GENOMIC EPIDEMIOLOGY AND ZOONOTIC POTENTIAL OF *STAPHYLOCOCCUS AUREUS* FROM PIGS AND HUMANS IN LUSAKA PROVINCE OF ZAMBIA

By

Mulemba Tillika Samutela

A Thesis Submitted to the University of Zambia in Fulfillment of the Requirements of the Award for the Degree of Doctor of Philosophy in Microbiology

The University of Zambia

Lusaka

Copyright Declaration

Declaration

I, Mulemba Tillika Samutela, do hereby declare that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for the degree award in this or any other institution.

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This thesis submitted by Mulemba Tillika Samutela has been approved as having fulfilled the requirements for the award of the degree of Doctor of Philosophy in Microbiology at the University of Zambia.

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	……	……
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Abstract

Pigs have been shown to be a reservoir for *Staphylococcus aureus* (*S. aureus*) including methicillin resistant strains which are collectively called Livestock-associated *S. aureus* (LA-SA) and Livestock-associated Methicillin-resistant *S. aureus* (LA-MRSA), respectively. These strains significantly colonise and cause infection in farmworkers and other individuals in contact with pigs. These persons are presumably the source of LA-SA transmission to household members and their associates. However, there is sparse information about LA-SA strains circulating in most African countries, including Zambia. This study aimed at determining the genomic epidemiology and zoonotic potential of *S. aureus* from pigs, farm and abattoir workers in selected districts of Lusaka province of Zambia. It was a cross-sectional study in which a total of 493 pig nasal swabs, 53 hand and nasal swabs each were collected from farm and abattoir workers. Forty-four human clinical isolates from a previous study were also included for selected genotypic investigation. Conventional microbiological methods were used to detect and identify *S. aureus* while the disc diffusion method was used to determine the antimicrobial susceptibility patterns. Polymerase chain reaction (PCR) with gene specific primers was used to confirm the species identity of *S. aureus* and detection of antimicrobial resistance and virulence genes. Genetic diversity of the strains was done using spa typing and whole genome sequencing (WGS). The overall prevalence of *S. aureus* in the study was 33.1%, specifically 37.8% for pigs and 11.8% for humans. The isolates were resistant to several anti-staphylococcal antibiotics including penicillin, tetracycline, and ciprofloxacin, with resistance rates ranging from 18% to 98%. However, the isolates showed considerable susceptibility to chloramphenicol, gentamicin, and co-trimoxazole. All isolates were susceptible to vancomycin. Although the *mec*A and *mec*C genes which encode resistance to methicillin were not detected, other resistance genes encoding resistance to tetracyclines (*tet*M, *tet*K, and *tet*L) and to erythromycin (*erm*B and *erm*C) were detected using PCR, while WGS showed the presence of other resistance genes which encode resistance to beta-lactams (*bla*Z), macrolides (*vga*(A)V), and fluroquinolones (*gry*A and *gyrl*A). More virulence genes were detected in silico via WGS compared to using PCR. These virulence genes included the aureolysin gene (*aur*), hemolysin genes (*hlg*A, *hlg*B, and *hlg*C) and enterotoxin genes (*seg*, *sei*, *sem*, *sen*, *seo* and *seu*). Immune evasion cluster genes (*sak* and *chp*) were also detected in some of the isolates from pigs. While several serine like protease genes (*spl*A to F) were detected in both human and pig isolates. Spa typing by both PCR and WGS revealed that most of the isolates belonged to the typical livestock-associated spa types (t1430 being the most common). Typical livestock-associated sequence types ST753 and ST9 were detected in two of the isolates. Novel spa and sequence types were detected among the isolates. Mobile genetic elements (plasmid, transposon and several insertion sequences) associated with the aforementioned resistance and virulence genes were also detected in silico in the isolates. Phylogenetic analysis based on WGS revealed that the isolates clustered together with typical livestock-associated ST398 MRSA isolates and were clonally related. The findings of our study show that LA-SA is present among pigs and workers who work closely with pigs in Zambia and there may be both zoonotic and anthropogenic transmission going on. Furthermore, these isolates pose a high risk to human health as they harbour both resistance and virulence genes which are possibly carried on mobile genetic elements and may thus spread easily. Therefore, continuous monitoring of *S. aureus* in this sector using a "One health" approach to combat *S. aureus* infections and prevention of the emergence and spread of antibiotic resistant strains is recommended.

Dedication

This thesis is dedicated to my mother, Elliet Banda, who encouraged me to love education early on in life, and to my siblings, whose love and support made it possible for me to complete my earlier education. It is also dedicated to my husband, Kateula Sichalwe, who made me realise my academic potential many years ago while in courtship. Last but not the least, I dedicate this thesis to my daughters, Lukundo and Chisomo Nachalwe, whom I believe will achieve all the good they will set out to do.

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List of Abbreviations

AMK	Amikacin
AMR	Antimicrobial Resistance
ATCC	The American Type Culture Collection
BURP	Based Upon Repeat Pattern
BHIB	Brian Heart Infusion Broth
Bp	Base Pairs
CAFOs	Concentrated Animal Feeding Operations
CA-MRSA	Community associated- Methicillin resistant *Staphylococcus aureus*
CC	Clonal Complex
chp	Chemotaxis Inhibitory Protein Gene
C	Chloramphenicol
CD	Clindamycin
CLSI	Clinical and Laboratory Standards Institute
CIP	Ciprofloxacin
CoPS	Coagulase-positive *Staphylococci*
CoNS	Coagulase-negative *Staphylococci*
cgMLST	core genome Multi Loci Sequence Typing
DDBJ	DNA Data Bank of Japan
DOX	Doxycycline
E	Erythromycin
ECDC	European Center for Disease Control and Prevention
EU	European Union
eMLST	Extended Multi Loci Sequence Typing
FOX	Cefoxitin
FUS	Fusidic Acid
CN	Gentamicin
HA-MRSA	Healthcare-associated-/ Hospital-acquired Methicillin resistant *Staphylococcus aureus*
HCAI	Health Care Associated Infections
hVISA	Heterogeneous-vancomycin-intermediate *S. aureus*
I	Intermediate
IEC	Immune Evasion Cluster
ISs	Insertion Sequences
LA	Livestock Associated
LA-MRSA	Livestock associated- Methicillin-resistant *Staphylococcus aureus*
LA-SA	Livestock associated- *Staphylococcus aureus*
LIN	Linezolid
Mbp	Mega base pairs
MDR	Multidrug-resistant
MGEs	Mobile Genetic Elements
MICs	Minimum inhibition concentrations
MLSB	Macrolide-lincosamide streptogramin B
MLST	Multi Locus Sequencing Typing
MLVA	Multiple-locus variable number tandem repeat analysis
MSSA	Methicillin susceptible *Staphylococcus aureus*
Mup	Mupirocin
MRSA	Methicillin resistant *Staphylococcus aureus*

NGS	Next Generation Sequencing
NT	Not Tested
OD	Optical Density
OXA	Oxacillin
P	Penicillin
PBP	Penicillin- binding Protein
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field gel electrophoresis
PVL	Panton-Valentine Leucocidin
R	Resistant
rMLST	ribosomal Multi Locus Sequence Typing
S	Susceptible
sak	Staphylokinase gene
SCC	Staphylococcal Cassette Chromosome
SCC*mec*	Staphylococcal Cassette Chromosome *mec*
scn	Staphylococcal Complement Inhibitor Gene
SEA	Staphylococcal Enterotoxin A
SEB	Staphylococcal Enterotoxin B
SEC	Staphylococcal Enterotoxin C
SED	Staphylococcal Enterotoxin D
SEE	Staphylococcal Enterotoxin E
SEF	Staphylococcal Enterotoxin F
SEG	Staphylococcal Enterotoxin G
SEH	Staphylococcal Enterotoxin H
SEI	Staphylococcal Enterotoxin I
SEJ	Staphylococcal Enterotoxin J
SEM	Staphylococcal Enterotoxin M
SEN	Staphylococcal Enterotoxin N
SEP	Staphylococcal Enterotoxin P
SER	Staphylococcal Enterotoxin R
SE*l*	Staphylococcal-like
SFP	Staphylococcal Food Poisoning
SID	Simpson's Index of Diversity
SNP	Single Nucleotide Polymorphism
SLST	Single Locus Sequence Typing
spa	Staphylococcus Protein A gene
spl	Serine protease like gene
SSR	Short Sequence Repeat
SXT	Trimethoprim-sulfamethoxazole
TET	Tetracycline
UNZABREC	University of Zambia Biomedical Research Ethics Committee
UNZAHSREC	University of Zambia Health Sciences Research Ethics Committee
V	Voltage
Van	Vancomycin
VISA	Vancomycin intermediate *S. aureus*
VNTR	Variable-number tandem repeats
wgMLST	Whole-genome Multi Locus Sequence Typing
WGS	Whole Genome Sequence

CHAPTER ONE

INTRODUCTION

1.0 Background of the Study

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen present in both healthy and diseased humans and animals (Deurenberg and Stobberingh, 2008, Corey, 2009). It causes a variety of diseases including mild infections such as skin and soft tissue infections, and potentially life-threatening infections such as pneumonia, and septicaemia (Gordon and Lowy, 2008). Moreover, these infections are associated with high morbidity, mortality and considerable economic impact. The medical importance of *S. aureus* has been heightened by its ability to adapt rapidly to the selective pressure of antibiotics and the resultant emergence and spread of methicillin-resistant *S. aureus* (MRSA) (Allegranzi et al., 2011). Methicillin-Resistant *S. aureus* is one of the most important causes of nosocomial and community-acquired infections worldwide described as Hospital-associated also known as healthcare-associated (HA-MRSA) and Community-associated (CA-MRSA), respectively (Gould et al., 2012). Although strains of *S. aureus* that are susceptible to methicillin so called Methicillin Susceptible *S. aureus* (MSSA) tend to be more susceptible to other antibiotics besides beta-lactams as well, they still contribute considerably to the pool of infections caused by *S. aureus* both in the hospital and community settings. Additionally, it is postulated that MSSA form the pool from which MRSA arise and thus it is important to study these strains too, especially those resistant to other antibiotics (Wardyn et al., 2015).

Recently, MRSA has emerged among livestock and has been described as Livestock-associated MRSA (LA-MRSA) (Huijsdens et al., 2006). Livestock-associated *S. aureus* (LA-SA), especially LA-MRSA, has been shown to not only increase infections in animals but also to have a greater zoonotic potential by being able to spread to humans in contact with animals (Smith, 2015). Studies of LA-MRSA around the globe have shown that it is prevalent in a wide range of animal species including pigs, cattle, poultry and non-human primates (Weese, 2010). However, relatively few studies on *S. aureus* and MRSA have been conducted in animals in Africa (Lozano et al., 2016). In most of these studies, molecular typing and antimicrobial resistance

profiling hasn't been described, thus, the information on these LA- strains is somewhat incomplete or limited (Lozano et al., 2016).

Pigs have been known to be asymptomatic carriers of *S. aureus* including MRSA although they rarely manifest clinical signs (Fluit, 2012). Of primary concern is that the MRSA in pigs can be transmitted to humans, especially those in close contact with livestock e.g., farm workers, people who work in slaughterhouses and veterinarians (Wulf et al., 2006, Van Loo et al., 2007, Van Cleef et al., 2010). A recent study conducted in China showed that contact with pigs, rather than pet contact was associated with carriage of LA-SA and LA-MRSA (Ye et al., 2016). Additionally, LA-MRSA has also been isolated from people without any previous contact with pigs or other animals (Reynaga et al., 2017). This may be attributed to transmission from household members who work in close contact with pigs or environmental transmission from air, water or manure (Smith, 2015). Furthermore, some strains of human origin have also been found in pigs (Sahibzada et al., 2017). These factors potentiate the need to study and monitor LA-SA strains.

In Zambia, pig farming is an important economic activity, with many pig farmers being smallholder farmers in the rural areas of the country in Eastern and Southern Provinces, who mostly use traditional rearing methods due to lack of resources (MAFF, 1998, Phiri et al., 2002). However, commercial pig farming has over the years become more common in the more urban parts of the country, especially in Lusaka Province. The shift to commercial pig farming could entail an increase of antibiotic use in pig rearing establishments, which has been shown to be a risk factor for the emergence of MRSA (van Duijkeren et al., 2008, Köck et al., 2009, Fang et al., 2014). Like other countries, Zambia, has recorded the presence of MRSA in hospitals as well as among animals. It is among the few African countries that has reported on *S. aureus* in animals. This data has come from very few studies (Youn et al., 2014, Samutela et al., 2015, Samutela et al., 2017, Phiri et al., 2022). Except for the most recent study by Phiri and colleagues that reported on *S. aureus* from milk from cattle (Phiri et al., 2022), the previous studies were not very comprehensive despite having included some genotyping and phenotyping such as antimicrobial profiling of the *S. aureus* strains isolated (Youn et al., 2014, Samutela et al., 2015, Samutela et al., 2017). Furthermore, there is still a paucity of data on LA-SA including LA-MRSA in Zambia, with no

studies conducted to check for the presence of *S. aureus* in pigs and workers on pig farms and abattoirs. The aim of this study, therefore, was to determine the genomic epidemiology and zoonotic potential of *S. aureus* from pigs and humans who work with pigs in Lusaka Province of Zambia.

1.1 Statement of the Problem

Zambia like other countries has reported cases of MRSA and MSSA especially in hospitals with increasing prevalence rates over the years from 23% to 43% (Samutela et al., 2015, Samutela et al., 2017). *S. aureus* has also been reported among companion animals and from milk and its environment (Youn et al., 2014, Phiri et al., 2022). Although studies from other countries have documented the presence of *S. aureus* in pigs and people who work closely with pigs, no studies have been conducted in Zambia to ascertain the prevalence of *S. aureus* in pig populations and the people who are at risk professionally MRSA (García-Álvarez et al., 2011). Further, data on the pathogenic potential and antimicrobial susceptility patterns of such isolates are also lacking in Zambia. Furthermore, the tranmissibility of such isolates are unknown too. Pigs act as carriers of S. aureus which can be passed on the humans. These *S. aureus* have been shown to not only colonise humans but can also cause infections and be passed on to other people such as household members in the community or indeed in the hospital. The later leads to the blurring of the epidemiology of *S. aureus* with implications of emergence and spread of antimicrobial resistance in the community and environment (Faldynova et al., 2013).

Pork being reported to be a vehicle for spread of pathogens that cause foodborne illnesses due to contamination during the slaughtering process including *S. aureus* which has been implicated as one of the major causes of foodborne diseases globally (Kadariya et al., 2014, Wu et al., 2016). However, there is still no data on staphylococcal enterotoxins expressed by enterotoxigenic strains of *Staphylococcus* in the Zambia pork production sector. It is therefore important to study the presence of such strains in pork production systems to ensure food safety. All the aforementioned factors put together potentiate the need for studies to determine the prevalence, phenotypes, genotypes, zoonotic and pathogenic potential of LA-SA circulating in pigs, and workers from pig farms and abattoirs in Zambia which are highly lacking.

1.2 Research Questions

1.2.1 What is the phenotypic and genetic diversity of *S. aureus* (MRSA and MSSA) in pigs and humans in Lusaka?

1.2.2 What are the susceptibility patterns and associated drug resistance genes carried by these strains?

1.2.3 What are the virulence factors of the *S. aureus* strains isolated from pigs and humans?

1.2.4 What is the zoonotic and anthroponotic potential of the strains isolated from pigs and humans?

1.3 Study Objectives

1.3.1 General Objective

To investigate the genomic epidemiology and zoonotic potential of *S. aureus* from pigs and humans in Lusaka Province of Zambia.

1.3.2 Specific Objectives

1.3.2.1 To assess the prevalence of methicillin-resistant and methicillin-susceptible *S. aureus* in pigs and humans in Lusaka.

1.3.2.2 To determine the antimicrobial susceptibility and presence of antimicrobial resistant genes in these *S. aureus* isolates.

1.3.2.3 To characterize virulence factor genes harbored by the *S. aureus* strains from pigs and humans.

1.3.2.4 To explore the genetic diversity, and zoonotic potential of the *S. aureus* isolates.

1.4 Significance of the Study

Staphylococcus aureus causes numerous infections that tend to be difficult to treat with consequent considerable mortality and morbidity, especially if multidrug resistant strains are involved. It is then important to control the spread of *S. aureus* including MRSA. Effective control measures are dependent on a thorough knowledge of the organism's epidemiology. This is achieved using reliable diagnostic and typing tools for detecting and tracking of sources and host reservoirs, pathways of spreading infections and studying population genetics. Thus, molecular diagnostic and typing tools are essential for the detection and monitoring of *S. aureus* infections and in

helping provide vital information useful in the implementation of appropriate control measures. To my knowledge this was the first study to detect *S. aureus* in pigs and humans in close contact with pigs in Zambia, thereby giving further insight into the epidemiology of *S. aureus* and MRSA in the country. The information generated in the study will enhance the infection prevention and control measures of this often-multidrug resistant organism by providing information on the molecular epidemiology and impact of *S. aureus* on livestock and humans in Zambia. Determining the burden of infections and or rates of carriage of the bacterium in pigs and humans will make it possible to trace potential zoonotic transmission of *S. aureus*. In addition, the findings about antimicrobial susceptibility and drug resistance genes will help in the establishment of appropriate guidelines on antimicrobial stewardship which can help in the prevention, management and control of *S. aureus* infections in the country as well as curb emergence of antimicrobial resistance. The information from the study could also be used to develop a rapid molecular diagnostic method by use of genetic markers that are more appropriate to the local situation in terms of specificity. These genetic markers could also be used as targets for alternative therapies and vaccine development.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview of the Genus *Staphylococcus*

The genus *Staphylococcus* belongs to the family *Staphylococcaceae*, under the order *Bacillales*, class *Bacillus* and phylum *Firmicutes*. Staphylococci are Gram-positive bacteria and comprises of several species, most of which are part of the normal flora of the skin and mucous membranes of man and animals (Ghebremedhin et al., 2008). Based on their ability to produce the enzyme coagulase, Staphylococci are classified into two groups namely coagulase-positive Staphylococci (CoPS) and coagulase-negative Staphylococci (CoNS). Notably, the CoPS have been recognized to cause infections compared to their CoNS counterparts which have been generally considered as being harmless to humans. *S. aureus* and *S. pseudintermedius* are the most important species in the CoPS group as they are major pathogens for both humans and animals, especially *S. aureus* (Marsilio et al., 2018). Interestingly, not all *S. aureus* species are coagulase positive. Although CoNS are saprophytic and rarely pathogenic, multidrug-resistant (MDR) strains of CoNS have been associated with severe cases of difficult to treat infections, especially in immunocompromised individuals (Piette and Verschraegen, 2009, Otto, 2013). *S. aureus* infections in pigs are infrequent even though pigs can be asymptomatic carriers of *S. aureus* including methicillin-resistant strains (van der Wolf et al., 2012). The central concern with MRSA in pigs is the risk of spread to humans as they can be reservoirs of these strains. *S. aureus* has been known to cause mastitis in cows, bumble foot in poultry and is a well-established pathogen in rabbits (McNamee and Smyth, 2000, Smith, 2015).

Staphylococcus was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint (Ogston, 1984). It was later called *S. aureus* by Friedrich Julius Rosenbach, who was credited by the official system of nomenclature at the time. Approximately 20% to 30% of the human populations are long-term carriers of *S. aureus*, which can be found as part of the normal skin flora and mucous membranes in the nostrils, axillae, perineum and vagina (Wertheim et al., 2005). Microscopically, *S. aureus* is a non-motile spherical Gram-positive bacterium that form grape-like clusters. They are facultative anaerobes that

grow mainly aerobically but can also grow anaerobically. Growth occurs within 24 to 48 hours of incubation at temperatures of about 35°C to 37°C. *S. aureus* form golden yellowish colonies although white variants are also common. The biochemical basis for the identification of *S. aureus* includes the production of catalase, coagulase and DNase (Bergeron et al., 2011).

2.2 Epidemiology of *Staphylococcus aureus*

S. aureus exists as a normal microbiota organism in humans and animals occupying mostly the relatively moist regions of the body such as the nasal tares, skin and groin areas. Despite this existence, it can cause infections especially when it enters sites that are not its normal habitat (Gordon and Lowy, 2008). Actually, colonization has been shown as a risk factor for subsequent infections (Kluytmans et al., 1997, Wertheim et al., 2005). The epidemiology of *S. aureus* can be fully discussed under the one health concept encompassing humans, animals and the environment. The one health concept (Figure 2.1) shows that human and animal health are interlinked and at the same time are both dependent on the environment (Cantas and Suer, 2014). Therefore, to achieve optimum health, all three aspects must be taken into consideration which signifies the need for continued consorted multidisciplinary efforts from human, animal and environmental experts (Cantas and Suer, 2014).

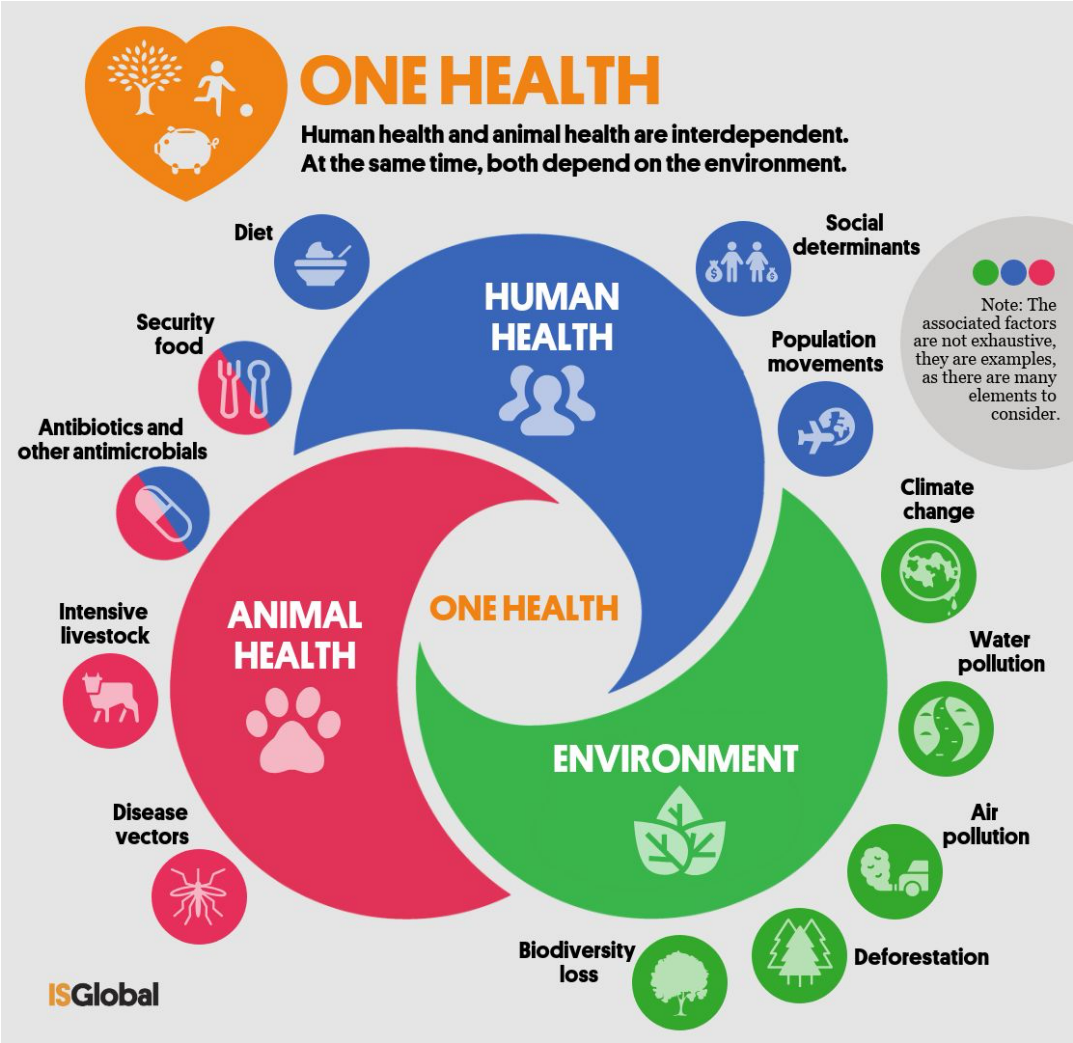


Figure 2.1 The One Health Model
Source of Image:(https://www.isglobal.org/)

The epidemiology of *S. aureus* which is currently classified into three as Hospital-associated (HA), Community-associated (CA) and Livestock-associated (LA), can be further described by the driving factors of antimicrobial resistance in the One Health concept (Figure 2.2). According to Smith (2015) these driving factors can be described as follows: the use of antibiotics as feed additives for growth promotion, disease prevention or treatment in animal husbandry, especially in industrial livestock and poultry may give rise to and evolution of LA-SA on farms. Many of the antibiotics used in agriculture include classes that are also used for treating humans such as tetracyclines, macrolides, penicillins, and sulfonamides. Antimicrobial resistant strains generated during animal husbandry may in turn spread to human populations in several different ways. These include occupational contact by farm workers, meat packers, butchers and veterinarians; handling or ingestion of meat products; potential secondary

spread into the community by those who are occupationally exposed, entry into and transmission via hospitals or other health care facilities; spread via environmental routes including air, water, or manure in areas in proximity to live animal farms or crop farms where manure has been used as a fertilizer. On the other hand, anthropogenic transmission of *S. aureus* from humans to the animals is also possible.

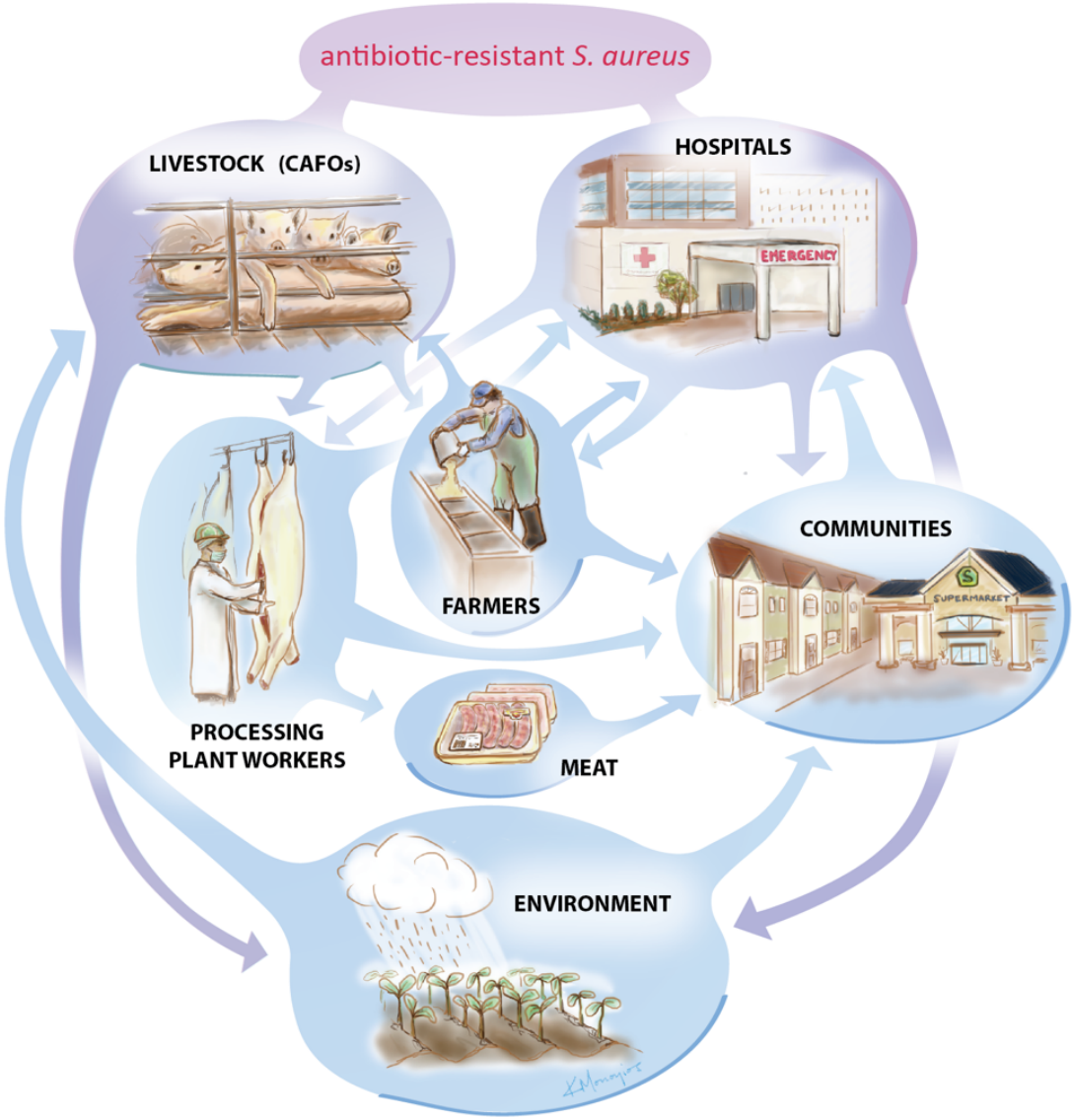


Figure 1.2 The Tangled Web of *S. aureus* in Relation to Humans, Animals and the Environment Adapted from (Smith, 2015) Abbreviations: CAFOs = Concentrated Animal Feeding Operations

2.2.1 Hospital-Associated *Staphylococcus aureus*

S. aureus is a common pathogen associated with hospital-associated infections. It is a major cause of bacteraemia which is associated with higher morbidity and mortality, compared with bacteraemia caused by other pathogens (Corey, 2009). Other serious infections caused by *S. aureus* include post-operative surgical wound infections,

infective endocarditis and deep-seated abscesses (Eisenstein, 2008, Sikora and Zahra, 2021).

According to the National Healthcare Safety Network data collected from approximately 2000 hospitals in the United States of America (USA), *S. aureus* was number one among the pathogens isolated from ventilator-associated pneumonia and in surgical site infections (Sievert et al., 2013). Furthermore, a variable portion (43 to 58 %) of the isolates were MRSA according to the type of infections or the hospital ward (Sievert et al., 2013).

In Europe, *S. aureus* is the first cause of surgical site infections and the second most commonly isolated microorganism after *E. coli* as shown by a point-prevalence survey carried out in acute care hospitals of 33 countries which was coordinated by the European Center for Disease Control and Prevention (ECDC) (Dulon et al., 2011, Johnson, 2011). Notably, the MRSA proportions greatly varied according to the country (Dulon et al., 2011, Johnson, 2011).

In Asia, *S. aureus* remain a significant microbial pathogen with more than one-half of hospital-related *S. aureus* infections being due to MRSA in most Asian countries. The detection of methicillin resistance in *S. aureus* in healthcare settings within Asia has increased significantly since the 1980s, with regional detection proportions ranging from 26% to 73% in 2011 (Mendes et al., 2013). Notably, *S. aureus* infections frequently present as severe or invasive diseases, especially in resource-limited Asian countries (Nickerson et al., 2009). In a study conducted in northeast Thailand, the overall mortality from *S. aureus* blood infection was 48% (Nickerson et al., 2006). This rate was almost double the mortality rate reported in a similar study in the USA (Fowler et al., 2003). Such findings potentiate the significant health burden on healthcare systems in the poor resource regions posed by *S. aureus* infections, thus the need to continuously monitor the epidemiology of *S. aureus*.

In Africa, although there is still a relatively paucity of data on HA-SA and HA-MRSA, staphylococcal infections were reported as early as 1978 with a hospital outbreak occurring in 1986 to 1987 in Johannesburg, South Africa (Park and Pearce, 1989). A systematic review by Irek and colleagues showed that *S. aureus* was one of the most

common causes of health care associated infections (HCAI) (Irek et al., 2018). Among the studies included in their review, the prevalence of MRSA isolates ranged from 3.9% to 58.6%. This is in congruence with Falagas and others' systematic review that reported a similar variability with the prevalence ranging from 1% to over 50% of *S. aureus* infections especially, MRSA in African countries (Falagas et al., 2013). This variability could be due to differences in the availability of antimicrobials but might as well be due to under reporting in some countries. Furthermore, extrapolation of relevant categorical conclusions is difficult using the available evidence on MRSA from disparate relevant studies which yielded variable findings (Falagas et al., 2013). Additionally, there is no readily available data on a pan-African wide scale on the mortality or financial burden of HA-MRSA.

In Zambia, the presence and prevalence of HA-SA and HA-MRSA have been reported to range from 14% to 67% by several studies (Lukwesa-Musyani et al., 2015, Samutela et al., 2015, Nagelkerke et al., 2017, Roth et al., 2021). However, these studies were not very comprehensive in the characterisation of *S. aureus* isolates in terms of its molecular epidemiology and impact on the health care systems. Therefore, there is need for more studies that can describe the epidemiology of *S. aureus* in Zambia.

2.2.2 Community- Associated *Staphylococcus aureus*

Community-associated *S. aureus*, particularly CA-MRSA were first reported in the late 1990s in healthy children and young adults in the community without prior exposure to the risk factors associated with MRSA infection in Australia, then New Zealand, then USA and Europe and have since spread throughout the world (Control and Prevention, 1999, Coombs et al., 2006, Elston, 2007, David and Daum, 2010). CA-MRSA is now a major cause of infections in the community as well as in healthcare facilities worldwide (Elston, 2007). They are known to mostly cause major skin and soft tissue infections and toxic shock syndrome in the children and young adults (Elston, 2007). Nevertheless, severe, life-threatening cases related to several clinical syndromes, such as necrotizing pneumonia and necrotizing fasciitis have been reported (David and Daum, 2010). The prevalence of CA-MRSA varies across the world and notably is higher in children than in adults. The rate of MRSA amongst CA-SA infection varies with ranges of up to 50% in different countries (Lakhundi and Zhang, 2018). Besides the USA, Taiwan, Canada and Australia were extensive

outbreaks of CA-MRSA have been reported (Adcock et al., 1998, Baggett et al., 2004, Begier et al., 2004, Aiello et al., 2006, Stemper et al., 2006, Diep et al., 2008), most other countries have reported small outbreaks or case series of CA-MRSA infected cases (Gardam, 2000, Baba et al., 2002, Coombs et al., 2006, David and Daum, 2010).

The incidence of CA-MRSA in Asian countries has been reported to vary markedly with rates being reported ranging from 2.5% to 39% (Chen and Huang, 2014). With the rates being greater than 30% for the Philippines, Vietnam, Taiwan, and Sri Lanka, and lower than 10% for India, Hong Kong, and Thailand (Chen and Huang, 2014). Relatively few studies from African countries have documented CA-SA including CA-MRSA. However, given the diverse geographical set ups and culture of Africa such as the relatively free interaction with livestock in some households, there is need for more studies and reports on CA-SA infections. A study conducted in Nigeria reported a 47.2% rate of CA-MRSA (Ghebremedhin et al., 2009). Another study from South Africa reported the prevalence of CA-SA at 2.3% while CA-MRSA accounted for 7.9% of all MRSA cases in the study (Perovic et al., 2017). In Zambia, there is a dearth of data on CA-SA including CA-MRSA with only one study reporting on CA-SA (Nagelkerke et al., 2017). This aspect of the epidemiology of *S. aureus* in Zambia is worth exploring in view of the reports that show that CA-MRSA maybe co-existing with or indeed replacing HA-MRSA.

2.2.3 Livestock-associated *Staphylococcus aureus*

Reports of LA-SA in pigs were first reported in the early 2000s on the European continent with reports coming from countries such as France and the Netherlands (Armand-Lefevre et al., 2005, Voss et al., 2005). In the reports from France, Armand-Lefevre and colleagues described the carriage of *S. aureus*, both methicillin-susceptible (MSSA) and methicillin-resistant, among pigs and pig farmers that shared certain Sequence types (ST) (Armand-Lefevre et al., 2005). However, the isolates came from separate, non-related collections. As such, the authors could not establish or confirm the transmission link between humans and pigs. The case report of the presence of MRSA in a 6-month-old baby from a pig farming family in July 2004 was the first report of pig associated MRSA in the Netherlands (Voss et al., 2005). This was followed by the subsequent isolation of MRSA from the father (who presumably transmitted it to the baby), some pigs on their farm, and other regional pig farmers

within the region in November 2004 (Voss et al., 2005). In January 2005, two new cases of MRSA were discovered in a son of a veterinarian who worked mostly with pigs and a pig farmer from a different region (Voss et al., 2005). Later the strains were found in the veterinarian (boy's father) and in a nurse who was working at a hospital unit were the boy was admitted (Voss et al., 2005). Overall, the MRSA strains were found to cluster into three groups based on the spa-types (including a novel spa-type) and were similar among the three epidemiological groups (Voss et al., 2005). Thus, it was demonstrated that transmission of MRSA between an animal (pig) and human (pig farmer), between family members (pig farmers and their families), and between a nurse and patient in the hospital was possible (Voss et al., 2005). Over the years, the presence of pig-related *S. aureus* especially MRSA has been reported in many European countries including Denmark, Belgium, Italy and Austria, Asia, America and Australia (van Cleef et al., 2011, Lim et al., 2012, Molla et al., 2012, Groves et al., 2014, Chuang and Huang, 2015, Parisi et al., 2019). The most recent study using Whole Genome Sequencing (WGS) data showed host adaptation and transmission from pigs into healthcare institutions in Denmark (Larsen et al., 2017). Therefore, there is need to monitor the emergence and spread of LA-SA strains.

Unfortunately, studies on the presence of *S. aureus* both MSSA and MRSA from animals and foods of animal origin in Africa are limited and there is only information on some countries that include Côte d'Ivoire, Egypt, Nigeria, Senegal, South Africa, Sudan, Tunisia and Zambia (Lozano et al., 2016). Most of these studies have been performed in the recent years, indicating an increased awareness of the role of animals in the evolution, epidemiology and dissemination of *S. aureus*. The lack of research on LA-SA in developing countries, including African countries is a great cause of alarm seeing the potential pathogenicity of these strains. Table 2.1 summaries the characteristics of studies that have reported on the presence of *S. aureus* between 2000 and 2019 in Africa. These studies showed that the prevalence of pig associated *S. aureus* in live pigs reported in Africa ranged from 0% to 55% (Fall et al., 2012, Adegoke and Okoh, 2014, Udegbunam et al., 2014, Chairat et al., 2015, Okunlola and Ayandele, 2015, Tanih et al., 2015, Katakweba et al., 2016, Igbinosa et al., 2016, Founou et al., 2018, Momoh et al., 2018, Odetokun et al., 2018, Otalu et al., 2018, Pekana and Green, 2018, Van Lochem et al., 2018, Adikwu et al., 2019, Dweba et al., 2019, Founou et al., 2019, Igbinosa and Beshiru, 2019, and Nwaogaraku et al., 2019).

Most of the studies from Africa reported relatively low to moderately high prevalences of *S. aureus* among live pigs ranging from 3.7% to 25% (Table 2.1). However, this prevalence is still somewhat lower than that reported in the US, Europe and Asia which ranges up to 85% (Fluit, 2012). The relatively low prevalence of pig associated *S. aureus* reported in Africa could be due to most studies not setting out to detect *S. aureus* specifically, but rather *Staphylococcus* species in general and/or other bacterial species. Furthermore, some of the studies sampled other animal species more than pigs. Notably, the studies that reported high *S. aureus* prevalence of 43.2% and 55% respectively, sampled pigs only or while they sampled other animals, they collected samples from more than one body part of the pigs, respectively (Odetokun et al., 2018, Dweba et al., 2019). Notably, both studies targeted *S. aureus* as the pathogen of interest and the sample sizes were also considerably larger than the studies that reported lower prevalence. This underscores the need for more studies that deliberately set out to study *S. aureus* in pig populations on the African continent.

Since *S. aureus* can also be transmitted from meat and meat products, it is essential to look at this aspect too. The prevalence of LA-SA associated with pork in the US, Europe and Asia has been reported at variable rates from as low as 5% to about 50% (Fluit, 2012). In Africa very few studies have reported on *S. aureus* in pork and/or carcases of pigs with the prevalence ranging from 0% to 53.9% (Table 2.1). The lowest prevalence was reported in a study from Tunisia that included a single pig sample since pork is rarely eaten in this country (Chairat et al., 2015). There was a marked difference (14% and 31.5%) in the prevalence of *S. aureus* from pork samples in the two studies from South Africa (Tanih et al., 2015, Pekana and Green, 2018). On the other hand, the prevalence of *S. aureus* among pork samples from the studies conducted in Nigeria were very high 35% (Adikwu et al., 2019) and 53% (Igbinosa et al., 2016). These findings show the importance of pork in the epidemiology of *S. aureus* and therefore more studies are needed in this area too.

Although LA- MRSA are often associated with asymptomatic colonisation, several cases of infections of variable clinical relevance, ranging from skin and soft tissue infections to endocarditis, pneumonia and necrotising fasciitis, have been described over the past few years in humans and mastitis in pigs (Huijsdens et al., 2006, Pantosti, 2012). Risk factors for colonisation include working in direct contact with animals on

farms and working with live pigs in slaughterhouses (Van Cleef et al., 2014, Grøntvedt et al., 2016, Sahibzada et al., 2017). In the systematic review by Samutela and others (2021), only seven studies sampled humans, besides pigs or pig carcasses and mostly involved either farm or abattoir workers and a few sampled from both places (Table 2.1). The prevalence of *S. aureus* among humans with professional contact with pigs (farm or abattoir workers) ranged from 0% to 30.8% (Samutela et al., 2021). Two of the studies did not detect any *S. aureus* among humans who had contact with pigs (Katakweba et al., 2016, Founou et al., 2018). Apart from one of the seven studies (Fall et al., 2012) which reported a high prevalence (30.8%), most studies reported relatively low prevalence (0% to 13.5%) of *S. aureus* among people who work with pigs. Livestock-associated MRSA may account for a significant proportion of human MRSA cases, particularly CA-MRSA but also HA-MRSA (Sahibzada et al., 2017). Therefore, there is need to incorporate this aspect of the epidemiology of *S. aureus* in Zambia as it is seriously lacking.

Table 2.1 Prevalence of Porcine-related *S. aureus* in Africa

Study Country	Sample Type	Method of Detection	*S. aureus* Prevalence			Reference
			Pigs	Humans with Contact	*S. aureus* Type (MRSA vs MSSA)	
Nigeria	Hand, water, meat, carcass swabs	Phenotypic, Serological	35% (50/200)	9.4% (3/32)	Not indicated	(Adikwu et al., 2019)
Nigeria	Nasal & rectal	Phenotypic, Molecular	14.9% 13/27	Not studied	MRSA	(Igbinosa and Beshiru, 2019)
Nigeria	Blood	Phenotypic, Molecular	25% (25/100)	Not studied	44% (11/25) MRSA	(Nwaogaraku et al., 2019)
South Africa	Oral, fecal, cloacal & environmental swabs	Phenotypic, Molecular (*nuc*)*	55% (15/27)	Not studied	MRSA	(Dweba et al., 2019)
Nigeria	Nasal	Phenotypic, Mass spectrometry	4.7% (20/425)	10.9% (6/55)	MRSA	(Otalu et al., 2018)
Nigeria	Nasal	Phenotypic, Serological, Mass spectrometry	5.3% (16/300)	12.9% (13/101)	MSSA	(Momoh et al., 2018)
South Africa	Nasal	Phenotypic, Serological, Mass spectrometry	12%	Not studied	12% MRSA	(Van Lochem et al., 2018)
South Africa	Meat and milk	Phenotypic, Molecular (*nuc*)*	14% (14/100)	Not studied	50% (7/14) MRSA	(Pekana and Green, 2018)
Nigeria	Nasal and Surface	Phenotypic, Serological Molecular (*nuc* & *tuf*)*	3.7% (3/8)	13.5% (10.4 % MSSA, 3.1% MRSA)	1.2% MRSA, 2.5 % MSSA in pigs)	(Odetokun et al., 2018)

* *nuc* and *tuf* genes were PCR targets in the particular studies

Table 2.1 Continued Prevalence of Porcine-related *S. aureus* in Africa

Study Country	Sample Type	Method of Detection	*S. aureus* Prevalence			Reference
			Pigs	Humans with Contact	*S. aureus* Type (MRSA vs MSSA)	
South Africa & Cameroon	Nasal, Rectal, Hand	Phenotypic	18.9% (7/37)	0%	100% MRSA in pigs	(Founou et al., 2018, Founou et al., 2019)
Tanzania	Nasal	Phenotypic, Molecular (*nuc*)*	4% (4/100)	0%[a]	4% MSSA (Pigs only)	(Katakweba et al., 2016)
Nigeria	Meat	Phenotypic, Molecular (nuc & 16rRNA)*	53.9% (14/26)	Not studied	100% MRSA	(Igbinosa et al., 2016)
Tunisia	Raw food samples of animal origin	Phenotypic, Molecular (*nuc*)*	0% (0/1)	Not studied	0%	(Chairat et al., 2015)
Nigeria	Nasal	Phenotypic	43.2% (41/95)	Not studied	43.9% (18/41) MRSA	(Odetokun et al., 2018)
South Africa	Rump, Flank, Brisket, & Neck swabs	Phenotypic, Serological	31.5% (20/64)	Not studied	31.5% [20/20 MRSA from (pigs)]	(Tanih et al., 2015)
Nigeria	Ocular swabs	Phenotypic	0% (0/2)	Not studied	0%	(Udegbunam et al., 2014)
South Africa	Nasal, Mouth wash & Ear	Phenotypic	23.3% (28/120)	Not studied	12.6% MRSA (Pigs alone)	(Adegoke and Okoh, 2014)
Senegal	Nasal	Phenotypic	12.3% (57/464)	30.8% (16/52)	10.5% (6/57) pig isolates only	(Fall et al., 2012)

* *nuc* gene were PCR targets in the particular studies

2.4 Antimicrobial Resistance in *Staphylococcus aureus*

Antimicrobial resistance (AMR) among bacteria is considered a serious threat to human health and is described as a crisis in waiting (Jensen, 2020). *S. aureus* is known to easily adapt and select for antimicrobial resistance in the presence of antibiotics. The discriminate use of antibiotics can lead to selective pressure for resistance. Multidrug resistant strains of *S. aureus* tend to be more difficult to deal with as there are fewer treatment options available (Diekema et al., 2001). It is therefore important to monitor the emergence and spread of AMR among *S. aureus* as well as the several mechanisms of AMR. This information is vital in informing the optimal use of antimicrobial agents in clinical practice and further underpins the critical features of antimicrobial stewardship programmes (Watkins, 2019).

2.4.1 Resistance to Beta-lactam Antibiotics

Beta-lactam antibiotics are a group of antibiotics that contain a beta-lactam ring as the major active site and they include penicillins, cephalosporins, monobactams and carbapenems. They act by inhibiting cell wall synthesis in bacteria. These antibiotics, especially the penicillins, are usually the main stay treatment of uncomplicated *S. aureus* infections (Lowy, 2003). However, resistance to beta-lactam antibiotics rapidly emerged in *S. aureus* with many strains being resistant to several antibiotics in this class with resultant difficult to treat infections. Resistance to methicillin is perhaps the most critical resistance occurrence in *S. aureus* as such strains are also often resistant to several other classes of antibiotics besides beta-lactam antibiotics (Lowy, 2003).

2.4.1.1 Methicillin-resistant *Staphylococcus aureus* and Methicillin-susceptible *Staphylococcus aureus*

Staphylococcus aureus can easily adapt to selective antibiotic pressure, and this led to the quick emergence of Methicillin resistant *S. aureus*. MRSA is *S. aureus* that is resistant to methicillin and cloxacillin. Conversely, *S. aureus* strains that are susceptible to methicillin and cloxacillin are called Methicillin susceptible *S. aureus* (MSSA). Methicillin and cloxacillin are semi-synthetic penicillins resistant to penicillinase and were introduced into therapeutic use to combat staphylococcal infectious that were resistant to penicillin due to the enzyme penicillinase. MRSA was first reported in 1961 in the United Kingdom, barely two years after the introduction

of methicillin in clinical practice (Barber, 1961). MRSA are resistant to all β-lactam antibiotics (Deurenberg et al., 2007). This poses a challenge to the health care system since β-lactam antibiotics are among the most commonly or widely used antibiotics. Moreover, MRSA are known to be resistant to other classes of antibiotics implying that they are usually multidrug resistant (Schmitz et al., 1997, Diekema et al., 2000). There remain little therapeutic options for treatment of MRSA. It is therefore important to be on the lookout on the emergence of MRSA strains to avoid their further spread i.e., surveillance of MRSA is key in prevention and control of MRSA (Hallin et al., 2007, Grøntvedt et al., 2016). This means there is need for a thorough knowledge of the epidemiology and dissemination of MRSA. MRSA strains do not account for a large proportion of staphylococcal infections compared to their counterparts that is MSSA. Moreover, MRSA is not necessarily more virulent than MSSA (Schmitz et al., 1997, Schlievert et al., 2010) although MRSA infections are more difficult and expensive to treat (Kinross et al., 2014). Severe MRSA infections cause significant morbidity and mortality (Corey, 2009). For example, mortality due to MRSA infections is twice as high compared to that due to MSSA infections (Moellering, 2012).

Nevertheless, MSSA are important in the evolution of *S. aureus*. Whole genome sequencing data has shown that LA-CC398-MRSA evolved from an ancestor which was a human-adapted HA-MSSA CC398 (Price et al., 2012). This CC398-MSSA ancestor could have acquired resistance to methicillin and tetracycline while losing the prophage that carries the IEC genes which protect *S. aureus* against the immune system in humans (van Wamel et al., 2006). Several studies from European countries show that the LA-CC398 MSSA have emerged as a subpopulation of causative agents of invasive infections in hospitals (Vandendriessche et al., 2011, Benito et al., 2014, Tavares et al., 2014, Bouiller et al., 2020, Mama et al., 2021). Of interest is a subset of the CC398 MSSA which is independent of livestock but is human adapted (Mama et al., 2021). However, there is sparse information on this clade especially from Africa. Therefore, more studies are warranted to further understand such lineages. Furthermore, given the role of human and animal interactions in the emergence of such lineages, it is critical to conduct such studies in a comprehensive manner using a "One Health" approach. Figure 2.3 shows the probable evolutionary relationships of MSSA and MRSA in humans and livestock (Laumay et al., 2021).

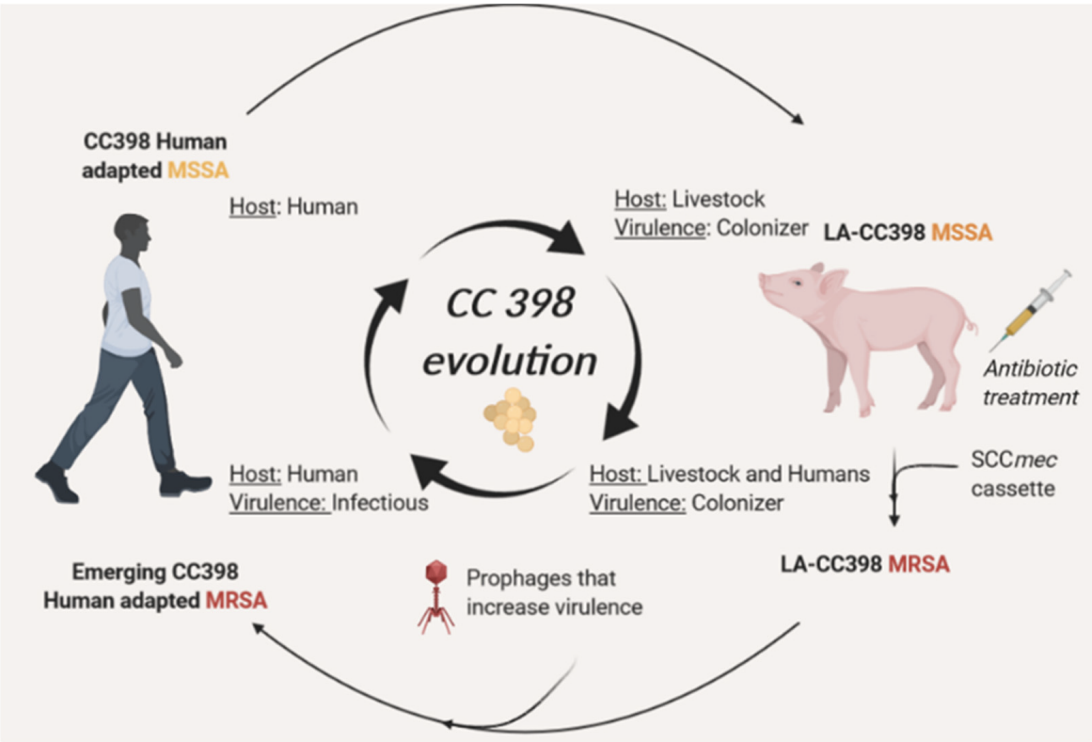


Figure 2.3: The Evolution of CC398 MRSA in Humans and Livestock
Adapted from (Laumay et al., 2021)

Relatively few studies have specifically looked at *S. aureus* associated with pigs in Africa. The systematic review by Samutela and others (2021) showed that, only five studies reported susceptibility profiles of the *S. aureus* against penicillin as shown in Table 2.2. Of these studies, four recorded high penicillin resistance of about 97% to 100% (Fall et al., 2012, Founou et al., 2018, Momoh et al., 2018, Otalu et al., 2018, Founou et al., 2019). Additionally, four out of the nine studies reported testing the isolates against cefoxitin (Table 2.2). Fall and others (Fall et al., 2012) reported the least (10.5%) resistance to cefoxitin while Nwaogaraku and colleagues (Nwaogaraku et al., 2019) reported the highest resistance to cefoxitin of 44%. Otalu and others (Otalu et al., 2018) and Faunou and colleagues (Founou et al., 2018, Founou et al., 2019) reported that all *S. aureus* isolates tested were resistant to cefoxitin. On the other hand, all nine studies reported susceptibilities of the *S. aureus* isolates to oxacillin (Table 2.2). Six of the nine studies reported different levels of resistance among the isolates with one study reporting the least resistance at 43.9% (Okunlola and Ayandele, 2015). The remaining three studies reported 100% susceptibility to oxacillin (Katakweba et al., 2016, Momoh et al., 2018, Otalu et al., 2018). Therefore,

methicillin resistance is present among pig related *S. aureus* isolates in Africa, albeit still at low levels.

2.4.1.2 Molecular Basis of Resistance to Beta-lactam Antibiotics

Methicillin resistance is mainly due to the acquisition of genes encoding a unique penicillin-binding protein (PBP2′ or PBP2a). PBP2a has decreased affinity for β-lactams and catalyses effective cell wall synthesis even in the presence of penicillins, cephalosporins and carbapenems. The *mecA* gene which encodes the PBP2a is carried on a mobile element known as the staphylococcal chromosome cassette *mec*, also called staphylococcal cassette chromosome *mec* (SCC*mec*) (Chambers, 1997). Several SCC*mecA* subtypes and their variants have been characterized (Deurenberg et al., 2007, Moellering, 2012). Currently, there are eleven (11) subtypes of SCC*mec* (type I-XI) (Shore et al., 2011). In the recent past, the *mecC* gene, a divergent homologue of the *mecA* gene, which was formerly called mecALGA251 and is 70% homologous to the *mecA* gene was detected and is associated with LA-MRSA (Harrison et al., 2013, Paterson et al., 2014, Lindgren et al., 2016). It encodes a penicillin-binding protein that differs from that encoded by *mecA*. This is important because while phenotypic methods will detect these strains, molecular methods such as PCR that detect the *mecA* gene will not detect these strains due to the differences in both target DNA sequences and the PBP encoded by *mecC* gene (Cartwright et al., 2013). The molecular bases of the mechanisms of resistance in *S. aureus* to various antibiotics is shown in Table 2.3 (Reygaert, 2018).

2.4.2 *Staphylococcus aureus* Resistance to other Antibiotic Classes

Emergence of resistance around the world to non-beta-lactam antibiotic options useful for treatment of staphylococcal infections such as the Macrolide-lincosamide streptogramin B (MLSB) family, quinolones, tetracylines, trimethoprim-sulfamethoxazole, and vancomycin among others is worrisome. Resistance rates seem to be higher in the hospital associated and MRSA strains compared with the community associated counterparts (Wang et al., 2012). For example, a study from China showed that HA-MRSA compared to CA-MRSA were more resistant to ciprofloxacin, gentamicin, rifampicin, tetracycline and trimethoprim–sulfamethoxazole with high resistance rates ranging between 84% to about 95% (Wang et al., 2012). This study also reported that CA-MSSA had lower resistance rates

compared to those of CA-MRSA to ciprofloxacin, chloramphenicol, gentamicin and tetracycline among the isolates (Wang et al., 2012). The study also reported high resistance rates of CA-MRSA, HA-MRSA and CA-MSSA to clindamycin (92.0, 77.9 and 64.1 %), respectively and erythromycin (85.9, 77.9 and 63.1 %), respectively (Wang et al., 2012). Furthermore, Wang and colleagues reported MDR rates (resistance to three or more non-b-lactams) of 49.6%, 100% and 14% in the CA-MRSA, HA-MRSA and CA-MSSA isolates, respectively (Wang et al., 2012). Overall resistance to mupirocin of 2.3 % was recorded (Wang et al., 2012). Mehdi et al 2020 in a study of clinical *S. aureus* from varied samples including wound, blood, pus, urine, sputum, conjunctivitis, and body fluids also reported that antibiotic resistance was higher in MRSA than MSSA strains (Goudarzi et al., 2020). These recorded a higher resistance rate to mupirocin of 18.3% and the lowest resistance rate in the study was to fusidic acid at 5.6% (Goudarzi et al., 2020). Furthermore, the study detected an overall MDR rate of 80.2%, and MDR rate for MRSA and MSSA isolates was 85.8% and 50%, respectively (Goudarzi et al., 2020). Similar findings of high resistance rates have been recorded in *S. aureus* isolates collected from retail food samples including meat and meat products (bacon/sausage, poultry, pork, mutton and beef) (Wu et al., 2019). Wu and others reported that 98.6% of the isolates (71/72) exhibited resistance to at least one antibiotic, including 47 multi-drug-resistant isolates (Wu et al., 2019). Resistance to erythromycin (83.4%), clindamycin (63.9%), kanamycin (61.1%), telithromycin (58.3%), streptomycin (51.4%), tetracycline (47.2%), chloramphenicol (27.8%), fusidic acid (27.8%) and other antibiotics (less than 20%) was observed (Wu et al., 2019). Generally, although resistance to vancomycin and other glycopeptide antibiotics as well as other newer antibiotic classes have been reported, highly susceptible results have been reported (Gould et al., 2012, Goudarzi et al., 2020).

A systematic review of pig-associated *S. aureus* isolates from Africa showed that the isolates had variable susceptibilities to the antimicrobials commonly used against *S. aureus* with some studies recoding high sensitivities rates of up to 100% to antibiotics such as trimethoprim-sulfamethoxazole, gentamicin, erythromycin, amikacin, ciprofloxacin, chloramphenicol, and vancomycin as shown in Table 2.2 (Katakweba et al., 2016, Founou et al., 2018, Otalu et al., 2018). Resistance rates to these antibiotics ranged from about 5% to 70%. On the other hand, resistance of as high as 100% to tetracyclines in some of the studies (Pekana and Green, 2018). Two studies

reported susceptibility to linezolid with resistances of 4% and 14. 3% respectively (Momoh et al., 2018, Pekana and Green, 2018). Only one study reported on mucipiron with resistance of 3.7% (Momoh et al., 2018). Fusidic acid was tested in only one study and all (100%) isolates were susceptible (Fall et al., 2012). Two studies reported resistances to more than one category of antibiotics; Fall and others (Fall et al., 2012) (6 antibiotics) and Mamoh and colleagues (Momoh et al., 2018) (7 antibiotics). The susceptibility profiles of the human *S. aureus* isolates from Fall and others study showed that the isolates were susceptible to cefoxitin thus were methicillin susceptible (Fall et al., 2012). The isolates were also susceptible to vancomycin, gentamicin and fusidic acid. They were resistant to penicillin (100%), trimethoprim-sulfamethoxazole (25%) and erythromycin (12.5%). This was notably the only study that reported the susceptibility of the isolates from humans with professional contact with pigs. A study from South Africa tested for heavy metal resistance in *S. aureus* isolates although the results were not separated according to animal species since other animals such as cattle were included in the study (Dweba et al., 2019). They found high resistance rates against the different heavy metals Cadmium (Cd), Copper (Cu), Lead (Pb), and Zinc (Zn) at 1500 µg/mL concentration as shown in Table 2.4.

Table 2.2 Antimicrobial Susceptibility Patterns of the Porcine-related *S. aureus* Isolates from Africa

FOX	P	SXT	CN	AMK	ERY	OXA	CD	TET & or/ DOX	C	LIN	MUP	VAN	CIP	FUS	Reference
R (11/25)	NT	NT	NT	NT	NT	R (11/11)	NT	NT	NT	NT	NT	NT	NT	NT	(Nwaogaraku et al., 2019)
R (100%)	R (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	NT	NT	NT	NT	NT	(Otalu et al., 2018)
NT	R (97%)	R (52%)	NT	NT	R (20%)	S (100%)	R (17%)	R (62%)	NT	R (4%)	R (3.4%)	NT	R (5%)	NT	(Momoh et al., 2018)
NT	R (7/14)	R (2/14)	R (0/14)	R (0/14)	R (4/14)	R (7/14)	R (8/14)	R (2/14) *	R (1/7)	R (2/14)	NT	NT	R (0/14)	NT	(Pekana and Green, 2018)
R (5/5)	R (5/5)	S (5/5)	S (5/5)	NT	R (4/5)	R (5/5)	R (4/5)	R (5/5)	NT	S (5/5)	S (5/5)	S (5/5)	S (5/5)	NT	(Founou et al., 2018, Founou et al., 2019)
NT	NT	S (100%)	R (25%)	NT	NT	S (100%)	NT	NT	R (25%)	NT	NT	NT	NT	NT	(Katakweba et al., 2016)

Key: FOX = Cefoxitin, P = Penicillin, SXT = Trimethoprim-sulfamethoxazole, CN = Gentamicin, AMK = Amikacin, ERY = Erythromycin, OXA = Oxacillin, CD = Clindamycin, TET/ DOX = Tetracycline/ Doxycycline, C= Chloramphenicol, LIN = Linezolid, MUP = Mupirocin, VAN = Vancomycin, CIP = Ciprofloxacin, FUS = Fusidic acid, NT= Not tested; * Also tested using Minocycline, ** Oxytetracycline was used

Adapted from (Samutela et al., 2021)

Table 2.2 Continued Antimicrobial Susceptibility Patterns of the Porcine-related *S. aureus* Isolates from Africa

Fox	P	SXT	CN	AMK	ERY	OXA	CD	TET & or/ DOX	C	LIN	MUP	VAN	CIP	FUS	Reference
NT	NT	R (45%)	R (70%)	NT	R (40%)	R (43.9%)	R (60%)	NT	NT	NT	NT	NT	S (100%)	NT	(Okunlola and Ayandele, 2015)
NT	NT	NT	S (100%)	NT	S (100%)	R (100%)	NT	S (88%) **	NT	NT	NT	S (100%)	NT	NT	(Tanih et al., 2015)
R [(10.5%) 6/57] pig isolates only	R (100%) Pigs and humans	R [(54.3%) 31/57 Pigs; (25%) 4/16 Humans]	S 100% Pigs and human isolates	NT	R [(12.5%) 2/16 Human isolates]	R [(10.5%) 6/57] pig isolates only	NT	R [(18%) Pigs; (2%) Humans)]	NT	NT	NT	S (100%) Both pig and human isolates	NT	S (100%) Both pig and human isolates	(Fall et al., 2012)

Key: FOX = Cefoxitin, P = Penicillin, SXT = Trimethoprim-sulfamethoxazole, CN = Gentamicin, AMK = Amikacin, ERY = Erythromycin, OXA = Oxacillin, CD = Clindamycin, TET/ DOX = Tetracycline/ Doxycycline, C= Chloramphenicol, LIN = Linezolid, MUP = Mupirocin, VAN = Vancomycin, CIP = Ciprofloxacin, FUS = Fusidic acid, NT= Not tested; * Also tested using Minocycline, ** Oxytetracycline was used

Adapted from (Samutela et al., 2021)

Table 2.3 *S. aureus* AMR Genes, Associated Antimicrobial Agent Classes, and Mechanism of Resistance

AMR Gene	Antimicrobial Class: Antimicrobial Agent	Mechanism of Resistance
*mec*A	β-lactams: Penicillins, Cephalosporins	Altered drug target, Penicillin binding protein 2 a (PBP2a)
*mec*C		
*bla*Z	Monobactams, carbapenems	Hydrolysis of β-lactam antibiotics by β-lactamase
*van*A	Glycopeptides: Vancomycin	Vancomycin Resistant *S. aureus* (VRSA) – Modified target
*mpr*F	Lipopeptides: Daptomycin	Change in cell membrane charge-decreased drug binding
aac, ant, aph	Aminoglycosides: Amikacin, Gentamicin, Tobramycin	Aminoglycosides modifying enzymes modify target
tet(K)	Tetracyclines: Tetracycline, Minocycline, Tigecycline	Active efflux pumping
tet(M)		Ribosomal protection by competitive binding
cat	Chloramphenicol	Inactivation by acetylation of the drug
erm(A)	Macrolides and Lincosamides: Erythromycin, Clindamycin	Methylation of ribosome to decrease binding
erm(B)		
erm(C)		
rrn, cfr	Oxazolidinones: Linezolid	Mutation of ribosome, Methylation of ribosome
erm(A), *erm*(B), *erm*(C)	Streptogramins: Quinupristin/Dalfopristin	Methylation of ribosome
*gyr*A	Fluoroquinolones: Ciprofloxacin	Gyrase modified target
*grl*A		Topoisomerase IV modified target
*nor*A	Norfloxacin, Levofloxacin, Gatifloxacin, Moxifloxacin	Active efflux
dhfr/ dhps	Metabolic Pathway Inhibitors: Trimethoprim/Sulfamethoxazole	Target enzyme modification

Adapted from (Reygaert, 2018)

Table 2.4 Genotypic Characteristics of African Pig-Associated *S. aureus* Isolates

										Virulence Factors					
Method	***mec*A**	***mec*C**	**SCC*mec* typing**	**Antibiotic Resistance genes**	**Heavy Metal Resistance genes**	**CC398 PCR**	**Spa typing**	**MLST**	**WGS Sequencing Similarity**	***pvl***	***scn***	**Enterotoxins**	***TST***	**Other Virulence factors**	Reference
Uniplex PCR	Pos10.5% (Pigs)	Not done	ST5-SCCmecIV (5), ST88-SCCmec IV (Steemers and Kevin L Gunderson1)	Not done	Not done	Not done	t355 (6), t1172 (1), t4235 (7), t4690 (3), t084 (8), t311 (7), t267 (5), t314 (3), t645 (1), t1476 (3), t1617 (1), t127 (2), t148 (2), t1510, t2700, t3489, t8481, t8482, t2915 from pig isolates; t335 (1), t1172 (1), t084 (4), t311 (3), t267 (2), t359 (1), t314 (1), t127 (1), t094, t8480 in human isolates	CC152 (26.0%), CC15 (19.2%), CC5(13.7%), and CC97 (10.9%)	Not done	49.1% (28) Porcine and 43.8% (7) human isolates	Not done	*sem* (13), *seh* (5), *sea* (5), *ser* (4), *eta* (3), *sed* (3), *sec* (2), *sep* (1) in Pigs; *sea* (3), *sem* (3), *sep* (2), *she* (2), *etb* (2), *eta* (1) in humans	*tst* (1) in both pigs and humans	Not done	(Fall et al., 2012)
PCR	Pos	Not done	Not done	vanA and vanB 0%, mphC*	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	(Adegoke and Okoh, 2014)

Table 2.4 Continued: Genotypic Characteristics of African Pig-Associated *S. aureus* Isolates

										Virulence Factors					
Method	***mec*A**	***mec*C**	**SCC*mec* typing**	**Antibiotic Resistance genes**	**Heavy Metal Resistance genes**	**CC398 PCR**	**Spa typing**	**MLST**	**WGS Sequencing Similarity**	***pvl***	***scn***	**Enterotoxins**	***TST***	**Other Virulence factors**	Reference
Uniplex PCR	0%	0%	Not done	Not done	Not done	Not done	t131 (4)	ST80 (4)	Not done	Not done	Not done	Not done	Not done	Not done	(Katakweba et al., 2016)
Multiplex PCR	100%	Not done	Not done	mphC, ermA, ermB, VanA	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	(Igbinosa et al., 2016)
Multiplex PCR	100%	Not done	Not done	Not done	Not done	Not done	t311 (1), t002(1), t442 (1), t084 (7), t5691 (1), t355 (4), t304 (1) in pigs; t311 (1), t084 (7), t2216 (3), t355 (1), t1931 (2), t127 (1), t5427 (1), t5126 (1), t5576 (1)	CC15, CC152, and CC5		0%	93.1% (27/29) 25 pig and 2 human isolates	41% (12/29), sea, seh, sei, sea and seh sed and sei	Not done	Not done	(Momoh et al., 2018)
Not done	Not done	Not done	Not done	Not done	Not done	Not done	t16571 (1/10) in pigs	Not done	Not done	Not done	Not done	Not done	Not done		(Odetokun et al., 2018)
Multiplex PCR	100%	Not done	SCCmecVIa	Not done	Not done	CC398 Poitive	t1603 (100%)	ST88	Highly similar (putative genes *scn*, *sak*, l*ukE*, *lukD*, gamma hemolysin, *aur*, *splA* and *splB*)	Negative	37/38 [(19 porcine, 6 human with contact, 12 human without contact	Not done	Not done	Not done	(Otalu et al., 2018)

Table 2.4 Continued: Genotypic Characteristics of African Pig-Associated *S. aureus* Isolates

										Virulence Factors					
Method	***mec*A**	***mec*C**	**SCC*mec* typing**	**Antibiotic Resistance genes**	**Heavy Metal Resistance genes**	**CC398 PCR**	**Spa typing**	**MLST**	**WGS Sequencing Similarity**	***pvl***	***scn***	**Enterotoxins**	***TST***	**Other Virulence factors**	**Reference**
REP-PCR & WGS	100%	Not done	SCC*mec*Vc				t011	ST398	Highly similar (several plasmids)	(*lukS*-PV (1)	Not done	*seb* (5)	Not done	*aur* (5), *hlb* (5), *hlg*A (5), *hlg*B (5), *hlg*C (5), vWbp (5), *clf*B (5), *fnb*A (5), *fnb*B (5), *ebp*S (5)	(Founou et al., 2018, Founou et al., 2019)
PCR	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	*sea* (7) & *sed*	Not done	Not done	(Adikwu et al., 2019)
Multiplex PCR	0%	Pos (7/15)	Not done	*aac* (1), *vanB* (7), *tet*K (12)	*cop*B	Not done	2	Not done	Not done	100%	Not done	*see* (1), *coa* (0), *sea* (11)	Not done	Not done	(Dweba et al., 2019)
PCR	0%	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	(Nwaogaraku et al., 2019)

2.5 Virulence Factors of *Staphylococcus aureus*

Staphylococcus aureus is a versatile pathogen capable of causing a wide range of diseases due to its extensive armamentarium of virulence factors (Lindsay and Holden, 2004). These virulence factors are generally not unique to MRSA which is not necessarily more virulent than MSSA. Nonetheless, some MRSA strains contain factors or genetic backgrounds that may enhance their virulence or may enable them to cause particular clinical syndromes (van Leeuwen et al., 2005). The virulent factors of *S. aureus* allow it to adhere to surfaces, invade or avoid the immune system, and cause harmful toxic effects to the host. *S. aureus* strains have special virulence factors such as leucocidins, enterotoxins, alpha toxin, protein A, and extracellular protein (Otto, 2014).

2.5.1 Protein A and other Adhesive Factors

The protein A has been shown to have a negative impact on the immune system by binding the FC fragment of immunoglobin G (neutrophil attachment region) instead of the Fab region (specific antigen attachment region) thereby inhibiting opsonization (Foster, 2005). Opsonization is required to attract neutrophils to the bacteria and to initiate phagocytosis and killing of the bacteria (Wertheim et al., 2005). The *spa* gene encodes for the regions of the protein A namely, the Fc-binding region, the X region, and the C-terminal region. Coagulase is an extracellular protein (exoenzyme) that binds to protein to form a molecular complex with thrombin-like activity which converts fibrinogen to fibrin around the infection site. The gene *coa* encodes for the coagulase enzyme. Hemolysis toxins, which lead to lysis of erythrocytes and results in cell death, are additional virulence factors of *S. aureus* (Otto, 2014). Several genes have been documented to encode for the hemolysin toxins including *hlg*A, *hlg*B and *hlg*C.

2.5.2 Enterotoxins

Staphylococcus aureus produces a wide variety of enterotoxins so called staphylococcal enterotoxins (SE) and staphylococcal-like (SE*l*) proteins which have been classified traditionally into two subgroups that is classical (SEA to SEE) and new (SEG to SE*l*U2) types. While the classical SEs have demonstrated emetic activity, and the SE*l* are not emetic in a primate model (SE*l*L and SE*l*Q) or have yet to be tested (SE*l*J, SE*l*K, SE*l*M to SE*l*P, SE*l*U, SE*l*U2 and SE*l*V) (Orwin et al., 2003, Omoe et al.,

2005). Both the SEs and SE*l* possess superantigenic activity and are encoded by accessory genetic elements, such as plasmids, prophages, pathogenicity islands, *v*Sa genomic islands, or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance (Zhang et al., 1998, Jarraud et al., 2001, Omoe et al., 2003, Noto and Archer, 2006, Novick and Subedi, 2007, Ono et al., 2008).

Staphylococcal enterotoxin producing *S. aureus* strains are among the major causes of food poisoning which is called staphylococcal food poisoning (SFP). SFP typically occurs after ingestion of different foods contaminated with *S. aureus* by improper handling and subsequent storage at elevated temperatures which leads to preformation of the SEs. Processed meat and dairy products are top on the list of foods that pose a greater risk (Wieneke et al., 1993). Symptoms include nausea and violent vomiting, with or without diarrhea with rapid onset. SFP illness is usually self-limiting but occasionally it may be severe enough leading to hospitalisation. SEA is the most common cause of SFP worldwide, although the other classical SEs also has been shown to be involved in some SFP cases and outbreaks (Argudín et al., 2010). Only SEH of the new SE/SE*l*s has clearly been associated with food poisoning (Pereira et al., 1996, Orwin et al., 2003, Ikeda et al., 2005, Jørgensen et al., 2005, Omoe et al., 2005).

Several enterotoxin genes including *sea, seb, sem, seh, ser, sed, sec,* and *sep,* have been detected from pig associated *S. aureus* in a number of studies in Africa (Fall et al., 2012b, Founou et al., 2018, Momoh et al., 2018, Adikwu et al., 2019a, Dweba et al., 2019b, Founou et al., 2019) shown in Table 2.4. However, no study has documented the presence of enterotoxins in pig associated *S. aureus* in Zambia. There is need for studies that will explore the presence of enterotoxins in such *S. aureus* strains, seeing pork is widely eaten in Zambia.

2.5.3 Immune Evasion Cluster

The immune evasion cluster (IEC), which is carried on a bacteriophage and encodes the secreted proteins staphylococcal complement inhibitor (scn), staphylokinase (sak), and chemotaxis inhibitory protein (chp) are thought to contribute to the immune evasion in humans (Falugi et al., 2013). Moreover, these genes are less prevalent in livestock adapted *S. aureus* lineages and are hence considered good genetic markers

for identification of human associated *S. aureus* clones (Lozano et al., 2016). Data on the virulence genes associated with LA-SA are important in that such data show the possible transmission routes or pathways of these strains. For example, a study conducted in Nigeria found a high incidence of *scn* amongst porcine isolates (19/20 isolates) and an additional occurrence of *sak* based on whole genome sequence (WGS) data (Momoh et al., 2018, Otalu et al., 2018) (Table 2.4). The presence of these IEC related genes suggests a possible human origin and that pigs were either transiently contaminated by farm workers or the result of a very recent human-to-pig transmission event. Human associated *S. aureus* lineages have been previously described in animals including pigs (Sahibzada et al., 2017, Guo et al., 2018). In Africa (Table 2.4), very few studies have documented IEC genes in pig associated *S. aureus* isolates. Detection of the *scn* genes has been reported in two studies (Momoh et al., 2018, Otalu et al., 2018). Notably, the chp has not been reported in pig associated *S. aureus* isolates in Africa.

2.5.4 Serine Like Proteases (*Spl*)

Notwithstanding the fact that many virulence factors of *S. aureus* have been characterized, other putative virulence factors produced by this organism are still poorly studied. The serine protease-like operon, which is found on the *v*Sa*ß* pathogenicity island or genomic island is an example of the understudied virulence factors of *S. aureus*. The *spl* operon carries six serine protease genes (*spl*A, *spl*B, *spl*C, *spl*D, *spl*E, and *spl*F) which encodes for *S. aureus Spl* proteases (Reed et al., 2001). The *spl* operon is found in most strains of *S. aureus* although some strains do not have the full operon (Reed et al., 2001, Baba et al., 2008, Paharik et al., 2016). Notably the *spl* operon is not found in the other staphylococci (Reed et al., 2001, Baba et al., 2008, Paharik et al., 2016). There is considerable variation in the number of the *spl* genes found in *S. aureus* strains as well as variations in the sequences of the *spl* genes.

The natural substrates and virulence roles of the *Spl* proteases are unknown although there is evidence that they are involved in colonization or infection of the host (Paharik et al., 2016). The *Spls* have been demonstrated to be immunogenic in individuals with *S. aureus* infections and in healthy individuals colonized by *S. aureus*. Recent studies identified the *Spl* proteases as immunogenic in the airway, particularly in patients with severe asthma, and stimulates human keratinocytes to increase their endogenous

protease activity including specific increases in trypsin activity (Paharik et al., 2016, Stentzel et al., 2017, Williams et al., 2017). These studies evidently show that *Spl* proteases are secreted *in vivo* and are potentially involved in *S. aureus*-host interaction. More studies are needed to investigate the role of the *Spl* proteases in different *S. aureus* infections, identify possible *Spl* cleavage targets and understand the genomic constitution of the *spl* operon and the pathogenicity islands that host them. This can be achieved using a combination of omics methods including proteomics and genomics. While no studies have reported on *spls* in pig-associated *S. aureus* isolates in Africa, one study has reported the presence of the *aur* gene in such isolates using WGS (Founou et al., 2019) (Table 2.4). The *aur* gene encodes for the metalloprotease aureolysin in *S. aureus* and its expression is regulated by the Agr virulence regulator that also regulates the *spls* and other proteases of *S. aureus* (Shaw et al., 2004).

2.6 Methods for Detection of *Staphylococcus aureus*

Diagnosis of infectious diseases including clinical manifestations of *S. aureus* is important in helping to deduce the causative pathogen and allows for further elucidation of the properties of the pathogen which in turn guides the treatment of the infection. Generally, phenotypic diagnostic methods tend to be more readily available as they are cheaper compared to their molecular diagnostic methods (Fluit et al., 2023). Nevertheless, molecular methods tend to be more robust. Timely and accurate diagnosis combined with prompt treatment is essential for combating infections.

2.6.1 Phenotypic Methods

Isolation and identification of bacterial pathogens from appropriate samples is generally considered as a definitive diagnosis of an infection or contamination. Nasal swabs are normally the sample of choice from pigs while blood, pus or swabs from the infection site are used as specimens from humans for *S. aureus*. Hand and nasal swabs from humans are normally used to determine *S. aureus* carriage and/or colonisation. Several conventional methods including culture, microscopy and biochemical tests are used to achieve this.

Culture of *S. aureus* on blood agar reveals golden yellow or white colonies within 24 to 48 hours at 37°C (Bergeron et al., 2011). *S. aureus* also have variable hemolytic activity on blood agar. Some strains show complete lysis which is called β-hemolysis

while some strains exhibit a partial hemolysis called α-hemolysis, δ-hemolysis has also been reported. Mannitol salt agar, a selective and differential media is also used to identify Staphylococci (Ugwu et al., 2015). Most *S. aureus* isolates can ferment mannitol and thus the colonies appear yellow with yellow zones although some strain that do not mannitol have been documented (Shittu et al., 2007). Therefore, it is recommended to use mannitol salt agar in combination with the coagulase tube test to overcome the chances of misidentification that occur with the use of either test (Koneman et al., 1997).

Various biochemical tests can be used singularly and in combination to identify *S. aureus*, including production of catalase, protein A, cell-bound clumping factor, extra-cellular coagulase and heat-stable nuclease (Foster, 1996). The tube coagulase test with rabbit sera and examination of tubes after incubation for 4 hours and 24 hours is the standard test for routine identification of *S. aureus* (Latif et al., 2015). In comparison the slide agglutination test for clumping factor is very rapid. Several commercial latex agglutination tests for identification of *S. aureus* are also available such as Staphaurex and Pastaurex (Foster, 2005, Di Giannatale et al., 2011). There are also many commercial kits and automated instruments which include identification of *S. aureus*, which though good are slower, technically more time-consuming, and more expensive (Cartwright et al., 2013). Some commercial biotype identification kits of *S. aureus* and indeed other staphylococci include API Staph Ident, API Staph-Trac, Vitek GPI Card and Microscan Pos Combo (Foster, 2005). However, due to some limitations or drawbacks in these phenotypic methods such as being time consuming, misidentification, requiring growth of an isolate, other more robust methods have been developed and used.

2.6.2 Molecular Methods

Due to the pitfalls in phenotypic identification of microbes such as cross-reactivity between species and the relatively long time taken to get to results, molecular methods have come in handy to compensate such shortfalls. Most molecular methods for identification of *S. aureus* are based on the polymerase chain reaction (PCR) which amplify a species-specific target gene using specific primers such as the nuclease (*nuc*), coagulase (Santos et al., 2021), protein A (*spa*), *femA* and *femB*, *Sa442*, 16S rRNA and surface-associated fibrinogen-binding protein genes (Mason et al., 2001).

Primers which target specific genes responsible for antimicrobial resistance have also been developed and used in determining the susceptibility of microbes including *S. aureus* (Shore et al., 2011). With the further advances in molecular and bioinformatic technologies such as whole genome sequencing, not only is it easier to identify *S. aureus* isolates but also to determine their similarity up to the genome level (Shopsin et al., 1999, Deurenberg et al., 2007, Shore et al., 2011). This allows for local and international comparisons of strains even during outbreaks and pandemics (Harmsen et al., 2003).

2.7 Typing of *Staphylococcus aureus*

Understanding the epidemiology and the evolution of *S. aureus* strains relies on typing methods which previously were mostly phenotypic, but in the recent past, molecular methods have been become more popular. Several molecular typing techniques have been employed including pulsed-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, whole-genome sequencing (WGS) and DNA microarrays. PFGE remains the gold standard, particularly for short-term surveillance despite the difficulties in reproducibility, interlaboratory reliability, and being laborious. MLVA is a high-throughput PCR-based method used to determine the genetic diversity and the emergence of *S. aureus* strains and seems quite useful. MLST is a good typing method for long-term and global epidemiological investigations, but it is not suitable for outbreak investigations. SCC*mec* typing has gained popularity for the evolutionary analysis of MRSA strains. spa typing is the most widely used method today for first line typing in the study of molecular evolution, and outbreaks investigations. WGS and DNA microarrays are relatively new DNA-based technologies that provide more information for tracking antibiotic resistant and virulent outbreak strains (Galhano et al., 2021). While they offer a higher discriminatory power, they have yet to be used routinely in both human and veterinary clinical medicine in most parts of the world.

2.7.1 Spa Typing

Spa typing targets the *spa* gene that encodes for the protein A and is conserved among *S. aureus* strains and thus provides a suitable short sequence repeating (SSR) regions to be used as a target for Single locus sequence typing (SLST) (Kumar et al., 2021). It

is the first DNA sequence-based typing method developed specifically for the characterisation of *S. aureus* that is based upon PCR amplification and sequencing of protein A gene specific for *S. aureus* (Frenay et al., 1996). The *spa* gene is approximately 2,150 bp in length and encodes three regions: the Fc-binding region, the X region, and the C-terminal region. The X region, also called the repeat region, consist of variable-number tandem repeats (VNTR) which contain 2 to 15 repetitive sequences consisting of 21 to 27 bp (mainly 24 bp). They are polymorphic and diverse due to deletions and duplications of the repeats and occasionally due to point mutations. This polymorphism is represented by the number, character, and order of repetitive sequences (Shopsin et al., 1999, Koreen et al., 2004, Kahl et al., 2005). In *spa* typing, each identified repeat is associated with a numerical or letter code, and the *spa* type is deduced from the order of the specific repeats and each repeat unit is given a unique identifier. A *spa* type denotes a collection of specific repeat units arranged in a precise pattern. Two strains with identical repeat sequences that is both content and organization are considered genetically related and are assigned the same *spa* type.

One of the advantages of *spa* typing is that it can be used for the investigation of both molecular evolution and hospital outbreaks (Koreen et al., 2004). Additionally, since it involves the interrogation of a single polymorphic locus, it is the most suitable typing method for local and short-term epidemiological studies (Shopsin et al., 1999, Harmsen et al., 2003). Furthermore, *spa* type clusters specifically associated with MRSA lineages seem to be stable over time, therefore *spa* typing is also valuable for long-term global epidemiological studies (Shopsin et al., 1999). Moreover, an algorithm called based upon repeat pattern (BURP) allows the exploration of spa typing for long-term epidemiological studies of MRSA (Mellmann et al., 2007, Mellmann et al., 2008), and has made cluster analysis based upon putting *spa* types into *spa* clonal complexes (CCs) possible. While the discriminatory power of spa typing falls between those of PFGE and MLST (Malachowa et al., 2005), it is cost-effective, easy to use, and rapid and has excellent reproducibility compared to both methods (Kumar et al., 2021). Spa typing is stable, with a standardized international nomenclature, and is amenable to high throughput using the StaphType software, and the data are fully portable via the Ridom database. These features make it the most useful instrument and method of choice for characterizing *S. aureus* isolates at local, national, and international levels (Harmsen et al., 2003, Hallin et al., 2007, Deurenberg

et al., 2009). The major disadvantage of spa typing is that since it is based upon single locus typing, misclassification of particular types due to recombination and/or homoplasy can easily occur (Sabat et al., 2013).

Different *spa* types have been found among the three main reservoirs of *S. aureus*. Among the LA-SA the most predominant types are t011, t034, t108, t567, t571, t899, t1197, t1250, t1451, t1456, t2510 which has been found in North America, Europe, Africa and Asia (Smith and Pearson, 2011). Other *spa* types associated with LA-SA include t002/t003, t311, t100, t411, t373, t4358. Studies on pig associated *S. aureus* from Africa have revealed that the isolates exhibited diverse *spa* types including t1603, t311, t002, t084, t5691, t1931, t5427 and t6571 (Fall et al., 2012, Katakweba et al., 2016, Momoh et al., 2018, Odetokun et al., 2018, Otalu et al., 2018, Founou et al., 2018, Founou et al., 2019).

2.7.2 Whole Genome Sequencing

Whole genome sequencing allows the ultimate or complete identification of DNA diversity in any organism. The first whole genomes of *S. aureus* to be published were the whole genomes of two HA-SA strains in 2001 (Kuroda et al., 2001). Then followed the publication of the whole genome of a CA-MRSA strain, MW2, by the same team (Baba et al., 2002). Insights into the genomes of *S. aureus* gained from these genome sequences were that the genomes were circular, having approximately 2,800,000 bp (2.8Mbp) which were coding about 2,600 proteins. Several *S. aureus* genomes (including the genomes of methicillin-resistant strains) are now publically available in such databases as the nucleotide database of the National Center for Biotechnology information (NCBI) and Bioinformation and DNA Database of Japan (DDBJ). With appropriate bioinformatics software, these genome sequences allow one to predict the number of open reading frames, eventually deducing the amino acid sequence of the whole proteome thus making the study of its biological systems possible (Lakhundi and Zhang, 2018). Additionally, sequencing has shown that the core genome of each specific lineage is genetically highly variable, especially in the carriage of surface and regulatory genes (Lakhundi and Zhang, 2018).

Next-generation sequencing has provided a cost-effective method of identifying genome-wide variations with its ability to generate millions of reads approximately 35

to 700 bp in length. Two mainstay NGS methods to generate WGS sequences have been developed namely, short and long reads (Kumar et al., 2021). The Illumina platforms is the champion for short read sequences generating reads between 200 to 600bps (Steemers and Kevin L Gunderson1, 2005). For long reads, the PacBio, a "third-generation" sequencer by Pacific Biosciences, which can generate average read lengths of 10 kb, with maximum read lengths of 60kb was first to be developed (Rhoads and Au, 2015). Another system, developed by Oxford Nanopore called nanopore sequencing technologies, can generate approximately 100-kb reads (English et al., 2012). To construct a complete genome, two methods are used namely de novo assembly and resequencing. In de novo assembly, multiple short sequence reads are assembled based on overlapping regions, while in resequencing, reads are assembled against a previously assembled genome sequence. Depending on the technology used, reads produced via WGS are sometimes short, making de novo assembly a challenge. Because of this, the term "whole genome sequence" sometimes refers to around 90% of the genome and it is represented in contigs (with gaps between assembled regions), which result from the occurrence of dispersed or tandemly arrayed repeats (Sabat et al., 2013). The approaches used to develop a genome-wide gene-by-gene analysis tool include extended MLST (eMLST) involving ribosomal MLST (rMLST), core genome MLST (cgMLST), whole-genome MLST (wgMLST), and a pangenome approach. Pangenome analysis was first coined by Tettelin and colleagues in 2005 denoting all or every gene that make up bacteria (Tettelin et al., 2005). However, the term pangenome has since been applied to eukaryotes for example plants too. Pangenome analysis allows for the comparison of the entire set of genes from all strains in a clade or taxa (species, serovar, phylum and kingdom) (Tettelin et al., 2005). Several analysis tools are available and examples of their use in different studies are reviewed by Vernikos (2020).

The major challenge with WGS is the rapid analysis of those data to interpret and extract relevant information which should enable one to directly compare results obtained from traditional typing methods and should be stored in a database that is publically accessible (Kumar et al., 2021). Notably, gaps between the contigs need to close completely in order for an in-silico restriction digest to simulate PFGE, therefore, PFGE profiles cannot be accurately predicted without complete closure of the genome. Although costs associated with next-generation sequencing continue to decline and

benchtop sequencers are now within the financial reach of many laboratories, the sequencing workflow remains too slow and genome assembly too technically laborious for implementation of routine clinical surveillance. In addition, NGS requires significant computer resources and well-trained bioinformaticians (Sabat et al., 2013).

WGS has proven to be a very good and highly attractive tool for epidemiological purposes including surveillance for antimicrobial resistance and outbreak investigations in clinical settings. This is so because it is able to accurately characterise transmission events and outbreaks due to its potential to compare different genomes with single-nucleotide resolution to determine the genetic relatedness among isolates. For example, Harris and colleagues used WGS to describe the intercontinental (through four decades of time) and local transmission (person-to-person) of MRSA (Harris et al., 2010). Additionally, Koser and others used WGS' detailed targeted analyses of variation within related species via methods based on single nucleotide polymorphism (SNPs) which allow illumination of the evolutionary histories of homogenous groups and are thus exceedingly informative markers such discrimination of MRSA outbreak and non-outbreak strains (Köser et al., 2012). Koser and others also created a "resistome" to demonstrate concordance between antibiotic resistance genes and the results of phenotypic susceptibility testing and a "toxome" to identify toxin genes, respectively (Köser et al., 2012). Further, Harris and colleagues confirmed that WGS is a reliable tool for fast, precise, and comprehensive recognition of MRSA transmission pathway in hospital and community settings (Harris et al., 2013). Furthermore, WGS has contradicted transmission events indicated through conventional methods and revealed unsuspected transmission events (Price et al., 2014). Therefore, Price and others recommended that WGS should ideally replace conventional methods to detect nosocomial transmission events (Price et al., 2014). Thus, in the near future, WGS is highly likely to take over routine investigation techniques currently used in clinical practice for the identification and characterization of bacterial isolates. Unfortunately, WGS is still not very widely used in Africa. Consequently, few studies have documented the use of WGS on pig associated *S. aureus* (Founou et al., 2018, Otalu et al., 2018, Founou et al., 2019) (Table 2.4). More studies, using WGS such as the current study, are needed to give further insight into the nature and epidemiology of *S. aureus* in Africa.

2.8 Control of *Staphylococcus aureus*

Strategies in the control of infectious diseases include the use of one or a combination of treatment and/ or prevention. Notably, infections caused by *S. aureus* especially MRSA are harder to treat compared to those caused by MSSA (Otto, 2012). This is so because besides being resistant to β-lactam antibiotics, MRSA are also resistant to other classes of antibiotics such as aminoglycosides and macrolides (Mathews, 2012). Thus, there are few effective treatment options for resistant gram-positive organisms including MRSA (Rybak et al., 2013). Commonly utilized or recently developed agents such as vancomycin, trimethoprim-sulfamethoxazole, daptomycin, linezolid, ceftaroline and telavancin are part of the treatment options. Older or less employed agents such as clindamycin, tigecycline, quinupristin-dalfopristin, tetracyclines, fosfomycin and chloramphenicol are also used to treat severe and systemic MRSA infections (Rybak et al., 2013). While vancomycin and teicoplanin (glycopeptide antibiotics) are currently considered the mainstay of therapy of most MRSA infections, isolates with reduced susceptibility to vancomycin called vancomycin intermediate *S. aureus* (VISA) and heterogeneous-vancomycin-intermediate *S. aureus* (hVISA) have emerged and steadily increased treatment failure (File Jr, 2011). Subsequently, new drugs e.g., daptomycin and linezolid developed. However, these drugs have limited usage for severe infections due to pharmacokinetic limitations or adverse reactions. Adaptive or cross-resistance to each of these new agents has also been documented (Rybak et al., 2013). Notably, drug development takes long, a handful of next-generation agents nearing approval include Solithromycin, Tedizolid, Dalbavancin and Oritavancin which may represent new options for the treatment of serious infections due to MRSA and other MDR organisms.

Prevention of *S. aureus* infections in humans including those caused by MRSA usually employs several strategies which include hand and farm hygiene (Allegranzi et al., 2011, Graveland et al., 2011), screening for colonisation and subsequent decolonization (Wertheim et al., 2004, Huijsdens et al., 2006). Antibiotic prophylaxis is also employed especially pre-surgery (Si et al., 2014). However, there is no vaccine for *S. aureus* available despite many attempts to develop one (Spaulding et al., 2014, Guo et al., 2018). It is necessary to know and understand the population structure and transmission dynamics of LA-SA including LA-MRSA within the pig production

system in order to come up with evidence-based interventions. However, very few studies have been conducted to investigate this important phenomenon. Various possible explanations for farm-to-farm transmission have been suggested including movement of positive animals, inadequate control measures, and spread by humans, contaminated fomites, wind, insects, rodents, and other alternative hosts (Sieber, 2018). Generally, animal movements have long been considered as a critical factor in the spread of livestock diseases, hence it has been suggested that animal movements may play a similar role in the dissemination of LA-MRSA CC398 (Crombé et al., 2013, Grøntvedt et al., 2016, Elstrøm et al., 2019). This was supported by findings in a study from Denmark which demonstrated that animal movements have played a critical role in the dissemination of LA-MRSA CC398 within the Danish pig production system (Sieber et al., 2018). Notably, the genetic relatedness of isolates from different farms was positively correlated with the number of animal movements between the farms. This underscores the need to include molecular typing and bioinformatics as we study *S. aureus* from animals as well as from humans.

2.9 The Economic Impact, Public Health Significance and Interspecies Transmission of *Staphylococcus aureus*

The economic importance of *S. aureus* is seen mostly from human infections caused by MRSA which are a serious public health concern and an economic burden to national health care systems and patients (Kinross et al., 2017). This is usually due to an increase in morbidity leading to prolonged stay in hospitals and extra cost in treatment of the multidrug resistant infections which entails the use of more expensive antibiotics. For example, in the European Union (EU) MRSA is estimated to affect more than 150000 patients annually resulting in attributable extra hospital cost of EUR 380 million (Köck et al., 2010). This impact has been heightened with the emergence of new MRSA strains as community and livestock associated human pathogens. Although *S. aureus* rarely causes infections in pigs, cases of possible infections have been reported (Meemken et al., 2010). However, pigs are frequently colonised by *S. aureus* and have been recognised as a main reservoir for MRSA and these strains are able to be passed on to persons in contact with these animals (Wulf and Voss, 2008, Köck et al., 2009, Harrison et al., 2013, Grøntvedt et al., 2016). Notably, LA-MRSA has been also isolated from persons without contact with animals (Lekkerkerk et al., 2015).

The public health significance of *Staphylococcus aureus* in animals has increased in the recent years (Lozano et al., 2016). This is so in part, because of the increase of infections caused by this pathogen (especially by MRSA strains) in animals. In addition, some clonal lineages associated with animals have emerged that have great zoonotic potential (Armand-Lefevre et al., 2005). An example is the case of the ST398, which has been identified as a colonizer or infectious agent in pigs, cattle, horses, and poultry, as well as in people in contact with these animals (farmers, veterinarians, and slaughterhouse workers) (Voss et al., 2005, Harrison et al., 2013, Grøntvedt et al., 2016). Other such lineages include CC1, CC5, CC9, CC97, and CC130, among others). Further, LA-MRSA infections have also been detected in relatives of farmers and sometimes even in people without contact with animals (the case of MRSA of Clonal Complex 398) (Lekkerkerk et al., 2015). Furthermore, these strains frequently exhibit multi drug resistance phenotypes (Perveen et al., 2013). Recently, there has been growing interest not only in the study of MRSA strains but also of MSSA strains, since these strains play an essential role in the evolution of different genetic lineages. In a study performed in Tunisia, nasal swabs of healthy people with different levels of interaction with animals were analyzed, and animal associated clonal lineages (CC30 and CC121) were found in some MSSA strains from people with frequent contact with animals (Ben Slama et al., 2011). In these cases, animal-to-human transmission might have happened.

On the other hand, human-to-animal transmission of MSSA and MRSA has been suggested in some studies in Western and African countries. Human related clonal lineages (CC15, CC72, CC80, CC101, and CC152) have been identified in MSSA strains from non-human primates, goats, sheep, poultry and pets. A number of studies have identified *S. aureus* with the same molecular types or traits e.g., MRSA CC88 strains with the same spa-type (t189) were identified in humans and sheep (samples were taken from humans and animals living in the same village) in Côte d'Ivoire (Schaumburg et al., 2015). Another human-to-animal case transmission was identified in a sanctuary in Africa in which a veterinarian and a chimpanzee showed MSSA strains with the same spa-type t279 (Lozano et al., 2016). The significance of this interspecies transmission is the blurring of the epidemiology of *S. aureus* where LA-

SA become established in humans and hospitals and HA-SA also become established in animals.

Another aspect on the significance of *S. aureus* in public health is its' isolation from food in different parts of the world including Africa (Vanderhaeghen et al., 2010, Di Giannatale et al., 2011, Hadjirin et al., 2015, Bangerter et al., 2016). Both MRSA and MSSA have been identified from various food staffs in varying prevalence. The prevalence rates of MSSA in some studies conducted from Africa were as follows in raw meat including raw chicken, meat products and cooked meat (3% to 81.8%); milk both raw milk and dairy products (6.3% to 100%); eggs both on the outer shell and inside (3%-18%) (Lozano et al., 2016). Other types of food that have been analyzed include the following: beans, corn flour, doughnut, fish roll, salted fish, maize flour porridge, mangoes, meat pie, salad, pawpaw, and cassava. MRSA prevalence from some African studies involving meat samples ranged 0.8%–4.6% (Lozano et al., 2016). Notably, the highest percentage was identified in a study in which MRSA strains were found in cooked meat samples. In a study from Egypt, methicillin resistance was observed in 12 of the 95 *S. aureus* strains tested (12.6%) from salted fish samples (Ezzeldeen et al., 2011). Another study performed in South Africa found the prevalence of MRSA was 81.2%–93.2% in milk samples from communal farms and 5.7%–7% in those from commercial farms (Ateba et al., 2010). It is important to note that this data has come from relatively very few studies conducted in a few African countries. Therefore, there is need for more such studies.

In Zambia, there is little or no data on such aspects of this organism. Further, most of the studies did not include extensive characterisation of the isolates for example, detection of the presence of the *mec*A gene and molecular typing to determine the clonal lineages of these strains. Thus, the need of more studies such as the current study that will provide insight into the clonal epidemiology of *S. aureus* from both humans and animals in Zambia. Several possible explanations of why *S. aureus* is present in food samples have been given e.g., the fact that animals (pets) are kept in kitchens where food is prepared; direct contamination by the food handlers through coughing and sneezing; storage of food at high temperature; and/or some processed foods, which constitute a good culture medium for bacteria. The source of

contamination would also be the animal in the case of raw meat samples. However, more systematic studies need to be done to investigate this aspect too.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was a descriptive cross-sectional study conducted between June 2020 to July 2022. The aim of the study was to assess the presence of S. aureus from pigs and workers at pig farms and abattoirs in Lusaka province of Zambia.

3.2 Study Site

The study was conducted on selected commercial and small-scale pig farms, and abattoirs in Lusaka province of Zambia. Lusaka province is the smallest province in Zambia with an area of 21,896 km^2. The provincial capital of the province is Lusaka city, which is also the country's capital city. Although Lusaka province has 8 districts, for this study, farms and abattoirs located in the following selected districts namely, Lusaka, Chilanga, and Chongwe were sampled (Figure 3.1), because most commercial pig farms and abattoirs are found in these districts.

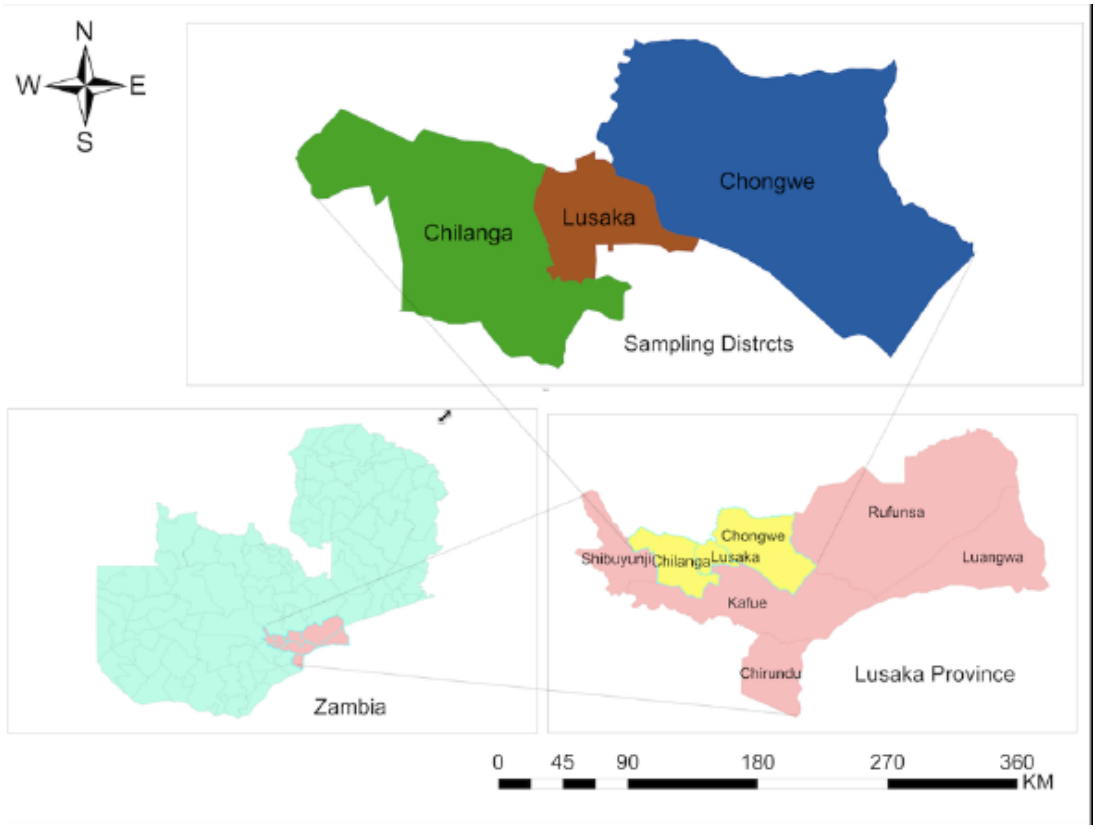


Figure 3.1 Map of Study Site Showing the Selected Districts of Lusaka Province, Zambia
Map was generated using the software ArcGIS version 10.3

3.3 Sampling Frame and Sample Collection

3.3.1 Farm level

Farm owners were asked for permission to conduct the study on their farms by consenting to the study after signing the consent form (Appendix A). Pigs of all ages and workers who worked closely with the pigs at a given farm were considered as part of the target population for the study. Nasal swabs were collected from pigs by gently rotating a dry swab in both anterior nares of each pig. Farm workers with close contact with the pigs were asked if they were willing to participate in the study. Nasal and hand swabs from all workers who consented by signing the consent form (Appendix A) were collected by gently rotating a sterile dry swab in both anterior nares of each consenting worker using one swab and then another sterile swab to rub both palms of the worker. This was done after explaining the purpose of the study in the language that the farm owners and workers understood. The swab samples were then put in Amies transport media and stored in a cool box containing ice packs at 4°C and then transported to the laboratory at the University of Zambia, School of Veterinary Medicine for culturing of *S. aureus.*

3.3.2 Abattoirs level

Abattoir owners were asked for permission to conduct the study on their abattoirs by consenting to the study after signing the consent form (Appendix A). The target population included all pigs brought to be slaughtered at a given abattoir. Nasal swabs were collected from pigs by gently rotating a swab in both anterior nares of each pig before stunning or before the jugular vein is cut in abattoirs where stunning was not performed. Abattoir workers with close contact with the pigs were asked if they were willing to participate in the study. Nasal and hand swabs from those who consented by signing the consent form (Appendix A) were collected by gently rotating a sterile dry swab in both anterior nares of each using one swab and then another sterile swab to rub both palms of the worker. This was done after explaining the purpose of the study in the language that the abattoir owners and workers understood. The swab samples were then put in Amies transport media and stored in a cool box containing ice packs at 4°C and transported to the laboratory at the University of Zambia, School of Veterinary Medicine for culturing of *S. aureus.*

3.3.4 Inclusion and Exclusion Criteria

3.3.4.1 Inclusion Criteria

Farms and abattoirs included in the study were those within the selected districts of Lusaka Province. Participants included in this study were pig farm owners and/ or farm workers and abattoir workers who were 18 years and above who gave consent to be part of the study.

3.3.4.2 Exclusion Criteria

Farms and abattoirs that handle less than 20 pigs were not included in the study since we had earlier determined that a minimum of 18 pigs per farm or abattoir was needed to establish the desired prevalence and precision based on a two-stage calculation of sample size adapted from (Van Lochem et al., 2018).

3.4 Sample Size

A total of 492 pig nasal swabs were purposively collected from 13 farms and three abattoirs. The distribution of the farms based on the scale of pig farming by districts were as follows, two medium farms in Chilanga, one commercial farm and four medium scale farms in Chongwe district and two medium scale and four commercial farms in Lusaka district. Abattoirs one and two were commercial facilities and received pigs from within their district, however, abattoir three which is located in Lusaka district possibly receives pigs from other districts as it allows for non-commercial farmers a place to sell their animals. The collection of pig nasal swabs was done purposively. Additionally, 53 nasal and 53 hand swabs each from humans (farm and abattoir workers) in close contact with the pigs were also collected.

3.5 Detection of *Staphylococcus aureus* in Pigs and Humans in Lusaka

3.5.1 Phenotypic Detection of *Staphylococcus aureus*

Standard microbiological methods were used to detect and identify *S. aureus* from the samples with slight modifications (Ugwu et al., 2015). Briefly, a pig nasal swab sample was placed in a 9 ml Mueller-Hinton Broth (Oxoid, Basingstoke, UK) supplemented with 6.5% NaCl and incubated at 37°C for 16 to 20 hours. The same was done for human swabs. Then a loopful of the broth was inoculated on Mannitol Salt Agar (MSA) (Oxoid, Basingstoke, UK) where the plates were then incubated for 16 to 20 hours at 37°C. Yellowish colonies were then inoculated onto Baird Parker Agar (BPA)

(Oxoid, Basingstoke, UK) supplemented with egg yolk tellurite and incubated for 24 to 48 hours at 37°C. Black or greyish colonies with or without a halo on the BPA plates were then grown in Brian Heart Infusion Broth (BHIB) (Oxoid, Basingstoke, UK) for 16 to 20 hours at 37°C. Using 0.5ml of the BHIB culture, a tube coagulase test using rabbit plasma was set up according to manufacturer's instructions (Sigma-Aldrich, Taufkirchen, Germany) and read every 30 minutes for four hours and at 24 hours after incubating at 37°C. All coagulase positive isolates were considered as *S. aureus* and stored in 20% glycerol at -20°C until further analysis. Therefore, in this study, the presumptive diagnosis of *S. aureus* was ability of an isolate to grow in the presence of sodium chloride, ferment mannitol, reduction of tellurite or production of egg yolk factor and be coagulase positive.

3.5. 2 Molecular Confirmation of *Staphylococcus aureus* Identification

The steps used to confirm the species identify are outlined below.

3.5.2.1 DNA Extraction

To extract DNA of all *S. aureus* isolates recovered, the crude DNA extraction method was used. Briefly, bacteria were grown overnight on nutrient agar, then 3 to 5 colonies of a pure culture were transferred into 200 µL of sterile molecular grade water in a 1.5mL Eppendorf tube. After lightly vortexing, the bacteria mixture was then heated in a heating block at 95°C for about 15 minutes. The mixture was then centrifuged at 13000 *xg* for 2 minutes and the extracted DNA aliquots (supernatant) were stored at -20°C until further analysis. This DNA was used for all the subsequent PCR analyses. For the whole genome analysis, DNA extraction was done using the QIAamp DNA Kit (QIAGEN, Germany) to extract the genomic DNA according to the manufacturer's instruction at a final elution volume of 50µl. Ultraviolet spectroscopy at 260nm was used to estimate the concentration of the DNA. An Optical density (OD) of 1 at 260nm corresponded to a DNA concentration of 50µg/ml of double-stranded DNA and a DNA/protein absorbance ratio of 260nm/280nm was used to determine the purity of the DNA sample using the Qubit 3.0 with the DNA High Sensitivity Kit (ThemoFisher Scientific, Invitrogen). The DNA was stored at -20°C until required for use.

3.5.3 *Staphylococcus aureus* Species Confirmation PCR

Species confirmation of the isolates was determined using the *nuc* gene PCR amplification as described previously (Zhang et al., 2004). Briefly, the *nuc* gene was amplified using the species-specific primers nuc 1 (5'- GCG ATT GAT GGT GAT ACG GTT -3') and nuc 2 (5'- AGCCAAGCCTTGACGAACTAAAGC -3') which result in amplicons of variable sizes of about 279bps (partial gene) (Zhang et al., 2004). A final volume of 25µl was used, containing 2µl bacterial DNA template, 2.5µl of each primer and 12.5µl of 2X PCR Master Mix and 5.5µl of nuclease free water (Thermo Scientific, Hanover, MD, USA), with a final MgCl concentration of 3mM. DNA isolated from ATCC 25923 *S. aureus* was used as a PCR positive control strain. PCR amplifications were performed with Veriti 9600 Well thermal cycler (Applied Biosystems, CA, USA), using the following cycle conditions: an initial denaturation step at 94°C for 5 min; 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, and a final extension step at 72 °C for 10 min. The PCR products were electrophoresed in 1.5% agarose gels with 1X Tris-acetate-EDTA buffer at 100V for 30 minutes and then gels were be stained with ethidium bromide (Cinnagen Co., Tehran, Iran) to see the amplified DNA fragments (279 bp) under UV light box by comparison with a molecular size marker (100 bp ladders, eurobio, UK).

3.6 Determination of Antimicrobial Susceptibility and Resistance Genes in the *Staphylococcus aureus* Isolates

3.6.1 Phenotypic Determination of Methicillin Resistance and Susceptibility to other Antibiotics

Resistance to methicillin was detected using the standard cefoxitin disc screen (CLSI, 2020). Determination of the antimicrobial susceptibility of the *S. aureus* isolates was also done by the disc diffusion test according to the M100 Clinical and Laboratory Standard Institute (CLSI, 2020). The antimicrobial agents tested included penicillin (P), cefoxitin (CX), erythromycin (E), clindamycin (CD), gentamicin (CN), tetracycline (TET), trimethoprim/sulphamethoxazole (SXT), chloramphenicol (C), and ciprofloxacin (CIP). A double-disc diffusion test (D-test) was performed on the isolates to detect inducible clindamycin resistance. Susceptibility to vancomycin was determined for the isolates resistant to methicillin using vancomycin E-strips to determine the minimum inhibition concentrations (MICs) according to the CLSI guidelines (CLSI, 2020).

3.6.2 Detection of Methicillin Resistance and Other Antimicrobial Resistance Genes

The presence of methicillin resistance genes in the isolates was determined by PCR as described in previous protocols (Milheiriço et al., 2007, Stegger et al., 2012) using primers for the *mec*A and *mec*C genes (Table 3.1). Other antimicrobial resistance genes tested included the genes responsible erythromycin-resistant genes (*erm*(A), *erm*(B); and *erm*(C), and tetracycline-resistant genes (*tet*(M), *tet*(K), *tet*(L), *tet*(O) and *tet*(T)), following previously described PCR protocols with gene-specific primers (Table 4) (Sutcliffe et al., 1996, Aarestrup et al., 2000). A final volume of 25µl was used, containing 2µl bacterial DNA template, 2.5µl of each primer and 12.5µl of 2X PCR Master Mix and 5.5µl of nuclease free water (Thermo Scientific, Hanover, MD, USA), with a final MgCl concentration of 3mM. DNA isolated from an in house known resistant *S. aureus* isolate was used as a PCR control strain. PCR amplifications were performed with Veriti 9600 Well thermal cycler (Applied Biosystems, CA, USA). The following cycle conditions were followed for the *mecA* gene: an initial denaturation step at 94°C for 4 min; 30 cycles of 94°C for 30 sec, 53 °C for 30 sec, and 72 °C for 1 min, and a final extension step at 72 °C for 4 min. For the *mecC* gene, the following cycle conditions were followed: an initial denaturation step at 94°C for 5 min; 30 cycles of 94°C for 30 sec, 59 °C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. For the *erm*(A), (B) and (C) genes the following PCR conditions were followed: an initial denaturation step at 93°C for 3 min; 35 cycles of 93 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. While for the *tet*(M), *tet*(K), *tet*(L), *tet*(O) and *tet*(T) genes the following PCR conditions were followed: an initial denaturation step at 94°C for 5 min; 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, and a final extension step at 72 °C for 10 min. The PCR products were electrophoresed in 1.5% agarose gels with 1X Tris-acetate-EDTA buffer at 100V for 30 minutes and then gels were be stained with ethidium bromide (Cinnagen Co., Tehran, Iran) to see the amplified DNA fragments under UV light box by comparison with a molecular size marker (100 bp ladders, eurobio, UK).

Table 3.1 Primer Sets for Determining Antimicrobial Resistance Genes of *S. aureus* Isolates

Primer name	**Target gene**	**Primer Sequence (5'-3')**	**Amplicon size**	**Reference**
mecA (F)	*mec*A	TCCAgATTACAACTTCACCAg	**162bp**	(Hanssen and Ericson Sollid, 2006)
mecA (R)		CCACTTCATATCTTgTAACg		
$mecA_{LGA251}$ MultiFP	*mec*C	GAAAAAAAGGCTTAGAACGCCTC	138bp	(Stegger et al., 2012)
$mecA_{LGA251}$ MultiRP		GAAGATCTTTTCCGTTTTCAGC		
ermA-1	*erm*(A)	TCTAAAAAGCATGTAAAAGAA	645bp	(Sutcliffe et al., 1996)
ermA-2		CTTCGATAGTTTATTAATATTAG		
ermB-1	*erm*(B)	GAAAAGTACTCAACCAAATA	639bp	(Sutcliffe et al., 1996)
ermB-2		AGTAACGGTACTTAAATTGTTTA		
ermC-1	*erm*(C)	TCAAAACATAATATAGATAAA	642bp	(Sutcliffe et al., 1996)
ermC-2		GCTAATATTGTTTAAATCGTCAAT		
tetK-1	*tet*(K)	TTAGGTGAAGGGTTAGGTCC	697bp	(Aarestrup et al., 2000)
tetK-2		GCAAACTCATTCCAGAAGCA		
tetM-1	*tet*(M)	GTTAAATAGTGTTCTTGGAG	576bp	(Aarestrup et al., 2000)
tetM-2		CTAAGATATGGCTCTAACAA		
tetL-1	*tet*(L)	CATTTGGTCTTATTGGATCG	456bp	(Aarestrup et al., 2000)
tetL-2		ATTACACTTCCGATTTCGG		
tetO-1	*tet*(O)	GATGGCATACAGGCACAGAC	615bp	(Aarestrup et al., 2000)
tetO-2		CAATATCACCAGAGCAGGCT		

3.7 Determination of the Genotypes of the *Staphylococcus aureus*

3.7.1 *Spa* typing of the *Staphylococcus aureus* Isolates

To determine the spa types of the *S. aureus* strains, spa typing was done following a previously described protocol (Shopsin et al., 1999). Briefly, the *spa* gene was amplified by PCR using the primers 1095F (5'-AgACgATCCTTCggTgAgC-3') and 1517R (5'-gCTTTTgCAATgTCATTTACTg-3') which result in amplicons of variable sizes ranging from about 240 bps to above 450 bps (Shopsin et al., 1999). The PCR reactions were performed on a Veriti 9600 Well thermal cycler (Applied Biosystems, CA, USA). A final volume of 25µl was used, containing 2µl bacterial DNA template, 2.5µl of each primer and 12.5µl of 2X PCR Master Mix and 5.5µl of molecular grade nuclease free water (Thermo Scientific, Hanover, MD, USA), with a final MgCl concentration of 3mM. DNA isolated from ATCC 25923 *S. aureus* was used as a PCR control strain. The cycle conditions used were as follows: initial denaturation at 95°C for 4 minutes, followed by 30 cycles of 95°C for 30 seconds (denaturation), 60°C for 30 seconds (annealing) and 72°C for 45 seconds (extension). The final extension time of 10 minutes at 72°C was used. Electrophoresis of 5µl of the PCR product was performed on a Tris-Acetate-EDTA agarose gel (wt/vol) (100V) containing 1µl ethidium bromide (10 mg/ml). A 50bp ladder (Thermo Scientific, Hanover, MD, USA) was used as a molecular weight standard and all gels were visualised using a Biotop Biosens SC - 645 Gel Documentation System (Biotech Co. Ltd, Shanghai, China).

3.7.1.1 Sanger DNA Sequencing for *spa* Gene

DNA fragments for sequencing were prepared from representative *spa* gene PCR positive samples based on the spa gene amplicon sizes using the QIA quick Gel Extraction Kit (Qiagen Inc. Valencia, CA, USA) according to the manufacturers' recommendations. Sequencing reactions were set-up as follows: 100ng of PCR product, 2µl Big Dye Terminator Reaction Mix (Applied Biosystems, Foster City, CA, USA), 1µl 5Å~sequencing buffer (Applied Biosystems) and 2µl of 5µM forward primer. The reaction was made up to 10µl with molecular grade water. The cycle conditions were 96°C for 1 minute, followed by 25 cycles of 96°C for 10 seconds, 50°C for 5seconds and 60°C for 4 minutes. The reaction was kept at 4°C until it was purified using ethanol precipitation. Ethanol precipitation was done as follows: The reaction was made up to 100µl with molecular grade water, where after 10µl of 3M

Sodium acetate (NaAOc) (Sigma-Aldrich) (pH 4.6) and 250µl of 100% molecular grade ethanol was added. The samples were then centrifuged at 11 000*xg* for 20 minutes. The supernatant was aspirated and 250µl of 70% ethanol was added. The samples were centrifuged at 11 000*xg* for 8 minutes. The supernatant was aspirated, and the samples were left to air dry for 15 minutes. Sequencing was performed on the forward and reverse strands by the dye terminator method using an ABI PRISM 3730XL DNA analyser (Applied Biosystems, Foster City, CA, USA). The DNA sequence reads were edited using the ATCG software ™ and submitted to online tool Center for Genomic Epidemiology to assign spa types (Bartels et al., 2014).

3.7.2 Whole Genome Sequencing

Whole genome sequencing using the Minion Nanopore platform following the protocol briefly described below was also used to determine sequence types, *spa* types, phylogenetic relationships, mobile genetic elements, plasmids and other virulence factors. Briefly, DNA was extracted using the QIAamp PowerFecal DNA Kit (see Section 3.5.2.1 DNA extraction) and its concentration measured using the Qubit 3.0 with the DNA High Sensitivity Kit (ThemoFisher Scientific, Invitrogen). This was then followed by DNA clean up and size selection by use of Ampure Beads binding and washing in freshly 70% Ethanol and elution in 50µl nuclease free water and measured again with the qubit 3.0 with a final DNA outcome of minimum DNA concentration of 1µg within 48µl. Then DNA repair and end preparation were done using the NEBNext FFPE DNA Repair mix and NEBNext END Repair/ dA-tailing Module reagents (New England Biolabs, UK) by mixing the DNA sample with the reagents and incubating at 20 °C for 30 minutes and 65 °C for 30 minutes and the put-on ice for 30 seconds. Cleanup was then done using 60µl Ampure beads and washing with freshly prepared 70% Ethanol and followed by barcoding using the Barcodes RT (Oxford Nanopore Technologies, EXP-NBD103). Adaptor ligation and clean up was then done followed by Flow Cell priming and addition of 75µl of the sample (DNA library mixed with sequencing buffer (Sequencing Kit (Oxford Nanopore Technologies, SQK-LSK 109) and loading beads) via the SpotON.

3.8 Determination of Virulence Factors Genes of the *Staphylococcus aureus* Isolates

PCR with gene-specific primers were performed to detect genes encoding several virulence factors of *S. aureus* including PVL, SEs, the human-specific IEC and *spl* (the primer sets used are listed in Table 3.2). The PCRs for all the genes except for the *spl* were performed with the QIAGEN PCR Kit (QIAGEN) according to manufacturer's instructions. Each reaction mix (25 µl) consisted of 24 µl of 2X QIAGEN PCR Master Mix (containing QIAGEN HotStartTaq DNA polymerase, QIAGEN PCR buffer, and dNTP mix), 0.2 µM of each primer, and 10 -100 ng of template DNA. GoTaq™ was used to perform the PCRs for the *spl* genes. To detect the PVL genes, a Singleplex PCR with the following cycling conditions was used: initial denaturation for 5 min at 94 °C, followed by 30 cycles of denaturation for 30 sec at 94 °C, annealing for 1 min at 59 °C, extension for 1 min at 72 °C, and final extension for 10 min at 72 °C (Stegger et al., 2012). PCR conditions for the SEs genes were as follows: an initial denaturation of DNA at 95 °C for 15 min was followed by 35 cycles of amplification (95 °C for 30 sec, 57 °C for 90 sec, and 72 °C for 90 sec), ending with a final extension at 72 °C for 10 min (Becker et al., 1998). The Singleplex PCR conditions for detecting the IEC genes were an initial denaturation at 94 °C for 5min, then 30 cycles of denaturation for 30 sec at 94 °C, annealing for 30 sec at 49 °C, then extension for 1 min at 72 °C and final extension for 10 min at 72 °C (van Wamel et al., 2006). PCR conditions used to amplify the *Spl* virulence genes were as follows: an initial denaturation at 95 °C for 2min, then 30 cycles of denaturation for 30 sec at 95 °C, annealing for 30 sec at 48 °C, then extension for 1 min at 72 °C and final extension for 5 min at 72 °C (Kläui et al., 2019). PCR products were resolved by electrophoresis in 1.5% agarose gel (Cambrex Bio Science Rockland, Inc., Rockland, ME) in 1X TAE (Tris-Acetate acid-EDTA) buffer and stained with 0.5 µg/ml of Ethidium Bromide (EtBr) and visualized on a transilluminator.

Table 3.2 Primer Sets for Determining the Virulence Genes of the *S. aureus* Isolates

Primer name	**Target gene**	**Primer Sequence (5'-3')**	**Amplicon size**	**Reference**
Luk-PV-1	LukS/F-PV (PVL)	ATCATTAGGTAAAATGTCTGGACATGATCCA	433 bp	(Moussa et al., 2012)
Luk-PV-2		GCATCAAGTGTATTGGATAGCAAAAGC		
Sak-1	*Sak*	AAGGCGATGACGCGAGTTAT	223bp	(van Wamel et al., 2006)
Sak-2		GCGCTTGGATCTAATTCAAC		
Chp-1	*Chp*	GAAAAAGAAATTAGCAACAACAG	410bp	(van Wamel et al., 2006)
Chp-2		CATAAGATGATTTAGACTCTCC		
Scn-1	*Scn*	AGCACAAGCTTGCCAACATCG	258bp	(van Wamel et al., 2006)
Scn-2		TTAATATTTACTTTTTAGTGC		
SEA-3	*Sea*	CCTTTGGAAACGGTTAAAACG	127bp	(Becker et al., 1998)
SEA-4		TCTGAACCTTCCCATCAAAAAC		
SEB-1	*Seb*	TCGCATCAAACTGACAAACG	477bp	(Becker et al., 1998)
SEB-4		GCAGGTACTCTATAAGTGCCTGC		
SEC-3	*Sec*	CTCAAGAACTAGACATAAAAGCTAGG	271bp	(Becker et al., 1998)
SEC-4		TCAAAATCGGATTAACATTATCC		
SED-3	*Sed*	CTAGTTTGGTAATATCTCCTTTAAACG	319bp	(Becker et al., 1998)
SED-4		TTAATGCTATATCTTATAGGGTAAACATC		
SEE-3	*See*	CAGTACCTATAGATAAAGTTAAAACAAGC	178bp	(Becker et al., 1998)
SEE-2		TAACTTACCGTGGACCCTTC		

Table 3.2 Continued Primer Set for Determining the Virulence of *S. aureus* Isolates

Primer name	Target gene	Primer Sequence (5'-3')	Amplicon size	Reference
OL5142 (SplA-Rev)	*spl*A	ATACGC GGATCC ATGAATAAAAATGTAATGGTTA	720bp	Designed for this study
OL5159 (SplA-Fwd)		ATACGCCTGCAGTTATTTTTCAATATTATTTTGAATAA		
OL5144 (SplB-Rev)	*spl*B	ATACGC GGATCC ATGAACAAAAACGTAGTCATC	720bp	Designed for this study
OL5160 (SplB-Fwd)		ATACGC CTGCAG TTATTTATCTATGTTTTCTGC		
OL5145 (SplC-Fwd)	*spl*C	ATACGC GGATCC TTATTGTTCAATGTGCTTTTG	720bp	Designed for this study
OL5146 (SplC-Rev)		ATACGCGGATCCATGAATAAAAATATAGTCATTAAAAGC		
OL5148 (SplD-Rev)	*spl*D	ATACGC GGATCC TAAACAGAGGAGCACAAAAATG	720bp	Designed for this study
OL5162 (SplD-Fwd)		ATACGCCTGCAGTTATTTATCTAAATTATCTGCAATAA		
OL5150 (SplE-Rev)	*spl*E	ATACGC GGATCC CTTAGAGGAGCAGAAAAATG	720bp	Designed for this study
OL5163 (SplE-Fwd)		ATACGC CTGCAG TTATTTATCTGTGTTATCTGC		
OL5152 (SplF-Rev)	*spl*F	ATACGCGGATCCATTATAATTATACAAATACTTAGAGG	720bp	Designed for this study
OL5164 (SplF-Fwd)		ATACGCCTGCAGTTATTTATCTAAATTATCTGCAATG		

3.9 Data Analysis

Data from the study were analysed using the statistical package SPSS version 21 (IBM Corp, Armonk, NY, USA). Univariate analysis was done for *S. aureus* detected in sample categories, descriptive results were presented as frequencies and percentages with 95% confidence intervals in tables and graphs. Analysis of the PCR gel pictures was done by comparing the amplicon size with the expected band sizes for each target gene for species identification, resistance, and virulence genes. For *spa* typing, the chromatograph sequence files of the isolates were edited using the ATCG software (Genetyx Corporation, Tokyo, Japan) and *spa* types were determined using the SpaFinder tool on the Center for Genomic Epidemiology (Bartels et al., 2014). Phylogenetic analysis based on *spa* types was done using the Ridom™ SeqSphere^{+} Software (Ridom GmbH, Münster, Germany). The *spa* sequences have been deposited in the DNA Data Bank of Japan (DDBJ) with the accession numbers LC710903 to LC710945. Genetic diversity was also analysed based on SNP analysis based on the whole genome sequencing data. Briefly, Sequence reads were trimmed to remove adaptors using Porechop (Wick et al., 2017) and assembled using flye (Kolmogorov et al., 2019). The assembled contigs were then mapped against a reference sequence using RagTag (Alonge et al., 2022). In silico species identification was done using the web-based tool KmerFinder (https://cge.food.dtu.dk/services/KmerFinder/), Other web-based tools, MLST Typer (Larsen et al., 2012), spaTyper (Bartels et al., 2014), ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), Virulence Finder (Joensen et al., 2014, Tetzschner et al., 2020) and MobileElement Finder (Johansson et al., 2021) were used to detect in silico the sequence types, spa types, AMR genes, virulence genes and mobile genetic elements (MGEs), respectively. Genome annotation was done using Prokka (Seemann, 2014). Contigs were aligned using muscle and phylogenetic analyses and trees were constructed using the CSI Phylogeny and MEGA software version 11 (Tamura et al., 2021).

3.10 Ethical Clearance

Ethical approval and authority to conduct the study was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) (Appendix B) approval number Ref: 613-2019 and National Health Research Authority (NHRA) (Appendix B). Consent was sought from farm owners to collect samples from their livestock and workers. Consent was also sought from abattoir managements to sample

the pigs and workers in their premises. Informed consent was obtained from individual farm workers and abattoir workers as wells as from the patients included in the study. The consent forms are listed in the appendices. Permission to use human isolates from a previous study (Mutalange, 2021) was also sought from the University Teaching Hospital, Lusaka. Confidentiality was ensured by anonymising the data before analysis using unique identifiers. Animal safety, comfort and biosecurity of the farms were also ensured during sample collection. There was minimal risk to the participants which was associated with sample collection. Notably, collection of nasal swabs caused minimal discomfort while blood samples were collected by professionally trained officers only. Another risk associated with the study was stress from answering questions from the questionnaires. However, the participants were free not to answer the questions they deemed personal or otherwise.

CHAPTER FOUR

RESULTS

4.1 Prevalence of *Staphylococcus aureus* in Pigs and Humans in Lusaka Province

Phenotypic analysis yielded a total of 212 *S. aureus* strains (Figure 4.1A). These isolates were not included in the calculation of the prevalences but were further characterized. Species identity was confirmed for all isolates based on detection of the *nuc* gene using PCR (Figure 4.1B). Appendix C shows the characteristics of samples that yielded more than one colony type of *S. aureus*.

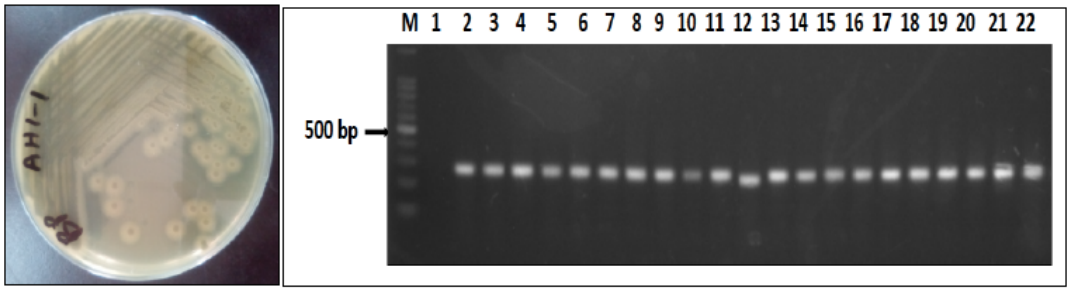


Figure 4.1 (A) *S. aureus* Isolates on Baird Parker Agar; (B) *Nuc* Gene Gel Electrophoresis of Controls and Selected Isolates

Key: MM = 100bp marker; Lane 1= Negative control; Lane 2 = Positive control; Lanes 3 to 22 = Samples

The *S. aureus* prevalence/ positivity rates determined in the study are shown in Table 4.1. The overall prevalence of *S. aureus* was found to be 33.1%, whereby it was 37.8% and 11.3% in pigs and workers, respectively. The positivity rate in both nasal and hand swabs from humans was 5.7%. Chilanga District showed the highest (66.4%) positivity rate among the three districts studied.

Table 4.1 *S. aureus* Positivity Rates from Pigs, Humans and Districts in Lusaka Province

Sample Type	Sample Source	*n* Tested	n Positive	Proportion (%)	95% CI
Overall Positivity	Negative	598	400	66.9	62.94-70.62
	Positive	598	198	33.1	29.4-37.1
Humans	Overall	106	12	11.3	6.2-19.3
	Hand Swabs	106	6	5.7	2.3-12.4
	Nasal Swabs	106	6	5.7	2.3-12.4
Pigs	Nasal swabs	492	186	37.8	33.5-42.3
Districts	Chongwe	250	60	24.0	18.9-29.9
	Lusaka	235	63	26.8	21.4-33.0
	Chilanga	113	75	66.4	56.6-74.8

Abbreviations: CI = Confidence interval; n = number of samples

With regards to study sites, the prevalences are shown in Table 4.2. The overall prevalence at farms and abattoirs was 27.2% and 65.9%, respectively. Generally, the prevalence of *S. aureus* in pigs was significantly higher than in humans at both farm and abattoir levels. While the prevalence of *S. aureus* was high for both pigs and humans at medium and large-scale facilities, the prevalence of *S. aureus* in humans was low in small-scale farms.

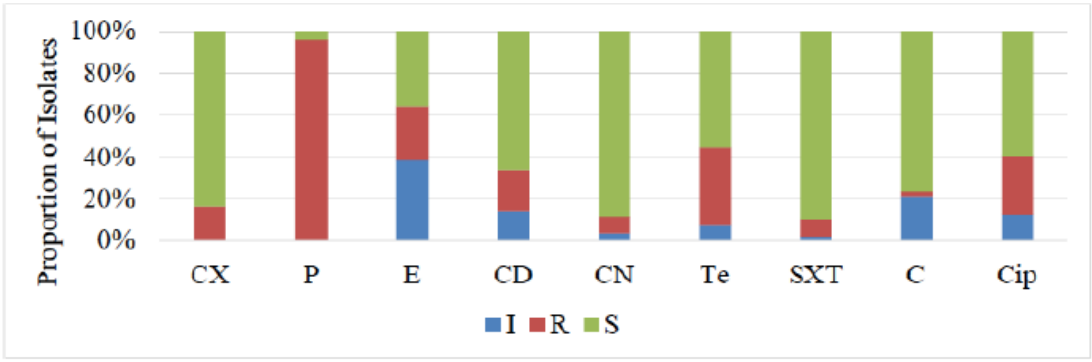
Table 4.2 Prevalence of *S. aureus* in Pigs and Humans at Farms and Abattoirs in Lusaka Province

Study Site	Species	Type[a] of Facility	*N*	Positives	Prevalence (%)	95% CI
Farms	Combined[b]	Small	53	13	24.5	14.2-38.6
		Medium	252	61	24.2	19.1-30.1
		Large	189	64	33.9	27.3-41.1
		Overall	494	138	27.9	24.1-32.1
	Pigs only	Small	45	13	28.9	16.8-44.5
		Medium	216	57	26.4	20.8-32.9
		Large	157	61	38.9	31.8-47.0
		Overall	418	131	31.3	27.0-36.1
	Humans only	Nasal	38	3	7.9	2.1-22.5
		Hand	38	4	10.5	3.4-25.7
		Overall	76	7	9.2	4.1-18.6
	Human Nasal	Small	4	0	0	0
		Medium	18	2	11.1	2.0-36.1
		Large	16	1	6.3	0.3-32.3
	Human Hand	Small	4	0	0	0
		Medium	18	2	11.1	2.0-36.1
		Large	16	2	12.5	2.2-39.6
Abattoirs	Combined[b]	Medium	20	4	20	6.6-44.3
		Large	71	56	78.9	67.3-87.3
		Overall	91	60	65.9	55.2-75.3
	Pigs only	Medium	20	4	20	6.6-44.3
		Large	54	51	94.4	83.7-98.6
		Overall	74	55	74.3	62.6-83.5
	Humans only[c]	Hand	8	2	25	4. 5-64.4
		Nasal	9	3	33.3	9.0-69.1
		Overall	17	5	29.4	11.4-56.0

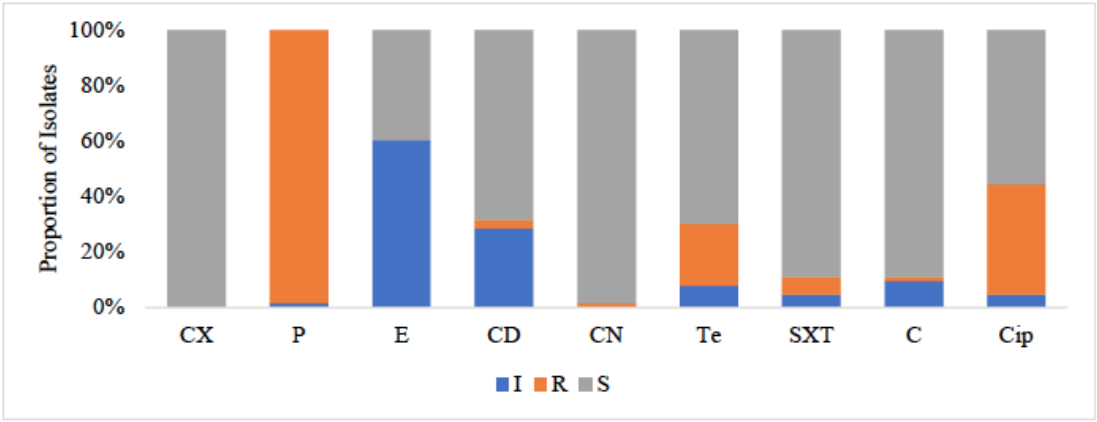
*Type of facility: Small scale (less than 100 pigs), medium scale (100 to 500 pigs) and commercial scale (greater than 500 pigs; Combined[b]: pigs & humans; [c]All human swabs from abattoirs were collected at the large facilities only. Abbreviations: CI = Confidence interval; n = number of samples

4.2. Antimicrobial Susceptibility Frequencies and Antimicrobial Resistance Genes Detected in the *Staphylococcus aureus* Isolates

The overall susceptibility Frequencies of the *S. aureus* isolates from samples collected from both pigs and humans are shown in Figure 4.2 (A) for isolates collected from farms and Figure 4.2 (B) for isolates from the abattoirs. The highest resistance of the *S. aureus* isolates from samples from the farms were to penicillin (98%). Resistance to tetracycline and ciprofloxacin was also recorded at 35% and 30%, respectively and about 18% of the isolates showed resistance to cefoxitin implying methicillin resistance. However, these isolates were more susceptible to co-trimoxazole (92%), gentamicin (90%) and chloramphenicol (79%). Forty percent of the isolates showed intermediate susceptibility to erythromycin while erythromycin-induced clindamycin resistance was detected in only one isolate. From abattoirs, the highest resistance was recorded to penicillin at 98%, followed by 35% and 25% to ciprofloxacin and tetracycline, respectively. Isolates from the abattoirs were susceptible to cefoxitin (100%), gentamicin (99%), co-trimoxazole (90%) and to chloramphenicol (88%). Intermediate results were highest for erythromycin (60%), while no erythromycin-induced clindamycin resistance was detected. Notably, all isolates tested against vancomycin were susceptible with MICs ranging from 1.5µg/l to 3µg/l.



(A)



(B)

Figure 4.2 (A) Overall Antimicrobial Susceptibility Frequencies of *S. aureus* Isolates from Pigs and Workers from Farms of Lusaka Province; (B) Overall Antimicrobial Susceptibility Frequencies of *S. aureus* Isolates from Pigs and Workers from Abattoirs of Lusaka Province

Abbreviations: P = Penicillin; CN = Gentamicin; E = Erythromycin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, SXT= Cotrimoxazole, C= Chloramphenicol, CX= Cefoxitin; I = Intermediate, R = Resistant, S = Susceptible

4.2.1 Multidrug Resistance Patterns of the *Staphylococcus aureus* Isolates

Antibiotic resistance patterns were assigned using designations P + Te + CN + E + CD + Cip + C + SXT (as defined in Figure 4.2 legend) to assess whether antibiotic resistance phenotypes clustered together. It was found that the isolates grouped into 23 antibiotic resistance patterns as shown in Table 4.3. Most of the isolates were resistant to at least one or two antibiotics besides penicillin, with the predominant phenotype being P+Cip (15.7%) and P+E+CD+Cip (14.2%). Isolates were classified as multi-drug resistant (MDR) if they were resistant to three different antibiotic classes. Based on this classification, 36 isolates from farms were MDR. Multi-drug resistance to three or more antibiotics was observed in 17.6% of the isolates.

Table 4.3 Antibiotic Resistance Patterns of *S. aureus* Isolates from Pigs and Workers from Pig Farms and Abattoirs in Lusaka Province

Resistance Pattern	Proportion of Isolates % (*n*)	
	Farm Isolates (*n* = 141)	Abattoir Isolates (*n* = 63)
P	34.8 (49)	42.9 (27)
Te	1.4 (2)	1.6 (1)
P + Te	20.6 (29)	7.9 (5)
P + Cip	7.1 (10)	34.9 (22)
P + CD	0.7 (1)	3.3 (2)
P + CN + Te	2.1 (3)	1.6 (1)
P + E + Te	1.4 (2)	-
P + Te + Cip	0.7 (1)	3.2 (2)
P + E + CD + Cip	14.2 (20)	-
P + E + CD + Te	1.4 (2)	-
P + E + C + Cip	1.4 (2)	-
P + CN + Te + SXT	5.0 (7)	-
P + E + CD + Te + SXT	0.7 (1)	-
P + E + CD + CN + Cip	1.4 (2)	-
P + E + CN + Te + Cip	0.7 (1)	-
P + E + CD + CN + Te + SXT	0.7 (1)	-
[1] Other	4.3 (6)	4.7 (3)

Abbreviations: *n* = number of samples, P = Penicillin; CN = Gentamicin; E = Erythromycin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, SXT= Cotrimoxazole, C= Chloramphenicol, - = Not detected; [1]Other = P+E, P+SXT, P+E+C, P+E+CD, P+E+SXT, P+CD+Te (farm isolates) and P+Te+SXT, P+CD+CN, P+C+Cip (Abattoir isolates). Each pattern was manifested in only one isolate.

4.2.2. Frequency of *Staphylococcus aureus* Isolates with Selected Antimicrobial Resistance Genes

The *mec*A and *mec*C genes that encode for methicillin resistance were not detected in all the isolates despite the phenotypic resistance to methicillin in some of the isolates. Detection rates of the genes encoding for tetracycline resistance were 19.3% (11/57) for *tet*(M), 12.3% (7/57) for *tet*(K) and 1.8% (1/57) for *tet*(L). Notably, all isolates

harbouring these genes were from pig nasal swabs. The *tet*(O) was not detected in any of the isolates tested while only one isolate harboured both the *tet*(M) and *tet*(L) genes. It was observed that isolates from the farms harboured more of the tetracycline resistance genes than those from the abattoirs. For genes that encode for erythromycin resistance, the *erm*(B) and *erm*(C) genes were detected in 19.2% (5/26) and 57.7% (15/26) of the isolates, respectively while the *erm*(A) gene was not detected in any of the isolates. Most of the isolates harbouring these resistance genes were from pigs sampled from the same farm. Only two human isolates harboured the erythromycin resistance genes. Figure 4.3 (A and B) below shows gel pictures of representative *tet*(M) and *erm*(C) positive samples, respectively.

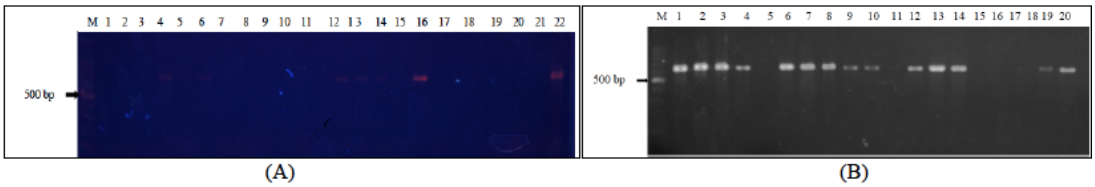


Figure 4.3 (A) Gel electrophoresis of *tet*(M) resistance gene; and (B) of *erm*(C) resistance gene
Key: M= 100bp ladder, Lanes 1 to 22 samples.

4.3 Virulence Genes Detected in the *Staphylococcus aureus* Isolates

For the genes encoding virulence factors, neither the PVL nor the SEs encoding genes were detected in any isolates in this present study. For the IEC genes, *sak*, *scn* and *chp*, were detected in 7.6 % (17/225), 1.3% (3/225) and 0.4% (1/225) isolates, respectively as shown in Table 4.6. All isolates positive for the IEC genes were from nasal swabs of pigs, and mostly from one farm (Farm 7). Figure 4.4 shows some representative *sak* positive isolates.

Table 4.4: IEC Genes Distribution Among the *S. aureus* Isolates (n=225)

Source (Farm or Abattoir)	Sample Type	IEC Gene		
		***scn* % (*n*)**	***sak*% (*n*)**	***chp* % (*n*)**
Farm 1	Pig nasal Swab	-	0.4 (1)	-
Farm 2	Pig nasal Swab	-	0.4 (1)	-
Farm 4	Pig nasal Swab	-	1.3 (3)	-
Farm 5	Pig nasal Swab	-	0.4 (1)	-
Farm 6	Pig nasal Swab	-	0.4 (1)	-
Farm 7	Pig nasal Swab	0.9 (2)	2.7 (6)	-
Farm 9	Pig nasal Swab	-	0.4 (1)	-
Farm 10	Pig nasal Swab	-	1.3 (3)	-
Abattoir 1	Pig nasal Swab	0.4 (1)	-	0.4 (1)
	Total	1.3 (3)	7.6 (17)	0.4 (1)

Abbreviation: n= number of isolates; - = None detected

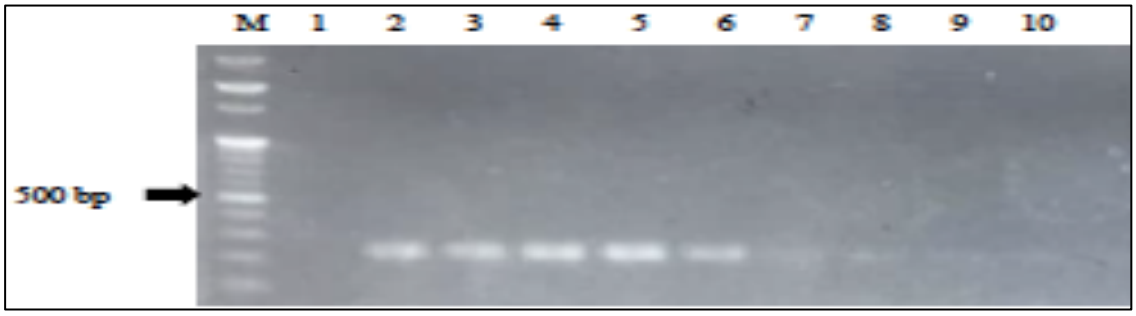


Figure 4.4 Gel Electrophoresis of the *sak* Gene
Key: MM: 100bp marker; Lane 1: Negative control; Lanes 2 to 10: Isolates

The *spl* genes detected using PCR are shown in the representative Figure 4.5 (A, B, C and D) below.

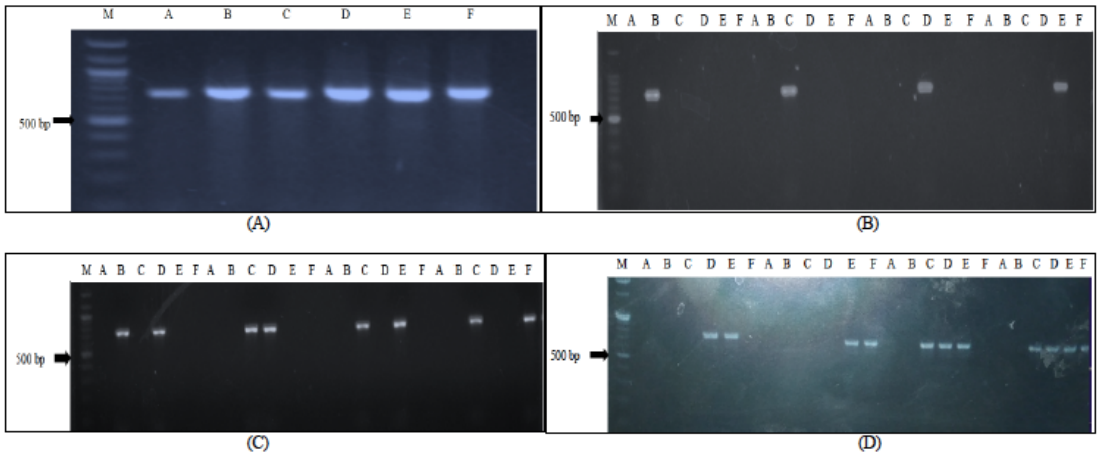


Figure 4.5: Electrophoresis Gel for *spl* Genes (A) Isolates Positive for All *spl* genes (B) Isolates Positive for B, C, D & E *spl* Genes (C) Isolates Positive for B & D, C & D, C & E, and C & F *spl* genes (D) Isolates Positive for D & E, E & F, C, D & E, and C, D, E & F *spl* genes
Key: M: 100bp marker; A: *spl*A; B: *spl*B; C: *spl*C; D: *spl*D; E: *spl*E; F: *spl*F

The *spl* genes were detected in 12.2% (26/213) isolates tested as shown in Table 4.5. The prevalence of *spl* genes was generally low in all specimen types tested even though *S. aureus* isolates from pigs yielded a relatively higher prevalence of the genes (Table 4.7). The most common *spl* genes detected were *spl*D (30.8%) followed by *spl*E (26.9%) while *spl*A was the least (3.9%) detected. Notably only two isolates from nasal swabs of workers from the same abattoir harboured all the six (6) *spl* genes. Another two isolates from pig nasal swabs harboured three *spl* genes namely, *spl*C, *spl*D and *spl*E. One isolate from a hand swab of an abattoir worker harboured the *spl*C and *spl*E. Only six out of 44 clinical isolates that were screened for the *spl* genes were positive for at least one *spl* gene. All these clinical isolates were from pus samples.

Table 4.5 Prevalence of *spl* Genes in *S. aureus* from Pigs, Workers, and Patients (n=213)

Sample type	**Positives**	**Prevalence (%)**	**95% CI**
Pus*	5	2.3	0.9-5.7
Pigs Nasal	17	8.0	4.9-12.7
Workers (Hand and Nasal)	4	1.9	0.6-5.1
Overall prevalence	26	12.2	8.3-17.6

*Pus samples from a previous study (Mutalange, 2021)

4.4 Genotypes of the *Staphylococcus aureus* Isolates

The genotypes of the *S. aureus* isolates as determined in the study are reported below.

4.4.1 *Spa* Types of the *Staphylococcus aureus* Isolates

All *S. aureus* isolates (n=225) were positive for the *spa* gene by PCR as shown by a representative gel picture in Figure 4.6.

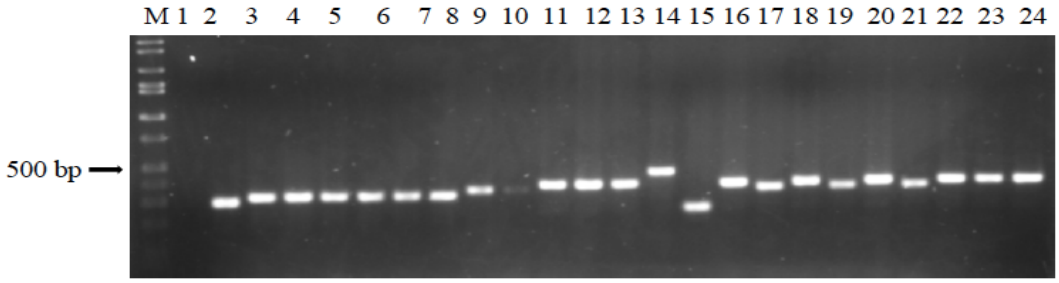


Figure 4.6 Gel electrophoresis of Spa Gene of Controls and Selected Isolates
Key: MM: 100bp marker; Lane 1: Negative control; Lane 2: Positive control (ATCC 25923); Lanes 3 to 24: Samples

The *spa* types determined in the present study are shown in Table 4.9, six already known spa types were detected namely, t1430 (n=12), t034 (n=8), t318 (n=4), t571 (n=1), t084 (n=1) and t899 (n=1). The most common spa type was t1430 followed by t034 (18.6%) as shown in Table 4.6. A total of 16/43 (37.3%) of the isolates were of unknown *spa* types (Table 4.6). The two most common spa types t1430 and t034 were found at both farms and abattoirs of medium and large scale from all the three districts (Table 4.6). Notably, t1430 was detected in most of the pig nasal swabs and one human hand swab at the abattoir 1 while the isolates with unknown spa types were mostly from medium scale facilities (Table 4.6).

Table 4.6 *Spa* Types Distribution among Representative Farm and Abattoir *S. aureus* Isolates

Species	Study Site	Spa type % (*n*)						
		t1430	**t034**	**t318**	**t571**	**t084**	**t899**	**Unknown**
Humans	Farms	0	0	0	0	0	2.3 (1)	4.7 (2)
	Abattoirs	4.7 (2)	0	0	0	2.3 (1)	0	0
Pigs	Farms	14.0 (6)	9.3 (4)	9.3 (4)	2.3 (1)	0	0	25.6 (11)
	Abattoirs	9.3 (4)	9.3 (4)	0	0	0	0	7.0 (3)
	Total	28.0 (12)	18.6 (8)	9.3 (4)	2.3 (1)	2.3 (1)	2.3 (1)	37.3 (16)

Abbreviation: n= number of isolates (*n*=43)

Phylogenetic analysis of the isolates based on the *spa* gene sequences showed that the isolates divided into two main clades with one of the clades separating into further two clusters as shown in Figure 4.7A and 4.7B.

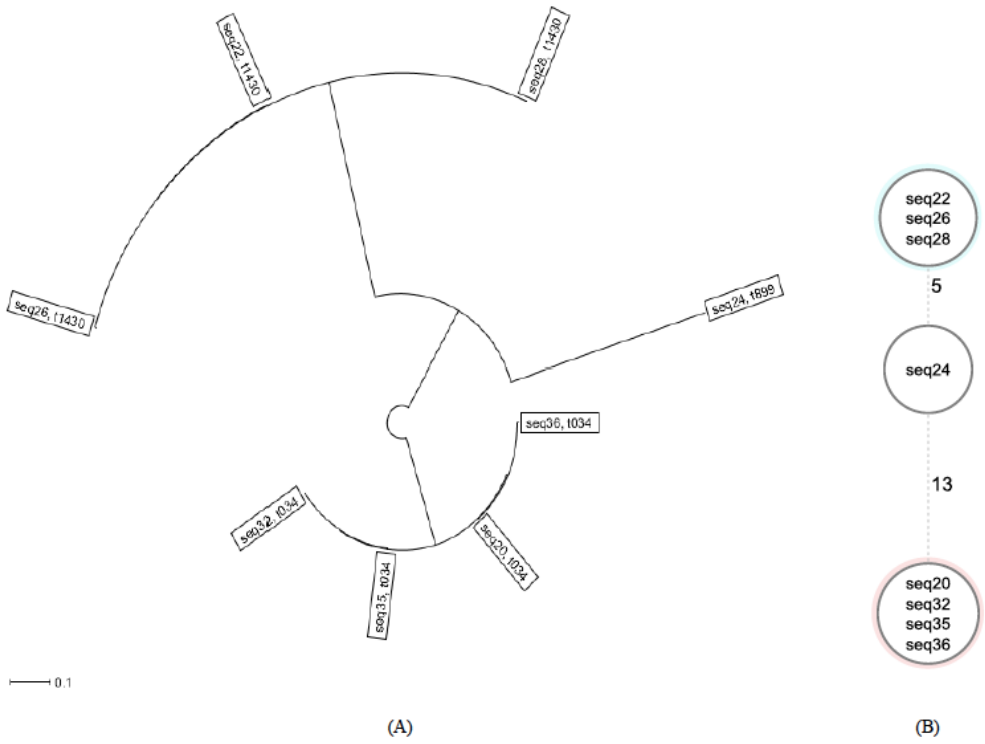


Figure 4.7: (A) Phylogenetic Tree of *S. aureus* Isolates Based on Detected Spa Types (B) Minimum Spanning Tree
Key: Seq20 to seq36 = Representative *S. aureus* isolates used to draw the trees

4.4.2 WGS Analysis of the *Staphylococcus aureus* Isolates

All the six isolates included in the WGS analysis were confirmed as *S. aureus* using the KmerFinder tool on the CGE website. The characteristics of the isolates are summarised in Table 4.7 below.

4.4.2.1 Spa and MLST Types Detected in Silico in the *Staphylococcus aureus* Isolates

The *spa* types of four of the isolates were detected as t1430 and for two as unknown (Table 4.7). This finding is similar to what was determined using the *spa* typing PCR and DNA sequencing method for these isolates. *In silico* determination of the MLST types of the six *S. aureus* revealed that one isolate (H1-2) belongs to ST573, and another isolate (H3-16-1) belonged to ST9 while the rest of the isolates were novel STs (Table 4.7).

4.4.2.2 AMR Genes Detected *in silico* in the *Staphylococcus aureus* Isolates

Several resistance genes were detected in silico in five of the six *S. aureus* isolates. The AMR genes were *bla*Z encoding for resistance to the beta-lactam antibiotics namely penicillin, amoxicillin, piperacillin, ampicillin, *glr*A and *gry*A genes encoding for resistance to the fluoroquinolone ciprofloxacin, *vga*(A)V gene encoding for resistance to macrolides, lincosamides, streptogramin b including clindamycin, tiamulin, virginiamycin, dulfupristrin and pristinamycin iia. One of the five isolates also had the *fus*A gene which encodes resistance to fusidic acid detected *in silico*. One of the six isolates had only *tet*M gene detected *in silico*. The *tet*M gene encoded for resistance to tetracylines including tetracycline, doxycycline and aminocycline. The AMR genes detected *in silico* generally corresponded well with the phenotypic susceptibility profiles as tested using the Kirby-Bauer disc diffusion method of all six isolates (Table 4.8).

Table 4.7 Summary of Sample Type and Genotypic Characteristics of *S. aureus* Isolates from Pigs and Humans Using in Silico Analysis

Isolate Name	District	Study Site	Source	Antibiotic Resistance genes	Virulence factor genes	MLST	Spa type
H1-2	Chilanga	Farm 1	Human hand swab	*tet*M	*aur, hlg*A, *hlg*B, *hlg*C	ST753	Unknown
H3-16-1	Chongwe	Farm 3	Human nasal swab	*bla*Z, *glr*A, *gry*A, *vga*(A)V	*aur, hlg*A, *hlg*B, *hlg*C, *seu, sen, seo, sem, seg, sei,*	ST9****	t1430
H3-13	Chongwe	Farm 3	Human hand swab	blaZ, *glr*A, *vga*(A)V	*aur, hlg*A, *hlg*B, *hlg*C, *seu, sen, seo, sem, seg, sei*	Unknown***	Unknown
H3-5	Chongwe	Farm 3	Human hand swab	*bla*Z, *glr*A, *vga*(A)V	*aur, hlg*A, *hlg*B, *hlg*C, *seu, sen, seo, sem, seg, sei*	Unknown**	t1430
P3-8	Chongwe	Farm 3	Pig nasal swab	*bla*Z, *glr*A, *gry*A, *vga*(A)V	*aur, hlg*A, *hlg*B, *hlg*C, *seu, sen, seo, sem, seg, sei,*	Unknown*	t1430
P3-14	Chongwe	Farm 3	Pig nasal swab	*blaZ, fus*A, *glr*A, *gry*A, *vga*(A)V	*aur, hlg*A, *hlg*B, *hlg*C, *seu, sen, seo, sem, seg, sei,*	Unknown	t1430

*Perfect hits (100%) for alleles; ** Novelle allele (yqiL), and Imperfect hit (99.5% for aroE); *** **yqiL**: Novel allele, ST may indicate nearest ST., tpi: Imperfect hit, ST cannot be trusted!, pta: Novel allele, ST may indicate nearest ST.; **** aroE: Novel allele, ST may indicate nearest ST, yqiL: Novel allele, ST may indicate nearest ST., glpF: Novel allele, ST may indicate nearest ST.

Table 4.8 Phenotypic Antimicrobial Susceptibility Profiles and *in silico* Resistance Genes of *S. aureus* Isolates

Isolate name	Beta-lactams			Non-Beta-lactams						Antibiotic resistance genes using Insilico detection
	CX	P	Ery	CD	CN	Tet	STX	C	Cip	
H1-2	S	R	I	S	S	R	S	S	S	*tet*M
H3-16-1	S	R	R	R	R	S	S	S	R	*blaZ, glr*A, *gry*A, *vga*(A)V
H3-13	S	R	R	R	S	S	S	S	R	*blaZ, glr*A, *vga*(A)V
H3-5	S	R	R	R	S	S	S	S	R	*blaZ, glr*A, *vga*(A)V
P3-8	S	R	R	R	S	S	S	S	R	*blaZ, glr*A, *gry*A, *vga*(A)V
P3-14	S	R	R	R	R	S	S	S	R	blaZ, *fus*A, *glr*A, *gry*A, *vga*(A)V

Abbreviations: P = Penicillin; CN = Gentamicin; E = Erythromycin; CD = Clindamycin; CIP = Ciprofloxacin; TET = Tetracycline, SXT= Cotrimoxazole, C= Chloramphenicol, CX= Cefoxitin; I = Intermediate, R = Resistant, S = Susceptible

4.4.2.3 Virulence Genes Detected *in silico* in the *Staphylococcus aureus* Isolates

In silico virulence gene analysis for the isolates showed that all six isolates harboured several virulence genes including *aur*, *hlg*A, *hlg*B, and *hlg*C which encode for aureolysin, gamma-hemolysin chain II precursor, gamma-hemolysin component B precursor, and gamma-hemolysin component C, respectively (Table 4.9). Five of the six isolates also harboured the *seu, sen, seo, sem, seg,* and *sei* genes which encode for the following *Staphylococcus aureus* enterotoxins, enterotoxin U, enterotoxin N, enterotoxin O, enterotoxin M, enterotoxin G and enterotoxin I, respectively (Table 4.9). Remarkably, none of the staphylococcal enterotoxin genes were detected using gene specific primers using PCR in any of the isolates as previously mentioned in section 4.3.

4.4.2.4 Plasmids and other Mobile Genetic Elements (MGEs) Detected *in silico* in the *Staphylococcus aureus* Isolates

Only one isolate harboured a plasmid (repUS43) and a transposon (Tn6009) as shown in Table 4.9. However, all six isolates harboured several Insertion Sequences (ISs) (Table 4.10). These MGEs had several AMR and virulence genes associated with them as shown in Table 4.9. Notably, these AMR and virulence genes are the same as when the isolates were analysed for *in silico* presence of the respective genes.

Table 4.9 Mobile Genetic Elements and Their Associated AMR and Virulence Genes Detected *in silico* in the *S. aureus* Isolates

Isolate ID	Total Number of MGEs Detected	Types of MGEs	Type and Family of The MGEs	Position of MGEs (% Identity)	Associated AMR Genes	Phenotype of Detected AMR genes	Associated Virulence Genes	Phenotype of Detected Virulence genes
H1-2	6 of 24	repUS43	Plasmid	450870-452075 (100%)	*tet*(M)	minocycline, tetracycline, doxycycline	*hlg*A, *hlg*C, *hlg*B, and *aur*	gamma-hemolysin chain II precursor, gamma-hemolysin component C, gamma-hemolysin component B precursor, and aureolysin
		Tn6009	None (Integrative Conjugative Element)	436870-438757 (99.89%)				
		ISSau8 also called ISRX	Insertion sequence (ISL3)	1568155-1569649 (98.5%)				
		ISSau1	Insertion sequence (IS30)	464002-465072 (99.53%)				
		ISSau1	Insertion sequence (IS30)	1714185-1715255 (99.53%)				
		ISSau1	Insertion sequence (IS30)	796589-797659 (99.53%)				
		ISSau1	Insertion sequence (IS30)	1852037-1853106 (99.63%)				

Key: MGEs= Mobile genetic elements, IS= Insertion sequences, AMR= Antimicrobial resistance

Table 4.9 Continued Mobile Genetic Elements and their Associated AMR and Virulence Genes Detected *in silico* in the *S. aureus* Isolates

Isolate ID	Total Number of MGEs Detected	Types of MGEs	Family of The MGEs	Position of MGEs (% Identity)	Associated AMR Genes	Phenotype of Detected AMR genes	Associated Virulence Genes	Phenotype of Detected Virulence genes
H3-16-1	2 f 28	ISSau5	Insertion sequence (IS30)	1847224-1848357 (99.03%)	*bla*Z,	piperacillin, amoxicillin, ampicillin, penicillin	*hlg*A, *hlg*B, *hlg*C, *aur*	hemolysin chain II precursor, gamma-hemolysin component B precursor, gamma-hemolysin
		ISSau3	Insertion sequence (IS1182)	1723335-1725336 (97.05%)	*vga*(A)V	clindamycin, lincomycin, dalfopristin, pristinamycin iia, virginiamycin m, tiamulin	*sei*, *sen*, *seg*, *seo*, *seu*, *sem*	Enterotoxin I, enterotoxin N, enterotoxin G, enterotoxin O, enterotoxin U, enterotoxin M,

Key: MGEs= Mobile genetic elements, IS= Insertion sequences, AMR= Antimicrobial resistance

Table 4.9 Continued Mobile Genetic Elements and their Associated AMR and Virulence Genes Detected *in silico* in the *S. aureus* Isolates

Isolate ID	Total Number of MGEs Detected	Other Types of MGEs	Family of The MGEs	Position of MGEs (% Identity)	Associated AMR Genes	Phenotype of Detected AMR genes	Associated Virulence Genes	Phenotype of Detected Virulence genes
H3-13	2 of 28	ISSau5	Insertion sequence (IS30)	1880269-1881404 (99.12%)	*bla*Z,	amoxicillin, piperacillin, ampicillin, penicillin	*hlg*A, *hlg*B, *hlg*C, *aur*	hemolysin chain II precursor, gamma-hemolysin component B precursor, gamma-hemolysin
		ISSau3	Insertion sequence (IS1182)	1756323-1758324 (96.73%)	*vga*(A)V	lincomycin, clindamycin, tiamulin, virginiamycin m, pristinamycin iia, dalfopristin	*sei*, *sen*, *seg*, *seo*, *seu*, *sem*	Enterotoxin I, enterotoxin N, enterotoxin G, enterotoxin O, enterotoxin U, enterotoxin M,
H3-5	1 of 26	ISSau5	Insertion sequence (IS30)	1347195-1348327 (98.77%)	*bla*Z, *vga*(A)V	penicillin, piperacillin, ampicillin, amoxicillin, lincomycin, clindamycin, tiamulin, virginiamycin m, pristinamycin iia, dalfopristin	*sei*, *hlg*A, *hlg*B, *sen*, *seg*, *seo*, *seu*, *sem*, *hlg*C, *aur*	Enterotoxin I, gamma-hemolysin chain II precursor, gamma-hemolysin component B precursor, enterotoxin N, enterotoxin G, enterotoxin O, enterotoxin U, enterotoxin M, gamma-hemolysin component C, aureolysin

Key: MGEs= Mobile genetic elements, IS= Insertion sequences, AMR= Antimicrobial resistance

Table 4.9 Continued Mobile Genetic Elements and their Associated AMR and Virulence Genes Detected *in silico* in the *S. aureus* Isolates

Isolate ID	Numbers of MGEs Detected	Type of MGEs	Family of MGEs	Position of MGEs (% Identity)	Associated AMR Genes	Phenotype of Detected AMR genes	Associated Virulence Genes	Phenotype of Detected Virulence genes
P3-8	2 of 27	ISSau5	Insertion sequence (IS30)	1846236-1847371 (99.21%)	*bla*Z	penicillin, piperacillin, amoxicillin, ampicillin	*hlg*A, *hlg*B, *hlg*C, *aur*	gamma-hemolysin chain II precursor, gamma-hemolysin component B precursor, gamma-hemolysin, aureolysin
		ISSau3	Insertion sequence (IS1182)	1722327-1724330 (96.96%)	*vga*(A)V	pristinamycin iia, virginiamycin m, dalfopristin, clindamycin, lincomycin, tiamulin	*sei*, *sen*, *seg*, *seo*, *seu*, *sem*	enterotoxin I, enterotoxin N, enterotoxin G, enterotoxin O, enterotoxin U, enterotoxin M
P3-14	2 of 26	ISSau5	Insertion sequence (IS30)	1942487-1943621 (99.12%)	*bla*Z,	penicillin, ampicillin, amoxicillin, piperacillin	*hlg*A, *hlg*B, *hlg*C, *aur*	gamma-hemolysin chain II precursor, gamma-hemolysin component B precursor, gamma-hemolysin component C, aureolysin
		ISSau3	Insertion sequence (IS1182)	1818571-1820573 (96.91%)	*vga*(A)V	tiamulin, clindamycin, lincomycin, dalfopristin, pristinamycin iia, virginiamycin m	*sei*, *sen*, *seg*, *seo*, *seu*, *sem*	enterotoxin I, enterotoxin N, enterotoxin G, enterotoxin O, enterotoxin U, enterotoxin M

Key: MGEs= Mobile genetic elements, IS= Insertion sequences, AMR= Antimicrobial resistance

4.4.2.5 Phylogenetic Analysis and Comparison of the *Staphylococcus aureus* Isolates

Phylogenetic analysis based on SNPs of the core genes using CSI Phylogeny of the six *S. aureus* together with a reference strain and pig strains from France and the Netherlands are shown in Figure 4.8. The phylogenetic analysis showed that the five isolates from farm 3 clustered together while the isolate from farm one clustered with the international strains which are part of the ST398 (Figure 4.8).

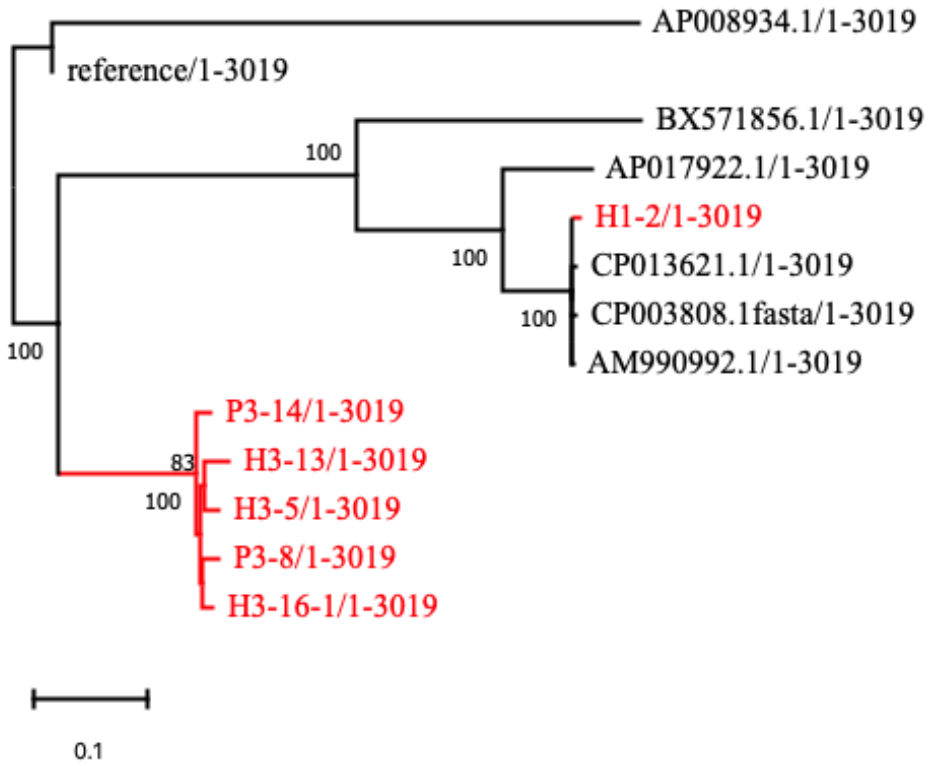


Figure 4.8 Phylogenetic Tree of *S. aureus* Isolates Based on CSI Phylogeny
Key*: S. aureus* from the present study are labeled in red. Isolates labeled in black are reference *S. aureus* isolates. P in the isolate ID means it is a pig-associated S. aureus isolates while H means it was a human-related isolate (for the isolates from present study). CSI Phylogeny = Call SNPs and Infer Phylogeny

The six *S. aureus* isolates were further analysed for similarity and differences using pangenome analysis against the reference strain based on the 100%, 70% and 50% identity similarity (Figure 4.9). Generally, this analysis showed that our isolates were highly similar with differences occurring in positions 1800 kbps and 2200 kbps (Figure 4.9). Notably, isolates H1-2 and H3-14 were more diverse as they showed lower percent identity when compared with the reference strain (Figure 4.9).

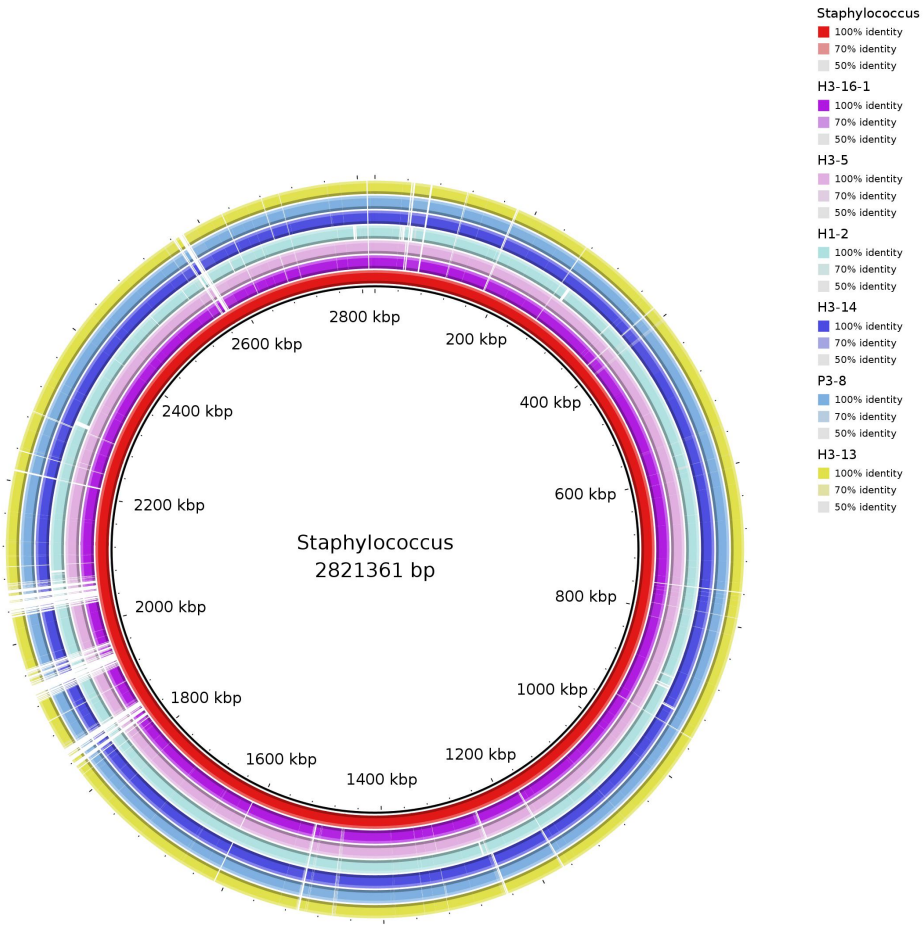


Figure 4.9 Pangenomic Representation of the *S. aureus* Isolates in Relation to a Reference Strain
Key: Red ring is the reference strain. The purple ring =Isolate H3-16-1, Pink ring =Isolate H3-5, Light Blue ring = Isolate H1-2, Dark Blue ring = Isolate H3-14, Sky Blue ring = Isolate P3-8 and Yellow ring =Isolate H3-13

CHAPTER FIVE

DISCUSSION

5.1 Discussion

This study aimed at determining the genomic epidemiology and zoonotic potential of *S. aureus* from pigs and workers from pig farms and abattoirs in the Lusaka Province of Zambia. It is the first report on the presence of *S. aureus* in pigs and workers from farms and abattoirs in Zambia. The overall prevalence rate of *S. aureus* (33.1 %) detected in the present study was relatively high and in congruent with similar studies on the African continent that have reported prevalence ranging up to 55% (Fall et al., 2012, Adegoke and Okoh, 2014, Okunlola and Ayandele, 2015, Tanih et al., 2015, Katakweba et al., 2016, Igbinosa et al., 2016, Founou et al., 2018, Momoh et al., 2018, Odetokun et al., 2018, Otalu et al., 2018, Pekana and Green, 2018, Adikwu et al., 2019, Dweba et al., 2019, Founou et al., 2019, and Nwaogaraku et al., 2019). Though specific comparisons of prevalence rates is difficult due to the variations in the conduct of these studies, for example, most studies have only studied isolates either from farms or abattoirs and not from both sites (Lozano et al., 2016). In addition, some studies may sample from more than one body part of the pigs (Lozano et al., 2016). While the nares are a good site to sample in both pigs and humans as *S. aureus* is known to be inhabit these sites in many animals including pigs and about 33% of humans, some studies have suggested that other body sites such as the skin around the groin and ear regions could also potentially habour *S. aureus* more than nasal tares (Agersø et al., 2014). Therefore, it is expected that sampling from more than one body part would lead to a higher yield of *S. aureus* and in turn, a higher prevalence rate.

A comparatively higher prevalence of *S. aureus* was detected in the pigs (37.8%) than in workers (11.3%) in the present study, similar to the findings from a recent study in Nigeria (Gaddafi et al., 2021). However, the studies from Nigeria and South Africa detected more *S. aureus* from pigs than in our study (Gaddafi et al., 2021, Sineke et al., 2021). This difference could be due to the fact that Sineke and others sampled pigs from intensive farms while Gaddafi and colleagues sampled more piglets than our present study and showed that these were more likely to be colonised with *S. aureus* than adult pigs (Gaddafi et al., 2021, Sineke et al., 2021). The current study further

showed that the hand and nasal prevalence of *S. aureus* was the same and relatively low among the workers sampled. The relatively lower prevalence of *S. aureus* found in the workers would indicate a low prevalence in this population in Zambia. It could also be due to the small number of people sampled as the study was conducted during the covid pandemic and few people were willing to participate in the study. Therefore, the prevalence determined among this population may not be the true representation of *S. aureus* carriage in such populations. Nevertheless, given the of zoonotic potential and transmissibility of *S. aureus*, regular screening of workers in pig farms and abattoirs is recommended. Furthermore, future studies could consider also screening other at-risk groups such as veterinarians and household members to gain even better insight into the prevalence of *S. aureus* among such individuals.

Antimicrobials remain a crucial part in tackling infectious diseases all over the world. However, the emergence and spread of AMR in microbes is worrisome and thus antimicrobial susceptibility and detection of AMR genes is crucial in the fight against AMR. This study revealed that most isolates from farms and abattoirs were resistant to several antibiotics with the highest resistance being to penicillin (above 90%). This finding is significantly higher than that from the study in Nigeria which reported a resistance to penicillin of 55% (Gaddafi et al., 2021). The high resistance to penicillin in our study suggests possible overuse of the antibiotic, as penicillin is generally among the most regularly used antibiotics in many farms in many countries including Zambia (Mainda et al., 2015, Holmer et al., 2019, Lekagul et al., 2020, Mudenda et al., 2022). Resistance to tetracycline, erythromycin and ciprofloxacin was also recorded in 25% to 35% of isolates in our present study. Notably, tetracycline is also commonly used to treat infections in both humans and animals and its resistance can be used as an indicative marker of LA-SA (Lozano et al., 2012, Benito et al., 2014). Although there are no documented studies on the use of tetracycline in Zambian pig farms, Mainda and colleagues have documented the use of tetracycline in dairy farms in Zambia (Mainda et al., 2015). On the other hand, ciprofloxacin and erythromycin are used mostly in treating human infections, in fact ciprofloxacin is considered a viable antibiotic for MRSA and other difficult to treat *S. aureus* infections (Gade and Qazi, 2013). Only 18% of farm isolates were resistant to cefoxitin implying methicillin resistance. However, all these isolates were susceptible to vancomycin with the MICs ranging between 1.5µg/l to 3µg/l. MRSA often causes difficult to treat infections and

contributes to increased mortality and morbidity of patients and adds to the health economic burden of countries. Efforts to prevent emergence and spread of MRSA are thus always a welcome move. Vancomycin is the drug of choice for MDR *S. aureus* infections in human health and is rarely used to treat animal infections (Pahadi et al., 2014). This finding which is similar to that of a previous study that studied vancomycin susceptibility of clinical *S. aureus* show that vancomycin is still a viable treatment option of *S. aureus* infections in Zambia (Mutalange, 2021).

In the present study, it was noted that most isolates were generally still susceptible to first line antibiotics including co-trimoxazole, gentamicin and chloramphenicol with susceptibility rates ranging from 79% to 92%. Inducible resistance to macrolides, lincosamides, and group B streptogramins (MLSBi) phenotype was only detected in one isolate. The MLSBi phenotype positive isolates appear to be erythromycin-resistant and clindamycin sensitive in vitro, but when given in vivo, they have constitutive *erm* mutations that render clindamycin ineffective (Prabhu et al., 2011). Relatively few studies in Zambia have documented the presence of the MLSBi phenotype and markedly these have involved *S. aureus* from clinical settings. The latest study at the largest referral hospital in Zambia found that none of the isolates had the MLSBi phenotype (Mutalange, 2021). Conversely, a high rate of the MLSBi phenotype of 68.3% was reported in an earlier study at the same hospital (Samutela et al., 2015). Many studies on *S. aureus* in animals have not reported on the MLSBi phenotype probably because clindamycin is not used to treat infections in animals. However, a study from South Africa reported the MLSBi phenotype among the studied isolates from pigs (Sineke et al., 2021). It is necessary to screen for the MLSBi phenotype since clindamycin is useful in treating difficult to treat *S. aureus* infections in humans. While multi-drug resistance was observed to two or more antibiotics in more than 40% of the isolates in the present study, generally our findings suggest that there are seemingly still several antibiotics that would be viable to treat infections caused by these isolates from the pig and pork production sector in Zambia.

Unexpectedly, despite the phenotypic resistance to methicillin that was detected in some of the isolates, neither the *mec*A nor *mec*C genes that encode for methicillin resistance were detected in any of the isolates. The phenotypic resistance to methicillin could possibly be due to the isolates being hyperproducers of penicillinases that confer

some resistance to cefoxitin (Montanari et al., 1990). Another possible explain could be that the mechanism of resistance is novel and therefore could not be detected using the available laboratory methods (Fluit et al., 2001). Whereas the *mec*A is the mainstay gene responsible for methicillin resistance in clinical isolates, the *mec*C gene is linked to livestock associated staphylococcus, especially LA-MRSA (Paterson et al., 2012). The *mec*C has been reported widely in many studies from pig related *S. aureus* and other animals from Europe and other continents (Petersen et al., 2013, Harrison et al., 2013, Porrero et al., 2014, Paterson et al., 2014, Kerschner et al., 2015, Dermota et al., 2015, Angen et al., 2016). On the other hand, few studies have reported on the presence of the *mec*C gene in animals in Africa. A recent study from South Africa reported the presence of the *mec*C in pig-associated *S. aureus* for the first time in Africa (Dweba et al., 2019). A study from Tunisia also reported the *mec*C gene in *S. aureus* isolates from chickens (Chairat et al., 2015). It is therefore imperative that future studies should include screening for the *mec*C gene in African *S. aureus* isolates. Furthermore, relatively few countries have reported typical LA-MRSA pig-related *S. aureus* in Africa (Founou et al., 2018, Dweba et al., 2019, Founou et al., 2019). Intensive pig farming methods and heavy use of antibiotics have been identified as risk factors for the emergence and spread of methicillin resistance as well as resistance to other antibiotics in *S. aureus* among pigs and attending workers at farms and slaughterhouses from other parts of the world such as Europe and America (van Duijkeren et al., 2008, Köck et al., 2009, Fang et al., 2014). However, none of the facilities included in the current study practises such intensive pig rearing which can potentially explain the absence of MRSA detected in the study. Markedly, MSSA cannot be overlooked as they form the reservoir from which MRSA arise (Price et al., 2012, Laumay et al., 2021). The present study also reports the presence of several antimicrobial resistance genes including genes encoding resistance for tetracycline (*tet*(M), *tet*(K) and *tet*(L)), erythromycin (*erm*(B) and *erm*(C)), ciprofloxacin (*gyr*A and *grl*A) and macrolides (*vga*(A)V) in some of the isolates. This indicates the need to closely monitor these strains as they may become a source of antimicrobial resistance given that some of these genes are harboured on mobile elements such as plasmids which can be easily transferred between microorganisms (Emaneini et al., 2013).

The PVL encoding genes were not detected in any of the isolates in the present study. A similar study conducted in Portugal did not detect any PVL genes in pig related *S. aureus* isolates (Conceição et al., 2017). Although this was the first study to look for the presence of these genes in isolates from pigs and workers associated with pigs in Zambia, the PVL has been reported in a previous study of clinical isolates howbeit only three out of 33 isolates were positive (Samutela et al., 2017). The low prevalence of the PVL genes in the previous study coupled with the lack of detection in our study would suggest that the PVL genes are generally low in the Zambian *S. aureus* population. Similarly, studies from South Africa detected the PVL gene in only one MRSA isolate from pigs from abattoirs (Founou et al., 2018, Founou et al., 2019). Conversely, a study in Senegal on pigs and workers at a commercial farm reported a high prevalence of the PVL gene (Fall et al., 2012). The PVL is associated with skin and soft tissue infections and has a provenance for humans, but our study indicates that it may be dispensable for pig colonisation.

While SE encoding genes were not detected in the present study by PCR, in silico analysis of WGS data of some of the isolates revealed the presence of some SE genes including the superantigens *sei* and *seg*. The role of SEs in staphylococcal foodborne disease has been documented in several studies (Bennet and Monday, 2003, Gallina et al., 2013, Kadariya et al., 2014). Other studies on the African pig associated *S. aureus* isolates have reported the *sea* and *seb* genes (Otalu et al., 2018, Founou et al., 2019). Several SEs including the classical SEs were detected in *S. aureus* isolates in a recent study on the diary food chain in Zambia (Phiri et al., 2022). The non-detection of SEs in the present study by PCR but detection by in silico analysis suggests the need to do more WGS studies and analyses into the relative safety of the pork and pork products on the Zambian market for consumers.

Remarkably, several isolates harboured the IEC genes as detected by PCR with the *sak* being the most prevalent in the present study, although WGS analysis did not reveal any IEC genes in the isolates. The staphylokinase and chemotaxis inhibitory proteins form the IEC and contribute to immune evasion in humans (van Wamel et al., 2006). While IEC genes are less prevalent in livestock-adapted *S. aureus* lineages, they are considered good genetic markers for identification of human-associated *S. aureus* clones (McCarthy et al., 2011). Therefore, the finding of IEC genes among *S.*

aureus isolates from pigs in the present study potentiate the notion of possible anthropogenic nature of some of the *S. aureus* in Africa but could also indicate the presence of LA-SA that are well adapted to human hosts (Mama et al., 2021).

The present study presents the first to detect *spl* genes in Zambian *S. aureus* isolates using PCR. Generally, the prevalence of *spl* genes was low, however, as no other studies have reported on the *spl* genes in Africa have not used PCR, it is difficult to make comparisons. Notably, only two isolates harboured all six *spl* genes and were from human healthy carriers, while the clinical isolates mostly carried one of the *spl* genes with only one clinical isolate harbouring four *spl* genes. Of interest again is that isolates from pigs had a relatively higher prevalence of *spl* genes. Our findings may suggest a propensity of the *spl* genes among healthy *S. aureus* carriers. However, since the number of clinical isolates included in the study was smaller than those from the healthy carriers, further studies are needed to confirm this speculation. The role of *spl* genes in infections have not been fully understood but they have been shown to be immunogenic and may be involved in some respiratory tract infections (Stentzel et al., 2017) and to play a role in colonization of hosts (Paharik et al., 2016). It worth mentioning that none of the isolates analysed by WGS showed the presence of the *spl* genes in our study. Other virulence genes, *hlg*A, *hlg*B, *hlg*C encoding for gamma hemolysins and *aur* encoding for aureolysin were detected in all six isolates via WGS in our study. The study by Founou and others also reported the presence of the gamma hemolysin genes among others (Founou et al., 2019). The gamma hemolysin genes were first reported among LA-MRSA from veterinarians by Wettstein and friends (Wettstein Rosenkranz et al., 2014). While Otalu and colleagues have reported the presence of the aureolysin gene in African LA-MRSA (Otalu et al., 2018). The gamma hemolysins and aureolysin are among the arsenal of staphylococcal toxins and enzymes, respectively which are implicated in some staphylococcal infections (Otto, 2014).

Our study also found several MGEs in the six isolates analysed by WGS. Only one isolate from a worker which harboured the *tet*(M) AMR gene, had the plasmid repUS43 and transposon Tn6009 in the present study. Studies from Africa have reported the presence of both Tn6009 and repUS43 in Gram positive bacteria including *Enterococcus faecalis* in humans (Founou et al., 2021), and chicken litter

(Fatoba et al., 2022) and in *Streptococcus pneumoniae* strains from humans (Manyahi et al., 2022). The Tn6009 is a non-composite transposon in the Tn916 family that carry the *S. aureus mer* genes directly linked to the *tet*(M) gene (Soge et al., 2008). It can be associated with both chromosomes as well plasmids and was first reported in Gram positive bacteria from Nigeria, and Gram positive and negative bacteria from Portugal (Soge et al., 2008). The repUS43 is a conjugative plasmid known to habour the *tet*(M) gene and carry Tn6009. The Tn6009 has been shown to participate in the dissemination of MDR determinants that could be transferred from numerous bacteria, such as *Klebsiella pneumoniae*, *Serratia liquefaciens*, *Pseudomonas species* and *Streptococcus species* (Soge et al., 2008). Therefore, there is need for serious monitoring of these *S. aureus* strains in the pig and pork production in Zambia to curb the spread of AMR genes among bacteria and of these bacteria into the health sector. Notably, this isolate had an abundance of insertion sequences which were also present in the other five isolates. Insertion sequences have been shown to play several roles in the plasticity and adaptability of bacterial genomes including gene inactivation as well as mobilization (Vandecraen et al., 2017).

Genetic diversity and relatedness analysis of the isolates showed that spa type t1430 was the most prevalent followed by t034 out of the six *spa* types detected in our present study. These *spa* types were mostly detected among *S. aureus* isolates from pigs both at the farms and abattoirs. Of significance is that t1430 and t034 are associated with CC9 and CC398 which are livestock-associated lineages of *S. aureus* in Asia and Europe, respectively (Smith and Pearson, 2011, Tegegne et al., 2017). The t1430 *spa* type was also found in some of the isolates from the workers. Therefore, our findings suggest that typical LA-SA lineages are present in pig and pork production facilities in Zambia. Previous studies on pig related *S. aureus* isolates on the African continent are relatively few but show that the isolates have diverse *spa* types some of which were also detected in the present study such as spa type t084 (Fall et al., 2012, Momoh et al., 2018).

WGS produces huge data that has the potential to provide great insight into the genomic characteristics and epidemiology of pathogens that may not be easily obtained with single typing methods. In the present study, the *S. aureus* analysed using WGS, revealed that two of the isolates belonged to the typical LA-SA lineages that is

ST753 of the clonal complex CC398 and ST9. ST753 and its close allies ST398 are most predominant in Europe and are reported in varying prevalences in other parts of the world including the US, Canada, Australia, South America and Asia (Voss et al., 2005, Van Loo et al., 2007, Khanna et al., 2008, Golding et al., 2010, Arriola et al., 2011, van Cleef et al., 2011, Lim et al., 2012, Groves et al., 2014, Sahibzada et al., 2017, Ahmad-Mansour et al., 2021). ST9 is common in Asia (Tegegne et al., 2017). Notably, four of the six isolates were of novel ST lineages. Therefore, the presence of novel STs may entail a more diverse genetic composition of *S. aureus* in Zambia. Additionally, these isolates had the same *spa* types by both *spa* type by sanger sequencing and WGS that is t1430 which is a typical livestock associated spa type. Previous studies on the African continent have reported the presence of ST398 (*spa* type t011) in *S. aureus* from pigs in South Africa and Cameron (Founou et al., 2019). Founou and others did comment that there finding signified the presence of LA-MRSA in Africa and bemoaned possible underreporting of these strains (Founou et al., 2019). Given that only a few isolates were analysed in the present study, we agree that there is need for more genomic surveillance in Africa. Remarkably, phylogenetic analysis showed that the isolates from farm 3 (analysed by WGS) closely clustered together. Additionally, all the five isolates had similar AMR, virulence genes and MGEs (associated with these AMR and virulence genes) implying clonal relatedness as well as a possibility of horizontal gene transfer between the isolates and indeed transmission from either the animals to humans or vice versa. However, as the current study design is not able to determine the transmission direction, future studies could explore this further.

5.3 Recommendations

Our study has revealed the presence of typical LA-SA in the pig and pork production systems in Zambia. Future studies could investigate risk assessment as well as investigate the presence of *S. aureus* in ready to eat foods of animal origin and the environment. Further, studies looking into the presence of LA-SA as a cause of disease among hospitalised patients in Zambia and indeed Africa at large are needed as this has not been reported yet but have a large impact on epidemiology of *S. aureus* infections. Furthermore, studies looking into the direction of transmission of *S. aureus* between the animals and humans could be helpful in strengthening infection prevention policies.

5.4 Conclusion

Although, MRSA was only phenotypically detected, the significance of MSSA as a potential source from which MRSA can arise cannot be overlooked. The presence of several resistance and virulence genes including plasmid mediated resistance genes and immune evasion genes among the isolates is worrisome as this denotes that the isolates are not only pathogenic but also carry resistance genes, subsequent infections with such strain may result in probable severe and difficult to treat infections. Furthermore, since the WGS analysis of the representative isolates revealed the presence of many MGEs associated with resistance and virulence genes which means that these genes can be easily shared between *S. aureus* and indeed other bacterial species. Additionally, the observed clonal similarity between human and pig associated *S. aureus* isolates and the presence of immune evasion genes in some pig associated *S. aureus* isolates observed in the study indicate potential transmissibility of the isolates between the pigs and humans in either a zoonotic or indeed zooanthroponotic manner. Further studies are needed to delineate this. We recommend continuous monitoring of *S. aureus* in this sector using a "One Health" approach to combat *S. aureus* infections and AMR in Zambia.

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Appendices

Appendix A Consent Forms and Information Sheets

Farm owner/ Farmworker/ Abattoir Manager/ Abattoir Worker Consent Form
(Please tick appropriate designation)

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature (**or thumbprint/mark)** on this form means:

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to participate in this study.
- You are free to withdraw or stop the study at any time.
- You are free to skip questions you may deem personal or otherwise.

Please indicate Yes or No

- I agree to be interviewed ☐Yes ☐No
- I agree to allow my hand or nasal samples to be used in this study ☐Yes ☐No
- I agree to my farm to be included in the study . ☐Yes ☐No
- I agree to my abattoir to be included in the study . ☐Yes ☐No

Name of participant: _____

_____ _____

Signature of participant or Thumb print Date

In signing here, I agree that I have read and understood the agreement/consent form and agree to participate in the study.

Name of Witness: _____

_____ _____

Signature of Witness Date

_____ _____

Signature of recipient of form Date

_____ _____

Signature of Witness Date

The signature of the witness above means that another person has observed the consenting of the participant. The witness must be impartial and not part of the study staff.

Translated Farm owner/ Farmworker/ Abattoir Manager/ Abattoir Worker Consent Form (Please tick appropriate designation)

Mwine Wapulazi/ Anchito Mupulazi / Abattoir Akapitao (Manager) / Anchito Ku Abattoir

Kodi kusaina kwanu papepalayi kapena kuika cala kutanthauza ciani?

Kusaina kwanu kutanthauza:

- Mwauzidwa colingo ca kafukufuku, mundandanda, ubwino wake ndi kuipa kwake.
- Mwapatsidwa danga la kunfunsa mafunso musana saine pepalayi.
- Mwadzipereka pakubvomera kutengako mbali pakafukufuku umeneyu.
- Ndinu ololedwa kucokamo kapena kuleka kafukufuku umeneyu nthawi iliyonse.
- Ndinu omasuka kusiya mafunso yomwe mwaona kwainu siyayenera.

Conde ikani Inde kapena Ayi

- Ndalola kufunsidwa mafunso

  ☐inde ☐ayi
- Ndalola twamukati mwaphuno ndi kumanja anga kutiapime kalombo kamene kabweletsa matenda kulinga ndi kafukufuku uyu

  ☐inde ☐ayi
- Ndabvomera pulazi langa kuikidwa kukafukufuku umeneyu ☐inde ☐ayi
- Ndabvomera kuti abattoir ayikidwemo mukafukufuku ☐inde ☐ayi

Dzina la otengaka mbali: _____

_____

Kusaina kwa otengaka mbali kapena kudinda cala canu

Tsiku_____

Pakusaina, ndabvomera ndawerenga ndi kumvetsetsa pangano yacipepala ndi kutengako mbali kukafukufuku umeneyu.

Dzina la umboni/ oimilira: _____

_____ _____

Kusaina mboni/ oimilira Tsiku

_____ _____

Kusaina kwa olandila pepalayi Tsiku

_____ _____

Kusaina mboni/ oimilira Tsiku

Kusaina kwa mboni/oimilira pamwambapa kutanthauza kuti munthu wina wayendera zokhuza otengako mbali. Mboni ifunika kukhala munthu amene Sali m'modzi mwa akulu a kafukufuku umeneyu.

Information Sheets

Farmer/ Farmworker/Abattoir Worker's Participant Information Sheet

Title of Study: Carriage and Characterisation of *Staphylococcus aureus* from Pigs and Humans in Lusaka Province of Zambia

Principal Investigator: Ms Mulemba Tillika Samutela
Co-Principal Investigators: Prof Bernard Hang'ombe, Dr Geoffrey Kwenda, Dr Edgar Simulundu and Prof Hidaki Higashi

We are conducting a study on a bacteria called *Staphylococcus aureus* from Pigs and Humans in Lusaka Province of Zambia. I would like to ask you for some of your time to explain the work that we are doing and to request for you to be one of the study participants. As we discuss the information below, please feel free to ask any questions.

Brief description of the Study
You may be aware that some germs called bacteria live in different parts of the body e.g., the skin of animals and people. When we come in contact with such animals or people, we may get these germs. *Staphylococcus aureus* is one such germ that can be passed on. It can cause infections which are difficult to treat sometimes.

For this study we are interested in *Staphylococcus aureus from* pigs and people who work on pig farms or in abattoirs where pigs are slaughtered. The information gained from this study will be used to know if the germs found in the pigs are similar to those found in the people who come in contact with the pigs, and the risk factors that contribute to the development of resistant germs.

Voluntary Participation and Withdrawal
Your participation in this study is voluntary. At any time, you may change your mind and choose not to participate, without penalty or loss of benefit whatsoever. You may withdraw from the study at any time you like. If you do not join the study, you will not be victimised in any way.

Furthermore, if you agree to take part in this study, we will ask a number of questions concerning you and your household. You will also be at liberty to answer all questions but should there be any questions that you feel uncomfortable with, you will be under no obligation to answer it. The questions that will be asked are general and not personal. This exercise will take about 20-30 minutes.

Risks

In addition, you will be asked if you will allow us to collect a swab from your nostrils and hands to test for the germs which cause infections as part of the study. The process of obtaining the samples is safe and will not cause you any pain. However, you may experience some discomfort that is associated with obtaining the nasal swabs.

Benefits

You will not directly benefit from this study. The information we collect will be used to try to stop the germs from being passed from animals to people.

Payment and Costs

You will receive no payment for participating in the study neither will you be required to pay anything in order to participate.

Confidentially

This study will be carried out in strict confidence, and you will not be required to give your name. The information that will be collected in this study will be confidential and will be used in a general manner and not necessarily linked to particular individuals. For example, names will not be used instead we will use study numbers. Once I/ we have completed the testing for this study we will not store your sample for testing in the future.

Contact Details

If you have any questions, complaints, or problems as a result of participating in this study, you may contact the PI: Ms. Mulemba Tillika Samutela the principal investigator at mobile +260 965621307 or email: mulembats@gmail.com; mulemba.samutela@unza.zm of Lot 32107/M Off Great North Road, Lusaka. All

research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, UNZABREC office at Telephone: 260-1-256067 or E-mail: unzarec@unza.zm or visit the office found at The University of Zambia Ridgeway Campus, Nationalist Road, Lusaka.

Translated Farmer/ Farmworker/Abattoir Worker's Participant Information Sheet

Mulimi/ Cikumbe/ Osewenza Mupulanzi/ Abattoir Anchito Odzipeleka Kapena Kugwapo Pa Uthenga Uyu

Mutu Wakafukufuku: Carriage ndi Characterisation of *Staphylococcus aureus* kucokera mu khumba ndi anthu muno Mudera ya la Lusaka mu Zambia

Oyanganila Ndi: Ms Mulemba Tillika Samutela
Oyanganila Enaomuleali Akulu Ndi: Aprofesa Bernard Hang'ombe, adotolo Edgar Simulundu, adotolo Geoffrey Kwenda ndi Aprofesa Hidaki Higashi

Tili kufufuzafufuza pakafufuku wa *Staphylococcus aureus* kucokera kunkumba ndi anthu muno mulusaka. Nimpemphako kuti munganipaseko kanthawi kotinifotokoze nchito yomweticita, ndiposo ndikupemphani kuti imwe mukhala amodzi mwaiwo otengako mbala pakafufuku omwetilinawo ise. Pomwe tikuffotokoza pa zauthenga womwe walembedwa pansi apa nkhalani omasuka kunfunsa mafuso yali onse yomwe mufuna imwe.

Kufotokoza Mwadule Pankhani Yanthu Yakafukufuku
Mungakhale odziwa kuti tuzilombo twina tumakhala mbali zanthupi zosiyana-siyana mukati mwanthupi, mwacizitsanzo kuzikumba kwa nyama ndi anthu omwe. Ngati takhala okhudzana ndi nyama kapena anthu omwe ali ndi tulombo utu tingatengeko ngati tilipafupi nao. Kalumbo kaStaphylococcus kapena kanthata aka ndikamodzi komuse kangapitendipo kangabweretse matenda yomwe yangakange kucilitsidwa nthawi zina.

Kukafukufuku uyu takhala cabe ndimbaliyaka nthata ka *Staphylococcus aureus* kucokera Kunkhumba ndi anthu omwe aweta ziweto izi. Tinga tengatenge kalombo aka ku anthu amene akhala mumapulazi kapena anthu amene akupha mkhumba. Uthenga uyu omwe udza tengedwa apa ndiofuna kudziwa ngati kalinganandi komwe kapezeka mu anthu amene amakhudzana ndi nkhumba, ndiponso ndi kuipa kwake komwe kumabwera kucokera kwa kanthata kapena kalombo aka. Tifunanso kudziwa

ngati kalombo aka kapezekanso ku anthu odwala amene ali muzipatala omwe akhala pafupi ndi mapulazi yazi weto zankhumba ngati alinako kalombo.

Kukwapo Kwakutengako Mbali Ndikwa Ulere Ndi Kucokamo

Kugwapo kwa kafukufuku uyu ndi kwaulere kopanda malipiro yaliyonse, panthawi iliyonse mungaleke kutengako mbali kapena kusintha manganizo yanu kusatengako mbali, kopanda ukulipilitsani ciliconse kapena cina ciliconse cabwino. Mungaleke kutengako mbali panthawi ili yonse mungafune kwa imwe ene ake. Ngati simunatengeko mbali pakafukufuku uyu kulibe cobvuta ciliconse comwe mungakherenaco ai.

Poikilapo, ngati mwakhala ocilora kutengako mbali kukafukufuku uyu, tidzakufunsakoni kuli imwe ndi pambanja yanu. Ndipo mudzakhala ndi ufulu oyankha mafunso yomwe muzafunidwa ngati pali mafunso ena omwe adzakhala obvuta palibe ciliconse comwe cidzakhala cobvuta kuli imwe. Nchito yonse yomwe tidzakhalanayo pakati pamphindi zikwanira makumi awiri (20) minutes kapena makumi atatu (30) minutes. Mafunso yomwe mudzafunsidza yadzakhala cabe mbali yomwe tabwerela osati ya munthu wina ai.

Kuipa Kwake

Kuikilapo /kuonjezera, mudzafunsidwa ngati munga cilole kutongako tuusako twamukati mwaphuno yanu kapena kumanja kwanu kuti tipime kalombo kamene kabweletsa matenda kulinga ndi kafukufuku uyu. Njira zotengelamo zomwe tifuna palibe zina zilizonse zomwe munza mvera kuwawa aikwa inu ngati titenga kucokera kuziwalo zanthupi zomwe takamba kale. Motero munzamverako twina tosamvekako bwino pang'ono tomwetuli ku ndi mbali kuti usako twa mphuno.

Ubwino

Simudzakhala ukhudzidwa ku ubwino wakafukufuku uyu. Uthenga womwe tidzakambapo udzatengedwa ndikusewenzesedwa kaleketsa kalombo aka kanthata kupita kumunthu kapena kunyama.

Mutengo Ndi Kalipilidwe

Simudzatenga ciliconse pakugwapokwa kafukufuku uyu kapena kulipila ciliconse ai. Kungwapo ndikwa ulele.

Mwakudzipeleka Mwacinsinsi

Kafukufuku uyu udzatengadwa mwaceru ndipo simulodwa kutipatsa zamaina yanu ai. Uthenga wamene udzatengedwa pakafukufuku uyu mwawakudzipile kakudzasewenzedwa mwanjirayo funikira ndiponso osati kudwiwika kwamunthu wina wace mwayekha. Mwacitsanzo, dzina, kapena maina siyasewenzesedwa, tizasewetsanamba la yanu yakafukufuku. Ngati tatsiliza kupima zomwetiza tenga kuli imwe sitidzakhalanso ndimpata wakuti tingansewenzetso kutsongolo ai.

Momwemungagwilizane Nao

Ngati mulindifunso iliyonse, zobvuta kapena zonunzitsa kapena zotuluka lake lakafukufuku wakugwapo mungatume kwa Ms Mulemba Tillika Samutela oyanganila kwanambala iyi +260965621307 kapena ku emelo: mulembats@gmail.com; mulemba.samutela@unza.zm kapena kuyumba yao pa plot Lot 32107/M pafupi ndinjira yakumpoto (Great North Road) muno mulusaka. Zonse zofunika kapena zofuna kucokerakwa azoyangila odzipeleka ndi kabungwe kanchito yoteteza zamaufulu ndiumbwinowake. Ngati mulindifunso kapena mandaulo pa ufulu, kukhala ngati otengako mbali pakafukufuku umenewu mungagwilizane, pazambili ngati mufuna, UNZABREC Ofisi pa nambala +260-1-256067 kapena emelo: unzarec@unza.zm kapena kufi ku ofisi yamwe ipezeka pa sukulu yapamwamba ya Zambia, Ridgeway Campus, Nationalist Road, muno mulusaka.

Appendix B Ethical Clearance Letters

UNZABREC Ethics Approval Letter

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

21st January, 2020.

Your **REF. No. 613-2019**.

Ms. Mulemba, Tillika Samutela,
Ministry of Health,
Department of National Malaria Elimination Centre,
Lusaka.

Dear Ms. Samutela,

RE: "CARRIAGE AND CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS FROM PIGS AND HUMANS IN LUSAKA PROVINCE OF ZAMBIA." (REF. NO. 613-2019)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 20th January, 2020. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

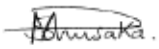
APPROVAL NUMBER : REF. 613-2019

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 20th January 2020**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 19th January 2021**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.

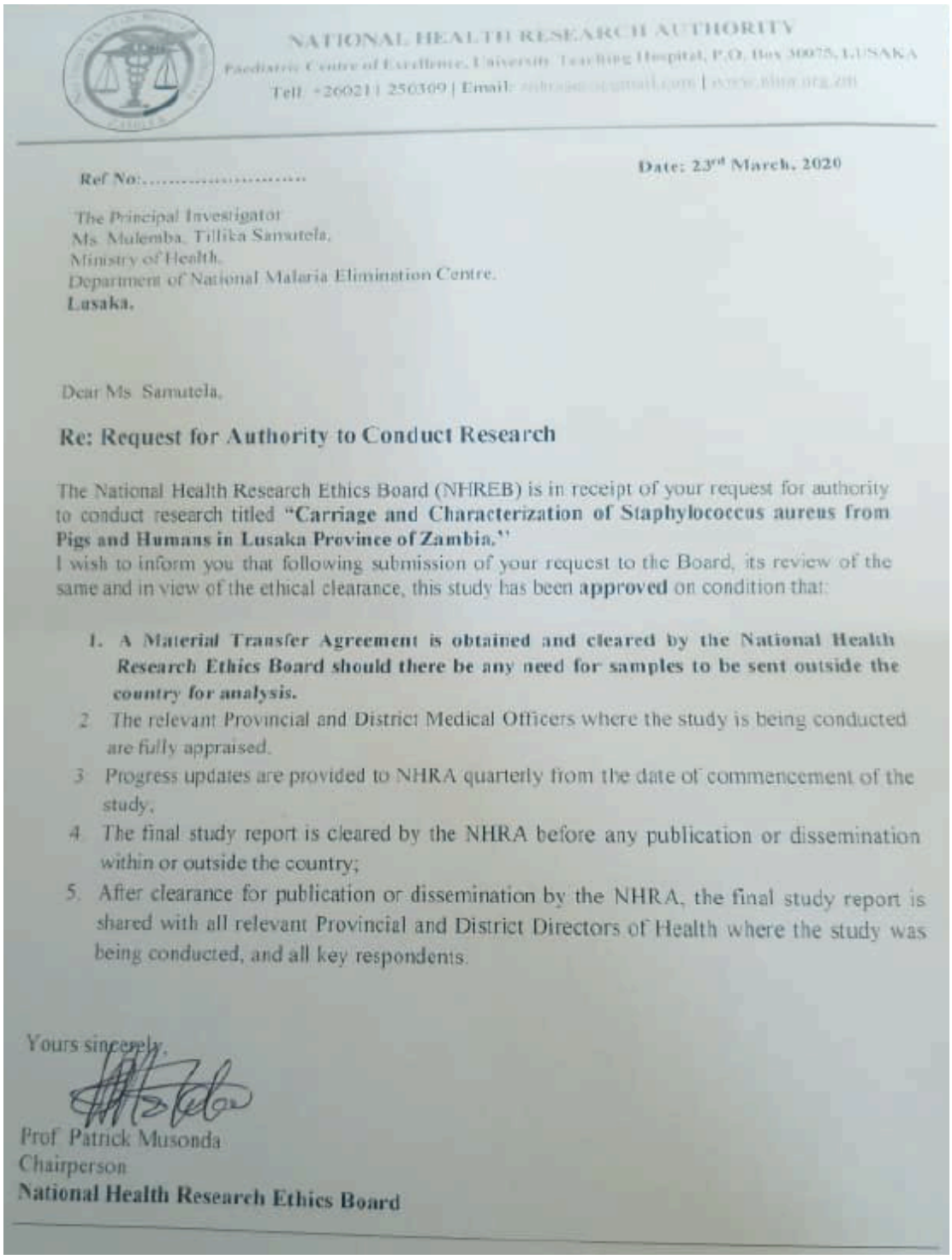
- **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No.256067 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You're also required to submit electronic copies of your publications in peer-reviewed journals that may Emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,



Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

NHRA Approval Letter



NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: [illegible] | www.nhra.org.zm

Ref No:.....

Date: 23rd March, 2020

The Principal Investigator
Ms. Mulemba. Tillika Samutela.
Ministry of Health.
Department of National Malaria Elimination Centre.
Lusaka.

Dear Ms Samutela,

Re: Request for Authority to Conduct Research

The National Health Research Ethics Board (NHREB) is in receipt of your request for authority to conduct research titled **"Carriage and Characterization of Staphylococcus aureus from Pigs and Humans in Lusaka Province of Zambia."**
I wish to inform you that following submission of your request to the Board, its review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. **A Material Transfer Agreement is obtained and cleared by the National Health Research Ethics Board should there be any need for samples to be sent outside the country for analysis.**
2. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised.
3. Progress updates are provided to NHRA quarterly from the date of commencement of the study.
4. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
5. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Prof. Patrick Musonda
Chairperson
National Health Research Ethics Board

Appendix C Characteristics of Samples for Which More than One *S. aureus* Colony Type was Isolated

Some samples yielded more than one colony morphology of *S. aureus* (*n*=27) on Baird Parker agar in the Table 1C below. These isolates were not included in the calculation of the prevalences but were further characterized.

Table 1C: Characteristics of Samples for Which More than One *S. aureus* Colony Type was Isolated

Study No.	District	Study Site	Type of Facility	Species	Sample Type	*S. aureus* Isolation
P4-4-1	Lusaka	Farm 4	Small Scale	Pig	Pig nasal swab	Yes
P4-8-1	Lusaka	Farm 4	Small Scale	Pig	Pig nasal swab	Yes
P5-14-1	Lusaka	Farm 5	Small Scale	Pig	Pig nasal swab	Yes
P6-8-1	Chongwe	Farm 6	Medium Scale	Pig	Pig nasal swab	Yes
P7-3-1	Chongwe	Farm 7	Medium Scale	Pig	Pig nasal swab	Yes
P10-4-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-10-1-1-2	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-10-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-7-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-6-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-17-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-23-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-26-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-9-1	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
P10-26-2	Chongwe	Farm 10	Medium Scale	Pig	Pig nasal swab	Yes
A1-6-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes

Table 1C Continued: Characteristic of Samples for Which More Than One *S. aureus* Colony Type was Isolated

Study No.	District	Study Site	Type of Facility	Species	Sample Type	*S. aureus* Isolation
A1-7-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes
A1-12-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes
A1-13-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes
A1-22-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes
AH1-1-1	Chilanga	Abattoir 1	Large Scale	Pig	Pig nasal swab	Yes
P11-20-1	Lusaka	Farm 11	Large Scale	Pig	Pig nasal swab	Yes
P11-42-1	Lusaka	Farm 11	Large Scale	Pig	Pig nasal swab	Yes
P11-51-1	Lusaka	Farm 11	Large Scale	Pig	Pig nasal swab	Yes
P12-13-1	Lusaka	Farm 12	Large Scale	Pig	Pig nasal swab	Yes
P13-14-1	Lusaka	Farm 13	Large Scale	Pig	Pig nasal swab	Yes
P13-16-1	Lusaka	Farm 13	Large Scale	Pig	Pig nasal swab	Yes

Appendix D List of Publications and Presentations

The following manuscripts, based on this thesis, have been published and or prepared for publication

1. Samutela, M.T., Kwenda, G., Simulundu, E., Nkhoma, P., Higashi, H., Frey, A., Bates, M. and Hang'ombe, B.M. (2021). Pigs as a potential source of emerging livestock-associated *Staphylococcus aureus* in Africa: a systematic review. *International Journal of Infectious Diseases*, *109*, pp.38-49.
2. Samutela, M.T.; Phiri, B.S.J.; Simulundu, E.; Kwenda, G.; Moonga, L.; Bwalya, E.C.; Muleya, W.; Nyirahabimana, T.; Yamba, K.; Kainga, H.; Kallu, S.A.; Mwape, I.; Frey, A.; Bates, M.; Higashi, H.; Hang'ombe, B.M. (2022). Antimicrobial Susceptibility Profiles and Molecular Characterisation of *Staphylococcus aureus* from Pigs and Workers at Farms and Abattoirs in Zambia. *Antibiotics*, *11*, 844. https://doi.org/10.3390/antibiotics11070844
3. Samutela, M.T.; Hang'ombe, B.M.; Simulundu, E.; Kwenda, G.; Mwenda, M.M.; Yamba, K.; Mutalange, M.; Tembo, J.; Bates, M. and Frey, A. Detection of the Serine Proteases-like (*spl*) Genes in *Staphylococcus aureus* from Pigs and Humans in Lusaka Province of Zambia. (In draft form)

Part of this work has been presented at the following scientific meetings

1. Samutela, M.T.; Kwenda, G.; Simulundu, E.; Phiri, B.S.J.; Moonga, L.; Bwalya, E.C.; Muleya, W.; Yamba, K.; Frey, A.; Bates, M.; Higashi, H.; Hang'ombe, B.M. Antimicrobial Susceptibility Profiles and Spa Types Distribution of Staphylococcus aureus from Pigs and Pig Farm and Abattoir Workers in Zambia. Poster presentation presented at the 10th EDCTP Forum held from 17 to 21 October 2021, in Maputo Mozambique and Virtual.

Appendix E Reprint of Published Articles

International Journal of Infectious Diseases 109 (2021) 38–49

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journal homepage: www.elsevier.com/locate/ijid

Review

Pigs as a potential source of emerging livestock-associated *Staphylococcus aureus* in Africa: a systematic review

Mulemba Tillika Samutela[a,1,*], Geoffrey Kwenda[b], Edgar Simulundu[c,2], Panji Nkhoma[b], Hideaki Higashi[d], Andrew Frey[e], Matthew Bates[f], Bernard M. Hang'ombe[a]

[a] Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[b] Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia
[c] Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[d] Division of Infection and Immunity, Research Centre for Zoonosis Control, Hokkaido University, Sapporo, Japan
[e] Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, USA
[f] School of Life Sciences, University of Lincoln, Lincoln, UK

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ABSTRACT

Objective: To assess the emergence of livestock-associated *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) in the pig and pork production systems in Africa for the past two decades.

Methods: PubMed and African Journals OnLine were searched for relevant primary studies from 2000 to 2019 using standardized key words. In total, 19 eligible articles were included in this review.

Results: The prevalence of *S. aureus* including MRSA ranged from 0% to 55% among live pigs and raw pork, and from 9.4% to 30.8% among pig farm and abattoir workers. Risk factors associated with *S. aureus* carriage among workers were: male gender, working in an abattoir, and medical-related occupation of a household member. *S. aureus* and MRSA from pigs and pork production systems in Africa are potentially pathogenic with diverse *spa* types and clonal complexes, with genes encoding antimicrobial resistance, heavy metal resistance, and virulence factors including secreted and enterotoxins, proteases and immune evasion cluster. The typical livestock-associated *S. aureus* CC398 and *mecC* genes were reported in two studies.

Conclusion: Pigs are a potential source of the emerging livestock-associated *S. aureus* in Africa. Continued monitoring using a 'One Health' approach is recommended for effective infection prevention and control of these infections in Africa.

Introduction

Staphylococcus aureus is one of the most clinically important multi-drug-resistant threats worldwide, according to the global priority pathogens list of antibiotic-resistant bacteria (WHO, 2017). *S. aureus*, a Gram-positive coccus, is an opportunistic pathogen found in both humans and animals that causes a wide range of diseases, including skin and soft tissue infections, infective endocarditis and toxic shock syndrome. These infections are associated with high morbidity, mortality and considerable economic impact (Köck et al., 2010). *S. aureus* virulence factors vary in their conservation between strains, and can be classified as toxins, proteases, adhesins, and microbial surface component recognizing adhesive matrix molecules.

The medical importance of *S. aureus* has been heightened by its ability to adapt rapidly to the selective pressure of antibiotics, and the resultant emergence and spread of methicillin-resistant *S. aureus* (MRSA). Methicillin resistance is mainly due to the acquisition of genes encoding a unique penicillin-binding protein (PBP2a). PBP2a has decreased affinity for β- lactam antibiotics, and catal-

* Corresponding author. Address: Department of Biomedical Sciences, School of Health Sciences, University of Zambia, P.O. Box 50110, Lusaka, Zambia. Tel.: +260965621307.
E-mail address: mulembats@gmail.com (M.T. Samutela).
[1] Permanent address: Department of Biomedical Sciences, School of Health Sciences, University of Zambia
[2] Present address: Macha Research Trust, Choma, Zambia

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yses effective cell wall synthesis even in the presence of these antibiotics. The *mecA* gene which encodes PBP2a is carried on a mobile element called the 'staphylococcal chromosome cassette *mec*' (SCC*mec*) which has several subtypes and variants. Currently, 13 subtypes (SCC*mec* type I-XIII) are recognized (Lakhundi and Zhang, 2018). Recently, *mecC*, a divergent homologue of *mecA*, was detected in Europe and is mainly associated with MRSA from livestock and wild animals, especially hedgehogs (Paterson et al., 2012; Monecke et al., 2013).

Previously, *S. aureus* including MRSA was known to be a major cause of nosocomial infections. It is now prevalent in the community, and has recently emerged among livestock, pets and wildlife (Cuny et al., 2015). It has been reported that pigs are frequently colonized by *S. aureus*, and they are recognized as a main reservoir for MRSA. These strains can be transmitted to people in contact with these animals (Grøntvedt et al., 2016). Notably, livestock-associated MRSA has been isolated from people without contact with animals, demonstrating the ability of these organisms to thrive in humans (Anker et al., 2018). This cross-transmission between animals and humans poses a considerable zoonotic threat and complicates treatment protocols. Based on DNA sequencing methods for typing *S. aureus* strains – *spa* typing and multi-locus sequence typing (MLST) – diverse *spa* types (t) and sequence types (STs) or clonal complexes (CCs) have been determined globally. Some types are commonly associated with livestock-associated *S. aureus*. Typically, livestock-associated *S. aureus* from pigs has been associated with CC398 in Europe, while CC9 is the predominant type in Asia (Tegegne et al., 2017). *Spa* types t011, t034, t108, t567, t571, t899, t1254, t1451, t2011 and t2510 are associated with CC398, and are among those more closely linked to livestock-associated *S. aureus* (Smith and Pearson, 2011).

Reports of livestock-associated *S. aureus* in pigs were first reported in the early 2000s in France and the Netherlands (Armand-Lefevre et al., 2005; Voss et al., 2005). Over the years, the presence of pig-related *S. aureus*, especially MRSA, has been reported in Europe, Asia, America and Australia (van Cleef et al., 2011; Groves et al., 2014; Chuang and Huang, 2015). Although initial reports involved isolates from separate, non-related collections, subsequent reports demonstrated the possibility of transmission of MRSA between pigs and pig farmers and their families, and between a nurse and a patient (Voss et al., 2005). A more recent study using whole-genome sequencing (WGS) data showed host adaptation and transmission from pigs into healthcare institutions in Denmark (Larsen et al., 2017). This underscores the need to monitor the emergence and spread of livestock-associated *S. aureus* strains. Unfortunately, studies on the presence of *S. aureus*, both methicillin-susceptible (MSSA) and methicillin-resistant strains, in animals and foods of animal origin in Africa are limited, and have only been undertaken in a few countries (Lozano et al., 2016). The lack of research on livestock-associated *S. aureus* in African countries is of great concern considering the potential pathogenicity of these strains.

Pork is one of the main protein sources for humans in many countries. However, pork has been reported to be a vehicle for the spread of pathogens that cause foodborne illnesses due to contamination during the handling process from the farm to the table. *S. aureus* is a potential pathogen that has been implicated as one of the major causes of foodborne diseases globally (Wu et al., 2016). A common staphylococcal foodborne disease is food poisoning resulting from ingestion of staphylococcal enterotoxins expressed by enterotoxigenic strains of *Staphylococcus* spp. (Kadariya et al., 2014). For food safety, it is thus important to study the presence of such strains in pork production systems.

Therefore, the aim of this systematic review was to assess the emergence of livestock-associated *S. aureus* including MRSA in the pig and pork production systems in Africa for the past two decades. The prevalence, antimicrobial susceptibility and genotypes of *S. aureus* of porcine origin have been reported, as well as the prevalence of *S. aureus* among pig farm and abattoir workers in Africa, and the risk factors associated with *S. aureus* carriage among such workers.

Methods

Information sources and search strategies

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2010). PubMed and African Journals OnLine were searched systematically to identify eligible studies published between 1 January 2000 and 31 December 2019. Studies were identified using the following keywords in the titles, abstracts or medical subject headings with the help of Boolean operators (AND, OR): *Staphylococcus aureus*, pig, porcine, swine, abattoir, slaughterhouse, antimicrobial resistance; abattoir worker, farm worker, livestock and MRSA. The last search date was 10 January 2020. In total, 5424 articles were identified. Furthermore, the reference lists of the identified studies were checked for additional studies. All the articles were stored in EndNote X9 (Thomson Reuters, New York, USA).

Eligibility criteria

The titles and abstracts of identified studies were screened and reviewed for eligibility based on the following inclusion criteria: English language; full-text journal article published between 2000 and 2019; any study design except experimental; and conducted in an African country. Studies were excluded if the animal source of the isolates was not listed, and if *S. aureus* was not specifically mentioned as one of the isolates or the results only mentioned '*Staphylococcus* spp.'.

Study quality

Eligible studies were assessed for quality of reporting and selecting for bias using the McMaster critical appraisal tools for quantitative studies and qualitative studies (Ducat and Kumar, 2015).

Data extraction process

The following information was extracted where possible: authors; study design; study setting or location; number of *S. aureus* isolates and proportion of MRSA and/or MSSA isolates; type of specimen or focal infection; method employed for detection; antimicrobial susceptibility profiles; and molecular types/genotypes of the *S. aureus* isolates. Risk factors associated with carriage of livestock-associated *S. aureus* were also extracted.

Data analysis

Data were entered in Microsoft Excel and analysed using Python 3.8 for Mac. As data were non-parametric, the Mann–Whitney *U*-test was used to compare the prevalence of *S. aureus* between the different animal species.

Results

Characteristics of eligible studies

In total, 19 articles (Figure 1) from six countries (Figure 2) were deemed relevant and included in this review. Only two of the articles reported multi-centre studies involving two countries. The

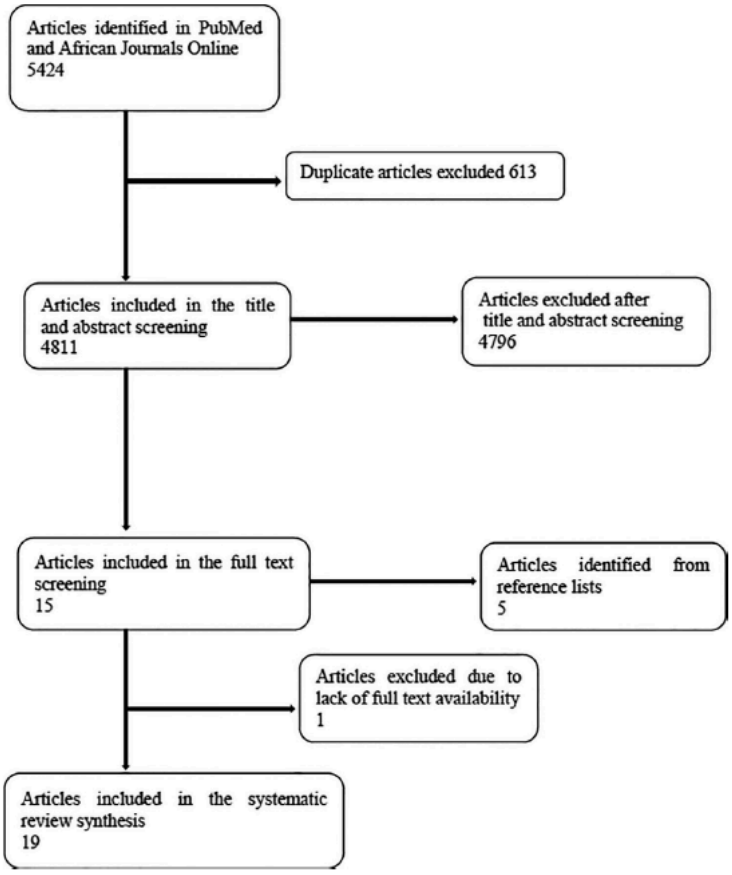

Figure 1. Flow diagram of the study selection process for inclusion in the review.

other 17 articles reported studies from a single country, with most studies being conducted in Nigeria and South Africa. Table S1 (see online supplementary material) shows an overview of the characteristics of the eligible studies.

Prevalence of S. aureus among live pigs, pork, and pig farm and abattoir workers in Africa

Eleven studies sampled live pigs, while five studies sampled pork and/or carcasses. The prevalence of *S. aureus* ranged from 0% to 55% among live pigs and from 0% to 53.9% in pork (Table 1). Most of the studies reported relatively low to moderately high prevalence rates of *S. aureus* among live pigs, ranging from 3.7% to 25% (Table 1). Most studies did not set out to detect *S. aureus* but rather *Staphylococcus* spp. in general and/or other bacterial species, while some of the studies sampled other animal species more than pigs (Table S1, see online supplementary material). Two studies that targeted *S. aureus* as the pathogen of interest reported very high prevalence rates of 43.2% (Okunlola and Ayandele, 2015) and 55% (Dweba et al., 2019). The sample sizes were also considerably larger in these two studies than in the studies that reported lower prevalence rates. There was a marked difference in the prevalence of *S. aureus* from pork samples in the two South African studies reviewed: 14% and 31.5% (Tanih et al., 2015; Pekana and Green, 2018). Conversely, the prevalence rates of *S. aureus* among pork samples from the studies conducted in Nigeria were very high: 35% and 53% (Igbinosa et al., 2016; Adikwu et al., 2019).

Eight studies sampled humans as well as pigs or pig carcasses, and mainly involved either pig farm or abattoir workers (a few studies sampled both) (Table 1). The prevalence of *S. aureus* among pig farm or abattoir workers ranged from 0% to 30.8%. Apart from one of the seven studies which reported a high prevalence rate (30.8%), most studies reported relatively low prevalence rates for *S. aureus* (<13.5%) among workers. Two studies did not detect any *S. aureus* among abattoir workers (Table 1).

Prevalence of porcine-related MRSA in Africa

Twelve studies recorded the presence of MRSA among *S. aureus* isolates, with prevalence rates of approximately 10% to 100% (Table 1). In a number of studies, it was difficult to deduce the proportion of MRSA isolates because this was either not detected or not reported. Notably, most studies used more than one method

Table 1
Prevalence of porcine-related *Staphylococcus aureus* in Africa.

Reference	Study country	Sample type	Method of detection	*S. aureus* prevalence		
				Pigs	Humans with contact with pigs	*S. aureus* type (MRSA vs MSSA)
Adikwu et al. (2019)	Nigeria	Hand, water, meat and carcass swabs	Phenotypic, serological	35% (50/200)	9.4% (3/32)	Not indicated
Igbinosa and Beshiru (2019)	Nigeria	Nasal and rectal	Phenotypic, molecular	14.9% 13/27	Not studied	MRSA
Nwaogaraku et al. (2019)	Nigeria	Blood	Phenotypic, molecular	25% (25/100)	Not studied	44% (11/25) MRSA
Dweba et al. (2019)	South Africa	Oral, faecal, cloacal and environmental swabs	Phenotypic, molecular (*nuc*)	55% (15/27)	Not studied	MRSA
Otalu et al. (2018)	Nigeria	Nasal	Phenotypic, mass spectrometry	4.7% (20/425)	10.9% (6/55)	MRSA
Momoh et al. (2018)	Nigeria	Nasal	Phenotypic, serological, mass spectrometry	5.3% (16/300)	12.9% (13/101)	MSSA
Van Lochem et al. (2018)	South Africa	Nasal	Phenotypic, serological, mass spectrometry	12%	Not studied	12% MRSA
Pekana and Green (2018)	South Africa	Meat and milk	Phenotypic, molecular (*nuc*)	14% (14/100)	Not studied	50% (7/14) MRSA
Odetokun et al. (2018)	Nigeria	Nasal and surface	Phenotypic, serological molecular (*nuc* and *tuf*)	3.7% (3/8)	13.5% (10.4% MSSA, 3.1% MRSA)	1.2% MRSA, 2.5% MSSA (in pigs)
Founou et al. (2019), Founou et al. (2018)	South Africa and Cameroon	Nasal, rectal and hand	Phenotypic	18.9% (7/37)	0%	100% MRSA (in pigs)
Katakweba et al. (2016)	Tanzania	Nasal	Phenotypic, molecular (*nuc*)	4% (4/100)	0%[a]	4% MSSA (in pigs)
Igbinosa et al. (2016)	Nigeria	Meat	Phenotypic, molecular (nuc and 16rRNA)	53.9% (14/26)	Not studied	100% MRSA
Chairat et al. (2015)	Tunisia	Raw food samples of animal origin	Phenotypic, molecular (*nuc*)	0% (0/1)	Not studied	0%
Okunlola and Ayandele (2015)	Nigeria	Nasal	Phenotypic	43.2% (41/95)	Not studied	43.9% (18/41) MRSA
Tanih et al. (2015)	South Africa	Rump, flank, brisket and neck swabs	Phenotypic, serological	31.5% (20/64)	Not studied	31.5% (20/20) MRSA (in pigs)
Udegbunam et al. (2014)	Nigeria	Ocular swabs	Phenotypic	0% (0/2)	Not studied	0%
Adegoke and Okoh (2014)	South Africa	Nasal, mouth wash and ear	Phenotypic	23.3% (28/120)	Not studied	12.6% MRSA (in pigs)
Fall et al. (2012)	Senegal	Nasal	Phenotypic	12.3% (57/464)	30.8% (16/52)	10.5% (6/57) (in pigs)

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2
Comparison of the prevalence of *Staphylococcus aureus* in pigs, other animal species and humans in Africa.

Animal species	*n*	Mean	SD	Median	Minimum	Maximum
Pigs alone	18	19.82	17.6	14.45	0.0	55
Other animals	18	17.24	25.51	3.25	0.0	83.3
Humans with contact with pigs	18	4.31	8.3	0.0	0.0	30.8
Humans without contact with pigs	18	1.56	5.26	0.0	0.0	22

SD, standard deviation.

to identify *S. aureus* and detect methicillin resistance among the isolates (Table 1).

Overall, a Mann–Whitney *U*-test indicated that the prevalence rate of *S. aureus* was significantly higher in pigs (median 14.45) than in humans with or without contact [median 0.0 (U=54.0, P<0.001); median 0.0 (U=33.0, P<0.001), respectively] (Table 2). The prevalence rate of *S. aureus* in other animals was also significantly higher (median 3.25) compared with humans without animal contact (median 0.0) (U=93.0, P=0.0039). However, there was no significant difference between other animals (median 14.45) and humans with animal contact (median 0.0) (U=117.5, P=0.056). The Mann–Whitney *U*-test was also performed to determine the difference in prevalence of *S. aureus* between humans with contact and those without contact with animals, and between pigs and other animals; no significant difference was found between humans with contact (median 0.0) and those without contact (median 0.0) (U=134.0, P=0.1). In addition, no significant difference was found between pigs (median 14.45) and other animals (median 3.25) (U=118.5, P=0.084).

Figure 2. Geographical distribution of studies on *Staphylococcus aureus* in pigs on the African continent. Red, countries that have reported the presence of *S. aureus* in pigs; grey, countries without any reports on the presence of *S. aureus* in pigs; white, water bodies.

Antimicrobial and heavy metal susceptibility patterns of porcine-related S. aureus

For the purposes of this review, analysis of the data was done for the anti-staphylococcal drugs commonly used for treatment (or testing) of infections in humans and animals. Following this criterion, 10 studies were included in this synthesis; notably only one of these 10 studies reported the susceptibility patterns of isolates from humans who had contact with pigs (Table 3). Table S2 (see online supplementary material) shows the general overview of the antimicrobial susceptibility data from all reviewed studies. The number of antibiotics tested ranged from one to 21 antibiotics. Most studies employed the disc diffusion method, while two used the Sensititre system and the other two used the Vitek 2 system to determine antimicrobial susceptibility (Table S2, see online supplementary material).

Antimicrobial susceptibility profiles

Five of the six studies that reported susceptibility profiles for *S. aureus* against penicillin recorded high resistance of approximately 97–100%, while the lowest and highest rates of cefoxitin resistance were 10.5% and 44%, respectively (Table 3). All 10 studies reported susceptibility of *S. aureus* isolates to oxacillin: three studies reported 100% susceptibility, while six studies reported different levels of oxacillin resistance, with the lowest at 43.9% (Table 3). Three of seven studies showed that the isolates were 100% sensitive to trimethoprim-sulfamethoxazole (Table 3). In total, eight studies reported sensitivity to gentamicin, five of which reported 100% sensitivity. Two studies tested amikacin, and all isolates were susceptible. Seven of 10 studies reported sensitivity for erythromycin, five of which reported resistance ranging from 12.5% to 80%. Clindamycin susceptibility was reported in six studies, four of which recorded resistance ranging from 17% to 80%. Tetracycline and/or doxycycline susceptibility profiles were recorded in seven studies, three of which showed resistance to these antibiotics ranging from 18% to 100% (Table 3).

Chloramphenicol susceptibility profiles were reported in three studies, two of which reported 92.9% and 100% sensitivity. Vancomycin was tested in three studies, all of which recorded 100% sensitivity. Three of four studies recorded 100% sensitivity results for ciprofloxacin. Two studies reported susceptibility to linezolid with resistance rates of 4% and 14.3% respectively. Only one study reported susceptibility to mupiriron, with resistance of 3.7%. Fusidic acid was tested in one study and all isolates were susceptible. Two studies reported resistance to more than one category of antibiotics namely six and seven antibiotics, respectively. The susceptibility profiles of *S. aureus* from workers showed that they were all susceptible to cefoxitin, vancomycin, gentamicin and fusidic acid, but resistant to penicillin (100%), trimethoprim-sulfamethoxazole (25%) and erythromycin (12.5%) (Table 3).

Susceptibility to heavy metals

Only one study tested for heavy metal resistance in the isolates. They found high resistance rates to cadmium, copper, lead and zinc at 1500 µg/mL concentration (Table 3). However, these results were not separated by animal species as other animals such as cattle were included in the study; as such, it is not possible to make further comment on this.

Genotypes of porcine-related S. aureus isolates

Twelve studies reported at least one genotypic characteristic of the *S. aureus* isolates (Table 4).

mecA, mecC and SCCmec types

mecA was detected in seven of 10 studies, while *mecC* was detected in 44% of the isolates in one study. Three studies reported SCC*mec* IV, SCC*mec*VIa and SCC*mec*Vc, respectively (Table 4).

Antibiotic and heavy metal resistance genes

The antibiotic resistance genes *mphc, ermA* and *ermB, vanA, vanB, aaC* and *tetK* were reported in at least one of the three studies which tested for these genes (Table 4). Only one study reported the presence of the heavy metal resistance gene *copB* (Table 4).

Spa typing and MLST

Spa types were reported in five of the six studies that conducted *spa* typing (Table 4). Three studies reported a single *spa* type, namely, t1603, t011 and t131. Two studies recorded a more diverse range of *spa* types, with *spa* types t084 and t311 being common between the studies. MLST was conducted in five studies, of which only one study reported the presence of CC398 among the isolates. Other CCs reported included CC5, CC152, CC15, CC97, CC80 and CC88 (Table 4).

Virulence factors

Genes to several virulence factors including the leukotoxins, enterotoxins and proteases were reported in a number of the reviewed studies (Table 4). The Panton–Valentine Leucocidin (PVL) gene *lukSF* was reported in three of four studies. The immune evasion cluster (IEC) gene (*scn*) was detected in two studies. Several enterotoxin genes (*sea, seb, sem, seh, ser, sed, sec* and *sep*) and exfoliative toxin genes (*eta* and *etb*) were detected in five studies. One study detected genes encoding other virulence factors, including *aur* (protease), *hlb, hlgA, hlgB* and *hlgC* (haemolysins), *vWb, clfB, FnbA, fnbB* and *ebpS* (adhesins). The toxic shock syndrome *tst* gene was detected in one study (Table 4).

Table 3
Antimicrobial susceptibility patterns of porcine-related *Staphylococcus aureus* isolates from Africa.

Reference	Fox	P	Sxt	Gen	Amk	Ery	Oxa	Cli	Tet and/or Dox	Chl	Lin	Mup	Van	Cip	Fus
Nwaogaraku et al. (2019)	R (11/25)	NT	NT	NT	NT	NT	R (11/11)	NT	NT	NT	NT	NT	NT	NT	NT
Oralu et al. (2018)	R (100%)	R (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	S (100%)	NT	NT	NT	NT	NT
Momoh et al. (2018)	NT	R (97%)	R (52%)	NT	NT	R (20%)	S (100%)	R (17%)	R (62%)	NT	R (4%)	R (3.4%)	NT	R (5%)	NT
Pekana and Green (2018)	NT	R (7/14)	R (2/14)	R (0/14)	R (0/14)	R (4/14)	R (7/14)	R (8/14)	R (2/14)[a]	R (1/7)	R (2/14)	NT	NT	R (0/14)	NT
Founou et al. (2019), Founou et al. (2018)	R (5/5)	R (5/5)	S (5/5)	S (5/5)	NT	R (4/5)	R (5/5)	R (4/5)	R (5/5)	NT	S (5/5)	S (5/5)	S (5/5)	S (5/5)	NT
Katakweba et al. (2016)	NT	NT	S (100%)	R (25%)	NT	NT	S (100%)	NT	NT	R (25%)	NT	NT	NT	NT	NT
Okunlola and Ayandele (2015)	NT	NT	R (45%)	R (70%)	NT	R (40%)	R (43.9%)	R (60%)	NT	NT	NT	NT	NT	S (100%)	NT
Tanih et al. (2015)	NT	NT	NT	S (100%)	NT	S (100%)	R (100%)	NT	S (88%)[b]	NT	NT	NT	S (100%)	NT	NT
Fall et al. (2012)	R [(10.5%) 6/57] pigs only	R (100%) pigs and humans	R [(54.3%) 31/57] pigs; [(25%) 4/16] humans	S 100% pigs and humans	NT	R [(12.5%) 2/16] humans only	R [(10.5%) 6/57] pigs only	NT	R (18%) pigs; (2%) humans	NT	NT	NT	S (100%) pigs and humans	NT	S (100%) pigs and humans

Fox, cefoxitin; P, penicillin; Sxt, trimethoprim-sulfamethoxazole; Gen, gentamicin; Amk, amikacin; Ery, erythromycin; Oxa, oxacillin; Cli, clindamycin; Tet/ Dox, tetracycline/doxycycline; Chl, chloramphenicol; Lin, linezolid; Mup, mupirocin; Van, vancomycin; Cip, ciprofloxacin; Fus, fusidic acid; NT, not tested; R, resistant; S, susceptible.

[a] Also tested using minocycline.

[b] Oxytetracycline was used.

Table 4
Genotypic characteristics of the *Staphylococcus aureus* isolates.

Reference	Method	*mecA*	*mecC*	SCC*mec* typing	Antibiotic resistance genes	Heavy metal resistance genes	CC398 PCR	*Spa* typing	MLST	WGS similarity	Virulence factors				
											pvl	scn	Enterotoxins and other toxins	*TST*	Other virulence factors
Adikwu et al. (2019)	PCR	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	*sea* (7), *sed* (1)	Not done	Not done
Igbinosa et al. (2016)	Multiplex PCR	100%	Not done	Not done	*mphC*, *ermA*, *ermB*, *vanA*	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done
	PCR	0%	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done
Nwaogaraku et al. (2019)															
Dweba et al. (2019)	Multiplex PCR	0%	Pos (7)	Not done	*aac* (1), *vanB* (7), *tetK* (12)	*copB* (8)	Not done	2	Not done	Not done	100%	Not done	*see* (1), *sea* (11)	Not done	*coa* (0)
Otalu et al. (2018)	Multiplex PCR	100%	Not done	SCCmecVIa	Not done	Not done	CC398 Positive	t1603 (100%)	CC88	Highly similar (putative genes *scn*, *sak*, *lukE*, *lukD*, gamma haemolysin, *aur*, *splA* and *splB*)	Negative	37/38 [(19 porcine, 6 human with contact, 12 human without contact	Not done	Not done	Not done
Momoh et al. (2018)	Multiplex PCR	100%	Not done	Not done	Not done	Not done	Not done	t311 (1), t002(1), t442 (1), t084 (7), t5691 (1), t355 (4), t304 (1) in pigs; t311 (1), t084 (7), t2216 (3), t355 (1), t1931 (2), t127 (1), t5427 (1), t5126 (1), t5576 (1)	CC15, CC152 and CC5		0%	93.1% (27/29) 25 pig and 2 human	41% (12/29). *seq*, *seh*, *sei*, *sea* and *seh* *sed* and *sei*	Not done	Not done
Odetokun et al. (2018)	Not done	Not done	Not done	Not done	Not done	Not done	Not done	t16571 (1/10) in pigs	Not done	Not done	Not done	Not done	Not done	Not done	

(continued on next page)

Table 4
(*continued*)

Reference	Method	*mecA*	*mecC*	SCC*mec* typing	Antibiotic resistance genes	Heavy metal resistance genes	CC398 PCR	*Spa* typing	MLST	WGS similarity	pvl	scn	Enterotoxins and other toxins	*TST*	Other virulence factors
Founou et al. (2019), Founou et al. (2018)	REP-PCR and WGS	100%	Not done	SCCmecVc				t011	CC398	Highly similar (several plasmids)	(lukS-PV (1)	Not done	*seb* (5)	Not done	aur (5), hlb (5), hlgA (5), hlgB (5), hlgC (5), vWbp (5), clfB (5), FnbA (5), fnbB (5), ebpS (5)
Katakweba et al. (2016)	Uniplex PCR	0%	0%	Not done	Not done	Not done	Not done	t131 (4)	CC80 (4)	Not done	Not done	Not done	Not done	Not done	Not done
Adegoke and Okoh (2014)	PCR	Pos	Not done	Not done	*mphC* Pos, but *vanA*, *vanB ermA*, *ermB* and *ermC* Neg.	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done
Fall et al. (2012)	Uniplex PCR	Pos10.5% (pigs)	Not done	ST5-SCCmecIV (5), ST88-SCCmec IV (1)	Not done	Not done	Not done	t355 (6), t1172 (1), t4235 (7), t4690 (3), t084 (8), t311 (7), t267 (5), t314 (3), t645 (1), t1476 (3), t1617 (1), t127 (2), t148 (2), t1510, t2700, t3489, t8481, t8482, t2915 from pigs; t335 (1), t1172 (1), t084 (4), t311 (3), t267 (2), t359 (1), t314 (1), t127 (1), t094, t8480 from humans	CC152 (26.0%), CC15 (19.2%), CC5(13.7%), and CC97 (10.9%)	Not done	49.1% (28) pigs; 43.8% (7) humans	Not done	*sem* (13), *seh* (5), *sea* (5), *ser* (4), *eta* (3), *sed* (3), *sec* (2), *sep* (1) from pigs; *sea* (3), *sem* (3), *sep* (2), *she* (2), *etb* (2), *eta* (1) from humans	tst (1) in both pigs and humans	Not done

PCR, polymerase chain reaction; MLST, multi-locus sequence testing; WGS, whole-genome sequencing.

Whole-genome sequencing

Two studies conducted WGS; the isolates were found to be highly similar in both studies (Table 4). One of these studies found several putative genes including *scn*, *sak*, *lukE*, *lukD*, gamma haemolysin, *aur*, *splA* and *splB*, while the other study detected several plasmids (Table 4).

Risk factors associated with carriage of S. aureus

Only three studies reported risk factors associated with carriage of *S. aureus* (Table 5). One study found medical-related occupation of the household to be significant (P=0.0087), while another study found male gender and working in a slaughterhouse were significant risk factors (P=0.034 and P=0.001, respectively). None of these risk factors were found to be of significance in the third study (Fall et al., 2012).

Discussion

This systematic review aimed to assess the emergence of livestock-associated *S. aureus* including MRSA in the pig and pork production systems in Africa for the past two decades. Many articles were retrieved, and 19 met the inclusion criteria for this review. These 19 studies were undertaken in six countries, with the majority being conducted in Nigeria and South Africa.

The prevalence rates of *S. aureus* in pigs, pork, and pig farm and abattoir workers in Africa were lower compared with those from other parts of the world, such as Belgium (37.8%), the Netherlands (39%) and Germany (70%) (De Neeling et al., 2007; Denis et al., 2009; Köck et al., 2009). Notably, most of the reviewed studies reported *S. aureus* rates below 25%, and the highest was 55%. This difference could be due to the subject being understudied in Africa. Additionally, most of the studies had relatively small sample sizes and focused on other animal or bacterial species besides pigs and *S. aureus*. Furthermore, several studies investigated members of the genus *Staphylococcus*, whereas two studies exclusively screened for *S. aureus* and showed higher prevalence rates (Okunlola and Ayandele, 2015; Dweba et al., 2019).

Most studies in this review reported low levels of MRSA (<12%) compared with levels reported in other continents (>30%) (De Neeling et al., 2007; Denis et al., 2009; Köck et al., 2009). However, a few studies recorded MRSA rates of >40%. Elsewhere, surveillance of livestock-associated MRSA has been included in hospitals, where it has been reported to cause serious infections in humans (Graveland et al., 2011). This underscores the need for continued surveillance of these strains on the African continent in case they emerge in the hospital environment and thereby compound the treatment of infections in already resource-limited healthcare settings. This review of antimicrobial susceptibility showed that most isolates were susceptible to the commonly used antimicrobials. The low prevalence of MRSA and resistance to other antimicrobials among the isolates could be due to the fact that most of the animals sampled came from small-scale farmers who perform 'backyard farming'. This type of farming utilizes low-cost resources as production is not intense and antibiotic use is rare. Conversely, studies from other continents have focused on large commercial farms, and the pig density per farm has been reported as a factor for the presence of antimicrobial resistance, including methicillin resistance (Van Duijkeren et al., 2008; Köck et al., 2009; Fang et al., 2014). Only two studies included in this review involved pigs reared in large commercial enterprises (in Senegal and South Africa) (Fall et al., 2012; Van Lochem et al., 2018). More studies involving large commercial pig farms need to be conducted in Africa, especially now that the industry is growing rapidly.

Notably, all isolates were susceptible to vancomycin, which is the drug of choice for multi-drug-resistant Gram-positive organisms including MRSA (Rybak et al., 2013). Therefore, probable infections that may result from these isolates could be treated successfully using vancomycin. However, the detection of high resistance to heavy metals and subsequent heavy metal resistance genes is of concern as the heavy metals can easily accumulate to high levels once the pork is consumed by humans. Selection for heavy metal resistance in bacteria coupled with antibiotic resistance may compound the treatment of infectious diseases. The studies included in this review used multiple tests for identification and detection of methicillin resistance in the isolates. This indicates improved laboratory diagnostic capacities in African countries and/or collaborations, which is of significance as misidentification of *S. aureus* has been reported (Ahmed et al., 2010; Omuse et al., 2014; Lee et al., 2018). The use of multiple methods provides more stringent speciation and identification of methicillin resistance.

Remarkably, only two of the reviewed studies reported the presence of typical livestock-associated *S. aureus* isolates in pigs on the African continent by reporting CC398 and its associated *spa* type t011 (Founou et al., 2019), and detection of the *mecC* gene among the isolates (Dweba et al., 2019). This presents evidence of the emergence of typical livestock-associated *S. aureus* isolates on the African continent among porcine isolates. The other report of CC398 was in isolates from chickens in Tunisia (Chairat et al., 2015). Taken together, these findings show the emergence of typical livestock-associated MRSA on the African continent. Therefore, concerted efforts and measures must be put in place to curb the spread of these strains. However, most CCs and *spa* types reported in the reviewed studies were associated with human lineages of *S. aureus* (Breurec et al., 2011b; Shittu et al., 2012; Egyir et al., 2014). Only two studies reported the clonal lineages of pig-associated MSSA on the African continent (from Cameroon and Nigeria): CC1, CC5, CC72, CC97, CC121, CC15, CC152 and CC8 (Fall et al., 2012; Momoh et al., 2018). More studies are needed as MSSA play a crucial role in the evolution of different genetic lineages of *S. aureus*.

Several enterotoxin genes were detected in *S. aureus* isolates from live pigs, carcasses and humans in the reviewed studies. Notably, *sea* was the most common Staphylococcus enterotoxin isolated, and *seb* was only detected in one study (from South Africa) (Founou et al., 2019). The presence of these genes implies that *S. aureus* isolates from pigs and humans in Africa pose a great risk as a cause of foodborne infections, especially to consumers of pork and pork products. The genes encoding PVL (associated with skin and soft tissue infections) were detected in three studies, and their prevalence rates were very high (Fall et al., 2012). PVL is normally detected in community-associated isolates of *S. aureus*, and has been shown to be species specific for humans and rabbits (O'Hara et al., 2008; Breurec et al., 2011a; Tam and Torres, 2019). The *scn* genes responsible for encoding the secreted protein staphylococcal complement inhibitor (scn) were detected in two studies, and may suggest possible human-to-animal transmission. scn, iecstaphylokinase and chemotaxis inhibitory protein form the IEC, which is thought to contribute to immune evasion in humans (van Wamel et al., 2006). Moreover, IEC genes are less prevalent in livestock-adapted *S. aureus* lineages, and are thus considered good genetic markers for identification of human-associated *S. aureus* clones (McCarthy et al., 2011). Taken together, the findings for PVL and *scn* potentiate the likelihood of the *S. aureus* originating from humans rather than pigs. The significance of interspecies transmission cannot be overemphasized as it leads to challenges in treatment and prevention of infections.

Only two of the studies included in this review conducted WGS. They reported high similarity among the isolates and found many putative virulence genes including *splA* and *splB*, and plas-

Table 5
Characteristics of studies that reported risk factors associated with *Staphylococcus aureus* carriage.

	Medical history			Occupation		Sex of humans		Age of humans (years), mean		Age of animals		Contact with pigs							Medical-related occupation of household
												Specific duty of workers in both farms and abattoirs							
Reference	Antibiotics in last 3 months,% (*n*)	Contact with HCC in last 12 months% (*n*)	Skin and soft tissue infections in the last 12 months,% (*n*)	Farm worker	Abattoir worker	Female	Male	Young	Adult	Young	Adult	Length of employment on farm , mean	Direct contact with pigs per day (*n*), mean	Cleaning on the farm	Cleaning of pigs	Feeding of pigs	Breeding of pigs	Slaughtering of pigs	
Momoh et al. (2018)	NR	NR	NR	69% (9)	31% (4)	62% (8)	38% (5)	31% (4)	69% (9)	NR	NR	<5 [31% (4), *P*=0.1828]; 5–10 [54% (7) *P*=0.2626]; >10 [155 (2), Ref]	NR	NR	NR	NR	NR	NR	62% (8); *P*=0.0087[a]
Oderokun et al. (2018)	NR	NR	NR	N/A	Yes	16.5% (17), *P*=0.034[a]	26.7% (46)	29.4% (5), *P*=0.525	53.7% (58)	NR	NR	NR	NR	N/A	NR	NR	N/A	21.8% (29), *P*=0.001[a]	NR
Fall et al. (2012)	12.5% (20) *P*=0.68	62.5% (10); *P*=0.77	37.5% (6); *P*=0.33	Yes	N/A	NR	31% (5) *P*=0.16	NR	44.4; *P*=0.59	NR	NR	10.6; *P*=0.61	2.4; *P*=0.63	62.5% (10)	0	NR	68.8% (11); *P*=0.23	18.7% (3); *P*=0.75	NR

ND, not done; NR, not reported. [a] Statistically significant

mids (Otalu et al., 2018; Founou et al., 2019). Notably, the serine-protease-like proteases (spls) encoded on the *spl* operon are rarely reported in *S. aureus* isolates in Africa. This may denote a lack of research in this aspect of the organism in Africa. The role of major staphylococcal proteases, such as the metallo proteinase aureolysin, in the pathogenic mechanisms of *S. aureus* including complement evasion have been documented (Jusko et al., 2014). Together, the findings of this review indicate that livestock-associated *S. aureus* isolates from Africa are potentially virulent and can cause infections once they gain access to human hosts.

There is an extreme paucity of data on the risk factors associated with the carriage of livestock-associated *S. aureus* from pigs to humans in Africa, with only three studies reporting on this aspect. None of the studies in this review indicated contact with pigs as a risk factor; people who have direct contact with livestock, particularly pigs, have been said to be at higher risk of either being colonized or infected by *S. aureus* (Ye et al., 2016). Interestingly, the risk factor of medical-related occupation of the household coupled with the genotypic findings of human-related CCs and *scn* strongly suggests the possibility of anthropogenic transmission.

One of the limitations of this study is that only two recognized electronic databases were searched, and only articles written in English were included, making it possible that studies or publications that were relevant to this review were omitted. Furthermore, the studies were only conducted in six countries, and therefore may not reflect the epidemiology of livestock-associated *S. aureus* in Africa.

Conclusion

This review assessed 19 studies of livestock-associated *S. aureus* in pig and pork production systems in Africa. The combined results of these studies indicate the presence of potentially highly virulent strains of *S. aureus* in live pigs and raw pork which may be passed on to high-risk individuals who have frequent contact with animals. Subsequent disease in these individuals or transmission to their community is a distinct possibility, as is foodborne transmission to pork consumers, with immediate illness due to the presence of enterotoxins or exposure to live *S. aureus*. One study reported the typical CC398 found in other continents, while most studies reported genotypes that are more frequently associated with humans than animals. Therefore, surveillance to better understand the epidemiology of livestock-associated *S. aureus*, together with an integrated 'One Health' approach in tackling livestock-associated *S. aureus*, is required to ensure effective prevention and control measures for *S. aureus* infections and curb antimicrobial resistance in both animals and humans.

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Conflict of interest statement

None declared.

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Ethical approval

Not required.

Author contributions

MTS, GK, ES and BMH conceived the idea and designed the study. MTS performed the literature search and selection, and extracted the data. MTS and PN analysed the data. MTS wrote the original manuscript draft. GK, ES, BMH, PN, AF, HH and BM critically reviewed the manuscript. GK, ES, HH, AF and BMH supervised the research. All authors read and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.06.023.

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Antimicrobial Susceptibility Profiles and Molecular Characterisation of *Staphylococcus aureus* from Pigs and Workers at Farms and Abattoirs in Zambia

Mulemba Tillika Samutela [1,2,*], Bruno Stephen July Phiri [3], Edgar Simulundu [4,5], Geoffrey Kwenda [1], Ladslav Moonga [2], Eugene C. Bwalya [6], Walter Muleya [7], Therese Nyirahabimana [7], Kaunda Yamba [4,8], Henson Kainga [4,9], Simegnew Adugna Kallu [4,10], Innocent Mwape [11], Andrew Frey [12], Matthew Bates [13], Hideaki Higashi [14] and Bernard Mudenda Hang'ombe [2]

1 Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka 10101, Zambia; jaffekwenda@gmail.com
2 Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia; ladslav.moonga@unza.zm (L.M.); bhangombe@unza.zm (B.M.H.)
3 Central Veterinary Research Institute, Lusaka 10101, Zambia; julypondayapa@yahoo.com
4 Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia; esikabala@yahoo.com (E.S.); kaundayamba@gmail.com (K.Y.); hkainga@bunda.luanar.mw (H.K.); adusim12@gmail.com (S.A.K.)
5 Macha Research Trust, Choma P.O. Box 630166, Zambia
6 Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia; eugene.bwalya@unza.zm
7 Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia; muleyawalter@gmail.com (W.M.); ntherese22@gmail.com (T.N.)
8 Department of Pathology and Microbiology, University Teaching Hospitals, Lusaka 10101, Zambia
9 Department of Veterinary Epidemiology and Public Health, Faculty of Veterinary Medicine, Lilongwe University of Agriculture and Natural Resources, Lilongwe 207203, Malawi
10 College of Veterinary Medicine, Haramaya University, Dire Dawa P.O. Box 138, Ethiopia
11 Center for Infectious Disease Research Zambia, Lusaka 10101, Zambia; innocent.mwape@cidrz.org
12 Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, FL 33620, USA; andrew.m.frey@gmail.com
13 School of Life & Environmental Sciences, University of Lincoln, Lincolnshire LN6 7TS, UK; mbates@lincoln.ac.uk
14 Division of Infection and Immunity, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan; hidea-hi@czc.hokudai.ac.jp
* Correspondence: mulembats@gmail.com; Tel.: +260-965621307

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Abstract: Pigs have been shown to be a reservoir for recently emerging livestock-associated *Staphylococcus aureus* (LA-SA), including methicillin resistant strains in many countries worldwide. However, there is sparse information about LA-SA strains circulating in Zambia. This study investigated the prevalence, phenotypic and genotypic characteristics of *S. aureus* from pigs and workers at farms and abattoirs handling pigs in Lusaka Province of Zambia. A total of 492 nasal pig swabs, 53 hand and 53 nasal human swabs were collected from farms and abattoirs in selected districts. Standard microbiological methods were used to isolate and determine antimicrobial susceptibility patterns of *S. aureus*. Polymerase Chain Reaction was used to confirm the species identity and detect antimicrobial resistance and virulence genes of isolates, whereas genetic diversity was evaluated using *spa* typing. Overall prevalence of *S. aureus* was 33.1%, 37.8% for pigs and 11.8% for humans. The isolates were resistant to several antibiotics with resistance ranging from 18% to 98% but were all susceptible to vancomycin. Typical LA-SA *spa* types were detected. The presence of plasmid mediated resistance genes such as *tet*M (12.8%), other resistance determinants and immune evasion cluster genes among the isolates is of great public health concern. Thus, continuous surveillance of *S. aureus* using a "One health" approach is warranted to monitor *S. aureus* infections and spread of antimicrobial resistance.

Keywords: antimicrobial resistance; *Staphylococcus aureus*; *spa* typing; swine; Zambia

1. Introduction

Staphylococcus aureus, a Gram-positive bacteria, is a pathobiont of humans and animals including pets, livestock and wildlife, with animal infections and reservoirs being a potential source for human infections and vice versa [1]. Currently, the epidemiology of *S. aureus* including methicillin resistant strains is classified into three hospital or healthcare-associated *S. aureus* (HA-SA and HA-MRSA, respectively), community-associated *S. aureus* (CA-SA) and livestock-associated *S. aureus* (LA-SA) [2]. *Spa* typing and multi-locus sequence typing (MLST) are widely used to derive *spa* types (t) and sequence types (STs) or clonal complexes (CCs), which have been determined globally. Some *spa* and ST types are commonly associated with LA-SA. Typically, LA-SA from pigs in Europe have been associated with CC398, whereas CC9 is the predominant type in Asia [3]. *Spa* types t011, t034, t108, t567, t571, t899, t1254, t1451, t2011 and t2510 are associated with CC398, and are among those more closely linked to LA-SA [4].

While a wide range of livestock are implicated with LA-SA, pigs are considered a major reservoir of these *S. aureus* strains [5]. Notably, the LA-SA have been detected in persons with occupational contact with pigs including farm and slaughterhouse workers and veterinarians [6,7]. Additionally, LA-SA has been isolated in persons without occupational contact to the animals [8]. Therefore, LA-SA are a source of concern as they can be passed on from animals to humans and from humans to humans. While LA-SA were mostly associated with colonisation and minor infections, blood stream infections (invasive infections) with livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) have been reported in Germany and Denmark [9,10]. This is worrisome as it shows that these strains are not only circulating in the community but are entering hospitals thereby blurring the distinctions between the epidemiological groups of *S. aureus*. Such transmission is of major concern, because while the use of antibiotics in farm animals such as pigs may select for antimicrobial resistance, LA-SA have generally been more susceptible to antibiotics compared to the HA-SA due to excessive use of antibiotics and or poor antibiotic stewardship in hospitals. Therefore, the entry of LA-SA into health care institutions may lead to these strains acquiring resistance which could be passed back into their communities of origin as adapted strains.

While emphasis is mainly placed on MRSA, methicillin susceptible *S. aureus* (MSSA) strains are equally important in the evolution of *S. aureus*. Using whole genome sequencing, it has been shown that LA-CC398-MRSA evolved from an ancestor which was a human-adapted HA-MSSA CC398 [11]. The CC398-MSSA ancestor could have acquired resistance to methicillin and tetracycline while losing the prophage that carries the immune evasion cluster genes (IEC). The IEC genes protect *S. aureus* against the immune system in humans [12]. The presence or absence of the IEC genes can indicate whether *S. aureus* strains are human or livestock-associated, respectively. Several studies from European countries show that the LA-CC398 MSSA have emerged as a subpopulation of causative agents of invasive infections in hospitals [13–17]. Of interest is a subset of the CC398 MSSA which is independent of livestock but is human adapted [15]. However, there is sparse information on this clade. Therefore, more studies are warranted to further understand such lineages. Furthermore, given the role of human and animal interactions in the emergence of such lineages, it is critical to conduct such studies in a comprehensive manner using a "One Health" approach. Despite heightened interest in the epidemiology of LA-SA across the globe, there is still a paucity of data on the prevalence and characteristics of pig related LA-SA on the African continent. A recent systematic review revealed that only 19 studies specifically reported on the prevalence or incidence, antimicrobial susceptibility profiles and genetic characteristics of pig-associated *S. aureus* in Africa between 2000 and 2019 [18].

Pig farming is an important economic activity in Zambia, with most pig farmers being smallholder farmers in the rural areas of the country. However, commercial pig farming has over the years become more common in the more urban parts of the country especially in Lusaka Province. This shift could entail an increase in antibiotic use in pig rearing establishments, which has been shown to be a risk factor for the emergence of MRSA.

Zambia, similarly to many other countries, has reported the presence of *S. aureus* infections in the clinical settings including multidrug resistant strains of MRSA [18,19], in pets [20] and wildlife [21]. However, there is scarce information on LA-SA. Therefore, this study aimed to determine the prevalence, phenotypic and genotypic characteristics of *S. aureus* in pigs and workers from farms and abattoirs handling pigs in Lusaka Province of Zambia.

2. Results

2.1. Prevalence of S. aureus in Pigs and Humans in Lusaka Province

The overall prevalence of *S. aureus* was 33.1% (Table 1). In pigs and workers, the prevalence was 37.8% and 11.3%, respectively. The positivity rate in both nasal and hand swabs from humans was 11.3% (Table 1). Chilanga District showed the highest (66.4%) positivity rate among the three districts studied (Table 1). Notably, some pig samples yielded more than one *S. aureus* isolate (n = 27) (Supplementary Materials Table S1) and these isolates were not included in the calculation of the prevalence but were further characterized. When broken down by district, the positivity rate of human samples was 30.4% (7/23), 8.7% (4/46) and 2.7% (1/37) for Chilanga, Chongwe and Lusaka districts, respectively. Therefore, positivity rate among humans was higher in Chilanga district compared with Chongwe and Lusaka districts (p = 0.005).

Table 1. *S. aureus* positivity rates from pigs, humans and districts in Lusaka Province.

Factor	Category	*n* Tested	*n* Positives	Prevalence (%)	95% CI
Overall Positivity	Positive	598	198	33.1	29.4–37.1
Humans	Overall	106	12	11.3	6.2–19.3
	Hand Swabs	53	6	11.3	4.7–23.7
	Nasal Swabs	53	6	11.3	4.7–23.7
Pigs	Nasal swabs	492	186	37.8	33.5–42.3
Districts	Chongwe	250	60	24.0	18.9–29.9
	Lusaka	235	63	26.8	21.4–33.0
	Chilanga	113	75	66.4	56.6–74.8

Abbreviations: CI = Confidence interval; n = number of samples.

With respect to study sites, the overall prevalence at farms and abattoirs was 27.2% and 65.9%, respectively. The prevalence at abattoirs was comparatively higher than that of farms (Table 2). Generally, the prevalence of *S. aureus* in pigs was significantly higher than in humans at both the farm and abattoir levels (Tables 1 and 2). While the prevalence of *S. aureus* was high for both pigs and humans at medium and large-scale facilities, the prevalence of *S. aureus* in humans was low in small-scale farms (Table 2).

2.2. Antimicrobial Susceptibility Profiles and Antimicrobial Resistance Genes Detected in the S. aureus Isolates

The highest resistance of the *S. aureus* isolates from samples collected from both pigs and humans at the farms were to penicillin (98%), whereas resistance to tetracycline, ciprofloxacin and cefoxitin was recorded at 35%, 30% and 18%, respectively (Figure 1A). In addition, these isolates were more susceptible to co-trimoxazole (92%), gentamicin (90%) and chloramphenicol (79%) (Figure 1A). Forty percent of the isolates showed intermediate susceptibility to erythromycin, whereas erythromycin-induced clindamycin resistance was detected in only one isolate. Notably, all isolates tested against vancomycin were susceptible with minimum inhibition concentrations (MICs) ranging from 1.5 µg/L to 3 µg/L (Supplementary Materials Table S2). From abattoirs, the highest resistance was recorded to penicillin at 98% followed by 35% and 25% to ciprofloxacin and tetracycline, respectively (Figure 1B). From all isolates, 100% susceptibility was observed to cefoxitin, 99% to gentamicin, 90% to co-trimoxazole and 88% to chloramphenicol. Intermediate results were highest for erythromycin, whereas no erythromycin-induced clindamycin resistance was detected.

Table 2. Prevalence of *S. aureus* in pigs and humans at farms and abattoirs.

Study Site	Species	Type of Facility *	*n* Tested	*n* Positives	Prevalence (%)	95% CI
Farms	Combined pigs and humans	Small	53	13	24.5	14.2–38.6
		Medium	252	61	24.2	19.1–30.1
		Large	202	64	31.7	25.4–38.7
		Overall	507	138	27.2	23.4–31.3
	Pigs only	Small	45	13	28.9	16.8–44.5
		Medium	216	57	26.4	20.8–32.9
		Large	157	61	38.9	31.8–47.0
		Overall	418	131	31.3	27.0–36.1
	Humans only	Nasal	38	3	7.9	2.1–22.5
		Hand	38	4	10.5	3.4–25.7
		Overall	76	7	9.2	4.1–18.6
	Human Nasal	Small	4	0	0	0
		Medium	18	2	11.1	2.0–36.1
		Large	16	1	6.3	0.3–32.3
	Human Hand	Small	4	0	0	0
		Medium	18	2	11.1	2.0–36.1
		Large	16	2	12.5	2.2–39.6
Abattoirs	Combined pigs and humans	Medium	20	4	20	6.6–44.3
		Large	71	56	78.9	67.3–87.3
		Overall	91	60	65.9	55.2–75.3
	Pigs only	Medium	20	4	20	6.6–44.3
		Large	54	51	94.4	83.7–98.6
		Overall	71	55	77.5	65.7–86.2
	Humans **	Hand	8	2	25	4. 5–64.4
		Nasal	9	3	33.3	9.0–69.1
		Overall	17	5	29.4	11.4–56.0

* Type of facility: Small scale (less than 100 pigs), medium scale (100 to 500 pigs) and commercial scale (greater than 500 pigs; ** All human swabs from abattoirs were collected at the large facilities only. Abbreviations: CI = Confidence interval; *n* = number of samples.

Figure 1. Overall Antimicrobial Susceptibility Profiles of *S. aureus* Isolates from pigs and workers from Farms (**A**) and Abattoirs (**B**) of Lusaka Province. Abbreviations: P = Penicillin; CN = Gentamicin; E = Erythromycin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, SXT = Cotrimoxazole, C = Chloramphenicol, CX = Cefoxitin; I = Intermediate, R = Resistant, S = Susceptible.

2.2.1. Multidrug Resistance Patterns of the *S. aureus* Isolates

To assess whether antibiotic resistance phenotypes clustered together, antibiotic resistance patterns were assigned using designations PG + Te + CN + E + CD + Cip + C + SXT (as defined in Figure 1 legend). Isolates were grouped into 23 antibiotic resistance patterns (Table 3). A majority of the isolates were resistant to at least one or two antibiotics besides penicillin, with the predominant phenotype being P + Cip (15.7%) and P + E + CD + Cip (14.2%). Multi-drug resistance to a combination of three, four, five, and six antibiotics was observed in 17.6% of the isolates. Isolates were classified as multi-drug resistant (MDR) if, in addition to the Beta-lactams, they were resistant to 3 classes based on susceptibility to erythromycin, clindamycin, chloramphenicol, ciprofloxacin, tetracycline and co-trimoxazole. Based on this classification, 36 isolates from farms were MDR (Table 3).

Table 3. Antibiotic resistance patterns of *S. aureus* isolates from pigs and workers from pig farms and abattoirs in Lusaka Province.

Resistance Pattern	Proportion of Isolates % (*n*)	
	Farm Isolates (*n* = 141)	Abattoir Isolates (*n* = 63)
P	34.8 (49)	42.9 (27)
Te	1.4 (2)	1.6 (1)
P + Te	20.6 (29)	7.9 (5)
P + Cip	7.1 (10)	34.9 (22)
P + CD	0.7 (1)	3.3 (2)
P + CN + Te	2.1	1.6 (1)
P + E + TE	1.4 (2)	-
P + Te + Cip	0.7 (1)	3.2 (2)
P + E + CD + Cip	14.2 (20)	-
P + E + CD + TE	1.4 (2)	-
P + E + C + CIP	1.4 (2)	-
P + CN + TE + SXT	5.0 (7)	-
P + E + CD + TE + SXT	0.7 (1)	-
P + E + CD + CN + Cip	1.4 (2)	-
P + E + CN + Te + CIP	0.7 (1)	-
P + E + CD + CN + Te + SXT	0.7 (1)	-
[1] Other	4.3 (6)	4.7 (3)

Abbreviations: *n* = number of samples, P = Penicillin; CN = Gentamicin; E = Erythromycin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, SXT = Cotrimoxazole, C = Chloramphenicol, - = Not detected; [1] Other = P + E, P + SXT, P + E + C, P + E + CD, P + E + SXT, P + CD + Te (farm isolates) and P + Te + SXT, P + CD + CN, P + C + Cip (Abattoir isolates). Each pattern was manifested in only one isolate.

2.2.2. Presence of Antimicrobial Resistance Genes in the *S. aureus* Isolates

The *mecA* and *mecC* genes that encode for methicillin resistance were not detected in all the isolates despite the phenotypic resistance to methicillin in some of the isolates. With respect to the genes encoding for tetracycline resistance, detection rates were 19.3% (11/57) for *tet*M, 12.3% (7/57) for *tet*K and 1.8% (1/57) for *tet*L. Notably, all isolates harbouring these genes were from nasal pig swabs. The *tet*O gene was not detected in any of the isolates tested. Only one isolate harboured both *tet*M and *tet*L genes. Farm isolates harboured more of the tetracycline resistance genes than those from the abattoirs. The *erm*B and *erm*C genes were detected in 19.2% (5/26) and 57.7% (15/26) of the isolates, respectively, whereas *erm*A was not detected in any of the isolates. Most of the isolates harbouring these resistance genes were from pigs sampled from farms. Only two human isolates harboured the resistance genes. Notably, all isolates resistant to erythromycin were from the same farm.

2.3. Virulence Genes Detected in the S. aureus Isolates

Neither the Panton-Valentine Leukocidin (PVL) nor the staphylococcal enterotoxin (SE) encoding genes were detected in any isolates in this study. For the IEC genes, *sak*, *scn* and *chp*, were detected in 7.6% (17/225), 1.3% (3/225) and 0.4% (1/225) isolates, respec-

tively (Table 4). All these isolates were from nasal swabs of pigs, mostly from one farm (Farm 7) (Table 4).

Table 4. IEC genes distribution among the *S. aureus* isolates (n = 225).

		IEC Gene		
Source (Farm or Abattoir)	**Sample Type**	***scn* % (*n*)**	***sak*% (*n*)**	***chp* % (*n*)**
Farm 1	Pig nasal Swab	-	0.4 (1)	-
Farm 2	Pig nasal Swab	-	0.4 (1)	-
Farm 4	Pig nasal Swab	-	1.3 (3)	-
Farm 5	Pig nasal Swab	-	0.4 (1)	-
Farm 6	Pig nasal Swab	-	0.4 (1)	-
Farm 7	Pig nasal Swab	0.9 (2)	2.7 (6)	-
Farm 9	Pig nasal Swab	-	0.4 (1)	-
Farm 10	Pig nasal Swab	-	1.3 (3)	-
Abattoir 1	Pig nasal Swab	0.4 (1)	-	0.4 (1)
	Total	1.3 (3)	7.6 (17)	0.4 (1)

Abbreviation: n = number of isolates; - = None detected.

2.4. *Spa* Typing of the *S. aureus* Isolates

All *S. aureus* isolates (n = 225) were positive for the *spa* gene by PCR and 43 representative isolates based on the most frequent resistance phenotypes were sequenced to determine the *spa* types (Supplementary Materials Table S3). Six *spa* types were detected namely, t1430 (n = 12), t034 (n = 8), t318 (n = 4), t571 (n = 1), t084 (n = 1) and t899 (n = 1). The most common *spa* type was t1430 (28.0%) followed by t034 (18.6%) (Table 5). Only *spa* type t1430 was detected in both humans and pigs, *spa* types t034, t318, t571 were found in pigs only while t084 and t899 were found in humans only (Table 5). A total of 16/43 (37.3%) of the isolates were of unknown *spa* types (Table 5). The two most common *spa* types, t1430 and t034, were found at both farms and abattoirs of medium and large scale from all the three districts (Supplementary Materials Table S3). Notably, t1430 was detected in most of the nasal pig swabs and one human hand swab at abattoir 1 (Supplementary Table S3). The isolates with unknown *spa* types were mostly from medium scale facilities (Supplementary Materials Table S3).

Table 5. *Spa* type distribution among representative farm and abattoir isolates (n = 43).

		***Spa* Type % (*n*)**						
Species	**Study Site**	**t1430**	**t034**	**t318**	**t571**	**t084**	**t899**	**Unknown**
Humans	Farms	0	0	0	0	0	2.3 (1)	4.7 (2)
	Abattoirs	4.7 (2)	0	0	0	2.3 (1)	0	0
Pigs	Farms	14.0 (6)	9.3 (4)	9.3 (4)	2.3 (1)	0	0	25.6 (11)
	Abattoirs	9.3 (4)	9.3 (4)	0	0	0	0	7.0 (3)
	Total	28.0 (12)	18.6 (8)	9.3 (4)	2.3 (1)	2.3 (1)	2.3 (1)	37.3 (16)

Abbreviation: n = number of isolates.

3. Discussion

This study aimed at determining the prevalence, phenotypic and molecular characteristics of *S. aureus* from pigs and workers from pig farms and abattoirs in the Lusaka Province of Zambia. This is the first report on the presence of *S. aureus* in pigs and workers from farms and abattoirs in Zambia. The overall prevalence rate (33.1%) of *S. aureus* in the present study was relatively high and is in congruence with similar studies on the African continent that have reported prevalences ranging from 0% to 55% [18]. However, specific comparisons of prevalences is difficult due to the variations in the conduct of these studies, for example, most studies have only studied isolates either from farms or abattoirs and not from both sites [1]. In addition, some studies may sample from more than one body part of the pigs [1]. A comparatively higher prevalence of *S. aureus* was detected in the pigs

(37.8%) than in workers (11.3%), similar to the findings from a recent study in Nigeria [22]. However, the studies from Nigeria and South Africa detected more *S. aureus* from pigs than in our study [22,23]. The current study further showed that hand and nasal prevalence of *S. aureus* was the same among workers.

The antimicrobial susceptibility profiles of the isolates revealed that most isolates from farms and abattoirs were resistant to several antibiotics, with the highest resistance being to penicillin (98%). This finding is significantly higher than that from the study in Nigeria which reported a lower resistance to penicillin of 55% [22]. The high resistance to penicillin reflects possible overuse of the antibiotic, as penicillin is generally among most frequently used antibiotics in many farms in many countries [22,24,25]. Resistance to tetracycline, erythromycin and ciprofloxacin was also recorded in 25% to 35% of isolates in our present study. Notably, tetracycline is also commonly used to treat infections in both humans and animals and its resistance can be used as an indicative marker of LA-SA [13,26]. Only 18% of farm isolates were resistant to cefoxitin implying methicillin resistance. However, all these isolates were susceptible to vancomycin with the MICs ranging between 1.5 µg/mL to 3 µg/mL. Vancomycin is the drug of choice for MDR *S. aureus* infections in human health and is rarely used to treat animal infections [27]. This finding which is similar to that of a previous study that studied vancomycin susceptibility of clinical *S. aureus* show that vancomycin is still a viable treatment option of *S. aureus* infections in Zambia [28].

The isolates in the present study were more susceptible to co-trimoxazole, gentamicin and chloramphenicol ranging from 79% to 92%. Inducible resistance to macrolides, lincosamides, and group B streptogramins (MLSBi) phenotype was only detected in one isolate. MLSBi phenotype positive isolates appear to be erythromycin-resistant and clindamycin sensitive in vitro, but when given in vivo, they have constitutive *erm* mutations that render clindamycin ineffective [29]. A recent study at the largest referral hospital in Zambia found that none of the isolates had the MLSBi phenotype [28]. However, an earlier study at the same hospital reported a high rate of the MLSBi phenotype of 68.3% [19]. Many studies on *S. aureus* in animals do not report on the MLSBi phenotype probably because clindamycin is not used to treat infections in animals, however, a study from South Africa reported the MLSBi phenotype among the studied isolates from pigs [23]. Although multi-drug resistance was observed to two or more antibiotics in more than 40% of the isolates, generally our findings suggest that there are seemingly still several antibiotics that would be viable to treat infections caused by these isolates from the pig and pork production sector in Zambia.

Unexpectedly, despite the phenotypic resistance to methicillin based on resistance to oxacillin using cefoxitin disc that was detected in some of the isolates, neither the *mecA* nor *mec*C genes that encode for methicillin resistance were detected in any of the isolates. A possible explanation to the phenotypic resistance could be that the isolates are hyperproducers of penicillinases that confer some resistance to cefoxitin [30]. While the *mecA* is the mainstay gene responsible for methicillin resistance in clinical isolates, the *mec*C gene is linked to livestock associated staphylococcus especially LA-MRSA [31]. A recent study from South Africa reported the presence of the *mec*C in pig-associated *S. aureus* for the first time in Africa [32]. Relatively few countries have reported typical LA-MRSA pig-related *S. aureus* in Africa [18]. Studies from other parts of the world such as Europe and America state that intensive pig farming methods and heavy use of antibiotics are risk factors for the emergence and spread of methicillin resistance as well as resistance to other antibiotics in *S. aureus* among pigs and attending workers at farms and slaughterhouses [9,33,34]. However, none of the facilities included in the current study practises such intensive pig rearing. Markedly, MSSA cannot be overlooked as they form the reservoir from which MRSA arise [11,35]. The presence of antimicrobial resistance genes in the present study including *tet*M, *tet*K and *tet*L genes encoding for tetracycline resistance and *erm*B and *erm*C genes encoding resistance to erythromycin in some of the isolates indicate the need to closely monitor these strains as they may become a source of

antimicrobial resistance given that some of these genes are harboured on plasmids which can be easily transferred between microorganisms [36].

Genes encoding the PVL and SEs were not detected in any of the isolates in the present study. While this was the first study to look for the presence of these genes in isolates from pigs in Zambia, the PVL has been reported in a previous study of clinical isolates howbeit only three out of 33 isolates were positive [37]. A study in Senegal on pigs and workers at commercial farm reported a high prevalence of the PVL gene [38]. The PVL is associated with skin and soft tissue infections and has a provenance for humans, but our study indicates that it is dispensible for pig colonisation. The role of SEs in Staphylococcal foodborne disease has been documented in several studies [39–41]. Therefore, the non-detection of SEs could indicate the relative safety of the pork and pork products on the Zambian market for consumers. Interestingly, several isolates harboured the IEC genes with the *sak* being the most prevalent. The staphylokinase and chemotaxis inhibitory proteins form the IEC and contribute to immune evasion in humans [12]. While IEC genes are less prevalent in livestock-adapted *S. aureus* lineages, they are considered good genetic markers for identification of human-associated *S. aureus* clones [42]. Therefore, the finding of IEC genes among *S. aureus* isolates from pigs in the present study potentiate the notion of possible anthropogenic nature of some of the *S. aureus* in Africa but could also indicate the presence of LA-SA that are well adapted to human hosts [15,18].

Our study found six *spa* types among which t1430 was the most prevalent followed by t304 mostly among *S. aureus* isolates from pigs both at the farms and abattoirs. Of significance is that t1430 and t034 are associated with CC9 and CC398 which are Livestock-associated lineages of *S. aureus* in Asia and Europe, respectively [3,4]. Therefore, our findings suggest that typical LA-SA lineages are present in pig and pork production facilities in Zambia. Generally, the *spa* types detected in pigs were different from those detected in humans in the present study, only t1430 was found in pigs and workers isolates. This would suggest distinct *S. aureus* lineages in the two populations. However, given that we could not identify the *spa* types of many isolates, we recommend further investigations into the clonal lineages using other molecular methods such as multilocus sequence typing (MLST) and whole genome sequencing (WGS) which could not be performed in the present study. Previous studies on pig related *S. aureus* isolates on the African continent are relatively few but show that the isolates have diverse *spa* types [18]. Furthermore, studies looking into the presence of LA-SA as a cause of disease among hospitalised patients in Africa are needed as this has not been reported yet but have a large impact on epidemiology of *S. aureus* infections.

4. Materials and Methods

4.1. Study Design and Sample Collection

The study was a cross sectional study carried out between June 2020 and September 2021 in three districts of the Lusaka Province of Zambia namely Chilanga, Lusaka and Chongwe districts (Figure 2). Lusaka Province hosts many of the commercial and semi-commercial (small and medium scale rearing of pigs meant solely for selling) pig farms in Zambia. Pig farms and abattoirs in selected districts within the province were included in the study following consent from the farm and abattoir owners. The farms and abattoirs were arbitrarily grouped into three following categories based on the number of pigs at the facility: small scale (less than 100 pigs), medium scale (100 to 500 pigs) and commercial scale (greater than 500 pigs). A total of 492 pig nasal swabs were randomly collected from 13 farms and three abattoirs by inserting a swab and gently rotating it in the anterior nares. Additionally, 53 nasal and 53 hand swabs each from humans (farm workers and abattoir workers) in close contact with the pigs were collected. The human nasal swabs were collected by inserting a swab and gently rotating it in the anterior nares, whereas a hand swab was collected by gently rubbing the swab in both palms.

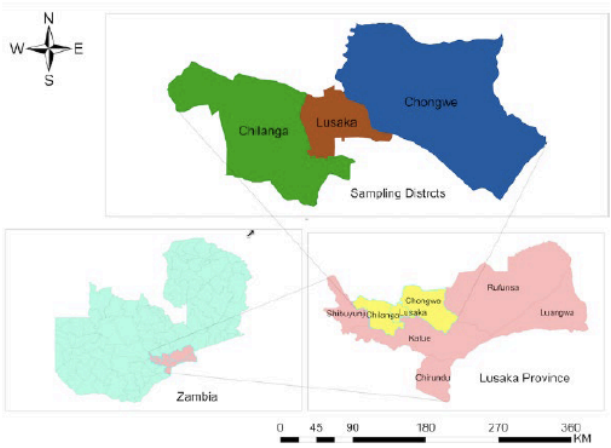

Figure 2. Map of the Study Sites Selected from Lusaka province in Zambia; Map was generated using the software ArcGIS version 10.3.

4.2. S. aureus Detection and Identification

Phenotypic detection and identification of *S. aureus* was carried out by conventional microbiological methods [43]. Briefly, each swab was transferred into 10 mL Mueller-Hinton broth (MHB) (Oxoid, Basingstoke, UK) supplemented with 6% Sodium Chloride (NaCl) and incubated at 37 °C for 16 to 20 h. Then, a loopful of the broth was inoculated on Mannitol Salt Agar (MSA) (Oxoid, Basingstoke, UK) and the plates were then incubated for 16 to 20 h at 37 °C. Resulting yellowish colonies were then inoculated onto Baird Parker Agar (BPA) (Oxoid, Basingstoke, UK) and incubated for 16 to 20 h at 37 °C. Resulting black or greyish colonies with or without a halo on the BPA plates were then grown in Brain Heart Infusion Broth (BHIB) (Oxoid, Basingstoke, UK) for 16 to 20 h at 37 °C. Using 0.5 mL of the BHIB culture, a tube coagulase test using rabbit plasma was set up according to manufacturer's instructions (Sigma-Aldrich, Taufkirchen, Germany) by incubating the tubes at 37 °C and reading after four (4) hours and at 24 h. All coagulase positive isolates were considered as *S. aureus* and stored in 20% glycerol at −20 °C until further analysis.

4.3. Determination of Antimicrobial Susceptibility Profiles

The antimicrobial susceptibility of *S. aureus* to antibiotic discs (Oxoid, Basingstoke, UK) of 10 µg gentamycin, 5 µg ciprofloxacin, 15 µg erythromycin, 10 µg clindamycin, 30 µg amikacin, 10 units penicillin G, 25 µg co-trimoxazole, 30 µg chloramphenicol and 30 µg tetracycline was determined using the Kirby-Baur disc diffusion method and interpreted according to the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines [44]. Methicillin resistance was detected by checking for resistance to oxacillin using the 30 µg cefoxitin discs following the 2020 CLSI guidelines [44]. The D-test using erythromycin and clindamycin discs was also used to detect inducible resistance to macrolides, lincosamides, and group B streptogramins (MLSBi) in the *S. aureus* isolates. Susceptibility to vancomycin was determined for the isolates resistant to methicillin using vancomycin E-strips to determine the minimum inhibition concentrations (MICs) according to the CLSI guidelines [44]. Briefly, using a swab, one to two pure colonies of the organism grown overnight on nutrient agar were suspended into 2 mLs of physiological normal saline to make a 0.5 McFarland density. These bacteria were then spread evenly on a Mueller Hinton agar plate using a sterile swab. After allowing the plate to air dry for a few minutes, antimicrobial discs were gently placed on the Mueller Hinton agar plate ensuring that discs were not closer than

24 mm from centre to centre. The plates were then incubated for 16 to 24 h at 37 °C for the other antibiotics and at 35 °C for 24 h for cefoxitin. For vancomycin, one E-strip was placed per plate of the isolate, which were incubated for 16 to 24 h at 37 °C.

4.4. Molecular Identification and Genotyping

4.4.1. DNA Extraction

Genomic DNA was prepared by thermo lysis of fresh *S. aureus* cells. Briefly, a loopful of *S. aureus* cells from Nutrient Agar (Oxoid, Basingstoke, UK) were transferred into a micro centrifuge tube containing 200 µL of 1X MiliQ water and boiled for 15 min. After cooling on ice, the DNA thermolysate were centrifuged at 14,000× *g* and then stored at −20 °C until required.

4.4.2. Molecular Identification of *S. aureus*

The species identification of the isolates was then confirmed by detection of the nuclease gene using *nuc* primers (Table 6) according to a previously described PCR protocol [45].

Table 6. Primer sets used in the study.

Primer Name	Target Gene	Primer Sequence (5′-3′)	Amplicon Size	Reference
Nuc1	*nuc*	GCG ATT GAT GGT GAT ACG GTT	279 bp	[45]
Nuc2		AGC CAA GCC TTG ACG AAC TAA AGC		
mecA P4	*mec*A	TCCAGATTACAACTTCACCAGG	162 bp	[46]
mecA P7		CCACTTCATATCTTGTAACG		
$mecA_{LGA251}$	*mecC*	GAAAAAAAGGCTTAGAACGCCTC	138 bp	[47]
$mecA_{LGA251}$		GAAGATCTTTTCCGTTTTCAGC		
ermA-1	*erm*[A]	TCTAAAAAGCATGTAAAAGAA	645 bp	[48]
ermA-2		CTTCGATAGTTTATTAATATTAG		
ermB-1	*erm*[B]	GAAAAGTACTCAACCAAATA	639 bp	[48]
ermB-2		AGTAACGGTACTTAAATTGTTTA		
ermC-1	*erm*[C]	TCAAAACATAATATAGATAAA	642 bp	[48]
ermC-2		GCTAATATTGTTTAAATCGTCAAT		
tetK-1	*tet*[K]	TTAGGTGAAGGGTTAGGTCC	697 bp	[49]
tetK-2		GCAAACTCATTCCAGAAGCA		
tetM-1	*tet*[M]	GTTAAATAGTGTTCTTGGAG	576 bp	[49]
tetM-2		CTAAGATATGGCTCTAACAA		
tetL-1	*tet*[L]	CATTTGGTCTTATTGGATCG	456 bp	[49]
tetL-2		ATTACACTTCCGATTTCGG		
tetO-1	*tet*[O]	GATGGCATACAGGCACAGAC	615 bp	[49]
tetO-2		CAATATCACCAGAGCAGGCT		
pvl-FP	*lukF-PV*	GCTGGACAAAACTTCTTGGAATAT	83	[47]
pvl-RP		GATAGGACACCAATAAATTCTGGATTG		
SEA-3	*sea*	CCTTTGGAAACGGTTAAAACG	127 bp	[50]
SEA-4		TCTGAACCTTCCCATCAAAAAC		
SEB-1	*seb*	TCGCATCAAACTGACAAACG	477 bp	[50]
SEB-4		GCAGGTACTCTATAAGTGCCTGC		
SEC-3	*sec*	CTCAAGAACTAGACATAAAAGCTAGG	271 bp	[50]
SEC-4		TCAAAATCGGATTAACATTATCC		
SED-3	*sed*	CTAGTTTGGTAATATCTCCTTTAAACG	319 bp	[50]
SED-4		TTAATGCTATATCTTATAGGGTAAACATC		
SEE-3	*see*	CAGTACCTATAGATAAAGTTAAAACAAGC	178 bp	[50]
SEE-2		TAACTTACCGTGGACCCTTC		
Sak-1	*sak*	AAGGCGATGACGCGAGTTAT	223 bp	[12]
Sak-2		GCGCTTGGATCTAATTCAAC		
Chp-1	*chp*	GAAAAAGAAATTAGCAACAACAG	410 bp	[12]
Chp-2		CATAAGATGATTTAGACTCTCC		
Scn-1	*scn*	AGCACAAGCTTGCCAACATCG	258 bp	[12]
Scn-2		TTAATATTTACTTTTTAGTGC		
1095F	*spa*	AGACGATCCTTCGGTGAGC	variable	[51]
1517R		GCTTTTGCAATGTCATTTACTG		

4.4.3. Detection of Methicillin Resistance Genes and Other Antimicrobial Resistance Genes

The presence of the *mecA* and *mecC* gene was checked by using previously described PCR protocols [46,47] and primers (Table 6). The erythromycin resistance encoding genes (*ermA, ermB* and *ermC*) and tetracycline resistance encoding genes (*tetK, tetL, tetM* and *tetO*) were detected using previously described protocols and primers shown in Table 6 [48,49].

4.4.4. Detection of PVL and SE Genes

PCR with gene-specific primers (Table 6) was performed according to previously described protocols to detect genes encoding several virulence factors of *S. aureus* including *lukS-PV* and *lukF-PV* genes encoding PVL [47], immune evasion cluster genes (IEC) *sak*, *scn* and *chp* [12] and staphylococcal enterotoxins: *sea*, *seb*, *sec*, *sed*, and *see* [52]. PCR conditions were as described in the previous protocols, respectively [12,47,52].

4.4.5. *Spa* Type Determination

Spa typing was performed using a previously described PCR protocol [51] with the primer sets shown in Table 6. Sequencing of the protein A gene (*spa*) was performed using BigDye terminator method with an ABI PRISM 3730XL DNA analyser (Applied Biosystems, Foster City, CA, USA). The DNA sequence reads were edited using the ATGC Software. The sequences obtained were then submitted to the online tool Center for Genomic Epidemiology to determine the *spa* types [53].

4.5. Data Analysis

Data from the study were entered into Microsoft™ excel spreadsheets, and then analysed using IBM SPSS version 25 (IBM Corp) and R. The frequencies of *S. aureus* in farm and abattoirs were presented as percentages and 95% confidence intervals. P values of less than 0.05 were considered statistically significant. The chromatograph sequence files of the isolates were analysed with the online tool Centre of Genomic Epidemiology (CGE) for *Spa* typing to determine the *spa* types [53].

5. Conclusions

The presence of *S. aureus* was high among pigs in Zambia. Furthermore, the detection of *S. aureus* on the hands and in nasal cavities of farm and abattoir workers is a public health concern. Although MRSA was only phenotypically detected, the significance of MSSA as a potential source from which MRSA can arise cannot be overlooked. The presence of plasmid mediated resistance genes and immune evasion genes among the isolates warrant continuous monitoring of *S. aureus* in this sector to combat *S. aureus* infections using a "One Health" approach in Zambia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antibiotics11070844/s1, Table S1: Samples for which more than one *S. aureus* colony type was isolated; Table S2: Vancomycin MICs of *S. aureus*; Table S3: Characteristics of *S. aureus* Isolates Sequenced for *Spa* typing. Reference [44] is cited in the supplementary materials.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the University of Zambia Biomedical Research Ethics Committee (UNZABREC) of The University of Zambia (protocol code 613-2019 approved on 20 January 2020) for studies involving humans or animals.

Informed Consent Statement: Written informed consent was obtained from all farm and abattoir workers as well as owners of the facilities involved in the study.

Data Availability Statement: Sequences for the *spa* types have deposited in the DDBJ.

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