MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL RESISTANCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PIGS AND HUMANS IN KOGI STATE, NIGERIA

BY

Otalu Jnr OTALU

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,

FACULTY OF VETERINARY MEDICINE,

AHMADU BELLO UNIVERSITY,

ZARIA

FEBRUARY, 2021

MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL RESISTANCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PIGS AND HUMANS IN KOGI STATE, NIGERIA

BY

Otalu Jnr OTALU
{B.Sc, 2006 (A.B.U); M.Sc, 2011(A.B.U)}
(P16VTPH9078)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA

FEBRUARY, 2021

DECLARATION

I declare that the work in the thesis entitled, **"MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL RESISTANCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*ISOLATED FROM PIGS AND HUMANS IN KOGI STATE, NIGERIA"**has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine under the supervision of Prof. J.K.P. Kwaga, Prof. E.C. Okolocha and Prof. B.O. Olayinka. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma in any university.

Otalu Jnr OTALU---------- ----------
Signature Date

CERTIFICATION

This thesisentitled **"MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL RESISTANCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PIGS AND HUMANS IN KOGI STATE, NIGERIA''** by Otalu Jnr OTALU meets the regulations governing the award of Doctor of Philosophy Degree in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

Prof. J.K.P. Kwaga ....
(Chairman, Supervisory Committee) Signature Date

-

Prof. E.C. Okolocha ....
(Member, supervisory committee) Signature Date

Prof. B.O. Olayinka ....
(Member, supervisory committe) Signature Date

Prof. M. Bello ....
Head of Department Signature Date

Prof. Sani A. Abdullahi ....
Dean, School of Post Graduate Studies Signature Date

DEDICATION

This work is dedicated to my beloved family.

ACKNOWLEDGEMENTS

My profound gratitude goes to the Almighty God, whose grace has brought me thus far.

I acknowledge with thanks, Prof. J. K. P. Kwaga, Chairman of my supervisory team, for mentoring me and access throughout the entire duration of this work.

I also acknowledge Prof. E. C. Okolocha and Prof. B. O. Olayinka, who are members of my supervisory team for their contributions towards the successful completion of this work as well as their friendship.

I appreciate the effort of the HOD and the entire staff of Vetrinary Public Health and Preventive Medicine Department towards the actualization of this project..

I thank immensely Dr. P. Zanger of Heidelberg University, Germany for his useful contributions towards the design of this work. Duly acknowleged also is Mr. O. Owonipa, who was a self voluntered personal assistant and driver especially during the sampling stage of this work.

Specially acknowledged is Dr. A. Moodley of University of Copenhagen, Denmark. Thank you for believing in this work and for making your lab available for me to carry out the molecular aspects of this study.

I could not have come all along without the assistance of great friends– Mr. C. Akpala, Mr. C. Osinlu, Mr. A. H. Tarfa, Mr. S. Ogbole, Mr. M. Odoba, Mr. K. Iwuanyanwu, Dr. U. Ghali, Dr. U. Akpabio, Mr. F. Babatunde, Mr. M. Olaomi, Engr. K. Melikam, Engr. A. Odeyele, and others too numerous to list who have been a source of inspiration and encouragement, I say God bless you all.

ABSTRACT

Staphylococcus aureus is a common cause of serious community and health care-associated infections and limited antimicrobial treatment options for methicillin resistant *Staphylococcus aureus* (MRSA) infections make their management challenging. This study was conducted to determine the molecular characteristics and antimicrobial resistance of MRSA isolated from pigs and humans in Kogi State, Nigeria. Using standard microbiological techniques, 680 nasal swab samples collected from 425 pigs, 55 'in-contact' and 200 'non-contact' humans were analysed. Risk factors associated with MRSA carriage among humans was also determined using a structured questionnaire. *Staphylococcus aureus*was recovered from 20 (4.7%) of the 425 pigs screened and 19 (7.5%) from humans. Six (2.4%) of the human *S. aureus* isolates were recovered from 55 'in-contact' while 13 (5.1%) were recovered from 200 'non-contact' humans. In addition 42 other isolates belonging to other *Staphylococcus* species (*S. hyicus, S. scuiri, S. intermedius, S. lentus, S. chromogens, S. warnari, S. lugdunensis, S. saprophyticus, S. haemolyticus, S. xylosus*) were recovered in the study. All of the *S. aureus* isolates (39; 100%) irrespective of source were resistant to β-lactams only. Also all of the *S. aureus*isolates had MIC values >4mg/ml for oxacillin and were termed methicillin resistant. Five (6.2%) and 3 (3.7%) of the other *Staphylococcus* species isolates from pigs and humans respectively showed multi-drug resistance patterns. Age and sex were found to be factors associated with nasal carriage of *S. aureus*within the study population. MRSA werecharacterized by a multiplex PCR to detect *spa*, *mec*A, *luk*F-PV(a marker of the Panton-Valentine leucocidin, PVL), *scn* and aCC398 specific band. Direct sequencing ofthe purified PCR reaction amplicon was used for *spa* typing. Four isolates (one pig, one pig-contact human fromthe same pig farm, one non-pig-contact human from the samedistrict, and one pig-contact human from a different district)were selected for further SCC*mec*, multilocus sequence typing and whole genomesequencing (WGS).All 39 MRSA isolates belonged to *spa* type t1603- sequence type (ST) 88, and *mec*A was associated with a SCC*mec*IVa element in

38/39 of the isolates. Core genome SNP analysis revealed high genetic similarity between strains (3-11 SNP differences), despite the temporal (2 year gap) and geographic (165km) differences between isolates. Furthermore, the MRSA isolates in this study form a distinct clade when compared with other African MRSA ST88 isolates. Nineteen (95%) of the porcine strains were positive for *scn*suggesting a possible human origin and that pigs were either transiently contaminated by humans or result of a very recent human-to-pig transmission event.

TABLE OF CONTENT

Content	Page
Cover Page - - - - - - - - - -	i
Title Page - - - - - - - - - -	iii
Declaration - - - - - - - - - -	iv
Certification - - - - - - - - - -	v
Dedication - - - - - - - - - -	vi
Acknowledgements - - - - - - - - - -.	vii
Abstract - - - - - - - - - -	viii
Table of Contents- - - - - - - - - -	x
List of Tables - - - - - - - - - -	xiv
List of Figures- - - - - - - - - -	xv
List of Plates- - - - - - - - - -	xvi
List of Appendices - - - - - - - - - -	xvii
Abbreviations - - - - - - - - - -	xviii
1.0 INTRODUCTION - - - - - - - -	-1
1.1 Background of the Study- - - - - - - -	-1
1.2 Statement of Research Problem - - - - - -	-3
1.3 Justification of the Study - - - - - - -	-4
1.4 Aim of the Study - - - - - - - -	-5
1.5 Objectives of the Study - - - - - - -	-5
1.6 Research Questions - - - - - - -	-6
2.0 LITERATURE REVIEW - - - - - - -	-7
2.1 The organism *Staphylococcus*- - - - - -	-7

2.2 *S. aureus* genome- - - - - - - - -8

2.2.1 The coregenome- - - - - - - - -9

2.2.2 The accessory genome- - - - - - - - -9

2.3 *Staphylococcus aureus* Transmission - - - - - -10

2.4 Overview of *S. aureus* Virulence Factors and Clinical Features- - -10

2.5 Methicillin Resistant *Staphylococcus aureus* (MRSA)- - - -13

2.5.1 Hospital-associated and Community-associated MRSA- - - -15

2.5.2 Livestock- associated MRSA - - - - - - -16

2.6 SCC*mec*- - - - - - - - - -17

2.7 MRSA in Animals - - - - - - - -18

2.7.1 MRSA in pigs - - - - - - - -18

2.7.2 MRSA in cattle - - - - - - - -19

2.7.3 MRSA in horses - - - - - - - -19

2.7.4 MRSA in poultry - - - - - - - -20

2.7.5 MRSAin companion animals - - - - - - -20

2.7.6 MRSA in food of animal origin - - - - - -21

2.8 Transmission of MRSA Between Humans and Animals - - -22

2.9 Laboratory Detection of MRSA - - - - - -23

2.9.1 Phenotypic methods of detecting methicillin resistance - - -24

2.9.2 Molecular Typing of MRSA - - - - - - -27

2.10 Antibiotic Resistance Mechanisms - - - - - -31

2.10.1 Mechanism of action of and resistance to penicillin - - - -32

2.10.2 Mechanism of resistance to methicillin and oxacillin - - - -33

2.10.3 Mechanism of resistance to vancomycin - - - - -34

2.10.4 Mechanism of resistance to drugs that bind to the 30S ribosomal subunit -35

2.10.5 Mechanism of resistance to drugs that bind to the 50S ribosomal subunit -36

2.10.6 Mechanism of resistance to drugs that inhibit nucleic acid synthesis - -37

2.10.7 Mechanism of resistance to drugs that are metabolic pathway inhibitors -38

2.11 Population Structure of MRSA in Animals in Africa - - - -38

2.12 Control of antibiotic resistance & spread of resistant *Staphylococcus aureus*-38

3.0 MATERIALS AND METHODS - - - - - -41

3.1 Materials - - - - - - - - -41

3.2 Methods - - - - - - - - - -41

3.2.1 Study Area - - - - - - - - -41

3.2.2 Isolation of methicillin resistant *Staphylococcus aureus* from pigs and Humans - - - - - - - - - -43

3.2.3 Determination of methicillin resistance and susceptibility of isolates to commonly used antibiotics - - - - - - -48

3.2.4 Molecular characterization of *S. aureus* isolates - - - -50

3.2.5 Data analysis - - - - - - - - -54

4.0 RESULTS - - - - - - - - -55

4.1 Detection of *S. aureus* in Pigs and Humans in Kogi State - - -55

4.2 Susceptibility of *S. aureus* Isolates to 12 Commonly Used Antimicrobials -55

4.3 Occurrence of *Staphylococcus* Species in Pigs and Humans and Antimicrobial Resistance - - - - - - - - -56

4.3.1 Prevalence of *Staphylococcus* species in pigs and humans - - -56

4.3.2 Antibiotic susceptibility of *Staphylococcus* species to 12 commonly used antimicrobials- - - - - - - - - -56

4.4 Molecular Characteristics of MRSA Isolates - - - - -56

5.0 Discussion - - - - - - - - -73

6.0 Conclusion and Recommendations - - - - - -82

6.1 Conclusion - - - - - - - - -82

6.2 Recommendations - - - - - - - -83

REFERENCES - - - - - - - - -85

LIST OF TABLES

Table	Title	Page
3.1	Primer sets used for detection of *mec*A, *spa, pvl* and *scn* genes -	-51
4.1	Isolation rates of *S. aureus* from pigs, pig farm workers and non-pig contact Humans in Kogi State - - - - - - - -	-58
4.2	Prevalence of *S. aureus* among different groups of pigs and humans in Kogi State- - - - - - - - - -	-60
4.3	Prevalence of *S. aureus* in humans with respect to demographic and risk factors- - - - - - - - - -	-61
4.4	Detection of *S. aureus* in pigs and humans in Kogi State based on three different methods- - - - - - - - -	-62
4.5	Antibiotic resistance profiles of *S. aureus* from pigs and humans in Kogi State- - - - - - - - - -	-64
4.6	Isolation of other *Staphylococcus* species from pigs and humans in Kogi State - - - - - - - - - -	65
4.7	Distribution of other *Staphylococcus* species among pigs and humans	66
4.8	Antibiotic resistance of other staphylococci species form pigs and humans in Kogi State to commonly used antibiotics - - -	67
4.9	Antibiotic resistance profiles of other *Staphylococcus* species from pigs and humans in Kogi State - - - - - -	68
4.10	Single nucleotide polymorphisms in MRSA isolates relative to the ST88 reference genome, AUS0325- - - - - -	-71

LIST OF FIGURES

Figure	Title	Page
3.1	Map of Kogi State showing Local Government Areasand sampling locations - - - - - - - - - -	42
4.1	Geographic locations of pig farms sampled - - - - -	59
4.2	Antibiotic resistance of *S. aureus* isolates from pigs, contact humans and non contact humans in Kogi State - - - - - - - -	63
4.3	Metadata describing sample origins, sampling location in Kogi State and isolation date. Phylogeny based on an alignment of 1,248 SNPs - -	70
4.4	Comparative analysis of the Nigerian and Ghanaian MRSA ST88. Core genomephylogeny based on an alignment of 1492 SNPs - - - - 72	

LIST OF PLATES

Plate	Title	Page
1	Multiplex PCR amplification of *spa*, *mec*A, and *scn* genes in MRSA isolates- - - - - - - -	-69

LIST OF APPENDICES

Appendix	Title	Page
1	Questionnaire to access information from humans - - -	-113
2	Questionnaire to access information from pig farms - - -	-115
3	Questionnaire to access information on pigs - - - -	-120

ABBREVIATIONS

agr	Accessory gene regulator
CA-MRSA	Community Associated methicillin resistant *Staphylococcus aureus*
CC	Clonal complex
ccr	Cassette chromosome recombinase
CHIP	Chemotaxis inhibitory protein
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase negative staphylococci
HA-MRSA	Hospital associated methicillin resistant *Staphylococcus aureus*
IEC	Immune evasion cluster
IgG	Immunoglobulin G
LA-MRSA	Livestock associated methicillin resistant *Staphylococcus aureus*
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrophotometry
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MSCRAMMS	Microbial surface components recognizing adhesive matrix molecules
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin susceptible *Staphylococcus aureus*
ORSAB	Oxacillin resistance screening agar base
PBP	Penicillin binding protein
PFGE	Pulse field gel electrophoresis
PVL	Panton Valentine Leukocidin
PTSAgs	Pyrogenic toxin superantigens
S. aureus	*Staphylococcus aureus*

SaPI	*S. aureus* pathogenicity island
spa	Staphylococcal protein A
SCC*mec*	Staphylococcal cassette chromosome *mec*
scn	Staphylococcal complement inhibitor
WGS	Whole genome sequencing

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Staphylococcus aureus(*S. aureus*) is a Gram-positive bacteria found as commensal flora of humans and various animalspecies (Vanderhaeghen*et al*., 2010). Multiple body sites canbe colonized in humans, but the anterior nares are the most frequently colonized sites (Wertheim *et al*., 2005).It can cause conditions ranging from minor skin infections to systemic, life-threatening illnesses, such as pneumonia, osteomyelitis, and endocarditis (Thammavongsa*et al*., 2015). Methicillin resistant *Staphylococcus aureus* (MRSA) evolved from methicillin-susceptible *S. aureus* (MSSA) by acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*)elementscontaining a *mec* gene (*mec*A, more rarely *mec*C), which codes for an additional penicillin bindingprotein that has low affinity for β-lactam antibiotics and therefore mediates resistance to nearly allcompounds from this antibiotic class (besides ceftobiprole and ceftarolin) (Cuny *et al*., 2015).The SCC*mec* cassette is a mobile genetic element consisting of the *mec* gene complex, the cassette chromosome recombinase (*ccr*) gene complex, and the junkyard regions (j-region) (Zhang *et al*., 2005). Based on the variability and combinations of these genetic elements, molecular typing of MRSA strains has revealed up to thirteen distinct SCC*mec* types (I–XIII) (Lakhundi and Zhang, 2018). SCC*mec* types I, II and III are characteristic hospital associated MRSA (HA-MRSA) strains and cause resistance to multiple classes of antibiotics due to the additional drug resistance genes integrated into SCC*mec.* SCC*mec* types IV, V and VI are generally associated with community acquired MRSA (CA-MRSA), which in most cases, do not contain additional antimicrobial resistance genes, and therefore, cause only β-lactam antibiotic resistance (Mediavilla *et al*., 2012).

Swine production plays a vital role in food security, poverty eradication, and employment generation in Nigeria (Adebisi, 2008).The pig population in Nigeria which stands at 7 million (Umeh *et al*., 2015) contributes significantly to national animal protien intake. The epidemiology of *S. aureus* in swine held little interest, until the sequence type (ST) 398 lineage of MRSA was found to be prevalent in pigs and pig farmers in the Netherlands in 2004 (Voss *et al*. 2005).MRSA ST398 has since been detected in multiple livestock species and in many countriesas well as in people in contact with MRSApositiveanimals(Petinaki and Spiliopoulou, 2012; Kock *et al*., 2013; Sahibzada *et al*., 2017). Besides direct exposure to livestock, potential sources fortransmission include farm visits or having a family member employed in farming (Bisdorff *et al*., 2011). There is the potential risk of MRSA introduction from the animal reservoirs into hospitals with humans serving as vectors. Meat and other foods of animal origin can also be contaminated and serve as sources of human infection (Smith and Pearson, 2011). Nasal carriage of *S. aureus*in humans has been reported across various settings in Nigeria (Nwankwo and Nasiru, 2011; Ayepola *et al*., 2018), and infections associated with *S. aureus*has also been reported in various parts of the country (Ayepola *et al*., 2015; Abdullahi and Iregbu, 2018; Abubakar and Suleimn, 2018).In the last decade, MRSA Sequence Type (ST)398 has found an ecological niche in pigs, horses, cattle and poultry industry, although other MRSA lineages (e. g. ST1, ST5, ST9, ST97, ST130, ST433), have been identified in farmed animals worldwide (Guardabassi*et al*., 2013). All these lineages are currently termed "livestock-associated MRSA" (LA-MRSA). In contrast to HA-MRSA and CA-MRSA, severe infection due to LA-MRSA in humans is rare (Cuny *et al*., 2009; Aires-de-Sousa, 2017). Only isolates belonging to ST5-MRSA-IV and ST88-MRSA-IV, and not ST398-MRSA, have so far been found in African livestock (Fall *et al*, 2012).

1.2 Statement of Research Problem

Intensive husbandry due to the frequency and intensity of antibacterial treatments is thought to facilitate clonal selection of antibiotic resistant strains (De Faveri*et al*., 2014).Further more,the close proximity of animal and human hosts has in turnincreased opportunities for the transmission and exchange of microbial flora (Guinane*et al*, 2011; Sarowska *et al*., 2019). Farm hygiene has also contributed to MRSA emergence, occurrence and spread in animals. MRSA is now responsible forconsiderable mortality, morbidity and health-careexpenditure both in hospital and community settings worldwide (Dweba *et al*., 2018; Kluytmans-Vandenbergh and Kluytmans, 2006).It is estimated that MRSA infections within the health care setting alone affected more than 150,000 patients annually in the European Union, with an additional cost of 380 million Euros (Kock*et al*., 2010). In the United States of America, 80,461 invasive MRSA infections and 11,285 related deaths occurred in 2011, and an estimated annual burden of between $1.4 billion and $13.8 billion was attributed to community-acquired MRSA (CDC, 2013; Lee *et al*., 2013a).Wherever there is aninterface between different host species, the opportunity exists for bacterial exchange and in mostcases, these exchanges lead to transient infections which are short lived due to the failure ofthe*S. aureus*strain to establish transmission pathways in the new host species (Shepheard *et al*., 2013).Bacterial transfer fromfarm animals as well as colonization or infection of humans is of significant concern tohuman medicine. The reverse scenario, which is often overlooked, warrants consideration.Humans may represent an important source of new bacterial strains, which can causedisease in livestock and, as such, pose a potential threat to food security (Fitzgerald, 2012). Typically, pigs carry MRSA without symptoms, although there are rare reports of clinical disease (van der Wolf *et al*., 2012). Thus the main problem with MRSA in pigs is the risk of spread to humans (Unnerstad *et al*., 2017). The zoonotic transmission of MRSA from colonized pigs to humans via direct animal contact, environmental

contamination or meat calls for concern. There is paucity of information on the occurrence and epidemiology of MRSA in animals and especially in pigs in Nigeria and Kogi State in particular.

1.3 Justification of the Study

MRSA is fast becoming a global epidemiological scourge with serious therapeutic implications as well as a potential cause of great economic loss in pig production systems (FAO, 2016). Pigs have been recognised as a reservoir of livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in Europe, Asia and North America. However, little is known about the presenceand distribution of MRSA in the Nigerian pig population.

Several molecular typing methods are widely accepted andimplemented for the characterization of *S. aureus* isolates. DNAsequence-based typing methods generate unambiguous andportable data, allowing the comparison of data from different geographic locations. Currently, multilocus sequence typing, basedon the sequencing of internal fragments of seven housekeepinggenes generating a sequence type (ST), and *spa* typing, based on thesequencing of a single gene (*spa* coding for protein A), are thesequence-based methods of choice to determine the geneticrelatedness of *S. aureus isolates*(Aires-de-Sousa, 2017). Detailed molecular characterization of clinical *S. aureus* isolates from Africa has not been accomplished.In the few studies which have been conducted to characterize ST and *spa* types in Africa, the majority of MRSA isolates has been assigned to ST8 and ST88 (Breurec*et al*., 2011; Fall *et al*., 2012; Schaumburg*et al*., 2015). To date there has been little genomic characterization of methicillin resistant*S. aureus* (MRSA) that are prevalent in swine populations in Nigeria. The occurrence and changes in types of *S. aureus*, clonal identities, and their geographic spread is essential for the establishment of adequate infectioncontrol programmes (Shittu *et al*., 2011). Therefore, the data to be generated from this study, which

seek to determine the concurrent detection of MRSA in pigs and humans could provide insight into the prevalence of MRSA in pigs and in humans with and without exposure to pigs in the study area and to implement appropriate control measures, particularly for those at risk.

1.4 Aim of the Study

This study was aimed at determining the prevalence of MRSA in pigs and humans in contact with and without contact with pigs in Kogi State and to molecularlycharacterize MRSA isolates.

1.5 Objectives of the Study

The objectives of this study were to;

i. determine the presence of MRSA in pigs, persons exposed to pigs and those without exposure to pigs.

ii. determine the susceptibility of the isolates by the standard disk diffusion methodto a panel of commonly used antibiotics.

iii. determine the M.I.C. of oxacillin using E-test among the MRSA isolates.

iv. determine risk factors associated with MRSA colonization in humans.

v. detect the presence of *spa*, *pvl*, *mec*A, *scn*, and CC398 specific genes in MRSA isolates using multiplex PCR.

vi. determine the genetic relatedness of the MRSA isolates using *spa*-typing and SCC*mec* typing, multilocus sequence typing and whole genome sequencing of 4 representative MRSA isolates.

1.6 Research Questions

i. What is the prevalence of MRSA in pigs and humans exposed to pigs and those without exposure to pigs in Kogi State?

ii. Do human MRSA isolates have the same resistance profiles as animal isolates?

iii. What are the risk factors associated with MRSA colonization in humans?

iv. Do MRSA isolates from pigs and humans possess *mec*A, *scn*, *pvl* and *spa* genes?

v. Are there similarities in genetic composition of MRSA isolates of porcine and human origin?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Organism *Staphylococcus*

*Staphylococcus*is a genus of Grampositive, facultatively anaerobic bacteria, which commensally inhabits the skin and mucosal membranes of humans and animals. They are approximately 1 μm in size and grow in pigmented yellow orwhite colonies (Kasper *et al*., 2016).Depending ontheir ability to coagulate plasma, staphylococci are classified as either coagulase-positive orcoagulase-negative (Pyorala and Taponen, 2009).*Staphylococcusaureus* (*S. aureus*) a coagulase positive cocci, is the foremost important and most studied human pathogen of thestaphylococcal genus and it is capable of causing a broad variety of infections rangingfrom minor skin infections to serious conditions such as osteomyelitis, bacteremia andinfective endocarditis (Tong *et al*., 2015). However, *S. aureus* is usually a harmless coloniser of about onethird of healthy humans without causing infections (Gorwitz *et al*., 2008).*S. aureus* has a clonal population structure and it does not undergo extensive genomicrecombination but rather, random nucleotide mutations and horizontal gene transfer are the mainfactor for diversity (Holmes and Zadoks, 2011). Morphologically, *S. aureus* can be observed as a golden yellow medium-size colony on solid media such as nutrient agar and can cause β-hemolysis on sheep blood agar (Sasaki *et al*, 2007). The production of golden pigmentation of *S. aureus* colonies is closely related to the presence of carotenoids which was previously reported as virulence factor protecting *S. aureus* from the immune system (Liu *et al*., 2012). Though *S. aureus* is non-spore forming, it is highly resistant to various environmental conditions. It is able to grow at a wide range of temperatures, from 7.0-48.5°C with an optimum growth temperature of 30-37°C. *S. aureus* is also tolerant of osmotic stress and highly salt resistant, as it can withstand salt at concentrations of 15% NaCl and readily grows at concentrations up to 10% NaCl. Additionally, it is tolerant to a pH range of 4.2-9.3,

with an optimum pH of 7.0-7.5 (Crossley *et al*., 2009). These characteristics allow *S. aureus* to grow in a variety of substrates, including various food products and epidermal tissue. Other coagulase-positive staphylococci that include *S. hyicus* and *S. flexneri have* been implicated in human and animal infections (Tormo *et al*., 2005; Coimbra-e-Souza*et al*., 2017).

The coagulase negative species (CoNS) constitute the majority of the genus and are opportunistic pathogens that can be isolated from the skin or mucous membrane as part of normal flora of humans and animals (He *et al*., 2013).Often dismissed as culture contaminants (Becker *et al*., 2014), they are becomingincreasingly recognized as pathogens with almost half of all CoNS identified till date implicated in human infections (Lowy, 2013).*S. saprophyticus* isa common cause of community-acquired urinary tract infection.*S. epidermidis* causes nosocomial bacteraemia and prostheticdevice-related infections. Other species that have been associatedwith infection include *S. haemolyticus*, *S.lugdunensis* and *S. schleiferi*. These organismsadhere to prosthetic material and become embedded in anexopolysaccharide matrix, forming a biofilm. The biofilmprotects the organisms from phagocytic cells and reduces thepenetration of antibiotics (Archer *et al*., 2011).

2.2 *S. aureus* Genome

In silico analysishas revealed that the *S. aureus* genome is approximately 2.8 Mb in size, exhibits a GC content of~33%, and includes approximately 2,800 proteinencoding sequences. The regions of staphylococcal genomes are well conserved, with many massive sequence blocks showing high variability (Diep *et al*., 2006; Ali *et al*., 2019). In order to describe evolutionary changes and phylogenetic relationships, the *S. aureus* genome can be divided into the core-genome and the accessory genome.

2.2.1The coregenome

The core genome accounts for approximately 85% of the entire *S. aureus* genome and includes both stable core genes (~75%) and accessory genes (~10%) (Mccarthy *et al*., 2012a). The stable core genome is highly conserved among all *S. aureus*strains and consists of genes associated with housekeeping functions such as central metabolism, growth and survival (Lindsay, 2010). Genetic diversity in the core-genome derives from single nucleotide variations (SNVs) and insertions/deletions. When a SNV becomes fixed within a population, it is referred to as a single nucleotide polymorphism (SNP). SNPs which do not change the encodedamino acid (synonymous SNPs) are phenotypically silent, while non-synonymous SNPs may result in functional effects. The latter includes mutations which may be retained under the selective pressure exerted on clinical strains by the use of antibiotics (Lindsay and Holden, 2006). Insertions/deletions can also have significant functional effects on a strain. They range in size from one nucleotide to several kilobases and may include complete genes or operons (Lindsay and Holden, 2006).

2.2.2 The accessory genome

The accessory genome constitutes the remaining ~15% of the *S. aureus* genome, and contains genes associated with non-essential functions such as virulence and resistance (Mccarthy *et al*., 2012a). These genes are typically located on mobile genetic elements (MGEs) which can be transferred horizontally between cells via conjugation, transduction or transformation (Lindsay, 2014). Five distinct types of MGE have been described in *S. aureus*, namely, lysogenic bacteriophage, plasmids, *S. aureus* pathogenicity islands (SaPIs), staphylococcal cassette chromosome (SCC) elements and transposons. Lysogenic bacteriophages and SaPIs generally harbour virulence genes, while resistance genes typically reside on plasmids, transposons and SCC elements (Lindsay and Holden, 2006).

2.3*Staphylococcus aureus* Transmission

Human carriers are the most important source of *S. aureus* among the human population, although animals also constitute a significant reservoir. *Staphylococcus aureus* is typically transmitted between individuals via direct physical contact, or through an environmental intermediary, such as a door handle (Sollid *et al*., 2014). Acquisition may also occur via droplets in the air, following dispersion from the nose or mouth of a carrier (Gehanno *et al*., 2009). In hospitals, patients are at risk of acquiring *S. aureus* from other patients, visitors and healthcare workers (Sollid *et al*., 2014).*S. aureus* persists in the environment, in milk, on the animal's body, and also in humans in livestock breeding (Harrison *et al*. 2014). During slaughter, sources of microbiological contamination on carcass may come from the hide or gastrointestinal tract of the animal or from the slaughter plant environment, including facilities and personnel (Lin *et al*., 2009). *S. aureus* has colonized diverse animal species following host-switching events and subsequent adaptation through acquisition and/or loss of mobile genetic elements (MGEs) as well as further host-specific mutations, allowing it to expand into new host populations. Close contact between animals and humans facilitates such host-switching events (Haag *et al*., 2019).

2.4 Overview of *S. aureus* Virulence Factors and Clinical Features

S. aureus virulence is dependent on a diverse array of virulence factors giving the bacteriacapacity for colonization, tissue destruction and invasion, immune evasion, and long termsurvival (Tuchscherr *et al*., 2019). To adhere to mucosal and other epithelial surfaces such as the anterior nares, axillae,oropharynx and perineum, *S. aureus* uses a number of cell surface bound adhesive proteinstermed MSCRAMMS (Microbial Surface Components Recognising Adhesive Matrix Molecules) (Ghasemian *et al*., 2015).These are anchored to the cell wall peptidoglycan by sortase and enable binding to a numberof different molecules such

as fibronectin (FnbA), fibrinogen (ClfA) and collagen (Cna) (Vazquez *et al*., 2011). Colonisation of the anterior nares in particular is facilitated by clumping factorB binding to epithelial cells, with the recently characterized SasX protein also having a role in some strains (Li *et al*, 2012; Li *et al*., 2015; Bakthavatchalam*et al*., 2019). Progression from colonisation to disease is usually dependent on breaches of the physicalbarrier provided by intact skin through abrasions, surgery or hair follicle trauma such thatbacteria can invade into tissue directly or via the bloodstream to establish abscesses (Cheng *etal*, 2011). Once in the bloodstream, MSCRAMMs are understood to play a vital role inendothelial adherence, along with wall teichoic acid (WTA) and a secreted, non-anchored,adhesive protein, the extracellular adherence protein (Eap) (Edwards and Massey, 2011).

Endovascular infections such as endocarditis are initiated by adherence to, often damaged,endothelium mediated by the fibronectin binding proteins with subsequent accumulation offibrin, erythrocytes, platelets and other cellular debris forming vegetations (Beynon *et al*, 2006; Tong*et al*, 2015). Following endothelial adherence at organ sites and other tissues, extravasation occurs via anumber of mechanisms including endocytosis, transport within professional phagocytes andperhaps paracytosis by interfering with tight junctions between cells (Edwards and Massey,2011). Staphylococci are notorious for their capacity to survive within various cell types, evenneutrophils, through inhibition of phagosome lysosome fusion, neutralization of phagosomecontents by staphyloxanthin, resistance to lysozyme and subsequent escape into the cellcytoplasm (Foster, 2005; Clauditz *et al*, 2006; Uribe-Querol and Rosales, 2017). Once bacteria have gainedaccess to tissues, *S. aureus* has a veritable arsenal of enzymes which can destroy surroundingcells and structures including proteases, lipases, hyaluronidase, nuclease, staphylokinase andhaemolysins (Burlak *et al*, 2007). Iron scavenging and uptake is also essential at this stage tomeet the nutritional requirements of the pathogen (Skaar *et al*, 2004). The inflammationgenerated by cell damage

would then generate a wider immune response, resulting in the influxof neutrophils to the site. Though *S. aureus* is adept at survival within neutrophils, however, numerous other strategies are employed to preventphagocytosis. The prophage encoded virulence factors CHIP (Chemotaxis inhibitory protein) and SCIN (Staphylococcal complement inhibitor) interfere withchemotaxis (van Wamel*et al*., 2006; Amissaha*et al*., 2017) and complement activationrespectively. Clumping factor A triggers staphylococcal agglutination and adherence to fibrin,presumably making opsonisation and phagocytosis more difficult (Higgins *et al*, 2006).ProteinA binds to the Fc region of IgG conferring additional antiphagocytic protection by providing a‘camouflage’ of ineffectively bound immunoglobulins (Kobayashi and DeLeo, 2013). Capsularpolysaccharide is also well characterized for its antiphagocytic properties (Mohamed*et al*., 2019).In addition to these defensive strategies, some strains of *S. aureus* are capable of directly lysingneutrophils through production of leukocidins and phenol soluble modulins (Bhattacharya *et al*., 2018).

While evasion of the immune response gives bacteria more time to replicate and disseminatethroughout the host, subversion of the immune response is a more pernicious strategy whichdirectly contributes to clinical syndromes. The best known example of this is the toxic shocksyndrome toxin 1 (TSST-1), although similar toxic shock syndromes can be caused by the otherpyrogenic toxin superantigens (PTSAgs) such as the staphylococcal enterotoxins. Superantigensinterfere with antigen presentation to cause a pathogenic ‘cytokine storm’ which leads to thefever, haemodynamic instability and the characteristic widespread blanching rash associatedwith toxic shock (Dinges *et al*., 2000). Abscess formation appears to be another form of immune subversion relying, in part, on theimmune response. Developing abscesses appear to be made up of a central nidus of replicating staphylococci surrounded by a fibrin deposit, promoted by coagulase and clumping factor Aand an outer ring of necrotic neutrophils (Cheng *et al*, 2011). Bacterial replication will

continuewithin an abscess capsule until rupture and dissemination of bacteria into the bloodstream or skin surface occurs, thus continuing the cycle. Abscess formation is the central component ofmany of the disease processes associated with *S. aureus* including skin and soft tissueinfections, cavitating pneumonia, osteomyelitis and other end organ abscesses such as in thekidneys and brain. The capacity for the bacteria to continually metastasize and disseminateback into the bloodstream at a later time is associated with recurrent infections. Bacterialendocarditis is a notorious source of continual bacteraemia and frequently occurs inconjunction with infections at other sites such as septic arthritis, discitis and infectedpulmonary emboli (Beynon *et al*, 2006). *S. aureus* tend to persist at former abscess sites through formingbiofilms within cellular debris or surviving within host cells. Biofilm formation promotes longtermbacterial survival by providing protection from the immune system and inhibitingpenetration of antibiotics (O'Neill *et al*, 2008). The formation ofbiofilms on the surface of medical devices, tissue engineering constructs (i.e, artificialheart valves) or damaged host tissue is associated with increased mortality in patients anddifficulty of antibiotic treatment. The last stage of biofilm, 'dispersion' is controlled bythe mechanism of quorum sensing regulated by *agr* genes (accessory gene regulator), isable to spread highly populated *S. aureus* inside of biofilm and cause bacteremia such asendocarditis, arthritis or osteomyelitis (Boles and Horswill, 2011).

2.5 Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin was put to clinical use in 1959 as the first semisynthetic β-lactamaseresistantpenicillin. It was designed for the treatment of β-lactamase-producing staphylococci.Within a year of its introduction, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged (Harkins *et al*., 2017). These strains were first reported in 1961 in the United Kingdom and acquired resistance via the incorporation of a *mecA* gene into the

chromosome at a specific site. The *mec*A gene is a DNA segment of 2.1 kb that is non-native to *S. aureus* and is inserted in a large block of exogenous DNA, known as the staphylococcal cassette chromosome mec (SCC*mec*) (Katayama *et al*. 2000).Genetic analyses of the first MRSA by multi-locus sequence typing (MLST) demonstrated that they were sequence type (ST) 250, a lineage belonging to clonal complex (CC) 8 and carried the type I SCC*mec* element. After emerging in the UK, this first epidemic MRSA clone (ST250-MRSA-I) spread across Europe during the 1960s and 70s, but by the late 1980s had become less prevalent and is now rarely reported (Enright *et al*., 2002; Harkins *et al*., 2017). *mec*Aencodes an alternative penicillin-binding protein (PBP2a orPBP2′) that has low affinity for β-lactam antibiotics and therefore mediates resistance to nearly allcompounds from this antibiotic class (besides ceftobiprole and ceftarolin) (Cuny *et al*. 2015). MRSA has nowglobally become a well-known risk of hospital admission in immunocompromisedpeople and a huge problem faced by numerous infection control teams.Intercountry and intercontinental spread of MRSA has occurred via infected patientsand staff. New strains of MRSA have continued to emerge and decline for decades forunknown reasons. Although global in its distribution, MRSA was once confined to onlyhospitals and health care settings. However, it has now invaded the community andemerged in livestock as well. Hence, the ever-increasing burden of MRSA infections hasled to the increased use of vancomycin, the last resort for the treatment of MRSAinfections, to which MRSA was susceptible. However, this increased use of vancomycinhas now resulted in the emergence of vancomycin-intermediate *S. aureus* (VISA) andvancomycin-resistant *S. aureus* (VRSA) strains. These strains are inhibited only at concentrationsabove 4 to 8 µg/ml, and 16 µg/ml, respectively (Tenover*et al*., 2001; McGuinness *et al*., 2017), causing a fear ofthem becoming completely resistant.

Homologues of *mec*A (*mec*C, *mec*B, and *mec*D) have recently been reported in *S. aureus* (Becker *et al*., 2018). *S. aureus* containing *mec*Chas been found in a range of multilocus

sequence type lineagesisolated from humans and other animal species (Cuny *et al*., 2011; García-Álvarez *et al*., 2011;Laurent *et al*., 2012; Harrison *et al*., 2013).Although *mecA* and *mecC*encoded proteins possess different biochemical properties,*mec*Cnevertheless confers methicillin resistance. Hence, the need for the correctidentification of these strains as MRSA is particularly important from a clinical diagnosticperspective (Lakhundi and Zhang, 2018).

2.5.1Hospital-associated and community-associated MRSA

MRSA infections were traditionally hospital-associated (HA-MRSA) and have been a major publichealth issue for the last 50 years causing severe nososcomial infections worldwide. However in the past decade and half, an increased number of infections due to community-associatedMRSA (CA-MRSA) have been reported in Europe and the US (DeLeo *et al*., 2010; Verkade and Kluytmans, 2013). The rapid dissemination of CA-MRSA strains among general populations in diverse communities has resulted in increasing reports of outbreaks worldwide (Kong *et al*., 2016).A number of factors have been found to be associated with a higher risk for nosocomial acquisition of MRSA and includesthe use of antimicrobials, underlying diseases, prior hospitalization, surgery, duration of hospitalization, central venous catheterization, endotracheal intubation, enteral feeding, admission to intensive care unit, nursing staff workload and compliance with hand disinfection procedures (Shuping *et al*., 2017).CA-MRSA infections are mainly seen in healthy individuals with no recentcontact to the healthcare system. Currently, more than 20 genetic lineages have been reported to be associated with CA-MRSA worldwide (Witte, 2009; Mediavilla *et al*., 2012).Typically CA-MRSA is genetically distinct from HA-MRSA (Diep and Otto, 2008; Kong *et al*., 2016). Theyfrequently carry the SCC*mec* type IV or V and they often harbour the Panton-Valentine leukocidin (PVL) that is associated with skin and soft tissue infections (DeLeo *et al*., 2010). The SCC*mec* types IV and V are smaller in comparison to the SCC*mec* types I, II and IIIoften

carried by HA-MRSA, which may facilitate a lower metabolic cost and a more efficientlytransferred element between CA strains(Lakhundi and Zhang, 2018).

The genetic differences seen between HA-MRSA and CA-MRSA correlate with the hostenvironment (Diep and Otto, 2008; David and Daum, 2010). HA-MRSA often infects immunecompromisedindividuals in hospital settings where a high antibiotic selective pressure are present,whereas CA-MRSA, infecting healthy individuals, requires fewer resistance genes, additionalvirulence factors, and a growth advantage in the more competitive environment outside the hospitalsetting (David and Daum, 2010).

2.5.2 Livestock- associated MRSA

In the last decade LA-MRSA has emerged as a challenge in some domestic animals such as horses (Guerin *et al*., 2017), cattle (Locatelli*et al*., 2017), and pigs (Reynaga *et al*., 2016). Pigs are the most commonly reported amongst livestock for LA-MRSA carriage that could transfer MRSA to people in direct contact (EFSA, 2009). Therefore, LA-MRSA is reported as an occupational health-hazard in many countries, especially those where pig production is common. A prevalence of LA-MRSA carriage as high as 86% has been reported in pigs in the Netherlands (Morcillo *et al*., 2015), and a similar proportion of carriage has also been reported in people working in direct pig contact in Germany (Cuny *et al*., 2009).

Populationgenetic studies have identified certain genotypes to be associated with specific host species.Multilocus sequence type ST71, ST97, ST126, ST133 and ST151 are often found among ruminants, and are the major cause of bovine mastitis, whereas ST5 is often associated with poultry (Lowder*et al*., 2009;Holmes and Zadoks, 2011; Moodley *et al*., 2012). The major MRSA sequence type colonizing pigs is ST398.Other STs described in pigs are ST541, ST1965, ST1966,ST1967, and ST1968 (Porrero *et al*., 2012). However, it has beenshown that there is a specific subgroup of human ST398strains, different from LA-

MRSA ST398, that can bereadily differentiated by SNP detection and the presence orabsence of scn and *tet*M(Butaye *et al*., 2016).

2.6 SCC*mec*

SCC*mec* elements range in size from approximately 20-60 kb, and integrate into the chromosome at the 3′ end of *orfX*, which encodes a ribosomal transferase (Shore and Coleman, 2013). Each SCC*mec* element includes a *mec* gene complex, a cassette chromosome recombinase (*ccr*) gene complex, and three joining (J) regions. The *ccr* complex comprises one or two *ccr* genes of four main types (*ccrA*, *ccrB, ccrC* and *ccrAA*) and various allotypes, and surrounding open reading frames (ORFs). While the function(s) of these ORFs remains unclear, the *ccr* genes are known to encode serine recombinases that mediate site- and orientation-specific integration into and excision from the chromosome. The *mec* gene complex includes *mecA* or *mecC*, regulatory genes *mec*R1and *mec*I, when present and associated insertion sequences. Five different classes (A-E) of the *mec* gene complex have been identified in *S. aureus* to date, four of which include *mecA* (classes A-D) and one of which includes *mec*C(class E). The J regions are the genomic regions surrounding the *ccr* and *mec* gene complexes. These are non-essential components of the cassette which are highly variable and which may harbour additional antimicrobial resistance determinants (Ito *et al*., 2009). To date, 13 different SCC*mec* types (I-XIII) have been identified in *S. aureus*, each of which harbours a unique *ccr* and *mec* gene complex combination (Ito *et al*., 2009; Wu *et al*., 2015; Baig *et al*., 2018). Many different subtypes have also been defined, based on variation in the J regions (Monecke *et al*., 2016). Sequence-based studies indicate that both SCC and the *mec*Acomplex evolved largely in *Staphylococcus sciuri*, a CoNS species prevalent in animals (Tsubakishita *et al*., 2010; Rolo *et al*., 2017). Evidence suggests that *mec*Aevolved from a harmless core gene (*mec*A1) encoding the penicillin binding protein D, and

subsequently acquired regulatory genes to form the *mec* complex. The *mec* complex was then integrated into SCC, which had evolved independently, at *orfX* (Rolo *et al*., 2017). This formed SCC*mec*, which diversified in other CoNS species (e.g. *Staphylococcus epidermidis, Staphylococcus hominis* and *Staphylococcus haemolyticus*), and was eventually acquired by *S. aureus* via horizontal gene transfer (Miragaia, 2018).

2.7MRSA in Animals

Staphylococcus. aureus is not only a colonizer of the upper respiratory tract mucosa of all mammals investigated so far, it has also been found in natural populations of birds, industrially- raised poultry and probably also in snakes (Christiane *et al*., 2010). Cases of MRSA infections in several animal species have been reported since mid 1970s (Walther *et al*., 2008)and among companion animals. MRSA has been isolated from horses, pigs, cattle, sheep,rabbits, birds,cats and dogs (Saleha and Zunita, 2010; Zomer *et al*., 2017).

2.7.1 MRSA in pigs

In 2005, pigs were reported as an animal reservoir of *S. aureus* in France, including MRSA ST398(Armand-Lefevre*et al*., 2005). MRSA ST398 was subsequently reported in different European countries, with higher rates in countries with higher densities of pig farming (EFSA 2009). The emergence of this strain wasalso reported in pigs and pig farmers in the Netherlands (van Duijkeren*et al*., 2008; Dierikx*et al*., 2016), Denmark (Schulz *et al*., 2019), Germany(Kock *et al*., 2013), Portugal (Conceicao*et al*, 2017), Spain (Reynaga *et al*., 2016) and Canada (Khanna*et al*., 2008; Narvaez-Bravo*et al*., 2016). Other ST types, human-associated MRSAST1 and cattle-associated ST97 in finishing holdings, and ST9 in the same animal species inEurope (Battisti*et al*., 2010), CC9, and CC49 in Switzerland (Oppliger*et al*., 2012) have also been reported. In Italy, CC1and CC97 lineages among MLST types that do not belong to CC398 were significantly high inthe primary production of pigs (Battisti*et*

al.,2010). A different swine-associated MRSA strain,CC9, is circulating among pigs and pig farmers in China and Malaysia (Neela*et al*., 2009; Zhou*et al*., 2018). In Thailand, porcineMRSA ST9 isolates had a unique *spa* type (t337) and SCC*mec* type (SCC*mec* IX) that were differentfrom other LA-MRSA ST9 strains found in Asian countries (Larsen *et al*., 2012). In contrast to studies in Europe, North America and Asia, the predominant strain as found in the majority of African pigs belonged to ST88. (Fall *et al*., 2012; Otalu *et al*., 2018).

2.7.2 MRSA in cattle

After the first report of MRSA in bovine mastitis in Belgium (Devriese *et al.* 1972), increasing numbers of cases of MRSA isolates in cows or milk have been reported from all over the world. Some MRSA lineages reported in cows are thought to be bovine-specific, such as CC130, ST425 and CC1943 but the detection of ST398 as a major clone in milk recovered from cases of bovine mastitis in Belgium confirmed that this clone has also emerged in cattle (Bardiau *et al*., 2013). Recently, a highly divergent *mec*Agene (*mec*C) was detected in isolates from both humans and dairy cattle. This *mecC* gene has been identified and localised to SCC*mec* type XI (García-Álvarez *et al*., 2011; Ito *et al*., 2012). MRSA strains shown to carry *mecC* typically belong to lineages reported in cattle (CC49, CC130, CC425, CC599 and CC1943), which suggests an animal origin (Petersen *et al*., 2013; Shore and Coleman, 2013). Besides cattle, *mec*C has also been found among other farm animals such as sheep and rabbits as well as among different companion animals (dogs, cats, horses, guinea pigs and rabbits) (Walther *et al*., 2012; Haenni *et al*., 2017).

2.7.3 MRSA in horses

The first report of an outbreak of MRSA infections in horses in a veterinary hospital came from the United States in 1999, and was followed by descriptions of clusters of MRSA infections in equine hospitals in Canada and in Central Europe a few years later (Cuny *et al*.,

2016). The majority of the Canadian MRSA isolates from horses and staff, has typically been identified as Canadian epidemic MRSA-5, equivalent to "USA500", belonging to ST8, a putatively equine clinic associated strain, which accounted for nearly 10% of MRSA in Canadian hospitals by the end of the 1990 (Simor*et al*., 2002). Other studies have demonstrated that horses are colonized and infected by MRSA clones that commonly belong to the sequence type (ST)8 and related STs within the clonal complex (CC)8 (Pantosi, 2012; Carfora *et al*., 2016). More recently, studies from Europe and Canada reported horses to be colonized by MRSA clones belonging to ST398, designated livestock-associated (LA)-MRSA, which is primarily recognized as a colonizer of pigs and pig farmers (Loncaric*et al*., 2014; Vinczee*t al*., 2014; Guerin *et al*., 2017).

2.7.4 MRSA in poultry

MRSA carriage in poultry was first reported in South Korea though not confirmed byPCR (Lee, 2003). Another study demonstrated the presence of MRSA in broilers atslaughterhouses in The Netherlands (Mulders *et al*., 2010). In Belgium, MRSA have beenrecovered from broilers occasionally (Persoons *et al*., 2009; Pletincks*etal.*, 2011; Nemeghaire*et al*., 2013). MRSA was rarely found in diseased breeder turkeys in France (Argudín *et al*.,2013) though a German study found 18 out of 20 fattening flocks of turkeys positive forMRSA (Richter *et al*., 2012). Otalu *et al*. (2015) reported the detection of *mec*A in MRSA isolate recovered from a slaughtered broiler chicken in Nigeria.

2.7.5 MRSAin companion animals

The first report on the emergence of MRSA in dogs came from the United Kingdom (Rich *et al*., 2005). Cats and dogs represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans (Guardabassi*et al*., 2004; Damborg *et al*., 2016). Cross transmission in veterinary

hospitals seems likely, and the finding of MRSA ST22, which represents the most frequent HA-MRSA in United Kingdom, suggested an origin from human hospitals (Loffler *et al*., 2005). MRSA ST22 from wound infections of dogs was also reported from Germany (Strommenger *et al*., 2006). Studies conducted in recent years have shown that staphylococci isolated from cats and dogs have become resistant to methicillin (Loeffler and Lioyd, 2010; Haenni *et al*., 2017).

2.7.6 MRSA in food of animal origin

Isolation of MRSA from food of animal origin has been reported in several studies from different continents, mostly from meat. For example, in the Netherlands, MRSA was isolated from 267 of 2217 (11.9%) raw meat samples from the retail trade, namely from beef (10.6%), veal (15.2%), lamb and mutton (6.2%), pork (10.7%), chicken (16.0%) and turkey (35.3%) and the majority of the isolates (85%) belonged to LA-MRSA ST398 (de Boer *et al*., 2009). A recent study from Brazil detected MRSA in beef (23.3%), chicken (23.3%), pork (37.5%), and also in fish (30%) samples but no further features of the isolates are known (Costa *et al*., 2015). In Africa, two MRSA were reported in Tunisia from raw meat samples and were assigned to ST30-V and ST398-IVa (Chairat *et al*., 2015). MRSA has also been isolated from fish (Hammad *et al*., 2012), although infrequently. Food of animal origin intended for human consumption is often contaminated with LA-MRSA ST398, particularly meat (de Boer *et al*., 2009), suggesting that LA-MRSA isolates present in the live animal may be later detected on the carcasses. Moreover, CA-MRSA and HA-MRSA may also be present, suggesting that humans are a likely source of contamination, probably during slaughtering or processing. In the USA, 22 (18.3%) MRSA isolates were detected among 120 retail meat samples from 30 grocery stores and the isolates belonged to two clones, USA300 (ST8-IV-PVL) currently widely spread in both the community and hospital settings, and USA100 (ST5-II) a frequent

clone circulating in hospitals in the USA (Pu *et al*., 2009). In Canada, MRSA was identified in 31 (7.7%) of 402 retail pork samples and the isolates belonged not only to LA-MRSA ST398, but also to two nosocomial Canadian clones (CMRSA-5; ST8 and CMRSA2; ST5) (Weese *et al*., 2010). Despite the fact that food may be contaminated by MRSA, to date there is no evidence that consumption increases the risk of human colonization or infection (Aires-de-Sousa, 2017).

2.8 Transmission of MRSA Between Humans and Animals

The role of animal populations in the transmission of pathogens to humans is obligatorily dependent not only on the possibility of transmission from animals tohumans, but also on the possibility of transmission between animals.Dogs and cats were initially indicated as a reservoir for MRSA years ago (Scott *et al*., 1988; Cefai *et al*., 1994). Thestrains involved in canine and feline cases are usually similar to those infecting humans,and the most obvious explanation for this veterinary problem is that the pets acquiredthe resistant bacteria from humans and that these strains can also be passed back from animals to humans (van Duijkeren*et al*., 2004; Loeffler*et al*., 2005). The zoonotic transmission of MRSA in horses was demonstrated in studies for which identical or a closely related strain of the organism was isolated from horses and horse personnel that differed from common human isolates (van Duijkeren*et al*., 2010). The presence of MRSA ST398 in environmental swabs indicates that direct contact withanimals is not necessarily needed for transmission to humans (van den Broek*et al*., 2008).

MSSA and MRSA human-to-animal transmission has been suggested in some African studies (Nagel *et al*., 2013; Schaumburg *et al*., 2015). 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 (Schaumburg *et al*., 2012; Schaumburg *et al*., 2015). Moreover,

MRSA CC88 strains with the same spa-type (t189) were identified in humans and sheep in Côte d'Ivoire (Fall *et al*., 2012). In the same vien, Otalu *et al*. (2018) found identical MRSA CC88 strains with identicsl spa-type (t1603) in human and pigs in Nigeria. 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 (Schaumburg *et al*., 2012). In addition, in the study of Nagel *et al*. (2013), interspecies transmission of a widely spread human associated *S. aureus* CC72 strain (spa-type t148) was observed; this strain was found as colonizer agent in three gorillas and caused infection in one of them. Strains with the same spa-type were identified in chimpanzees in contact with the infected gorilla. These strains presented only one different band in Pulsed-Field Gel Electrophoresis (PFGE) compared with the strains obtained from gorillas (Nagel *et al*., 2013). On the other hand, animal-to-human transmission might have occured in one study performed in Tunisia where 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).

2.9 Laboratory Detection of MRSA

Phenotypic detection of MRSA has been problematic ever since its discovery in the 1960s. Heterogenous expression of methicillin resistance complicates detection of these strains in clinical microbiology laboratories. Methods successful for other antibiotics can be insensitive for detection of methicillin resistance. Resistance can easily be missed if inappropriate or improperly stored β-lactam antibiotic is tested, if the incubation is too brief (e.g. 18 hours instead of 24 hours), if temperature of 37 ^{0}C is used or if the inoculum is too low (Panda*et al*, 2016). On the other hand, methods to enhance sensitivity in detection of methicillin resistance, such as increase salt concentration of media and prolonged (48hours) incubation,

can lead to erroneous categorization of susceptible strains as resistant. Genotypic detection of *mec*Agene or its product, PBP-2a is however now considered the 'gold standard' for MRSA confirmation.

2.9.1 Phenotypic methods of detecting methicillin resistance

The methods for detection of methicillin resistance most commonly used in the clinical laboratories are the disk diffusion, broth dilution, agar screen, Epsilometer test (E-test) and automated systems.

2.9.1.1 *Disk diffusion test*

Disk diffusion testing is widely used to detect methicillin resistance in staphylococci, and cefoxitin is currently considered thebest marker for *mec*A-mediated methicillin resistance (Bonjean *et al*., 2016). The Clinical and Laboratory Standards Institute specifies optimum temperature of 35 ^{0}C for testing of staphylococci to oxacillin and full 24 hours incubation. Recommended culture medium for disk diffusion susceptibility testing is Mueller-Hinton agar (Cookson and Philips, 1990) although Iso-Sensitest (O'Mahony *et al*., 2005) and Mannitol salt agar (Smyth *et al*., 2001) have been reliably used. Disk diffusion test can be performed using 1µg oxacillin disk, 1 µg nafcillin disk, 5µg methicillin disk and very recently 10µg or 30µg cefoxitin disk (Panda*et al*., 2016). Cefoxitin is considered as a better inducer of *mec*A gene expression than oxacillin or methicillin, and can be used to screen heterogeneous MRSA populations (Anand *et al*., 2009).Heterogeneous strains are composed of two populations of bacteria; one is relatively susceptible and other highly resistant population. Thus only few (one in 10^4 -10^6) of them express the phenotype (Datta *et al*., 2011).

2.9.1.2 *Broth microdilution test*

The standard broth microdilution test uses bacterial inoculum of approximately 5 x 10^5 CFU/ml and cation-supplemented Mueller Hinton broth containing 2% sodium chloride to determine the minimum inhibitory concentration (MIC) of oxacillin for the isolate. Incubation is at 35 ^{0}C for 24 hours and oxacillin, methicillin or nafcillin can be used butcephalosporins and cloxacillin should not be used because they give inaccurate results. Although addition of 5% NaCl may slightly improve detection of resistance; at this concentration, higher rates of borderline or falsely resistant results occur for susceptible strains. Incubation for 18 hr renders the test too insensitive and because of β-lactamase effect, incubation for 48 hr is not recommended (Andrews *et al*., 2000; Lallemand *et al*., 2016).

2.9.1.3 *Epsilometer test (E-test)*

In this method, an E-test strip containing graded concentration of β-lactam and other antibiotics is aseptically placed on an agar plate, which entire surface has been inoculated with a standardized inoculum (0.5 McFarland standard) of the bacterium. The agar plate is incubated for 18–24 hours at 35-37 ^{0}C and the MIC is read as the point of intersection between edge of zone of inhibition and the E-test strip. Oxacillin E-test for detection of methicillin-resistance in *S. aureus* has been standardized for use with Mueller-Hinton agar supplemented with 2% NaCl and incubation at 35 ^{0}C for full 24 hours but recently cefoxitin E-test strip, which has comparable sensitivity and specificity with oxacillin E-test, is performed with unsupplemented Mueller Hinton agar and incubation temperature of 35 ^{0}C or 36 ^{0}C for 18 hours (Skov *et al*., 2006). E-test method has been shown to have excellent correlation with broth microdilution and the agar screen method for the detection of MRSA.

2.9.1.4 *Agar screen test*

The agar screen test is performed by inoculating 10^4 CFU onto Mueller-Hinton agar supplemented with 4% NaCl containing 6 mg of oxacillin per ml. After a 24 hour-incubation at 35°C, the agar is inspected for growth. The presence of even one colony is indicative of resistance. The sensitivity of this method approaches 100% for the detection of methicillin-resistant *S. aureus* and 95% for the coagulase-negative strains (Chambers, 1993; Kumar *et al*., 2017).

2.9.1.5 *Chromogenic media*

The most frequent and laboratory verified selective chromogenic media include:

MRSASELECTis a selective chromogenic medium designed for isolation and direct identification of the methicillin resistant *Staphylococcus aureus* strain (MRSA). Pink colonies grow within 18-24 hours and all the other microorganisms are inhibited. The test specificity is 99.8 % and its sensitivity is 98.9 % (Nsira *et al*., 2006; Brennan *et al*., 2016).

Oxacillin Resistance Screening Agar Base (ORSAB) is intended as a medium for the screening for methicillin resistant *Staphylococcus aureus* (MRSA) directly from routine swab samples. The screening of patients and staff for the early detection of MRSA colonization is essential if epidemics are to be prevented. ORSAB is a novel medium which uses aniline blue to detect mannitol fermentation creating intense blue colonies of presumptive MRSA (Kluytmans *et al*, 2002). Using this cultivation medium, after 24 hrs (48 hrs), Apfalter *et al*. (2002) found a sensitivity of 68 % and a specificity of 95%. Within the context of the detected values they drew attention to the existence of mannitol-negative *S. aureus* strains and mannitol-positive methicillin-resistant coagulase-negative staphylococci.

ChromID MRSAis a new chromogenic medium for the rapid and reliable screening of methicillin-resistant *Staphylococcus aureus* (MRSA). Direct identification of MRSA strains

is based on spontaneous green coloration of alpha-glycosidase-producing colonies and the presence of an antibiotic, cefoxitin. Immediate identification of MRSA (green colonies) is possible after 18-24 hours of incubation (Compernolle *et al*., 2007). Using this cultivation medium, Yang *et al*. (2010) found a sensitivity of 99% and a specificity of 99.5% after 24hr.

2.9.1.6 *Automated systems*

Several automated systems for detection of methicillin resistance, mostly based on broth microdilution methods, are in use. These include Autobac, Sceptor, AutoMicrobic, Vitek GPS-SA Card systems, BD Phoenix PMIC/ID-60 panel, MicroScan Pos MIC type 31 panel and Sensititre system (Putland and Guiness, 1985; Kolenda *et al*., 2017). Variable results have been reported regarding the accurate detection of methicillin-resistance with these commercial kits due to changes in software over time, variations in reference methodology used and poor definition as to the relative distribution of isolates expressing homogenous and various degrees of heterogeneous resistance (Sakoulas *et al*., 2001).

2.9.2 Molecular Typing of MRSA

MRSA can be typed by several different genetic typing methods, including Multilocus sequence typing (MLST), *S.aureus* protein A (*spa*) gene typing, SCC*mec* typing, and macro-restriction pulsed-field gel electrophoresis (PFGE) (EFSA, 2012). Molecular typing helps in the understanding of the epidemiology of MRSA as well as the molecular evolution of MRSA. Typing also helps in defining and comparing local or regional epidemics and potential sources of MRSA spread.

2.9.2.1 MLST

MLST is currently considered to be the gold standard in *S.aureus* molecular typing and is the preferred method for epidemiological studies because of harmonised protocols and nomenclature that allows high inter-laboratory reproducibility, and facilitates the comparison of a large number of STs globally (Williamson *et al*., 2015; Kinross *et al*., 2017). MLST classifies *S.aureus* on the basis of nucleotide allelic variation in the seven housekeeping genes, and identical isolates are assigned a ST (http://saureus.mlst.net). Closely related STs are grouped in a clonal complex (CC) (Enright *et al*., 2000) such as CC398. Almost all nosocomial MRSA strains detected worldwide belong to five clonal complexes (CCs): 5, 8, 22, 30 and 45(Lindsay, 2013).Although MLST is a widely used method, it may not be useful to investigate local outbreaks due to a lack of discriminatory power within the strains.

2.9.2.2Spa typing

Spa typing is another powerful technique used in epidemiological investigations. This typing is based on sequencing a single gene, *S.aureus* protein A gene, which is a highly polymorphic X-region containing different short tandem repeats whose combination results in different *spa* types (Harmsen *et al*., 2003). Different *spa* types can also be grouped within CCs. The method is supported by a central *spa* server (http://www.seqnet.org) which hosts thousands of *spa* types and allows for comparison of local MRSA epidemiology in relation to other regions and countries. *Spa* typing is thought to be a more useful tool for epidemiological investigation compared to MLST as it is more discriminatory, for example, a single ST398 contains different *spa* types like t011, t034, t108, and t01254 (Ballhausen *et al*., 2014). However, CC cannot always be deduced from *spa* type results because the same *spa* type can belong to unrelated lineages (Cuny *et al*., 2010).

2.9.2.3 SCCmec typing

The SCC*mec* is another way to group MRSA that is based on the variation across different SCC*mec* elements (http://www.sccmec.org). The SCC*mec* cassette is a mobile genetic element consisting of the *mec* gene complex, the cassette chromosome recombinase (*ccr*) gene complex (i.e. *ccr*A, *ccr*Bor *ccr*C), and the junkyard regions (j-region) (Zhang *et al*., 2005). Based on the variability and combinations of these genetic elements, molecular typing of MRSA strains has revealed up to thirteen distinct SCC*mec* types (I–XIII) (Lakhundi and Zhang, 2018) which differ from each other in antibiotic resistance markers. The SCC*mec* typing is of minimal epidemiological importance as they are unable to detect a single-nucleotide polymorphism that helps to distinguish the major type of MRSA, for example, LA-MRSA vs CA-MRSA. Therefore, SCC*mec* typing is usually used in conjunction with MLST such as ST398-V, ST93-IV.

2.9.2.4 PFGE

PFGE is a typing technique used for the separation of large DNA molecules using rare-cutting restriction enzymes, such as SmaI and Cfr9I, that produce different banding patterns on agarose gel in the presence of electric field with alternative voltage gradient (Bosch *et al*., 2010). PFGE can be a useful tool in a local outbreak investigation because of high discriminatory power (Argudín *et al*., 2010); however, it has limited use in epidemiological studies. As protocols and nomenclature are not harmonised for PFGE, it makes it difficult to compare inter-laboratory results (EFSA, 2012). Although PFGE typing is still used in the USA to describe the major MRSA clones (e.g. USA100, USA300); *spa* and MLST typing are now the most commonly used methods in Europe and other parts of the world (Kinross *et al*., 2017).

2.9.2.5 DNA microarray analysis

DNA microarray analysis, also known as biochip or DNA chip analysis, is a techniqueemployed for typing studies using a collection of DNA probes attached in an orderlyfashion to a solid surface. The probes provide a medium for matching known andunknown DNA samples, thereby automating the process of identifying unknowns (Ramsy, 1998).The probes could be oligonucleotides (up to 70-mers) or gene segments (PCR ampliconsof >200 bp) that can be used to detect the presence of complementary nucleotidesequences in a particular bacterial species. Based on the number of probes on thechip, one can distinguish low-density (hundreds) or high-density (hundred thousands)DNA microarrays. Microarrays represent a tool for spotting genes that aid as markers forspecific bacterial strains or to uncover allelic variants of a gene present in all strains of a particular species (Sabat *et al*., 2013).Previouslyreported microarrays could covalently immobilize probes for approximately 180genes and 300 alleles of *S. aureus* (Moneck*et al*., 2008; Sanchini*et al*., 2011). These include genes for antimicrobialresistance, toxins, surface components of microorganisms, and typing targets, givingthem the potential for simultaneous detection of large number of loci.Microarrays appear to be well suited for bacterial typing and are widely used for theanalysis of genomic mutations such as single-nucleotide polymorphisms (SNPs). Theyare an efficient tool for the detection of extragenomic elements, including uncommonantibiotic resistance genes, as well as complex patterns of virulence genes, and therefore have the potential to detect new epidemiological markers for clones (McCarthy *et al*., 2012b). Using microarray-based technology, regulons of regulatory systems of *S. aureus*which help us to better understand the molecular mechanisms of pathogenesis havealso been elucidated (Bischoff*et al*., 2004; Yan *et al*., 2012). Comparativegenomics using whole-genome microarrays, has revealed 10 major *S. aureus* lineages responsible for causing the majority of human infections (Lindsay *et al*., 2006).Each lineage has a unique combination of surface proteins and regulators called core variable (CV)

genes, and corresponds to the clonal complexes (CCs) identified by MLST typing. Each lineage has not evolved independently, but is the result of multiple recombinations of CV genes between lineages. Furthermore, all strains carry a variety of mobile genetic elements (MGE) encoding resistance and putative virulence genes, and there is much variation within lineages indicating frequent horizontal transfer (Sung *et al*., 2008).

2.9.2.6 Whole genome sequencing

Whole genome sequencing (WGS) is a technique used for typing of bacteria including *S.aureus*. The application of WGS has gained traction during the past decade as technological advances have improved the accessibility and cost-effectiveness of high-throughput sequencing (Trees *et al*., 2015). At present however, WGS still remains unaffordable in resource-limited settings. WGS can be utilized for species identification, evolutionary clustering (Price *et al*., 2012), identification of resistance (Zankari *et al*., 2012) and virulence markers (Joensen *et* al., 2014), just to mention afew of the many applications.WGS of MRSA isolates has revealed that these isolates aresignificantly similar to their MSSA counterparts from the same lineage, apart from theacquired SCC*mec* gene region (Miragaia, 2018). In addition, sequencing has revealed that the coregenome of each specific lineage is genetically highly variable, especially in the carriageof surface and regulatory genes. Within lineages, up to 20% of the genome consists ofmobile genetic elements (MGEs), which vary substantially between isolates, and thereis evidence of horizontal transfers of these elements between isolates of the same lineage (McCarthy and Linsay, 2010).

2.10Antibiotic Resistance Mechanisms

S. aureus develops resistance to antimicrobials by differentmechanisms. These mechanisms include limiting uptake of the drug, modification of the drug target, enzymatic inactivation of the drug, and active efflux of the drug. Depending on the antimicrobial involved, the bacteria

may use one or several of these resistance mechanisms. In particular, the localization of resistance genes on transferable genetic elements such as plasmids and transposons facilitates horizontal transfer of resistance between bacteria (van Hock *et al*., 2011).From an evolutionary perspective, bacteria use two major genetic strategies to adapt to antibiotic challenge namely, i) mutations in gene(s) often associated with the mechanism of action of the compound, and ii) acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer (Munita and Arias, 2016).Among all the antibiotic resistance achieved by *Staphylococcus aureus*, two most remarkable ones are methicillin and vancomycin resistance. The methicillin resistance was achieved by interspecies transfer of *mec*A gene from an ancestral *Staphylococcus*species to *S. aureus* mediated by a unique staphylococcal mobile genetic element. Vancomycin resistance was achieved by horizontal transfer of a plasmid-born *van*A-gene transposon from vancomycin-resistant *Enterococcus* to *S. aureus*across the genus barrier (Hiramatsu*et al*., 2014).

2.10.1 Mechanism of action of and resistance to penicillin

The major inhibitory target for *β*-lactam antibiotics in *S. aureus*is the bifunctional transglycolylase-transpeptidase PBP2(Giesbrecht *et al.* 1998; Walsh and Wencewicz,2016). The transglycosylase domainof the enzyme is responsible for transferring the disaccharidepentapeptide building block of peptidoglycan frommembrane-bound lipid II to growing polysaccharide chainswhile the transpeptidase (TP) domain cross-links the glycinecross-bridge of the fourth D-alanine of an adjacent chain.*β*-Lactam antibiotics bind at the active site serine of the TP in PBP2 by acting as structural analogues of D-Ala4-D-Ala5. The *β*-lactam bond is broken and a penicilloyl-O-serine intermediate is formed. The difference between this intermediate and the peptidoglycan acyl enzyme intermediate is that the former is extremely stable, taking 1–4 h for the addition of water to regenerate the active

site Ser and release the penicilloic acid product. This compares to themilliseconds for the natural reaction to be completed. In essence, the active site of the TP enzyme is blocked and peptidoglycan biosynthesis ceases (Walsh and Wencewicz,2016). The bactericidal effect is more complex than simply inhibiting PBP activity. The drug induces toxic malfunctioning of the wall biosynthetic apparatus which involves a continuing cycle of synthesis and autolysis (Cho *et al*., 2014). The *S. aureus* β-lactamase responsible for resistance to penicillin is a typical serine β-lactamase (BlaZ) that forms the same type of acyl enzyme intermediate as the TP of PBP2 (Lowy,2003). The catalytic difference between BlaZ and TP is the kinetics of deacylation. With BlaZ, the addition of water is rapid resulting in regeneration of the active site serine and release of the ring-opened penicilloic acid, a hydrolytic degradation product with no inhibitory activity.

The β-lactamase structural gene *blaZ* gene is carried by the transposon Tn*552* or Tn*552*-like elements (Jensen and Lyon, 2009). The transposon is either located on a large plasmid, the prototype of which is pI524, or is integrated into the bacterial chromosome. Expression of the enzyme is inducible, being controlled by the *Bla*I repressor and the *Bla*R sensor (Zhang *et al.*2001; Lowy,2003).

2.10.2 Mechanism of resistance to methicillin and oxacillin

The mechanistic basis of resistance to methicillin and oxacillin is through acquisition of a gene that encodes a homologue of the PBP2 called PBP2a (Peacock and Paterson,2015) which is not susceptible to drug action. This is because the active site serine of the TP of PBP2a is located in a deep pocket which is not accessible to β-lactams (Lim and Strynadka, 2002). The enzyme can therefore take over peptidoglycan (PG) biosynthesis if the housekeeping PBP2 TP is inactivated. In fact, the PBP2 transglycosylase activity is required for PG biosynthesis becausethe PBP2a moiety is non-functional (Pinho *et al*., 2001). Thus,

PG biosynthesis is a cooperative effort between the two proteins when the TP of PBP2 is inactivated.

When an MRSA strain is grown in the presence of *β*-lactams, the PG formed is poorly cross-linked. One of the consequences of this is that the peptidoglycan has stronger pro-inflammatory effects which could contribute to pathology during infection if the MRSA strain is exposed to *β*-lactams (Muller *et al.* 2015). The altered PG structure might also account for the inability of the global accessory gene regulator (Agr) to be induced in some MRSA strains (Rudkin *et al.*,2012).

PBP2a is encoded by the *mec*Agene which is located within a family of distinct but related staphylococcal cassette chromosome(SCC) elements (Jensen and Lyon,2009; Peacock and Paterson 2015). However, a distinct PBP2a called *mec*C with only 63% residue identity to *mec*A was discovered recently. It occurs predominantly in a single lineage of MRSA in Europe (Paterson *et al*., 2014).

2.10.3 Mechanism of resistance to vancomycin

The introduction of antibiotics into clinical setting is usually followed by the fairly rapid emergence of resistant bacteria. In this respect, vancomycin was somewhat atypical, because for almost 30 years following its introduction, resistance to this glycopeptide was reported only rarely and appeared to have little clinical significance. However, in the late 1980s, the emergence of acquired glycopeptides resistance was recognized for the first time (Leclercq *et al*., 1988; Johnson *et al*., 1990). This vancomycin resistance resulted from the production of modified peptidoglycan precursors ending in D-Ala–D-Lac (*van*A, *van*B, and *van*D) or D-Ala–D-Ser (*van*C, *van*E, and *van*G), to which glycopeptides exhibit low binding affinities. Vancomycin kills bacteria by binding to the C-teminal D-Ala–D-Ala residues of the peptidoglycan precursor lipid II in the cytoplasmic membrane to form a stable, non-covalent

complex, which prevents the use of the precursor for cell wall synthesis. The steric hindrance imparted by vancomycin may further inhibit the glycosyltransferase and transpeptidase activities of penicillin-binding proteins (PBPs) (Kahne *et al*., 2005).Classification of glycopeptide resistance is based on the primary sequence of the structural genes for the resistance-mediating ligases. The *van*A and *van*B operons are located on plasmids or on the chromosome, whereas the *van*C1, *van*C2/3, *van*D, *van*E, and *van*G have so far been found exclusively on the chromosome (Klare *et al*., 2003; Depardieu *et al*., 2007).

Vancomycin resistant *Staphylococcus aureus* (VRSA) arises when vancomycin resistance genes (e.g., the *van*A operon, which codes for enzymes that result in modification or elimination of the vancomycin-binding site) from vancomycin resistant *Enterococcus* (VRE) are transferred to *S. aureus*. The *van*A gene cluster is often located in the plasmidborne transposon Tn1546, which can transfer from vancomycinresistant *Enterococcus faecalis* to *S. aureus* and produce a VRSA isolate (Zhu *et al*., 2013). The mechanism for VRSA resistance is well-characterized. The coded product enables VRSA to replace the D-Ala–D-Ala terminal dipeptide with D-Ala–D-Lac dipeptide, thereby altering the binding target of vancomycin and often mediating high level resistance to vancomycin. Although VRSA may have been underestimated (Moravvej *et al*., 2013), the limited cases of VRSA suggest that *van*A-mediated vancomycin resistance is significant but has not evolved or quickly spread; this trend is probably caused by the high fitness cost imparted by *van*A-type transposon (Foucault *et al*., 2009).

2.10.4 Mechanism of resistance to drugs that bind to the 30S ribosomal subunit

The main aminoglycoside drugs used to treat *S. aureus* infections are gentamicin and tobramycin. Resistance to these drugs has been observed since the 1960's. The resistance mechanism is similar for all aminoglycoside drugs; production of an aminoglycoside-

modifying enzyme (AME) that chemically modifies the drug (quite often by transfer of an acetyl group), decreasing the drug's ability to bind to the 30S ribosomal subunit (Hauschild*et al*., 2008). The *aac* (6')-Ie + *aph* (2''), *ant* (4')-Ia, *aph* (3')-IIIa, and *ant* (6)-Ia genes that encode aminoglycoside-6'-N-acetyltransferase/2''-O-phosphoryltransferase, aminoglycoside-4'-O-nucleotidyltransferase I, aminoglycoside-3'-O-phosphoryltransferase III, and streptomycin modifying enzyme, respectively, are hence the most important genes in this regard. Resistance to gentamicin, kanamycin, and tobramycin in staphylococci is mediated by a bi-functional enzyme displaying AAC (6') and APH (2'') activity. The ANT (4')-IA enzyme inactivates neomycin, kanamycin, tobramycin, amikacin, and kanamycin, while the APH (3')-III, enzyme inactivates neomycin (Rahimi, 2016).

Resistance to the tetracycline drugs has also been observed for many years. The resistance mechanisms are similar for all bacteria. There are two mechanisms used: active efflux of the drug (the *tet*Kgene), and ribosomal protection by competitive binding to the 30S subunit by a ribosomal protection protein (RPP) encoded by the *tet*Mgene. Binding ofthe RPP interferes with the binding of the drug. The *tet* genes are contained within conjugative transposons that can be transferred horizontally and expressed in Gram-positive and Gram-negative bacteria. (Chopra and Roberts, 2001).

2.10.5 Mechanism of resistance to drugs that bind to the 50S ribosomal subunit

Lincosamides, streptogramins, phenicols, and pleuromutilins represent four structurally different classes of antimicrobial agents that inhibit bacterial protein synthesis by binding to particular sites on the 50S ribosomal subunit of the ribosomes. Members of all four classes are used for different purposes in human and veterinary medicine in various countries worldwide (Schwarz *et al*., 2016).Resistance to the lincosamides (clindamycin), macrolides (erythromycin), and streptogramins (quinupristin/dalfopristin) is mediated by the same

mechanism. Three related genes, *erm*A, *erm*B, and *erm*C, that encode for erythromycin resistance methylases are acquired via plasmids. These enzymes methylate a site on the ribosome resulting in a conformational change. This decreases the ability of these drugs to bind to the ribosome (Gul *et al*., 2008).

Resistance to chloramphenicol is mediated by acquisition of a plasmid that carries a chloramphenicol acetyltransferase gene (*cat* gene). The enzyme produced by this gene inactivates chloramphenicol by acetylation (chloramphenicol can no longer bind to the 50S subunit) (Schwarz *et al*., 2004).

The oxazolidinones are newer drugs, and the only one currently in use is linezolid. Resistance to linezolid has been foundto happen via two mechanisms: mutation of the ribosomal RNA (*rrn* gene), and methylation of the ribosomal RNA (*cfr*gene). The *cfr* gene which encodes an RNA methyltransferase is plasmid-borne, and has also been shown to confer resistance to chloramphenicol, clindamycin, and streptogramin drugs (Arias *et al*., 2008; Schwarz*et al*., 2016).

2.10.6 Mechanism of resistance to drugs that inhibit nucleic acid synthesis

The fluoroquinolone drugs act by inhibiting gyrase or topoisomerase IV which halts DNA replication and transcription. Resistance to these drugs is a result of mutations in either the GyrA subunit of gyrase (encoded by *gyr*Agene), or the GrlA subunit of topoisomerase IV (encoded by *grl*Agene) which reduce the ability of the drugs to bind to their targets.In addition, some moderate resistance to norfloxacin (and possibly ciprofloxacin) is caused by induction of the NorA efflux pump (*nor*Agene) (Winston and Chambers, 2009).

2.10.7 Mechanism of resistance to drugs that are metabolic pathway inhibitors

The combination drug sulfamethoxazole/trimethoprim targets the folate biosynthesis pathway in bacteria by competitive inhibition. Sulfonamides inhibit dihydropteroate synthase in a competitive manner with higher affinity for the enzyme than the natural substrate, p-amino benzoic acid. Agents such as trimethoprim act at a later stage of folic acid synthesis and inhibit the enzyme dihydrofolate reductase (Yoneyama and Katsumata, 2006). Resistance to sulfamethoxazole is due to a mutation in the *dhps* (dihydropteroate synthase enzyme) genewhich allows pABA to bind but has greatly reduced binding of sulfamethoxazole. Resistance to trimethoprim occurs by a mutation in the *dhfr* (dihydrofolate reductase enzyme) gene with reduced binding of trimethoprim (Houvinen, 2001; Kapoor*et al*., 2017).

2.11 Population Structure of MRSA in Animals in Africa

Most of the studies in which MRSA strains have been identified in animals in Africa has been by phenotypic detection and onlyin three of them there is information about the ST or the CC detected (Lozano *et al*., 2016). The CCs identified were CC5 in pigs (Fall *et al*., 2012, CC80 in sheep (Gharsa *et al*., 2012) and CC88 in pigs and sheep (Fall *et al*., 2012; Schaumburg *et al*., 2015; Otalu *et al*., 2018). All three CCs found in MRSA of animals in Africa have been frequently detected amonghuman clinical MRSA isolates in this continent (Abdulgader *et al*., 2015; Schaumburg *et al*., 2015). MRSA isolates with the *mec*C gene is yet to be reported in Africa.

2.12Control of Antibiotic Resistance and Spread of Resistant *Staphylococcus aureus*

Overuse of antimicrobial agents and poor compliance with infection-control measures have been identified as the major reasons for increasing trends in antimicrobial resistance. It is estimated that 25 to 50% of all antibiotic prescriptions are inappropriate as a result of

incorrect choices in drug, dose, or duration (Atif *et al*., 2019). Hospitals have considerable potential for the spread of antimicrobial resistance. Poor compliance with basic infection-control techniques is largely responsible for the dissemination of resistant organisms. Caregivers, for example, may not wash their hands or wear and discard gloves appropriately, and efforts to modify caregiver behavior in this regard have proved unsuccessful over the long term (Boyce, 1999; Ayukekbong*et al*., 2017).

Environmental contamination with organisms such as vancomycin resistant *Enterococcus* and MRSA also adds to the problem of resistance control. A large reservoir of patients colonized with resistant organisms may be difficult to identify in the absence of costly surveillance measurements, unless they have an active infection, which would then further increase the likelihood of person-to-person spread in the hospital setting (Rekha, 2001).

Expert groups agree that the major focus on MRSA control is the prevention of hand transfer of MRSA which can be achieved by performing hand hygiene directly before and after each and every physical contact with a patient or their immediate environment, before performing aseptic procedures, before the handling or manipulation of any invasive device including injection through venous catheters and when emptying drains or catheters. Also, hand hygiene is required before entering and upon leaving critical care units, isolation rooms, and open rooms used for cohorting of MRSA cases (Pittet *et al*., 2000; Marimuthu*et al*., 2014).

Specific measures to control and prevent MRSA in the hospital environment will include surveillance and screening of patients and hospital staff, patient isolation and cohorting as well as decolonization. Decolonization regimens vary, but usually include the application of topical mupirocin ointment to the anterior nares twice daily for 5 days along with adjunctive chlorhexidine baths, using either 4% chlorhexidine gluconate-containing soap or 2% chlorhexidine gluconate wipes (Daum, 2007). Skin to skin, and skin to formite contact are

important routes of CA-MRSA acquisition (Miller and Diep, 2008),measures to limit its spread therefore would focus on wound coverage, hand washing, laundering of clothes, linens, and towels,and environmental decontamination.

For farm environments, controlled use of antibiotics for either therapeutic or as growth promoters should be encouraged in a bid to check the spread of multi-resistant microorganisms as well as the use of protective clothing when working with livestock in the farm and routine use of disinfectant and proper cleaning of the environment.

Confronted with a shortage of new antimicrobials, current drugs must be used more prudently. Reducing and improving use can diminish resistance and permit a drug to resurface eventually as an effective therapy. The appropriate use of the antibiotics not only can help to reverse high resistance frequencies, but also can curb the emergence of resistance to newer agents (Barbosa and Levy, 2000; Lee *et al*., 2013b).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The following materials were used in this study;

Incubator, weighing balance, centrifuge, binocular microscope, autoclave, deep freezer, hot air oven, refridgerator, thermocycler (PCR) machine, heating block, water bath, electrophoresis machine, computer, photographic machine, votex machine, eppendorf centrifuge, bijou bottles, glass slides, petri dishes, test tubes, measuring cylinder, conical flasks, pipettes, bunsen burner,Mannitol Salt Agar (Oxoid Ltd, Basingstoke, UK), Trypticase Soy Broth (Oxoid Ltd, Basingstoke, UK), Oxidation-Fermentation Medium (Oxoid Ltd, Basingstoke, UK), Mueller Hinton Agar (Oxoid Ltd, Basingstoke, UK), distilled water,sucrose, glucose, mannitol, xylose, antibiotic disks and antibiotic disk dispenser (Oxoid Ltd, Basingstoke, UK).

3.2 Methods

3.2.1 Study Area

Sampling for this study covered 16 out of the 21 Local Government Areas of Kogi State.Kogi State is bordered by the states of Nassawara to the northeast; Benue to the east; Enugu, Anambra, and Delta to the south; Ondo, Ekiti, and Kwara to the west; and Niger to the north. Abuja Federal Capital Territory also borders Kogi to the north.Kogi state consists of a wooded savanna region bisected by the southward-flowing Niger River; the Benue River, a major tributary of the Niger, forms part of the state's northeastern border. It has its coordinates as longitude $E6^0\ 42^{|}$ (East of Greenwich meridian) and latitude $N7^0 30^{|}$ (North of equator) (Fig 3.1).

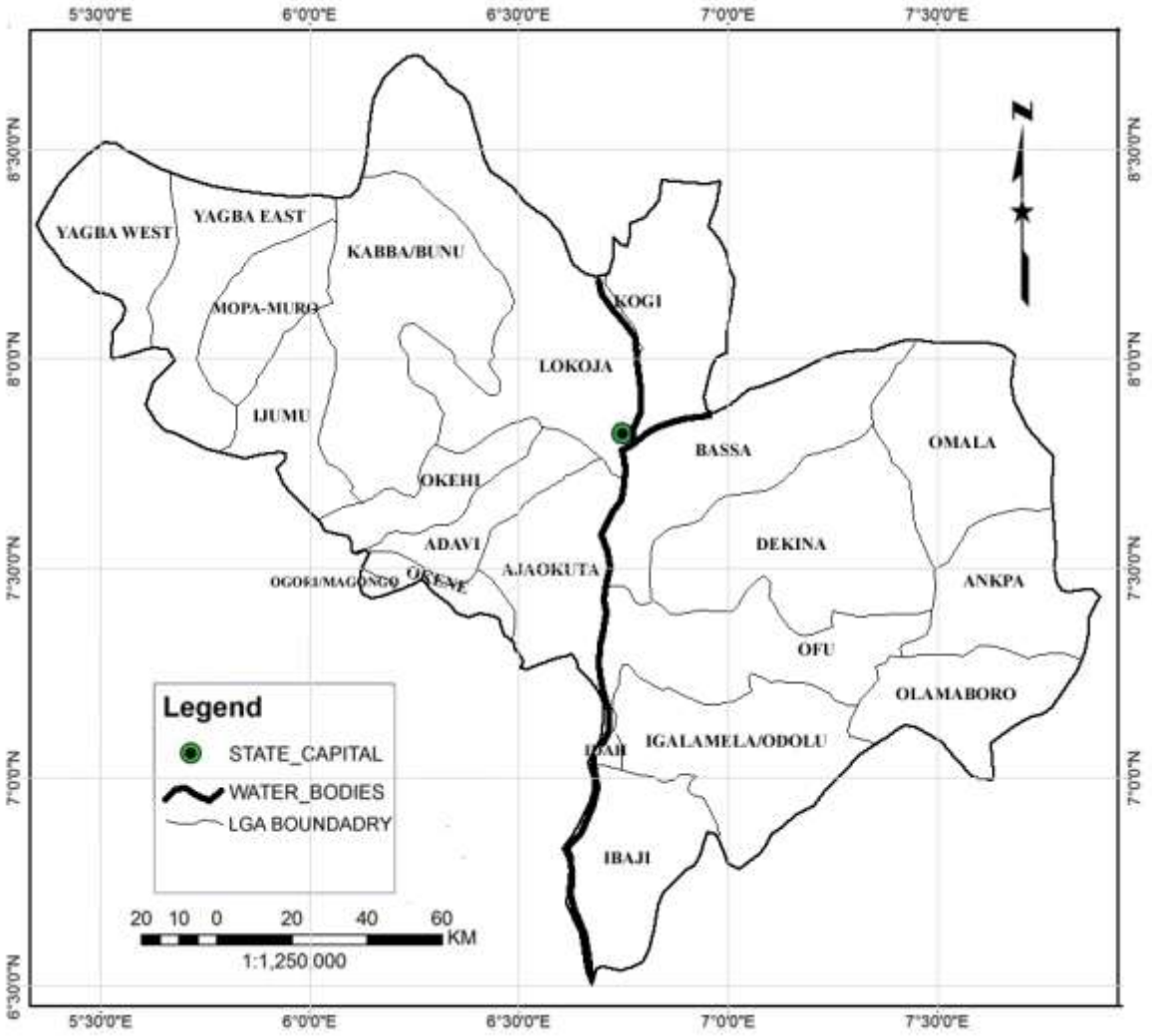


Fig 3.1 Map of Kogi State showing Local Government Areas and sampling locations

Source: Geographical Information Service centre, Kogi State University, Anyigba.

3.2.2 Isolation of methicillin resistant *Staphylococcus aureus* from pigs and humans

3.2.2.1 Study design and sample collection

A cross-sectional study was conducted using snowball sampling method to select 35 pig farms in the study area while systematic random sampling was employed in selecting pig samples using 5 interval. Nasal swab samples were collected from 35 pig farms housingbetween 28 and 275 pigs per farm. Between 10 and 15 pigs weresampled per farm. When pigs were housed in pens(i.e., on 5/35 farms), two pigs per pen were sampled.Nasal swab samples from humans on farms were collected based on consent and availability from each of the sampled locations. Also, nasal swab samples were collected from students in Kogi State University, Anyigba representing the 'non-contact' group based on simple random sampling technique (lottery method). The study and protocol was approved by the Institutional ReviewBoard of Kogi State University Teaching Hospital, Anyigba. For humans,a standardized specific questionnaire was administered to collectdemographic data, medical history over the last 12 months, and information about contact with pigs.A self administered questionnaire was used to collect information about each pig including age, sex, current healthstatus, and history of antimicrobial therapy in the last 6 monthsprior to sampling. Information was also retrieved about farming system (Intensive, Semi-intensive), and presence ofother animals on the farm.

3.2.2.2 Sample size determination

Sample size was arrived at using the formula outlined by Thrusfield (2005).

$n = Z^2Pq/ d^2$

Where Z = 1.9

P = prevalence

q = 1-P

d^2 = allowable error (5%)

A prevalence of 5.3% for pigs and 12.8% for humans was recently reported in Plateau State (Momoh *et al*., 2018), giving a minimum of 77 pigs and 172 humans to be sampled. However, 425 samples were collected from pigs and 255 human samples in this study.

3.2.2.3 Criteria used for sample collection

3.2.2.3.1 Inclusion criteria

a. Pig owner's consent
b. Oral consent from farm workers and students of Kogi State University
c. Pigs that were adequately restrained for sample collection
d. Farms and households with minimum of 10 pigs

3.2.2.3.2 Exclusion criteria

a. Sick pigs
b. Sick humans
c. Pigs that could not be restrained for samples to be collected
d. Farms and households with less than 10 pigs

3.2.2.4 Sample collection

Nasal swab Samples were collected by inserting a sterile swab stick in both anterior nares of each pig and human and gently rubbing the swab against the mucosa surface for approximately 5-10 seconds.These were placed in 3ml Mueller-Hinton broth (Oxoid, UK).

All the samples were transported immediately on ice to the Department of Microbiology Laboratory, Kogi State University, Anyigba for processing.

3.2.2.5 Culture on media

All the media were prepared in the laboratory according to the manufacturers' instructions and included Mannitol Salt agar (Oxoid), Trypticase Soy broth (Oxoid), Mueller Hinton agar (Oxiod), Oxidation-Fermentation medium (Oxiod), and Blood agar base (Oxiod), Dnase agar (Oxiod), Brillance agar (Oxiod), and Oxacillin resistance screening agar base (ORSAB) (Oxoid).

3.2.2.6 Isolation of Staphylococcus

Nasal swabs were collected from eachpig and human and were placed into 3ml Mueller Hinton broth (Oxoid, UK) supplemented with6.5% sodium chloride and incubated at 37 ^{0}C for 24h. A loopful of the inoculum from the enrichment broth was streaked onto mannitol salt agar and incubated at 37^{0}C for 24h.

3.2.2.7 Identification of isolates

Suspected *S. aureus* isolates (golden yellow colonies)on mannitol salt agar were tested using conventional methods that included Gram staining, catalase, coagulase, Dnase and sugar fermentation tests.

3.2.2.7.1 Catalase test

An 18-24 hour old pure colony was placed on a clean microscope slide using a loop. By using a Pasteur pipette or a dropper, a drop of 3% H_2O_2 was immediately dropped over the colony on the slide and observed for immediate bubbling as evidence of gas production.

3.2.2.7.2 Coagulase test

The tube method for coagulase test was carried out as follows: 0.5ml of rabbit serum diluted at 1:9ml of normal saline was pipetted into a test tube. 0.1ml of the test isolate was added to the test tube. After mixing, the test tube was incubated at 37°C for 4-24 h,and examined for clotted plasma which indicated positive result.

3.2.2.7.3 Haemolysis on blood agar

Haemolysis was carried out by plating the isolates onto 5% sheep blood agar and incubated for 24 hrs at 37ºC. Complete clearing of the blood was indicative of beta haemolysis; partial clearing was alpha haemolysis while no clearing was indicative of gamma or no haemolysis.

3.2.2.7.4 Deoxyribonuclease (Dnase) test

The Dnase agar was prepared one day before it was used and stored in the fridge at 4 °C. A sterile wire loop was dipped into an overnight broth culture of the *S. aureus* and inoculated onto Dnase agar plate and incubated for 24 hrs at 37 ^{0}C, following which, the plates were flooded with 1 N HCl and the excess decanted. The HCl was left to act for about ten minutes after which the plates were observed for clear zones around each streak of *S. aureus.* The presence of a clear zone indicates a positive result, showing that DNase was produced and it digested the DNA.

3.2.2.*7*.5*Sugar fermentation test*

These sugars (mannitol, sucrose, glucose, xylose) were prepared at 1% concentration i.e. one gram of each of the sugars in 100ml of Oxidation-Fermentation medium, with 1ml of sterile paraffin added to each tube. These were inoculated with the test organisms and incubated at 37 ^{0}C for four days and checked for color change.

3.2.2.8Confirmation of Staphylococcus isolates

3.2.2.8.1 Microgen 1DStaph confirmation

The test was carried out and interpreted based on the instructions of the manufacturer(Microgen Bioproducts, U.K). A homogenous suspension was prepared by mixing 2-5 isolated colonies in 3ml staphylococci suspending medium provided in the kit, then four drops of the bacterial suspension were added to each well with a Pasteur pipette; well no 7 indicated by a black circle on the test strip coated with arginine was overlaid with two drops of mineral oil. The inoculated test strips were incubated at 35°C ± 2ºC for 24hr. After incubation, two drops of fast blue reagent was added to well no 12 indicated by a green circle on the test strip (Beta galactosidase). A colour change within 5-10seconds to plum purple was observed for positive test. All results were recorded on the organism ID report forms provided in the kit and interpreted using the microgen identification package.

3.2.2.8.2 Matrix-assisted laser desorption/ionizationtime-of-flight mass spectrophotometry (MALDI-TOF MS) (BioMérieux, France).

All *S. aureus* isolates were confirmed using MALDI-TOF technique. Sample preparation for MALDI-TOF MS: Ethanol/ formic acid extraction procedure was followed according to the manufacturer's instruction (Bruker Daltonik GmbH, Bremen, Germany). Briefly, 2-3 isolated colonies were transferred into the 1.5 ml screw cap extraction tube containing 300 µl of double distilled water with an inoculation loop and mixed thoroughly. Absolute ethanol (0.9 ml) was added, the contents of the tube were carefully mixed, and the tubes were then centrifuged at 13,000 × g for 2 min, then 1 µl of the supernatant was placed onto a ground steel MALDI target plate and allowed to dry at room temperature. Subsequently, each sample was overlaid with 1 µl of matrix solution, which consisted of a saturated solution of α-cyano-4 hydroxy-cinnamic acid (HCCA) in 50 percent acetonitrile and 2.5 percent trifluoroacetic

acid (final concentration: 10 mg HCCA/ml) and air dried at room temperature. The MALDI-TOF MS target was subsequently introduced into the MALDI-TOF MS mass spectrometer (Brucker Daltonik GmbH, Bremen, Germany) for automated measurement and data interpretation using the MALDI Biotyper software package with the reference database v.2.0 by AHL.

3.2.3 Determination of methicillin resistance and susceptibility of isolates to commonly used antibiotics

3.2.3.1 *Antibiotic susceptibility test*

Each *S. aureus* isolate was inoculated into trypticase soy broth (Oxoid) at 37^0C for 24hours before testing. The turbidity of the actively growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. Subsequently 0.1ml of the suspended culture was inoculated onto Mueller Hinton agar plates (90mm diameter disposable Petri dishes) and spread over the surface with sterile cotton swabs. Six antimicrobial disks were then placed on the surface of each plate by means of antibiotic disk dispenser and incubated at 37 ^{0}C for 18hrs. Inhibition zone diameters were measured using a transparent ruler, and the interpretative breakpoints for resistance determined by comparing zone diameters according to the Clinical and Laboratory Standards Institute (CLSI, 2014) guidelines. *S. aureus* ATCC 25923 was used for quality control.*S. aureus* strains were tested using a panel of twelve antimicrobials namely, sulphamethoxazole/trimethoprim (25µg), tetracycline (30µg), gentamicin (10µg), cefoxitin (30µg), amikacin (30µg), amoxycillin/clavulanic acid (30µg), penicillin (10µg), chloramphenicol (30µg), erythromycin (5µg), vancomycin (30µg), oxacillin (1µg) and ciprofloxacin (5µg).

3.2.3.2 Minimum Inhibitory Concentration (M.I.C) Determination

M.I.Cs of oxacillin was determined using the Episolometer test strips (Etest strip) (Biomerieux, France). This was carried out as described by the manufacturer and interpreted using the CLSI guidelines (CLSI, 2014). Procedure: 0.5 McFarland standard bacterial suspension was made from an overnight culture plated on Mueller Hinton agar on which the Etest strips were placed with a forcep one on each plate. The plates were incubated for 24 h at 37ºC. After the period of incubation, an ellipse corresponds to the antimicrobial concentration no longer inhibitory to the growth of the organism. The corresponding concentration of antibiotic at the point of intersection on the strip was read as the MIC in μg/ml.If the growth intersects on the line between two sections, the MIC was read as the value in the lower section. If there was growth along the entire length of the strip, the MIC was read as greater than the highest value on the M.I.C.E scale.MIC values were interpreted as susceptible (S), intermediate (I) and resistant (R).

The *S. aureus* isolates were further subjected to antibiotic susceptibility using the Sensititre system and COMPAN1F MIC plate (ThermoFisher Scientific, USA). The antibiotic susceptibility of the isolates to penicillin, amoxycillin/clavulanic acid, clindamycin, gentamicin, oxacillin, vancomycin, tetracycline, ciprofloxacin, ticarcillin, trimethoprim/sulfamethoxazole, ceftiofur, erythromycin, rifampicin, amikacin, imipenem, enrofloxacin, ceforecin, cefpoxodime, marbofloxacin, cefoxitin, doxycycline, chloramphenicol, cefazoline was determined andsusceptibility was interpreted according to CLSI (CLSI, 2014).The reference strain used for quality control was *S. aureus* ATCC 29213.

3.2.3.3 ORSAB and Brilliance test for MRSA screening

An overnight broth culture of each *S. aureus* isolate was also streaked on ORSAB medium as well as brilliance medium and incubated at 37^0 C for 24h. All isolates that grew on ORSAB

medium with intense blue colonies and demin blue colonies on brilliance agar were considered MRSA.

3.2.4 Molecular characterization of *S. aureus* isolates

3.2.4.1 DNA extraction

Twenty five µL of 100µg/mL lysostaphin solution was added to a 1.5mL Eppendorf tube and 3-4 colonies (~1µL) resuspend in thelysostaphin solution. This was incubated at 37°C for 10min. Thereafter, 25 µL of a 100µg/mL proteinase Ksolution and 75µL of Tris-HCl 1M (pH8.0) was added, mixed well and incubated at 37°C for 10min. The suspension was further incubated at 97°C for 5min and centrifuged at 15,000 x g for 5min. The supernatant containing DNA was transferred to a new tube and stored at-20°C.

3.2.4.2 Multiplex polymerase chain reaction (PCR)

Amultiplex PCR to detect *spa*, *mec*A, the CC398-specific *sau*1-*hsd*S1 variant,*luk*F-PV(a marker of the Panton-Valentine leucocidin, PVL),and *scn* was carried out as previously described (Islam *et al*., 2017).The PCR reactionswere carried out in a final volume of 13µl containing 1 × QiagenMultiplex PCR Master Mix (Qiagen, Germany), 2µM ofeach primer, and 1µl of bacterial DNA. The following PCRconditions were used: initial denaturation at 94^0C for 15 min,followed by 25 cycles consisting of denaturation (94^0C for 30s), annealing (59^0C for 60s), and extension (72^0C for 60 s),and a final extension step at 72^0C for 10 min.The resultant PCR products were visualized on electrophoresis gels 2% w/v (Invitrogen, Carlsbad, CA, USA).Positive control DNA was derived from a known MRSA strain bearing *spa*, *pvl*, *mec*A, *scn*, and CC398 specific genes.

Table 3.1: Primer sets used for detection of *mec*A, *spa*, *pvl* and *scn* genes

Target gene		Primers	Amplicon size (bp)	Reference
*mec*A	P4	5'-TCCAGATTACAACTTCACCAG-3'	162	Larsen *et al*., (2008)
	P7	5'-CCACTTCATATCTTGTAACG-3'		
*spa*FP		5'-TAAAGACGATCCTTCGGTGAGC-3'	Variable	Larsen *et al*., (2008)
	RP	5'-CAGCAGTAGTAGTGCCGTTTGCTT-3'	(165-550)	
pvl	FP	5'-GCTGGACAAAACTTCTTGGAATAT-3'	80	Larsen *et al*., (2008)
	RP	5'-GATAGGACACCAATAAATTCTGGATTG-3'		
scn	FP	5'-TACTTGCGCGAACTTTAGCAA-3'	130	
	RP	5'-AATTCATTAGCTAACTTTTCGTTTTGA-3'		
CC 398	FP	5′-GAGAATGATTTTGTTTATAACCCTAG-3′	106	Stegger *et al*., (2011)
	RP	5′ CAGTATAAAGAGGTGACATGACCCCT-3′		

3.2.4.3 Multiplex PCR amplicon clean-up prior to sequencing

A reaction mixture comprising 10µl PCR mixture (directly after completion of PCR), 1µ1 Exonuclease I (Exo I) and 2µl FastAP^{TM} Thermosensitive Alkaline Phosphatase was mixed well and incubated at 37^0C for 15 min. The reaction was stopped by heating the mixture at 85^0C for 15 min.

3.2.4.4 spa typing

Direct sequencing of the cleaned PCR product was used for *spa* typing (Harmsen *et al*., 2003).The x region of the *spa* gene was amplifiedby PCR with primers 1095F (5'-AGACGATCCTTCGGTGAGC-3') and 1517R (5'-GCTTTTGCAATGTCATTTACTG-3'). PCR amplification was accomplished by adding 1 µl of a 1:200 dilution of genomic DNA and 24 µl of water to 25 µl of PCR master mixture in 0.2 ml PCR tubes (Perkin-Elmer Applied Biosystems Division [PE-ABI]). Master mixture buffer contained 0.5 µM PA 1095F forward PCR primer, 0.5 µM PA 1517R reverse primer, 2.5 U of AmpliTaq Gold DNA polymerase, 2 mM $MgCl_2$, 350 µM total dNTPs, and 25 mM KCl. Tubes were capped and placed in a GeneAmp PCR System 9600 Thermocycler (PE-ABI). Thermal cycling parameters included an initial 10 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 60°C, and 45 s at 72°C; and a final extension at 72°C for 10 min. PCR products were purified and concentrated twofold prior to sequencing in distilled water with a Microcon-100 (Millipore) micro-concentrator. Completed reaction mixtures were stored at -20°C.

DNA cycle sequencing reaction mixtures had a total volume of 20 µl, and reactions were carried out with a GeneAmp PCR System 9600 with the following reaction conditions: 13 µl of AmpliTaq FS Dye Terminator ready reaction mixture and sequencing primer (PA 1095F and PA 1517R), 3 µl of purified PCR product, and 4 µl of deionized water. The cycle sequencing profile consisted of a 96°C denaturation step for 10 s followed by an

annealing/extension step starting at 65°C and decreasing 1°C every six cycles until a touchdown temperature of 55°C was reached, for a total of 66 cycles. Dye terminator cycle sequencing reaction products were purified with a Sephadex column (Pharmacia) and a Silent Screen filter plate (Nalge Nunc). The column filtrate was evaporated in avacuum centrifuge and resuspended in 2 µl of blue dextran-EDTA–deionizedformamide (1:5). Sequences were determined by electrophoresis with the ABI PRISM 377 DNA sequencer. Consensus sequences were assembled from both orientations with PE-ABI software.

Isolates were assigned to *spa* type using Ridom StaphType software (Ridom GmbH, Germany). Associations of the assigned *spa* type with multilocus sequence type (MLST) were determined using the Ridom spa server database (www.spaserver.ridom.de/).

3.2.4.5 Whole genome sequencing

Four isolates (one pig, one pig-contact human from the same pig farm, onenon pig-contact human from the same district, and one pig-contact human from a different district)were selected for further SCC*mec* (Kondo *et al*., 2007), multilocus sequencing typing (Enright *et al*., 2000), and whole genome sequencing (WGS). For WGS, overnight cultures were grown in tryptic soy broth at 37°C with 200-rpm shaking. Genomic DNA was extracted from cultures by using the automated Maxwell DNA instrument using the Maxwell®RSC Cultured Cells DNA Kit (Promega, United Kingdom). Library preparation was carried outusing the Nextera XT kit and paired end 2 x 250bp sequencing on the MiSeq, all following standardIllumina protocols (Illumina, Inc., United States). All raw reads have been deposited in theSequence Read Archive database in the European Nucleotide Archive under the study accessionnumber PRJEB26533.

3.2.4.6 Phylogenetics and comparative genomics

All analyses were performed in CLC Genomics Workbench v. 11.0 using the tools within the MicrobialGenomics Module. First, Kmer analysis was performed against the NCBIRefSeq database and including only complete staphylococcalgenomes. This analysis was performed to determine if therewas any contamination of the DNA prior to sequencingand as a secondary confirmation of the *S. aureus* speciesidentification. Secondly, adapter trimmed, high quality reads were mapped against the reference genomeAUS0325, a closed ST88 methicillin-susceptible, penicillin-resistant genome (NCBI accession no.LT615218) to identify single nucleotide polymorphisms (SNPs). A maximum likelihood tree wasgenerated from core genome SNPs. To further investigate the clonal relationship of MRSA ST88from the African continent, MRSA strains in this study were compared to the 17 publically available ST88 MRSAisolated in Ghana (Kpeli *et al*., 2017). The Resfinder v.3.0 and Virulence finder v.1.5 web-basedpipelines were used to search for the presence of known virulence and antibiotic resistance genes(Zankari *et al.*,2012; Joensen *et* al., 2014).

3.2.5 Data analysis

The results obtained are presented in tables and percentages were computed using Microsoft Excel Programme version 2010. Chi-square test, Fisher's exact test and odds ratio from logistic regression was used to analyze data for associations with variables where necessary. Values of $P < 0.05$ were considered significant. Stata version 12.0 was used to analyze all data generated from the study.

CHAPTER FOUR

4.0 RESULTS

4.1Detection of *S. aureus* in Pigs and Humans in Kogi State

Out of 680 samples collected from pigs (425 across 35 farms) and humans (55 pig-contact humans and 200 non pig-contact humans), 39 (5.7%) *S. aureus* isolates were recovered. Twenty of the *S. aureus* isolates were obtained from pigs in 12 of the 35 farms sampled, while 19 were from humans in contact with pigs (6/55) and no pig-contact humans (13/200) (Table 4.1). The distribution of *S. aureus* in pigs according to geographical location is shown in Fig 4.1, which showed that there was at least a farm habouring a MRSA positive pig in 8 out of the 16 Local Government Area sampled. There were no statisticallysignificant differences in occurrence of *S. aureus* in the various age groups of pigs and in humans with or without exposure to pig farming sampled in this study (Table 4.2). Age and sex were found to be the only factors associated with nasal carriage of *S. aureus*within the study population (Table 4.3).MALDI-TOF was shown to be a much more reliable method in the confirmation of *S. aureus*, followed by microgen (Table 4.4).

4.2 Susceptibility of *S. aureus* Isolates to 12 Commonly Used Antimicrobials

All 39 (100%) *S. aureus* isolates from pigs and humans were resistant to β-lactams (penicillin, oxacillin, cefoxitin and amoxicillin/clavulanic acid) only, and were susceptible to other classes of antibiotics tested (Fig 4.2). Also the most reoccurring resistance profile pattern of *S. aureus* isolates from pigs and humans was found to be P, OX, AMC (Table 4.5). Furthermore, all *S. aureus* isolates from pigs and humans had MIC values ranging from 8mg/ml to >256mg/ml for oxacillin and were termed methicillin resistant. The *S. aureus* isolates were further subjected to antibiotic susceptibility using the Sensititre system and COMPAN1F MIC plate (ThermoFisher Scientific, USA) that corroborated the results of

sensitivity using disk diffusion method i.e. 100% of the *S. aureus* isolates from pigs and humans were resistant to β-lactams (penicillin and cefoxitin) only.

4.3 Occurrence of Other *Staphylococcus* Species in Pigs and Humans and Antimicrobial Resistance

4.3.1 Prevalence of other *Staphylococcus* species in pigs and humans

Aside *S. aureus*, other *Staphylococcus* species (n=42) were isolated in the course of the study. The species were identified as *S. aureus*, *S. hyicus*, *S. scuiri*, *S. intermedius*, *S. lentus*, *S. chromogens*, *S. warneri*, *S. lugdunensis* and *S. saprophyticus*, *S. haemolyticus* and *S. xylosus* based on microgen test (Table 4.6). The recovery rate was 100% *S. hyicus* in pigs only, (75%, 25%) for *S. scuiri* and *S. chromogens* respectively in pigs and humans and 100% for *S. haemolyticus* and *S. xylosus* in humans only (Table 4.7).

4.3.2 Antibiotic susceptibility of other *Staphylococcus* species to 12 commonly used antimicrobials

The majority of analysed isolates displayed penicillin resistance (61.9%). They were however, susceptible (100%) to vancomycin and amikacin while only 4.8% of the isolates were resistant to cefoxitin (Table 4.8). Nineteen unique susceptibility profiles were identified among the *Staphylococcus species* isolates while 5 (11.9%) and 3 (7.1%) of these isolates from pigs and humans respectively showed multi-drug resistance (Table 4.9).

4.4 Molecular Characteristics of MRSA Isolates

The results of the multiplex PCR to detect *spa*, *mec*A, CC398, *pvl*,and *scn* confirmed all the 39 isolates as *S. aureus* by the presence of *spa*. The results also showed that *mec*A was presnt in 38 (97.4%) of the isolates, presence of *scn*in all but one isolate from a pig and absence of *pvl* in all 39 (100%) of the isolates (Plate 1). Sequencing of the *spa* product demonstratedthat

all strains had the same *spa* type, t1603 and associated to ST88. The *mecA*was harbored on anSCC*mec* type IVa cassette.

Core genome single nucleotide polymorphism (SNP) analysis from whole genome sequencing (WGS) of fourisolates from a pig, a pig-contact human from the same farm, a pig-contact human from a differentfarm with no MRSA positive pigs, and a non pig-contact human using AUS0325 as the reference genomerevealed high genetic similarity between strains (3-11 SNP differences), despite the temporal (2 year gap) and geographic (165km) differences between isolates (Fig. 4.3). SNP in MRSA isolates relative to ST88 reference genome is shown in Table 4.10. Based on the analysis ofthe WGS data in Virulence Finder, all four strains contained the putative virulence factors*scn*, *sak*, *luk*D, E, *hlg*A, B, C, *aur*, and*spl*A, B. Furthermore, these isolates form a distinct clade when compared to MRSA ST88 isolates from Ghana (Fig. 4.4).

Table 4.1: Isolation rates of *S. aureus* from pigs, pig farm workers and non-pig contact humans in Kogi State

	No. of sampled pigs/humans	No. of pig farms	No. of *S. aureus* positive farms	No. of *S. aureus*positive samples
Pigs	425	35	12	20
Pig farm workers	55	35	6*	6
Kogi State University Students	200	-	-	13
Total	680	35	15	39

*3/6 farms harboured *S. aureus* positive pigs

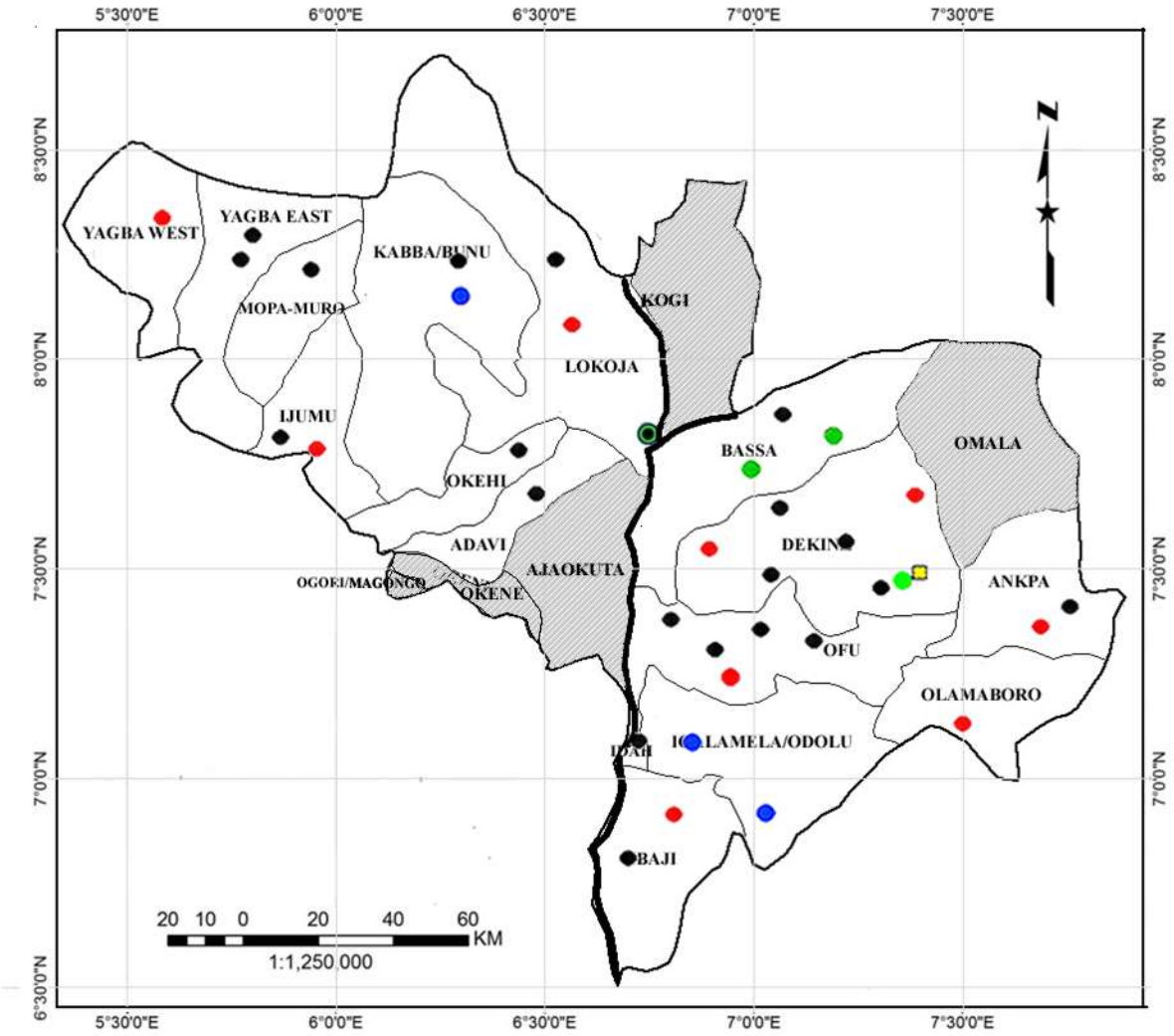


Figure 4.1: Geographic locations of pig farms sampled

Source: Geographical Information Service Centre, Kogi State University.

Legends:

- MRSA negative farms.
- MRSA positive pig farms where only pigs were positive.
- Pig farms where only humans were positive.
- Pig farms harboring both MRSA positive pigs and humans.
- Kogi State University

Table 4.2: Prevalence of *S. aureus* among different age groups of pigs and humans in Kogi State.

Group	% (Positive/Total)	x^2	P-value	OR
Pigs	4.7(20/425)			
<1month	18.2(2/11)	5.0435	0.081	7.7273
1-3months	2.4(2/85)	0.8217	0.459	0.4706
>3-7months	5.0(5/100)	0.0053	0.9999	1.0409
>7months	4.8(11/229)	0.6699	0.454	0.7281
Humans	7.5(19/255)	0.608	0.423	0.683
Contact	10.9(6/55)	1.0238	0.389	1.6783
No-Contact	6.5(13/200)	1.7032	0.210	2.7972

Table 4.3: Prevalence of *S. aureus* in humans with respect to demographic and risk factors

Characteristics	No.(%) of subjects		OR	p value
	MRSA (n=19)	Non-MRSA (n=236)		
Age				
<20 yrs	12(63.12)	88(37.29)	5.0909	0.013*
21-30 yrs	3(15.79)	112(47.46)	0.6964	0.574
31-40 yrs	1(5.26)	26(8.90)	0.1154	0.124
41-50 yrs	2(10.53)	6(2.54)	1.3333	0.999
>50 yrs	1(5.26)	4(1.69)	0.5455	0.491
Gender				
Male	7(53.85)	143(60.59)	0.3794	0.050*
Female	12(63.16)	93(39.41)		
Occupation				
Pig farm worker	6(31.58)	49(20.76)	1.7614	0.258
Student	13((68.42)	187(79.24)		
Antibiotic Use within 6 months				
Yes	0(0.00)	23(9.75)	0.000	0.233
No	19(100.00)	213(90.25)		

*Statistically significant

Table 4.4: Detection of *S. aureus* in pigs and humans in Kogi State based on three different methods

Method	Source		No. Positive (%)
	Human (%)	Pigs (%)	
Conventional	24 (48)	26 (52)	50 (7.3)
Microgen	21 (46)	25 (54)	46 (6.7)
MALDI-TOF	19 (49)	20 (51)	39 (5.7)
Overall Prevalence	19/255 (7.5)	20/425 (4.7)	39/680 (5.7)

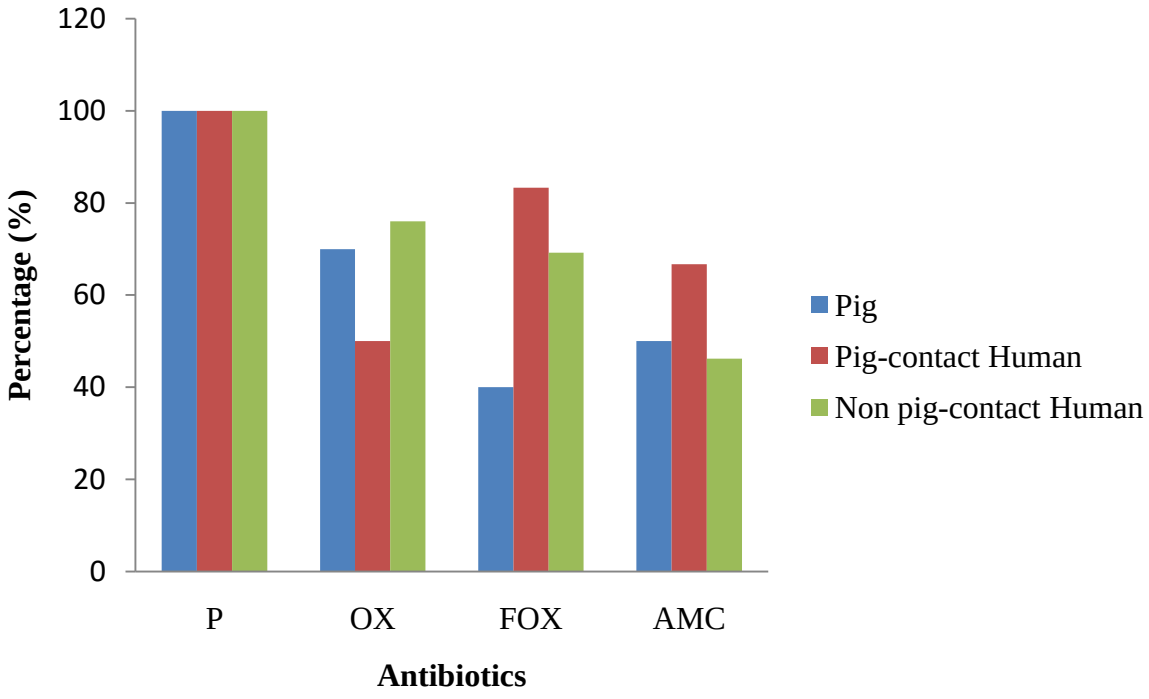


Figure 4.2: Antibiotic resistance of *S. aureus* isolates from pigs, contact humans and non-contact humans in Kogi State

P= Penicillin; OX= Oxacillin; Fox= Cefoxitin; AMC= Amoxycillin/Clavulanic acid.

Table 4.5: Antibiotic resistance profiles of MRSA from pigs and humans in Kogi State

Resistance profile	Number of isolates	
	Humans	Pigs
P, FOX	2	2
P, OX	1	7
P, OX, FOX	6	2
P, FOX, AMC	4	4
P, OX, AMC	4	5
P, OX, FOX, AMC	2	0
Total	19	20

Table 4.6: Isolation of other *Staphylococcus* species from pigs and humans in Kogi State

Staphylococcus species	Number	Percentage
S. hyicus	8	19.0
S. scuiri	8	19.0
S. intermedius	5	11.9
S. lentus	5	11.9
S. chromogens	4	9.5
S. warneri	3	7.1
S. lugdunensis	3	7.1
S. saprophyticus	3	7.1
S. haemolyticus	2	4.8
Staphylococcus xylosus	1	2.4
Total	42	100

Table 4.7: Distribution of other *Staphylococcus* species among pigs and humans

Staphylococcus species	Pigs (%)	Humans (%)
S. hyicus	8 (100.0)	0 (0.0)
S. scuiri	6 (75.0)	2 (25.0)
S. intermedius	4 (80.0)	1 (20.0)
S. lentus	2 (40.0)	3 (60.0)
S. chromogens	3 (75.0)	1 (25.0)
S. warneri	1 (33.3)	2 (66.7)
S. lugdunensis	2 (66.7)	1 (33.3)
S. saprophyticus	1 (33.3)	2 (66.7)
S. haemolyticus	0 (0.0)	2 (100.0)
S. xylosus	0 (0.0)	1 (100.0)
Total	27 (64.3)	15 (35.7)

Table 4.8: Antibiotic resistance of other staphylococci species form pigs and humans in Kogi State to commonly used antibiotics

Antibiotic	Disk Potency (µg)	Number (%)Sensitive	Number (%)Resistant
Amikacin	30	42 (100.0)	0 (0.0)
Amoxycillin/Clavulanic acid	30	39 (92.9)	3 (7.1)
Cefoxitin	30	40 (95.2)	2 (4.8)
Chloramphenicol	30	37 (88.1)	5 (11.9)
Ciprofloxacillin	5	39 (92.9)	3 (7.1)
Erythromycin	5	34 (81.0)	8 (19.0)
Gentamicin	10	37 (88.1)	5 (11.9)
Oxacillin	1	35 (83.3)	7 (16.7)
Penicillin	10	16 (38.1)	26 (61.9)
Sulphamethoxazole/ Trimethoprim	25	41 (97.6)	1 (2.4)
Tetracycline	30	29 (69.0)	13 (31.0)
Vancomycin	30	42 (100.0)	0(0.0)

Table 4.9: Antibiotic resistance profiles of other*Staphylococcus* species from pigs and humans in Kogi State

Resistance Profile	Number of isolates	
	Humans	Pigs
P	4	6
TE	0	1
C	0	1
P, TE	1	1
P, E	1	0
P, AMC	1	0
P, OX	0	1
P, CIP	0	2
CN, TE	0	1
P, OX, TE	1	1*
P, E, TE	1*	0
P, C, TE	1*	0
P, OX, AMC	0	1
CN, E, TE	1*	0
CIP, TE, AMC	0	1*
P, OX, E, FOX	0	1
P, OX, CN, E	0	1*
P, SXT, C, E, TE	0	1*
P, OX, CN, E, TE	0	1*
Total	11	20

*Multi-drug resistant

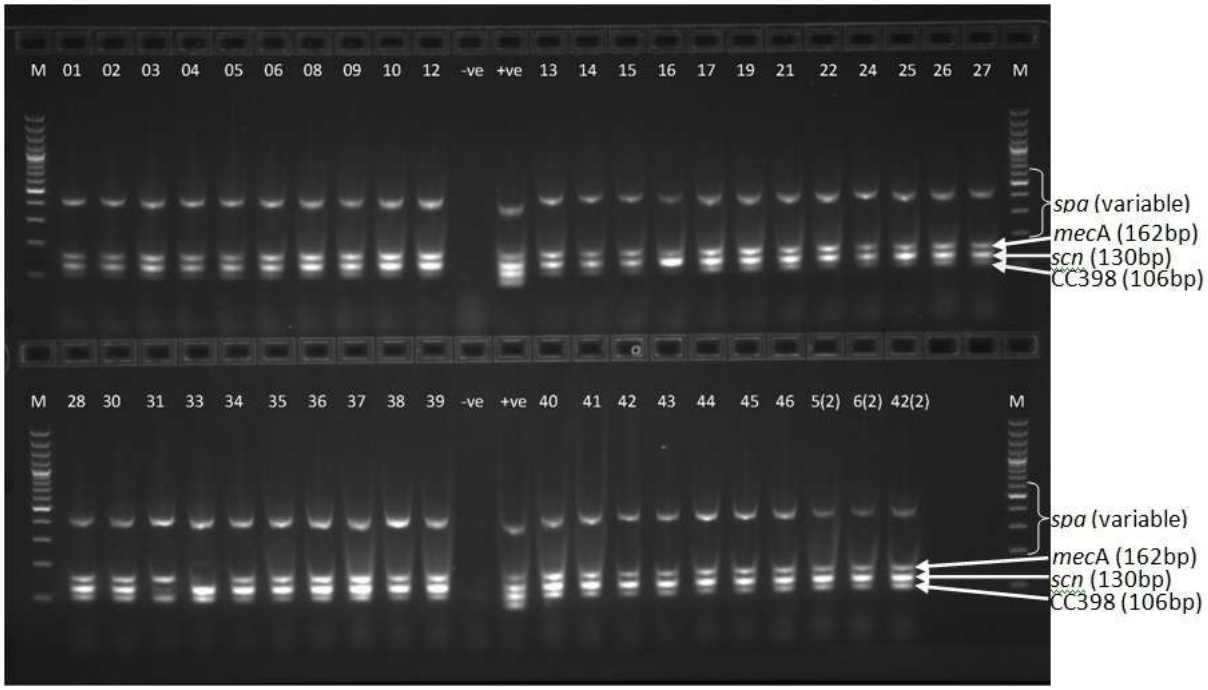


Plate 1: Multiplex PCR amplification of *spa*, *mec*A, and *scn* genes in MRSA isolates

M=100bp marker; -ve=negative control; +ve=positive control with *spa*, *mec*A, *scn*, CC398 and *pvl*, 1=KSU3, 2=AJA1, 3=SHE2, 4=AGU1, 5=KAB5, 6=KSU10, 8=EJU1, 9=KSU10, 10=EGB3, 12=OKP3, 13=OKU1, 14=OKU2, 15=OKP6, 16=SHE4, 17=AGU2, 19=LOK5, 21=ITA1, 22=OKU6, 24=ANK9, 25=OKP8, 26=KSU5, 27=SHE5; 28=ECH2; 30=SHE8; 31=OGI2; 33=63; 34=30; 35=12; 36=20; 37=2; 38=83; 39=49; 40=19; 41=ELE1, 42=164; 43=75; 44=102; 45=13; 46=35

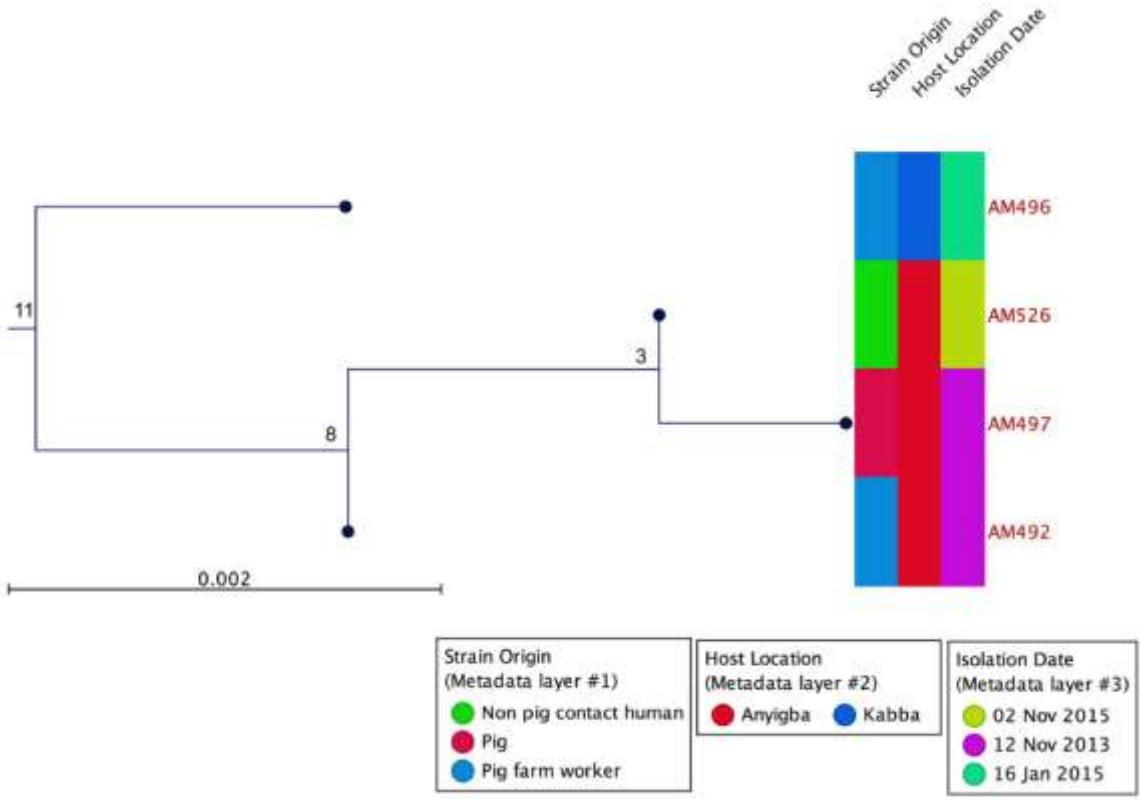


Figure 4.3: Metadata describing sample origins, sampling location in Kogi State andisolation date. Phylogeny based on analignment of 1,248 SNPs.

AM496=KAB5; AM526=164; AM497=KSU10; AM492=KSU3

Table 4.10: Single nucleotide polymorphisms in MRSA isolates relative to the ST88 reference genome, AUS0325

Nucleotide position	164	KSU10	KSU3	KAB5
853428	T	T	C	C
853440	C	Gap	Gap	Gap
853452	C	Gap	Gap	Gap
1349949	T	G	T	T
2673340	G	G	G	A
2673364	A	A	A	G
2673388	G	G	Gap	Gap
2673400	G	G	Gap	Gap
2673412	Gap	Gap	Gap	G
2673424	Gap	Gap	G	G
2673436	Gap	Gap	G	G

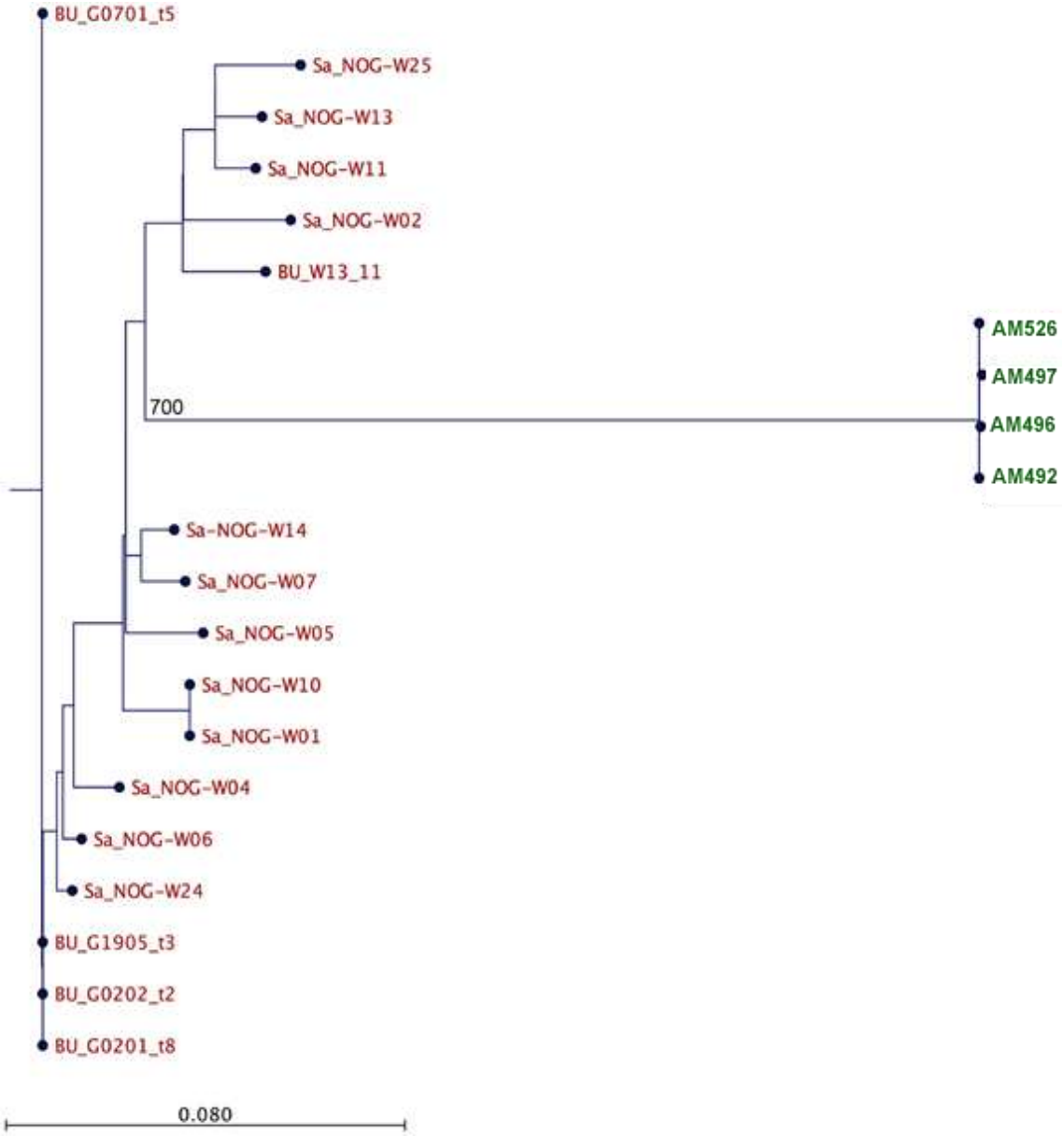


Figure 4.4: Comparative analysis of the Nigerian and Ghanaian MRSA ST88. Core genome phylogeny based on an alignment of 1492 SNPs.

AM496=KAB5; AM526=164; AM497=KSU10; AM492=KSU3

CHAPTER FIVE

5.0 DISCUSSION

Colonization with*S. aureus* may occur on mucous membranes of the respiratory and/or intestinaltract, or on other body surfaces, without causing disease or harming their hosts(Verkaik*et al*., 2009). Colonization with *S. aureus*usually precedes infection, and is mostly caused by the same isolate (Mertz *et al*., 2009). A prevalence of 4.7% (20/425) and 10.9% (6/55) *S. aureus* carriage was found in pigs and in-contact humans respectively in this study. This was lower than the prevalence of 12.3% (57/464) and 30.8% (16/52) *S. aureus* carriage in pigs and pig farm workers respectively reported in Senegal by Fall *et al*. (2012) but similar to the prevalence of 5.3% and 12.9% *S. aureus* carriage in pigs and pig farm workers respectively reported in Plateau State, Nigeria by Momoh *et al*. (2018). The prevalence of 6.5% (13/200) *S. aureus* colonization in apparently healthy pupils in this study was lower than 32.9% (72/219) reported by Ouedraogo*et al*., (2016). The factors responsible for the observed differences in *S. aureus* carriage in pigs and humans in this study is unknown, but may not be unconnected with antibiotic use and personal hygiene especially among humans. When the opportunity arises, *S. aureus* can cause a wide range of infections (Shittu *et al*., 2011) with methicillin resistant strains adding to the disease burden.

MRSA in pigs and pig farmers in Africa was first reported in Senegal (Fall *et al*., 2012) however, no MRSA was isolated from the farm workers. In Nigeria, there has been to our knowledge only one report of phenotypic oxacillin-resistant *S. aureus*in pigs from Oyo State in South Western, Nigeria (Okunlola and Ayandele, 2015). Only 18/200 (9%) pigs were positive for presumptive MRSA on 7 of the 11 farms sampled. Similar to Okunlola and Ayandele, (2015), 20/425 MRSApositive animals were observed onfarms sampled in this study.MRSA prevalence of 4.7% (20/425) in pigs in this study was lower than 14.4%

(89/641) reported by Fang *et al*. (2014) in Taiwan, but higher than 1.3% (6/464) reported in Senegal by Fall *et al*. (2012). The prevalence of MRSA in pigs in this study and Senegal (Fall *et al*., 2012), a fellow West African country, is lower than what was reported in industrialized countries such as the Netherland, Spain and Portugal (Aberu *et al*., 2019; Conceicao *et al*., 2017; Dierikx *et al*., 2016). This difference may be a consequence of the intensity of antibiotic use in pig farming in industrialised countries and may also be attributed to the fact that majority of the pigs sampled in this study were reared in semi-intensive farming system and is in accordance with previous reports demonstrating a correlation between MRSA isolation and conditions that subsist in intensive farming such as crowded environment (Alt *et al*., 2011; Conceico *et al*., 2017). Also, MRSA prevalence of 10.9% (6/55) in pig farm workers (in-contact humans) in this study is lower than 60% (31/52) reported by Sahibzada *et al*. (2017) in pig farmers in Australia and 13% (13/100) reported by Fang *et al*. (2014) in pig farmers in Taiwan. Prevalence of MRSA positive farms 42.8% (15/35) in this study was lower than 59.1% (13/22) reported by Fang *et al*. (2014). Furthermore, MRSA carriage of 25% (3/12) for on-farm workers with MRSA colonized pigs in this study was lower than 50% reported by Denis *et al*. (2009). These observed differences may be due to differences in factors associated with pig husbandry such as movement of animals between different types of pig farms, use of antimicrobials and intensity of contact between animals and farm personnel (van Duijkeren*et el*., 2008).People inclose contact with pigs (farmers, veterinarians, transporters, and slaughterhouse workers) have been shown to be at a greater risk of being colonized with livestock associated MRSA (LA-MRSA) (Denis *et al*., 2009; Liu *et al*., 2015). There was no statistically significant difference in MRSA carriage between humans in contact with pigs and those without exposure to pigs in this study. The reason for this is unknown, but may not be unconnected with the fact that other domestic animals and humans also could serve as source of the pathogen. Previous studies indicated that higher

density of pigs on pig farms increased the risk of MRSA colonization (Battisti *et al*, 2010; Graveland *et al*., 2010). MRSA was isolated from more persons without antibiotic treatment than in persons with previous antibiotic treatment as none of the MRSA positive individuals had reported takingantibiotics and only one pig farm worker had reported beinghospitalized in the last 12 months prior to sampling in this study. This finding agrees with Cuny *et al*. (2009) who reported that antibiotic treatment had no influence on MRSA colonization in case of humans exposed to MRSA colonized pigs. Risk factors associated with human carriage of MRSA in this study were male sex and age < 20yr. This finding is in agreement with that of Sangvik *et al*. (2011) who reported that male sex and younger age were positively associated with nasal MRSA colonization and carriage rates in their study population. It has also been previously established that children/younger adults and the elderly were much more susceptible to colonization by MRSA (Huang *et al*., 2007).

Inrecentyearsmatrixassisted laser desorption ionization timeofflightmassspectrometry(MALDI-TOFMS)hasemergedasapotentialtoolformicrobialidentificationanddiagnosis (Singhal *et al*., 2015) and numerous studies have shown that MALDI-TOF MS is a rapid, reliable and cost-effective technique for identification of bacteria (Burillo *et al*., 2014; Song *et al*., 2017; Momoh *et al*., 2018). MALDI-TOF MS was shown to be a much more reliable method in the confirmation of MRSA in this study and is in agreement with Guembe *et al*. (2014) who reported that MALDI-TOF MS performed better than conventional culture methods.

MRSA isolates found in this study expressed phenotypic resistance to β-lactams only, possessed *bla*Z (100%) and *mec*A (97.4%) genes and no other acquired antimicrobial resistance genes based on whole genome sequencing (WGS) of selected strains. All MRSA isolates further subjected to antibiotic susceptibility testing using the Sensititre system and

COMPAN1F MIC plate (ThermoFisher Scientific, USA) were resistant to penicillin and cefoxitin only and there was agreement between phenotypic and inferred genotypic resistance. Although multidrug resistance is presently defined as resistance to three or more classes of antibiotics (Seiler and Berendonk, 2012), it should be noted that any lack of therapeutic effectiveness due to resistance to the administered substance can be devastating. The main dangers associated with these infections are aggravation of disease due to unexpected ineffectiveness of antibiotic therapy in a severely ill patient and the uncontrolled spread of these organisms in the hospital environment(Schmithausen*et al*., 2015).The absence of *mec*A gene in an isolate phenotypically described as MRSA may suggest that the expression of methicillin resistance could be due to over production of β-lactamase controlled by *bla*Z. That isolate also had the highest MIC value for oxacillin resistance (8µg/ml). Resistance to methicillin may be extrinsic, non-*mecA* mediated, in *S. aureus* strains with low-level resistance to oxacillin, known as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) (Leahy *et al*., 2011). BORSA exhibit oxacillin MIC of 1-8µg/ml, but lack *mec*A, which encodes the low-affirnity penicillin-binding protein (Nadarajah *et al*., 2006). Typically, this borderline phenotype results from excess production of β-lactamase. It was described initially by McDougal and Thornsberry(1986) in the US. According to these authors, these strains were neither hetero-resistant nor multi-resistant, and they produced large amounts of normal staphylococcal β-lactamase, which partially hydrolyze oxacillin and became fully susceptible to oxacillin in the presence of β-lactamase inhibitors. However, the borderline phenotype has been attributed to other mechanisms, i.e., the production of an inducible, plasmid-mediated methicillinase or different modifications in the PBP genes due to spontaneous amino acid substitutions in the transpeptidase domain (Argudin *et al*., 2018).*mec*A was harboured on an SCC*mec*type IVa cassette in MRSA isolates in this study. SCC*mec* types IV, V and VI are generally associated with community acquired MRSA (CA-

MRSA), which in most cases, do not contain additional antimicrobial resistance genes, and therefore, cause only β-lactam antibiotic resistance (Mediavilla *et al*., 2012). Panton-Valentine leucocidin (PVL) genes (*luk*S/*luk*F) was not detected in any of the MRSA isolates tested in this study and this is in agreement with the findings of Kolawole *et al*. (2013) that reported absence of PVL in all MRSA isolates in a study conducted in Nigeria. Also, Pirolo *et al*. (2019) reported that none of the MRSA isolates in their study harboured the PVL genes, while Yanagihara *et al*.(2012) reported a low prevalence (2.3%) carriage of PVL genes among MRSA SCC*mec* IV isolates.

Molecular epidemiology-based methods are essential tools for the study of clonal relatedness, genetic diversity, and also tracking the dissemination of *S. aureus* infections (Wang *et al*., 2018). All MRSA isolates found in this study belonged to ST88,which has been isolated from both hospital and community acquired infections in humans in several Sub-Saharan African countries, including Nigeria (Ghebremedhin *et al*., 2009; Shittu *et al*., 2012; Kolawole *et al*., 2013; Raji *et al*., 2013; Abdulgader *et al*., 2015). ST88 appears to be primarily associated with the African continent, but has also been reported in Asia and sporadically in other parts of the world (Schaumburg *et al*., 2014). The association of ST88 and livestock has only been described in Africa. Specifically, MRSA ST88 has been identified in one pig isolate from Senegal and healthy sheep in the Ivory Coast (Fall *et al*., 2012; Schaumburg *et al*., 2015). In China, MRSA ST88 has been found in food of animal origin and recently in livestock workers (Wang *et al*., 2014; Ye *et al*., 2016). Also all MRSA ST 88 isolates in this study belonged to *spa* type t1603 a unique *spa* type from those (t168, t186, t667, t690, t729, t1289, t1855, t3489, t7237) previously reported on the African continent (Breurec *et al*., 2011; Fall *et al*., 2012; Moremi *et al*., 2012; Kolawol *et al*., 2013; Kpeli *et al*., 2017). MRSA isolates belonging to the same sequence type/clonal complex were found to share similar virulence gene profiles (Jamrozy *et al*., 2012). MRSA isolates in this study belonged to the same

sequence type irrespective of the source (pig, pig-contact human or non-pig contact human) and shared genetic similarities based on WGS, raising questions about the origin of these strains. The immuneevasion cluster (IEC), which is carried on a bacteriophageand encodes the secreted proteins staphylococcal complementinhibitor (*scn*), staphylokinase (*sak*), and chemotaxis inhibitoryprotein (*chp*), are thought to contribute to the immune evasionin humans (van Wamel *et al*., 2006). Moreover, these genesare less prevalent in livestock adapted *S. aureus*lineages andare hence considered good genetic markers for identificationof human associated *S. aureus*clones (McCarthy *et al*., 2012a; Uhlemann *et al*., 2012). Most of the porcine isolates (19/20 isolates) in this study possessed*scn* and the additionaloccurrence of *sak*based onWGS data. The presence of these IECrelated genes suggest a possible human origin and that pigs wereeither transiently contaminated by farmworkers or the result of avery recent human-to-pig transmission event. Human associated*S. aureus*lineages have been previously described in animalsincluding pigs (Arriola *et al*., 2011; Schaumburg *et al*., 2012; Nagel *et al*., 2013; Baez *et al*., 2017).All strains were positivefor the CC398 specific band. However, *spa*sequencing demonstrated that all strains had the same *spa*type, t1603, which is unrelated to *spa*types typically associated with CC398. Furthermore, MLST demonstrated that the strains belongedto ST88. *In silico*PCR using the CC398 specific primers, which target the C398-specific *sau*1-*hsd*S1 variant, (FP2*sau*1: 5′GAGAATGATTTTGTTTATAACCCTAG3′ and CC398r1: 5′CAGTATAAAGAGGTGACATGACCCCT-3′) and *denovo*assembled contigs in this study, showed that the primers shared 100% complementarity to the target and produced the expected 106 bp amplicon. The primers band to an open reading frame (ORF) with 99% nucleotide identity to *hsd*S, which is the sequence specificity gene of the *Sau*1 type I restriction-modification system. The CC398 specific PCR is an excellent tool to rapidly identify*S. aureus*belonging to CC398, which was shown to be 100% specific for CC398 only

(Stegger *et al*., 2011). This PCR is based on *sau*1-*hsd*S since it was previously shown that variations in this gene is associated with specific *S. aureus*clonal lineages, but maintained a high sequence homology within the lineage (Waldron and Lindsay, 2006). This approach has also been used for rapid identification of hospital-acquired MRSA lineages (Cockfield *et al*., 2007). Unfortunately, at the time of the study by Stegger *et al*. (2011)there were no publically available ST88 genomes and strains belonging to ST88 were not included in that study, since ST88 is the not the typical LA-MRSA and it is not a globally disseminated human associated clone.

Based on WGS, the core genome consisted of 2407 coding DNA sequences (CDS) with *S. aureus*AUS0325 used as reference. A core genome phylogeny was inferred by mapping reads from the four sequenced isolates to AUS0325, and a phylogenetic tree was constructed using the core genome SNPs and maximum likelihood estimation. Relative to AUS0325, 1,248 SNPs were identified, but only 11 SNPs were found between the four isolates in this study despite the temporal and geographic differences signifying high genetic relatedness. Also, isolates from the same region i.e., Anyigba, differed only by 3–8 SNPs despite a 2-year gap. A phylogenetic comparison with the 17 ST88 from Ghana indicates that the Nigerian isolates formed a separate, distinct clade with 700 SNPs differences. WGS analysis revealed a limited content of virulence determinants (*scn*, *sak*, *lukE*, *lukD*, gamma hemolysin, *aur*, *splA* and *spl*B) among representative MRSA ST88 isolates in this study as compared to ST 88 isolates from a previous study (Kpeli *et al*., 2017). The absence of significant virulence factors among isolates belonging to ST88 has been reported previously in Nigeria (Ghebremedhin *et al*., 2009; Kolawole *et al*., 2013).

Most of the staphylococci species (*S. hyicus, S. scuiri, S. intermedius, S. lentus, S. chromogens, S. warnari, S. lugdunensis, S. saprophyticus, S. haemolyticus, S. xylosus*)

isolated in this study have been reported previously in Nigeria (Ugwu *et al*., 2015; Momoh *et al*., 2016) and elsewhere (Nagase *et al*., 2002). The prevalence of 6.4% (27/425) and 5.9%(15/255) for CoNS, *S. hyicus* and *S. intermedius*isolated from pigs and humans respectively in this study was lower than the 100% (12/12) and 88.9% (80/90) for pigs and humans respectively reported by Nagase *et al*. (2002). Also, the combined prevalence of 6.2% (42/680) for CoNS, *S. hyicus* and *S. intermedius* isolated from pigs and humans in this study was lower than the 22.4% (90/401) reported by Momoh*et al*. (2016). This observed difference may reflect variations in intensity of antibiotic usage on farms sampled. Of all described staphylococcal species, only *S. aureus*, *S. epidermidis*, *S. hyicus* and *S. pseudintermedius* are responsible for significant disease conditions in animals (Aarestrup and Schwarz, 2006). Other *Staphylococcus* species are predominantly associated with opportunistic infections in different animal species. Two CoNS, *S. lugdunensis* and *S. schleferi*, have been described as emerging zoonotic pathogens (Davis *et al*., 2013).*S. sciuri* has been reported to cause fatal exudativeepidermitis in piglets in China (Chen *et al.*, 2007). This specieshas also been associated with endocarditis (Wallet *etal.*, 2000), urinary tract infections (Stepanovic *et al.*, 2003)and wound infections (Coimbra *et al.*,2011) in humans.*S.hyicus* was the predominant species found among the pig *Staphylococcus*isolates in this study followed by *S. scuiri*. This finding differs from those of Ugwu *et al*. (2015) who reported that *S. scuiri* was the predominant species in their study and Tulinski *et al*., (2011) who reported *S. cohnii* as the predominant CoNS. Also Momoh *et al*. (2016) reported that *S. xylosus* and *S. haemolyticus* were the predominant species detected in pigs and in-contact humans. The resistance of CoNS, *S. hyicus* and *S. intermedius* isolates to Oxacillin, Tetracycline, Chloramphenicol and Ciprofloxacin (16.7%, 31%, 11.9%, 7.1%) respectively in this study was lower than that observed by Ugwu *et al*. (2012) who reported resistance of 100%, 74%, 19%, 48% to Oxacillin, Tetracycline, Chloramphenicol and Ciprofloxacin

respectively. This difference may be due to differences in management systems. That 61.9% of CoNS, *S. hyicus* and *S. intermedius*isolates were resistant to penicillin is in agreement with the report of Detwiller *et al*. (2013) who reported that CoNS are often resistant to beta-lactams, and such high level of resistance has been reported previously in Nigeria (Ugwu *et al*., 2015). Resistance to penicillin calls for concern as they are probably the most commonly used antibiotics in pig production system both in treatment of infections and as growth promoter (Rajic *et al*., 2006). Apart from the beta-lactams, 26.2% of CoNS, *S. hyicus* and *S. intermedius*isolates were multi-drug resistant by expressing resistance to 3 or more classes of antimicrobial agents. This could be associated with the fact that CoNS are often reported as methicillin resistant with co-resistance to different classes of antibiotics (Huber *et al*., 2010). Also, 63.6% of the multi-drug resistant isolates were of porcine origin and this is in agreement with Nagase *et al*. (2002) who reported that staphylococci from animals including pigs showed higher incidence of multi-drug resistance when compared to those isolated from humans in the same study.

Molecular characterization of CoNS, *S. hyicus* and *S. intermedius* was not carried out in this study. Hence, genetic markers such as antibiotic resistance genes and virulence genes possessed by them could not be elucidated. They are thought to be reserviors of antibiotic resistance genes and unique virulence factors that could be transferred to methicillin susceptible *S. aureus* thereby contributing to its pathogenecity (Otto, 2013). It has been reported previously in studies on staphylococci isolated from pigs in Nigeria that coagulase negative species possessed several antibiotic resistance genes including *mec*A and a vast array of virulence factors (Ugwu *et al*., 2015).Horizontal gene transfer of the SCC*mec* element is thought tocontribute to the generation of new methicillin-resistant staphylococci, including MRSA (Tulinski *et al*., 2011).

CHAPTER SIX

6.0CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study provides insights into the carriage of MRSA as well as CoNS species, *S. hyicus*, and *S. intermedius* among pigs, pig farm workers (in-contact humans) and students (non-contact humans) of a tertiary institution in Kogi State, Nigeria and highlights molecular characteristics of the MRSA isolates. Conclusions made from this study are outlined below;

1. There was a relatively low prevalence (4.7% and 7.5%) of MRSA among pigs and humans in the study area.

2. The MALDI-TOF MS was more sensitive in the detection of MRSAisolates in the study than Microgen and conventional methods.

3. MRSA isolates were resistant to β-lactams only, while 26.2% of CoNS, *S. hyicus* and *S. intermedius* isolates were multi-drug resistant by expressing resistance to 3 or more classes of antimicrobial agents.

4. *mec*A wasdetected in 38/39 of the MRSAisolates and its absence was recorded in an isolate from human. *mec*A was associated with a SCC*mec* IVa element in 38/39 of the isolateswhile *pvl* was absent in all isolates tested.

5. All MRSA isolates belonged to the same *spa* type t1603 associated with ST88 irrespective of origin.

6. *scn* was detected in 38/39 of the isolates being absent in a pig isolate only. The absence of*scn*in only 1 porcine strain suggest a possible human origin and that pigs

were either transiently contaminated by humans or as a result of very recent human-to-pig transmission event.

7. Core genome SNP analysis of four isolates revealed high genetic similarities between strains (3-11 SNP differences), despite the temporal (2 year gap) and geographic (165km) differences between isolates.

8. MRSA isolates in this study formed a distinct clade when compared to other African MRSA ST88 isolates.

6.2 Recommendations

Based on the findings of this study, the following recommendations are made;

1. Although an expensive technique, the possibility of a more sensitive confirmatory test like the use of MALDI-TOF MS should be considered for correct and quick identification of *S. aureus* in research and in clinical settings in Nigeria because of its sensitivity.

2. There is the need to carry out periodic screening of pigs for resistant staphylococci species as CoNS are a reservoir of antibiotic resistance genes with the potential to transfer to *S. aureus*, as has been demonstrated with the plasmid-mediated transfer of *mup*A, conferring mupirocin resistance, from *S. haemolyticus* to *S. aureus*.

3. Pig workers should always wear protective equipment when working with pigs to reduce human sources of pig colonization.

4. For effective monitoring and continuous improvement of antimicrobial policies both in the human and animal populations, it would be useful to conduct follow-up studiesperiodically, extending to other geographical areas not included in this study in

order to determine prevalent sequence types and to monitor changes in antimicrobial resistance over time.

5. Further research is required in the study area to determine whether MRSA carriage amongst pigs was transient or persistent, establish the exact transmission routes on pig farms and explore biosecurity measures for preventing the spread of MRSA in the farm.

REFERENCES

Aarestrup, F. M. and Schwarz, S. (2006). Antimicrobial resistance in staphylococci and streptococci of animal origin. In: Antimicrobial resistance in bacteria of animal origin. Aarestrup F.M. ed. ASM Press, Washington, Pp. 187–212.

Abdulgader, S. M., Shittu, A. O., Nicol, M. P. and Kaba, M. (2015). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*in Africa: a systematic review. *Frontiers in Microbiology*, 30: 348.

Abdullahi, N. and Iregbu, K. C. (2018). Methicillin-resistant *Staphylococcus aureus* in a Central Nigeria teaching hospital. *Annals of Tropical Pathology*, 9: 6-10.

Abreu, R., Rodriguez-Alvarez, C., Lecuona, M., Castro, B., Gonzalez, C. J., Aguirre-Jaime, A. and Arias, A. (2019). Increased antimicrobial resistance of MRSA strains isolated from pigs in Spain between 2009 and 2018. *Veterinary Sciences*, 6: 38-45.

Abubakar, U. and Suleiman, S. A. S. (2018). Prevalence, trend and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* in Nigeria: a systemic review. *Journal of Infection and Public Health*, 11: 763-770.

Adebisi, O. R. (2008). Gastrointestinal helminths and public health: Overview of a negleted sector. *The internet Journal of Veterinary Medicine*, 4:72-78.

Aires-de-Sousa, M. (2017). Methicillin-resistant *Staphylococcus aureus* among animals: current overview. *Clinical Microbiology and Infection*, 23: 373-380.

Ali, M. S., Isa, N. M., Abedelrham, F. M., Alyas, T. B., Mohammed, S. E., Ahmed, A. E., Ahmed, Z. S. A., Lau, N., Garbi, M. I., Amiru, A. A., Seed, A. O., Omer, R. A. and Mohamed, S. B. (2019). Genomic analysis of methicillin-resistant *Staphylococcus aureus* strain SO- 1977 from Sudan. *BMC Microbiology*, 19:126-135.

Alt, K., Fetsch, A., Schroeter, A., Guerra, B., Hammeri, J. A., Hertwig, S, Senkov, N., Geinets, A., Mueller-Graf, C., Braeunig, J., Kaesbohrer, A., Appel, B., Hensel, A. and Tenhagen, A. (2011). Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. *BMC Veterinary Research*, 7: 69.

Amissaha, N. A., Chlebowicz, M. A., Ablordey, A., Tetteh, C. S., Prah, I., van der werf, T. S., Friedrich, A. W., van Dijl, J. M., Stienstra, Y. and Rossen, J. W. (2017). Virulence potential of *Staphylococcus aureus* isolates from Buruli ulcer patients. *International Journal of Medical Microbiology*, 307: 223-232.

Anand, K., Agrawal, P., Kumar, S. and Kapila, K. (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian Journal of Medical Microbiol*ogy, 21:27–29.

Andrews, J. M., Boswell, F. J. and Wise, R. (2000). Establishing MIC breakpoints for coagulase-negative *Staphylococci* to oxacillin. *Journal of Antimicrobial Chemotherapy*, 45: 259-61.

Apfalter, P., Assadian, O., Kalczyk, A., Lindenmann, V., Makristathis, A. and Mustafa, S. (2002). Performance of a new chromogenic oxacillin resistance screen medium (Oxoid) in the detection and presumptive identification of methicillin resistant *Staphylococcus aureus.Diagnostic Microbiology and Infectious Diseases*, 44: 209-211.

Archer, K. N., Mazaitis, J. M., Costerton, W. J., Leid, G. F., Powers, E. M. and Shirtliff, E. M. (2011). *Staphylococcus aureus* biofilms: properties, regulation and roles in human disease. *Virulence*, 2: 445-459.

Argudín, M. A., Cariou, N., Salandre, O., Le Guennec, J., Nemeghaire, S. and Butaye, P. (2013). Genotyping and antimicrobial resistance of *Staphylococcus aureus* isolates from diseased turkeys. *Avian Pathology*, 42: 572-580.

Argudín, M. A., Fetsch, A., Tenhagen, B. A., Hammerl, J. A., Hertwig, S., Kowall, J., Rodicio, M. R., Käsbohrer, A., Helmuth, R., Schroeter, A., Mendoza, M. C., Bräunig, J., Appel, B. and Guerra, B. (2010). High heterogeneity within methicillin-resistant *Staphylococcus aureus* ST398 isolates, defined by Cfr9I macro restriction pulsed-field gel electrophoresis profiles and *spa* and SCC*mec* types. *Applied and Environmental Microbiology*, 76**:** 652-658.

Argudin, M. A, Roisin, S., Nienhaus, L., Dodémont, M., de Mendonça, R., Nonhoff, C., Deplano, A. and Denis, O. (2018). Genetic diversity among *Staphylococcus aureus* isolates showing oxacillin and/or cefoxitin resistance not linked to the presence of *mec* genes. *Antimicrobial Agents and Chemotherapy*, 62: 1-6.

Arias, C. A., Vallejo, M., Reyes, J., Panesso, D., Moreno, J., Castaneda, E., Villegas, M. V., Murray, B E. and Quinn, J. P. (2008). Clinical and microbiological aspects of linezolid resistance mediated by the *cfr* gene encoding a 23S rRNA methyltransferase. *Journal ofClinical Microbiology*. 46: 892-896.

Armand-Lefevre, L., Ruimy, R. and Andremont, A. (2005). Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerging Infectious Diseases*,11:711-714.

Arriola, C. S., Güere, M. E., Larsen, J., Skov, R. L., Gilman, R. H., Gonzalez, A. E. and Silbergeld, E. (2011). Presence of methicillin-resistant *Staphylococcus aureus*in pigs in Peru. *PLoS One*. 6: e28529.

Atif, M., Asghar, S., Mushtaq, I., Malik, I., Amin, A., Babar, Z. and Scahill, S. (2019). What drives inappropriate use of antibiotics? A mixed methods study from Bahawalpur, Pakistan. *Infection and Drug Resistance*, 12: 687-699.

Ayepola, O. O., Olasupo, N. A., Egwari, L. O., Becker, K. and Schaumburg, F. (2015). Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from clinical infection and asymptomatic carriers in Southwest Nigeria. *PLoS One*, 10: e0137531.

Ayepola, O. O., Taiwo, O. S., Anifowose, A. and Onile-ere, O. (2018). Nasal Carriage of *Staphylococcus aureus* and associated risk factors among students in a Nigerian University. *Acta Scientific Microbiology*, 1: 6-8.

Ayukekbong, J. A., Ntemgwa, M. and Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance and Infection Control*, 6: 47.

Baez, M., Collaud, A., Espinosa, I., and Perreten, V. (2017). MRSA USA300, USA300-LV and ST5-IV in pigs, Cuba. *International Journal of Antimicrobial Agents*. 49, 259-261.

Baig, S., Johannesen, T. B., Overballe-Petersen, S., Larsen, J., Larsen, A. R. and Stegger, M. (2018). Novel SCC *mec* type XIII (9A) identified in an ST152 methicillin-resistant *Staphylococcus aureus*. *Infection, Genetics and Evolution*, 61: 74-76.

Bakthavatchalam, Y. D., Triplicane, D. H., Munusamy, E., Jennifer, L. and Veeraraghavan, B. (2019). A distinct geographic variant of SasX in methicillin-resistant *Staphylococcus aureus* ST239 and ST368 lineage from South India. *Microbial Drug Resistance*, 25: 413-420.

Ballhausen, B., Jung, P., Kriegeskorte, A., Makgotlho, P. E., Ruffing, U., von Müller, L., Köck, R., Peters, G., Herrmann, M., Ziebuhr, W., Becker, K. and Bischoff, M. (2014). LA-MRSA CC398 differ from classical community acquired-MRSA and hospital acquired-MRSA lineages: functional analysis of infection and colonization processes. *International Journal of Medical Microbiology*, 304**:** 777-786.

Barbosa, T. M. and Levy, S. B. (2000). The impact of antibiotic use on resistance development and persistence. *Drug Resistance Update*,3: 303–311.

Bardiau, M., Yamazaki, K., Duprez, J. N., Taminiau, B., Mainil, J. G. and Ote, I. (2013). Genotypic and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk of bovine mastitis. *Letters in Applied Microbiology*, 57:181-186.

Battisti, A., Franco, A., Merialdi, G., Hasman, H. and Iurescia, M. (2010). Heterogeneity among methicillin resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Veterinary Microbiology*, 142: 361-366.

Becker, K., Heilmann, C., and Peters, G. (2014). Coagulase-negative staphylococci. *Clinical Microbiology Reviews*, 27: 870-926.

Becker, K., van Alen, S., Idelevich, E. A., Schleimer, N., Seggewiss, J., Mellmann, A., Kaspar, U. and Peters, G. (2018). Plasmid-encoded transferable *mecB* mediated methicillin resistance in *Staphylococcus aureus*. *Emerging Infectious Diseases*, 24: 242-248.

Ben Slama, K., Gharsa, H., Klibi, N., Jouini, A., Lozano, C., Gómez-Sanz, E., Zarazaga, M., Boudabous, A. and Torres, C. (2011). Nasal carriage of *Staphylococcus aureus* in healthy humans with different levels of contact with animals in Tunisia: genetic lineages, methicillin resistance, and virulence factors. *European Journal of Clinical Microbiology and Infectious Diseases*, 30: 499–508.

Beynon, R. P, Bahl, V. K. and Prendergast, B. D. (2006). Infective endocarditis. *British Medical Journal*,333:334-339.

Bhattacharya, M., Berends, E. T. M., Chan, R., Schwabd, E., Royd, S., Send, C. K., Torres, V. J. and Wozniak, D. J. (2018). *Staphylococcus aureus* biofilms release leukocidins to elicit extracellular trap formation and evade neutrophil-mediated killing. *Proceedings of the National Association of Science USA*, 115: 7416-7421.

Bischoff, M., Dunman, P., Kormanec, J., Macapagal, D., Murphy, E., Mounts, W., Berger-Bachi, B., Projan, S. (2004). Microarray-based analysis of the *Staphylococcusaureus* sigmaB regulon. *Journal of Bacteriology*, 186: 4085-4099.

Bisdorff, B., Scholholter, J. L., Claussen, K., Pulz, M. and Nowak, D. (2011). MRSA ST398 in livestock farmers and neighboring residents in a rural area in Germany. *Epidemiology and Infection*, 140: 1800-1808.

Boles, B. R. and Horswill, A. R. (2011). Staphylococcal biofilm disassembly. *Trends in Microbiology*, 19: 449-455.

Bonjean, M., Hodille, E., Dumitrescu, O., Dupieux, C., Mongo, C. N., Allam, C., Beghin, M., Paris, M., Borrel, O., Chardon, H., Laurent, F., Rasigade, J. and Lina, G. (2016). Disk diffusion testing for detection of methicillin-resistant staphylococci: does moxalactam improve upon cefoxitin? *Journal of Clinical Microbiology*, 54: 2905-2909.

Bosch, T., de Neeling, A. J., Schouls, L. M., van der Zwaluw, K. W., Kluytmans, J. A., Grundmann, H. and Huijsdens, X. W. (2010). PFGE diversity within the methicillin-resistant *Staphylococcus aureus* clonal lineage ST398. *BMC Microbiology*, 10**:** 1-7.

Boyce, J. M. (1999). It is time for action: improving hand hygiene in hospitals. *Annals of Internal Medicine*, 130: 153–155.

Brennan, G. I., Herra, C., Coleman, D. C., O'Conell, B. and Shore, A. C. (2016). Evaluation of commercial chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. *The Journal of Hospital Infection*, 92: 287-292.

Breurec, S., Zriouil, S. B., Fall, C., Boisier, P., Brisse, S., Djibo, S., Etienne, J., Fonkoua, M. C., Perrier-Gros-Claude, J. D., Pouillot, R., Ramarokoto, C. E., Randrianirina, F., Tall, A., Thiberge, J. M., the Working Group on *Staphylococcus aureus* infections, Laurent, F. and Garin, B. (2011). Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. *Clinical Microbiology and Infection*, 17: 160-165.

Burillo, A., Rodríguez-Sanchez,B.,Ramiro,A.,Cercenado, E., Rodríguez-Creixems, M. andBouza,E.(2014).Gram-stainplusMALDI-TOFMS (Matrix-assisted laser desorption ionization-timeof flight mass spectrometry) for arapiddiagnosisofurinarytractinfection. *PLoSONE*,9:e86915.

Burlak, C., Hammer, C. H., Robinson, M. A., Whitney, A. R., McGavin, M. J., Kreiswirth, B. N. and Deleo, F. R. (2007). Global analysis of community-associated methicillin-resistant *Staphylococcus aureus* exoproteins reveals molecules produced in vitro and during infection. *Cell Microbiology*,9:1172-1190.

Butaye, P., Argudin, M. A. and Smith, T. C. (2016). Livestock-associated MRSA and its current evolution. *Current Clinical Microbiology Reports*, 3: 19-31.

Carfora, V., Caprioli, A., Grossi, I., Pepe, M., Alba, P., Lorenzetti, S., Amoruso, R., Sorbara, L., Franco, A. and Battisti, A. A. (2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) Sequence Type 8, spa type t11469 causing infection and colonizing horses in Italy. *Pathogens and Disease*, 74: ftw025.

Cefai, C., Ashurst, S. and Owens, C. (1994). Human carriage of methicillin-resistant *Staphylococcus aureus* linked with pet dog. *The Lancet*, 344: 539-540.

Centers for Disease Control and prevention (CDC). (2013). Antibiotic resistance threats in the United States, 508. Available online at: http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf.

Chairat, S., Gharsa, H., Lozano, C., Gomez-Sanz, E., Gomez, P., Zarazaga, M., Boudabous, A., Torres, C. and Ben Slamak, K. (2015). Characterization of *Staphylococcus aureus* from raw meat samples in Tunisia: detection of clonal lineage ST398 from the African continent. *Foodborne Pathogens and Disease*, 12: 686-692.

Chambers, H. F. **(**1993). Detection of methicillin resistant staphylococci. *Infectious Disease Clinics of North America*, **7:** 425-433.

Chen, S., Wang, Y., Chen, F., Yang, H., Gang, M. and Zheng, S. J. (2007). A highly pathogenic strain of *Staphylococcus sciuri* caused fatal exudative epidermitis in piglets. *PLoS One*, 1: e147.

Cheng, A. G., DeDent, A. C., Schneewind, O. and Missiakas, D. (2011). A play in four acts: *Staphylococcus aureus* abscess formation. *Trends in Microbiology*,19:225-232.

Cho, H., Uehara, T. and Bernhardt, T. G. (2014). Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. *Cell*, 159: 1300–1311.

Chopra, I. and Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, 65: 232–260.

Christiane, C., Alexander, F., Svetlana, K., Franziska, L. Urlich, N., Knut, O., Birgits, S., Birgit, W., Lothar, W. and Wolfgang, W. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* in different animal species. *International Journal of Medical Microbiology*, 300: 109-117.

Clauditz, A., Resch, A., Wieland, K. P., Peschel, A. and Götz, F. (2006). Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. *Infection and Immunity*,74: 4950-4953.

Clinical and Laboratory Standards Institute (CLSI). (2014). Performance standards for antimicrobial susceptibility testing; Twenty-fourth information suplement. CLSI document M100-24S, Vol. 34 No. 1, (ISBN 1-56238-897-5 [Print]; ISBN 1-56238-898-3 [Electronic]), Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, PA 19087 USA.

Cockfield, J. D., Pathak, S., Edgeworth, J. D., and Lindsay, J. A. (2007). Rapid determination of hospital-acquired meticillin-resistant *Staphylococcus aureus* lineages. *Journal of Medical Microbiology*. 56, 614–619.

Cohen, M. L. (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science*, 257: 1050-1055.

Coimbra, D. G., Almeida, A. G., Junior, J. B., da Silva, L. A., Pimentel, B. J., Gitai, D. L., Moreira, L. S., Silva-Filho, E. A. and de Andrade, T. G. (2011). Wound infection by multi-resistant *Staphylococcus sciuri* identified by molecular methods. *New Microbiology*, 34: 425-427.

Coimbra-e-Souza, V., Brito, M. A. V. P., Chamon, R. C., Laport, M. S. and Giambiagi-deMarval, M. (2017). Characterization of *Staphylococcus* spp. strains in milk from buffaloes with mastitis in Brazil: the need to identify to species level to avoid misidentification. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 69: 1669-1675.

Compernolle, V., Verschraegen, G. and Claeys, G (2007). Combined Use of Pastorex Staph-Plus and Either of Two New Chromogenic Agars, MRSA ID and CHROMagar MRSA, for Detection of Methicillin resistant *Staphylococcus aureus. Journal of Clinical Microbiology*, 45 (1): 154-158.

Conceicao, T., de Lencastre, H. and Aires-de-Sousa, M. (2017). Frequent isolation of methicillin resistant *Staphylococcus aureus* (MRSA) ST398 among healthy pigs in Portugal. *PLoS One*, 12: e0175340.

Cookson, B. and Philips, I. (1990). Methicillin resistant *Staphylococci*. *Journal of Bacteriology Symposium Supplement*, 69: 55S-70S.

Costa, W. L., Ferreira, J. S., Carvalho, J. S., Cerqueira, E. S., Oliveira, L. C. and Almeida, R. C. (2015). Methicillin-resistant *Staphylococcus aureus* in raw meats and prepared foods in public hospitals in Salvador, Bahia, Brazil. *Journal of Food Science*, 80: M147-M150.

Crossley, K. B., Archer, G. L., Jefferson, K. K. (2009). Staphylococci in human disease. John Wiley and Sons, Inc. Hoboken, New Jersey. Pp 65-68.

Cuny, C., Abdelbary, M. M. H., Köck, R., Layer, F., Scheidemann, W., Werner, G. and Witte, W. (2016). Methicillin-resistant *Staphylococcus aureus* from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans. *One Health*, 2: 11-17.

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nübel, U., Ohlsen, K., Strommenger, B., Walther, B., Wieler, L. and Witte, W. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *International Journal of Medical Microbiology*, 300**:** 109-117.

Cuny, C., Layer, F., Strommenger, B. and Witte, W. (2011). Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mec*A homologue in humans in Germany. *PLoS One*, 6: e24360.

Cuny, C., Nathaus, R., Layer, F., Strommenger, B., Altmann, D. and Witte, W. (2009). Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. *PLoS One*, 4(8): e6800.

Cuny, C., Strommenger, B., Witte, W. and Stanek, C. (2008). Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. *Microbial Drug Resistance,* 14:307-310.

Cuny, C., Wieler, H. L. and Wolfgang, W. (2015). Livestock-associated MRSA: the impact on humans. *Antibiotics,* 4: 521-543.

Damborg, P., Broens, E. M., Chomel, B. B., Guenther, S., Pasmans, F., Wagenaar, J. A., Weese, J. S., Wieler, L. H., Windahl, U., Vanrompay, D. and Guardabassi, L. (2016). Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. *Journal of comparative pathology*, 155: S27-S40.

Datta, P., Gulati, N., Singla, N., Vasdeva, H. R., Bala, K., Chander, J., and Gupta, V. (2011). Evaluation of various methods for the detection of meticillin resistant *Staphylococcus aureus* strains and susceptibility patterns. *Journal of Medical Microbiology*, 60: 1613–1616.

Daum, R. S. (2007). Skin and soft-tissue infections caused by methicillin resistant *Staphylococcus aureus. New England Journal of Medicine*, 357: 280-290.

David, M. Z. and Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*, 23, 616-687.

Davis, M. F., Cain, C.L., Brazil, A. M. and Rankin, S.C. (2013). Two coagulase-negative staphylococci emerging as potential zoonotic pathogens: wolves in sheep's clothing? *Frontiers in Microbiology*, 4: 123.

de Boer, E., Zwartkruis-Nahuis, J. T., Wit, B., Huijsdens, X. W., de Neeling, A. J., Bosch, T., van Oosteron, R. A., Vila, A. and Heuvelink, A. E. (2009).. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology*, 134: 52-56.

De Faveri, E., Rimoldi S., Pagani C. and Sala, V. (2014). Interspecific epidemiology of MRSA in pig Farming. *Journal of Infectious Diseases and Therapy,* 2: 135-138.

DeLeo, F. R., Otto, M., Kreiswirth, B. N. and Chambers, H. F. (2010). Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet*, 375: 1557-1568.

Denis, O., Suetens, C., Hallin, M., Catry, B., Ramboer, I., Dispas, M., Willems, G., Gordts, B., Butaye, P. and Struelens, J. M. (2009). Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerging Infectious Diseases*, 15: 1098-1101.

Depardieu, F., Podglajen, I., Leclercq, R., Collatz, E., and Courvalin, P. (2007). Modes and modulations of antibiotic resistance gene expression. *Clinical Microbiology Reviews*, 20: 79-114.

Detwiler, A., Bloom, P., Petersen, A. and Rosser, E. J. (2013). Multi-drug and methicillin resistance of staphylococci from canine patients at a veterinary teaching hospital (2006-2011). *Veterinary Quarterly,* 33: 60-67.

Devriese, L. A., Vandamme, L. R. and Fameree, L. (1972). Methicillin-resistant *Staphylococcus* strains isolated from bovine mastitis cases. *Zentralblatt fur Veterinarmedizin*, B19: 598-605.

Diep, B. and Otto, M. (2008). The role of virulence determinants in community-associated MRSA pathogenesis. *Trends in Microbiology*, 16: 361-369.

Diep, B. A., Gill, S. R., Chang, R. F., Phan, T. H., Chen, J. H., Davidson, M. G., Lin, F., Lin, J., Carleton, H. A., Mongodin, E. F., Sensabaugh, G. F. and Perdreau-Remington, F. (2006). Complete genome sequence of USA300, an epidemic clone of acquired methicillin-resistant *Staphylococcus aureus*. *Lancet*, 367:731-739.

Dierikx, C. M., Hengeveld, P. D., Veldman, K. T., de Haan, A., van der Voorde, S. and Dop, P. Y. (2016). Ten years later: still a high prevalence of MRSA in slaughter pigs despite significant reduction in antimicrobial usage in pigs in the Netherlands. *Journal of Antimicrobial Chemotherapy*, 71: 2414-2418.

Dinges, M. M., Orwin, P. M. and Schlievert, P. M. (2000). Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*,13:16-34.

Dweba, C. C., Zishiri, O. T. and El Zowalaty, M. E. (2018). Methicillin-resistant *Staphylococcus aureus*: livestock-associated, antimicrobial, and heavy metal resistance. *Infection and Drug Resistance*, 11: 2497-2509.

Edwards, A. M. and Massey, R. C. (2011). How does *Staphylococcus aureus* escape the bloodstream? *Trends in Microbiology*,19: 184-190.

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J. and Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of Clinical Microbiology*,38**:** 1008-1015.

Enright, M. C., Robinson, D. A., Randle, G., Feil, E. J., Grundmann, H. and Spratt, B. G. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of the National Academy of Science USA*, 99: 7687–7692.

European Food Safety Authority (EFSA). (2009). Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant *Staphylococcus aureus* (MRSA) in livestock, companion animals and foods. *EFSA Journal*, 7**:**1-10.

European Food Safety Authority (EFSA). (2012). Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food. *EFSA Journal*, 10**:** 1-56.

Fall, C., Seck, A., Richard, V., Ndour, M., Sembene, M., Laurent, F. and Breurec, S. (2012). Epidemiology of *Staphylococcus aureus*in pigs and farmers in the largest farm in Dakar, Senegal. *Foodborne Pathogens and Diseases*. 9, 962-965.

Fang, H. W., Chiang, P. H. and Huang, Y. C. (2014). Livestock-associated methicillin resistant *Staphylococcus aureus* ST9 in pigs and related personnel in Taiwan. *PLoS One*, 9: e88826.

Fitzgerald J. R. (2012). Human origin for livestock-associated methicillin-resistant *Staphylococcus aureus*. *mBio*, 3(2): e00082-12.

Food and Agricultural Organization (2016). Drivers, dynamics and epidemiology of antimicrobial resistance in animal production. Food and Agricultural Organization-Rome.

Foster, T. J. (2005). Immune evasion by staphylococci. *Nature Reviews Microbiology*,3: 948-958.

Foucault, M. L., Courvalin, P. and Grillot-Courvalin, C. (2009). Fitness cost of VanA-type vancomycin resistance in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy*, 53: 2354–2359.

García-Álvarez, L., Holden, M. T. G., Lindsay, H., Webb, C. R., Brown, D. F. J., Curran, M. D., Walpole, E., Brooks, K., Pickard, D. J., Teale, C., Parkhill, J., Bentley, S. D., Edwards, G. F., Girvan, E. K., Kearns, A. M., Pichon, B., Hill, R. L. R., Larsen, A. R., Skov, R. L., Peacock, S. J., Maskell, D. J. and Holmes, M. A. (2011). Meticillin-resistant *Staphylococcus aureus* with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infectious Diseses*, 11: 595-603.

Gehanno, J., Louvel, A., Nouvellon, M., Caillard, J. F. and Pestel-Caron, M. (2009). Aerial dispersal of meticillin-resistant *Staphylococcus aureus* in hospital rooms by infected or colonised patients. *Journal of Hospital Infection*,71:256-262.

Gharsa, H., Ben Slama, K., Lozano, C., Gómez-Sanz, E., Klibi, N., Ben Sallem, R., Gómez, P., Zarazaga, M., Boudabous, A. and Torres, C. (2012). Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. *Veterinary Microbiology*, 156: 367–373.

Ghasemian, A., Peerayeh, S. N., Bakhshi, B. and Mirzaee, M. (2015). The microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) genes among clinical isolates of *Staphylococcus aureus* from hospitalized children. *Iranian Journal of Pathology*, 10: 258-264.

Ghebremedhin, B., Olugbosi, M. O., Raji, A. M. (2009) Emergence of a community associated methicillin resistant *Staphylococcus aureus* strain with a unique resistant profile in southwest Nigeria. *Journal of Clinical Microbiology*, 47: 2975-2980.

Giesbrecht, P., Kersten, T. and Maidhof, H. (1998).Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. *Microbial and Molecular Biology Reviews*, 62: 1371–1414.

Gorwitz, R. J., Kruszon-Moran, D., McAllister, S. K., McQuillan, G., McDougal, L. K., Fosheim, G. E., Jensen, B. J., Killgore, G., Tenover, F. C. and Kuehnert, M. J. (2008). Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. *The Journal of Infectious Diseases*, 197**:** 1226-1234.

Graveland, H., Wagenaar, J. A., Heesterbeek, H., Mevius, D. and van Duijkeren, E. (2010) Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human

MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One*, 5: e10990.

Guardabassi, L., Larsen, J., Weese, J. S., Butaye, P., Battisti, A., Kluytmans, J., Lloyd, D. H. and Skov, R. L. (2013). Public health impact and antimicrobial selectionof meticillin-resistant staphylococci in animals. *Journal of Global AntimicrobialResistance*, 1: 55-62.

Guardabassi, L., Stefan, S. and Llyod, D, H. (2004). Pet animals as reservoirs of antimicrobial resistant bacteria. *Journal of Antimicrobial Chemotherapy*, 54: 321-332.

Guembe,M.,Rodríguez-Sanchez,B.,Ruiz,A.,Martín-Rabadán,P., Rodríguez- Creixems, M. andBouza,E.(2014).CanMALDI-TOFmassspectrometrybe usedwithintravascularcatheters? *Enfermedades Infecciosas y Microbiologia Clinica*,32: 372-374.

Guerin, F., Fines-Guyon, M., Meignen, P., Delente, G., Fondrinier, C., Bourdon, N., Cattoir, V. and Leon, A. (2017). Nationwide molecular epidemiology of methicillin-resistant *Staphylococcus aureus* responsible for horse infections in France. *BMC Microbiology*, 17: 104-110.

Guinane, C. M., Penades J. R. and Fitzgerald, J. (2011). The role of horizontal gene transfer in *Staphylococcus aureus* host adaptation. *Virulence*, 2(3): 241-243.

Gul, H. C., Kilic, A., Guclu, A. U., Bedir, O., Orphon, M. and Basustaoglu, A. C. (2008). Macrolide-lincosamide-streptogramin B resistant phenotypes and genotypes for methicillin-resistant *Staphylococcus aureus* in Turkey, from 2003 to 2006. *Polish Journal ofMicrobiology*, 57: 307-312.

Haag, A. F., Fitzgerald, J. R. and Penadés, J. R. (2019). *Staphylococcus aureus* in animals. *Microbiology Spectrum*, 7(3):GPP3-0060-2019.

Haenni, M., Chatre, P., Dupieux-Chabert, C., Metayer, V., Bes, M., Madec, J. and Laurent, F. (2017). Molecular epidemiology of methicillin resistant *Staphylococcus aureus* in horses, cats and dogs over a 5-year period in France. *Frontiers in Microbiology*, 8:2493.

Hammad, A. M., Watanabe, W., Fujii, T. and Shimamoto, T. (2012). Occurrence and characteristics of methicillin-resistant and -susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. *International Journal of Food Microbiology*, 156:286-289.

Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., de Lencastre, H., Bentley, S. D., Kearns, A. M., Holden, M. T. G. (2017). Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. *Genome Biology*, 18: 130.

Harmsen, D., Claus, H., Witte, W., Rothgänger, J., Claus, H., Turnwald, D. and Vogel, U. (2003). Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *Journal of Clinical Microbiology*, 41: 5442-5448.

Harmsen, D., Claus, H., Witte, W., Rothgänger, J., Claus, H., Turnwald, D. and Vogel, U. (2003). Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *Journal of Clinical Microbiology*, 41: 5442-5448.

Harrison, E. M., Paterson, G. K., Holden, M. T. G., Larsen, J., Stegger, M., Larsen, A. R., Petersen, A., Skov, R. L., Christensen, J. M., Bak Z. A., Heltberg, O., Harris, S. R., Zadoks, R. N., Parkhill, J., Peacock, S. J. and Holmes, M. A. (2013). Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mec*A homologue *mec*C. *EMBO Molecular Medicine,* 5: 509-515.

Harrison, E. M., Weinert, L. A., Holden, M. T. G., Welch, J. J., Wilson, K., Morgan, F. J. E., Harris, S. R., Loeffler, A., Boag, A. K., Peacock, S. J., Paterson, G. K., Waller, A. S., Parkhill, J. and Holmes, M. A. (2014). A shared population of epidemic methicillin-resistant *Staphylococcus aureus* circulates in humans and companion animals. *mBio*, 5: 0985-13.

Hauschild, T., Sacha, P., Wieczorek, P., Zalewska, M., Kaczynska, K. and Tryniszewska, E. (2008). Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. *Folia Histochemica et Cytobiologica*, 46(2): 225–228.

He, W., Chen, H., Zhao, C., Zhang, F., Li, H., Wang, Q., Wang, X. and Wang, H. (2013). Population structure and characterisation of *Staphylococcus aureus* from bacteraemia at multiple hospitals in China: association between antimicrobial resistance, toxin genes and genotypes. *International Journal of Antimicrobial Agents*, 42: 211–219.

Higgins, J., Loughman, A., van Kessel, K. P., van Strijp, J. A. and Foster, T. J. (2006). Clumping factor A of *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. *FEMSMicrobiology Letters*,258:290-296.

Hiramatsu, K., Katayama, Y., Matsuo, M., Sasaki, T., Morimoto, Y., Sekiguchi, A. and Baba, T. (2014). Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy. *Journal of Infection and Chemotherapy*, 20: 593-601.

Holmes, M. and Zadoks, R.N. (2011). Methicillin resistant *S. aureus* in human and bovine mastitis. *Journal of Mammary Gland Biology and Neoplasia*, 16: 373-382.

Houvinen, P. (2001). Resistance to trimethoprim-sulfamethoxazole. *Clinical Infectious Diseases*, 32: 1608-1614.

Huang, Y. C., Su, C. L. H., Chen, C. J. and Lin, T. Y. (2007). Prevalence of methicillin resistant *Staphylococcus aureus* nasal colonization among Taiwanese children in 2005 and 2006. *Journal of Clinical Microbiology*, 45: 3992-3995.

Huber, K., Koller, S., Giezendanner, N., Stephan, R. and Zweifel, C. (2010). Prevalence and characteristics of methicillin-resistant *Staphyloccocus aureus* in humans in contact with farm animals, in livestock, and in food of animals' origin, Switzerland, 2009. *European Surveillance,* 15: 19542.

Islam, M. Z., Espinosa-Gongora, C., Damborg, P., Sieber, R. N., Munk, R., Husted, L., Moodley, A., Skov, R., Larsen, J. and Guardabassi, L. (2017). Horses in Denmark are

a reservoir of diverse clones of methicillin-resistant and susceptible *Staphylococcus aureus*. *Frontiers in Microbiology*. 8: 543.

Ito, T., Hiramatsu, K., Oliveira, D. C., De Lencastre, H., Zhang, K., Westh, H., Giffad, P. M., Colemen, D., Tenover, F. C., Boyle-Vavra, S., Skov, R. L., Enright, M. C., Kreiswirth, B., Ko, K. S., Grundmann, H., Laurent, F., Sollid, J. E., Kearns, A. M., Goering, R., John, J. F., Daum, R. and Soderquist, B. (2009).Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): Guidelines for reporting novel SCC*mec* elements. *Antimicrobial Agents and Chemotherapy.* 53, 4961-4967.

Ito T, Hiramatsu, K., Tomasz, A., de Lencastre, H., Perreten, V., Holden, M. T. G., Coleman, D. C., Goering, R., Giffard, P. M., Skov, R. L., Zhang, K., Westh, H., O'Brien, F., Tenover, F. C., Oliveira, D. C., Boyle-Vavra, S., Laurent, F., Kearns, A. M., Kreiswirth, B., Ko, K. S., Grundmann, H., Sollid, J. E., John, J. F., Daum, R., Soderquist, B. and Buist, G. (2012). Guidelines for reporting novel *mecA* gene homologues. *Antimicrobial Agents and Chemotherapy*. 56: 4997-4999.

Jamrozy, D. M., Fielder, M. D., Butaye, P. and Coldham, N. G. (2012) Comparative genotypic and phenotypic characterisation of methicillin resistant *Staphylococcus aureus* ST398 isolated from animals and humans. *PLoS One*, 7: e40458.

Jensen, S. O. and Lyon, B. R. (2009). Genetics of antimicrobial resistance in *Staphylococcus aureus*. *Future Microbiology*, 4: 565–582.

Joensen, K. G., Scheutz, F., Lund, O., Hasman, H., Kaas, R. S., Nielsen, E. M. and Aarestrup, M. F. (2014). Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *Journal of Clinical Microbiology*, 52: 1501-1510.

Johnson, A. P., Uttley, A. H. C., Woodford, N. and George, R. C. (1990). Resistance to vancomycin and teicoplanin: an emerging clinical problem. *Clinical Microbiology Reviews*, 3: 280–291.

Kahne, D., Leimkuhler, C., Lu, W. and Walsh, C. (2005). Glycopeptide and lipoglycopeptide antibiotics. *Chemical Reviews*, 105: 425–448.

Kapoor, G., Saigal, S. and Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: a guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33: 300-305.

Kasper, U., Kriegeskorte, A., Schubert, T., Peters, G., Rudack, C., Pieper, D. H., Wos-Oxley, M. and Becker, K. (2016). The culturome of human nose habitats reveals individual bacterial fingerprint patterns. *Environmental Microbiology*, 18: 2130-2142.

Katayama, Y., Ito, T. and Hiramatsu, K. (2000). A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy*, 44: 1549–1555.

Khanna, T., Friendship, R., Dewey, C. and Weese, J. S. (2008). Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary Microbiology*, 128: 298–303.

Kinross, P., Petersen, A., Skov, R., van Hauwermeiren, E., Pantosti, A., Laurent, F., Voss, A., Kluytmans, J., Struelens, M. J., Heuer, O., Monnet, D. L. and The European Human, L. A. M. S. G. (2017). Livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA) among human MRSA isolates, European Union/European Economic Area countries, 2013. *European Surveillance*,22**:** 1-13.

Klare, I., Konstabel, C., Badstübner, D., Werner, G. and Witte, W. (2003). Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. *International Journal of Food Microbiology*, 88: 269-290.

Kluytmans, J., Van Griethuysen, A., Willemse, P. and Van Keulen, P. (2002). Performance of Chromagar selective medium and Oxacillin resistance screening agar base for identifying *Staphylococcus aureus* and detecting methicillin resistance. *Journal of Clinical Microbiology*, 40: 2480-2482.

Kluytmans-Vandenbergh, M. F. and Kluytmans, J. A. (2006). Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. *Clinical Microbiology and Infection*, 12 (S1): 9-15.

Kobayashi, S. D. and DeLeo, F. R. (2013). *Staphylococcus aureus* protein A promotes immune suppression. *mBio*, 4: e00764-13.

Kock, R., Becker, K., Cookson, B., van Gemert-Pijnen, J. E, Harbarth, S., Kluytmans, J., Mielke, M., Peters, G., Skov, R. L., Struelens, M. J., Tacconelli, E., Navarro, T. A., Witte, W. and Friedrich, A. W. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *European Surveillance*, 15(41): 19694.

Kock, R., Schaumburg, F., Mellmann, A., Koksal, M., Jurke, A., Becker, K. and Friedrich, A. W. (2013) Livestock-associated methicillin resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. *PLoS One*, 8(2): e55040.

Kolawole, O. D., Adeyanju, A., Schaumburg, F., Akinyoola, L. A., Lawal, O. O., Amusa, B. Y., Kock, R. and Becker, K. (2013). Characterization of colonizing *Staphylococcus aureus* isolated from surgical ward's patients in a Nigerian university hospital. *PLoS One*, 8: e68721.

Kolenda, C., Dupieux, C., Decousser, J., Larsen, A. R., Pichon, B., Holmes, M., Bès, M., Teale, C., Dickson, E., Hill, R., Skov, R., Kearns, A. and Laurent, F. (2017). Comparison of automated antimicrobial susceptibility testing systems to detect mecC positive methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 55: 3554-3556.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J. and Hiramatsu, K. (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrobial Agents Chemotherapy*, 51, 264-274.

Kong, E. F., Johnson, J. K. and Jabra-Rizk, M. A. (2016). Community-associated methicillin resistant *Staphylococcus aureus*: an enemy amidst us. *PLoS Pathogens*, 12: e1005837.

Kpeli, G., Buultjens, A. H., Giulieri, S., Owusu-Mireku, E., Aboagye, S.Y., and Baines, S. L. (2017). Genomic analysis of ST88 community acquired methicillin resistant *Staphylococcus aureus* in Ghana. *Peer Journal*, 28: e3047.

Kumar, A., Kashyap, B. and Mehndiratta, A. (2017). Comparison of rapid and conventional methods for the detection of methicillin resistance in *Staphylococcus aureus* from blood cultures. *Tropical Journal of Medical Research*, 20: 196-200.

Lakhundi, S. and Zhang, K. (2018). Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution and epidemiology. *Clinical Microbiology Reviews*, 31: e00020-18.

Lallemand, E. A., Lacroix, M. Z., Toutain, P., Boullier, S., Ferran, A. A. and Bousquet-Melou, A. (2016). *In vitro* degradation of antimicrobials during use of broth microdilution method can increase the measured minimum inhibitory and minimal bactericidal concentrations. *Frontiers in Microbiology*, 7:2051.

Larsen, J., Imanishi, M., Hinjoy, S., Tharavichitkul, P., Duangsong, K., Davis, M. F., Nelson, K. E., Larsen, A. R. and Skov, R. L. (2012). Methicillin-resistant *Staphylococcus aureus* ST9 in pigs in Thailand. *PLoS One*, 7(2): e31245.

Laurent, F., Chardon, H., Haenni, M., Bes, M., Reverdy, M., Madec, J., Lagier, E., Vandenesch, F. and Tristan, A. (2012). MRSA harboring *mec*A Variant Gene. *Emerging Infectious Diseases*, 18: 1465–1467.

Leahy, T. R., Yau, Y. C. W., Atenafu, A., Ratjen, F. and Waters, V. **(**2011). Epidemiology of borderline oxacillin-resistant *Staphylococcus aureus* in pediatric cystic fibrosis. *Pediatric Pulmonology*, 46: 489-496.

Leclercq, R., Derlot, E., Duval, J. and Courvalin, P. (1988). Plasmid mediated resistance to vancomycin and teicoplanin in Enterococcus faecium. *New England Journal of Medicine*, 319: 157–161.

Lee, B.Y., Singh, A., David, M. Z., Bartsch, S. M., Slayton, R. B., Huang, S. S., Zimmer, S. M., Potter, M. A., Macal, C. M., Laudardale, D. S., Miller, L. G. and Daum, R. S. (2013a). The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *Clinical Microbiology and Infection*, 19: 528-536.

Lee, C., Cho, I. H., Jeong, B. C. and Lee, S. H. (2013b). Strategies to minimize antibiotic resistance. *International Journal of Environmental Research and Public Health*, 10: 4274-4305.

Lee, J. H. (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied Environmental Microbiology*, 69: 6489-6494.

Li, M., Du, X., Villaruz, A. E., Diep, B. A., Wang, D., Song, Y., Tian, Y., Hu, J., Yu, F., Lu, Y. and Otto, M. (2012). MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nature Medicine*,18**:** 816-819.

Li, Q., Du, X., Hong, X., Li, T., Zheng, B., He, L., Wang, Y., Otto, M. and Li, M. (2015). Targeting surface protein SasX by active and passive vaccination to reduce *Staphylococcus aureus* colonization and infection. *Infection and Immunity*, 83: 2168-2174.

Lim, D. and Strynadka, N. C. (2002). Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nature Structural Biology*, 9: 870–876.

Lin, J., Yeh, K., Liu, H. and Lin, J. (2009). Staphylococcus aureus isolated from pork and chicken carcass in Taiwan: prevalence and antimicrobial susceptibility. *Journal of Food Protection*, 72: 608-611.

Lindsay, J. A. (2010). Genomic variation and evolution of *Staphylococcus aureus*. *International Journal of Medical Microbiology*, 300: 98–103.

Lindsay, J. A. (2013). Hospital-associated MRSA and antibiotic resistance-What have we learned from genomics? *International Journal of Medical Microbiology*, 303: 318-323.

Lindsay, J. A. (2014). *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. *International Journal of Medical Microbiology*,304: 103–109.

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

Liu, D., Chai, T., Xia, X., Gao, Y., Cai, Y., Li, X., Miao, Z., Hao, H., Roesler, U. and Wang, J. (2012). Formation and transmission of *Staphylococcus aureus* (including MRSA) aerosols carrying antibiotic-resistant genes in poultry farming environment. *Science of the Total Environment*, 426: 139-145.

Liu, W., Liu, Z., Yao, Z., Fan, Y., Ye, X. and Chen, S. (2015). The prevalence and influencing factors of methicillin-resistant *Staphylococcus aureus*carriage in people in contact with livestock: a systematic review. *American Journal of Infection Control*, 43: 469-475.

Locatelli, C., Cremonese, P., Caprioli, A., Carfora, V., Lanzano, A., Barberio, A., Morandi, S., Casula, A., Castiglioni, B., Bronzo, V. and Moronill, P. (2017). Occurrence of methicillin-resistant *Staphylococcus aureus* in dairy cattle herds, related swine farms, and humans in contact with herds. *Journal of Dairy Science*, 100: 608-619.

Loeffler, A. and Lloyd, D. H. (2010). Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiology and Infection*, 138: 595-605.

Loeffler, A., Boag, A. K., Sung, J., Lindsay, J. A., Guardabassi, L., Dalsgaard, A., Smith, H., Stevens, K. B. and Lloyd, D. H. (2005). Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *Journal of Antimicrobial Chemotherapy*, 56: 692–697.

Loncaric, I., Künzel, F., Licka, T., Simhofer, H., Spergser, J. and Rosengarten, R. (2014). Identification and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from Austrian companion animals and horses, *Veterinary Microbiology*, 168: 381–387.

Lowder, B. V., Guinane, C. M., Ben Zakour, N. L., Weinert, L. A., Conway-Morris, A., Cartwright, R. A., Simpson, A. J., Rambaut, A., Nubel, U. and Fitzgerald, J. R. (2009). Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proceedings of the National Academy of Science U. S. A*, 106: 19545-19550.

Lowy, F. (2013). Staphylococcal infections, In: *Harrison's Principles of Internal Medicine*. Fauci, A., Braunwald, E. and D. Casper, eds. 18th Ed. McGraw-Hill, New York, Pp. 386–399.

Lowy, F. D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *Journal of Clinical Investigation*, 111: 1265-1273.

Lozano, C., Gharsa, H., Ben Slama, K., Zarazaga, M. and Torres, C. (2016). *Staphylococcus aureus* in animals and food: methicillin resistance, prevalence and population structure. A review in the African Continent. *Microorganisms*, 4: 12.

Marimuthu, K., Pittet, D. and Harbarth, S. (2014). The effect of improved hand hygiene on nosocomial MRSA control. *Antimicrobial Resistance and Infection Control*, 3: 34.

McCarthy, A. J., Breathnach, A. S. and Lindsay, J. A. (2012b). Detection of mobile genetic element variation between colonizing and infecting hospital-associated methicillin-resistant *Staphylococcus aureus* isolates. *Journal of Clinical Microbiology*, 50: 1073-1075.

McCarthy, A. J., Lindsay, J. A. (2010). Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implicationsfor vaccine design and host-pathogen interactions. *BMC Microbiology*, 10: 173.

Mccarthy, A. J., Lindsay, J. A. and Loeffler, A. (2012a). Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. *Veterinary Dermatology*,23: 267-275.

McDougal, L. K. and Thornsberry, C. **(**1986). The role of β-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *Journal of Clinical Microbiology*, 23: 832-839.

McGuinness, W. A., Malachowa, N. and DeLeo, F. R. (2017). Vancomycin resistance in *Staphylococcus aureus. Yale Journal of Biology and Medicine*, 90: 269-281.

Mediavilla, J. R., Chen, L., Mathema, B. and Kreiswirth, N. B. (2012). Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CAMRSA). *Current Opinion in Microbiology*, 15: 588-595.

Mertz, D., Frei, R., Periat, N., Zimmerli, M., Battegay, M., Fluckiger, U. and Widmer, A. F. (2009). Exclusive *Staphylococcus aureus* throat carriage: at-risk populations. *Archives of Internal Medicine*, 169: 172-178.

Miller, L. G. and Diep, B. A. (2008). Colonization, fomites, and virulence: rethinking the pathogenesis of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Diseases*, 46: 752-760.

Miragaia, M. (2018). Factors contributing to the evolution of *mecA*-mediated β-lactam resistance in staphylococci: update and new insights from whole genome sequencing (WGS). *Frontiers in Microbiology*.9: 1-16.

Mohamed, N., Timofeyeva, Y., Jamrozy, D., Rojas, E., Hao, L., Silmon de Monerri, N. C., Hawkins, J., Singh, G., P., Cai, B., Liberator, P., Sebastian, S., Donald, R. G. K., Scully, I. L., Jones, C. H., Creech, C. B., Thomsen, I., Parkhill, J., Peacock, S. J., Jansen, K. U., Holden, M. T. G. and Anderson, A. S. (2019). Molecular epidemiology and expression of capsular polysaccharides in *Staphylococcus aureus* clinical isolates in the United States. *PLoS One*, 14: e0208356.

Momoh, A. H., Kwaga, J. K. P., Bello, M. and Sackey, A. K. B. (2016). Prevalence and antimicrobial resistance pattern of coagulase negative staphylococci isolated from pigs and in-contact humans in Jos metropolis, *Nigeria. Nigerian Veterinary Journal*, 37: 140-147.

Momoh, A. H., Kwaga, J. K. P., Bello, M., Sackey, A. K. B., Larsen, A. R. (2018). Antibiotic resistance and molecular charcteristics of *Staphylococcus aureus* isolated from backyard-raised pigs and pig workers. *Tropical Animal Health and Production*, 50: 1565-1571.

Monecke, S., Jatzwauk, L., Müller, E., Nitschke, H., Pfohl, K., Slickers, P., Reissig, A., Ruppelt-Lorz, A. and Ehricht, R. (2016). Diversity of SCC*mec* elements in *Staphylococcus aureus* as observed in south-eastern Germany. *PLoS One*,11: 1–24.

Monecke, S., Slickers, P. and Ehricht R. (2008). Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis andpattern recognition. *FEMS Immunology and Medical Microbiology*, 53: 237–251.

Moodley, A., Espinosa-Gongora, C., Nielsen, S. S., McCarthy, A. J., Lindsay, J. A and Guardabassi, L. (2012). Comparative host specificity of human- and pig- associated *Staphylococcus aureus* clonal lineages. *PLoS One*, 7: e49344.

Moravvej, Z., Estaji, F., Askari, E., Solhjou, K., Naderi Nasab, M. and Saadat, S. (2013). Update on the global number of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains. *International Journal of Antimicrobial Agents*, 42: 370-371.

Morcillo, A., Castro, B., Rodríguez-Álvarez, C., Abreu, R., Aguirre-Jaime, A. and Arias, A. (2015). Descriptive analysis of antibiotic-resistant patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 isolated from healthy swine. *International Journal of Environmental Research and Public Health*,12**:** 611-622.

Moremi, N., Mshana, E. S., Kamugisha, E., Kataraihya, B. J., Tappe, D., Vogel, U., Lyamuya F. E. and Claus, H. (2012). Predominance of methicillin resistant *Staphylococcus*

aureus ST88 and new ST1797 causing wound infection and abscesses. *Journal of Infection in Developing Countries*, 6: 620-625.

Mulders, M. N., Haenen, A. P., Geenen, P. L., Vesseur, P. C., Poldervaart, E. S., Bosch, T., Huijsdens, X. W., Hengeveld, P. D., Dam-Deisz, W. D., Graat, E. A., Mevius, D., Voss, A. and Van De Giessen, A. W. (2010). Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. *Epidemiology and Infection*,138: 743-755.

Muller, S., Wolf, A. J. and Iliev, I. D. (2015). Poorly cross-linked peptidoglycan in MRSA due to *mec*A induction activates the inflammasome and exacerbates immunopathology. *Cell Host and Microbe*, 18: 604–612.

Munita, J. M. and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, 4: 1-24.

Nagase, N., Sasaki, A., Yamashita, K., Shimizu, A., Wakita, Y., Kitai, S. and Kawano, J. (2002). Isolation and distribution of staphylococci from human and animal skin. *Journal of Veterinary Medical Sciences*, 64: 245-250.

Nagel, M., Dischinger, J., Türck, M., Verrier, D., Oedenkoven, M., Ngoubangoye, B., Flohic, G., Drexler, J. F., Bierbaum, G. and Gonzalez, J. (2013). Human-associated *Staphylococcus aureus*strains within great ape populations in Central Africa (Gabon). *Clinical Microbiology and Infection*, 19: 1072–1077.

Narvaez-Bravo, C., Toufeer, M., Weese, S. J., Diarra, M. S., Deckert, A. E., Reid-Smith, R. and Aslam, M. (2016). Prevalence of methicillin-resistant *Staphylococcus aureus* in Canadian commercial pork processing plants. *Journal of Applied Microbiology*, 120: 770-780.

Neela, V., Mohd Zafrul, A., Mariana, N. S., van Belkum, A., Liew, Y. K. and Rad, E. G. (2009). Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. *Journal of Clinical Microbiology*, 47(12): 4138-4140.

Nemeghaire, S., Roelandt, S., Argudın, M. A., Haesebrouck, F. and Butaye, P. (2013). Characterization of methicillin-resistant *Staphylococcus aureus* from healthy carrier chickens. *Avian Pathology*, 42: 342-346.

Nsira S. B., Dupuis M. and Leclerq, R. (2006). Evaluation of MRSA *Select*, a new chromogenic medium for the detection of nasal carriage of methicillin resistant *Staphylococcus aureus*. *International Journal of Antimicrobial Agents*, 27: 561-564.

Nwankwo, E. O. and Nasiru, M. S. (2011). Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. *The Pan African Medical Journal*, 8:4-10.

O'Mahony, R., Abbott, Y., Leonard, F. C., Markey, B. K., Quinn, P. J., Pollock, P. J., Fanning, S. and Rossney, A. S., (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology*, 109: 285–296.

Okunlola, I. O., and Ayandele, A. A. (2015). Prevalence and antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus*(MRSA) among pigs in selected farms in Ilora, South Western Nigeria. *European Journal of Experimental Biology*, 5: 50–56.

O'Neill, E., Pozzi, C., Houston, P., Humphreys, H., Robinson, D. A., Loughman, A., Foster, T. J. and O'Gara, J. P. (2008). A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. *Journal of Bacteriology*,190:3835-3850.

Oppliger, A., Moreillon, P., Charriere, N., Giddey, M., Morisset, D. and Sakwinska, O. (2012). Antimicrobial resistance of *Staphylococcus aureus* strains acquired by pig farmers from pigs. *Applied Environmental Microbiology*, 78(22): 8010–8014.

Otalu, O. Jr., Kabir, J., Okolocha, E. C. and Umoh, V. J., Kwaga, J. K. P. and Owolodun, A. O. (2015). Detection of methicillin resistant *Staphylococcus aureus* in chicken carcasses and live birds in Zaria, Nigeria. *FUTA Journal of Research in Sciences*. 11: 132-138.

Otalu, O. J., Kwaga, J. K. P., Okolocha, E. C., Islam, M. Z. and Moodley, A. (2018). High genetic similarity of MRSA ST88 isolated from pigs and humans in Kogi State, Nigeria. *Frontiers in Microbiology*. 9: 3098.

Otto, M. (2013). Coagulase-negative staphylococci as reserviors of genes facilitating MRSA infection: staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays*, 35: 4-11.

Ouedraogo, A., Dunyach-Remy, C., Kissou, A., Sanou, S., Poda, A., Kyelem, G. C., Solassol, J., Banuls, A., van de Perre, P., Ouedraogo, R., Jean-Pierre, H., Lavigne, J. and Godreuil, S. (2016). High nasal carriage rate of *Staphylococcus aureus* containing Panton-Valentine leukocidin and EDIN encoding genes in community and hospital settings in Burkina Faso. *Frontiers in Microbiology*, 7: 1406.

Panda, R. K., Mahapatra, A., Mallick, B. and Chayani, N. (2016). Evualation of genotypic and phenotypic methods for detection of methicillin resistant *Staphylococcus aureus* in a tertiary care hospital of Eastern Odisha. *Journal of Clinical and Diagnostic Research*, 10: 19-21.

Pantosti, A. (2012). Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. *Frontiers in Microbiology*, 3:127.

Paterson, G. K., Harrison, E. M. and Holmes, M. A. (2014). The emergence of *mec*C methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiology*,22: 42–47.

Peacock, S. J. and Paterson, G. K. (2015). Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annual Reviews of Biochemistry*, 84: 577–601.

Persoons, D., van Hoorebeke, S., Hermans, K., Butaye, P., de Kruif, A., Haesebrouck, F. and Jeroen, D. (2009). Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerging Infectious Diseases*, 15 (3):452-453.

Petersen, A., Stegger, M., Heltberg, O., Christensen, J., Zeuthen, A., Knudsen, L. K., Urth, T., Sorum, M., Schouls, L., Larsen, J., Skov, R. and Larsen, A. R. (2013). Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir withtransmission to humans. *Clinical Microbiology and Infection*, 19: E16-22.

Petinaki, E. and Spiliopoulou, I. (2012). Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. *Clinical Microbiology and Infection*, 18: 626-634.

Pinho, M. G., de Lencastre, H. and Tomasz, A. (2001). An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proceedings of the National Academy of Science USA*, 98:10886-10891.

Pirolo, M., Gioffre, A., Vissagio, D., Gherardi, M., Pavia, G., Samele, P., Ciambrone, L., Natale, R. D., Spatari, G., Casalinuovo, F. and Visca, P. (2019). Prevalence, molecular epidemiology, and antimicrobial resistance of methicillin resistant *Staphylococcus aureus* from swine in southern Italy. *BMC Microbiology*, 19: 51-62.

Pittet, D., Hugonnet S., Harbarth, S., Mourouga, P., Sauvan, V. and Touveneau, S. (2000). Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. *Lancet*, 356:1307-1312.

Pletincks, L. J., Verhegghe, M., Dewulf, J., Crombe, F., De Bleecker, Y., Rasschaet, G., Goddeeris, B. M. and De Man, I. (2011). Screening of poultry-pig farms for methicillin resistant *Staphylococcus aureus*: sampling methodology and within herd prevalence in broiler flocks and pigs. *Infection Genetics and Evolution*,11: 2133-2137.

Porrero, M. C., Wassenaar, T. M., Gomez-Barrero, S., Garcia, M., Barcena, C., Alvarez, J., Saez-Llorente, J. L., Fernandez-Garayzabal, J. F., Moreno, M. A. and Dominguez, L. (2012). Detection of methicillin-resistant *Staphylococcus aureus* in Iberian pigs. *Letters in Applied Microbiology*, 54: 280-285.

Price, L., Stegger, M., Hasman, H. and Aziz, M. (2012). *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *Mbio*, 3: e00305–11.

Pu S, Han F, Ge B. (2009). Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. *Applied Environmental Microbiology*, 75: 265-267.

Putland, R. A., and Guinness, M. D. (1985). Autobac susceptibility testing of methicillin resistant and susceptible *Staphylococcusaureus* isolated in an Australian hospital. *Journal of Clinical Microbiology*.22: 822–827.

Pyorala, S. and Taponen, S. (2009). Coagulase-negative staphylococci emerging mastitis pathogens. *Veterinary Microbiology*, 134: 3–8.

Rahimi, F. (2016). Characterization of Resistance to Aminoglycosides in methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary care hospital in Tehran, Iran. *Jundishapur Journal of Microbiology*, 9(1): e29237.

Raji, A., Ojemhen, O., Umejiburu, U., Ogunleye, A., Blanc, D. S. and Basset, P. (2013). High genetic diversity of *Staphylococcus aureus*in a tertiary care hospital in Southwest Nigeria. *Diagnostic Microbiology and Infectious Diseases*, 77: 367–369.

Rajic, A., Reid-Smith, R., Deckert, E. A., Dewey, E. C. and McEwen, A. S. (2006). Reported antibiotic use in 90 swine farms in Alberta. *Canadian Veterinary Journal*, 47: 446-452.

Ramsay, G. (1998). DNA chips: state-of-the art. *Nature Biotechnology*, 16: 40-44.

Rekha, M. (2001). Implementation of strategies to control antimicrobial resistance. *Chest*, 119: 405S- 411S.

Reynaga, E., Navarro, M., Vilamala, A., Roure, P., Quintana , M., Garcia-Nunez, M., Figueras, R. Torres, C., Lucchetti, G. and Sabria, M. (2016). Prevalence of colonization by methicillin resistant *Staphylococcus aureus* ST398 in pigs and pig farm workers in an area of Catalonia, Spain. *BMC Infectious Diseases*, 16: 716.

Rich, M., Roberts, L. and Kearns, A. (2005). Methicillin-resistant staphylococci isolated from animals. *Veterinary Microbiology*, 105: 313-314.

Richter, A., Sting, R., Popp, C., Rau, J., Tenhagen, B. A., Guerra, B., Hafez, H. M. and Fetsch, A., (2012). Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals. *Epidemiology and Infection*,10, 1-10.

Rolo, J., Worning, P., Nielsen, J. B., Sobral, R., Bowden, R., Bouchami, O., Damborg, P., Guardabassi, L., Perreten, V., Westh, H., Tomasz, A., de Lancastre, H. and Miragaia, M. (2017). Evidence for the evolutionary steps leading to *mecA*-mediated β-lactam resistance in staphylococci. *PLoS Genet*,13: e1006674.

Rudkin, J. K., Edwards, A. M., Bowden, M. G., Brown, E. L., Pozzi, C., Waters, E. M., Chan, W. C., Williams, P., O'Gara, J. P. And Massey, R. C. (2012). Methicillin resistance reduces the virulence of healthcare-associated methicillin resistant *Staphylococcus aureus* by interfering with the agr quorum sensing system. *Journal of Infectious Diseases*, 205: 798–806.

Sabat, A. J., Budimir, A., Nashev, D., Sa-Leao, R., van Dijl, J., Laurent, F., Grundmann, H., Friedrich, A. W. and ESCMID Study Group of Epidemiological Markers. (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance*, 18: 20380.

Sahibzada, S., Abraham, S., Coombs, G. W., Pang, S., Hernández-Jover, M., Jordan, D. and Heller, J. (2017). Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. *Scientific Reports*, 7: 5273.

Sakoulas, G., Gold, H. S., Venkataraman, L., Degirolami, P. C., Eliopoulos, G. M. and Qian, Q. (2001). Methicillin-resistant *Staphylococcus aureus*: Comparison of susceptibility testing methods and analysis of *mecA*-positive susceptible strains. *Journal of Clinical Microbiology*, 39: 3946-3951.

Saleha, A. and Zunita, Z. (2010). Methicillin resistant *Staphylococcus aureus*: an emerging veterinary and zoonotic pathogen of public health concern and some studies in Malaysia. *Journal of Animal and Veterinary Advances*, 9 (7): 1094-1098.

Sanchini, A., Campanile, F., Monaco, M., Cafiso, V., Rasigade, J. P., Laurent, F., Etienne, J., Stefani, S. and Pantosti, A. (2011). DNA microarray-based characterisation of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* from Italy. *European Journal of Clinical Microbiology and Infectious Diseases*, 30:1399–1408.

Sangvik, M., Olsen, S. R., Olsen, K., Simonsen, S. G., Furberg, A., and Sollid, E. J. (2011). Age and gender associated *Staphylococcus aureus spa* types found among nasal carriers in a general population: the Tromso staph and skin study. *Journal of Clinical Microbiology*, 49: 4213-4218.

Sarowska, J., Futoma-Koloch, B., Jama-kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G. and Choroszy-Krol, I. (2019). Virulence fctors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. *Gut Pathogens*, 11: 10-26.

Sasaki, T., Kikuchi, K, Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K. (2007). Reclassification of phenotypically identified *Staphylococcus intermedius* strains. *Journal of Clinical Microbiology*, 45: 2770-2778.

Schaumburg, F., Alabi, A. S., Peters, G., and Becker, K. (2014). New epidemiology of *Staphylococcus aureus*infection in Africa. *Clinical Microbiology and Infection*, 20: 589-596.

Schaumburg, F., Mugisha, L., Peck, B., Becker, K., Gillespie, T. R., Peters, G. and Leendertz, F. H. (2012). Drug-resistant human *Staphylococcus aureus*in sanctuary apes pose a threat to endangered wild ape populations. *American Journal of Primatology*. 74: 1071–1075.

Schaumburg, F., Pauly, M., Anoh, E., Mossoun, A., Wiersma, L., Schubert, G., Flammen, A., Alabi, A. S., Muyembe-Tamfum, J., Grobusch, M. P., Karhemere, S., Akoua-Koffi, C., Couacy-Hymann, E., Kremsner, P. G., Mellmann, A., Becker, K., Leendertz, F. H. and Peters, G. (2015). *Staphylococcus aureus*complex from animals and humans in three remote African regions. *Clinical Microbiology and Infection*, 21: e1-e8.

Schmithausen, R. M., Schulze-Geisthoevel, S. V., Stemmer, F., El-Jade, M., Reif1, M., Hack, S., Meilaender, A., Montabauer, G., Fimmers, R., Parcina, M., Hoerauf, A., Exner, M., Petersen, B., Bierbaum, G. and Bekeredjian-Ding, I. (2015) Analysis of Transmission of MRSA and ESBLE among pigs and farm personnel. *PLoS One*, 10: e0138173.

Schulz, J., Boklund, A., Toft, N. and Halasa, T. (2019). Efects of control measures on the spread of LA-MRSA among Danish pig herds between 2006 and 2015 – a simulation study. *Scientific Reports*, 9: 691.

Schwarz, S., Kehrenberg, C., Doublet, B., and Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews*, 28: 519-542.

Schwarz, S., Shen, J., Kadlec, K., Wang, Y., Michael, G. B., Febler, A. T. and Vester, B. (2016). Lincosamides, Streptogramins, Phenicols, and Pleuromutilins: mode of action and mechanisms of resistance. *Cold Spring Harbor Perspectives in Medicine*, 6: a027037.

Scott, G. M., Thomson, R., Malone-Lee, J. and Ridgway, G. L. (1988). Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans? *The Journal of Hospital Infection*, 12: 29-34.

Seiler, C. and Berendonk, T. U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology*, 3: 399.

Shepheard M., Flemin V., Connor T., Corander J., Feil, E.J. and Fraser C. (2013). Historial zoonoses and other changes in host tropism of *Staphylococcus aureus*, identified by phylogenetic analysis of a population dataset. *PLoS One*. 8(5): e62369.

Shittu, A., Oyedara, O., Abegunrin, F., Okon, K., Raji, A., Taiwo, S., Ogunsola, F., Onyedibe, K. and Elisha, G. (2012). Characterization of methicillin-susceptible and -resistant staphylococci in the clinical setting: a multicentre study in Nigeria. *BMC Infectious Diseases*, 12:286.

Shittu, O. A., Okon, K., Adesida, S., Oyedara, O., Witte, W., Strommenger, B., Layer, F. and Nübel, U. (2011). Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. *BMC Microbiology*, 11: 92-99.

Shore, A. C. and Coleman, D. C. (2013). Staphylococcal cassette chromosome *mec*: recent advances and new insights. *International Journal of Medical Microbiology*,303: 350-359.

Shuping, L. L., Kuonza, L., Musekiwa, A., Iyaloo, S. and Perovic, O. (2017). Hospital-associated methicillin-resistant *Staphylococcus aureus*: a cross-sectional analysis of risk factors in South African tertiary public hospitals. *PloS One*, 12: e0188216.

Simor, A. E., Ofner-Agostini, M., Bryce, E., McGeer, A., Paton, S. and Mulvey, M. R., Canadian Hospital Epidemiology Committee and Canadian Nosocomial Infection Surveillance Program, Health Canada. (2002). Laboratory characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: results of 5 years of National Surveillance, 1995–1999. *The Journal of Infectious Diseases*, 186: 652–660.

Singhal, N., Kumar, M., Kanaujia, P. K. and Virdi, J. S. (2015). MALDI-TOF mass spectometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, 6: 791.

Skaar, E. P., Humayun, M., Bae, T., DeBord, K. L. and Schneewind, O. (2004). Iron-source preference of *Staphylococcus aureus* infections. *Science*,305:1626-1628.

Skov, R., Smyth, R., Larsen, A. R., Bolmstrom, A., Karlsson, A. and Mills, K.(2006). Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and E-test on Mueller-Hinton agar. *Journal of Clinical Microbiology*,44:4395-4399.

Smith, T.C. and Pearson, N. (2011). The emergence of *Staphylococcus aureus* ST398. *Vector-Borne Zoonotic Diseases*. 11(4): 327-339.

Smyth, R. W., Kahlmeter, G., Liljequist, B. O. and Hoffman, B. (2001). Methods for identifying methicillin resistance in *Staphylococcus aureus. Journal of Hospital Infection*,48: 103–107.

Sollid, J. U. E., Furberg, A. S., Hanssen, A. M. and Johannessen, M. (2014). *Staphylococcus aureus*: determinants of human carriage. *Infection, Genetics and Evolution*,21, 531-541.

Song, Z., Liu, X., Zhu, M., Tan, Y. and Wu, K. (2017). Using MALDI-TOF-MS to *Staphylococcus aureus* infected vitreous. *Molecular Vision*, 23: 407- 415.

Stegger, M., Lindsay, J. A., Moodley, A., Skov, R., Broens, E. M., and Guardabassi, L. (2011). Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying *sau*1-*hsd*S1. *Journal of Clinical Microbiology*, 49: 732–734.

Stepanovic S, Jezek P, Vukovic D., Dakic, I. and Petras, P. (2003). Isolation of members of the *Staphylococcus sciuri* group from urine and their relationship to urinary tract infections. *Journal of Clinical Microbiology*, 41: 5262-5264.

Strommenger, B., Kehrenberg, C., Kettlitz, C., Cuny, C., Verspohl, J., Witte, W. and Schwarz, S. (2006). Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *Journal of Antimicrobial Chemotherapy*, 57: 461-465.

Sung, J. M., Llyod, D. H. and Linsay, J. A. (2008). *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. *Microbiology*, 154: 1949-1959.

Tenover, F. C, Biddle, J. W. and Lancaster, M. V. (2001). Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. *Emerging Infectious Diseases*, 7: 327–332.

Thammavongsa, V., Kim, H. K., Missiakas, D. and Schneewind, O. (2015). Staphylococcal manipulation of host immune responses. *Nature Reviews Microbiology*, 13: 529-543.

Thrusfield, M. (2005). *Veterinary Epidemiology*. 5thed, Blackwell Scientific Publications, Oxford. Pp. 233-235.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. and Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28:603-661.

Tormo, M. A., Knecht, E., Gotz, F., Lasa, I. and Penades, J. R. (2005). Bap-dependent biofilm formation by pathogenic species of *Staphylococcus*: evidence of horizontal gene transfer? *Microbiology*,151: 2465-2472.

Trees, E., Rota, P. A., MacCannell, D., and Gerner-Smidt, P. (2015). Molecular epidemiology. In: *Manual of Clinical Microbiology*. Jorgensen, J. H., Pfaller, M. A., Caroll, K. C., Funke, G., Landry, M. L. and Richter, S. S. eds. 11th Edn. ASM Press. Washington, DC, Pp. 131–160.

Tsubakishita, S., Kuwahara-arai, K., Sasaki, T. and Hiramatsu, K. (2010). Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. *Antimicrobial Agents and Chemotherapy*.54: 4352-4359.

Tuchscherr, L., Pöllath, C., Siegmund, A., Deinhardt-Emmer, S., Hoerr, V., Svensson, C., Figge, M. T., Monecke, S. and Loffler, B. (2019). Clinical *S. aureus* isolates vary in their virulence to promote adaptation to the host. *Toxins*, 11: 135.

Tulinski, P., Fluit, C. A., Wagenaar, A. J., Mevius, D., van de Vijver, L and Duim, B. (2011). Methicillin-resistant coagulase-negative staphylococci on pig farms as a reservoir of heterogeneous staphylococcal cassette chromosome *mec* elements. *Applied and Environmental Microbiology*, 78: 299-304.

Ugwu, C., Gomez-Sanz, E., Agbo, C. I., Torres, C. and Chah, K. F. (2015). Characterization of mannitol-fermenting methicillin-resistant staphylococci isolated from pigs in Nigeria. *Brazilian Journal of Microbiology*, 46:885-892.

Uhlemann, A. C., Porcella, S. F., Trivedi, S., Sullivan, S. B., Hafer, C. and Kennedy, A. D. (2012). Identification of a highly transmissible animal-independent *Staphylococcus aureus*ST398 clone with distinct genomic and cell adhesion properties. *mBio*. 3: e00027-e00012.

Umeh, C. J., Ogbane, C. and Adejo, A. M. (2015). Technical efficiency of pig production: A sustainable animal protein augmentation for Nigerians. *Journal of Advanced Agricultural Technologies*, 2: 19-24.

Unnerstad, E. H., Wahlstrom, H., Molander, B. and Bengtsson, B. (2017). Methicillin-resistant *Staphylococcus aureus* not detected in Swedish nucleus and multiplying pig herds. *Infection Ecology and Epidemiology*, 7: 1313068.

Uribe-Querol, E. and Rosales, C. (2017). Control of phagocytosis by bacterial pathogens. *Frontiers in Immunology*, 8: 1368.

van den Broek, I. V. F., van Cleef, B. A. G. L., Haenen, A., Broens, E. M., van der Wolf, P. J., van den Broek, M. J. M., Huijsdens, X. W., Kluytmans, J. A. J. W., van de Giessen, A. W. and Tiemersma, E. W. (2008). Methicillin resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiology and Infection*, 137: 700-708.

van der Wolf, P. J., Rothkamp, A. and Junker, K. (2012). *Staphylococcus aureus* (MSSA) and MRSA (CC398) isolated from post-mortem samples from pigs. *Veterinary Microbiology*. 158: 136-141.

van Duijkeren, E., Ikawaty, R., Broekhuizen-Stins, M. J., Jansen, M. D. and Spalburg, E. C. (2008). Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. *Veterinary Microbiology*, 126: 383-389.

van Duijkeren, E., Moleman, M., Sloet, M. M., Multem, J., Troelstra, A., Fluit, A. C., van Wamel, W. J., Houwers, D. J., de Neeling, A. J. and Wagenaar, J. A. (2010). Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. *Veterinary Microbiology*, 141: 96-102.

van Duijkeren, E., Wolfhagen, M. J., Box, A. T., Heck, M. E., Wannet, W. J. and Fluit, A. C. (2004). Human-to-dog transmission of methicillin resistant *Staphylococcus aureus*. *Emerging Infectious Diseases*, 10: 2235-2237.

van Hock, A. H., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P. and Aarts, A. J. (2011). Acquired antibiotic resistance genes: an overview. *Frontiers in Microbiology*, 2:203.

van Wamel, W. J., Rooijakkers, S. H., Ruyken, M., van Kessel, K. P., and van Strijp, J. A. (2006). The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcusaureus*are located on beta-hemolysin-converting bacteriophages. *Journal of Bacteriology*, 188: 1310–1315.

Vanderhaeghen, W., Hermans, K., Haesebrouck, F. and Butaye, P. (2010). Methicillin resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiology and Infection*, 138: 606–625.

Vazquez, V., Liang, X., Horndahl, J. K., Ganesh, V. K., Smeds, E., Foster, T. J. and Hook, M. (2011). Fibrinogen is a ligand for the *Staphylococcus aureus* microbial surface components recognizing adhesive matrix molecules (MSCRAMM) bone sialoprotein-binding protein (Bbp). *The Journal of Biological Chemistry*, 286: 29797-29805.

Verkade, E. and Kluytmans, J. (2013). Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. *Infecion, Genetics and Evolution*, 21: 523-530.

Verkaik, N. J., de Vogel, C. P., Boelens, H. A., Grumann, D., Hoogenboezem, T., Vink, C., Hooijkaas, H., Foster, T. J., Verbrugh, H. A., van Belkum, A. and van Wamel, W. J. (2009). Anti-staphylococcal humoral immune response in persistent nasal carriers and non-carriers of *Staphylococcus aureus*. *Journal of Infectious Diseases*, 199: 625-632.

Vincze, S., Stamm, I., Kopp, P. A., Hermes, J., Adlhoch, C., Semmler, T., Wieler, L. H., Lübke-Becker, A. and Walther, B. (2014). Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010–2012. *PLoS One*, 9: e85656.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C. and Wulf, M. **(**2005). Methicillin resistant *Staphylococcus aureus* in pig farming. *Emerging Infectious Diseases*, 11: 1965-1966.

Waldron, D. E., and Lindsay, J. A. (2006). Sau1: a novel lineage-specific typeI restriction-modification system that blocks horizontal gene transfer into *Staphylococcus aureus*and between *S. aureus*isolates of different lineages. *Journal of Bacteriology*. 188: 5578–5585.

Wallet, F., Stuit, L., Boulanger, E. Roussel-Delvallez, M., Dequiedt, P. and Courcol, J. R. (2000). Peritonitis due to *Staphylococcus sciuri* in a patient on continuous ambulatory peritoneal dialysis. *Scandanavian Journal of Infectious Diseases*, 32: 697-698.

Walsh, C. T. and Wencewicz, T. A. (2016). Antibiotics: Challenges, mechanisms, opportunities. ASM Press , Washington, DC, Pp. 215.

Walther, B., Wieler, L. H., Friedrich, A. W., Hanssen, A. M., Kohn, B., Brunnberg, L. and Lubke-Becker, A. (2008). Methicillin resistant *Staphylococcus aureus* isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Veterinary Microbiology*, 127: 171-178.

Walther, B., Wieler, L. H., Vincze, S., Antao, E. M., Brandenburg, A., Stamm, I., Kopp, P. A., Kohn, B., Semmler, T. and Lubke-Becker, A. (2012). MRSA variant in companion animals. *Emerging Infectious Diseases*, 18:2017 2020.

Wang, W., Lin, X., Jiang, T., Peng, Z., Xu, J., Yi, L., Li, F., Fanning, S. and Baloch, Z. (2018). Prevalence and Characterization of *Staphylococcus aureus* cultured from milk taken from dairy cows with mastitis in Beijing, China. *Frontiers in Microbiology*, 9: 1123.

Wang, X., Li, G., Xia, X., Yang, B., Xi, M., and Meng, J. (2014). Antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* in retail foods in Shaanxi, China. *Foodborne Pathogens and Diseases*, 11: 281–286.

Weese, J. S., Reid-Smith, R., Rousseau, J. and Avery, B. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of retail pork. *Canadian Veterinary Journal*, 51:749-752.

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A. and Nouwen, J. L. (2005). The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infectious Diseases*, 5: 751-762.

Williamson, D. A., Heffernan, H. and Nimmo, G. (2015). Contemporary genomic approaches in the diagnosis and typing of *Staphylococcus aureus*. *Pathology*,47**:** 270-275.

Winston, L. G., Chambers, H. F. (2009). Antimicrobial resistance in staphylococci: mechanisms of resistance and clinical implications. In: *Antimicrobial Drug Resistance, Clinical and Epidemiological Aspects*. Mayers, D. L. ed.2nd Ed. Humana Press, New York. Pp. 735-748.

Witte, W. (2009). Community-acquired methicillin-resistant *Staphylococcus aureus*: what do we need to know? *Clinical Microbiology and Infection*,15: 17-25.

Wu, Z., Li, F., Liu, D., Xue, H. and Zhao, X. (2015). Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinase, CcrC2. *Antimicrobial Agents and Chemotherapy.* 59: 7597-7601.

Yan, M., Hall, J. W., Yang, J. and Ji, Y. (2012). The essential yhcSR two-component signal transduction system directly regulates the *lac* and *opuCABCD* operons of *Staphylococcus aureus*. *PLoS One*, 7: e50608.

Yanagihara, K., Araki, N., Watanabe, S., Kinebuchi, T., Kaku, M., Maesaki, S., Yamaguchi, K., Matsumoto, T., and Mikamo, H. (2012). Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant *Staphylococcus aureus* isolates from 16 medical centers in Japan (2008-2009): nationwide survey of community-acquired and nosocomial MRSA. *Diagnostic Microbiology and Infectious Disease*, 72: 253-257.

Yang, H., Suh, J. and Lee, H. (2010). Evaluation of commercial selective agars in screening for methicillin resistant Staphylococcus aureus. *Annals of Clinical and Laboratory Science*, 40: 252-256.

Ye, X., Wang, X., Fan, Y., Peng, Y., Li, L., Li, S., Huang, J., Yao, Z. and Chen, S. (2016). Genotypic and phenotypic markers of livestock-associated methicillin-resistant *Staphylococcus aureus*CC9 in Humans. *Applied Environmental Microbiology*, 82: 3892–3899.

Yoneyama, H. and Katsumata, R. (2006). Antibiotic resistance in bacteria and its future for novel antibiotic development. *Bioscience, Biotechnology and Biochemistry*, 70:1060-1075.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, M. F. and Larsen, V. M. (2012). Identification of acquired antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, 67:2640-2644.

Zhang, H. Z., Hackbarth, C. J. and Chansky, K. M. (2001).A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci. *Science*, 291: 1962–1965.

Zhang, K., McClure, J. A., Elsayed, S., Louie, T. and Conly, J. M. (2005). Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*.*Journal of Clinical Microbiology*, 43: 5026-5033.

Zhou, W., Li, X., Osmundson, T., Shi, L., Ren, J. and Yan, H. (2018). WGS analysis of ST9-MRSA-XII isolates from live pigs in China provides insights into transmission among porcine, human and bovine hosts. *Journal of Antimicrobial Chemotherapy*, 73: 2652-2661.

Zhu, W., Clark, N. and Patel, J. B. (2013). pSK41-like plasmid is necessary for Inc18-like *van*A plasmid transfer from *Enterococcus faecalis* to *Staphylococcus aureus* in vitro. *Antimicrobial Agents Chemotherapy*. 57, 212–219.

Zomer, T. P., Wielders, C. C. H., Veenman, C., Hengeveld, P., van der Hoek, W., de Greeff, S. C., Smit, L. A. M., Heederik, D. J., Yzermans, C. J., Bosch, T., Maassen, C. B. M. and van Duijkeren, E. (2017). MRSA in persons not living or working on a farm in a livestock-dense area: prevalence and risk factors. *Journal of Antimicrobial Chemotherapy*, 72: 893-899.

APPENDICES

APPENDIX I: Questionnaire to access information from humans

Date of visit (FDAT) (dd/mm/yy): Farm ID (FID)

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

I. QUESTIONNAIRE FOR HUMANS

Dear Respondent,

This questionnaire is designed as part of a scientific study of molecular epidemiological studies on *S. aureus* isolated from pigs and in-contact humans in two Local Government areas of Kogi State, Nigerian. Please, all information obtained will be treated strictly and confidential and information provided would be solely for this purpose!

Thank you for your cooperation.

Otalu Otalu Jnr.

Date……..

Sex…….

1. How old are you?

   (i)10-20

   (ii)21-30

   (iii)31-40

   (iv)41-50

2. Resident at:……..
3. What is your profession?.....

4. Do you have any history of skin infection in the last 12months? Yes……. No……
5. Have you taken any antibiotics in the last 6months? Yes…… No…….

   If Yes, please specify antibiotic……..
6. For how long did you take the antibiotic?…….
7. Have you had close contact with pigs, other domestic animals and pets in the last 3months? Yes….. No……. If Yes, please specify species of animal…….
8. Do you handle raw meat? Yes….. No……
9. Have you been hospitalized in the last 12months? Yes….. No……

   If Yes, please specify duration……...
10. Do you have family member with history of skin lesion or abraisions in the last 6months? Yes…… No…….
11. Do you know of family member with history of hospitalization in the last 6months? Yes…. No…… If Yes, please specify duration……

APPENDIX II: Questionnaire to access information from pig farms

Date of visit (FDAT) (dd/mm/yy): Farm ID (FID)

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

II. QUESTIONNAIRE FOR THE FARM

Dear Respondent,

This questionnaire is designed as part of a scientific study of molecular epidemiological studies on *S. aureus* isolated from pigs and in-contact humans in two Local Government areas of Kogi State, Nigerian. Please, all information obtained will be treated strictly and confidential and information provided would be solely for this purpose!

Thank you for your cooperation.

Otalu Otalu Jnr.

Name of farm…….

Town/Village……..

Local Government Area…….

1. System of management practiced on the farm (FMAN):

   (1)Intensive system

   (2)Semi-Intensive system

2. Source of pigs (FPSORC)

   (1)Locally sourced

   (2)Bought from a commercial farm

   (3)Others please specify

3. No of pigs on farm:…….. (FNUM)

4. Source of feed (FFPSORC):

   (1)Locally compounded

   (2)Commercial

   (3)Scavenge for feed

   (4)Others please specify……..

5. Inclusion of feed additives (FADD):

   (1) Yes

   (2) No

6. What type of additive is added? (FTADD):

   (1)Vitamins…….

   (2)Minerals……

   (3)Antibiotics please specify……

   (4)Hormones

7. How do you obtain antibiotics? (FANT):

   (1)Direct purchase

   (2)Prescribed by a veterinarian

   (3)Prescribed by Animal Health Officer

   (4)Self prescription

8. We always start our pigs on antibiotic ……. beginning with the age of ……

9. Who adds the feed additive? (FWADD):

   (1)Manufacturer

   (2)Farmer

10. Source of drinking water (FWSORC):

    (1)Well

    (2)Bore hole

(3)Pipe borne

(4)Others please specify

11. How often do you clean your drinking and feeding troughs? (FCLN):

(1)Daily

(2)Weekly

(3)Others please specify

12. How often do you change your pen? (FPEN):

(1)Weekly

(2)Monthly

(3)Others please specify

13. How do you dispose your waste? (FWAST):……

14. How do you dispose dead pigs?

(i)Bury

(ii)Feed to pets

(iii)Sell

15. Are there pests on the farm? (FPEST):

(1)No

(2)Yes, please specify……..

16. Are there other animals on the farm? (FANIM):

(1)No

(2)Yes, please specify……..

17. Nature of personnel on the farm (FPERSON):

(1)Permanent

(2)Casual

18. Number of personnel on the farm (FPNUM):……..

19. What kind of personal protective equipment do the personnel use on the farm? (FPEQIP):

(1)Overall

(2)Jump Suit

(3)Rain boot

(4)Nose mask

(5)Gloves

20. Do you practice “all in all out” system? (FINOUT):

(1)No

(2)Yes

21. Do you disinfect premises before bringing in new pigs? (FDIS):

(1)No

(2)Yes

22. Presence of footbath (FBATH):

(1)No

(2)Yes

Chemical content of footbath if known ……..

23. Are visitors allowed into the farm? (FVISIT):

(1)No

(2)Yes

24. Do you keep any record of disease outbreak? (FDIS):

(1)No

(2)Yes

If yes, please specify diseases recorded in the past three months……..……

……..……

……..……

……..……

…….

25. What preventive and control measures do you take to reduce disease outbreaks? (FPREV): .....…….....

……....

……

APPENDIX III: Questionnaire to access information on pigs

Date of visit (FDAT) (dd/mm/yy): PIG ID (PID)

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

III. QUESTIONNAIRE ON PIGS

1. Breed of pig (PBREED):

   (1)Local

   (2)Improved/Foreign

   (3)Cross

2. Age of pig in months…….. (PAGE)
3. Sex of pig (PSEX):

   (1)Male

   (2)Female

4. Were the pigs vaccinated against any disease and at what age? (PVAC):

   Specify vaccine…….

   ……..

5. Who administered the vaccine? (PWVAC):

   (1)Self

   (2)Veterinarian

   (3)Others please specify…….