFQ

Jamison, D. T., Summers, L. H., Alleyne, G., Arrow, K. J., Berkley, S., Binagwaho, A., Bustreo, F., Evans, D., Feachem, R. G. A., Frenk, J., Ghosh, G., Goldie, S. J., Guo, Y., Gupta, S., Horton, R., Kruk, M. E., Mahmoud, A., Mohohlo, L. K., Ncube, M., . . . Yamey, G. (2013). Global Health 2035: A World Converging Within a Generation. *The Lancet*, *382*(9908), 1898-1955. https://doi.org/https://doi.org/10.1016/S0140-6736(13)62105-4

Jee, Y., Carlson, J., Rafai, E., Musonda, K., Huong, T. T. G., Daza, P., Sattayawuthipong, W., & Yoon, T. (2018). Antimicrobial resistance: a threat to global health. *The Lancet Infectious Diseases*, *18*(9), 939-940. https://doi.org/10.1016/S1473-3099(18)30471-7

Jindal, A. K., Pandya, K., & Khan, I. D. (2015). Antimicrobial resistance: A public health challenge. *Medical Journal Armed Forces India*, *71*(2), 178-181. https://doi.org/10.1016/j.mjafi.2014.04.011

Johnson, B., Stephen, B. M., Joseph, N., Asiphas, O., Musa, K., & Taseera, K. (2021). Prevalence and bacteriology of culture-positive urinary tract infection among pregnant women with suspected urinary tract infection at Mbarara regional referral hospital, South-Western Uganda. *BMC Pregnancy and Childbirth*, *21*(1), 159. https://doi.org/10.1186/s12884-021-03641-8

Jolley, K. A., Bray, J. E., & Maiden, M. C. J. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Research*, *3*, 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Jolley, K. A., Chan, M.-S., & Maiden, M. C. J. (2004). mlstdbNet – distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics*, *5*(1), 86. https://doi.org/10.1186/1471-2105-5-86

Jonas, O. B., Irwin, A., Berthe, F. C. J., Le Gall, F. G., & Marquez, P. V. (2017). *Drug-resistant infections: a threat to our economic future: final report*. http://documents.worldbank.org/curated/en/323311493396993758/final-report

Kajumbula, H., Fujita, A. W., Mbabazi, O., Najjuka, C., Izale, C., Akampurira, A., Aisu, S., Lamorde, M., Walwema, R., Bahr, N., Meya, D., Boulware, D., & Manabe, Y. (2018). Antimicrobial Drug Resistance in Blood Culture Isolates at a Tertiary Hospital, Uganda. *Emerging infectious diseases*, *24*(1), 174. https://doi.org/10.3201/eid2401.171112

Karer, M., Kussmann, M., Ratzinger, F., Obermueller, M., Reischer, V., Winkler, H., Kriz, R., Burgmann, H., Jilma, B., & Lagler, H. (2020). Different Types of Coagulase Are Associated With 28-Day Mortality in Patients With Staphylococcus aureus Bloodstream Infections [Original Research]. *Frontiers in Cellular and Infection Microbiology*, *10*(236). https://doi.org/10.3389/fcimb.2020.00236

Kateete, D. P., Asiimwe, B. B., Mayanja, R., Najjuka, C. F., & Rutebemberwa, E. (2020). Species and drug susceptibility profiles of staphylococci isolated from healthy children in Eastern Uganda. *PLoS One*, *15*(2). https://doi.org/http://dx.doi.org/10.1371/journal.pone.0229026

Kateete, D. P., Namazzi, S., Okee, M., Okeng, A., Baluku, H., Musisi, N. L., Katabazi, F. A., Joloba, M. L., Ssentongo, R., & Najjuka, F. C. (2011). High prevalence of methicillin resistant *Staphylococcus aureus* in the surgical units of Mulago hospital in Kampala, Uganda. *BMC Research Notes*, *4*, 326. https://doi.org/10.1186/1756-0500-4-326

Kemnic, T. R., & Coleman, M. (2021). Trimethoprim Sulfamethoxazole. In *StatPearls*. StatPearls Publishing LLC.

Kengmo Tchoupa, A., Watkins, K. E., Jones, R. A., Kuroki, A., Alam, M. T., Perrier, S., Chen, Y., & Unnikrishnan, M. (2020). The type VII secretion system protects *Staphylococcus aureus* against antimicrobial host fatty acids. *Scientific Reports*, *10*(1), 14838. https://doi.org/10.1038/s41598-020-71653-z

Khoramrooz, S. S., Dolatabad, S. A., Dolatabad, F. M., Marashifard, M., Mirzaii, M., Dabiri, H., Haddadi, A., Rabani, S. M., Shirazi, H. R. G., & Darban-Sarokhalil, D. (2017). Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of *Staphylococcus aureus* in the Southwest of Iran. *Iranian Journal of Basic Medical Sciences*, *20*(8), 912-919. https://doi.org/10.22038/ijbms.2017.9114

Kityamuwesi, R., Muwaz, L., Kasangaki, A., Kajumbula, H., & Rwenyonyi, C. M. (2015). Characteristics of pyogenic odontogenic infection in patients attending Mulago Hospital, Uganda: a cross-sectional study. *BMC Microbiology*, *15*, 46. https://doi.org/10.1186/s12866-015-0382-z

Kluytmans, J., van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev*, *10*(3), 505-520. https://doi.org/10.1128/cmr.10.3.505

Koplan, J. P., Bond, T. C., Merson, M. H., Reddy, K. S., Rodriguez, M. H., Sewankambo, N. K., & Wasserheit, J. N. (2009). Towards a common definition of global health. *The Lancet*, *373*(9679), 1993-1995. https://doi.org/10.1016/s0140-6736(09)60332-9

Koreen, L., Ramaswamy, S. V., Graviss, E. A., Naidich, S., Musser, J. M., & Kreiswirth, B. N. (2004). spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *Journal of clinical microbiology*, *42*(2), 792-799. https://doi.org/10.1128/JCM.42.2.792-799.2004

Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: An Overview. *Cold Spring Harbor Perspectives in Medicine*, *6*(6). https://doi.org/10.1101/cshperspect.a027029

Kumar, N., Raven, K. E., Blane, B., Leek, D., Brown, N. M., Bragin, E., Rhodes, P. A., Parkhill, J., & Peacock, S. J. (2020). Evaluation of a fully automated bioinformatics tool to predict antibiotic resistance from MRSA genomes. *The Journal of Antimicrobial Chemotherapy*, *75*(5), 1117-1122. https://doi.org/10.1093/jac/dkz570

Kushner, B., Allen, P. D., & Crane, B. T. (2016). Frequency and Demographics of Gentamicin Use. *Otology and Neurotology*, *37*(2), 190-195. https://doi.org/10.1097/mao.0000000000000937

Kwak, Y. G., Truong-Bolduc, Q. C., Bin Kim, H., Song, K.-H., Kim, E. S., & Hooper, D. C. (2013). Association of norB overexpression and fluoroquinolone resistance in clinical isolates of *Staphylococcus aureus* from Korea. *The Journal of Antimicrobial Chemotherapy*, *68*(12), 2766-2772. https://doi.org/10.1093/jac/dkt286

Kyany'a, C., Nyasinga, J., Matano, D., Oundo, V., Wacira, S., Sang, W., & Musila, L. (2019). Phenotypic and genotypic characterization of clinical *Staphylococcus aureus* isolates from Kenya. *BMC Microbiology*, *19*(1), 245. https://doi.org/10.1186/s12866-019-1597-1

Li, M., Diep, B. A., Villaruz, A. E., Braughton, K. R., Jiang, X., DeLeo, F. R., Chambers, H. F., Lu, Y., & Otto, M. (2009). Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(14), 5883. https://doi.org/10.1073/pnas.0900743106

Li, S.-M., Zhou, Y.-F., Li, L., Fang, L.-X., Duan, J.-H., Liu, F.-R., Liang, H.-Q., Wu, Y.-T., Gu, W.-Q., Liao, X.-P., Sun, J., Xiong, Y.-Q., & Liu, Y.-H. (2018). Characterization of the Multi-Drug Resistance Gene cfr in Methicillin-Resistant *Staphylococcus aureus (*MRSA) Strains Isolated From Animals and Humans in China [Original Research]. *Frontiers in microbiology*, *9*(2925). https://doi.org/10.3389/fmicb.2018.02925

Lindsay, J. A., & Holden, M. T. G. (2004). *Staphylococcus aureus:* superbug, super genome? *Trends in Microbiology*, *12*(8), 378-385. https://doi.org/https://doi.org/10.1016/j.tim.2004.06.004

Lindsay, J. A., Moore, C. E., Day, N. P., Peacock, S. J., Witney, A. A., Stabler, R. A., Husain, S. E., Butcher, P. D., & Hinds, J. (2006). Microarrays reveal that each of the ten dominant lineages of Staphylococcus aureus has a unique combination of surface-associated and regulatory genes. *Journal of Bacteriology*, *188*(2), 669-676. https://doi.org/10.1128/JB.188.2.669-676.2006

Liu, B., Zheng, D., Jin, Q., Chen, L., & Yang, J. (2019). VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic acids research*, *47*(D1), D687-d692. https://doi.org/10.1093/nar/gky1080

Liu, C., & Chambers, H. F. (2003). *Staphylococcus aureus* with Heterogeneous Resistance to Vancomycin: Epidemiology, Clinical Significance, and Critical Assessment of Diagnostic Methods. *Antimicrobial Agents and Chemotherapy*, *47*(10), 3040-3045. https://doi.org/doi:10.1128/AAC.47.10.3040-3045.2003

Löffler, B., Hussain, M., Grundmeier, M., Brück, M., Holzinger, D., Varga, G., Roth, J., Kahl, B. C., Proctor, R. A., & Peters, G. (2010). *Staphylococcus aureus* Panton-Valentine Leukocidin Is a Very Potent Cytotoxic Factor for Human Neutrophils. *PLOS Pathogens*, *6*(1), e1000715. https://doi.org/10.1371/journal.ppat.1000715

Lozano, R., Wang, H., Foreman, K. J., Rajaratnam, J. K., Naghavi, M., Marcus, J. R., Dwyer-Lindgren, L., Lofgren, K. T., Phillips, D., Atkinson, C., Lopez, A. D., & Murray, C. J. (2011). Progress towards Millennium Development Goals 4 and 5 on maternal and child mortality: an updated systematic analysis. *The Lancet*, *378*(9797), 1139-1165. https://doi.org/10.1016/s0140-6736(11)61337-8

Ma, E. S. K., Wong, C. L. P., Lai, K. T. W., Chan, E. C. H., Yam, W. C., & Chan, A. C. W. (2005). *Kocuria kristinae* infection associated with acute cholecystitis. *BMC Infectious Diseases*, *5*, 60-60. https://doi.org/10.1186/1471-2334-5-60

Maiden, M. C. J., Van Rensburg, M. J. J., Bray, J. E., Earle, S. G., Ford, S. A., Jolley, K. A., & McCarthy, N. D. (2013). MLST revisited: the gene-by-gene approach to bacterial genomics. *Nature Reviews. Microbiology*, *11*(10), 728-736. https://doi.org/http://dx.doi.org/10.1038/nrmicro3093

Manishimwe, R., Nishimwe, K., & Ojok, L. (2017). Assessment of antibiotic use in farm animals in Rwanda. *Tropical Animal Health and Production*, *49*(6), 1101-1106. https://doi.org/http://dx.doi.org/10.1007/s11250-017-1290-z

Manyahi, J., Matee, M. I., Majigo, M., Moyo, S., Mshana, S. E., & Lyamuya, E. F. (2014). Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili national hospital, Tanzania. *BMC Research Notes*, *7*(1), 500. https://doi.org/10.1186/1756-0500-7-500

Manzi, A., Hirschhorn, L. R., Sherr, K., Chirwa, C., Baynes, C., Awoonor-Williams, J. K., Hingora, A., Mboya, D., Exavery, A., Tani, K., Manzi, F., Pemba, S., Phillips, J., Kante, A. M., Ramsey, K., Baynes, C., Awoonor-Williams, J. K., Bawah, A., Nimako, B. A., . . . the, A. H. I. P. P. C. (2017). Mentorship and coaching to support strengthening healthcare systems: lessons learned across the five Population Health Implementation and Training partnership projects in sub-Saharan Africa. *BMC Health Services Research*, *17*(3), 831. https://doi.org/10.1186/s12913-017-2656-7

Marwa, K. J., Mushi, M. F., Konje, E., Alele, P. E., Kidola, J., & Mirambo, M. M. (2015). Resistance to Cotrimoxazole and Other Antimicrobials among Isolates from HIV/AIDS and Non-HIV/AIDS Patients at Bugando Medical Centre, Mwanza, Tanzania. *AIDS Res Treat*, *2015*, 103874. https://doi.org/10.1155/2015/103874

Mawalla, B., Mshana, S. E., Chalya, P. L., Imirzalioglu, C., & Mahalu, W. (2011). Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. *BMC Surgery*, *11*, 21. https://doi.org/10.1186/1471-2482-11-21

Mbonye, A. K., Asimwe, J. B., Kabarangira, J., Nanda, G., & Orinda, V. (2007). Emergency obstetric care as the priority intervention to reduce maternal mortality in Uganda. *International*

Journal of Gynecology & Obstetrics, *96*(3), 220-225. https://doi.org/10.1016/j.ijgo.2006.12.017

McKenna, M. (2013). Antibiotic resistance: The last resort. *Nature*, *499*(7459), 394-396. https://doi.org/10.1038/499394a

Mediavilla, J. R., Chen, L., Mathema, B., & Kreiswirth, B. N. (2012). Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Current Opinion in Microbiology*, *15*(5), 588-595. https://doi.org/https://doi.org/10.1016/j.mib.2012.08.003

Medina-Pizzali, M. L., Hartinger, S. M., Salmon-Mulanovich, G., Larson, A., Riveros, M., & Mäusezahl, D. (2021). Antimicrobial Resistance in Rural Settings in Latin America: A Scoping Review with a One Health Lens. *International Journal of Environmental Research and Public Health*, *18*(18), 9837. https://doi.org/http://dx.doi.org/10.3390/ijerph18189837

Mingeot-Leclercq, M.-P., Glupczynski, Y., & Tulkens, P. M. (1999). Aminoglycosides: Activity and Resistance. *Antimicrobial Agents and Chemotherapy*, *43*(4), 727-737. https://doi.org/doi:10.1128/AAC.43.4.727

Mitchell, J., Purohit, M., Jewell, C. P., Read, J. M., Marrone, G., Diwan, V., & Stålsby Lundborg, C. (2021). Trends, relationships and case attribution of antibiotic resistance between children and environmental sources in rural India. *Scientific Reports*, *11*(1), 22599. https://doi.org/10.1038/s41598-021-01174-w

Mitiku, A., Aklilu, A., Biresaw, G., & Gize, A. (2021). Prevalence and Associated Factors of Methicillin Resistance *Staphylococcus aureus* (MRSA) Among Urinary Tract Infection Suspected Patients Attending at Arba Minch General Hospital, Southern Ethiopia. *Infection and drug resistance*, *14*, 2133-2142. https://doi.org/10.2147/IDR.S306648

Morinaga, N., Kaihou, Y., & Noda, M. (2003). Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. *Medical Microbiology and Immunology*, *47*(1), 81-90. https://doi.org/10.1111/j.1348-0421.2003.tb02789.x

Mrochen, D. M., Trübe, P., Jorde, I., Domanska, G., Brandt, C. v. d., & Bröker, B. M. (2021). Immune Polarization Potential of the *S. aureus* Virulence Factors SplB and GlpQ and Modulation by Adjuvants [Original Research]. *Frontiers in Immunology*, *12*(1301). https://doi.org/10.3389/fimmu.2021.642802

Mshana, S., Kamugisha, E., Miramb, M., Chalya, P., Rambau, P., Mahalu, W., & Lyamuya, E. (2009). Prevalence of clindamycin inducible resistance among methicillin-resistant Staphylococcus aureus at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania Journal of Health Research*, *11*(2).

Mshangila, B., Paddy, M., Kajumbula, H., Ateenyi-Agaba, C., Kahwa, B., & Seni, J. (2013). External ocular surface bacterial isolates and their antimicrobial susceptibility patterns among pre-operative cataract patients at Mulago National Hospital in Kampala, Uganda. *BMC Ophthalmology*, *13*(1), 71. https://doi.org/10.1186/1471-2415-13-71

Mwambi, B., Iramiot, J., Bwanga, F., Nakaye, M., Itabangi, H., & Bazira, J. (2014). Clindamycin Resistance among *Staphylococcus aureus* Isolated at Mbarara Regional Referral Hospital, in South Western Uganda. *British Microbiology Research Journal*, *4*(12), 1335-1344. https://doi.org/10.9734/bmrj/2014/10572

Najjuka, C. F., Kateete, D. P., Kajumbula, H. M., Joloba, M. L., & Essack, S. Y. (2016). Antimicrobial susceptibility profiles of *Escherichia coli* and *Klebsiella pneumoniae* isolated from outpatients in urban and rural districts of Uganda. *BMC Research Notes*, *9*, 235-235. https://doi.org/10.1186/s13104-016-2049-8

National Population Council. (2017). *State of Uganda Population Report 2017: Transforming Uganda's Economy: Opportunities to Harness the Demographic Dividend for Sustainable Development*.

http://library.health.go.ug/sites/default/files/resources/State%20of%20Uganda%20Population%20Report%202017.pdf
Nawrotek, P., Karakulska, J., & Fijałkowski, K. (2018). Chapter 9 - The *Staphylococcal* Panton-Valentine Leukocidin (PVL). In V. Savini (Ed.), *Pet-To-Man Travelling Staphylococci* (pp. 117-125). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-813547-1.00009-1
Neyfakh, A. A., Borsch, C. M., & Kaatz, G. W. (1993). Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrobial Agents and Chemotherapy*, *37*(1), 128-129. https://doi.org/10.1128/aac.37.1.128
Ngonzi, J., Tornes, Y. F., Mukasa, P. K., Salongo, W., Kabakyenga, J., Sezalio, M., Wouters, K., Jacqueym, Y., & Van Geertruyden, J. P. (2016). Puerperal sepsis, the leading cause of maternal deaths at a Tertiary University Teaching Hospital in Uganda. *BMC Pregnancy and Childbirth*, *16*(1), 207. https://doi.org/10.1186/s12884-016-0986-9
Nicholson, B. D., McKimm, J., & Allen, A. (2015). *Global Health*. SAGE Publications. http://ebookcentral.proquest.com/lib/salford/detail.action?docID=4714068
Nishino, K., & Yamaguchi, A. (2001). Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. *Journal of Bacteriology*, *183*(20), 5803-5812. https://doi.org/10.1128/jb.183.20.5803-5812.2001
Nishtar, S. (2007). Politics of health systems: WHO's new frontier. *The Lancet*, *370*(9591), 935-936. https://doi.org/10.1016/s0140-6736(07)61442-1
Nishtar, S. (2010). The mixed health systems syndrome. *Bulletin of the World Health Organization*, *88*(1), 74-75. https://doi.org/10.2471/BLT.09.067868
Novak, I., & Kovač, B. (2015). Electronic structure of antibiotic erythromycin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *138*, 550-552. https://doi.org/10.1016/j.saa.2014.11.076
Nurjadi, D., Olalekan, A. O., Layer, F., Shittu, A. O., Alabi, A., Ghebremedhin, B., Schaumburg, F., Hofmann-Eifler, J., Van Genderen, P. J. J., Caumes, E., Fleck, R., Mockenhaupt, F. P., Herrmann, M., Kern, W. V., Abdulla, S., Grobusch, M. P., Kremsner, P. G., Wolz, C., & Zanger, P. (2014). Emergence of trimethoprim resistance gene dfrG in Staphylococcus aureus causing human infection and colonization in sub-Saharan Africa and its import to Europe. *Journal of Antimicrobial Chemotherapy*, *69*(9), 2361-2368. https://doi.org/10.1093/jac/dku174
Nworie, A., Onyema, A. S., Okekpa, S. I., Elom, M. O., Umoh, N. O., Usanga, V. U., Ibiam, G. A., Ukwah, B. N., Nwadi, L. C., Ezeruigbo, C., Olayinka, B. O., Ehinmidu, J. O., Onaolapo, J. A., Hanson, B. M., Wardyn, S. E., & Smith, T. C. (2017). A Novel Methicillin-Resistant *Staphylococcus aureus* t11469 and a Poultry Endemic Strain t002 (ST5) Are Present in Chicken in Ebonyi State, Nigeria. *Biomed Research International*, *2017*, 2936461. https://doi.org/10.1155/2017/2936461
O'Niel, J. (2015). *Securing New Drugs for Future Generations: The Pipeline of Antibiotics*. https://amr-review.org/sites/default/files/SECURING%20NEW%20DRUGS%20FOR%20FUTURE%20GENERATIONS%20FINAL%20WEB_0.pdf
O'Niel, J. (2016). *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*. https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf
Obakiro, S. B., Kiyimba, K., Paasi, G., Napyo, A., Anthierens, S., Waako, P., Royen, P. V., Iramiot, J. S., Goossens, H., & Kostyanev, T. (2021). Prevalence of antibiotic-resistant bacteria among patients in two tertiary hospitals in Eastern Uganda. *Journal of Global Antimicrobial Resistance*, *25*, 82-86. https://doi.org/https://doi.org/10.1016/j.jgar.2021.02.021
Obasuyi, O., McClure, J., Oronsaye, F. E., Akerele, J. O., Conly, J., & Zhang, K. (2020). Molecular Characterization and Pathogenicity of *Staphylococcus aureus* Isolated from Benin-City, Nigeria. *Microorganisms*, *8*(6), 912. https://doi.org/10.3390/microorganisms8060912
Odongo, C. O., Anywar, D. A., Luryamamoi, K., & Odongo, P. (2013). Antibiograms from community-acquired uropathogens in Gulu, northern Uganda - a cross-sectional study. *BMC Infectious Diseases*, *13*(1), 193. https://doi.org/10.1186/1471-2334-13-193

Ohr, R. J., Anderson, M., Shi, M., Schneewind, O., Missiakas, D., & Silhavy, T. J. (2017). EssD, a Nuclease Effector of the *Staphylococcus aureus* ESS Pathway. *Journal of Bacteriology*, *199*(1), e00528-00516. https://doi.org/doi:10.1128/JB.00528-16

Ojulong, J., Mwambu, T. P., Joloba, M., Bwanga, F., & Kaddu-Mulindwa, D. H. (2009). Relative prevalence of methicilline resistant *Staphylococcus aureus* and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzania Journal of Health Research*, *11*(3), 149-153. https://doi.org/10.4314/thrb.v11i3.47703

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., Pablos-Mendez, A., & Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet Infectious Diseases*, *5*(8), 481-493. https://doi.org/10.1016/S1473-3099(05)70189-4

Okon, K. O., Basset, P., Uba, A., Lin, J., Oyawoye, B., Shittu, A. O., & Blanc, D. S. (2009). Co-occurrence of predominant Panton-Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 *Staphylococcus aureus* clones in Nigerian hospitals. *Journal of clinical microbiology*, *47*(9), 3000-3003. https://doi.org/10.1128/JCM.01119-09

Omulo, S., Thumbi, S. M., Njenga, M. K., & Call, D. R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? *Antimicrobial Resistance and Infection Control*, *4*(1), 1. https://doi.org/10.1186/s13756-014-0041-4

Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T., Fookes, M., Falush, D., Keane, J. A., & Parkhill, J. (2015). Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics*, *31*(22), 3691-3693. https://doi.org/10.1093/bioinformatics/btv421

Pagès, J. M., James, C. E., & Winterhalter, M. (2008). The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol*, *6*(12), 893-903. https://doi.org/10.1038/nrmicro1994

Panda, A. K., Bisht, S. S., DeMondal, S., Senthil Kumar, N., Gurusubramanian, G., & Panigrahi, A. K. (2014). Brevibacillus as a biological tool: a short review. *Antonie van Leeuwenhoek*, *105*(4), 623-639. https://doi.org/10.1007/s10482-013-0099-7

Paterson, G. K., Harrison, E. M., & Holmes, M. A. (2014). The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiology*, *22*(1), 42-47. https://doi.org/10.1016/j.tim.2013.11.003

Phaku, P., Lebughe, M., Strauß, L., Peters, G., Herrmann, M., Mumba, D., Mellmann, A., Muyembe-Tamfum, J. J., & Schaumburg, F. (2016). Unveiling the molecular basis of antimicrobial resistance in *Staphylococcus aureus* from the Democratic Republic of the Congo using whole genome sequencing. *Clinical Microbiology and Infection*, *22*(7), 644.e641-645. https://doi.org/10.1016/j.cmi.2016.04.009

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R., & Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, *53*(1), 28-52. https://doi.org/10.1093/jac/dkg483

Pietsch, F., Bergman, J. M., Brandis, G., Marcusson, L. L., Zorzet, A., Huseby, D. L., & Hughes, D. (2017). Ciprofloxacin selects for RNA polymerase mutations with pleiotropic antibiotic resistance effects. *Journal of Antimicrobial Chemotherapy*, *72*(1), 75-84. https://doi.org/10.1093/jac/dkw364

Pinchuk, I. V., Beswick, E. J., & Reyes, V. E. (2010). Staphylococcal enterotoxins. *Toxins*, *2*(8), 2177-2197. https://doi.org/10.3390/toxins2082177

Plata, K., Rosato, A. E., & Wegrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. *Journal of the Polish Biochemical Society and of the Committee of Biochemistry and Biophysics*, *56*(4), 597-612.

Prescott, K. (2020). *Chinese manufacturing hits record low amid coronavirus outbreak*. British Broadcasting Company (BBC). Retrieved 18/06/2021 from https://www.bbc.co.uk/news/business-51689178

Price, J. R., Didelot, X., Crook, D. W., Llewelyn, M. J., & Paul, J. (2013). Whole genome sequencing in the prevention and control of *Staphylococcus aureus* infection. *Journal of Hospital Infection*, *83*(1), 14-21. https://doi.org/https://doi.org/10.1016/j.jhin.2012.10.003

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*(7), 1641-1650. https://doi.org/10.1093/molbev/msp077

Ramalingam, A. J. (2015). History of Antibiotics and Evolution of Resistance. *Research journal of pharmacy and technology*, *8*(12), 1719-1724. https://doi.org/10.5958/0974-360X.2015.00309.1

Rasmussen, G., Monecke, S., Ehricht, R., & Söderquist, B. (2013). Prevalence of clonal complexes and virulence genes among commensal and invasive Staphylococcus aureus isolates in Sweden. *PLoS One*, *8*(10), e77477-e77477. https://doi.org/10.1371/journal.pone.0077477

Reddy, E. A., Shaw, A. V., & Crump, J. A. (2010). Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *10*(6), 417-432. https://doi.org/10.1016/s1473-3099(10)70072-4

Reinhart, K., Daniels, R., Kissoon, N., Machado, F. R., Schachter, R. D., & Finfer, S. (2017). Recognizing Sepsis as a Global Health Priority — A WHO Resolution. *The New England Journal of Medicine*, *377*(5), 414-417. https://doi.org/10.1056/NEJMp1707170

Roberts, M. C. (1996). Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews*, *19*(1), 1-24. https://doi.org/10.1111/j.1574-6976.1996.tb00251.x

Rochford, C., Sridhar, D., Woods, N., Saleh, Z., Hartenstein, L., Ahlawat, H., Whiting, E., Dybul, M., Cars, O., Goosby, E., Cassels, A., Velasquez, G., Hoffman, S., Baris, E., Wadsworth, J., Gyansa-Lutterodt, M., & Davies, S. (2018). Global governance of antimicrobial resistance. *The Lancet*, *391*(10134), 1976-1978. https://doi.org/10.1016/s0140-6736(18)31117-6

Rodloff, A., Bauer, T., Ewig, S., Kujath, P., & Müller, E. (2008). Susceptible, intermediate, and resistant - the intensity of antibiotic action. *Deutsches Arzteblatt international*, *105*(39), 657-662. https://doi.org/10.3238/arztebl.2008.0657

Rouch, D. A., Byrne, M. E., Kong, Y. C., & Skurray, R. A. (1987). The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn4001 from *Staphylococcus aureus*: expression and nucleotide sequence analysis. *Journal of General Microbiology*, *133*(11), 3039-3052. https://doi.org/10.1099/00221287-133-11-3039

Ruffing, U., Alabi, A., Kazimoto, T., Vubil, D. C., Akulenko, R., Abdulla, S., Alonso, P., Bischoff, M., Germann, A., Grobusch, M. P., Helms, V., Hoffmann, J., Kern, W. V., Kremsner, P. G., Mandomando, I., Mellmann, A., Peters, G., Schaumburg, F., Schubert, S., . . . Herrmann, M. (2017). Community-Associated Staphylococcus aureus from Sub-Saharan Africa and Germany: A Cross-Sectional Geographic Correlation Study. *Scientific Reports*, *7*(1), 154-154. https://doi.org/10.1038/s41598-017-00214-8

Ruimy, R., Maiga, A., Armand-Lefevre, L., Maiga, I., Diallo, A., Koumaré, A. K., Ouattara, K., Soumaré, S., Gaillard, K., Lucet, J.-C., Andremont, A., & Feil, E. J. (2008). The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. *Journal of Bacteriology*, *190*(11), 3962-3968. https://doi.org/10.1128/JB.01947-07

Santos Costa, S., Viveiros, M., Amaral, L., & Couto, I. (2013). Multidrug Efflux Pumps in *Staphylococcus aureus*: an Update. *The Open Microbiology Journal*, *7*, 59-71. https://doi.org/10.2174/1874285801307010059

Satta, G., Ling, C. L., Cunningham, E. S., McHugh, T. D., & Hopkins, S. (2013). Utility and limitations of Spa-typing in understanding the epidemiology of *Staphylococcus aureus* bacteraemia isolates in a single University Hospital. *BMC Research Notes*, *6*(1), 398. https://doi.org/10.1186/1756-0500-6-398

Say, L., Chou, D., Gemmill, A., Tunçalp, Ö., Moller, A. B., Daniels, J., Gülmezoglu, A. M., Temmerman, M., & Alkema, L. (2014). Global causes of maternal death: a WHO systematic analysis. *The Lancet Global Health*, *2*(6), e323-333. https://doi.org/10.1016/s2214-109x(14)70227-x

Schaumburg, F., Alabi, A. S., Peters, G., & Becker, K. (2014). New epidemiology of *Staphylococcus aureus* infection in Africa. *Clinical Microbiology and Infection*, *20*(7), 589-596. https://doi.org/https://doi.org/10.1111/1469-0691.12690

Schaumburg, F., Köck, R., Friedrich, A. W., Soulanoudjingar, S., Ngoa, U. A., von Eiff, C., Issifou, S., Kremsner, P. G., Herrmann, M., Peters, G., & Becker, K. (2011). Population Structure of *Staphylococcus aureus* from Remote African Babongo Pygmies. *PLOS Neglected Tropical Diseases*, *5*(5), e1150. https://doi.org/10.1371/journal.pntd.0001150

Schaumburg, F., Ngoa, U. A., sters, K. K., Köck, R., Adegnika, A. A., Kremsner, P. G., Lell, B., Peters, G., Mellmann, A., & Becker, K. (2011). Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. *Clinical Microbiology and Infection*, *17*(10), 1507-1513. https://doi.org/https://doi.org/10.1111/j.1469-0691.2011.03534.x

Schumann, H., Rubagumya, K., Rubaihayo, J., Harms, G., Wanyenze, R. K., & Theuring, S. (2020). The incidence of HIV and associated risk factors among pregnant women in Kabarole District, Uganda. *PLoS One*, *15*(6), e0234174. https://doi.org/10.1371/journal.pone.0234174

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14), 2068-2069. https://doi.org/10.1093/bioinformatics/btu153

Seemann, T. (2015). *snippy: fast bacterial variant calling from NGS reads*. https://github.com/tseemann/snippy

Seni, J., Bwanga, F., Najjuka, C. F., Makobore, P., Okee, M., Mshana, S. E., Kidenya, B. R., Joloba, M. L., & Kateete, D. P. (2013). Molecular characterization of Staphylococcus aureus from patients with surgical site infections at Mulago Hospital in Kampala, Uganda. *PLoS One*, *8*(6), e66153. https://doi.org/10.1371/journal.pone.0066153

Seni, J., Najjuka, C. F., Kateete, D. P., Makobore, P., Joloba, M. L., Kajumbula, H., Kapesa, A., & Bwanga, F. (2013). Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Uganda. *BMC Research Notes*, *6*(1), 298. https://doi.org/10.1186/1756-0500-6-298

Serio, A. W., Keepers, T., Andrews, L., & Krause, K. M. (2018). Aminoglycoside Revival: Review of a Historically Important Class of Antimicrobials Undergoing Rejuvenation. *EcoSal Plus*, *8*(1). https://doi.org/10.1128/ecosalplus.ESP-0002-2018

Sharma, M., Sanneving, L., Mahadik, K., Santacatterina, M., Dhaneria, S., & Stålsby Lundborg, C. (2013). Antibiotic prescribing in women during and after delivery in a non-teaching, tertiary care hospital in Ujjain, India: a prospective cross-sectional study. *Journal of Pharmaceutical Policy and Practice*, *6*(1), 9. https://doi.org/10.1186/2052-3211-6-9

Shore, A. C., Lazaris, A., Kinnevey, P. M., Brennan, O. M., Brennan, G. I., O'Connell, B., Feßler, A. T., Schwarz, S., & Coleman, D. C. (2016). First Report of *cfr* -Carrying Plasmids in the Pandemic Sequence Type 22 Methicillin-Resistant Staphylococcus aureus Staphylococcal Cassette Chromosome *mec* Type IV Clone. *Antimicrobial Agents and Chemotherapy*, *60*(5), 3007-3015. https://doi.org/doi:10.1128/AAC.02949-15

Sköld, O. (2011). *Antibiotics and Antibiotic Resistance*. John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/salford/detail.action?docID=697907

Smith, K. J., Neafie, R., Yeager, J., & Skelton, H. G. (1999). *Micrococcus folliculitis* in HIV-1 disease. *British Journal of Dermatology*, *141*(3), 558-561. https://doi.org/10.1046/j.1365-2133.1999.03060.x

Smith, T. C. (2015). Livestock-associated *Staphylococcus aureus*: the United States experience. *PLOS Pathogens*, *11*(2), e1004564. https://doi.org/10.1371/journal.ppat.1004564

Souza, R. R., Coelho, L. R., Botelho, A. M. N., Ribeiro, A., Rito, P. N., Vieira, V. V., Teixeira, L. A., Ferreira-Carvalho, B. T., & Figueiredo, A. M. S. (2009). Biofilm formation and prevalence of lukF-pv, seb, sec and tst genes among hospital- and community-acquired isolates of some

international methicillin-resistant Staphylococcus aureus lineages. *Clinical Microbiology and Infection*, *15*(2), 203-207. https://doi.org/https://doi.org/10.1111/j.1469-0691.2008.02118.x
Spízek, J., & Rezanka, T. (2004). Lincomycin, clindamycin and their applications. *Applied Microbiology and Biotechnology*, *64*(4), 455-464. https://doi.org/10.1007/s00253-003-1545-7
Stefani, S., Chung, D. R., Lindsay, J. A., Friedrich, A. W., Kearns, A. M., Westh, H., & MacKenzie, F. M. (2012). Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *International Journal of Antimicrobial Agents*, *39*(4), 273-282. https://doi.org/https://doi.org/10.1016/j.ijantimicag.2011.09.030
Storberg, V. (2014). ESBL-producing Enterobacteriaceae in Africa - a non-systematic literature review of research published 2008-2012. *Infection Ecology & Epidemiology*, *4*. https://doi.org/http://dx.doi.org/10.3402/iee.v4.20342
Strauß, L., Stegger, M., Akpaka, P. E., Alabi, A., Breurec, S., Coombs, G., Egyir, B., Larsen, A. R., Laurent, F., Monecke, S., Peters, G., Skov, R., Strommenger, B., Vandenesch, F., Schaumburg, F., & Mellmann, A. (2017). Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(49), E10596-E10604. https://doi.org/10.1073/pnas.1702472114
Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. (2017). Antimicrobial resistance in Africa: a systematic review. *BMC Infectious Diseases*, *17*(1), 616. https://doi.org/10.1186/s12879-017-2713-1
Takayama, Y., Tanaka, T., Oikawa, K., Fukano, N., Goto, M., & Takahashi, T. (2018). Prevalence of blaZ Gene and Performance of Phenotypic Tests to Detect Penicillinase in *Staphylococcus aureus* Isolates from Japan. *Annals of Laboratory Medicine*, *38*(2), 155-159. https://doi.org/10.3343/alm.2018.38.2.155
Tanaka, M., Wang, T., Onodera, Y., Uchida, Y., & Sato, K. (2000). Mechanism of quinolone resistance in *Staphylococcus aureus*. *Journal of Infection and Chemotherapy*, *6*(3), 131-139. https://doi.org/https://doi.org/10.1007/s101560070010
Tankeshwar, A. (2021). *Inducible Clindamycin Resistance (D Test)*. MICROBE ONLINE. Retrieved 31/08/21 from https://microbeonline.com/inducible-clindamycin-resistance-d-test-principle-procedure-and-interpretation/
Thammavongsa, V., Kim, H. K., Missiakas, D., & Schneewind, O. (2015). Staphylococcal manipulation of host immune responses. *Nature Reviews Microbiology*, *13*(9), 529-543. https://doi.org/10.1038/nrmicro3521
Thomas, S., Liu, W., Arora, S., Ganesh, V., Ko, Y. P., & Höök, M. (2019). The Complex Fibrinogen Interactions of the *Staphylococcus aureus* Coagulases. *Frontiers in Cellular and Infection Microbiology*, *9*, 106. https://doi.org/10.3389/fcimb.2019.00106
Thomer, L., Schneewind, O., & Missiakas, D. (2016). Pathogenesis of Staphylococcus aureus bloodstream infections. *Annual Review of Pathology: Mechanisms of Disease*, *11*, 343-364.
Tiong, J. J. L., Loo, J. S. E., & Mai, C.-W. (2016). Global Antimicrobial Stewardship: A Closer Look at the Formidable Implementation Challenges. *Frontiers in microbiology*, *7*, 1860-1860. https://doi.org/10.3389/fmicb.2016.01860
Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, *28*(3), 603-661. https://doi.org/10.1128/cmr.00134-14
Tumuhamye, J., Sommerfelt, H., Bwanga, F., Ndeezi, G., Mukunya, D., Napyo, A., Nankabirwa, V., & Tumwine, J. K. (2020). Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk. *PLoS One*, *15*(8), e0237085. https://doi.org/10.1371/journal.pone.0237085
Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler, V. G. (2019). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), 203-218. https://doi.org/10.1038/s41579-018-0147-4

Turyasiima, M., Nduwimana, M., Kiconco, G., Egesa, W. I., Manuel, S. A., Kalubi, P., & Ortiz, Y. E. A. (2020). Bacteriology and Antibiotic Susceptibility Patterns among Neonates Diagnosed of Omphalitis at a Tertiary Special Care Baby Unit in Western Uganda. *International Journal of Pediatrics*, *2020*, 4131098. https://doi.org/10.1155/2020/4131098

UBOS. (2019a). *District Projected population by single age and sex (2015-2025)*. Government of Uganda Retrieved from https://www.ubos.org/?pagename=explore-publications&p_id=20

UBOS. (2019b). *Housing Monograph*. Uganda: Government of Uganda Retrieved from https://www.ubos.org/?pagename=explore-publications&p_id=20

Udou, T. (2004). Dissemination of nosocomial multiple-aminoglycoside-resistant *Staphylococcus aureus* caused by horizontal transfer of the resistance determinant (*aacA/aphD*) and clonal spread of resistant strains. *American Journal of Infection Control*, *32*(4), 215-219. https://doi.org/10.1016/j.ajic.2003.11.002

Uganda Bureau of Statistics. (2016). *The National Population and Housing Census 2014 - Main Report*. https://unstats.un.org/unsd/demographic-social/census/documents/Uganda/UGA-2014-11.pdf

Uganda National Academy of Sciences. (2015). *Antibiotic Resistance in Uganda: Situation Analysis and Recommendations*. https://www.cddep.org/wp-content/uploads/2017/06/uganda_antibiotic_resistance_situation_reportgarp_uganda_0-1.pdf

Ugandan National Academy of Sciences. (2015). Antibiotic Resistance in Uganda: situation analysis and recommendations. Kampala, Uganda: Uganda National Academy of Sciences. *Center for Disease Dynamics, Economics & Policy*, 107.

Ugandan National Academy of Sciences. (2018). Antimicrobial Resistance National Action Plan (NAP). *Center for Disease Dynamics, Economics & Policy*.

United Nations. (2015). *The Millennium Development Goals Report 2015*. https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%201).pdf

United Nations. (2019). *World Population Prospects 2019*. United Nations, Department of Economic and Social Affairs, Population Dynamics. Retrieved 11/01/2022 from https://population.un.org/wpp/Download/Standard/Population/

Varshney, A., Ansari, Y., Zaidi, N., Ahmad, E., Badr, G., Alam, P., & Khan, R. H. (2014). Analysis of binding interaction between antibacterial ciprofloxacin and human serum albumin by spectroscopic techniques. *Cell Biochemistry and Biophysics*, *70*(1), 93-101. https://doi.org/10.1007/s12013-014-9863-1

Vialle-Valentin, C., Lecates, R., Zhang, F., Desta, A., & Ross-Degnan, D. (2012). Predictors of antibiotic use in African communities: evidence from medicines household surveys in five countries. *Tropical Medicine & International Health*, *17*(2), 211-222.

Vignaroli, C. (2009). Methicillin-resistant *Staphylococcus aureus* USA400 Clone, Italy. *Emerging infectious diseases*, *15*(6), 995-996. https://doi.org/10.3201/eid1506.081632

Votintseva, A. A., Fung, R., Miller, R. R., Knox, K., Godwin, H., Wyllie, D. H., Bowden, R., Crook, D. W., & Walker, A. S. (2014). Prevalence of *Staphylococcus aureus* protein A (spa) mutants in the community and hospitals in Oxfordshire. *BMC Microbiology*, *14*, 63. https://doi.org/10.1186/1471-2180-14-63

Walker, R. J., Campbell, J. A., & Egede, L. E. (2014). Effective strategies for global health research, training and clinical care: a narrative review. *Global Journal of Health Science*, *7*(2), 119-139. https://doi.org/10.5539/gjhs.v7n2p119

Wangai, F. K., Masika, M. M., Maritim, M. C., & Seaton, R. A. (2019). Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa: red alert or red herring? *BMC Infectious Diseases*, *19*(1), 596. https://doi.org/10.1186/s12879-019-4245-3

Wekesa, Y. N., Namusoke, F., Sekikubo, M., Mango Dennis, W., & Bwanga, F. (2020). Ceftriaxone- and ceftazidime-resistant *Klebsiella* species, *Escherichia coli*, and methicillin-resistant

Staphylococcus aureus dominate caesarean surgical site infections at Mulago Hospital, Kampala, Uganda. *Sage Open Medicine*, *8*. https://doi.org/http://dx.doi.org/10.1177/2050312120970719

Welsh, J. (2019). *Providing an evidence base for antibiotic stewardship for midwives in the Kabarole District of Uganda : a modified action research study* [PhD, University of Salford]. http://usir.salford.ac.uk/id/eprint/52326/

Wendlandt, S., Shen, J., Kadlec, K., Wang, Y., Li, B., Zhang, W.-J., Feßler, A. T., Wu, C., & Schwarz, S. (2015). Multidrug resistance genes in *Staphylococci* from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. *Trends in Microbiology*, *23*(1), 44-54. https://doi.org/https://doi.org/10.1016/j.tim.2014.10.002

William, B., Rwenyonyi, C. M., Swedberg, G., & Kironde, F. (2012). Cotrimoxazole Prophylaxis Specifically Selects for Cotrimoxazole Resistance in *Streptococcus mutans* and *Streptococcus sobrinus* with Varied Polymorphisms in the Target Genes *folA* and *folP*. *International Journal of Microbiology*, *2012*, 916129. https://doi.org/10.1155/2012/916129

World Health Organisation. (2014). Antimicrobial resistance: global report on surveillance 2014. https://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/

World Health Organisation. (2015a). *Global Action Plan on Antimicrobial Resistance*. W. D. P. Services. https://ahpsr.who.int/publications/i/item/global-action-plan-on-antimicrobial-resistance

World Health Organisation. (2015b). *Global Antimicrobial Resistance Surveillance System: Manual for Early Implementation*. W. D. P. Services. https://www.who.int/publications/i/item/9789241549400

World Health Organisation. (2016a). *Life expectancy and Healthy life expectancy: Data by country*. Retrieved 01/02/2022 from https://apps.who.int/gho/data/node.main.688

World Health Organisation. (2016b). *WHO Country Cooperation Strategy 2016-2020: Uganda*. https://apps.who.int/iris/handle/10665/255018

World Health Organisation. (2017). *Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early implementation 2016-17*. https://www.who.int/publications/i/item/9789241513449

World Health Organisation. (2018a). *Antimicrobial resistance and primary health care (No. WHO/HIS/SDS/2018.57)*. https://apps.who.int/iris/bitstream/handle/10665/326454/WHO-HIS-SDS-2018.56-eng.pdf

World Health Organisation. (2018b). *Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early implementation 2017-18*. https://www.who.int/publications/i/item/9789241515061

World Health Organisation. (2018c). *Tackling Antimicrobial Resistance (AMR) Together. Working Paper 1.0: Multisectoral coordination*. https://www.who.int/antimicrobial-resistance/publications/Tackling-AMR-multisectoral-coordination-june2018.pdf?ua=1

World Health Organisation. (2019). *World health statistics 2019: monitoring health for the SDGs, sustainable development goals*. World Health Organisation. Retrieved 09/09/2021 from https://apps.who.int/iris/handle/10665/324835

World Health Organisation. (2020, 17/06/2021). *Antimicrobial Resistance, Key Facts*. https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance

World Health Organisation. (2021). *WHO AWaRe Antibiotic Categorisation*. Retrieved 25/08/2021 from https://aware.essentialmeds.org/groups

World Health Organization, Food and Agriculture Organization of the United Nations, & World Organisation for Animal Health. (2016). *Antimicrobial Resistance – A Manual for Developing National Action Plans*. https://apps.who.int/iris/bitstream/handle/10665/204470/9789241549530_eng.pdf;jsessionid=6D2D729D85612B7D086937E0F6CB35B2?sequence=1

Wu, C., Zhang, X., Liang, J., Li, Q., Lin, H., Lin, C., Liu, H., Zhou, D., Lu, W., Sun, Z., Lin, X., Zhang, H., Li, K., Xu, T., Bao, Q., & Lu, J. (2021). Characterization of florfenicol resistance genes in the coagulase-negative *Staphylococcus* (CoNS) isolates and genomic features of a multidrug-resistant *Staphylococcus lentus* strain H29. *Antimicrobial Resistance and Infection Control*, *10*(1), 9. https://doi.org/10.1186/s13756-020-00869-5

Wyllie, D. H., Walker, A. S., Miller, R., Moore, C., Williamson, S. R., Schlackow, I., Finney, J. M., O'Connor, L., Peto, T. E., & Crook, D. W. (2011). Decline of meticillin-resistant Staphylococcus aureus in Oxfordshire hospitals is strain-specific and preceded infection-control intensification. *BMJ Open*, *1*(1), e000160. https://doi.org/10.1136/bmjopen-2011-000160

Xaplanteri, M. A., Andreou, A., Dinos, G. P., & Kalpaxis, D. L. (2003). Effect of polyamines on the inhibition of peptidyltransferase by antibiotics: revisiting the mechanism of chloramphenicol action. *Nucleic acids research*, *31*(17), 5074-5083. https://doi.org/10.1093/nar/gkg686

Yun, M.-K., Wu, Y., Li, Z., Zhao, Y., Waddell, M. B., Ferreira, A. M., Lee, R. E., Bashford, D., & White, S. W. (2012). Catalysis and Sulfa Drug Resistance in Dihydropteroate Synthase. *Science*, *335*(6072), 1110. https://doi.org/10.1126/science.1214641

Chapter 8 Appendix

Certificate of Ethical Approval Acquired from Makerere University

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: biomedicalresearch62@gmail.com
Phone: 256 752575050
Fax: 256 414 532204
COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
HIGHER DEGREES RESEARCH AND ETHICS COMMITTEE

1st Oct 2018

File: SBS 584

To: Mr Garvin Ackers
Principal Investigator
Department of Pharmacology and Therapeutics

Category of review
[ ] Initial review
[ ] Continuing review
[X] Amendment
[ ] Termination of study
[ ] SAEs

Re: Approval of an amendment to the proposal entitled **"Assessing the resistance patterns of bacteria isolated from cases of maternal Sepsis at Fort Portal Regional Referral Hospital, Uganda and further investigating the potential mechanisms of antibiotic resistance in the staphylococcus aureus isolates obtained"**

Your proposal entitled **"Assessing the resistance patterns of bacteria isolated from cases of maternal Sepsis at Fort Portal Regional Referral Hospital, Uganda and further investigating the potential mechanisms of antibiotic resistance in the staphylococcus aureus isolates obtained"** was initially reviewed and approved by School of Biomedical Sciences Higher Degrees Research and Ethics Committee in a letter dated 13th Aug 2018.

On 29th Oct 2018, you requested for permission to make the following modifications:

1. To revise the consent form to include a provision for a thumbprint for illiterate participants
2. To indicate in the study procedures that the samples collected will not be stored for use beyond the scope of the project.

The committee considered these amendments and found them appropriate since they will help to address consenting concerns better.

On behalf of the committee, iam glad to inform you that these amendments have been approved. You may now proceed with the study but forward regular reports to the committee.

Final approval is to be granted by Uganda National Council for Science and Technology.

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
★ 01 OCT 2018 ★
RESEARCH & ETHICS COMMITTEE
P.O. BOX 7072, KAMPALA

Signed

Dr. Ema Mwaka
Chairperson, School of Biomedical Sciences Higher Degrees Research and Ethics Committee.

Ethical Approval Acquired from the University of Salford

University of Salford MANCHESTER

Research, Innovation and Academic Engagement Ethical Approval Panel

Research Centres Support Team
G0.3 Joule House
University of Salford
M5 4WT

T +44(0)161 295 5278

www.salford.ac.uk/

17 November 2017

Chloe James

Dear Chloe,

RE: AMENDED ETHICS APPLICATION STR1617-104: Comparing the antibiotic resistance profiles of bacteria isolated from different sources in the UK and Uganda (1. Healthy skin; 2. Maternal sepsis cases)

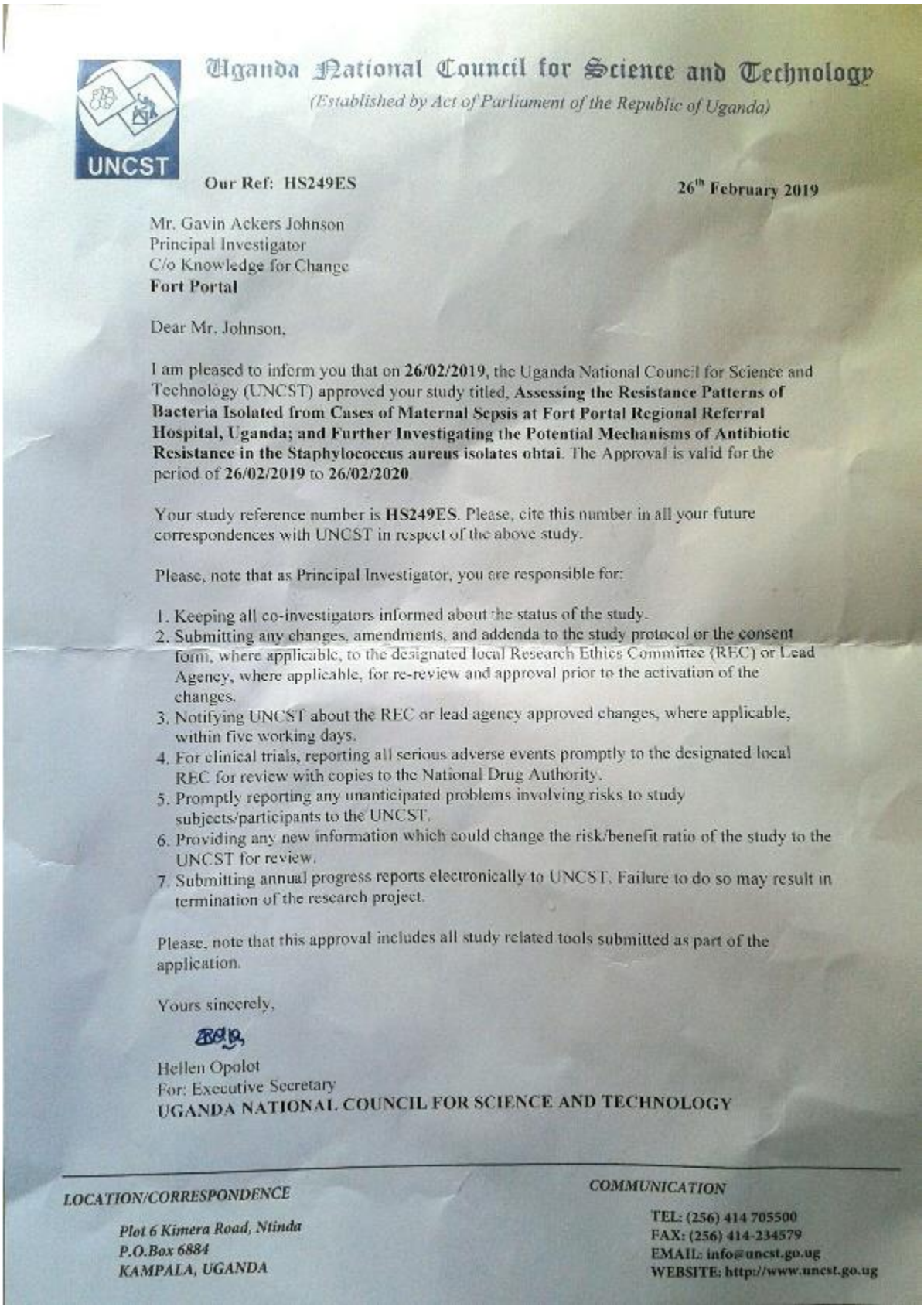
Based on the information you provided, I am pleased to inform you that your amended application STR1617-104 has been approved.

If there are any changes to the project and/ or its methodology, please inform the Panel as soon as possible by contacting S&T-ResearchEthics@salford.ac.uk

Yours sincerely,

Dr Anthony Higham
Chair of the Science & Technology Research Ethics Panel

Certificate of Approval from Uganda National Council for Science and Technology



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

UNCST

Our Ref: HS249ES **26th February 2019**

Mr. Gavin Ackers Johnson
Principal Investigator
C/o Knowledge for Change
Fort Portal

Dear Mr. Johnson,

I am pleased to inform you that on **26/02/2019**, the Uganda National Council for Science and Technology (UNCST) approved your study titled, **Assessing the Resistance Patterns of Bacteria Isolated from Cases of Maternal Sepsis at Fort Portal Regional Referral Hospital, Uganda; and Further Investigating the Potential Mechanisms of Antibiotic Resistance in the Staphylococcus aureus isolates obtai**. The Approval is valid for the period of **26/02/2019** to **26/02/2020**.

Your study reference number is **HS249ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above study.

Please, note that as Principal Investigator, you are responsible for:

1. Keeping all co-investigators informed about the status of the study.
2. Submitting any changes, amendments, and addenda to the study protocol or the consent form, where applicable, to the designated local Research Ethics Committee (REC) or Lead Agency, where applicable, for re-review and approval prior to the activation of the changes.
3. Notifying UNCST about the REC or lead agency approved changes, where applicable, within five working days.
4. For clinical trials, reporting all serious adverse events promptly to the designated local REC for review with copies to the National Drug Authority.
5. Promptly reporting any unanticipated problems involving risks to study subjects/participants to the UNCST.
6. Providing any new information which could change the risk/benefit ratio of the study to the UNCST for review.
7. Submitting annual progress reports electronically to UNCST. Failure to do so may result in termination of the research project.

Please, note that this approval includes all study related tools submitted as part of the application.

Yours sincerely,

Hellen Opolot
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P.O.Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug