MOLECULAR EPIDEMIOLOGY OF LIVESTOCK-ASSOCIATED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (LA-MRSA) FROM POULTRY IN EBONYI STATE, NIGERIA

BY

Amos NWORIE

DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

JUNE, 2014

MOLECULAR EPIDEMIOLOGY OF LIVESTOCK-ASSOCIATED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (LA-MRSA) FROM POULTRY IN EBONYI STATE, NIGERIA

BY

Amos NWORIE, B. Sc, UNIVERSITY OF PORTHARCOURT, NIGERIA, M. Sc. UNIVERSITY OF MANCHESTER, UNITED KINGDOM
Ph. D/PHARM-SCI/10465/2010-2011

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA,

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHILOSOPHY DEGREE (Ph.D) IN PHARMACEUTICAL MICROBIOLOGY.

DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

JUNE, 2014

DECLARATION

I, Amos NWORIE, hereby declare that the work in this dissertation entitled "MOLECULAR EPIDEMIOLOGY OF LIVESTOCK-ASSOCIATED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM POULTRY IN EBONYI STATE, NIGERIA" was carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria under the supervision of Professors J.A. Onaolapo, J.O. Ehinmidu and Dr B.O. Olayinka. This work has not been presented in any previous application for degree at any University. The information derived from literature and the works of other investigators have been duly acknowledged both in the text and reference list accordingly.

_____ _____

Amos NWORIE
Department of Pharmaceutics and
Pharmaceutical Microbiology,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria, Nigeria

Date

CERTIFICATION

This dissertation entitled "MOLECULAR EPIDEMIOLOGY OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM POULTRY IN EBONYI STATE, NIGERIA", by Amos NWORIE meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) in Pharmaceutical Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Chairman Supervisory Committee — Date
Professor J.A. ONAOLAPO
Department of Pharm. and Pharm. Microbiology
Faculty of Pharmaceutical Sciences
Ahmadu Bello University Zaria.

_____ _____

Member Supervisory Committee — Date
Professor J.O. EHINMIDU.
Department of Pharm. and Pharm. Microbiology
Faculty of Pharmaceutical Sciences
Ahmadu Bello University Zaria.

_____ _____

Member Supervisory Committee — Date
Dr B.O. OLAYINKA.
Department of Pharm. and Pharm. Microbiology
Faculty of Pharmaceutical Sciences
Ahmadu Bello University Zaria.

_____ _____

Head of Department — Date
Dr A.B. ISAH
Department of Pharm. and Pharm. Microbiology
Faculty of Pharmaceutical Sciences
Ahmadu Bello University Zaria.

_____ _____

Dean School of Postgraduate Studies — Date
Prof. A. JOSHUA
Ahmadu Bello University Zaria

DEDICATION

To my precious wife Amina; for her support and care.

ACKNOWLEDGEMENT

I thank God for his enduring mercies, protection and guidance throughout the period of this program and research.

I am most grateful to Prof. J.A. Onaolapo, the chairman of my supervisory team whose mentoring, constructive criticisms and contributions and personal involvement gave this work its quality and peculiarity. I also thank other members of the Supervisory Committee, Prof. J.O. Ehinmidu, who was always on hand to guide me and Dr B.O. Olayinka who provided the platform and framework for this research. I am highly indebted to these wonderful academics. I also appreciate the contributions of my Head of Department, Dr. A.B. Isah and other members of staff of the Department of Pharmaceutics and Pharmaceutical Microbiology for their immense constructive criticisms, contributions and support.

I wish to acknowledge members and staff of the Department of Medical Laboratory Science, Ebonyi State University, especially Mr. Azi Simon who was always there for me even at odd times.

I wish to thank Prof. Tara C. Smith, Director of the Center for Emerging Infectious Disease (CEID), Department of Epidemiology, College of Public Health, University of Iowa, United States of America for providing a space in her Laboratory for the molecular analysis of this work. I appreciate her mentoring, criticisms and guidance in the course of molecular analysis of the isolates. I also thank Blake Hanson, Shylo Warden, Ashley Kates, Dipendra Thapilla, Sean O'Malley and all other members of the Center for Emerging Infectious Diseases research team who guided and saw me through the rigorous and challenging molecular analysis stage of this research.

Furthermore, I thank all the faculty members of the Department of Epidemiology, College of Public Health, University of Iowa, United States of America especially Michele Hogue, the

human resource officer for their assistance and the University of Iowa for offering me a visiting scholarship.

I must acknowledge the contribution of the Vice Chancellor of Ebonyi State University Prof. F.I Idike, the Deputy Vice Chancellor, Prof Egwu U. Egwu and the Registrar of the University Mr. Sam N. Egwu who through the Tertiary Education Trust Fund (TETFUND) provided me the grant with which I travelled to the United States of America for the molecular analysis of this work.

I also wish to thank my immediate family Amina, Bob, George, Richard and Amuche whose understanding and support encouraged me through this program. I also acknowledge my parents, Mr. and Mrs. Lazarus Nworie Njaka, my brothers Romanus, Titus, Ifeanyi, Effiong and my friend Igwe James who contributed their own little quota to the success of this program.

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is now a leading cause of staphylococcal infections in humans and animals. In this study, cloacae and nostrils of 1800 poultry birds selected at random in 9 farms spread across Ebonyi State and 19 poultry farm workers from these farms were screened for *Staphylococcus aureus*. In addition, 150 clinical isolates of staphylococci from Microbiology Laboratories in 3 hospitals, one in each senatorial district of Ebonyi State were screened for *Staphylococcus aureus* using standard bacteriological techniques. The number of birds positive for *S. aureus* was 247, representing a percentage carriage of 14% (247/1800). Out of the 150 clinical isolates of staphylococci screened, 78 of them were identified as *S. aureus* representing a percentage incidence of 52%. Only 4 (21%) of the 19 volunteer poultry farm workers were found to carry *S. aureus*. More *S. aureus* were recovered by swabbing both cloacae and nostrils than either of cloacae or nostrils used singly. Antibiotic resistance profile of all the 329 *S. aureus* isolates using disk agar dilution method (DAD) showed that 8.5% (28/329) of the isolates were methicillin resistant. Percentage carriage of MRSA amongst poultry birds in the State was 6% (15/247). MRSA from broilers constituted 73% (11/15) of the total MRSA in poultry. Only one poultry farm worker had MRSA, representing 25% (1/4) carriage. The percentage of MRSA amongst the clinical isolates was 15.4% (12/78). The highest antibiotic resistance exhibited by the isolates was recorded against tetracycline with 47.7% (157/329) followed by Trimethoprim/Sulfamethoxazole with 42.5% (140/329). All the isolates were sensitive to Quinupristin/Dalfopristin, Linezolid, Rifampin and Vancomycin. Multidrug resistance (defined as resistance to at least one antibiotic agent in three classes of antibiotics) was recorded amongst *S. aureus* and MRSA in Ebonyi State. All the MRSA in this study were multidrug resistant. Multiplex PCR showed a correlation between phenotypic recovery of MRSA isolates and genotypic detection of *mecA* gene. All the isolates that were cefoxitin resistant by disk agar dilution (DAD) method were found to carry the *mecA* gene. Molecular

epidemiological analysis revealed that 4 *spa* types (t002, t084, t2304 and t11469) were circulating in Ebonyi State with *spa* type t002 being the most prevalent, constituting 47% (22/46) of all the isolates. *spa* type t084 and t2304 were not found in poultry farm workers. Novel *spa* type t11469 was recorded in poultry (57%), poultry farm worker (14%) and hospital isolates (29%). Molecular screening for PVL gene (*lukS-lukF*) showed that only 8 out of 54 isolates were positive, representing a prevalence of 14.8%. All the PVL positive isolates were from poultry. Molecular screening for *tetM* gene among the tetracycline resistant isolates showed that only one isolate (n=32) harbored the *tetM* gene which could mean that other tetracycline resistant genes might be responsible for the huge tetracycline resistance observed in this study. Multilocus sequence typing of 9 isolates selected at random including the novel *spa* type showed that the *spa* types fell into 3 sequence types (ST5, ST15, ST121). *spa* types belonging to sequence type ST398 and ST9, the main sequence types of livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) were not found in poultry in this study. However, a poultry endemic strain t002 belonging to ST5 was found. The SCC*mec* typing of the isolates carrying the *mecA* gene showed that all the MRSA isolates typed were type V. The finding of the same *spa* types in poultry, poultry farm worker and hospital isolates in the State suggests possible zoonosis and interspecies transmission although the actual route of transmission was not investigated. It was also an indication of the involvement of poultry associated *S. aureus* and MRSA in staphylococcal infections in Ebonyi State.

TABLE OF CONTENTS

Title Page - - - - - - - - - - i

Declaration - - - - - - - - - - ii

Certification - - - - - - - - - - iii

Dedication - - - - - - - - - - iv

Acknowledgement - - - - - - - - - - v

Abstract - - - - - - - - - - vii

Table of contents - - - - - - - - - - x

List of tables - - - - - - - - - - xiv

List of figures - - - - - - - - - - xv

List of Appendixes - - - - - - - - - - xvi

List of Abbreviation - - - - - - - - - - xviii

CHAPTER ONE: INTRODUCTION

1.0 Introduction - - - - - - - - - - 1

1.1 Statement of Research Problems - - - - - - - 4

1.2 Justification of Research - - - - - - - - 6

1.3 Aim and Objectives - - - - - - - - - 7

1.3.1 Aim - - - - - - - - - - 7

1.3.2 Specific Objectives - - - - - - - - - 7

1.4 Research Hypothesis - - - - - - - - - 7

1.4.1 Null Hypothesis (H_o) - - - - - - - - - 7

1.4.2 Alternate Hypothesis (H_a) - - - - - - - - 8

1.5 Research Limitations - - - - - - - - - 8

CHAPTER TWO

2.0 Literature Review - - - - - - - - - 9

2.1 *Staphylococcus Aureus* - - - - - - - - 9

2.1.1 Carriage of *Staphylococcus aureus* - - - - - - - 11

2.1.2 Laboratory Identification of *S. aureus* - - - - - - 12

2.1.3 Pathogenesis of *Staphylococcus aureus* - - - - - - 14

2.2 Antibiotic Resistance - - - - - - - - 18

2.2.1 Mechanisms of Antibiotic Resistance- - - - - - - 21

2.3 Multidrug Resistance in *Staphylococcus aureus* - - - - 24

2.4 Methicillin Resistant *Staphylococcus aureus* - - - - - 26

2.4.1 Mechanism of Methicillin Resistance- - - - - - - 28

2.5 Hospital Associated Methicillin Resistant *Staphylococcus aureus* - - 34

2.6 Community Associated Methicillin Resistant *Staphylococcus aureus* - - 35

2.7 Livestock Associated Methicillin Resistant *Staphylococcus aureus*. - - 39

2.8 Transmission of Methicillin Resistant *Staphylococcus aureus.* - - 42

2.9 Molecular Typing of Methicillin Resistant *Staphylococcus aureus* - - 45

CHAPTER THREE

3.0 Materials and Methods - - - - - - - - 49

3.1 The Study Area - - - - - - - - 49

3.2 The Study Population - - - - - - - - 51

3.3 Ethical Considerations - - - - - - - - 51

3.4 Methodology - - - - - - - - - 51

3.4.1 Collection of Nasal and Cloacae Swabs of Poultry - - - - 51

3.4.2 Collection of Nasal Swabs of Poultry Farm Workers - - - 52

3.4.3 Clinical Isolates of Staphylococci - - - - - - 52

3.5 Preparation of Media - - - - - - - - 52

3.5.1 Preparation of Blood Agar - - - - - - - 53

3.6 Phenotypic Screening to Identify *Staphylococcus aureus* - - - 54

3.6.1 Gram Staining - - - - - - - - 54

3.6.2 Catalase Test - - - - - - - - 54

3.6.3 Coagulase Test - - - - - - - - 54

3.7 Antibiotic Sensitivity Testing (AST) - - - - - - 55

3.8 Transportation of Isolates to USA - - - - - - 56

3.9 Sub culturing of the Isolates - - - - - - - 56

3.10 Re-Screening of the Isolates at CEID - - - - - - 56

3.10.1 Catalase and coagulase tests - - - - - - - 57

3.10.2 Staph Latex agglutination Assay (Pastorex Staph-Plus) - - - 57

3.11 Glycerol Stock of Isolates - - - - - - - 58

3.12 Molecular Screening - - - - - - - - 59

3.12.1 DNA Extraction - - - - - - - - 59

3.12.2 Detection of *S. aureus* 16S rRNA, *nuc* and *mecA* gene by Multiplex PCR 60

3.12.3 *S. aureus spa* typing - - - - - - - - 64

3.12.4 Detection of PVL (*lukS-lukF*) gene present in *S. aureus* by PCR - - 67

3.12.5 Detection of tetracycline resistance genes (*tetM*) present in *S. aureus* by PCR 69

3.12.6 Multilocus sequence typing (MLST) of novel *S. aureus* in the study - 71

3.12.7 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of MRSA - 73

CHAPTER FOUR

4.0 Result - - - - - - - - - - 76

4.1 Sample Population - - - - - - - - 76

4.2 Isolation and Identification of *S. aureus* from Poultry Farms - - 78

4.3 Recovery of *Staphylococcus aureus* in Nostrils and Cloacae of Birds Screened 81

4.4 Isolation of *S. aureus* From Poultry Farm Workers - - - - 84

4.5 Clinical Isolates of *S. aureus* - - - - - - - 87

4.6 Antibiotic Sensitivity Testing (AST) - - - - - - 91

4.7 Multidrug Resistance - - - - - - - - 102

4.8 Molecular Analysis of the Isolates - - - - - - 105

4.8.1 Detection of *S. aureus* 16SrRNA, *nuc* and *mecA* gene Multiplex PCR - 105

4.8.2: *S. aureus* spa Typing - - - - - - - - 107

4.8.3 Detection of PVL (lukS-lukF) gene - - - - - - 113

4.8.4 Detection of tetracycline resistance gene (*tetM*) present in *S. aureus* by PCR 115

4.8.5 Multilocus sequence typing (MLST) of *S. aureus* - - - - 117

4.8.6 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of MRSA Isolates - - - - - - - - - 119

CHAPTER FIVE

5.0 Discussion - - - - - - - - - 123

CHAPTER SIX

6.0 Summary - - - - - - - - - 139

6.1 Conclusion - - - - - - - - - 140

6.2 Recommendations - - - - - - - - 141

References - - - - - - - - - 143

Appendixes - - - - - - - - - 165

LIST OF TABLES

Table 3.1: 16S rRNA, *nuc* and *mecA* gene Multiplex PCR Primers - - - 62

Table 3.2: PCR Primers for *spa* typing of the *S. aureus* - - - - 65

Table 3.3: Primers for PVL (*lukS-lukF)* gene - - - - - 68

Table 3.4: Primers for tetracycline resistance gene (*tetM)* - - - - 70

Table 3.5: Primer for the Multilocvus sequence typing (MLST) - - - 72

Table 3.6: Primers for the Staphyloconccal cassette chromosome *mec* (SSC*mec)* typing - - - - - - - 74

Table 4.1: Summary of the number of farms and Birds Screened in Ebonyi State- 77

Table 4.2: *Staphylococcus aureus* Recovered from Broilers and Layers in the farms 79

Table 4.3: Recovery of *S. aureus* from cloacae and nostrils, cloacae, nostrils - 82

Table 4.4: Isolation of *Staphylococcus aureus* from poultry farm workers - 85

Table 4.5: Clinical isolates of *S. aureus* in the 3 selected hospitals - - - 88

Table 4.6: Summary of *Staphylococcus aureus* isolates from poultry, poultry farm workers and hospitals - - - - - - - 90

Table 4.7: Antibiotic resistance profiles of the *S. aureus* isolates - - - 93

Table 4.8: Comparism of antibiotic resistance of *S. aureus* from poultry, clinics and poultry farm workers - - - - - - - 94

Table 4.9: Prevalence of MRSA in Broilers and Layers in each of the Farms- - 98

Table 4.10: Prevalence of MRSA amongst *S. aureus* in each of the 3 hospitals Screened - - - - - - - - - 100

Table 4.11: Antibiotic resistance index of *S. aureus* isolates in Ebonyi State - 103

Table 4.12: Multidrug resistance (MDR) pattern of the isolates with MARI OF 0.3 and above - - - - - - - - - 104

Table 4.13: *spa* types identified amongst *S. aureus* isolates from poultry, hospitals and poultry workers - - - - - - - 110

Table 4.14: The Sequence types (ST) of sequenced *S. aureus* isolates - - 118

LIST OF FIGURES

Figure 3.2: Map of Ebonyi State showing Abakaliki (Ebonyi North), Onueke (Ebonyi Central) and Afikpo (Ebonyi South) districts where the poultry Farms sampled were located - - - - - - 50

Figure 4.1: Percentage of birds positive for *S. aureus* - - - - 80

Figure 4.2: Percentage of birds with *S. aureus* recovered from both cloacae and nostrils, cloacae only and nostrils only - - - - - 83

Figure 4.3: Percentage carriage of *S. aureus* amongst poultry farm workers- - 86

Figure 4.4: Percentage of *S. aureus* from the 3 hospitals - - - - 88

Figure 4.5: Comparison of percentage antibiotic resistance of poultry and clinical isolates 95

Figure 4.6: Percentage prevalence of MRSA in poultry - - - - 96

Figure 4.7: Percentage composition of MRSA from broilers and layers - - 97

Figure 4.8: Percentage of MRSA amongst hospital isolates - - 99

Figure 4.9: Percentage distribution of MRSA amongst the 3 Hospitals - - 101

Figure 4.10: Multiplex PCR amplification of *S. aureus* 16SrRNA, *nuc* and *mecA* Gene 106

Figure 4.11: *spa* PCR of 17 MRSA isolates showing the amplified base pairs - 108

Figure 4.12: The *spa* PCR of *S. aureus* isolates - - - - - 109

Figure 4.13: Percentage of each *spa* type of *S. aurous* type - - - 111

Figure 4.14: Percentage distribution of the *spa* types of *S. aureus* in Poultry, hospital and poultry farm workers - - - - - - 112

Figure 4.15: PVL PCR showing amplification of *lukS-lukF* gene in positive isolates of *S. aureus* - - - - - - - - - 114

Figure 4.16: PCR amplification of *tetM* gene of a tetracycline positive isolate - 116

Figure 4.17: SCC*mec* typing of *mecA* positive isolates - - - - 120

Figure 4.18: SCC*mec* typing of *mecA* positive isolates - - - - 121

Figure 4.19: SCC*mec* typing of a *mecA* positive isolate - - - 122

LIST OF APPENDIXES

Appendix 1a: Federal Teaching Hospital Abakaliki Ethical Clearance - 165

Appendix 1b: CDC Permit to Import or Transfer Etiological or Vectors of Human Disease 166

Appendix 2: Media preparation 167

Appendix 3: Phenotypic Identification of isolates of *S. aureus* in hospital, poultry and poultry farm workers 168

Appendix 4: Isolation of *S. aureus* in both cloacae and nostril of poultry birds in Ebonyi State 176

Appendix 5a: *Staphylococcus aureus* recovered from broilers and layers in the poultry farm 177

Appendix 5b: Recovery of *S. aureus* from cloacae and nostrils, cloacae, nostrils, 178

Appendix 5c: Prevalence of MRSA in each of the farms screened 179

Appendix 5d: ANOVA of prevalence of MRSA amongst s.aureus in each of the hospital Screened 180

Appendix 6: *S. aureus* from isolates of staphylococci from 3 hospitals in Ebonyi State 181

Appendix 7: Antibiotics, Strength, Zone of Inhibition and Classes of Antibiotics 182

Appendix 8: Antibiotic resistance profiles of the *S. aureus* isolates from hospital, poultry and poultry farm workers 183

Appendix 9: Number of *S. aureus* resistant to each of the antibiotics tested 195

Appendix 10: Antibiotics resistance patterns of all the isolates 196

Appendix 11: Reagents used in molecular analysis 199

Appendix 12: *spa* types of 46 isolates typed in this study 201

Appendix 13a: Calculation of the amplicon sizes of *S.aureus* 16rRNA, *nuc* and *mecA* gene 202

Appendix 13b: Calculation of the *spa* amplicon size of 17 MRSA isolates 203

Appendix 13c: Calculation of the *spa* amplicon size of 26 *S.aureus* isolates from poultry 204

Appendix 13d: Calculation of the amplicon size of *lukS-lukF* gene in PVL positive isolates of *S.aureus* 205

Appendix 13e: Calculation of the amplicon size of the *tetM* gene of a tetracycline resistant isolate 206

Appendix 13f: Calculation of the amplicon size of SCC*mec* of *mecA* positive isolates 207

Appendix 13g: Calculation of amplicon size of SCC*mec* of 5 *mecA* positive isolates 208

Appendix 13h: Calculation of the amplicon size of SCC*mec* of AMC 9 *mecA* positive Isolates 209

Appendix 14: *S. aureus* on blood agar plate, equipments used and farms screened 210

LIST OF ABBREVIATIONS

AIP: *agr*-encoded auto inducing peptide

AST: Antibiotic sensitivity testing

ATCC: American type culture collection

arcC Carbamate kinase

aroE Shikimate dehydrogenase

BA: Blood agar

bp: base pair

BHIA: Brain heart infusion agar

CA-MRSA: Community acquired methicillin resistant *Staphylococcus aureus*

ccr: Cassette chromosome recombinase

CDC: Centre for Disease Control

CEID: Centre for Emerging Infectious Diseases

CHIPS: Chemotaxis inhibitory protein of *Staphylococcus aureus*

CLSI: Clinical and Laboratory Standards Institute

CC: Clonal complex

CoNS: Coagulase-negative staphylococci

CAN: Columbia agar

DAD: Disk agar dilution

DNA: Deoxyribonucleic acid

EDTA: Ethylene diamine tetra acetic acid

EtBr: Ethidium bromide

ETA and ETB: exfoliative toxins A and B

Eap: Extracellular adherence protein

Efb: Extracellular fibrinogen binding protein

ECM: Extracellular matrix

FAME: Fatty acid modifying enzymes

FnbpA and FnbpB: Fibronectin-binding proteins A and B

FLIPr: Formyl peptide receptor-like-1 inhibitory protein

glp Glycerol kinase

gmk Guanylate kinase

HA-MRSA: Hospital acquired methicillin resistant *Staphylococcus aureus*

HGT: Horizontal gene transfer

ICU: Intensive Care Units

J: Junkyard

LAB: Lactic acid bacteria

LA-MRSA: Livestock associated methicillin resistant *Staphylococcus aureus*

LukS-lukF: Panton valentine leukocidin gene.

MCA: MacConkey agar

MecA: Methicillin resistance gene A

MIC: Minimum inhibitory concentration

MLST: Multilocus sequence typing

MLEE: Multilocus enzyme electrophoresis

MGEs: Mobile genetic elements

MRSA: Methicillin resistant *Staphylococcus aureus*

MSSA: Methicillin-susceptible *Staphylococcus aureus*

MSCRAMMs: Microbial surface component recognizing adhesive matrix molecules

MDR: Multidrug resistance

MLEE: Multilocus enzyme electrophoresis

MLST: Multilocus sequence typing

NT-MRSA: Non-typable (NT)-MRSA

ORF: Open reading frame

PBP: Penicillin binding protein

PCR: polymerase chain reaction

PFGE: Pulsed field gel electrophoresis.

PEA: Phenyl ethyl alcohol

PVL: Panton Valentine Leukocidin

pta phosphate acetyltransferase

PTSAg: Pyrogenic toxin superantigens

RNA: Ribonucleic acid

SAK: Staphylokinase

sarA: Staphylococcal accessory regulator

SCC*mec*: Staphylococcal cassette chromosome *mec*.

SCIN: Staphylococcal complement inhibitor

SEB: Staph enrichment broth

Spa: Staphylococcal protein A

SIG: *Staphylococcus intermedius* group

SrRNA: Staphylococcal ribosomal RNA

SSSS:	Staphylococcal scalded skin syndrome
SSTIs:	Skin and soft -tissue infections
STI:	Soft tissue infection
ST:	Sequence type
tetM:	tetracycline resistance gene M
tpi	Triosephosphate isomerase
t:	*spa* type
TAE:	tris acetate-ethylene diamine tetra acetic acid
TETFUND:	Tertiary education trust fund.
TSA:	Tryptic soy agar
UV:	Ultraviolet
VISA:	Vancomycin intermediate resistant *Staphylococcus aureus*
VRSA:	Vancomycin resistant *Staphylococcus aureus*
WGST:	whole genome sequence typing
yqiL	Acetyl coenzyme A acetyltransferase

CHAPTER ONE

1.0 INTRODUCTION

Staphylococcus aureus is a well-known commensal of man. About a third of healthy individuals carry the organism on their skin and nose (Grundmann *et al*., 2002). The organism has established itself as a leading and most frequent cause of a wide range of both hospital and community-acquired infections (Zetola *et al*., 2005; Mwangi *et al*., 2007) and bacteraemia associated with it can be fatal (Nabera, 2009). Pathologies associated with *S. aureus* include osteomyelitis, pneumonia, meningitis, arthritis, endocarditis, septicemia, deep tissue abscesses, skin and soft -tissue infections (SSTIs) as well as toxic shock syndrome amongst others (Zetola *et al*., 2005; Jensen and Lyon, 2009). It is also a common cause of wound and urinary tract infections (Nonie and Mush 2010; Sani *et al*., 2011; Shittu *et al*., 2011).

Shortly after the introduction of methicillin, a β-lactamase stable antibiotic in the early 1960s, Methicillin resistant *staphylococcus aureus* (MRSA) emerged, predominantly associated with infections in clinics and health-care settings (de Sousa and de Lencastre, 2004). MRSA infections in the hospitals and clinical settings are referred to as hospital associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) infections. However, in the 1990s, the epidemiology of MRSA changed with the emergence of MRSA infection in those without the risk factors of HA-MRSA (Chen, 2012). This new group of MRSA is more invasive and causes mainly skin and soft tissue infections in otherwise healthy individuals especially amongst the young and has been designated as community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) (Skov *et al*., 2012).

MRSA is the leading cause of hospital and community acquired infections worldwide (Mwangi *et al*., 2007; Reyes *et al*., 2009; David and Daum, 2010; Wu *et al*., 2011; Shittu *et al*., 2011). It remains one of the most common agents of nosocomial infections, increasing the

cost and mortality associated with staphylococcal infections as compared to methicillin sensitive *Staphylococcus aureus* (MSSA) infections (de Sousa and de Lencastre, 2004). Nosocomial MRSA carries staphylococcal cassette chromosome *mec* (SCC*mec*) type I-III as opposed to CA-MRSA with a characteristic staphylococcal cassette chromosome *mec* type IV or V, lacking in non β-lactam determinants and possessing a distinct necrotizing toxin; Panton-Valentine Leukocidin (PVL). It is associated with staphylococcal diseases in otherwise healthy individuals without HA-MRSA risk factors such as recent hospitalization, residence in care homes, surgery, catheterization or contact with colonized people (Salgado *et al*., 2003).

Staphylococcus aureus as a pathogen is not limited to man. Infections and colonization of livestock have been documented (Waters *et al*., 2011; Spanu *et al*., 2012). It has now been established that livestock can harbour and constitute a reservoir and source of a novel and rapidly emerging type of MRSA (Soavi *et al*., 2010). This new type of MRSA belonging to clonal complex (CC) 398 is predominant in livestock (Smith *et al*., 2009). It is associated with diseases in livestock but has also been implicated in human infections (Witte *et al*., 2007; Hunter *et al*., 2011). This new clonal complex has become known as livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) due to its spread in most animal species. Its disease causing propensities have been established in feed and companion animals (Persoons *et al*., 2009; Smith *et al*., 2009; Hunter *et al*., 2011) and are implicated in a wide range of diseases (Quinn *et al*., 2000). The most common member of this clonal complex is ST398. Initially, it was thought to be limited to pigs and calves but this sequence type which is non-typable by Pulse Field Gel Electrophoresis (PFGE) with *Sma1* is found in most livestock (Soavi *et al*., 2010).

Staphylococcus aureus is also a common cause of food spoilage and in particular meat and meat products where they have been associated with food poisoning and severe diseases of man (Lewis *et al*., 2008; Fanoy *et al*., 2009).

Staphylococcus aureus is often referred to as "superbug" due to its versatility and ability to cause myriad of diseases in otherwise healthy individuals and its multiple drug resistance propensities (Basset *et al*., 2009); a phenomenon that continues to pose great challenges in treatment of infections associated with MRSA (Foster, 2004).

Multi-drug resistant *S. aureus* evolved following acquisition of discrete preformed antimicrobial resistance genes by horizontal gene transfer (HGT) and resistance determinants generated by chromosomal mutation (Jensen and Lyon, 2009) which poses great challenges in treatment of staphylococcal infections (Chopra *et al*., 1997).

MRSA seem to have arisen by multiple introductions of a novel mobile genetic element known as staphylococcal cassette chromosome*mec* (SCC*mec*) (Ito *et al*., 2001) carrying *mecA* gene encoding penicillin binding protein (PBP2a) with reduced binding affinity for β-lactam antibiotics (Zetola *et al*., 2005; Hunter *et al*., 2011) into successful methicillin-susceptible *S. aureus* (MSSA) lineages probably from coagulase-negative staphylococci with intrinsic resistance to methicillin (Zhang *et al*., 2009). They are thought to have gradually acquired other resistance genes carried on plasmids and transposons which were integrated into this mobile element resulting in multidrug resistance and in some cases resistance to all antibiotics available in clinical practice (David *et al*., 2007; Jensen and Lyon, 2009). This scenario is now complicated by the emergence of *vanA* mediated vancomycin-resistant *S. aureus* (VRSA) (de Sousa and de Lencastre, 2004; Basset *et al*., 2009).

1.1 STATEMENT OF RESEARCH PROBLEMS

Staphylococcus aureus is a dangerous and difficult pathogen because of a combination of acquired antibiotic resistances and virulence which accounts for its high incidence in hospital and community-acquired infections (Chopra *et al*., 1997; Enright *et al*., 2002; Otto, 2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are increasingly problematic to treat because of emerging resistance to most currently available antibiotic classes (Enright *et al*., 2002; Lowy, 2003). The changing epidemiology of methicillin-resistant *Staphylococcus aureus* from its natural niche in the hospitals and clinical settings to the community remains the greatest challenge to man.

Community acquired MRSA is the major cause of skin and soft tissue infections (SSTIs) that has become increasingly problematic in management due to high virulence and the ease with which it spreads in the community (Reyes *et al*., 2009). Several genes found in *S. aureus* are associated with its high virulence and resistance to several classes of antibiotics. They include *mecA* gene which codes for penicillin resistance, the *tetM* gene coding for tetracycline resistance and Panton-Valentine Leukocidin gene *lukS–lukF* responsible for widespread skin and soft tissue infections commonly associated with CA-MRSA (Crum-Cianflone *et al*., 2006; David *et al*., 2007).

There are abundant reports that there is a specific type of multidrug *S. aureus* in 'feed animals' and pets (Witte *et al*., 2007; Hunter *et al*., 2011) whose occurence in these farm and companion animals is attributed to the use of antibiotics in animal feeds (Smith *et al*., 2009; Graveland *et al*., 2010). According to Nemati *et al* .(2008) who reported 12.4% MRSA in poultry in Belgium, the emergence of MRSA and multi-antibiotic resistant strains of *S. aureus* in farm and companion animals was due to antibiotic pressure used as food supplements and growth promoters. Livestock acquired methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been reported to cause infections amongst farm workers and their

relatives and transmission of infection from pet animals to their owners and handlers have also been documented (Smith *et al*., 2009; Hunter *et al*., 2011; Graveland *et al*., 2010).

The increasing prevalence of MRSA of clonal complex CC398 in pigs, dogs, horse and their products as well as in poultry has been reported (Smith *et al*., 2009; Hasman *et al*., 2010; Mulders *et al*., 2010). Zoonotic transmission of this clone of MRSA has also been documented especially among farm workers and their families (Smith *et al*., 2009; Graveland *et al*., 2010; van Cleef *et al*., 2011). Reports of LA-MRSA (ST398) involvement in staphylococcal infection in the community and in hospital settings has also been documented (Graveland *et al*., 2010). Although the mechanisms, risk factors, and other information about the emergence of MRSA in animals are rather poorly understood, the close contact between humans and various animal species and antimicrobial use in animals presumably facilitate the emergence and spread of MRSA (Wesse and van Duijkeren, 2010).

The presence of LA-MRSA in farmers constitutes a major threat to public health and the health care system (Smith *et al*., 2009; Golding *et al*., 2010; Waters *et al*., 2011). MRSA prevalence in humans has been reported to be strongly associated with prevalence in animals and intensity of contact with animals positive for methicillin-resistant *Staphylococcus aureus* (Graveland *et al*., 2010).

Several reports show that *S. aureus* is a common pathogen in Nigeria causing a plethora of infections in hospital and community settings with varying prevalences (Yah *et al*., 2007; Adegoke and Komolafe, 2009; Ghebremedhin *et al.*, 2009; Nworie and Umeh, 2010; Sani *et al*., 2011; Shittu *et al*., 2011).

Nasal carriage of *S. aureus* and MRSA has been reported across settings in Nigeria. Lamikanra *et al*. (1985) reported 56.4% nasal carriage of *S. aureus* amongst health Nigerian students in different institutions in Ile Ife, Nigeria. Onanuga and Tremedie reported 33.3%

nasal carriage of *S. aureus* in Ammasoma community in Niger Delta region of Nigria while Nworie *et al*. (2013b) reported 23% of nasal carriage of *S. aureus* amongst meat sellers in Abakaliki metropolis, Ebonyi State, Nigeria.

Although the presence of methicillin resistant *Staphylococcus aureus* in farm animals and poultry has been documented in Nigeria (Olayinka *et al*., 2010, Umaru, *et al.*, 2011), there has not been any molecular characterization of LA-MRSA in Ebonyi State, Nigeria nor a documentation of its involvement in staphylococcal infections in community and in hospital settings in the State to the best of our knowledge.

Nigeria is an agrarian society (Obansa and Maduekwe, 2013). Pasturing and livestock grazing is a major farm activity. Poultry farming is common in rural communities in Ebonyi State, where they are practiced at subsistence level in addition to commercial poultry farms scattered across the State. It is possible that these birds carry methicillin-resistant *S. aureus* as reported by Olayinka *et al*. (2010) and may transmit them to those working in these farms as reported by Graveland *et al*. (2010) and Umaru *et al*. (2011). Persons that work in these poultry farms may acquire and transmit them across community settings where they may cause human staphylococcal infection. As the information on this is not documented in Ebonyi State, Nigeria, this study is aimed at investigating the presence of MRSA in livestock and its probable involvement in human staphylococcal infections in Ebonyi State, Nigeria.

1.2 JUSTIFICATION OF RESEARCH

The changing epidemiology of MRSA in recent time, leaving its niche in the hospital as a nosocomial pathogen to the community and in livestock requires an understanding of its molecular epidemiology. The emergence and involvement of a distinct clone of MRSA (CC398) associated with livestock in human diseases in many countries which are thought to have arisen from increasing use of antibiotics in animal feeds especially in pigs and poultry,

gives good reason for this study since subsistence and commercial poultry farming is a common practice in Ebonyi State and Nigeria in general.

Since MRSA has been reported in these animals elsewhere and in Nigeria, and since their involvement in human diseases is no longer in doubt, chances are that they are already in poultry farms in Nigeria. This study will provide information on the prevalence of this clone of MRSA in poultry in Ebonyi State and its impact on staphylococcal infections. Furthermore, since ST398 of clonal complex (CC) 398 has been implicated in invasive diseases of man in both hospital and community acquired infections, there is an obvious need to characterize *Staphylococcus aureus* isolates in these settings to establish the molecular epidemiology of this organism in the country.

1.3 AIM AND OBJECTIVES

1.3.1 Aim

To determine the molecular epidemiology of livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) in Ebonyi State, Nigeria

1.3.2 Specific Objectives

1. To screen poultry birds in selected poultry farms in Ebonyi State, Nigeria for *S. aureus* and MRSA.
2. To screen poultry farm workers for *S. aureus* and MRSA.
3. To screen clinical isolates of staphylococci in selected hospitals in Ebonyi State for *S. aureus* and MRSA.
4. To carry out molecular characterization of the *S. aureus* using Polymerase Chain reaction (PCR) for methicillin resistance gene (*mecA*).
5. To carry out *spa*-sequence typing of the polymorphic region of protein A (*spa* typing).
6. To carry out staphylococcal cassette chromosome *mec* (SCC*mec*) typing.

7. To screen for panton valentine leukocidin gene (*lukS-lukF*) and tetracycline resistance gene (*tetM*).
8. To carry out multilocus sequence typing (MLST) of the isolates.

1.4 RESEARCH HYPOTHESIS

1.4.1 Null Hypothesis (Ho)

There is no incidence of LA-MRSA in Ebonyi State and LA-MRSA is not involved in staphylococcal infection in Ebonyi State, Nigeria.

1.4.2 Alternate Hypothesis (Ha)

There are incidences of LA-MRSA in Ebonyi State and LA-MRSA is involved in staphylococcal infection in Ebonyi State.

1.5 Research Limitations

- Specimen collection is limited to poultry, poultry farm workers and hospital isolates in Ebonyi State.
- Molecular characterization to be done for selected isolates and genes
- Problems of storage and shipment of the isolates to United States of America for molecular analysis.
- Lack of adequate funds to carry out molecular analysis of large number of isolates

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Staphylococcus aureus*

Staphylococcus aureus is a Gram positive opportunistic pathogen of both animals and humans which has generated global health concern. Several species of staphylococci have been recognized and some of them have been isolated as commensals in humans with enormous pathogenic potentials (Rodrı´guez-Noriega *et al*., 2010).

On blood agar, *Staphylococcus aureus* appears as a large white to creamy white colony while in mannitol salt agar it grows as golden yellow colonies (Olayinka *et al*., 2010). It is a facultative anaerobe, growing well under aerobic conditions within 24 hours (Cheesbrough, 2000). It is found in people and frequently colonizing the nares, the armpit, the perineum, skin fold and the vagina without giving rise to disease (Lamikanra *et al*., 1985; Charlier *et al*., 2009; Rodrı´guez-Noriega *et al*., 2010; Lupo *et al*., 2012).

Staphylococcus aureus sometimes colonize, persist and express virulence factors and diseases in environment where lactic acid bacteria (LAB) exist as the normal dominant microbiota, such as in fermented food or in the vaginal ecosystem, producing food poisoning or urogenital infections, respectively (Charlier *et al*., 2009). It is a major opportunistic pathogen that is responsible for a wide range of chronic and acute diseases of man and animals (Jensen and Lyon, 2009) and a leading cause of hospital and community acquired infections worldwide (Mwangi *et al*., 2007; Reyes *et al*., 2009; David and Daum, 2010; Wu *et al*., 2011; Shittu *et al*., 2011). It is associated with skin and soft tissue infections such as furunculosis and carbunculosis, post-operative and urinary tract infections, otitis media, arthritis, toxic shock syndromes, osteomyelitis, endocarditis, acute respiratory syndromes, pneumonia and

many others (Cheesbrough, 2000; Jarraud *et al*., 2002; Zetola *et al*., 2005; Nworie and Umeh, 2010).

Speciation of staphylococci isolates is essential to distinguish *Staphylococcus aureus* from coagulase-negative staphylococci (CoNS). The production of coagulase, an enzyme that enables the organism to clot plasma is a feature peculiar to pathogenic staphylococci and often associated with increased virulence of *S. aureus.* This has been used to classify *Staphylococcus* species into two groups; those that produce coagulase – the coagulase positive *Staphylococcus* and those that lack the enzyme–the coagulase negative group (Baba *et al*., 2002). These two attributes (catalase and coagulase production) have been exploited in the identification of *S. aureus* (Isenberg, 1998; Kateete *et al*., 2010).

Both coagulase and non-coagulase *Staphylococcus* are involved in human and animal infections (Jensen and Lyon, 2009). Coagulase-positive staphylococci that include *S. hyicus* and *S. flexneri have* been implicated in human and animal infections (Tormo *et al*., 2005). They also include the *Staphylococcus intermedius* group (SIG) namely *S. intermedius, S. pseudintermedius*, and *S. delphini* A and B which are rarely in humans and originally thought to be part of the normal microflora of the skin and mucosal membranes of dogs and cats but now found in a variety of other animals (Tormo *et al*., 2005).

Coagulase negative staphylococci are rarely pathogenic and are considered to be opportunistic pathogens of humans (Tormo *et al*., 2005; Devriese *et al*., 2005). They include *Staphylococcus epidermidis, S. chromogenes, S. xylosus, S. simulans, S. saprophyticus, S. haemolyticus, S. warneri* and *Staphylococcus hominis* group (Foster, 1996).

2.1.1 Carriage of *Staphylococcus aureus*

Staphylococcus aureus is a well known commensal of man (Lowy, 1998). A fundamental biological property of *Staphylococcus aureus* is its ability to asymptomatically colonize both healthy and hospitalized individuals; most often involving the anterior nares (Henderson, 2006; Chambers and Deleo, 2009; Davies and Davies 2010; Bien *et al*., 2011). In addition, they can be found on the skin, throat, axillae, groin, and gastrointestinal tract without causing damage to the host and from where it has emerged to cause community and hospital infections (Foster, 2004; Gordon and Lowy, 2008). In hospitals, the asymptomatic host can disseminate *S. aureus* to immunocompromised patients (Martins and de Lourdes Ribeiro de Souza da Cunha, 2012). Asymptomatic carriers of *S. aureus* are at higher risk of infection and are presumed to be an important source of *S. aureus* strains that spread among individuals (Chambers and Deleo, 2009).

Humans are the main source of *Staphylococcus* species with diverse clonality and toxigenicity (Gomez-Sanz *et al*., 2013). Humans and animals have persistent, intermittent or transient nasal colonization from where *Staphylococcus aureus* and MRSA can cause infection or be transmitted to other persons (Murray *et al*., 2002; Rodrı´guez-Noriega *et al*., 2010). A carrier of *S. aureus* or MRSA is at potential risk of developing an infection involving the colonizing pathogen especially when admitted to hospital and of transmitting the same to other patients with risk factors for infection (Gordon and Lowy, 2008; Rodrı´guez-Noriega *et al*., 2010).

Staphylococcus aureus carriage is higher among healthcare workers than general population (Murray *et al*., 2002). Almost 25% of healthcare workers are stable nasal carriers, and 30% to 50% of them also carry the bacteria on their hands (Farzana *et al*., 2008). Furthermore, high carriage rates of *S. aureus* and MRSA in the nares have been associated with increase in the

transfer and spread from patient to healthcare worker, patient to patient, or healthcare worker to patient (Henderson, 2006).

Factors that enhance colonization by *S. aureus* are complex and are not fully understood. However, it does appear to involve the host's contact with other carriers; *Staphylococcus aureus* ability to adhere to host cells and evade the immune response; and the antimicrobial properties of the nasal fluid which allows it to be transmitted among individuals in both health care and community settings (Murray *et al*., 2002; Kumar *et al*., 2011). Consequently, a combination of bacterial resistance mechanisms, defective nasal fluid and lack of alternative clearance mechanisms (host associated determinants) results in nasal colonization by *S. aureus* (Wertheim *et al*., 2005).

Mortality associated with staphylococcal bacteraemia can be very high (Zetola *et al*., 2005; Nabera, 2009), usually higher in non-carriers than among carriers as infections often arise from carrier strains; a situation thought to confer some protective immunity (Gordon and Lowy, 2008).

MRSA infections in pets are often associated with the owner being previously colonized which is essentially a consequence of previous human colonization in the household (Faires *et al*., 2009). Furthermore, exposure to livestock is an important factor for MRSA colonization (van Cleef *et al.*, 2010).

2.1.2 Laboratory Identification of *Staphylococcus aureus*

The disease causing propensity of *Staphylococcus aureus* which has remained a major challenge to healthcare worldwide requires constant screening of risk subjects and its accurate diagnosis. Several standard laboratory procedures (phenotypic and biochemical) have been developed for the identification of *S. aureus*. Testing for the presence and type of haemolysis on blood agar plates represents a simple and rapid method currently used to

differentiate *Staphylococcus aureus* from other staphylococci in primary non selective cultures though with limited sensitivity (Barrow and Feltham, 1993; Boerlin *et al*., 2003). Selective agar like modified Baird Parker agar has been used successfully for the detection and identification of *Staphylococcus aureus* and other coagulase-positive staphylococci (Kateete *et al*., 2010).

All staphylococci grow well in the presence of bile salt and on media with 4-6.5% NaCl (Brooks *et al*., 2007). *Staphylococcus aureus* is isolated by streaking clinical specimen or materials for epidemiological survey onto solid media such as blood agar (BA), tryptic soy agar (TSA) or brain heart infusion agar (BHIA), MacConkey agar (MCA) (Cheesbrough, 2000). Specimens likely to be contaminated with other microorganisms can be cultured on mannitol salt agar containing 4-6.5% sodium chloride, which allows the salt-tolerant staphylococci to grow (Foster, 1996; Brooks *et al*., 2007). Both Phenylethanol agar (PEA) and Staph enrichment broth (SEB) which selects for Gram positive cocci in mixed cultures or cultures contaminated with Gram negative and free living organisms have been used for higher recovery and identification of *S. aureus* (Atlas and Snyder, 2006).

The simplest biochemical test that is used to differentiate between *Staphylococcus* species and other Gram positive cocci is the catalase test (Isenberg, 1998; Freney *et al*., 1999). Catalase production, an enzyme that hydrolysis hydrogen peroxides into water and oxygen during metabolism is used to differentiate *S. aureus* from streptococci; both of which are Gram positive cocci (Cheesborough, 2000). The production of catalase enzyme by staphylococci is characterized by the production of bubbles or effervescence (Cheesbrough, 2000).

Mannitol fermentation, protein A, DNase, phosphatase and coagulase production is commonly used for the phenotypic identification of *Staphylococcus aureus* (Brown *et al*., 2005; Luczak-Kadlubowska *et al*., 2006).

Coagulase production has been a useful tool in the identification of *S. aureus*. The test utilizes two methods for the identification of coagulase enzyme; the slide agglutination test that detects bound coagulase and the tube agglutination test that detects the free coagulase (Foster, 1996). *Staphylococcus aureus* can be confirmed by testing colonies with tube coagulase for clumping factor and latex particles coated with immunoglobulin G for agglutination (Foster, 1996; Kateete *et al*., 2010). Pastorex Staph-Plus$^{(R)}$ is a latex agglutination kit that allows simultaneous detection of fibrinogen affinity factor, protein A and capsular polysaccharides of *Staphylococcus aureus*.

In spite of these, there is no single phenotypic test including the tube coagulase test that can guarantee reliable results in the identification of *Staphylococcus aureus* (Kateete *et al*., 2010). However, molecular methods have been developed to identify *Staphylococcus aureus* especially methicillin resistance (Brown *et al*., 2005).

2.1.3 Pathogenesis of *Staphylococcus aureus*

Staphylococcus aureus is a highly pathogenic organism capable of causing a wide range of human diseases. Its success as commensal organism and pathogen is because of its versatility and rapid adaptability to selective pressures imparted by the human host (Otto, 2010). Mobile genetic elements (MGEs) as means of transfer of genetic information among and within bacterial species play central role in adaptation process (Malachowa and Deleo, 2010).

The virulence potential of *S. aureus* is attributed to virulence determinants, several of which are involved in the development of staphylococcal infections in which some clonal types are more equipped to cause disease across the globe, whereas others simply cause disease in the community (Gordon and Lowy, 2008; Otto, 2010).

Various host factors predispose individuals to infection, including the loss of the normal skin barrier, the presence of underlying diseases such as diabetes or AIDS and defects in

neutrophil function (Moran *et al*., 2006; Nabera, 2009; Chambers and Deleo, 2009). The primary mode of transmission of *S. aureus* is by direct contact, usually skin-to-skin contact with a colonized or infected individual, hands and probably fomites (Foster, 1996; Shaffer, 2013). In addition, nutrients promote *S. aureus* ability to colonize, persist, and expresses virulence factors that aid infections (Charlier *et al*., 2009).

Infections caused by *S. aureus* can be classified into three major groups: i) superficial wound infections and abscesses; ii) toxinoses such as food poisoning, scalded skin syndrome and toxic shock syndrome; iii) systemic and life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, and bacteremia (Jarruad *et al*., 2002; Nabera, 2009).

Genome sequencing of *S. aureus* strains has helped to identify a number of virulence factors, but the key determinants for infection are still unknown (Feng *et al*., 2008). *S. aureus* infections are attributed to a broad array of virulence and pathogenic determinants that promote tissue colonization and adherence, tissue damage, immune system evasion and invasion including a variety of non-professional phagocytes such as fibroblasts, osteoblasts, endothelial and epithelial cells and harmful toxic effects to the host (Holden *et al*., 2004; Bien *et al*., 2011). These include pore-forming toxins (alpha-hemolysin, Panton-Valentine leukocidin), superantigens (enterotoxins, toxic shock syndrome toxin-1), phagocytosis inhibitors (polysaccharide capsule, protein A), and immune evasion molecules (chemotaxis inhibitory protein, staphylokinase, aureolysin) (Bartlett and Hulten, 2010).

On entry into a host, *S. aureus* will either persist, escape host defenses and antibacterial agents, or multiply and further disseminate; a behavior triggered by a wide range of global regulators which allow the bacteria to propagate (Pratten *et al*., 2001; Bronner *et al*., 2004; Bien *et al*., 2011). Virulent strains of *S. aureus* produce large quantities of extracellular proteins involved in pathogenesis, producing a typical staphylococcal lesion involving pus

formation in contrast to strains that cause toxic shock syndrome which secrete very small quantities of exoproteins and high levels of toxic shock syndrome toxin-1 (Vojtov *et al*., 2002).

The expression of a variety of extracellular proteins and polysaccharides by *S. aureus*, whose combined effect results in virulence during infection (Foster, 1996; Bronner *et al*., 2004) are coordinated by a network of global regulators and transcription factors that allow optimal adaptation of the pathogen to a changing environment and stress conditions encountered during the various stages of infection (Bien *et al*., 2011). Central among this regulatory element is the accessory gene regulator *agr*, a bi-component quorum sensor regulating gene whose activating ligand is an *agr*-encoded auto inducing peptide (AIP) (Jarraud *et al*., 2002; Schulthess *et al*., 2012), staphylococcal accessory regulator (sarA) and a number of sarA homologues (Ferry *et al*., 2005; Oscarsson *et al*., 2005). These regulators constitute an important network that coordinate the expression of *S. aureus* virulence genes in which one virulence gene can be under the influence of several regulators that "cross talk" to ensure that the specific gene is expressed only when conditions are favorable (Bien *et al*., 2011).

The colonization process of *S. aureus* is mediated by several adhesins comprising proteins covalently anchored to cell peptidoglycans that specifically attach to the plasma or extracellular matrix (ECM) components collectively termed the microbial surface component recognizing adhesive matrix molecules (MSCRAMMs). They include staphylococcal protein A (SpA), fibronectin-binding proteins A and B (FnbpA and FnbpB), collagen-binding protein, and clumping factor (Clf) A and B proteins are members (Foster, 1996; Fery *et al*., 2005; McCarthy and Lindsay, 2010; Bien *et al.*, 2011).

Staphylococcal protein A (SpA), a cell wall anchored protein of *S. aureus* with capability to interact with several host components is a virulence factor in staphylococcal infections (Palmqvist *et al*., 2002). The production of staphylococcal protein A is controlled by several

global regulators including *agr*, *sarA*, *sarS*, *sarT*, *rot* and *mgrA*, which appear to form a regulatory network in which *sarS*, an activator of *spa*, is a key regulator (Lindsay and Foster, 1999; Oscarsson *et al*., 2005). Most strains of *S. aureus* produce exoproteins; exotoxins and enzymes including nucleases, proteases, lipases, hyaluronidase, and collagenase whose main functions may be to convert local host tissue into nutrients required for bacterial growth. An exception is the fatty acid modifying enzymes (FAME) that may be important in abscesses, where it could modify anti-bacterial lipids and prolong bacterial survival (Foster, 1996).

Panton-Valentine leukocidin (PVL), α-hemolysin, β-hemolysin, γ-hemolysin, leukocidin, are among cytolytic exotoxins produced by *Staphylococcus aureus.* These cytolytic exotoxins provoke inflammation when they insert and oligomerize to form β-barrel pores in the plasma membrane, causing leakage of the cell's content and lysis of the target cell (Ferry *et al*., 2005). Alpha hemolysin causes osmotic cytolysis with cytotoxicity to human platelets, endothelial cells, thrombocytes and monocytes (Foster, 1996). PVL is a bi-component cytolysin (*lukS-lukF*) with high affinity to leukocytes, while other bicomponent toxins, γ-hemolysin and leukocidin show cytotoxicity to erythrocytes and leukocytes, respectively (Ferry *et al*., 2005). *S. aureus*, SpA, α-, and β-toxins are involved in the pathogenesis of staphylococcal pneumonia (Bien *et al*., 2011)

S. aureus in addition, produces exfoliative toxins (ETA and ETB) involved in staphylococcal scalded skin syndrome (SSSS) and pyrogenic toxin superantigens (PTSAgs) that stimulate proliferation of T-lymphocytes (Foster, 1996; Vojtov *et al*., 2002; Wu *et al*., 2010). The staphylococcal enterotoxins (SEA, SEB, SECn, SED, SEE, SEG, SEH, and SEI) are associated with food poisoning (Foster, 1996; Wu *et al*., 2010).

Other specific proteins associated with *S. aureus* that can have profound impact on the innate and adaptive immune system include staphylococcal complement inhibitor (SCIN), chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS), staphylokinase (SAK),

extracellular fibrinogen binding protein (Efb), extracellular adherence protein (Eap), and formyl peptide receptor-like-1 inhibitory protein (FLIPr) (Bien *et al*., 2011).

SCIN is C3 convertase inhibitor which blocks the formation of C3b on the surface of the bacterium thereby hindering the ability of human neutrophils to phagocytose *S. aureus* (Jongerius *et al*., 2010). On the other hand, CHIPS and FLIPr block neutrophil receptors for chemoattractants (Prat *et al*., 2009). Migration of neutrophils from blood vessels into the tissue may be blocked by Eap. SAK binds to α-defensins eliminating their bactericidal properties while Efb inhibits both classical and alternative pathways of complement activation (Foster, 2005; McCarthy and Lindsay, 2010).

2.2 Antibiotic Resistance

Bacterial resistance to antibiotics has emerged as a global problem involving all known classes of both natural products and synthetic compounds. Its emergence is a set back and increases the challenges posed by infectious diseases. Soil-dwelling bacteria produce and encounter myriad antibiotics and consequently have evolved a multitude of sensing and evading strategies (Dantas and Sommer, 2012). The soil microbiota therefore represents a reservoir of resistance genes, the soil antibiotic resistome, which has the potential to be mobilized into the greater microbial community (D' Costa *et al*., 2006).

Antibiotic resistance in an antibiotic free environment was a rare event (Ayliffe, 1997). According to Davies and Davies (2010), development of generations of antibiotic-resistant microbes and their distribution in microbial populations are the results of many years of incessant selection pressure from human applications of antibiotics through under use, overuse, and misuse.

Antibiotics denote any class of organic molecule that inhibits or kills microbes by specific interactions, including synthetic therapeutics which interact with receptors and provoke

specific cell responses and biochemical mechanisms of cross-resistance in pathogens (Davies and Davies, 2010). In modern terms, an antibiotic has broadly come to mean any synthetic or naturally occurring low molecular weight molecule that inhibits bacterial growth at low concentration (Russell, 1998).

There are several classes of antibiotics with different modes of action and target sites. They include the β-lactams (penicillins and cephalosporins) that inhibit cell wall synthesis, the macrolides that inhibit protein synthesis, glycopeptides that inhibit peptidoglycan synthesis, tetracyclines that inhibit aminoacyl tRNA, quinolones that inhibit DNA gyrase, aminoglycosides that inhibit protein synthesis, lipopeptides that cause membrane depolarization, oxazolidinones and chloramphenicols that inhibit protein synthesis and others (Lambert, 1998).

Antibiotic-resistant pathogens constitute an important and growing threat to the general public and antibiotic resistance occurs when a microbe acquires a gene (Muto *et al*., 2003) or undergoes changes (Jensen and Lyon, 2009) which allow the microbe to inactivate the antibiotic or otherwise nullify its antimicrobial activity. This may occur as a result of spontaneous genetic mutation or involve acquisition of mobile genetic element such as plasmids, transposons, integrons, or gene cassettes (Roberts, 2012). Antibiotic resistant organisms have resulted in prolonged suffering, serious infections and higher risk of death than with antibiotic-susceptible strains of the same species. The consequence is an increasing cost of medical care, prolonged hospital stay and associated morbidity and mortality (Muto *et al*., 2003).

Acquired resistance in pathogens with introduction of new drugs is a common feature of antibiotic use. Antibiotic resistances had been regarded as a problem of human and animal pathogens following the observation of a correlation between the introduction of new

antibiotics and the emergence of resistance in the clinics in previously susceptible populations of bacteria (Wright, 2012).

Staphylococcal infection arises from a combination of bacterial factors, e.g. production of toxins and host factors, including underlying patient disease, e.g. diabetes mellitus (Humphreys, 2012). What makes *S. aureus* a dangerous pathogen is the combination of antibiotic resistance and high virulence (Otto, 2010). The relative ease with which *S. aureus* exchanges genetic material encoding antibiotic resistance and virulence determinants amongst strains and other species such as *Staphylococcus epidermidis* suggests an emerging hypervirulent, multidrug-resistant superbug (Davies and Davies 2010).

As a "superbug" *S. aureus* has higher morbidity and mortality due to multiple mutations endowing it with high levels of resistance to different antibiotic classes specifically recommended for their treatment (Davies and Davies 2010). The complexity and sophistication of the staphylococcal genome has enabled it to change and adapt to varying circumstances such as exposure to a new antibiotic, adherence to a clinical device and transfer from an animal to a human host (Humphreys, 2012).

Resistance of *S. aureus* to antibiotics was presumed to have occurred in 4 waves, the last being the emergence of community acquired methicillin resistant *S. aureus* (Lowy, 2003). Prior to the use of antibiotics to treat *S. aureus* infections, resistant strains outside of hospital settings were rarely encountered (Ayliffe, 1997). After World War II and following widespread use of antibiotics, penicillin-resistant *Staphylococcus aureus* prevalence increased. Resistance to penicillin is associated with production of penicillinase, a predominantly extracellular enzyme that hydrolyzes the β-lactam rings of β-lactam carrying antibiotics encoded by *blaZ* gene controlled by two adjacent regulatory genes, the antirepressor *blaR1* and the repressor *blaI* (Lowy, 2003).

A great majority of staphylococcal isolates now produce penicillinase, regardless of the setting. The gene for β-lactamase is part of a transposable element located on a large plasmid, often with additional antimicrobial resistance genes (genes for gentamycin and erythromycin) and spread of penicillin resistance primarily occurs by spread of resistant strains (Lowy, 2003).

The introduction of methicillin in 1959 led to a drop in the prevalence of penicillin-resistant *S. aureus* (Haley *et al*., 1982). However, in less than a year of its introduction methicillin-resistant *S. aureus* was identified (Haley *et al*., 1982: Chambers, 2001; Deleo *et al*., 2009). In addition to use of antibiotics in humans, antibiotic use in animals for therapeutics, for food production and disease prevention has also promoted antibiotic resistance in humans (Ndi and Barton, 2012). Under doses of antibiotics to food-producing animals can result in bacterial resistance in livestock, elevating the potential for resistant bacterial strains to cross species boundaries, especially livestock imported from countries where antibiotic use is indiscriminate (Smith, 2005). Several studies have shown that people having close contact with animals are more at risk of zoonotic diseases (Smith *et al*., 2009; van Cleef *et al*., 2010). Furthermore, antibiotic resistance in animals is related to antibiotic use (Smith *et al*., 2009).

2.2.1 Mechanisms of Antibiotic Resistance

Antibiotics act on bacteria by inhibition of cell wall synthesis, protein synthesis, nucleic acid synthesis, and metabolic pathways (Donham, 2010). Antibiotic resistant bacteria prevent the action of antibiotics by innate or acquired resistance (Schwartz, 2012). Antibiotic therapy cause microbes with gene encoding inducible resistance to induce synthesis of enzymes in the presence of certain antibiotics to inactivate it and/or other antibiotics giving them selective advantage to survive, proliferate, and spread (Muto *et al*., 2003).

Mechanisms conferring antimicrobial resistance include membrane permeability alterations, active antimicrobial extrusion or efflux, antimicrobial target alteration, metabolic bypass and enzymatic modification of the antimicrobial (Miao *et al*., 2012). Membrane permeability alterations may prohibit influx of certain antimicrobials, e.g., aminoglycosides (Schwartz, 2012). Antimicrobial target alterations lower affinity for the antimicrobial agent, e.g., β-lactams, macrolides while enzymatic modification of antimicrobials may inhibit membrane permeability or ability to interact with the target of action, e.g., β-lactams and chloramphenicol (Smith and Jarvis, 1999).

In addition, genes conferring antimicrobial resistance often have a metabolic purpose in that organism or in a different organism. For example, *mecA,* which confers methicillin resistance to *Staphylococcus aureus,* probably is a cell wall transpeptidase from a non-staphylococcal organism (Smith and Jarvis, 1999).

Different genetic elements found in bacteria are capable of acquiring resistance genes and promoting their transmission which vary from pathogen to pathogen and more broadly, between the Gram-positive and Gram-negative bacteria (Roberts, 2012). However, plasmid-mediated transmission has been shown to be the most common mechanism of horizontal gene transfer (HGT) (Norman *et al*., 2009). Although bacteriophages carrying antibiotic resistance genes are hardly identified in the environment or in hospital isolates of resistant bacteria, there seem to be an association between phages with the insertional mechanisms required for the formation of mobile resistance elements and the functions of chromosomally associated resistance genes frequently seen as phage "fingerprints" flanking genes encoding resistance or virulence on different vectors which appear quite common in *S. aureus* (Skurray and Firth, 1997).

The transmission and expression of resistance genes in pathogenic bacteria has been prompted by human use and misuse of antibiotics which has added to the bigger random gene

acquisition events (Pruden and Arabi, 2012). All *Staphylococcus aureus* for instance, were sensitive to penicillin, but penicillin use rapidly selected for β-lactamase- (penicillinase-) producing strains and this has remained a major clinical problem ever since.

Acquired resistance occurs when a change in nucleic acid allows the bacteria to prevent the actions of antibiotics on the bacteria. Mechanisms involved with acquired resistance are transformation involving uptake and exchange of nucleic acid, transduction in which insertion of resistance gene into bacteria is mediated by bacteriophage, conjugation which involves plasmids or transposons and mutation (Sapkota *et al*., 2006). Acquired resistance mechanism of MRSA involves chromosomal mutation of the DNA that encodes a unique penicillin-binding-protein (PBP2a) (Smith and Jarvis, 1999).

Staphylococcus aureus employs multiple layers of gene regulation to ensure protection against environmental stresses and host defenses. They include metabolic adaptation to changing nutrient conditions, the coordinated expression of virulence factors and modification of core genomic regulatory networks by imported regulators present on mobile elements such as plasmids, phages and genomic islands (McCallum *et al*., 2010).

2.3 Multidrug Resistance in *Staphylococcus aureus*

Staphylococcus aureus is predominant in most developed countries where they occur as multi-drug resistant pathogen (Enright *et al*., 2000). Multidrug-resistant bacteria such as MRSA are endemic in healthcare environment and serve as a potential source for outbreaks in these settings (Henderson, 2006). MRSA is said to be multidrug resistant because of its 'non-susceptibility to at least one antimicrobial agent in three or more categories and whose resistance to oxacillin or cefoxitin predicts non-susceptibility to all categories of β-lactam antimicrobials with the exception of the anti-MRSA cephalosporins (Magiorakos *et al*., 2012).

Antimicrobial resistance in *S. aureus* is a major public health threat, compounded by emergence of strains with resistance to vancomycin and daptomycin; both last line antimicrobials (Howden *et al*., 2011). The improved medicare due to antibiotic discovery in the 20th century has been eclipsed by the upsurge of antibiotic resistance in hospitals, community and the environment attributed to extraordinary genetic versatility of microbes that have capitalized on anthropogenic activities to utilize every source of resistance genes and horizontal gene transfer mechanism to develop multiple mechanisms of resistance for each and every antibiotic introduced into clinical and agricultural practice (Davies and Davies, 2010).

Resistance to antimicrobials in bacteria is now known to originate from variation in DNA, some of which evolved long ago in soil bacteria (Kayser, 1993). The huge prescription of antibiotics and their uncontrolled extensive usage has resulted in the widespread of antibiotic resistance in microorganisms (Lamikanra *et al*., 1985; Davies and Davies, 2010). Antibiotic use creates conditions that allow bacteria with mechanisms for resistance to compete better. These mechanisms arise not only by mutation of microbial genes which code for antibiotic uptake into cells or the binding sites for antibiotics, but by horizontal spread or longitudinal

transfer of resistance determinants (Kayser, 1993) and has accounted in part to antibiotic resistance and virulence among *Staphylococcus* (Chan *et al*, 2011).

Mobile genetic elements (MGEs) encode putative virulence factors and molecules that confer resistance to antibiotics, including the gene that confers resistance to β-lactam antibiotics in MRSA (Malachowa and Deleo, 2010). They include bacteriophages, pathogenicity and composite island, plasmids and transposon, staphylococcal cassette chromosome *mec* (SCC*mec*) and genomic islands (Malachowa and Deleo, 2010; Monecke *et al.*, 2012; Stefani *et al.*, 2012).

The environment, and especially freshwater, constitutes ambiance for the evolution and rise of new resistances as it provides 'environmental-hotspots' for antibiotics and other pollutants from different sources, environmental species with intrinsic antibiotic resistance mechanisms, and bacteria from different antibiotic sources to interact to acquire resistance determinants which may involve phages and integrons and introducing same into the clinics (Lupo *et al*., 2012).

The problems posed by the increasing spread of multidrug-resistant *S. aureus* (MDR-SA) in clinical setting is compounded by the spectacular adaptive capacity of this pathogen resulting in the emergence and worldwide spread of lineages that acquired resistance to the majority of available antimicrobial agents, narrowing choice of therapy to a few antibacterial agents, among them the glycopeptide antibiotic vancomycin, which has become the mainstay of therapy worldwide (Mwangi *et al*., 2007).

2.4 Methicillin Resistant *Staphylococcus aureus*

There has been a global increase of MRSA over the past few decades and hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) has remained endemic in hospitals in most countries and regions. Strains of MRSA which had been largely confined to hospitals and long-term care facilities are emerging in the community with prevalence varying from country to country (Reyes *et al*., 2009). And though the origin (hospital or the community) of the emerging MRSA strains is not known, the prevalence of these strains in the community seems likely to increase substantially (Chambers, 2001). On the other hand, community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) clones have been spreading rapidly in the community and also infiltrating healthcare in many regions of the world. Similarly, livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) has remained prevalent in certain high-risk groups of workers in direct contact with live animals (Monecke *et al.*, 2012, Stefani *et al*., 2012).

Prolonged administration of antibiotic therapy or inadequate dosing of antibiotics selects for methicillin-resistant isolates in an overall population of *S. aureus* which may colonize and subsequently cause infection and/or serve as a reservoir for transmission to other patients in hospital setting (Henderson, 2006). Resistance in MRSA is associated with the production of PBP2a encoded by the *mecA* gene which acts by binding β-lactams to the cell wall and inactivating the β-lactam which prevents the antibiotic from acting on the bacteria (Thorne *et al*., 2010).

The evolutionary origins of MRSA are poorly understood and there is no consensus on the nomenclature, the number of major MRSA clones or the relatedness of clones described from different countries (Enright *et al*., 2002). The first MRSA isolate evolved from a sensitive, epidemic strain prevalent in Europe and its progeny, the first MRSA clone, rapidly spread to other continents (Robinson and Enright, 2004). Analyses of epidemic MRSA isolates from

hospitals in different countries by molecular methods, including multilocus sequence typing (MLST) and DNA microarray analysis, reveal that MRSA strains have evolved separately within five distinct epidemic, sensitive lineages (Robinson and Enright, 2004; Johnson, 2011). The over 3000 MRSA isolates from different regions of the world characterized, as reported by de Sousa and de Lencastre, (2004), confirms the existence of only a few epidemic clones worldwide, namely the Iberian, Brazilian, Hungarian, New York/Japan and Pediatric clones. This suggests that acquisition of the staphylococcal cassette chromosome SCC*mec*, the mobile genetic element harboring the *mecA* gene that confers methicillin resistance, has been a rare event (Brurec *et al*., 2011).

Methicillin resistant *Staphylococcus aureus* (MRSA) seemed to have arisen by multiple introduction of SCC*mec* into successful methicillin-susceptible *Staphylococcus aureus* (MSSA) lineages which gradually acquired resistance to most antibiotics available in clinical practice by transfer of resistance genes probably from the environment or by mutation (de Sousa and de Lancastre, 2004; Gordon and Lowy, 2008; Jensen and Lyon, 2009; Hunter *et al*., 2011). Horizontal interspecies transfer of this element could be an important factor in the dissemination of MRSA, which evidence has shown is occurring amongst staphylococcal species (Bloemendaal *et al*., 2010).

Methicillin resistance is different from penicillinase-mediated resistance because in addition to lack of drug inactivation, methicillin resistance is broad, conferring resistance to the entire β-lactam class of antibiotics, which include penicillins, cephalosporins and carbapenems unlike penicillinase-mediated resistance, which is narrow in its spectrum of activity, (Chambers and Deleo, 2009).

The continued evolutionary changes of MRSA have resulted in its continuing threat to public health. The ever-increasing burden of MRSA infections in hospitals has led to the increased use of vancomycin, the last remaining antibiotic to which MRSA strains were reliably

susceptible (Chambers and Deleo, 2009). Emerging strains of *S. aureus* with reduced susceptibility to glycopeptides is presumed to be due to altered cell wall architecture arising from thickened and or poorly cross-linked cell wall peptidoglycan resulting in sequestered glycopeptides that are unable to reach their lethal target at the site of cell division (Howden *et al*., 2010). Or it could be as a result of intrinsic glycopeptides resistance which has been shown to evolve as a result of an accumulation of multiple genetic alterations including genomic mutations and regulatory changes (McCallum *et al*., 2010). In addition, chromosomally mediated resistance for vancomycin intermediate resistant *Staphylococcus aureus* (VISA) and vancomycin resistant *Staphylococcus aureus* (VRSA) strains acquire resistance by conjugal transfer of the *vanA* operon from an *Enterococcus faecalis* (Lowy, 2003). Vancomycin (or glycopeptide) intermediate resistant *S. aureus* (VISA/GISA) have vancomycin minimum inhibitory concentration (MICs) in the range of 8–16mg/l (Hiramatsu, 2001). Elevated MICs of up to or over 32 lg/mL is indicative of VRSA (Smith, 2005).

Furthermore, quinolone (fluoroquinolone) resistance among *S. aureus* is associated with spontaneous chromosomal mutations in the target site of the antibiotic, topoisomerase IV or DNA gyrase, or by the induction of a multidrug efflux pumps (Lowy, 2003).

2.4.1 Mechanism of Methicillin Resistance

Methicillin resistance is conferred by the *mecA* gene, which encodes an aberrant penicillin-binding protein (PBP2a) with decreased affinity for β-lactam antibiotics, so that PBP2a are able to catalyze the transpeptidation reaction required for peptidoglycan cross-linking, enabling cell wall synthesis in the presence of high concentrations of β-lactams which otherwise would inhibit the endogenous PBPs, and by so doing confer resistance to all β-lactam antibiotics (Berger-Barchi, 1995; Hunter *et al*., 2011).

Resistance attributed to high levels of penicillin-binding protein (PBP2a) depends, in addition to PBP2a, chromosomally encoded factors that are involved in the synthesis and degradation of the peptidoglycan so that any mutations that reduce peptidoglycan precursor formation or change the chemical composition of the muropeptide precursor result in lowered resistance (Berger-Barchi, 1995).

Expression of PBP2a is under dual regulatory control of its own *mecR*1 and *mecI* (Berger-Barchi, 1995; Lewis and Dyke, 2000). The *mec* complex is carried by a novel mobile genetic element called staphylococcal cassette chromosome (SCC*mec*) which has a characteristic unique structure at the extremities and cassette chromosome recombinase (*ccr*) gene (*ccrA*/*ccrB* or *ccrC*) that allow horizontal transfer and acquisition of SCC*mec* and *mec* complexes amongst species and strains (Ito *et al*., 2001).

The *mecA* structural gene is a small part of a much larger genetic element which is inserted precisely into the *S. aureus* chromosome. The SCC*mec* varies in size from 20 to 68 kb (Ito *et al*., 2001). The element is characterized by flanking terminal direct and, in most cases, inverted repeat sequences, the *mec* and *ccr* gene complexes, and their surrounding DNA in which unique combinations of the *mec* and *ccr* gene complexes generate various SCC*mec* types (Zhang *et al*., 2009). Based on the organization of *mec* and associated genes within the SCC*mec* complex, five (A–E) different classes of SCC*mec* have been defined, of which three (A–C) are the most common in *Staphylococcus aureus.* Amongst the three classes, only class A SCC*mec* consists of the complete *mecA* regulon (*mec1-mecR1-mecA*) because the regulatory genes in class B and C are disrupted by insertional sequences; SCC*mec*–IS1272-D*mecR1-mecA* in class B and IS431-D*mecR1-mecA* in class C SCC*mec* elements (Malachowa and Deleo, 2010).

The cassette chromosome recombinase (*ccr*) genes which four allotypes have been defined are located within the SCC*mec* element whose function is to integrate into, or excise SCC*mec*

from the chromosome at a specific site; *attBssc* at the 3' end of an open reading frame (ORF) of unknown function known as *orfX* (Ito *et al.*, 1999). The regions outside the *mec* and *ccr* complexes are designated the (J) Joining or junkyard region and are divided into 3 (Ito *et al*., 2004; Deurenberg *et al*., 2007) which constitutes non-essential components of the cassette that can carry additional antimicrobial resistance determinants (Hanssen & Sollid, 2007; IWG-SCC, 2009; Malachowa and Deleo, 2010). The SCC*mec* J1 region is located between the chromosome right junction to the *ccr* genes, J2 region from the *ccr* genes to the *mec* complex, and J3 region from the *mec* complex to the left extremity of *orfX* (Chongtrakool *et al*., 2006).

Eleven SCC*mec* types have so far been described (IWG-SCC, 2011). Types I, II and III described by Ito *et al*. (2001) are significantly larger than types IV and V, and are mainly responsible for nosocomial infections. According to Ito *et al*. (2001), the type-I cassette with a size of 34.364 bp does not have any transposons or plasmids that provide resistance to other drugs besides methicillin or to heavy metals. Type- II cassette which is 53.017 bp, in addition to the *mecA* and *mecRI* genes, contains transposon Tn554 responsible for the resistance of this SCC*mec* type to erythromycin and streptomycin. Type-III cassette has a size of 66.896 bp and contains genes *mecA*, *mecRI*, transposons Tn554, and plasmid pt181. Transposon Tn554 induces cadmium resistance, and plasmid pt181 is responsible for tetracycline and mercury resistance.

Type-IV SCC*mec* described by Ma *et al*. (2002) in community samples is mainly responsible for community infections. It is a small element that does not carry other resistance genes, except *mecA*. It is also divided into multiple subtypes arising from different sequences on the left extremity of the *ccr* complex, known as L – C region, which suggests that type-IV SCC*mec* is highly transmittable (Ito *et al*., 2004).

Type-V SCC*mec* with size of 27.624 bp identified by Ito *et al*. (2004) in an Australian isolate is larger than SCC*mec* IV with only genes that code for methicillin resistance similar to SCC*mec* IV. It has a new *ccr* gene type that is characterized as type C and is individually found, unlike other elements that have a pair of these genes. It has a restriction and modification system coded for by genes *V22* and *V23* commonly associated with community infections.

Type-VI SCC*mec* as described by Oliveira *et al*. (2006) in pediatric clone of MRSA differs in the *ccrAB* complex identified as type 4, and the presence of the type-B *mec* complex, which does not have the *mecl* gene.

Type-VII SCC*mec* as described by Higuchi *et al*. (2008) through a detailed analysis of community *Staphylococcus aureus* samples isolated in Taiwan, has a size of 41.347 bp, and a *mec* complex homologous to that found in type V. However, it presents substitutions, insertions and rearrangements that differentiate it. The cassette is characterized by the presence of the *ccrC* complex, transposon ΨIS431 and a unique sequence on the right side of the cassette which is referred to as *orf*35.

The SCC*mec* VIII described by Zhang *et al*. (2009) in an MRSA sample from a hospital has a size of 32.168 base pairs. It also possesses genes *mecA*, *mecR1* and *mecI* of the *mec* complex, c*crA*4 *ccrB*4of the *ccr* complex and transposon Tn554, which is also present in type II.

Types SCC*mec* IX (class C2, *ccr1 mec* gene complex), SCC*mec* typeX (class C 1, *ccr*7 *mec* gene complex) and SCCmec type XI (class E, *ccr*8 *mec* gene complex) have also been described (IWG-SCC, 2011).

The initial reservoir of SCC*mec* is not very clear but is suspected to have arisen from coagulase-negative staphylococcal species (Gordon and Lowy, 2008). According to Ito *et al*. (2001), "SCC*mec* may have evolved from a primordial mobile element, staphylococcal

cassette chromosome (SCC) into which the *mec* complex was inserted and it is likely that SCC*mec* serves as the carrier of the *mecA* gene moving across staphylococcal species, since *mecA* genes in other staphylococcal species have never been found without the accompaniment of SCC*mec*-like structure"

Staphylococcal cassette chromosomes (SCCs) are relatively large fragments of DNA that always insert into the orfX gene on the *Staphylococcus aureus* chromosome and can encode antibiotic resistance and/or virulence determinants (Malachowa and Deleo, 2010). The SCC*mec* types differ from one another by the number of genes in their gene architecture (Hiramatsu *et al*., 2001). Some SCC*mec* are carriers of resistance genes that are determinants of multiple antibacterial drugs which includes β-lactam antibiotics, macrolides, lincosamides, streptogramins, aminoglycosides and tetracycline and bacterial cell acquiring such SCC*mec*, acquires a multiple-resistance phenotype (Ito *et al*., 2004). SCC*mec* type I, IV, V and VI encode resistance to β-lactam antibiotics only, while SCC*mec* type II and III carry multi-resistant genes some on plasmids and transposons (Deurenberg *et al*., 2007).

The levels of resistance amongst methicillin resistant *Staphylococcus aureus* do not correlate with the amount of PBP2 produced but are determined by the strain specific genetic background which is *mecA*-independent (Berger-Bachi, 1995). Among this is the pathogenicity island which three types (vSAα, vSAβ, and vαSγ) carrying different resistant genes have been described (Malachowa and Deleo, 2010).

Although methicillin-resistant *Staphylococcus aureus* (MRSA) strains are not necessarily more virulent than methicillin-sensitive *Staphylococcus aureus* strains (MSSA), some MRSA strains contain factors or genetic backgrounds that may enhance their virulence or may enable them to cause particular clinical syndromes (Gordon and Lowy, 2008).

Staphylococcus aureus has a formidable ability to adapt to varying environmental conditions and the phenotypic expression of *mecA* gene is affected by various factors including pH, temperature and osmolarity (Sakoulas *et al*., 2001). It has an extraordinary capacity to rapidly become resistant to virtually all antibiotics which develops either through mutations and rearrangements within the staphylococcal genome, or by the acquisition of resistance determinants. According to McCallum *et al*. (2010), antibiotic resistance often imposes a fitness burden on the host and such biological costs can be reduced by tight regulation and antibiotic-inducible expression of resistance genes, or by compensatory mutations. Resistance induction by antibiotics can be mediated by dedicated, antibiotic-recognizing signal transducers or by mechanisms relieving translational attenuation (Balazy *et al*., 2006).

The ease of acquisition of methicillin resistance by *S. aureus* has seriously threatened the effectiveness of antibiotic treatment of both nosocomial and community acquired infections (Chopra *et al*., 1997; Enright *et al*., 2000) and although the proportion of *S. aureus* bacteraemia due to MRSA is declining in many countries, data from the European Antimicrobial Resistance Surveillance System (EARSS) for 2008, showed that in more than one third of countries, the proportion remained 25% (Johnson, 2011).

2.5 Hospital Associated Methicillin Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aurous* (MRSA) remains an important pathogen in clinical settings due to development of multiple-drug resistant strains, which makes it increasingly difficult to treat, and accounting for its leading role in nosocomial infections globally (Grundman *et al*., 2002; Enright *et al*., 2002). Infections with HA-MRSA remain a significant cause of morbidity and mortality in hospitals worldwide, with a small number of clones accounting for a large number of hospital acquired infections (Elston and Barlow 2009; Howden *et al*., 2011), whose incidence vary from country to country (Brurec *et al*., 2011). However by the 1990s it had spread into community worldwide (Persoons *et al*., 2009).

Methicillin resistant *Staphylococcus aureus* (MRSA) nosocomial infections were originally detected in large tertiary hospitals and in intensive care units, where colonized and infected patients as well as colonized health care workers were a significant source of cross-infection. Presently however, MRSA is one of the most common pathogens in hospitals of all sizes with increasing prevalence (de Sousa and de Lencastre, 2004). Only people with direct exposure to health-care setting such as hospitalization, outpatient visit, nursing home admission, antibiotic exposure, chronic illness, surgery or indwelling catheters or in close contact with people with these risk factors were at risk of HA-MRSA infection (Foster, 2004; Zetola *et al*., 2005). Colonization increases risk of infection and nasal carriage is one of the important sources of staphylococcal nosocomial infection among hospital personnel (Farzana *et al*., 2008). Patient-to-patient transmission of MRSA within healthcare settings primarily occurs via carriage on the hands of healthcare workers (Henderson, 2006).

The multidrug resistance propensity of MRSA commonly found in clinical settings is due to a relatively large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to types I, II, and III containing the *mecA* gene, which is nearly universal among MRSA isolates (Zetola *et*

al., 2005). HA-MRSA strains seldom carry the genes for the Panton-Valentine leukocidin (PVL) and are frequently resistant to many classes of non-β-lactam antimicrobials (David and Daum, 2010).

While only few clonal types have been identified to be responsible for most of hospital acquired infections worldwide and disseminating across borders (Gordon and Lowy, 2008), the hospital epidemiology of MRSA has changed in the past few years due to the encroachment of community-associated MRSA (CA-MRSA) strains into health care settings (Valsesia *et al*., 2010). Study using pulsed-field gel electrophoresis (PFGE) showed that USA100, USA700 and USA 800 were found to be mostly associated with nosocomial infections (Pu *et al*., 2009)

2.6 Community Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA).

Methicillin resistant *S. aureus* (MRSA) traditionally associated with hospitals and other health care settings has now emerged as a widespread cause of community infections whose outbreaks have been reported worldwide especially among healthy individuals (Chambers and Deleo, 2009). The increasing incidence of MRSA infections has been attributed to this new MRSA clones known as community-associated MRSA (CA-MRSA) (Baba *et al*., 2002; An Diep *et al*., 2006).

Methicillin resistant *S. aureus* (MRSA) as a community pathogen has emerged in several countries, and multilocus sequence typing (MLST) and SCC*mec* typing have shown that the genotypes of community-acquired MRSA (CA-MRSA) strains are remotely related to hospital MRSA strains, and thus represent novel acquisitions of SCC*mec* (Robinson and Enright, 2004). CA-MRSA strains are therefore different from those in health care facilities (Nabera, 2009).

The origin of CA-MRSA is not clear. It was thought to have emanated only from patients discharged from hospitals into the community (de Sousa and de Lencastre, 2004) but the discovery of MRSA strains with distinct genetic and diverse properties indicates that MRSA strains may also have originated from the community (Charlebios *et al*., 2004) causing skin and soft tissue infections in people, mostly children, with no underlying classical risk factors (Hunter *et al*., 2011). Furthermore, it appears that MRSA transmission from the hospital setting to the community and new acquisitions of SCC*mec* DNA in susceptible backgrounds may account for its emergence (de Sousa and de Lencastre, 2004).

Outbreaks and epidemics of CA-MRSA now occur worldwide with similar epidemiology and although the specific clones that have emerged vary with geographical location, the rapid dissemination of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) since the early 2000s and the appearance of new successful lineages is a matter of concern (de Sousa and de Lencastre, 2004). CA-MRSA is susceptible to numerous antibiotics to which hospital strains are routinely resistant. Two molecular markers that are not found in typical hospital MRSA strains are strongly associated with CA-MRSA regardless of geographical origin: a specific cassette element encoding *mecA* and genes encoding PVL (Chambers and Deleo, 2009). This new and distinct strain of *Staphylococcus aureus* with high virulence is associated with high morbidity and mortality in the community (Baba *et al*., 2002; Nabera, 2009; Hunter *et al*., 2011).

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in Western Australia in the early 1990s from indigenous peoples living in remote areas (Coombs *et al*., 2005). This unique MRSA clones with more susceptibility to non β-lactam antibiotics, carrying type IV staphylococcal cassette chromosome *mec* gene and Panton-Valentine leukocidin (PVL) genes have been identified in many other countries including United States (Lina *et al*., 1999; Okuma *et al*., 2002; Reyes *et al*., 2009). Though

non multidrug-resistant initially, many of these CA-MRSA clones have acquired plasmids and chromosomal resistance determinants in recent years allowing some strains to become resistant to 5 or more non β-lactam antimicrobial agents, including erythromycin, tetracycline, trimethoprim, ciprofloxacin, gentamicin, rifampin, fusidic acid, and mupirocin (Coombs *et al*., 2005).

According to Centre for Disease Control (CDC)(2005), Fey *et al*. (2003) and David *et al*. (2007), community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) infections do not have typical MRSA risk factors such as recent hospitalization, kidney dialysis, surgery, haemodialysis, indwelling catheter, long-term residence in a health care facility or intravenous drug use. Clinically, CA-MRSA differs from HA-MRSA by its specific involvement in skin and soft tissue infections, occurrence in diverse and often healthy populations especially children without clear risk factors, difference in antimicrobial susceptibility patterns, genetic markers and its frequent association with PVL genes (Foster, 2004; David and Daum, 2010; Hunter *et al*., 2011).

The strain possesses more invasive capabilities and is associated with reported cases of epidemic furunculosis and severe invasive pulmonary infections in the young and otherwise healthy people (Zetola *et al*., 2005; Elston and Barlow, 2009). Though all CA-MRSA strains share a type IV SCC*mec* cassette and the Panton-Valentine leukocidin locus, the toxin genes associated with it vary from continent to continent (Vandenesch *et al*., 2003).

Panton-Valentine leukocidin (PVL) and its genes (*lukS-lukF*) which elicit tissue necrosis are the most well-known community-acquired methicillin resistant *Staphylococcus aureus* virulence factor and though present in most cases of CA-MRSA infection worldwide (Lina *et al*., 1999; Zetola *et al*., 2005; Elston and Barlow, 2009), it may not to be essential for the evolution of other community-associated strains of MRSA, including ST8:USA300 and

ST59:USA1000 (An Diep *et al*., 2006). Its occurrences in urban regions with large populations of lower socio-economic status, overcrowding and young children has become a global problem (Moran *et al*., 2006; David *et al*., 2007; Ghebremedhin *et al*., 2009; Nabera, 2009).

The burden of CA-MRSA infections varies widely between different groups of individuals and in different regions of the world. In Taiwan, skin and soft-tissue infection (SSTI) was the most common presentation of CA-MRSA infection including staphylococcal scalded skin syndrome, bacteraemia/septicaemia, necrotizing pneumonitis, osteomyelitis/arthritis with or without septic pulmonary embolism, deep-seated soft tissue infection (STI) necrotising fasciitis, peritonitis and orbital cellulites (Huang and Chen, 2011). In Europe, the predominant strains are ST80, ST8 and ST5; in the US, the commonest clone of methicillin resistant *Staphylococcus aureus* associated with community acquired infection is USA300 and USA400 strains while in Australia they are ST93 and ST1(Pu *et al*., 2009, Chen, 2012). In Nigeria however, ST88 and ST152 have been reported (Ghebremedhin *et al*., 2009; Shittu *et al*., 2011). Studies in Nigerian have revealed that 40% of methicillin susceptible *Staphylococcus aureus* (MSSA) isolates of sequence type ST152 from wound infections and 8.13 % MSSA from diverse biological specimens from hospitalized and extra-hospitalized patients carry Panton-Valentine leukocidin (PVL) genes (Sani *et al*., 2011; Shittu *et al*., 2011). This finding indicates that Panton-Valentine leukocidin (PVL) may not be an exclusive marker of CA-MRSA and that both methicillin-susceptible *Staphylococcus aureus* (MSSA) and community-associated methicillin-resistant *S. aureus* (CA-MRSA) could express PVL (Mine *et al*., 2012).

While HA-MRSA strain types are rarely transmitted in the community, CA-MRSA strains are now transmitted in healthcare facilities, making it difficult to differentiate both on the basis of their epidemiology (Otter and French, 2011). Genetic characterization using

multilocus sequence typing (MLST), *spa* typing of the polymorphic region X of *Staphylococcus aureus* protein A and SCC*mec* has provided the best way to differentiate CA-MRSA from HA-MRSA (Coombs *et al*., 2012). Community strains of *S. aureus* are distinct from HA-MRSA by the nature of the staphylococcal cassette chromosome *mec*, the *spa* types and although phenotypic markers such as carriage of Panton-Valentine leukocidin or antimicrobial susceptibility profiles can be useful indicators of CA-MRSA combining them with genotypic typing provides a more robust differentiation of the two (Mediavilla *et al*., 2012).

2.7 Livestock Associated Methicillin Resistant *Staphylococcus aureus*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been detected in several species of animals and animal-derived products (Waters *et al*., 2011). Animals can act as a reservoir and source for the emergence of novel methicillin-resistant *Staphylococcus aureus* (MRSA) clones in humans (Garcia-Alvarez *et al*., 2011). Recently, a new livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) sequence type 398 (ST398) isolate with high prevalence rates in pigs and calves has emerged worldwide causing invasive disease in humans (Shijffelen *et al*., 2010). And since its discovery in the early 2000s, MRSA of clonal complex 398 (CC398) has become a rapidly emerging cause of human infections, most often associated with livestock exposure (Price *et al*., 2012). There is also a report of high prevalence of methicillin susceptible *S. aureus* (MSSA) of clonal complex (CC398) among pigs in Europe (Hasman *et al*., 2010). Studies provide strong evidence that CC398 originated in humans as MSSA and then spread to livestock, where it acquired resistance to methicillin and tetracycline and with the jump from human to animals came the decreased capacity for human colonization, transmission, and virulence (Price *et al*., 2012)

The sequence type ST398 has also been isolated from other animal species and has become known as livestock-associated methicillin resistant *Staphylococcus aureus* (van Cleef *et al*.,

2011). These include veal calf (Wulf *et al*., 2006), cattle (Vanderhaeghen *et al*., 2010), horses and dogs (Witte *et al*., 2007). There is strong evidence that MRSA of ST398 from horses are unrelated to the other ST398 isolates and probably have evolved independently by acquisition of a different SCC*mec* element as they contained SCC*mec* elements of group IVa (Witte *et al*., 2007).

Apart from CC398/ST398 with (t011, t034, t108, t567, t571, t1451, t2011, 2510) livestock indicator spa types, other LA-MRSA clonal lineages have also been detected in animals in Europe and elsewhere (Kock *et al*., 2013). These include CC9/ST9 with t1430 and t5487 as livestock indicator *spa* types; CC5/ST5 with t002 as the livestock indicator spa type; CC97/ST97 with t3992 as the livestock indicator *spa* type; CC30/ST39 with t007 as livestock indicator *spa* type. All these clonal and sequence types have been reported in Germany in different animal species according to European baseline study as cited by Kock *et al*. (2013).

The first case of livestock associated methicillin resistant *Staphylococcus aureus* strains ST398 emerging in healthy poultry in Europe were reported in Belgium in 2008 (Nemati *et al*., 2008). *spa* type t1456 of ST398 has been reported in poultry and more specifically in broiler chickens in Belgium that are different from *spa* types found in other animal species but it is not clear whether this *spa* type is peculiar to chicken (Persoons *et al*., 2009). In the Netherlands, Mulders *et al*. (2010) reported both ST398 and ST9 in broiler chickens and slaughter house personnel. While Hasman *et al*. (2010) found no ST398 in clinical isolates from poultry in the Netherlands. Other studies in addition have reported ST5 as dominant strain in poultry rather than ST398 (Lowder *et al*., 2009; Hasman *et al*., 2010).

Antibiotics such as bacitracin, Chlortetracycline, erythromycin, penicillin and fluoroquinolones licensed to treat diseases in man and animals are used in the treatment and control of bacterial diseases in poultry (Kilonzo-Nthenge *et al*., 2008) and may account for the emergence of antibiotic resistance in poultry (Nemati *et al*., 2008).

While in Europe and Americas as reported by Smith and Pearson (2011), ST398 is the predominant sequence type associated with pigs and calves, the dominant type in Asia is ST9. Furthermore, ST398, ST9 and ST5 have also been reported in poultry in Europe and Asia (Nemati *et al*., 2008, Lowder *et al*., 2009; Hasman *et al*., 2010; Mulders *et al*., 2010).

MRSA ST398 is non-typable (Soavi *et al*., 2010). According to Huijsdens *et al.* (2007), 30% of all human isolates of MRSA sent to the National Institute for Public Health and the Environment in 2007 in the Netherlands could not be typed by pulsed-field gel electrophoresis (non-typable (NT)-MRSA). Molecular characterization of the NT-MRSA isolates revealed 27 different *spa* types and 2 distinct SCC*mec* types: type IV and V which were closely related based on *spa* and multi-locus sequence typing. They belonged to ST398. Furthermore, Price *et al*., (2012) identified 15 different *spa* types among 89 clonal complex (CC398) isolates from 19 countries and 4 continents using whole genome sequence typing (WGST).

Initially, MRSA ST398 isolates did not cause frequent invasive disease in humans, which may be due to the absence of several common virulence factors, however, the enhanced ability of S0385 a MRSA ST398 isolate from human endocarditis to acquire mobile elements suggests a possible rapid acquisition of determinants which contribute to virulence in human infections in this species (Shijffelen *et al*., 2010).

2.8 Transmission of Methicillin resistant *Staphylococcus aureus.*

Staphylococcus aureus is a ubiquitous organism whose niche remains the anterior nares; however, skin, throat, intestinal tract and vagina are other potential habitats making human to human contact one way of transmitting the bacterium. MRSA is harbored sporadically and patients do not have to be placed in contact isolation based on a history of previously being MRSA positive (Spence *et al*., 2009).

Transmission of MRSA occurs by direct contact with infected and colonized individuals shedding the bacterium, through contaminated formites and air. While frequent hand washing is a protective factor against MRSA infection, sharing of personal items with other persons such as nail clippers, towels, razors, underwear or cosmetics had more than three times higher risk of CA-MRSA infection than those who had not; a good proof of the importance of fomites in the transmission of MRSA (Leung *et al*., 2012).

MRSA is principally a human pathogen, and molecular typing has shown that certain clones have spread widely between humans and from humans to animals and have emerged in pigs, poultry and cattle (Hunter *et al*., 2011). There are various reports of transmission of MRSA or MSSA between human patients, their family members and dogs living in the same household or those in contact with livestock (Voss *et al*., 2005; Witte *et al.*, 2007; Waters *et al*., 2011). There is also a report of transmission between swine and swine workers, an indication that agricultural animals might be a major reservoir (Smith *et al*., 2009).

MRSA carriage can be a serious health risk to pet owners with susceptibility to infection since pet animals can disseminate acquired resistant bacteria by faecal shedding, enhancing their spread in humans and in the environment (Waters *et al*., 2011). The most frequent MRSA isolates from animals are ST398, and although pigs and veal calves constitute the main reservoirs, multidrug resistant PVL positive clones have also been isolated from

chickens and horses and can be transferred to humans and people working in food animal production contributing greatly to CA-MRSA cases (Van-loo *et al*., 2007; Hunter, *et al*., 2011). Studies elsewhere reports direct associations between animal and human carriage of ST398; between antibiotic resistance in animals and antibiotic usage; between hygiene and prevalence; and between farmers and family members which may be attributed to occasional human to human transmission (Smith *et al*., 2009; Graveland *et al*., 2010).

In Europe (Netherlands, Denmark, Belgium) and Canada, there appear to be an increasing problem involving transmission of MRSA ST398 from colonized livestock, particularly pigs, to farm workers, abattoir workers and veterinarians who are in contact with such animals (Johnson, 2011; Hunter *et al*., 2011). The isolation of MRSA ST398 showing the same characteristics from a wound infection in a dog and from nasal colonization of the staff of a veterinary practice where this dog had been treated suggests that interspecies transmission may occur (Witte *et al.,* 2007). MRSA carriage of 33% and 8% in farmers and their relatives respectively have been reported in 102 farms study in the Netherlands where 88% of the farms were found to harbor MRSA while 28% of the calves were positive (Graveland *et al*., 2010). On the average, MRSA carriage in pig and veal calf farmers in the Netherlands is estimated at 25 to 35% (van Cleef *et al*., 2011).

Clone of MRSA ST398 exhibiting similar *spa* types with SCC*mec* elements of types IVa or V has also been isolated from colonized and infected humans and companion animals in Germany, Austria and Netherlands (Witte *et al*., 2007; van Cleef *et al*., 2011). MRSA sequence type ST398 with SCC *mec* V has also been identified in patients with skin and soft tissue infection in Canada though the prevalence rates in the general human population were unknown (Golding *et al*., 2010). A study in Midwestern US reported methicillin-resistant *Staphylococcus aureus* ST398 prevalence of 49% in swine and 45% in swine workers (Smith *et al*., 2009). Lozano *et al.* (2011) reported *spa* type t1451 of MRSA ST398 with SCC*mec*

type V in a skin lesion and nasal sample of a pig-farmer, his brother, and nine pigs from his farm, suggesting a possible transmission from animals to humans.

The presence of Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in farmers constitutes a major threat to public health and health care system (Smith *et al*., 2009; Golding *et al*., 2010; Waters *et al*., 2011). MRSA prevalence in humans is strongly associated with prevalence in animals and intensity of contact with animals positive for methicillin-resistant *Staphylococcus aureus* (Graveland *et al*., 2010). Human MRSA clones have also been reported in animals in Upper Midwestern and Northeastern United States which suggests possible reverse zoonotic transmission (Lin *et al*., 2010).

Although the rate of transfer of ST398 from animals to humans may be low, its presence in most animals and in most countries may nevertheless prove it as a reservoir of CA-MRSA (Hunter *et al*., 2011).

Incidence of MRSA causing serious infections amongst hospitalized and non-hospitalized patients has been reported in several places in Nigeria and elsewhere (Okesola *et al*., 1999; Adegoke and Komolafe, 2009; Ghebremedhin *et al*., 2009; Sani *et al*., 2011; Orji *et al*., 2012). Several sequence types including ST5, ST8, ST94 and ST241 have been reported in Nigeria (Shittu *et al*., 2011). In Southwest Nigeria, a study in Ibadan involving 188 cases of wound infection in University College Hospital Ibadan between December 1994 and April 1995 showed that 78 strains from 188 isolates were methicillin resistant (Okesola *et al*., 1999). Similarly, Adegoke and Komolafe, (2009) reported 50% methicillin resistance amongst isolates of *Staphylococcus aureus* in Ile-Ife with 70% producing β lactamases. In another study still in Southwest Nigeria, 20.2% of 346 isolates were methicillin resistant while 47.15% of them met the criteria for CA-MRSA (Ghebremedhin *et al*., 2009). Furthermore, MRSA and MSSA producing PVL and vancomycin resistant *S. aureus* (VISA) have also been reported in Nigeria (Sani *et al*., 2011; Orji *et al*., 2012).

2.9 Molecular typing of Methicillin resistant *Staphylococcus aureus*

Molecular methods have been developed to identify *Staphylococcus aureus* where results are ambiguous and where clinical samples have a high suspicion of MRSA infection (Brown *et al*., 2005). A combination of different typing methods, namely pulsed-field gel electrophoresis, multi-locus sequence typing and SCC*mec* typing have been used to characterize MRSA (de Sousa and de Lencastre, 2004). In the USA and elsewhere for instance, pulsed-field gel electrophoresis (PFGE) technique has identified 8 MRSA types: USA100 to USA800, which are involved in staphylococcal infections (Pu *et al*., 2009).

The phenotypic expression of *mecA* gene is affected by various factors including pH, temperature and osmolarity and when proper conditions for laboratory MRSA detection, together with Mueller-Hinton agar supplemented with NaCl and adequate temperature and time, as recommended by Clinical and Laboratory Standards Institute (CLSI) are applied, detection is easily achieved (Sakoulas, 2001).

Staphylococcus aureus presents a highly clonal structure initially revealed by multilocus enzyme electrophoresis (MLEE) and pulsed field gel electrophoresis (PFGE), and afterwards by multilocus sequence typing (MLST) (Feng *et al*., 2008). Molecular typing of MRSA is required to study the routes and rates of transmission of this pathogen (Schouls *et al*., 2009). Several molecular typing methods with standardization and optimization of sequence-based techniques offering broad applicability and high throughput have been described and used for the identification of MRSA (Struelens *et al*., 2009).

Typing methods are either phenotypic or genotypic. The phenotypic methods involves products of gene expression and includes biotyping that exploits biochemical profiles, serotyping that utilizes series of antibodies to detect surface antigens on bacterial cells, phage typing that exploit phage infectivity profile of the organism and its antibiotic

susceptibility pattern (Tenover *et al*., 1997). The phenotypic methods in general are easier to perform, easier to interpret, cost effective and are widely available, however, it is less discriminatory (Mehindratta and Bhalla, 2012).

Genotypic methods involves the analysis of the genetic structure of the organism polymorphism DNA restriction pattern based on enzymatic cleavage of the chromosomal DNA into few or several fragments (Tenover *et al*., 1997). Genotyping includes plasmid finger printing in which the number and size of plasmid present in a cell form basis for its identification, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR), Mutilocus sequence typing (MLST) which though cumbersome, expensive and technically demanding are more discriminatory (Tenover *et al*., 1997; Mehindratta and Bhalla, 2012).

Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), randomly amplified polymorphic DNA analysis, *spa* typing, SCC*mec* typing and phage typing are epidemiological tools that have been used to characterize MSRA (Tenover *et al*., 1997; Grundmann *et al*., 2002; Derenberg *et al*., 2007; Mehindratta and Bhalla, 2012). PFGE which is based on the digestion of chromosomal DNA with the restriction enzyme *Sma*I, followed by agarose gel electrophoresis is still considered to be the gold standard for typing of MRSA isolates though the method is time-consuming and expensive (Stranden *et al*., 2003). It is one of the most discriminative typing methods and is used to study MRSA outbreaks in hospitals and hospital-to-hospital transmission (Deurenberg *et al*., 2007). As PFGE is hampered by inadequate laboratory-to-laboratory reproducibility (Chung *et al*., 2000), DNA sequencing methods including MLST, *spa* typing, SCC*mec* typing and toxin gene profile typing are more practical methods for detecting evolutionary changes and transmission events (Pfaller, 2001).

Isolates of bacteria that are indistinquishable in genotype are assigned as a clone, having descended from the same ancestor. As recombinational and replacement events results in

diversification of the anscestral genotypes or clonal complex which varies from bacteria to bacteria with some more stable, clone and clonal complexes needs to be assigned by indexing the genetic variation that is selectively neutral (Spratt, 2004). This has been achieved using MLST.

MLST is currently the most popular typing method that sequences the seven housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi, and yqiL*) in which, the difference in each gene sequences are assigned as alleles and the alleles at the seven loci provide an allelic profile, which unambiguously defines the sequence type (ST) of each isolate and in which isolates with at least six of seven matching genes are considered to belong to the same clonal complex (CC) (Feng *et al*., 2008). It involves sequencing DNA fragments of about 500 bp of the seven housekeeping genes which are compared to known alleles at each locus via the MLST website (http://www.mlst.net), where every isolate is described by a seven-integer allelic profile that defines a sequence type (ST) (Robinson and Enright, 2004; Deurenberg *et al*., 2007).

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing in combination with genotyping of the *Staphylococcus aureus* chromosome has become essential for defining methicillin-resistant *Staphylococcus aureus* (MRSA) clones in epidemiological studies (Kondo *et al*., 2007; Derenberg *et al*., 2007). SCC*mec* elements are currently classified into different types based on the nature of the *mec* and *ccr* complexes, and subtypes according to their junkyard (joining) region DNA segments (Zhang *et al*., 2005); a feature very important in understanding MRSA epidemiology). SCC*mec* type assignment system developed by Kondo *et al*, (2007) consists of 6 multiplex PCRs for identifying the *ccr* gene complex, the *mec* gene complex and specific structures in the junkyard (J) regions and therefore can be applied in the typing of SCC*mec* elements. The 3 methods mainly used to characterize SCC*mec* are; i) a multiplex PCR for SCC*mec* types in which *mecA* and different loci on SCC*mec* are detected

as developed by Oliveira *et al.* (2002); multiplex PCR in which the structure of the *mec* complex and the *ccr* genes are amplified by PCR as developed by Ito *et al.* (2001), and PCR for the specific structures in the junkyard (J) regions as developed by Kondo *et al*. (2007).

The sequences of the polymorphic region X of the *Staphylococcus aureus* protein A (*spa*) gene have been used to develop a single-locus sequence typing technique (*spa* typing) for MRSA whose locus consists of a number of mainly 24-bp repeats, attributed to deletions and duplications of the repeats, and more seldom, to point mutation (Deurenberg *et al*., 2007). As a result, *spa* typing can be used to investigate both the molecular evolution and hospital outbreaks of MRSA

Despite all the success recorded with these techniques, there is no consensus regarding the single best method for typing of MRSA strains (Mehindratta and Bhalla, 2012), and while there is no single typing method that meets all the above criteria, there have been recent trends towards the use of *spa*, MLST and SCC*mec* methods in preference to PFGE and MLVA (Monecke *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Area

This research work was carried out in Abakaliki, Onueke and Afikpo areas of Ebonyi State, Nigeria. The State is located within the rain forest belt at coordinates 6˚15″ N 8˚ 05″E with two distinct seasons: the wet and dry seasons. While the rainy season occurs between April and October, the dry season is usually between November and March. Ebonyi State is a mainland state, inhabited and populated primarily by Igbo speaking people. Its capital, Abakaliki is the largest city in the State followed by Afikpo. It covers a total area of 5,530 km^2, (Uneke and Ibeh, 2009).

The State lacks grazing land and because of this, grazing is practiced at subsistence level. Poultry farming is a big enterprise in the area. There are many poultry farms scattered all over the State in addition to the traditional chicken farming system practiced in the rural communities where chicken are reared and raised for food in homes and households.

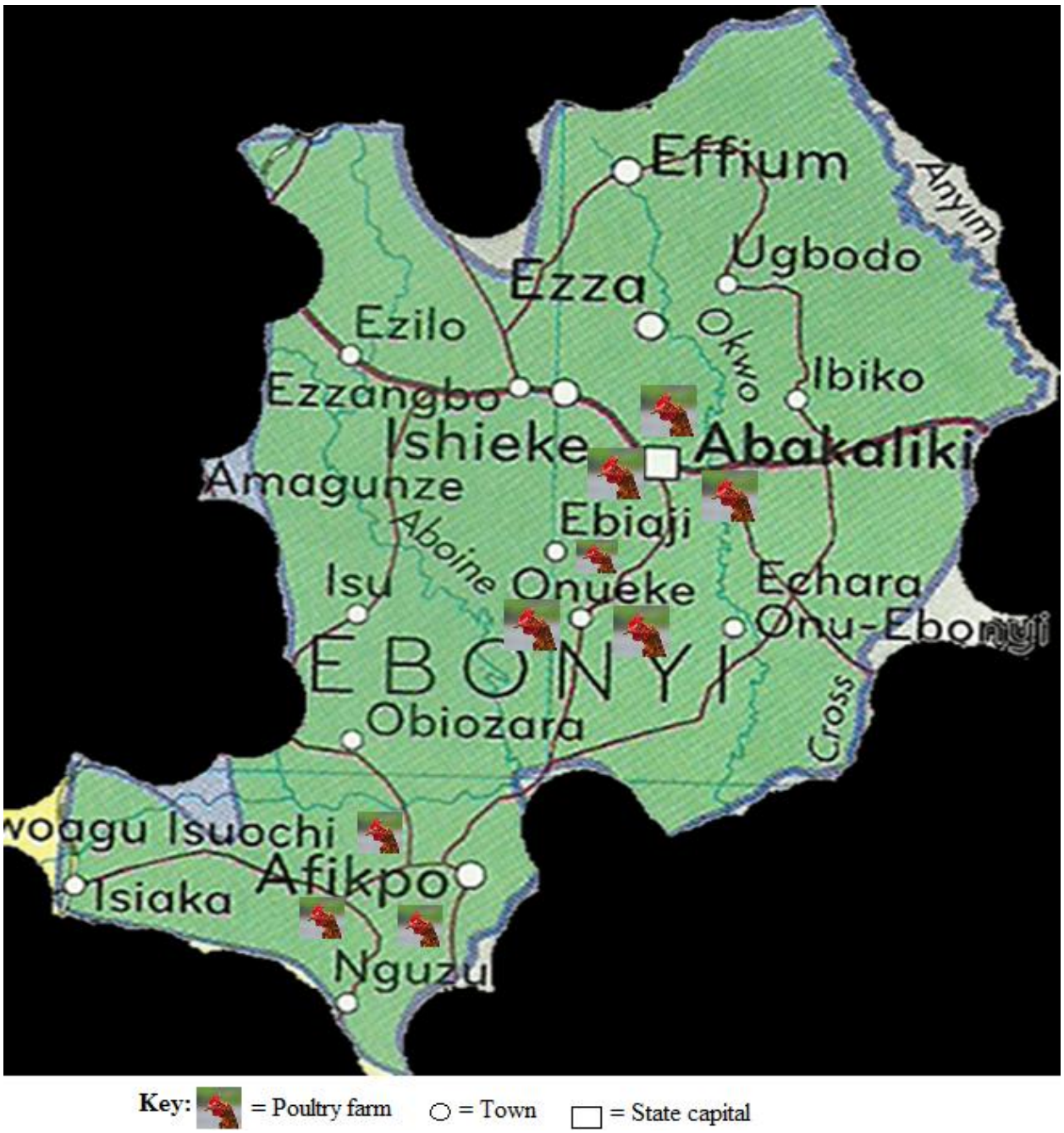


Figure 3.1: Map of Ebonyi State showing Abakaliki (Ebonyi North), Onueke (Ebonyi Central) and Afikpo (Ebonyi South) districts where the poultry farms sampled were located.

3.2 The Study Population

Nine poultry farms spread across the 3 senatorial districts (3 farms from each senatorial district) of Ebonyi State were screened for *S. aureus* and methicillin resistant *Staphylococcus aureus* (MRSA). Two hundred (200) birds selected at random (100 broilers and layers each) from each of the poultry farms were screened. All the farms selected were of comparative size with similar features; i) all the farms had more than 400 birds; ii) the buildings in the farms were similar; iii) each farm had between 2 to 5 members of staff.

Consenting Poultry farm workers in each of the selected farms were screened for MRSA carriage. Likewise, clinical isolates of staphylococci from the microbiology unit of three hospitals selected, one from each of the senatorial districts in the State were screened for methicillin resistance.

3.3 Ethical Considerations

The study protocol was approved by the Department of Pharmaceutics and Pharmaceutical Microbiology of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. Informed consent was obtained from each of the participating poultry farm worker for inclusion in the study. Ethical clearance was obtained from Federal Teaching Hospital Abakaliki which in addition, covered the other two hospitals that used the teaching hospital as their referral center. (Appendix 1a). These other two hospitals had no ethical committee.

3.4.0 Methodology

3.4.1 Collection of Nasal and Cloacae Swabs of Poultry

The nostrils and cloacae of the chicken (layers and broilers) selected at random were swabbed using improvised sterile cotton tipped swab sticks (smaller than standard swab sticks) moistened with normal saline for the nostrils because of their small size, and a standard swab

stick moistened with normal saline for the cloacae. These were taken, processed and analyzed as described by Persoons *et al*. (2009).

3.4.2 Collection of Nasal Swabs of Poultry Farm Workers

A modified nasal collection method as described by Nworie *et al*. (2013b) was used in the collection of the nasal samples. In this method, the anterior nares of each poultry farm worker consenting to the study was swabbed with a sterile cotton swab dampened with sterile normal saline by rubbing (rolling) the swab stick in both nostrils one after the other using the same swab stick. This was transferred in ice to the Department of Medical Laboratory Science Laboratory where they were analyzed using standard bacteriological techniques for the presence of *Staphylococcus aureus*.

3.4.3 Clinical Isolates of Staphylococci

Clinical isolates of staphylococci species from different sources (wounds, urine, sputum and otitis media) from each of the selected hospitals in the State were collected from December, 2011 to August, 2012 and screened for *Staphylococcus aureus* using standard bacteriological procedures.

3.5.0 Preparation of Culture Media

All media were prepared in the laboratory according to the manufacturers' instructions and directions and sterilized at 121^{o}C for 15 minutes. The media include: peptone water, nutrient agar, Brain Heart infusion broth, Mueller Hinton agar, Baired Parker agar and Mannitol Salt agar all from Oxoid Co UK). (Appendix II).

3.5.1 Preparation of Blood Agar

Appropriate quantity of nutrient agar powder was dissolved in corresponding volume of water and autoclaved at 121^{o}C for 15 minutes. The medium was allowed to cool to 50^{o}C in a water bath. Sterile expired human blood obtained from Federal Teaching Hospital Abakaliki was aseptically added to the molten nutrient agar and mixed properly. The prepared blood agar was then dispensed aseptically into sterile Petri-dishes and the surface flamed to remove air bubbles.

3.12. Phenotypic Screening to Identify *Staphylococcus aureus*

The phenotypic screening to identify *Staphylococcus aureus* and MRSA was carried out in the research laboratory of the Department of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University, Abakaliki, Nigeria.

Staphylococcus aureus was isolated and identified by exploiting its culture characteristics and biochemical activities using standard bacteriological techniques. General and selective culture media, Gram reaction, catalase production, slide and tube coagulation assay and latex agglutination tests as described by Barrow and Feltham (1993), Isenberg (1998), Cheesbrough (2000), Jensen and Lyon (2009) and Nwankwo and Nasiru (2011) were utilized.

All the chicken nasal and cloacae swabs collected from the selected poultry farms and nasal swabs collected from anterior nares of the consenting Poultry farm workers were inoculated first into brain heart infusion broth as described elsewhere (Persoons *et al*., 2009; Olayinka *et al*., 2010) and the overnight culture were then subcultured onto 5% blood agar, mannitol salt agar and Baird Parker agar. After overnight incubation at 37^{o}C, colonies showing appropriate β-haemolysis and growth pattern that resembled staphylococci and producing the characteristic golden yellow colonies on mannitol salt agar and black colonies surrounded by

clear hallow on Baird Parker agar were isolated as presumptive *Staphylococcus aureus* (Boerlin *et al*., 2003; Olayinka *et al*., 2010). The colonies were also tested for their Gram reaction, catalase, coagulase (slide and tube) and latex agglutination reaction. Isolates that tested positive in all these tests were documented.

3.6.1 Gram Staining

The isolates were stained for Gram reaction using the standard staining procedures as described by Isenberg, (1998) and Cheesbrough, (2000). The stained preparation was viewed using the oil immersion (x100) objective. The reaction, morphology and arrangement of the isolates were recorded.

3.6.2 Catalase Test

Catalase test was carried out as described by Ochei and Kohlhatkar, (2000). A colony of each Gram positive isolate was placed on a clean grease-free slide. Two drops of hydrogen peroxide (H_2O_2) were added using Pasteur pipette and observed for effervescence.

3.6.3 Coagulase Test

The slide agglutination test was carried out as described elsewhere (Cheesbrough, 2000). A drop of sterile normal saline was placed on one end of a clean grease-free slide and a drop of plasma on the other end. A colony of the test isolate was collected with a sterile wire loop and emulsified on the sterile normal saline and on the plasma. The sterile normal saline was used as a negative control. The two suspensions were observed for clumping. A confirmatory tube coagulase test was performed according to standard microbiological procedures as described by Boerlin *et al*. (2003) and results were recorded after 4 and 24 hours of incubation at 37 ^{o}C.

3.12. Antibiotic Susceptibility Testing (AST)

Susceptibility of nare (chicken and volunteers), cloacae and clinical isolates of *S. aureus* to Gentamicin (10 µg), Rifampicin (5 µg), Cefuroxime (30 µg), Trimethoprim/Sulphamethoxazole (1.25/23.75 µg), Linezolid (30 µg), Chloramphenicol (30 µg), Dalfopristin (15 µg), Doxcycline (30 µg), tetracycline (30µg), Erythromycin (15 µg), Levofloxacin (5 µg), Augmentin (30 µg), Vancomycin (30 µg) and Cefoxitin (30 µg) (all from Oxoid Co. UK), was determined on Mueller-Hinton agar using the Kirby-Bauer-CLSI modified disc agar diffusion (DAD) method as described elsewhere (Isenberg, 1998; Grundmann *et al*., 2002; Nwankwo and Nasiru, 2011) with standard type *Staphylococcus aureus* (ATCC 33252). Results were interpreted in accordance with the CLSI guideline M2-A9 and M7-A7 (CLSI, 2008).

McFarland turbidity standard of 0.5 suspensions containing about $1.5X10^8$ CFU/ml of bacteria of each confirmed *Staphylococcus aureus* isolate was prepared in peptone water. Standard type *Staphylococcus aurous* (ATCC 33252), obtained from Centre for Emerging Infectious Disease (CEID), University of Iowa, United States of America was used as control. All test *Staphylococcus aureus* isolates were screened for methicillin resistance by inoculation onto Mueller-Hinton agar supplemented with 4% Sodium chloride (NaCl). Cefoxitin discs (5 µg) were aseptically placed on the surface of the inoculated plates and incubated aerobically at 35 ^oC for 18-24 hrs. For antibiotic susceptibility to other antibiotics, the test staphylococci were also inoculated onto the surfaces of plain Mueller-Hinton agar plates and the panel of antibiotic discs were placed on the plates and allowed to stand for 30 minutes. The plates were then incubated at 37 ^oC for 24 hrs to determine their susceptibility to the panel of test antibiotics.

The zones of inhibition were measured, documented and interpreted according to Clinical and Laboratory Standards Institutes guidelines (CLSI, 2008).

3.8 Transportation of Isolates to United States of America

A total of 28 MRSA determined by their resistance to cefoxitin and 42 MSSA were shipped to Center for Emerging Infectious Disease (CEID), University of Iowa, United States of America for molecular analysis. The isolates were sent on cryovial nutrient agar slants (a colony of each of the isolates was streaked on nutrient agar slant made on cryovials and incubated for 24 hrs). The slants were sent through export permit obtained from Centre for Disease Control (CDC) (Appendix 1b). On receipt of the isolates, they were immediately transferred to cold room at a temperature of 5 ^{o}C.

3.9 Subculturing of the Isolates

The isolates were subcultured onto Columbia Nalidixic agar (I) with 5% sheep blood and Baird Parker agar (BPA) with tellurite enrichment and incubated at 35 ^{o}C for 18-24 hours and examined for growth. Presumptive *S. aureus* (black colonies surrounded by clear halos on BPA and typical β-haemolysis on I plate) were further assayed biochemically. Some of the cultures which were found to be contaminanted were subcultured onto Phenylethanol Agar (PEA) plate.

3.10 Re-screening of the Isolates at CEID

All the recovered isolates showing characteristic *Staphylococcus aureus* colonies on Columbia Nalidixic acid agar (I) plates were subjected to biochemical test to confirm their identity as *Staphylococcus aureus*. The following biochemical tests were carried out;

3.10.1 Catalase and coagulase tests

These were carried out as described in 3.6.2 and 3.6.3 and according to the SMITH LAB standard operating procedure (SOP) of the Center for Emerging Infectious Disease, Department of Epidemiology, College of Public Health, University of Iowa, entitled USDA FOLLOW-UP PROTOCOLS.

3.10.2 Staph Latex agglutination Assay (Pastorex Staph-Plus)

All the test isolates that were catalase and coagulase positive obtained from culture on Columbia Nalidixic agar with 5% sheep blood were tested with *Staphylococcus aureus* latex agglutination assay kit (Pastorex Staph-plus, Bio Rad) for agglutination. The *Staph* Latex agglutination test, determines the presence of clumping factor, Protein A and capsular polysaccharides on the cell wall of *S. aureus*. This test was performed according to the manufacturer's directives. Visible clumping was indicative of a positive *S. aureus* isolate.

3.12. Glycerol Stock of Isolates

Two sets of tubes containing 5 ml Tryptic Soya Broth (TSB) were inoculated with recovered and re-screened isolates of *S. aureus* from Nigeria (two tubes for each isolate) and incubated at 37 ^{o}C for 24 hrs. At the end of the incubation period, they were centrifuged at 2000 rpm for 10 minutes to form pellet. The supernatants were discarded into a disinfecting jar. The tubes with the pellets were taken to the safety hood that had been disinfected with ultraviolet (UV) light for 15 minutes including racks, beakers, pipets and their tips. Prior to UV sterilization, these items had been scrubbed with 70% ethanol (EtOH). The pellets were re-suspended with 2 ml mixture of equal volume of Triptic Soy Broth (TSB) and glycerol solution and vortexed. This was done under aseptic condition. It was vortexed until the pellets were all dissolved. The 2 ml TSB/Glycerol suspension of each tube were transferred to 2 cryo-vials (1ml for each cryo-vial). The cryo-vials containing the glycerol stock of the isolates were properly labeled, boxed and stored in a -80^{o}C chest freezer (MDF-U5486SC-PA, USA).

3.12 Molecular Screening

A total of 28 MRSA isolates and 26 MSSA were subjected to molecular typing. *S. aureus* DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison WI) following manufacturer's instructions. The presence of *mecA* was detected by a multiplex PCR as described by Milheirico *et al*. (2007). The *spa* typing was for the polymorphic region X of protein A (repeat polymorphism of the X-region of the *spa* gene) as described elsewhere (Shopsin *et al*., 1999) and multilocus sequence typing (MLST) of the seven house-keeping genes for clonality was carried out for all novel *spa* types as described elsewhere (Enright *et al*., 2000). All the isolates were screened for Panton valentine leukocidin (PVL) gene (*lukS-lukF*) by Polymerase Chain Reaction (PCR) as described by Lina *et al*. (1999). Isolates that were resistant to tetracycline were screened for the tetracycline resistance gene (*tetM*) as described by Weigel *et al*. (2007), while isolates that were positive for the *mecA* gene were typed for the staphylococcal cassette chromosome *mec* (SCC*mec*) according to a protocol described by Ghaznavi-Rad *et al*. (2010).

3.12.1 DNA Extraction

An overnight TSB culture of each of the *S. aureus* isolates was centrifuged at 2000 rpms for 10 minutes and the supernatant discarded into a disinfecting jar. To the pellet in each tube, 480 µl of 50 mM EDTA at pH8.0 was added and vortexed to resuspend the cells. A master mix containing 30 µl of lysostaphin, 15 µl of mutanolysin and 75 µl of lysozyme per reaction (multiplied by the number of isolates) were vortexed to mix. Aliquots of 120 µl from the master mix were made in 1.5 ml microcentrifuge tubes labeled to correspond with the isolates. This pre-treatment was done to weaken the cells to allow for efficient cell lysis. The cell and EDTA mixture was poured into corresponding labeled 1.5 ml microcentrifuge tube. The samples were incubated at 37 ^{o}C for 60 minutes and centrifuged for 3 minutes at 13,500 rpms. The supernatants were decanted into a disinfecting jar. To the pellet, 600 µl Nuclei

Lysis Solution was added and inverted severally to re-suspend. It was further incubated at 75 ^{o}C for 5 minutes in heat block to lyse the cells. On cooling to room temperature, 2 µl of Rnase Solution was again added to the cell lysate, inverted 2-5 times to mix and further incubated at 37 ^{o}C for 45-60 minutes. Finally, 200 µl of Protein Precipitation Solution was added to the Rnase-treated cell lysate and vortexed vigorously at high speed for 20 seconds to mix.

The Protein Precipitation Solution/cell lysate were incubated on ice for 5 minutes and brought to room temperatures and then centrifuged at 13,500 rpms for 3 minutes. The supernatant (~800 µl) containing the DNA was transferred into a clean 1.5 ml microcentrifuge tube containing 600µl of isopropanol (2-propanol) labeled with their corresponding DNA label. They were gently mixed by inversion until the thread-like strands of DNA formed a visible mass. It was further centrifuged at 13,500 rpms for 3 minutes, decanted and the tube drained on clean absorbent paper. To wash the DNA pellets, 600 µl of 70% ethanol at room temperature were added to each of the tube and gently inverted several times. It was again centrifuged at 13,500 rpms for 3 minutes and the ethanol carefully aspirated. The tubes were drained on clean absorbent paper and the pellets were air-dried for 15 minutes. The DNA was rehydrated by adding 100 µl of DNA rehydration solution to the tube and incubated at 60 ^{o}C for 1 hour with periodic tapping to mix. The extracted DNA was stored at -20 ^{o}C freezer till required for Polymerase Chain Reaction (PCR).

3.12.2: Detection of the *S. aureus* 16S rRNA, *nuc* and *mecA* gene by Multiplex PCR

A multiplex PCR analysis was carried out to detect *S. aureus* 16S rRNA, *nuc* and *mecA* gene. *mecA*, is a determinant of methicillin resistance; *nuc*, encodes the *S. aureus*-specific region of the thermonuclease gene; and a genus-specific 16S rRNA sequence used as an internal amplification control for staphylococcal DNA (Maes *et al*., 2002).

A master mix was prepared in a 1.5 ml eppendorf tube and the final volume of the master mix was determined by the number of samples that were processed (see Appendix 11).

The combined volume of reactants per reaction was 24 µl. This was multiplied by the number of samples to arrive at the total volume of the mastermix. The mastermix was vortexed to mix thoroughly before the addition of the HotStartTaq enzyme. The HotStartTaq enzyme was added after the vortex (because of its fragility) and inverted several times to mix. The master mix was then aliqouted using repeater pipette in volumes of 24 µl into the PCR tubes numbered to correspond with the samples. The DNA template of 1µl for each sample was then added to the corresponding tubes except for the negative control where distilled water was used in place, bringing the final volume of each PCR tube to 25µl. The positive control was MRSA USA300 DNA. The PCR tubes were then agitated to mix using an agitator. This was to ensure complete mix of the DNA template and to expel any air that might have been trapped in the aliquots.

The following primers (forward and reverse) were used as designed and described by Milheirico *et al*. (2007).

Table 3.1: **16S rRNA, *nuc* and *mecA* gene Multiplex PCR Primers**

Gene	Primer sequence	Expected amplicon size
mecA forward	GGGATCATAGCGTCATTATTC	
mecA reverse	AACGATTGTGACACGATAGCC	500bp
nuc forward	TCAGCAAATGCATCACAAACAG	
nuc reverse	CGTAAATGCACTTGCTTCAAG	200bp
16S rRNA forward	GTGCCAGCAGCCGCGGTAA	
16S rRNA reverse	AGACCCGGGAACGTATTCAC	800bp

The sizes of the *nuc* multiplex (forward and reverse) primers were 200 base pairs (bp). The *mecA* multiplex (forward and reverse) primers were 500 bp while the 16S rRNA (forward and reverse) primers were 800 bp.

The thermocycler was programmed according to the manufacturer's instructions. The temperatures and cycling times were optimized for each new DNA template target and primer pairs to achieve maximum yield and specificity. Each PCR program started with an initial heat-activation step at 95 ^{o}C for 15 minutes. This was to activate the HotStarTaq DNA polymerase. This was followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94 ^{o}C; annealing for 30 seconds at a temperature of 68 ^{o}C; and extension step for 1 minute at 72 ^{o}C. The total number of cycles was 35 and the final extension time was 10 minutes at 72 ^{o}C.

After PCR amplification of the gene product, they were subjected to electrophoresis and viewed in the illumination system. First, tris acetate-ethylene diamine tetracetic acid (TAE) gel was prepared. The volume of the TAE and the weight of the agarose used were dependent on the number of wells or gene product. For a 30 well gel that was used, 3 g agarose was added to 200 ml 1X TAE and warmed to dissolve in a microwave for 2 minutes. As soon as the crystalline nature of the viscous TAE appeared, it was removed from the microwave and allowed to stand for a few minutes before 8µl of ethidium bromide (EtBr) was added to stain the DNA product (Ethidium Bromide is an intercalating agent and it was treated with utmost care because of its toxicity and mutagenicity).

After the addition of the Ethidium Bromide to the viscous agarose and shaken to mix, it was poured into the gel mould with 30 well comb and allowed to stand for 30 minutes to harden. The comb was carefully pulled out of the gel. The clamp of the mould was removed and the gel was transferred into the electrophoretic tank. The tank was filled with 1X TAE buffer to

the maximum limit and the PCR products were loaded into the wells. The first well was loaded with the ladder followed by the PCR products in that order. A negative control (reactants plus sterile distilled water) was loaded after all the PCR products had been load followed by a positive control which was MRSA USA300. The last well was again loaded with the ladder and the gel was run at 120V for 1hr.

At the end of the electrophoresis, the gel was removed and viewed in the transilluminator (Bio-rad Gel Doc XR). The PCR products appeared as bands corresponding to the equivalent molecular weight of the ladder.

3.12.4 *spa* typing of the *S. aureus*

The staphylococcal protein A (*spa*) sequence typing was done for the polymorphic region of protein A (repeat polymorphism of the X-region of the *spa* gene) and the protocol used was as described by Shopsin *et al.* (1999). A master mix was prepared based on the number of DNA templates. The reagents and the amount used were as presented in Appendix 11

The volume of all the reagents was 48 µl for each reaction before the 2 µl DNA templates was added. The number of isolates for *spa* typing was multiplied by the volume of each reaction to give the final volume of the master mix. Before the addition of the Taq polymerase as was the case in the multiplex PCR, the mastermix was vortexed to mix for a few seconds. On addition of the Taq polymerase, the master mix was inverted several times to mix and dispensed using auto-repeater into PCR tubes at a volume of 48 µl. Finally, 2 µl of each DNA template was added to the corresponding PCR tube. The PCR tubes were agitated and placed in the thermocycler. The following primers as designed by Shopsin *et al*. (1999) were used.

Table 3.2: PCR Primers for *spa* typing of the *S. aureus*

Gene	Primer sequence	Expected amplicon size
spa 2F	GAACAACGTAACGGCTTCATCC	
spa 1514R	CAGCAGTAGTGCCGTTTGCCT	(250-637bp)

The thermocycler was programmed according to the manufactures' instructions. Optimization of the conditions was necessary for maximum yield. Each *spa* PCR program started with an initial heat-activation step at 95 ^{o}C for 10 minutes to activate the Taq DNA polymerase. This was followed by 3-step cycling: denaturation for 30 seconds at a temperature of 95 ^{o}C; annealing for 30 seconds at a temperature of 60^{o}C; and extension step for 1 minute at 72 ^{o}C. The total number of cycles was 30 and the final extension time was 10 minutes at 72 ^{o}C.

Agarose gel was prepared as described in 3.12.2, loaded with the *spa* PCR product and electrophoresed. Following electrophoresis, it was viewed using the transilluminator (Bio-rad Gel Doc XR) and the picture of the bands was taken.

The *spa* products were purified before sequencing. The purification protocol as described by the manufacturer was followed. The Qiagen PCR purification kit (QIAquick) was used. To 1 volume of the *spa* PCR reaction, 5 volumes of buffer PB (binding buffer) were added and mixed. A QIAquick column was placed in a 2 ml collection tube and the PCR DNA was applied to it and centrifuged for 1minute for the DNA to bind to it. The flow-through was discarded and the QIAquick column was placed back in the same tube. To wash the DNA, 750 µl of buffer PE (washing buffer) was added to the QIAquick column and centrifuged for 1 minute. The flow-through was again discarded and the QIAquick was returned back in the same tube. This was centrifuged for another I minute to remove the entire residual wash buffer. After the centrifugation, each QIAquick column was placed in a clean 1.5 ml microcentrifuge tube. To elute the DNA, 50 µl buffer EB (eluting buffer) consisting of 10 mM Tris-Cl with pH of 8.5 was added at the centre of the QIAquick membrane and centrifuged for 1 minute. This was allowed to stand for 1 minute and re-centrifuged. The flow-through was collected for quantification.

The concentration of each PCR sample was determined using a Spectrophotometer (Spectrum-lab 21A, Texas USA). This was to determine the amount of each sample needed in each reaction to include 5 ng/100bp of the amplified product. The amplified *spa* region was ~425 bp in length and so 20 ng of product was required. The sequencing protocol required two wells (one for forward primer and the second for reverse primer) as the DNA core utilizes cycle sequencing. The molecular grade water added to each reaction was determined using the formula: Template + primer A + dH_2O = 11 µl total volume. The dH_2O needed for each reaction was aliquoted into the respective well of 96 well plates. To each well, 1 µl of the appropriate primer was added which were the same with forward and reverse used in the PCR. The amount of DNA determined earlier was then added to the corresponding wells. After loading the wells, a tinfoil lid was placed on the plate and sealed and sent off to the DNA core laboratory where the sequencing was done.

The *spa* types were determined with RidomStaph software, version 1.3 (Ridom GmbH, Wu¨rzburg, Germany) (http://spaserver.ridom.de/). The repeats varied according to strains, forming bases for its usefulness as a molecular tool.

3.12.4 Detection of PVL (*lukS-lukF*) gene present in the *S. aureus* by PCR

The PVL PCR was carried out to detect the toxic gene *lukS-lukF* that is associated with necrosis in *Staphylococcus aureus* infection. The protocol as described by Lina *et al*. (1999) was followed. The master mix was prepared based on the number of DNA templates. The volume of the reagents for each reaction was 48 µl before the 2 µl of DNA templates was added. The master mix was vortexed to mix for a few seconds before the addition of the Taq DNA polymerase. Thereafter, the mastermix was inverted several times to mix and dispensed using auto-repeater into PCR tubes at a volume of 48 µl. Finally, 2 µl of each DNA template was added to the corresponding PCR tube which were agitated and placed in the thermocycler. MRSA, USA300 was used as a positive control. The following primers as designed by Lina *et al*. (1999) were used.

Table 3.3: Primers for PVL (*lukS-lukF*) gene

Gene	Primer sequence	Expected amplicon size
PVL Forward	ATCATTAGGTAAAATGTCTGGACATGATCCA	
PVL Reverse	GCATCAASTGTATTGGATAGCAAAAGC	451bp

The optimization of the PCR conditions was required for maximum yield of the PCR product and involved initial heat activation of the Taq DNA polymerase at 94 ^{o}C for 5 minutes. This was followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94 ^{o}C; annealing for 30 seconds at a temperature of 55 ^{o}C; and extension step for 1 minute at 72 ^{o}C. The total number of cycles was 30 and the final extension time was 5 minutes at 72 ^{o}C.

The amplified DNA product was run on a large gel in which 2.4 grams of agarose was gradually heated to dissolve in 200ml of TAE buffer and 8µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophorectic machine at 120 V for 1.5 hrs. At the end, the bands were viewed using the Bio-rad Gel Doc XR trans-illuminator.

3.12.5: Detection of tetracycline resistance gene (*tetM*) present in the *S. aureus* by PCR

A PCR was carried out to detect a tetracycline resistant gene, *tetM* using a protocol described by Weigel *et al*. (2006). The master mix was prepared based on the number of DNA templates. A master mix was prepared for 32 isolates under study. The master mix was vortexed to mix for a few seconds before the addition of the Taq polymerase. It was then inverted several times to mix and dispensed using auto-repeater into PCR tubes at a volume of 48 µl. Finally, 2 µl of each DNA template was added to the corresponding PCR tube, agitated and placed in the thermocycler.

The following primers by Weigel *et al*. (2006) were used (table 3.4).

Table 3.4: Primers for tetracycline resistance gene (*tetM*)

Gene	Primer sequence	Expected amplicon size
tetM Forward	GAACTCGAACAAGAGGAAAGC	
tetm Reverse	ATGGAAGCCCAG AAAGGAT	740bp

The optimization process was required for maximum yield of the PCR product involving initial heat activation of the Taq DNA polymerase at 95 ^{o}C for 5 minutes followed by 3-step cycling: denaturation for 2 minutes at a temperature of 94 ^{o}C; annealing for 60 seconds at a temperature of 60 ^{o}C; and extension step for 2 minute at 72 ^{o}C. The total number of cycles was 30 and the final extension time was 10 minutes at 72 ^{o}C.

The amplified DNA product was run on a large gel in which 3 grams of agarose was gradually heated to dissolve in 200 ml of TAE buffer and 8µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophorectic machine at 120 V for 1.5 hrs. At the end, the bands were viewed using the Bio-rad Gel Doc XR transilluminator.

3.12.6: Multilocus sequence typing (MLST) of the *S. aureus*.

A multiplex PCR was carried out to detect the seven housekeeping genes: *rc, aroE, glpF, gmk, pta, tpi, and yqiL*. The protocol used was as described by Enright *et al*. (2000). Seven mastermixes were prepared one for each house keeping gene. The final volume of the mastermix was determined by the number of reaction DNA templates. The master mix was votexed to mix before the addition of the Taq polymerase which was inverted severally to mix and aliquoted in volumes of 48 µl into PCR tubes. Finally, the template DNA was added, agitated to mix and then loaded in the thermocycler. The positive control used was MRSA USA300.

Table 3.5: Primers for the Multilocus sequence typing (MLST)

Gene	Primer sequence	Expected amplicon size
rc-Forward	TTG ATT CAC CAG CGC GTA TTG TC	
rc-Reverse	AGG TAT CTG CTT CAA TCA GCG	456bp
AroE-Forward	ATC GGA AAT CCT ATT TCA CAT TC	
AroE-Reverse	GGT GTT GTA TTA ATA ACG ATA TC	456bp
glpF Forward	CTA GGA ACT GCA ATC TTA ATC	
glpF Reverse	TGG TAA AAT CGC ATG TCC AAT TC	465bp
gmk, Forward	ATC GTT TTA TCG GGA CCA TC	
gmk, Reverse	TCA TTA ACT ACA ACG TAA TCG TA	429bp
pta, Forward	GTT AAA ATC GTA TTA CCT GAA GG	
pta, Reverse	GAC CCT TTT GTT GAA AAG CTT AA	474bp
tpi Forward	TCG TTC ATT CTG AAC GTC GTG AA	
tpi Reverse	TTT GCA CCT TCT AAC AAT TGT AC	402bp
yqiL Forward	CAG CAT ACA GGA CAC CTA TTG GC	
yqiL Reverse	CGT TGA GGA ATC GAT ACT GGA AC	516bp

The optimization process involved initial heat activation of the Taq DNA polymerase at 94 ^{o}C for 5 minutes followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94 ^{o}C; annealing for 30 seconds at a temperature of 55^{o}C; and extension step for 60 seconds at 72 ^{o}C. The total number of cycles was 30 and the final extension time was 5 minutes at 72 ^{o}C.

The amplified DNA product for each of the seven housekeeping genes was run on a large gel in which 3 grams of agarose was gradually heated to dissolve in 200 ml of TAE buffer and 8µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophorectic machine at 120 V for 1.5 hrs. At the end, the gel bands were viewed using the Bio-rad Gel Doc XR transilluminator.

The PCR products were purified and quatified as described in 3.12.3 and sent out for sequencing. Results of the sequencing was analysed by the Multi Locus Sequence Typing facility (http://www.mlst.net) (Hallin *et al*., 2007).

3.12.7 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of the MRSA

A multiplex PCR was carried out to detect *mecA* gene for methicillin resistance and to determine the type of staphylococcal cassette chromosome *mec* (SCC*mec*). Primers (forward and reverse) used were for type I to type V SCC*mec* according to a protocol previously described (Ghaznavi-Rad *et al*., 2010).

The mastermix was prepared to include the *mecA* and the SCC*mec* types I-V pimers according to the number of DNA templates. The mastermix was votexed to mix before the addition of the HotStarTaq which on addition was inverted severally to mix and aliquoted in volumes of 48µl into PCR tubes. Finally, the template DNA was added, agitated to mix and then loaded in the thermocycler. The positive control used was MRSA USA300.

Table 3.6: Primers for the Staphylococcal cassette chromosome *mec* (SCC*mec*) typing

Gene	Primer sequence	Expected amplicon size
Type I Forward	GCTTTAAAGAGTGTCGTTACAGG	
Type I Reverse	GTTCTCTCATAGTATGACGTCC	613bp
Type II Forward	GATTACTTCAGAACCAGGTCAT	
Type II Reverse	TAAACTGTGTCACACGATCCAT	287bp
Type III Forward	CATTTGTGAAACACAGTACG	
Type III Reverse	GTTATTGAGACTCCTAAAGC	243bp
Type IVa Forward	GCCTTATTCGAAGAAACCG	
Type IVa Reverse	CTACTCTTCTGAAAAGCGTCG	776bp
Type IVb Forward	AGTACATTTTATCTTTGCGA	
Type IVb Reverse	AGTCATCTTCAATATGGAGAAAGTA	1000bp
Type IVc Forward	TCTATTCAATCGTTCTCGTATT	
Type IVc Reverse	TCGTTGTCATTTAATTCTGAACT	677bp
Type IVd Forward	AATTCACCCGTACCTGAGAA	
Type IVd Reverse	AGAATGTGGTTATAAGATAGCTA	1242bp
Type IVh Forward	TTCCTCGTTTTTTCTGAACG	
Type IVh Reverse	CAAACACTGATATTGTGTCG	663bp
Type V Forward	GAACATTGTTACTTAATGAGCG	
Type V Reverse	TGAAAGTTGTACCCTTGACACC	325bp
mecA Forward	TCCAGATTACAACTTCACCAGG	
mecA Reverse	CCACTTCATATCTTGTAACG	162bp
Sa442 Forward	AATCTTTGTCGGTACACGATATTCTTCACG	
Sa442 Reverse	CGTAATGAGATTTCAGTAGATAATACAACA	108bp

The optimization process involved initial heat activation of the Taq DNA polymerase at 95 ^{o}C for 15 minutes followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94 ^{o}C; annealing for 90 seconds at a temperature of 57^{o}C; and extension step for 90 seconds at 72 ^{o}C. The total number of cycles was 30 and the final extension time was 10 minutes at 72 ^{o}C.

The amplified DNA product was run on a large gel in which 3 grams of agarose was gradually heated to dissolve in 200 ml of TAE buffer and 8 µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophorectic machine at 120 V for 1.5 hrs. At the end, the bands were viewed using the Bio-rad Gel DocXR trans-illuminator.

CHAPTER FOUR

4.0 RESULT

4.1 Sample Population

Two hundred birds (100 broilers and 100 layers) selected at random from each of the 9 farms in this study were screened for *Staphylococcus aureus* giving a total of 1800 birds. Two samples (nostril and cloacae swabs) were collected from each of the birds screened, giving a total of 200 samples for broilers and 200 samples for layers and an overall sample size of 3600 in the 9 farms as shown in table 4.1.

Table 4.1: Summary of the Number of Farms and Birds Screened in Ebonyi State.

	Broilers: N-900		Layers: N-900		
Source	Cloacae	Nostril	Cloacae	Nostril	Total no. of samples
Farm 1	100	100	100	100	400
Farm 2	100	100	100	100	400
Farm 3	100	100	100	100	400
Farm 4	100	100	100	100	400
Farm 5	100	100	100	100	400
Farm 6	100	100	100	100	400
Farm 7	100	100	100	100	400
Farm 8	100	100	100	100	400
Farm 9	100	100	100	100	400
Total	900	900	900	900	3600

Key: N= no. of birds

4.2: Isolation and identification of *S. aureus* from the poultry farms

Staphylococcus aureus was isolated from 9 farms (Appendix 3). The number recovered varied from farm to farm and between broilers and layers (Table 4.2). In 4 farms (farms 1, 5, 7 and 8) more *S. aureus* were recovered from broilers while in 5 farms (farms 2, 3, 4, 6 and 9) more *S. aureus* were recovered from layers. A total of 125 *S. aureus* were recovered from layers while 122 *S. aureus* were recovered from broilers.

Out of the 1800 birds screened in the 9 farms, 247 of the birds had *S. aureus* constituting 14% (247/1800) of the total number of birds (Figure 4.1).

Table 4.2: *Staphylococcus aureus* recovered from Broilers and Layers in the Poultry Farms.

Source	**Number of isolates**	
	Broilers	Layers
Farm 1	19	14
Farm 2	13	18
Farm 3	11	19
Farm 4	11	12
Farm 5	17	8
Farm 6	10	13
Farm 7	19	16
Farm 8	12	10
Farm 9	10	15
Total	122	125

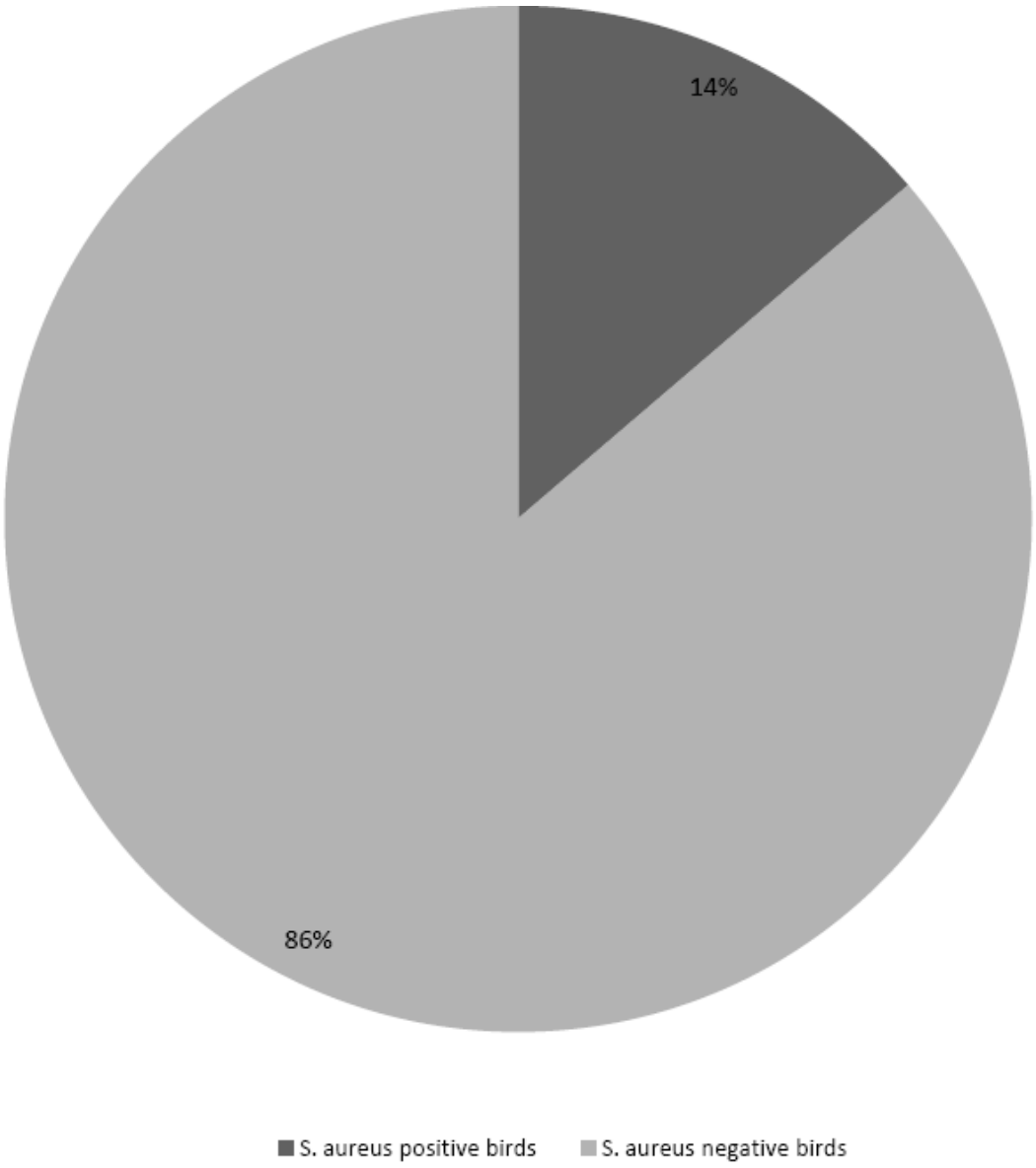


Figure 4.1: Percentage of birds positive for *S. aureus*

4.3: Recovery of *Staphylococcus aureus* in Nostrils and Cloacae of birds

Staphylococcus aureus was isolated from all the farms. Both nostrils and cloacae screened yielded growth of *Staphylococcus aureus* but the frequency of isolation varied from farm to farm. Positive sample was taken as any yield of *S. aureus* in any or both of the sites from one bird (cloaca and nostril).

Farm to farm analysis show that more *S. aureus* were recovered when both cloacae and nostrils were screened compared to cloacae and nostril screened singly (Table 4.3).

Percentage recovery of *S. aureus* as shown in figure 4.2 was cloacae and nostrils 52% (129), cloacae 27% (67) and nostrils 21% (51).

Table 4.3: Recovery of *S. aureus* from cloacae and nostrils, cloacae, nostrils

Source	Birds with *S. aureus* in cloacae & nostril (%)	Birds with *S. aureus* in cloacae (%)	Birds with *S. aureus* in nostrils (%)
farm 1	17 (50.5%)	10 (30.4%)	6 (18.2%)
farm 2	14 (45.2%)	9 (29%)	8 (25.8%)
farm 3	14 (46.6%)	8 (26.7%)	8 (26.7%)
farm 4	16 (69.6%)	5 (21.7%)	2 (8.7%)
farm 5	14 (56%)	8 (32%)	3 (12%)
farm 6	11 (48%0	7 (30%)	5 (22%)
farm 7	22 (63%)	8 (23%)	5 (14%)
farm 8	10 (45.4%)	6 (27.3%)	6 (27.3%)
farm 9	11 (44%)	6 (24%)	8 (32%)
Total	**129**	**67**	**51**

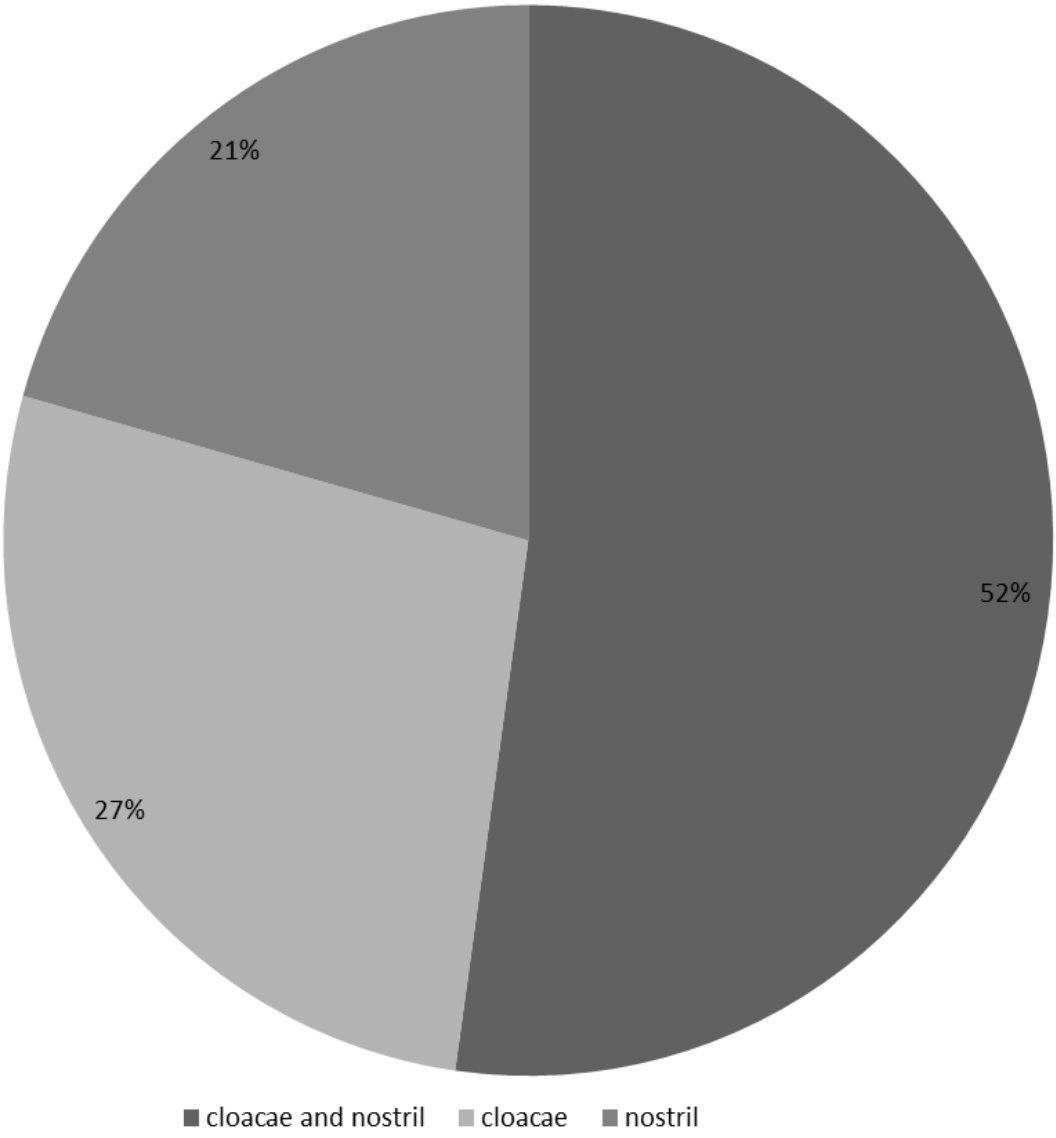


Figure 4.2: Percentage of birds with *S. aureus* recovered from both cloacae and nostrils, cloacae only and nostrils only.

4.4: Isolation of *S. aureus* from Poultry Farm Workers

Volunteer Poultry farm workers were swabbed after informed consent was obtained from each of them. The number of workers from whom *S. aureus* was recovered from their nasal swabs in the poultry farms was 4, (one worker from each farm) (Table 4.4)**.** The percentage recovery of *S. aureus* from these workers was shown in figure 4.3. The percentage of poultry farm workers that harbored *S. aureus* in the 9 farms was 21% (n=19).

Table 4.4: Isolation of *Staphylococcus aureus* from Poultry Farm Workers

Source	**Nasal swab of workers**	***S. aureus***
farm 1	2	1
farm 2	3	0
farm 3	2	1
farm 4	3	0
farm 5	1	0
farm 6	2	0
farm 7	1	0
farm 8	2	1
farm 9	3	1
Total	**19**	**4**

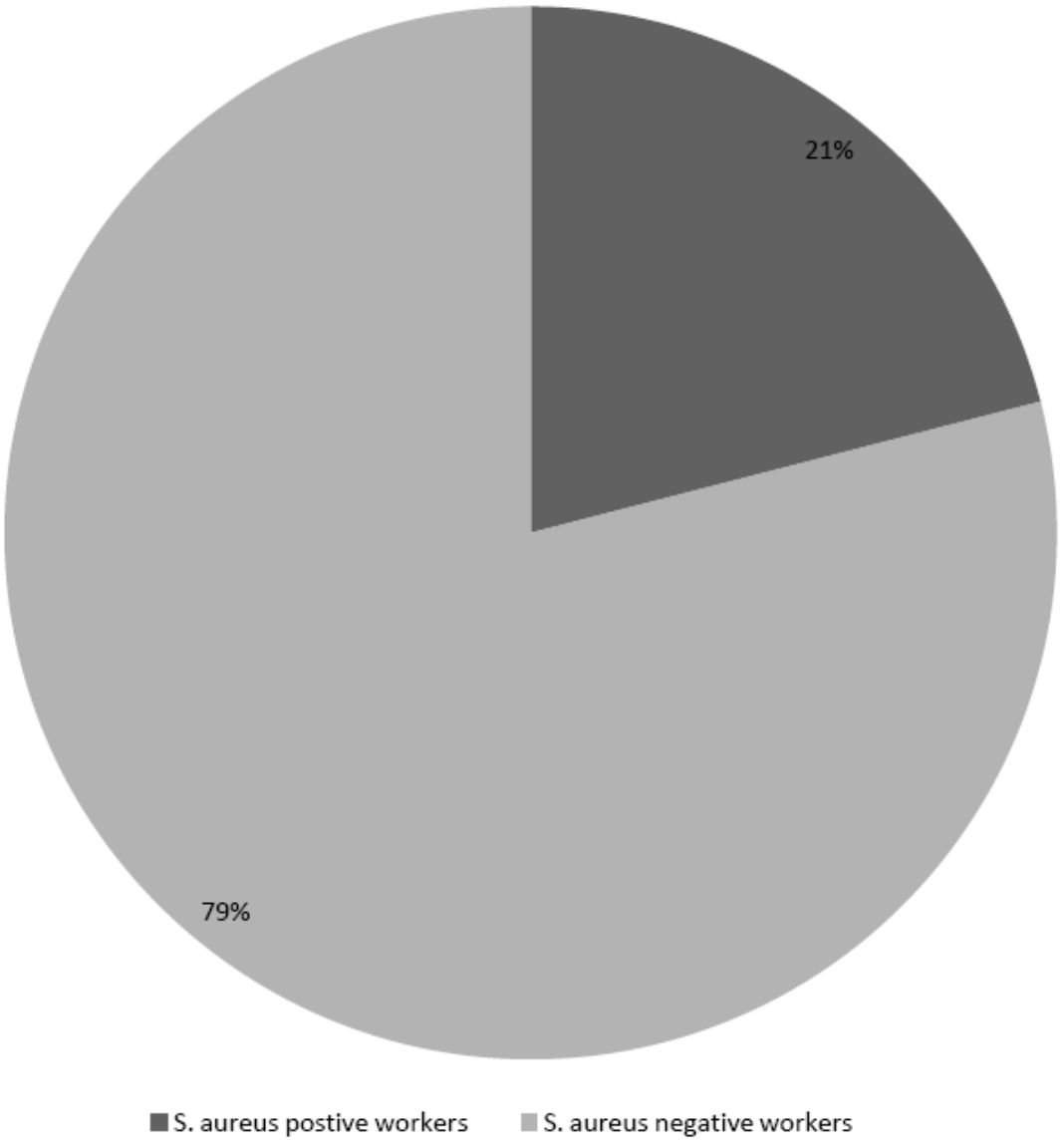


Figure 4.3: Percentage carriage of *S. aureus* amongst poultry farm workers.

4.5: Clinical Isolates of *S. aureus*

Clinical isolates of staphylococci were obtained from 3 hospitals in Ebonyi State. The hospitals were Federal Teaching hospital Abakaliki, Onueke General Hospital and Afikpo Medical Center. In each of these hospitals, 50 isolates of staphylococci were screened for *S. aureus*. A total of 150 clinical isolates were screened and 78 isolates were found to be *S. aureus*. The hospital by hospital percentage incidence showed that Afikpo Medical Center had the highest incidence of 62%. The percentage incidence in Federal Teaching Hospital Abakaliki was 56% while Onueke General Hospital had 38% (Table 4.5).

The percentage distribution of *S. aureus* from the three hospitals showed that 40% of them were from Afikpo Medical Center and 36 % from Federal Teaching Hospital. Onueke General Hospital had 24% (Figure 4.4).

The total number of *S. aureus* recovered from the three sources (poultry. Poultry farm workers and hospitals) were 329 (Table 4.6). .

Table 4.5: Clinical isolates of *S. aureus* in 3 selected hospitals

Hospital	**Staphylococci**	***S. aureus***
Fed. Teaching Hospital Abakaliki	50	28 (56%)
Onueke General Hospital	50	19 (38%)
Afikpo Medical Center	50	31 (62%)
Total	150	78 (52%)

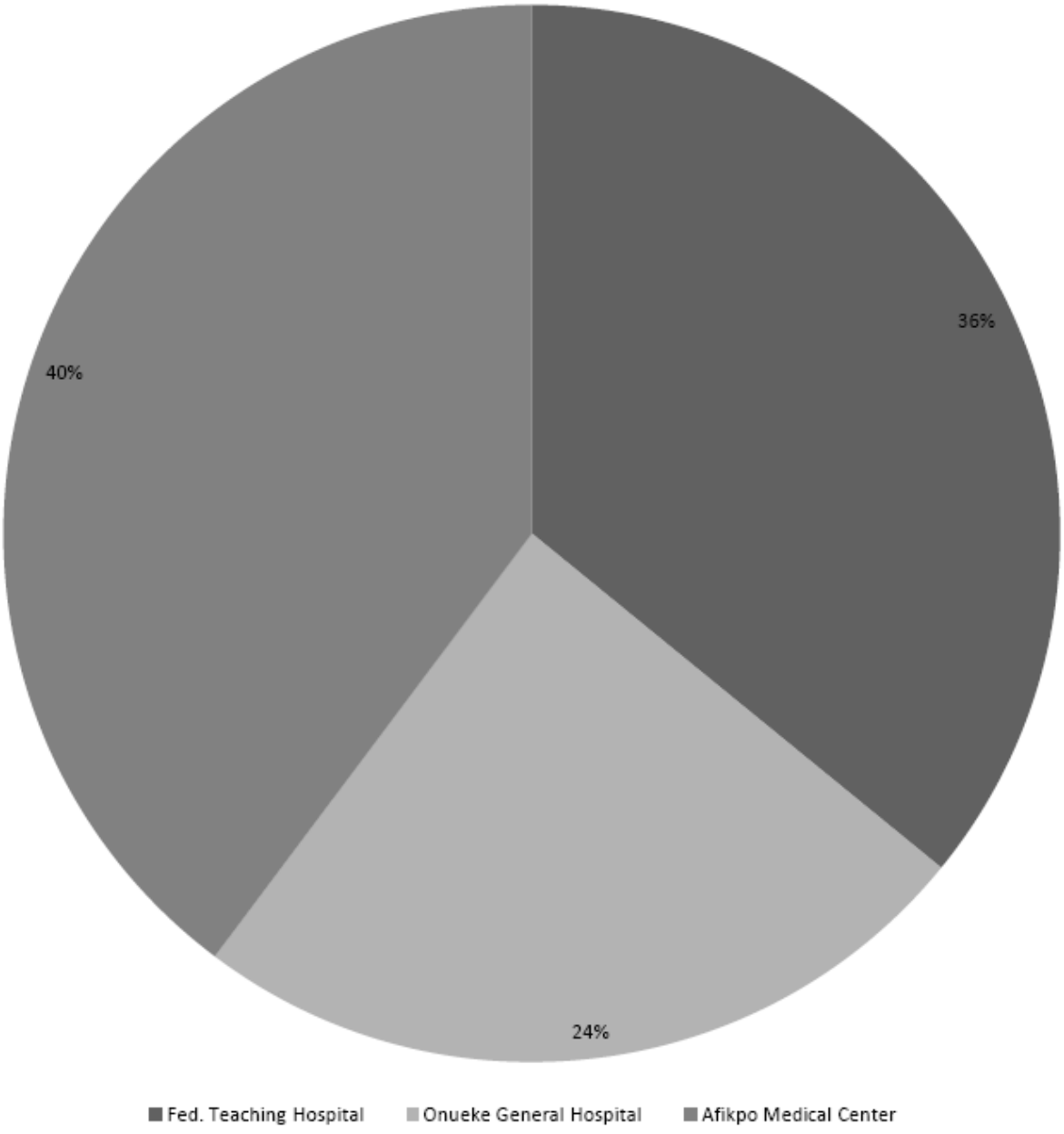


Figure 4.4: Percentage of *S. aureus* from the 3 hospitals

Table 4.6: Summary of *Staphylococcus aurous* recovered from Poultry, Poultry farm workers and Hospitals.

Source	*S. aureus*
Poultry	247
poultry workers	4
Hospital	78
Total	329

4.6: Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing (AST) was carried out to obtain the antibiotic susceptibility profiles of *Staphylococcus aureus* isolates from the poultry farms, poultry farm workers and hospitals screened (Appendix 3). The *Staphylococcus aureus* were tested against a panel of 14 antibiotics which cut across 11 categories with varying strengths (Appendix 7).

The number of isolates resistant to each of the antibiotics is shown in table 4.7. Out of the 329 isolates of *S. aureus* recovered, only 28 (8.5%) of them were resistant to cefoxitin. There was no resistant isolate to Quinupristin-dalfopristin, Linezolid, Rifampicin and Vancomycin. The highest percentage resistance of 47.7% (157/329) was recorded against Tetracycline followed by Trimethoprim/sulfamethoxazole with 42.6% (140/329). Resistance to Chloramphenicol and Erythromycin was 19.1% and 18.2% respectively.

The number of *S. aureus* from the 3 sources (poultry, hospital and poultry workers) that were resistant to each of the antibiotics tested was compared as shown in table 4.8. Isolates from the clinics showed higher percentage of resistance to each of the antibiotics where resistance was observed compared to poultry isolates (Figure 4.5).

There were more MRSA in broilers (n=11) than in layers (n=4). In farms 4, 8 and 9, no MRSA was detected amongst broilers while only 3 farms (farms 1, 6 and 7) had MRSA in layers. Carriage of MRSA in broilers was 9% (11/122) while in layers it was 3.2% (4/125). The prevalence of MRSA in broilers and layers is shown in table 4.9. Percentage carriage of MRSA in poultry was 6% (Figure 4.6). MRSA from broilers constituted 73% of the total MRSA in poultry (Figure 4.7).

The percentage carriage of MRSA in the hospitals was 15% (11/78) (Figure 4.8). All the hospitals harbored MRSA and this varied from hospital to hospital. Afikpo Medical Center had a total of 31 *S. aureus* out of which 19.4% (6) were MRSA.

MRSA from Afikpo Medical Center constituted 50% (6/12) of all MRSA isolates from the 3 hospitals (Figure 4.9). This was followed by Federal Teaching Hospital Abakaliki with MRSA prevalence of 33% (4/12) and Onueke General Hospital with 17% (2/12). Hospital to hospital MRSA composition is shown in table 4.10.

Table 4.7: Antibiotic resistance profiles of the *S. aureus* isolates

Antibiotic	Strength	No. of *S. aureus* (%)
Augmentin®	30µg	11 (3.3%)
Cefoxitin	30µg	28 (8.5%)
Cefuroxime	30µg	22 (6.7%)
Chloramphenicol	30µg	63 (19.1%)
Doxcycline	30µg	26 (7.9%)
Erythromycin	15µg	60 (18.2%)
Gentamycin	10µg	19 (5.8%)
Levofloxacin	5µg	9 (2.7%)
Linezolid	30µg	0 (0%)
Quinupristin-dalfopristin	15µg	0 (0%)
Rifampicin	5µg	0 (0%)
Tetracycline	30µg	157 (47.7%)
Trimethoprim-sulfamethoxazole	1.25/23.75µg	140 (42.6%)
Vancomycin	30µg	0 (0%)

Table 4.8: Comparison of antibiotic resistance of *S. aureus* from Poultry, Clinics and Farm workers.

Antibiotic	Poultry (%) isolates	Clinical isolates (%)	Poultry workers (%)
Augmentin®	1(0.4%)	9(11.5%)	1 (25%)
Cefoxitin	15(6.1%)	12(15.3%)	1 (25%)
Cefuroxime	13(5.3%)	9(11.5%)	0 (0%)
Chloramphenicol	30 (12.1%)	32((41.0%)	1 (25%)
Doxycycline	19(7.7%)	7(9.0%)	0 (0%)
Erythromycin	48(19.4%)	12(16.7%)	0 (0%)
Gentamycin	13(5.2%)	5(6.4%)	1 (25%)
Levofloxacin	2(0.8%)	6(7.7%)	1 (25%)
Linezolid	0(0%)	0(0%)	0(0%)
Quinuspristin/dalfopristin	0(0%)	0(0%)	0(0%)
Rifampicin	0(0%)	0(0%)	0(0%)
Tetracycline	113(45.0%)	42(53.8%)	2 (50%)
Trimethoprim/sulfamethoxazole	101(40.9%)	38(48.7%)	1 (25%)
Vancomycin	0(0%)	0(0%)	0(0%)

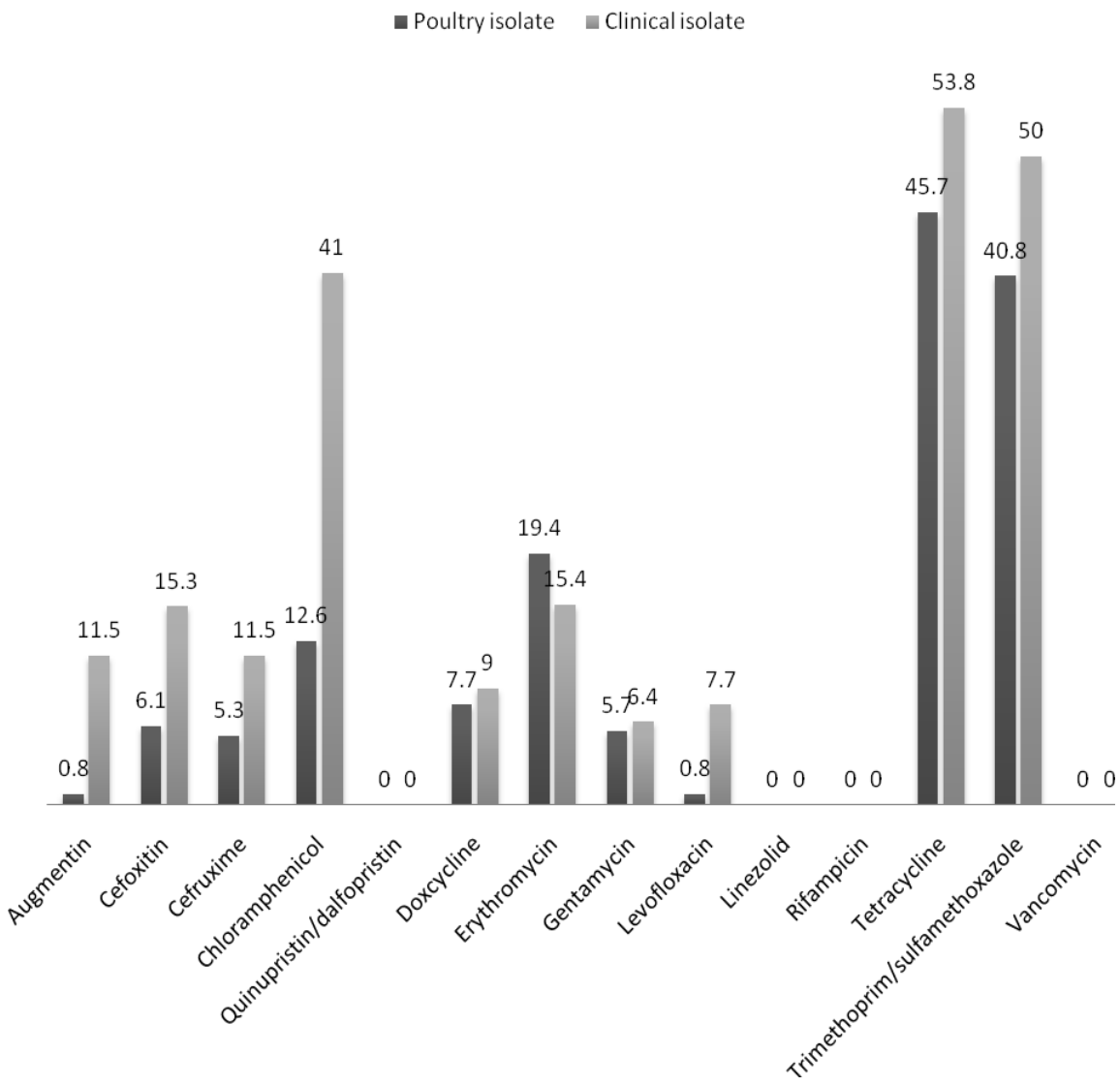


Figure 4.5: Comparison of percentage antibiotic resistance of poultry and clinical isolates.

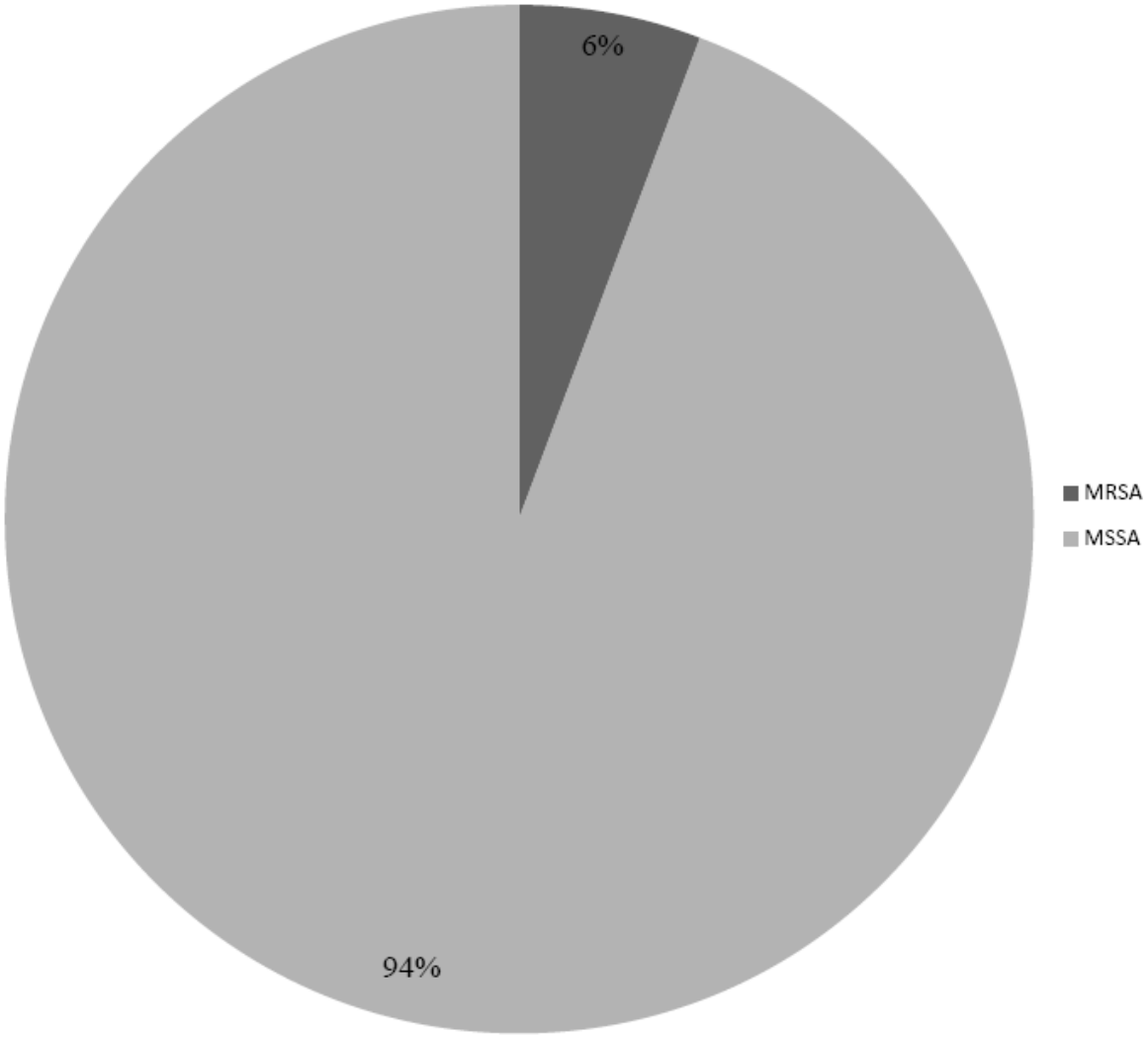


Figure 4.6: Percentage prevalence of MRSA in poultry

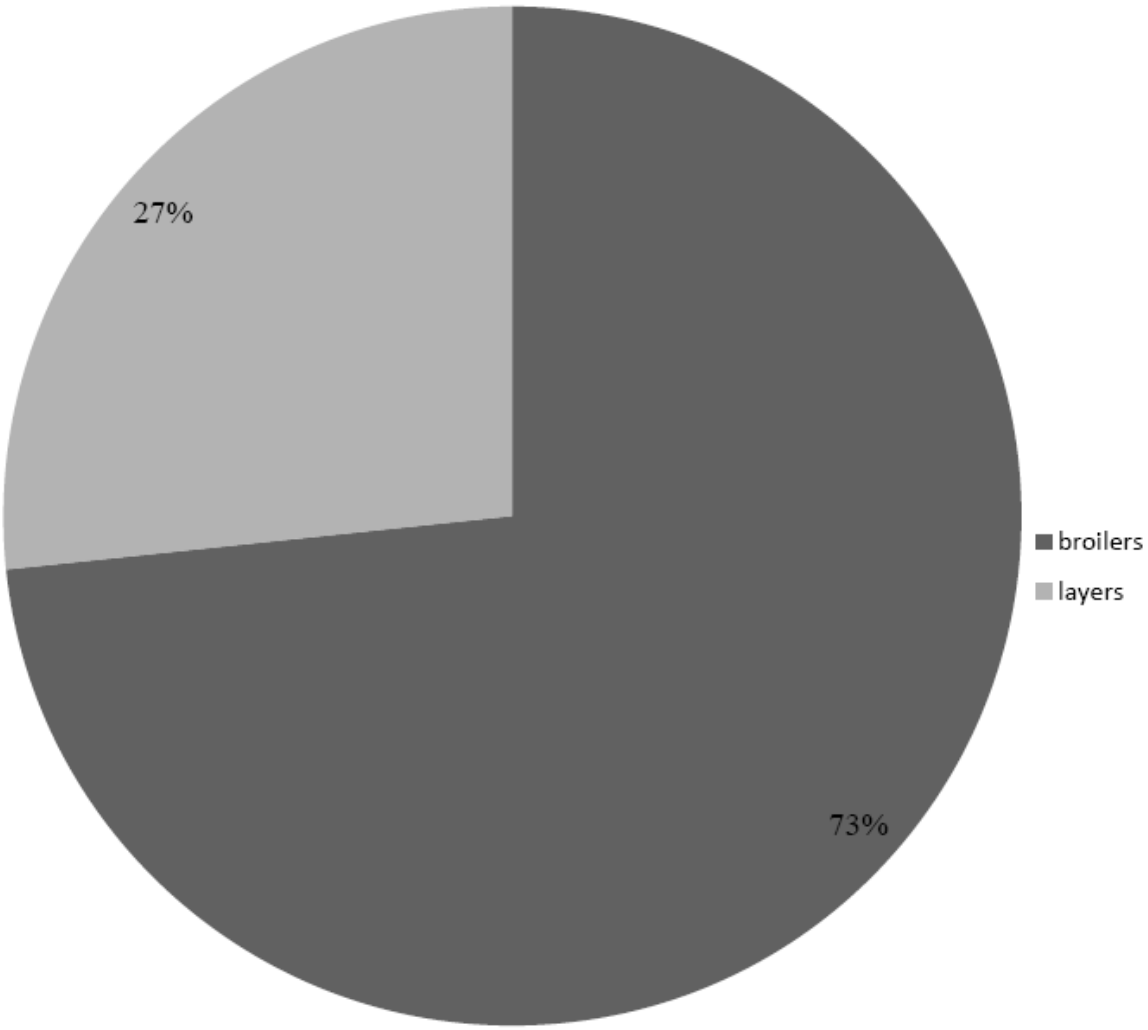


Figure 4.7: Percentage composition of MRSA from broilers and layers.

Table 4.9: Prevalence of MRSA in Broilers and Layers in each of the Farms.

Source	Broilers		Layers	
	MSSA	MRSA	MSSA	MRSA
farm 1	15 (78.9%)	4 (21.1%)	13 (92.6%)	1 (7.4%)
farm 2	12 (92.3%)	1 (7.7%)	18 (100%)	0 (0%)
farm 3	10 (90.9%)	1 (9.9%)	19 (100%)	0 (0%)
farm 4	11 (100%)	0 (0%)	12 (100%)	0 (0%)
farm 5	15 (88.2%)	2 (11.8%)	8 (100%)	0 (0%)
farm 6	8 (80%)	2 (20%)	11 (84.6%)	2 (15.45)
farm 7	18 (94.7%)	1 (5.3%)	15 (93.8%)	1 (6.2%)
farm 8	12 (100%)	0 (0%)	10 (100%)	0 (0%)
farm 9	10 (100%	0 (0%)	15 (100%)	0 (0%)

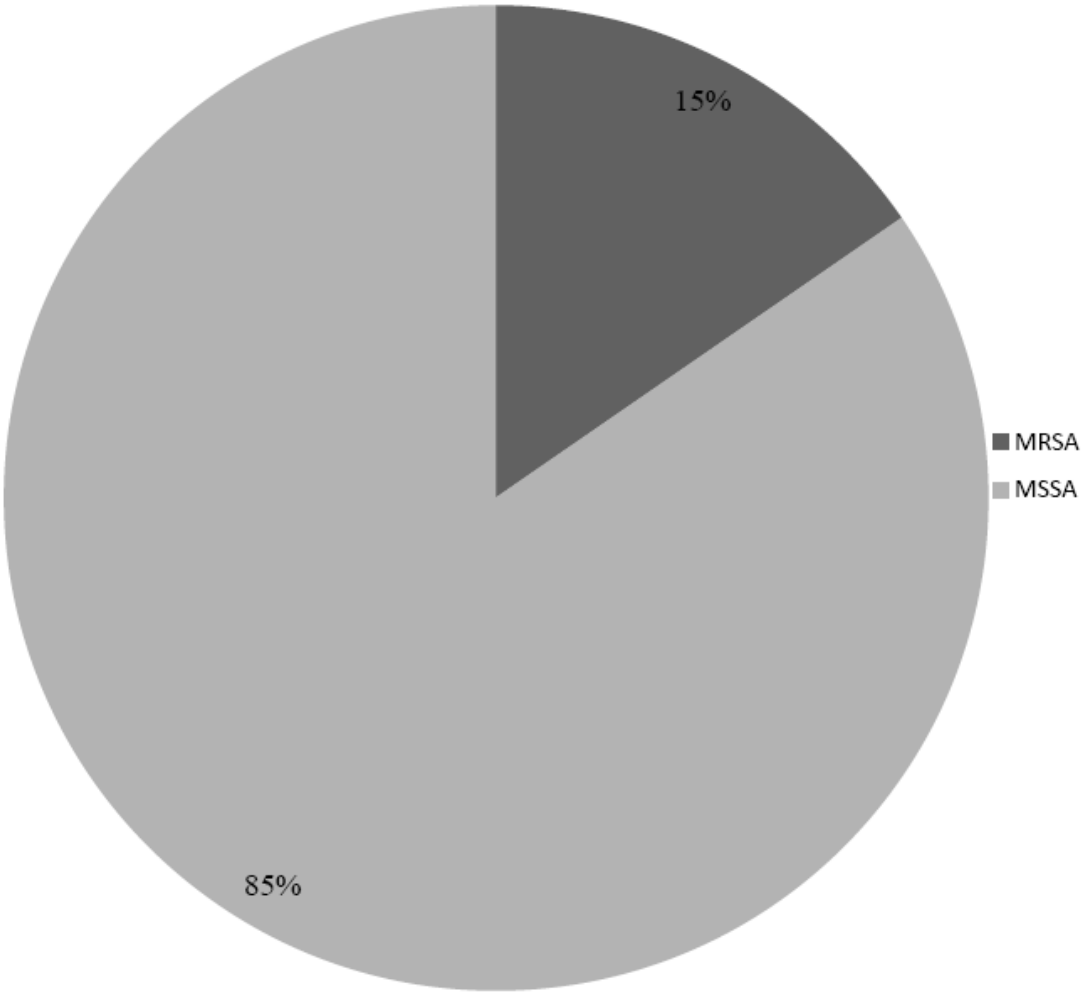


Figure 4.8: Percentage of MRSA amongst hospital isolates.

Table 4.10: Prevalence of MRSA amongst *S. aureus* in each of the 3 hospitals screened.

Hospital	*S. aureus*	
	MSSA	MRSA
Fed. Teaching Hospital	24 (85.7%)	4 (14.3%)
Onueke General Hospital	17 (89.5%)	2 (10.5%)
Afikpo Medical Centre	25 (80.6%)	6 (19.4%)

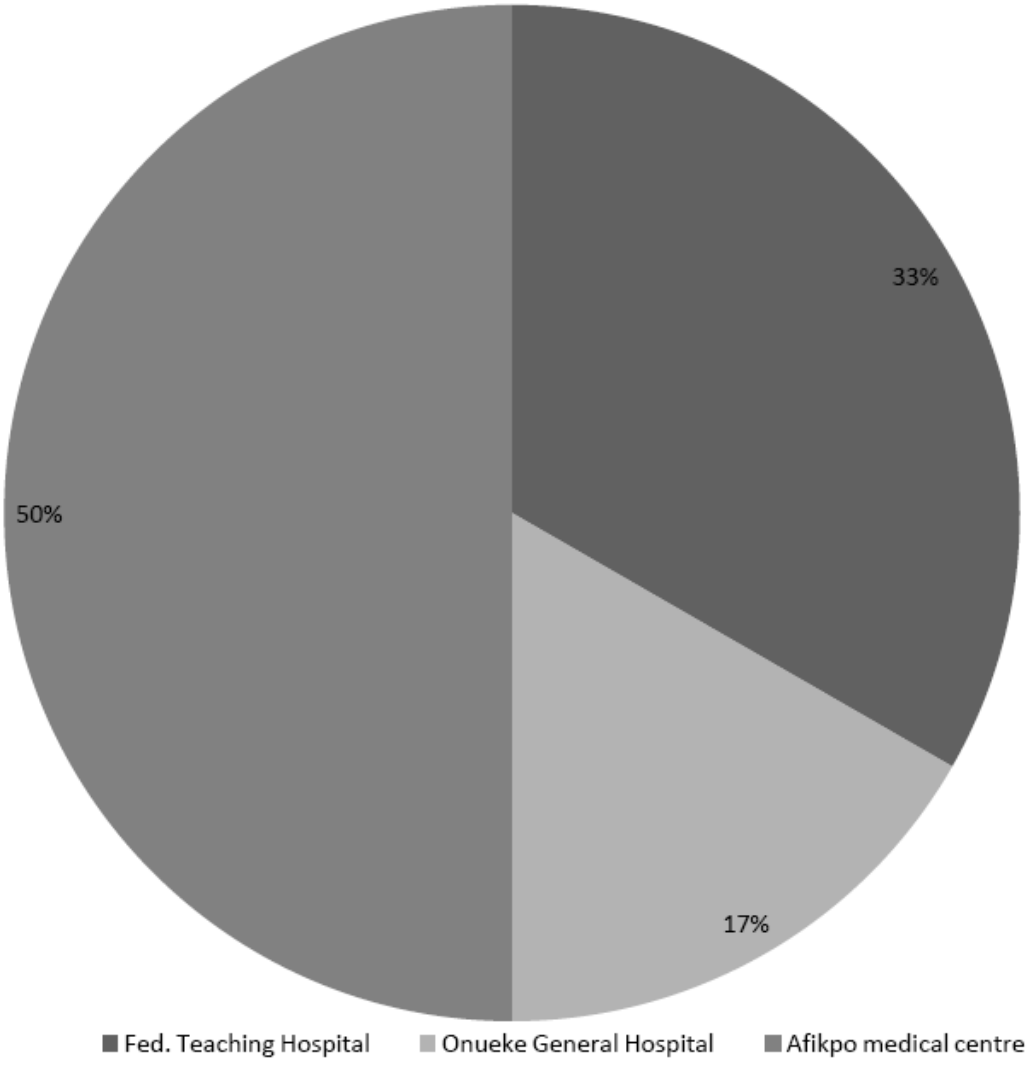


Figure 4.9: Percentage distribution of MRSA amongst the 3 hospitals

4.7 MULTIDRUG RESISTANCE

Multidrug resistance (MDR) of all the 329 isolates of *S. aureus* was determined. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. The multi antibiotic resistance index (MARI) which is the number of antibiotics an isolate was resistant to, divided by the total number of antibiotics used was used to determine the multi-antibiotic resistance index of all the isolates (table 4.11). The multidrug resistance pattern is as shown in (table 4.12).

Table 4.11: Antibiotic resistance index of *S. aureus*

MAR Index	Resistant isolates	%MARI	
0	109	33.13	
0.1	128	38.90	
0.2	46	13.98	
0.3	29	8.81	13.97% (Multidrug Resistant)
0.4	17	5.16	

Table 4.12: Multidrug Resistance (MDR) Pattern of the isolates with MARI of 0.3 and above.

Antibiotics Resistances pattern	Number of Isolate	Percentage (%)
Aug, Cef, Levo, Tetra	1	2.2
Aug, Cef, Cefx, Tetra	1	2.2
Aug, Cef, ,Chl, Trim/Sulf	1	2.2
Chl, Levo, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Genta, Tetra	1	2.2
Chl, Ery, Tetra, Trim/Sulf	7	15.2
Doxy, Genta, Tetra, Trim/Sulf	2	4.0
Cefx, Chl, Genta, Tetra	1	2.2
Cefx, Chl, Doxy, Trim/Sulf	1	2.2
Cefx, Chl, Tetra, Trim/Sulf	1	2.2
Doxy, Ery, Tetra, Trim/Sulf	3	6.5
Cef, Chl, Tetra, Trim/Sulf	3	6.5
Cef, Cefx, Genta, Trim/Sulf	1	2.2
Ery, Genta, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Tetra, Trim/Sulf	1	2.2
Chl, Genta, Tetra, Trim/Sulf	1	2.2
Chl, Doxy, Ery, Trim/Sulf	1	2.2
Cef, Doxy, Genta, Levo	1	2.2
Aug, Cef, Ery, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Chl, Ery, Tetra	1	2.2
Chl, Ery, Levo, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Chl, Tetra, Trim/Sulf	1	2.2
Cefx, Chl, Doxy, Tetra, Trim/Sulf	2	4.0
Cef, Cefx, Doxy, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Genta, Tetra	1	2.2
Cefx, Ery, Genta, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Ery, Genta, Trim/Sulf	1	2.2
Cef, Doxy, Genta, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Chl, Genta, Tetra	1	2.2
Aug, Cef, Ery, Levo, Tetra, Trim/Sulf	1	2.2
Cef, Cefx, Chl, Genta, Tetra, Trim/Sulf	1	2.2
Aug, Cef, Cefx, Doxy, Levo, Tetra	1	2.2
Aug, Cef, Chl, Ery, Tetra, Trim/Sulf	1	2.2
Total	46	100

The percentage occurrence of these patterns ranged from 15.2% > 6.5% > 4.3% > 2.1%

Key: Cef-Cefoxitin, Cefx-Cefuroxime, Chl-Chloramphenicol, Doxy- Doxycycline, Ery-Erythromycin, Genta-Gentamycin, Levo-Levofloxacin, Tetra-Tetracycline, Trim/sul-Trimethoprim/sufamethoxazole.

4.8 MOLECULAR ANALYSIS OF THE ISOLATES

Out of 70 isolates shipped from the laboratory at the Department of Medical Laboratory Science, Ebonyi State University Abakaliki, Nigeria to the Center for Emerging Infectious Disease (CEID), Department of Epidemiology, College of Public Health, University of Iowa, United States of America 54 (77%) were recovered.

Several molecular techniques were used to analyse some of the samples and results obtained in each technique were as presented below.

4.8.1 Detection of *S. aureus* 16SrRNA, *nuc* and *mecA* gene

Multiplex PCR was carried out to detect *mecA* gene in cefoxitin resistant isolates from poultry, poultry farm workers and clinics. *mecA* is a determinant of methicillin resistance; *nuc* encodes the *S. aureus*-specific region of the thermonuclease gene; and 16S rRNA is a genus-specific sequence used as an internal amplification control for staphylococcal DNA.

All the isolates that were cefoxitin resistant carried the *mecA* gene (figure 4.10).

The 16S rRNA, the *nuc* and *mecA* gene were amplified and detected. MRSA USA300 was used as positive control.

Isolates in lane 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 were clinical isolates. Isolates in lanes 13, 14, 15, 16 and 18 were poultry isolates while isolate in lane 17 was a nasal isolate from a poultry farm worker.

The multiplex PCR showed the amplification of a 216.9 bp *nuc* gene, 821.94 bp 16S rRNA and 518.7 bp *mecA* gene in both the positive control (USA300) and all the test isolates (figure 4.11).

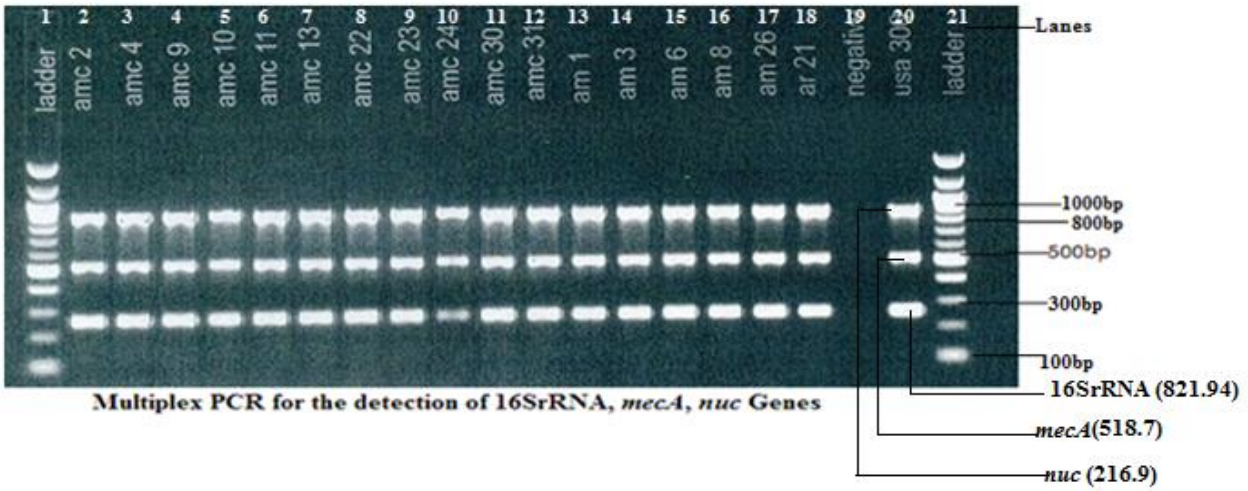


Figure 4.10: Multiplex PCR amplification of *S. aureus* 16SrRNA, *nuc* and *mecA* gene

Lane 1: Ladder

Lane 2-12: Hospital MRSA isolates

Lane 13-16: Poultry MRSA isolates

Lane 18: Poultry MRSA isolate

Lane 17: MRSA isolate from Poultry farm worker

Lane 19: Negative control (SDW)

Lane 20: Positive control (MRSA, USA300)

4.7.2: *S. aureus spa* Typing

All the 54 isolates taken for molecular analysis were sequenced for *spa* types. The *spa* PCR amplification of the isolates was as shown in figure 4.11 and figure 4.12.

In figure 4.11, lanes 1 and 21 were the ladder while lanes 2-18 were the various MRSA isolates. Mastermix with sterile distilled water (SDW) was used as negative control while MRSA USA300 was used as positive control. The amplified *spa* sequence of the positive control, MRSA USA300 was 476.986 bp while the test isolates had the same *spa* sequence of 412.368 bp

In figure 4.12, the ladder was in lane 1 and 30 while lanes 2 – 27 were various isolates of *S. aureus.* Lane 28 was the negative control and lane 29 was the positive control. The *spa* PCR showed amplification of a 505.5 bp of the positive control MRSA USA300. There was variation in the base pairs amplified. *Spa* sequence varies amongst isolates depending on the repeats which ranges from 250 bp to 637 bp. Test isolates in lanes 4, 26 and 27 had *spa* sequence of 416.55 bp while isolate in lanes 3, 5-9 and 11-25 had a *spa* sequence of 535.15 bp. Lane 10 isolate had a *spa* sequence of 297.95 bp.

The sequencing result showed that the isolates fell into 4 *spa* types; t002, t084, t2304 and t11469 (table 4.13).

The percentage of each *spa* type was as presented in figure 4.13. *spa* type (t002) had the highest percentage (48%) followed by *spa* type (t084) with 24% and t11469 with 15%. The least was *spa* type (t2304) with percentage of 13%.

The percentage distribution of these *spa* types amongst poultry, poultry farm workers and hospitals was as shown in figure 4.14.

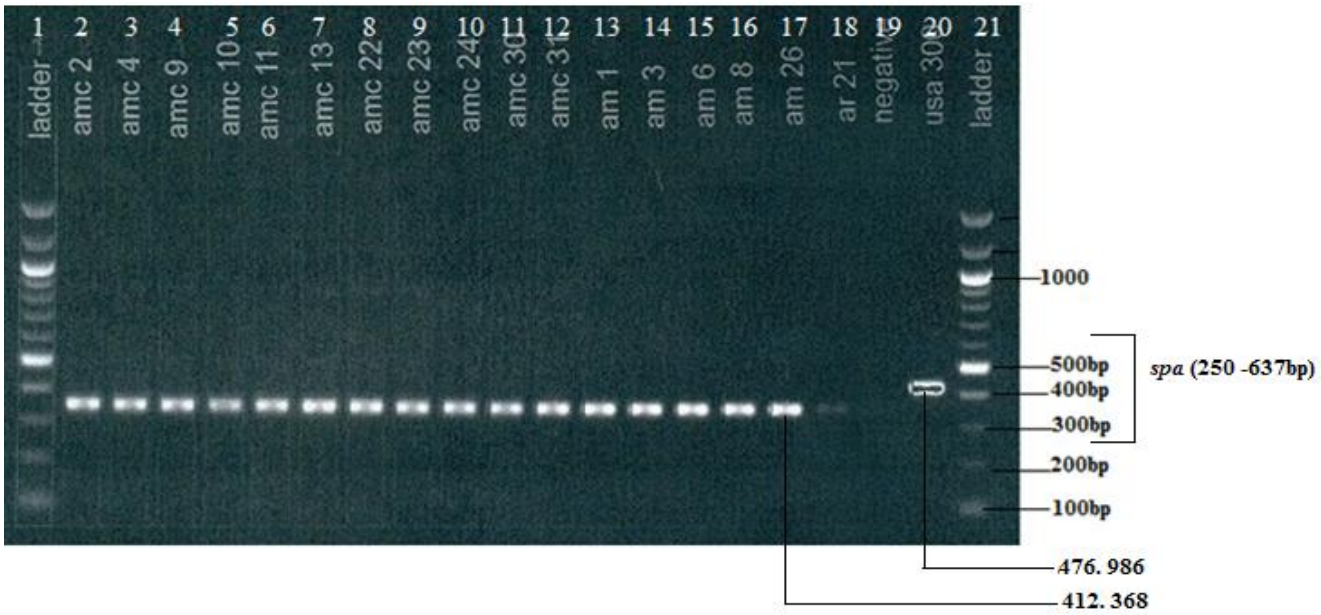


Figure 4.11: *spa* PCR of 17 MRSA isolates showing the amplified base pairs

Lane 1: Ladder

Lane 2-12: Hospital MRSA isolates

Lane 13-16: Poultry MRSA isolates

Lane 18: Poultry MRSA isolate

Lane 17: Poultry farm workers MRSA isolate

Lane 20: Positive control (USA 300)

Lane 19: Negative control (SDW)

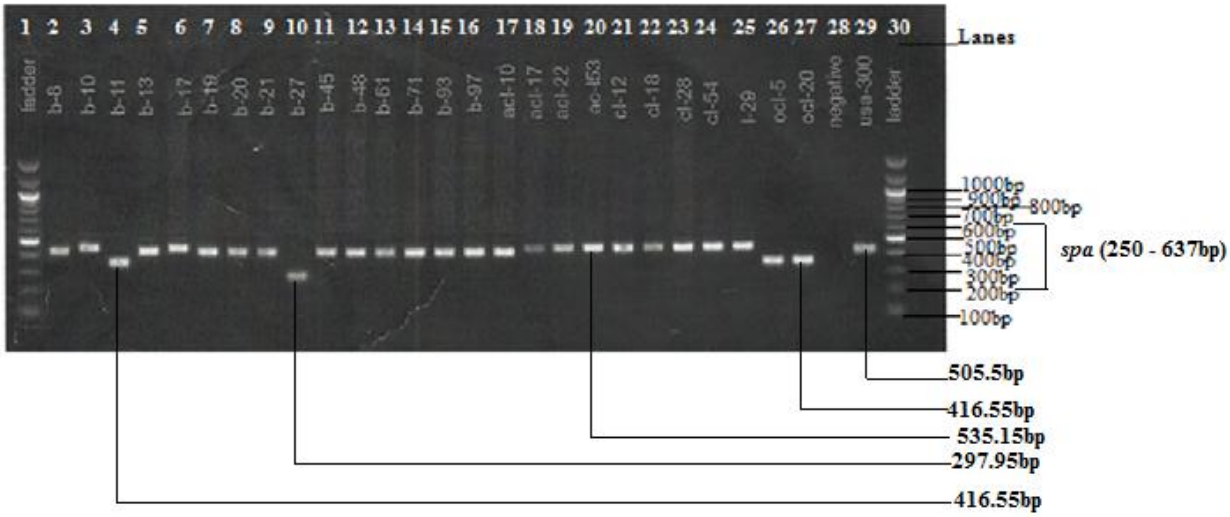


Figure 4.12: The *spa* PCR of 26 *S. aureus* isolates

Lane 1: ladder

Lane 2-3: poultry isolate of *S. aureus*

Lane 4: poultry MRSA isolate

Lane 5-25: poultry isolate of *S. aureus*

Lane 26: poultry MRSA isolate

Lane: 27-28: poultry *S. aureus* isolate

Lane 28: negative control (SDW)

Lane 29: positive control (MRSA300)

Lane 30: ladder.

Table 4.13: *spa* types identified amongst *S. aureus* isolates from Poultry, Hospitals and Poultry Farm workers.

***spa* type**	**Number**	**(%)**
t002	22	(48%)
t084	11	(24%)
t2304	6	(13%)
t11469	7	(15%)
Total	**46**	

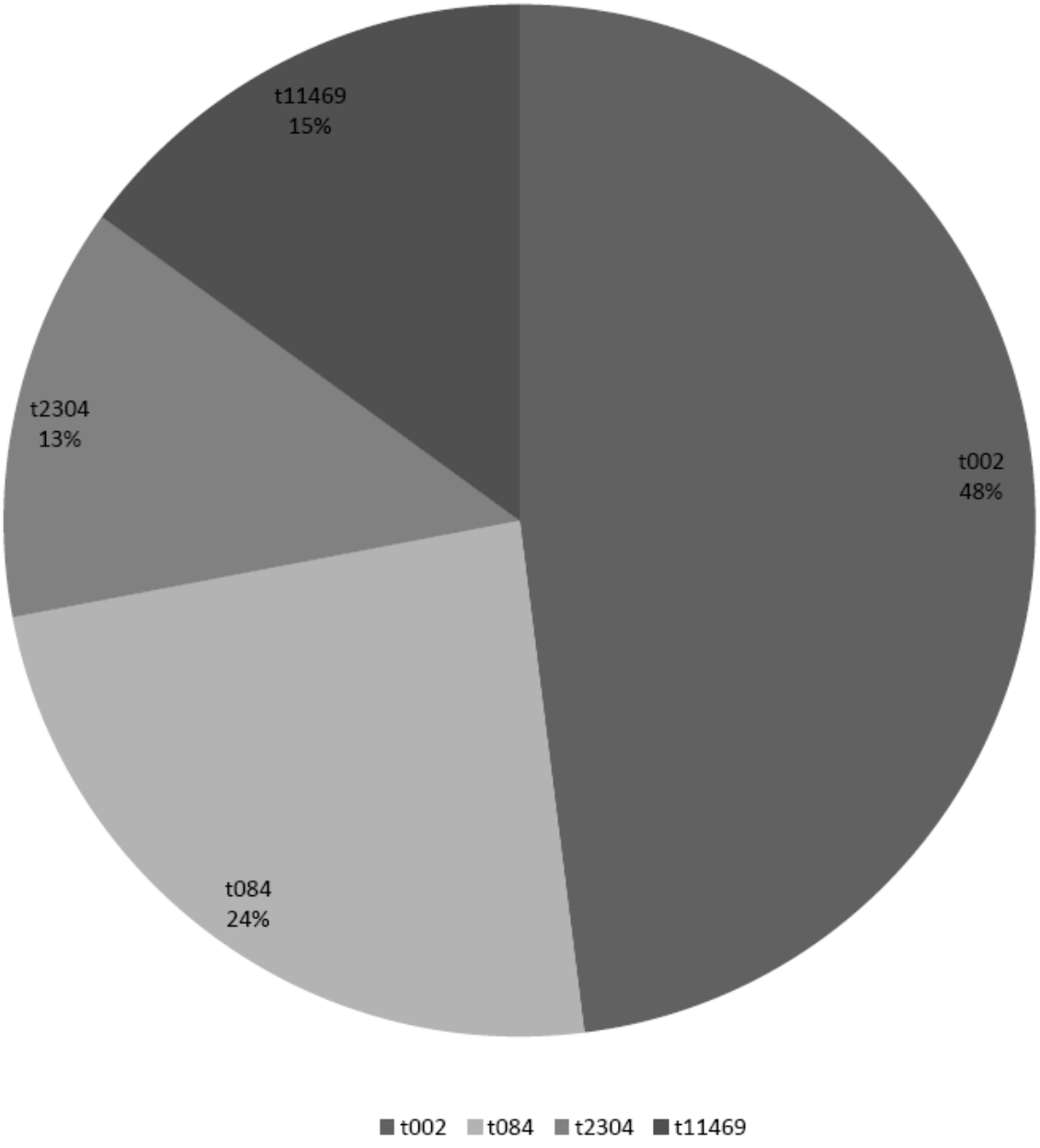


Figure 4.13: Percentage of each *spa* type of *S. aureus*.

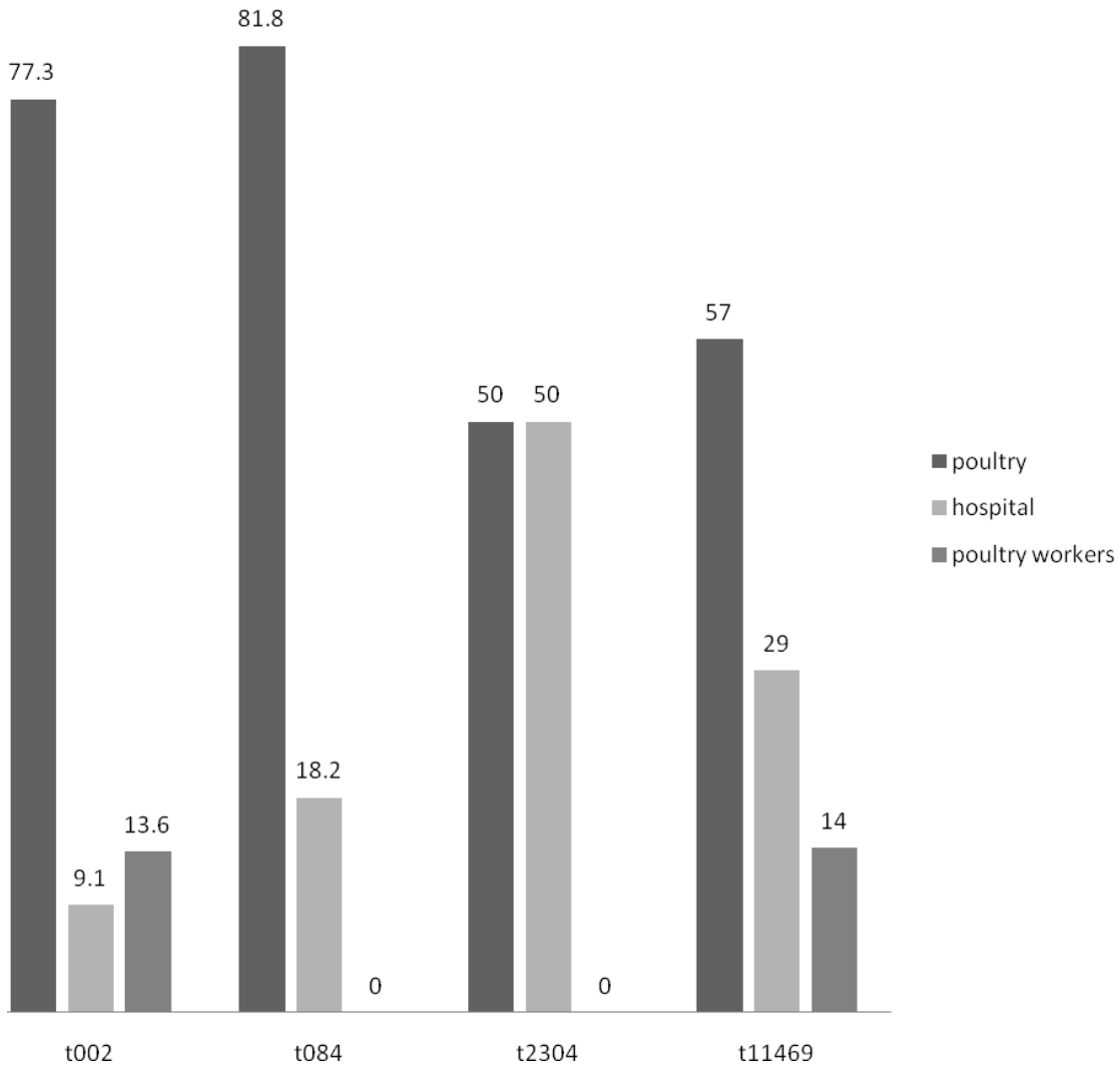


Figure 4.14: Percentage distribution of the *spa* types of *S. aureus* in poultry, hospitals and poultry farm workers

4.7.3: Detection of PVL (*lukS-lukF*) gene

The isolates were screened for panton valentine leukocidin (PVL) genes (*lukS-lukF*) associated with tissue necrosis. Out of the 54 isolates screened, only 8 isolates were PVL positive (Figure 4.15). The *lukS-lukF* gene carrying isolates were lane-3, 10, 19 and lanes 21-26. All the PVL positive isolates were recovered from poultry.

The amplified band of the PVL gene of MRSA, USA300 (lane 29) used as positive control was 424.25 bp. The size of the amplified band of the 8 PVL positive test isolates (lanes 3, 10, 19, and 21-25) was also 424.25 bp.

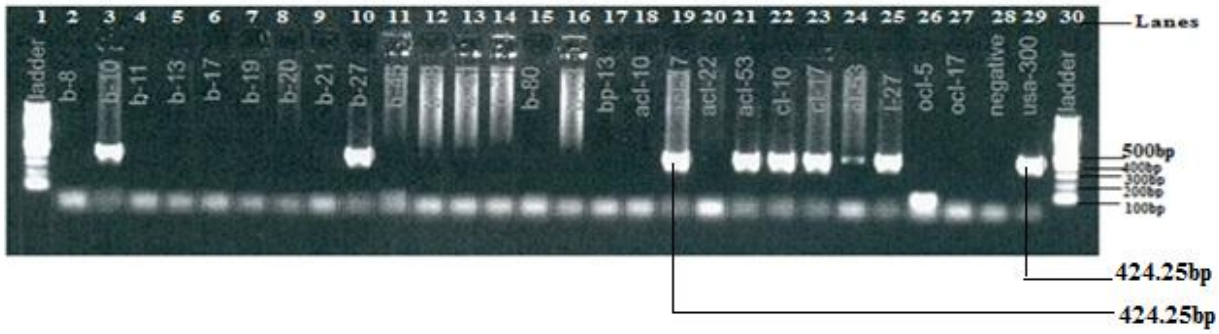


Figure 4.15: PVL PCR showing amplification of *lukS-lukF* gene in positive isolates of *S. aureus*

Lane 1: ladder

Lane 2: pvl negative isolate

Lane 3: pvl positive isolate

Lane 4-9: pvl negative isolates

Lane 10: pvl positive isolate

Lane 11-18: pvl negative isolates

Lane 19: pvl positive isolate

Lane 20: pvl negative isolate

Lane 21-25: pvl positive isolates

Lane 26-27: pvl negative isolates

Lane 29: positive control (USA300)

Lane 28: negative control (SDW) Lane 30: ladder.

4.7.4 Detection of tetracycline resistance gene (*tetM*).

The isolates were screened for tetracycline resistance gene (*tetM*). One isolate; l-137, a poultry isolate was positive for the (*tetM*) gene (Figure 4.16). The amplified band of the *tetM* gene of the positive control was 733.87 bp. The amplified band of the *tetM* gene of the positive test isolate was also 733.87 bp.

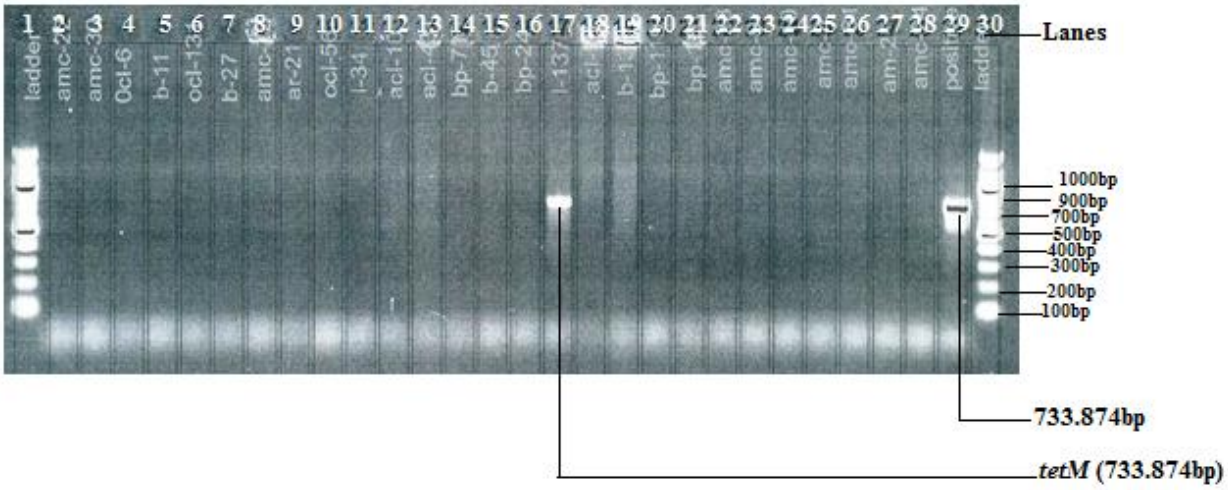


Figure 4.16: PCR amplification of the *tetM* gene of the positive isolate

Lane 1: Ladder. Lane 2-3: hospital MRSA isolates

Lane 4: poultry isolate of *S. aureus* Lane 5; poultry MRSA isolate

Lane 6-7: poultry MRSA isolate Lane 8: hospital MRSA isolate

Lane 9: poultry MRSA isolate Lane 10-16: poultry *S. aureus* isolate

Lane 17: positive *tetM* poultry isolate Lane 18-21: poultry *S. aureus* isolates

Lane 22-26: hospital MRSA isolates Lane: lane 27: poultry MRSA isolate

Lane 28: hospital MRSA isolates Lane 28: negative control (sterile distilled water)

Lane 29: positive control. Lane 30: ladder.

4.7.5 Multilocus sequence typing (MLST) of *S. aureus*

Results obtained from multilocus sequence typing of the seven housekeeping genes of 9 of the isolates showed that the isolates were of 3 sequence types (ST5, ST15 and ST121).

ST121 had only one *spa* type t002. ST5 had 2 *spa* types t002 and t11469. ST15 had 2 *spa* types; t002 and t084 (Table 4.14).

Table 4.14: The Sequence types (ST) of sequenced *S. aureus* isolates.

Isolate		Allelic profiles	
	spa type (t)	arc,aro,glp,gmk,pta,pti,yqi	sequence type (ST)
amc-10	t084	13, 13, 1, 1, 12, 11, 13	ST15
amc-23	t11469	1, 4, 1, 4, 12, 1, 10	ST5
am-26	t002	1, 4, 1, 4, 12, 1, 10	ST5
ar-15	t002	6, 5, 6, 2, 7, 14, 5	ST121
b-13	t11469	1, 4, 1, 4, 12, 1, 10	ST5
b-93	t11469	1, 4, 1, 4, 12, 1, 10	ST5
bp-13	t11469	1, 4, 1, 4, 12, 1, 10	ST5
acl-10	t002	13, 13, 1, 1, 12, 11, 13	ST15
cl-10	t11469	1, 4, 1, 4, 12, 1, 10	ST5

4.7.6: Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of MRSA isolates.

The MRSA isolates were typed to determine the staphylococcal cassette chromosome *mec* (SCC*mec*) for which 11 types have been reported. All the isolates typed were SCC*mec* type V. This was as shown in figures 4.17- 4.19

Figure 4.17 showed the SCC*mec* typing of 11 MRSA isolates from poultry (lanes 2-3, 5, 8) and hospital isolates (lanes 4, 5, 7 and lanes 9-13). MRSA, USA300 used as a control has SCC*mec* type IVc. The SCC*mec* type V MRSA, HPH1 was also used as control. AMC 9 failed to amplify.

The amplified band of the MRSA USA300 used as control for type IVc SCC*mec* was 738.53bp. The amplified band of the type V positive control was 364.64bp. The amplified base pair of the test isolates were 364.64 bp.

Figure 4.18 is the SCC*mec* typing of 5 MRSA isolates, 3 from poultry (lanes 2, 3 and 7) and 2 from hospital (lanes 4 and 5). All the MRSA were SCC*mec* type V. The amplified bands of the control and test isolates were 364.64 bp. The amplified band of the *mecA* gene was 166.45 bp.

Figure 4.19 was a repeat SCC*mec* typing of MRSA AMC 9. It was also found to be SCC*mec* type V. MRSA. The amplified base pair of the test control (HPH1) and test isolate (AMC 9) was 359.557 bp.

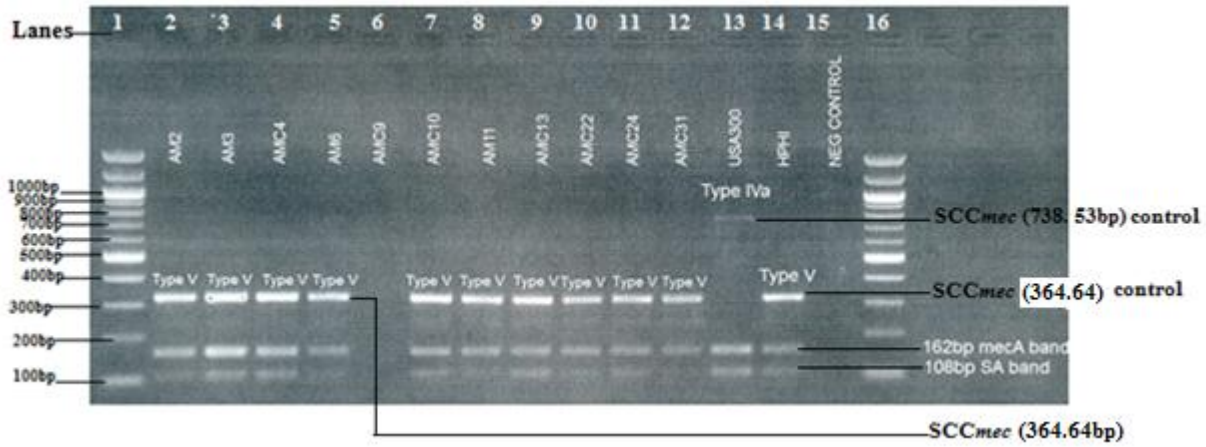


Figure 4.17: SCC*mec* typing of 11 *mecA* positive isolates

Lane 1: ladder Lane 2-3: MRSA isolates from poultry

Lane 4: MRSA isolate from hospital Lane 5: MRSA isolate from poultry

Lane 6: Unamplified hospital MRSA isolate Lane 7: MRSA isolate from hospital

Lane 8: MRSA isolate from poultry Lane 9-13: MRSA isolate from hospital

Lane 13: positive control (USA300) Lane 14: positive control (HPHI)

Lane 15: negative control (sterile distilled water) Lane 16: 1Kb ladder.

.

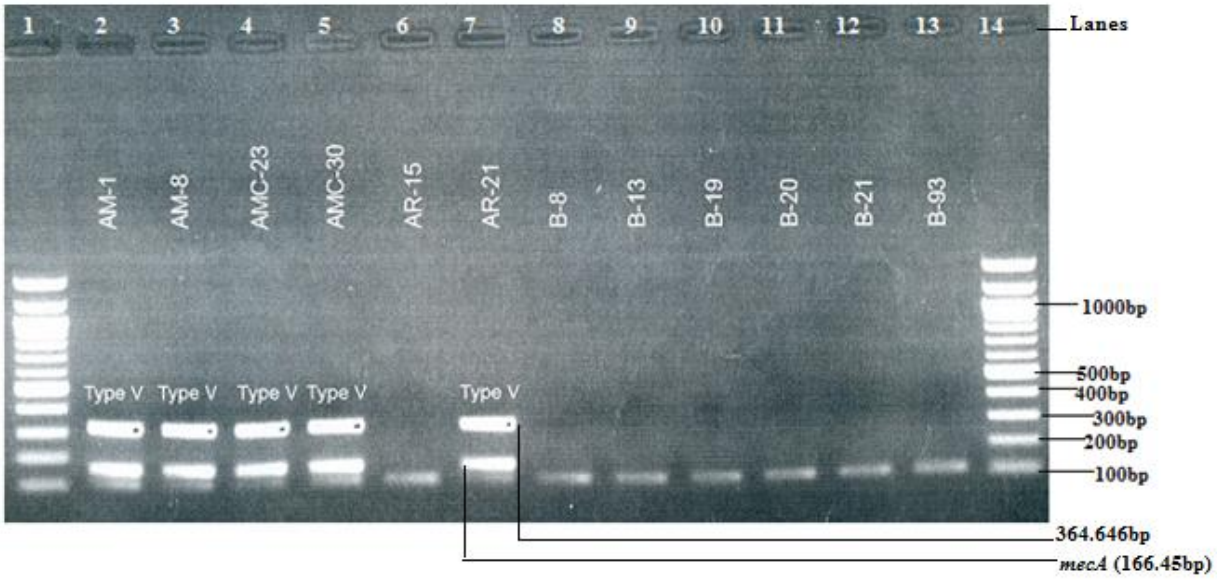


Figure 4.18: SCC*mec* typing of 5 of the *mecA* positive isolates

Lane 1: ladder

Lane 2-3: poultry MRSA isolate

Lane 4-5: hospital MRSA isolates

Lane 7: poultry MRSA isolate

Lane 8-13: poultry MSSA

Lane 14: 1Kb ladder

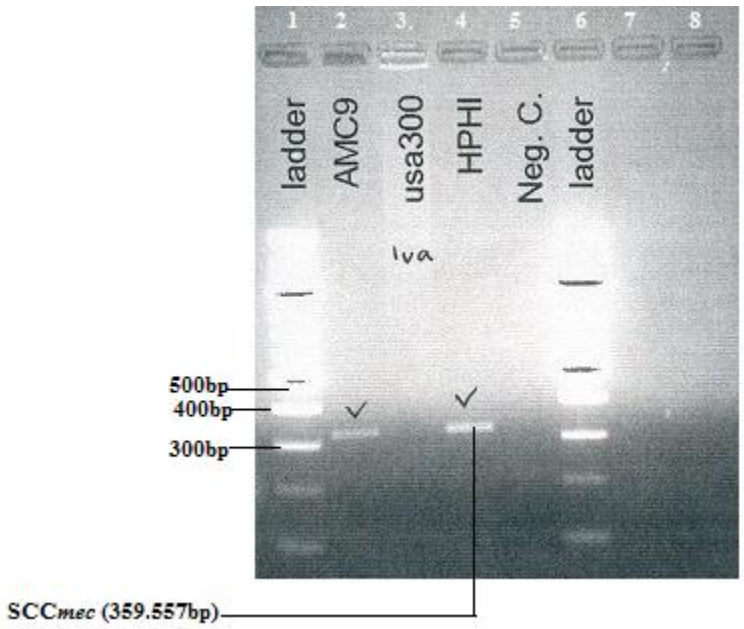


Figure 4.19: SCC*mec* typing of AMC 9, a *mecA* positive isolate

Lane 1: ladder Lane 2: hospital MRSA isolate

Lane 3: positive control (USA300) Lane 4: positive control (HPH1)

Lane 5: negative control (sterile distilled water) Lane 6: ladder.

CHAPTER FIVE

5.0 DISCUSSION

In this study, *S. aureus* was recovered from 247 chicken from 9 farms spread across Ebonyi State and 4 poultry farm workers amongst 19 workers recruited and screened for *S. aureus*. Several studies have reported *Staphylococcus aureus* in animals (Cuny *et al*., 2010; Umaru *et al*., 2011) especially in their nares (Persoon *et al*., 2009; Szabó *et al*., 2012) and in chicken droppings (Olayinka *et al*., 2010). In addition, 78 *S. aureus* were identified amongst clinical isolates of staphylococci from 3 hospitals across the State.

All the farms screened harbored *S. aureus*. The *S. aureus* were isolated from cloacae and nostrils of the poultry birds; the two sites screened for the carriage of *S. aureus* in poultry. In some of the broilers and layers screened, both sites yielded growth of *S. aureus*. This was consistent with the reports of Williams (1963) and Basset *et al*, (2011) that *S. aureus* resides asymptomatically on the skin and in the nose of animals.

The number of *S. aureus* isolated varied from farm to farm. This could be attributed to a number of factors including; people through their footwears and clothings, feed bags and litters, contaminated equipments and machines, sick and wild birds, rodents and day old chicks infected in the hatcheries. The differences in the recovery of *S. aureus* in the farms could be accounted for by the variation in the levels of contamination of these birds from these sources and the hygiene level of each of the farm. Statistical analysis of our data showed that there was no significant difference in prevalence of *S. aureus* among layers and broilers in this study ($p = 0.862 > 0.05$).

Poultry birds cloacae and nostrils were screened for *S. aureus*. In most of the chicken found to harbor *S. aureus*, recovery of the bacterium was from both cloacae and nostrils. A chicken was termed positive if any of the sites screened yielded growth of *S. aureus.* Farm to farm

analysis showed that the percentage of recovery from both cloacae and nostrils was higher than either recovery from cloacae or nostril alone. The number of *S. aureus* recovered by sampling both the cloacae and the nostril were higher (52%). This finding was in concordance with the findings of Persoons *et al*. (2009) who recovered *S. aureus* in all cloacae and nares screened. In other chickens, *S. aureus* was recovered only from cloacae while in some others it was from nostril only. More *S. aureus* were isolated from cloacae compared to nostrils. The percentage of *S. aureus* recovered from cloacae alone was 27% (n=67) while the ones that were isolated only in nostril was 21% (n=51). The reason for recovery of more *S. aureus* in cloacae (27%) than in nostril (21%) was not clear, but might be associated with the feeds which has been reported to carry contaminants including *S. aureus* (Crump *et al*., 2002) and are shed in droppings, part of which could be retained in the cloacae. Study has reported droppings as a good specimen for recovery of *S. aureus* in poultry (Olayinka *et al*., 2010). Chicken droppings were not screened in this study because these birds were not caged as such it was difficult to establish that a particular dropping was from a particular chicken. Statistical evaluation of *S. aureus* recovered from various sites using single factor Anova, showed that at p level ≤ 0.01, there was a significant difference between *S. aureus* recovered from the poultry sites (Appendix 5b). This was in conformity with the findings of Pletinckx *et al*, (2011), who also reported that the percentage of *S. aureus* recovered from both cloacae and nostrils as compared to cloacae or nostril only were not only higher but statistically significant. Furthermore, the recovery of more *S. aureus* by sampling both cloacae and nostril in the same bird indicated that multisite sampling in poultry gave better yield of *S. aureus* than single site. It could therefore, be inferred that cloacae was a better site for recovery of *S. aureus* in poultry than the nostrils; an inference in agreement with Pletinckx *et al*. (2011). The overall carriage of *S. aureus* amongst poultry birds in this study was 14% (247/1800). Percentage MRSA prevalence of 25.9% has been reported in turkey in Germany (Friese *et al*., 2013).

Staphylococcus aureus was isolated from nasal swabs of workers in poultry farms screened. In humans, the nasopharynx remains major niche of *Staphylococcus aureus* where they exist as normal commensal (Bien *et al*., 2011). Out of 19 workers screened across the State, only 21 % (4/19) harbored *S. aureus* in their nostrils. Though the sample size in this study (n=19) was small, the percentage carriage was higher compared to the finding of Mulders *et* a*l*. (2010), who reported a nasal carriage of 13.8% among broiler rearers but lower than the findings of Smith *et al.* (2009), who reported 45% carriage amongst swine workers. Carriage of 25-30% of *S. aureus* has been reported amongst pig farmers in the Netherlands (van-Loo *et al*., 2007). As reported elsewhere nasal carriage of *S. aureus* remains a major threat to human health as it may emerge from colonized sites to cause infection or be transmitted to other persons (Rodriguez-Noriega *et al*., 2010). A carrier of *S. aureus* or MRSA is at potential risk, especially when admitted to hospital, of developing an infection involving the colonizing pathogen and of transmitting the MRSA to other patients with risk factors for infection (Rodriguez-Noriega *et al*., 2010).

The incidence of *S. aureus* varied from hospital to hospital. Out of the 50 isolates obtained from Federal teaching hospital Abakaliki (FETHA), 28 of them were *S. aureus*. In General Hospital Onueke (GHO) 19 isolates were found to be *S. aureus* while at Afikpo Medical Center 31 were *S. aureus*. The high incidence of *S. aureus* amongst clinical isolates of staphylococci analyzed in this study showed the versatility of the bacterium and the frequency of its involvement in staphylococcal infections. Out of the 78 *S. aureus* from the 3 hospitals, Afikpo Medical Center had the highest percentage incidence of (40%) followed by Federal Teaching Hospital Abakaliki (36%) and Onueke General Hospital with 24%. The differences in the incidence have been statistically evaluated and were found to be insignificant. (Appendix 5c). *Staphylococcus aureus* have been implicated in several clinical situations worldwide (Humphreys, 2012).

Antibiotic susceptibility testing (AST) was carried out to obtain antibiotic resistance profiles of the 329 *Staphylococcus aureus* isolates (Appendix 8). All the *S. aureus* were found to be susceptible to Quinupristin/dalfopristin, Linezolid, Rifampicin and Vancomycin; a result similar to reports elsewhere (Shittu *et al*., 2011; Adwan *et al*., 2012). This finding was also consistent with earlier reports in the same environment (Nworie *et al*., 2013a, Nworie *et al*., 2013b). Vancomycin has remained last line treatment for MRSA infections although cases of vancomycin resistance has been reported in many places (Sievert *et al*., 2008; Olayinka *et al*., 2010; Howden *et al*., 2011; Orji *et al*., 2012). Out of the 329 isolates that were screened for methicillin resistance, 28 of them were methicillin resistant, representing a prevalence of 8.5% in this environment. This finding was consistent with report elsewhere (Liu *et al*., 2012). Varying MRSA prevalence has been reported both in the clinics and in farms (van Cleef *et al*., 2010; Rafee *et al*., 2012). Data obtained in this study showed that most of the MRSA were resistant to 4 or more antibiotics. Infact, 4 of the MRSA isolates were resistant to 6 antibiotics.

The highest percentage resistance of 47.7% was recorded against tetracycline followed by trimethoprim/sulfamethoxazole with 42.6%. Comparison of the percentage resistance of poultry and clinical isolates of *S. aureus* showed that the clinical isolates displayed higher percentages of resistance than poultry isolates except to erythromycin. This may be attributed to the constant use of these antibiotics in clinical therapy resulting in the emergence of resistance to most of them. As reported by Davies and Davies (2010), the huge prescription and extensive use of antibiotics without control and caution may account for widespread of antibiotic resistant organisms. In Federal Teaching Hospital some of these antibiotic are rountinely used (Orji *et al*., 2012). The higher resistance of poultry isolates to erythromycin is attributable to heavy use of these antibiotics in animal husbandry and veterinary (Kilonzo-Nthenge *et al*., 2007; Nemati *et al*., 2008). Resistance to fluoroquinolones, tetracycline and

macrolides has been reported to be frequent amongst broilers in the Denmark (Aarestrup *et al*., 2000).

The number of poultry isolates of *S. aureus* that were cefoxitin resistant was 15 (n=247), representing an overall MRSA carriage of 6% amongst poultry isolates. This finding was similar to that of Persoons *et al*. (2009) who reported low prevalence of MRSA amongst poultry in Belgium. MRSA from broilers constituted 73% of all MRSA recovered from poultry.

Compared to reports elsewhere in other animal species, the result obtained in this study was very low. Similar study amongst Iberian pigs reported 28% prevalence of MRSA (Porrero *et al*., 2012). There were more MRSA in broilers 73% (n=11) than in layers 27% (n=4) in this study. This was statistically evaluated and was found to be insignificant (Appendix 5d). This finding was similar to the report of Persoons *et al*. (2009) who found MRSA amongst broilers and none in layers. The reason for this was not clearly understood but it could be associated with the sources of contamination. Friese *et al*. (2013) had reported that hatchery, air emmissions, environment and rodents are common sources of MRSA in animals. All the farms had MRSA except farm 4, 8 and farm 9. The number of MRSA isolates ranged from one in some farms to 5 in others. Reasons for this are not clear but it could be as a result of factors that contaminate the farm environment and the chicken themselves. It could also be due to zoonoses or reverse zoonoses or even contamination of feeds by MRSA. It could be due to improved hygiene in the MRSA free-farms.

Resistance to tetracycline and erythromycin were very high, 45.7% and 19.4% respectively; a finding consistent with findings of De Neeling *et al*. (2007) in pigs. This could be attributed to increasing use of these antibiotics in animal husbandry as earlier reported (Kilonzo-Nthenge *et al*., 2008; Nemati *et al*., 2008; Persoons *et al*., 2009) and high incidence of resistance to them has also been reported elsewhere (Persoons *et al*., 2009; Liu *et al*., 2012).

The low resistance to the other antibiotics except trimethoprim/sulfamethoxazole (40.9%) observed could be attributed to their seldom use in animal husbandry.

Compared to poultry isolates, only one isolate from a nasal swab of a poultry farm worker was MRSA, representing a percentage carriage of 25% (n=4). Nasal carriage of *Staphylococcus aureus* has been identified as a risk factor for community-acquired and nosocomial infections (Datta and Huang, 2008). Varying MRSA prevalence has been reported worldwide amongst animal farm workers. Smith *et al*. (2009) reported 45% carriage of MRSA amongst swine workers in the Midwestern United States of America. In a study in 3 large pig slaughterhouses to determine the prevalence and determinants of nasal MRSA carriage in pig slaughterhouse workers in the Netherlands, van Cleef *et al*. (2010) reported 5.6% overall prevalence of nasal MRSA carriage amongst this population. This was higher than in the general population, suggesting that working with live pigs was the single most important factor for being MRSA positive (van Cleef *et al*. 2010). In a study of high diversity of *S. aureus* and *S. pseudintermedius* lineages and toxigenic traits in healthy pet-owning household members, Gomez-Sanz *et al*. (2013), reported that *S. aureus* was present in 51.2% of households studied while 28 owners (41.8%) carried *S. aureus* and one MRSA. In an international study of the prevalence of MRSA among veterinarians, Wulf *et al*. (2008) reported that 12.5% participants from 9 countries carried MRSA and transmission of MRSA from pigs to staff tending to these animals appears to be an international problem, creating a new reservoir for community-acquired MRSA (CA-MRSA) in humans in Europe, and possibly worldwide. Faires *et al*. (2009), in a study of the prevalence of concurrent MRSA colonization in people and pets in the same household, reported that 17.9% humans, 8.3% dogs, and 10% cats were colonized with MRSA.

All the isolates from the poultry farm workers in this study were susceptible to quinupristin/dalfopristin, doxycycline, erythromycin, gentamycin, linezolid, rifampin and

vancomycin. However, 2 of the isolates were tetracycline resistant representing tetracycline resistance of 50% (n=4).

All the 78 clinical isolates of *S. aureus* from the hospitals were susceptible to quinupristin/dalfopristin, linezolid, rifampin and vancomycin. This was consistent with reports elsewhere (Persoons *et al*., 2009; Shittu *et al*., 2011; Liu *et al*., 2012). However, unlike the poultry isolates, the clinical isolates displayed higher percentage of resistance to most of the antibiotics. This was not surprising as these antibiotics were routinely used in clinical environment resulting in the generation of high level of resistance. This was consistent with findings of Shittu *et al*. (2011). Cefoxitin resistance varied from hospital to hospital. The overall percentage prevalence of MRSA in the hospitals in this study was 15%; a finding similar to the report of Shittu *et al*, (2011) who documented 16% oxacillin resistance amongst *S. aureus* from clinics and healthy individuals in Southwest Nigeria. Higher prevalence has been reported in many other populations and amongst clinical isolates of *S. aureus* in Ngeria. In Southwest Nigeria, a study in Ibadan involving 188 cases of wound infection in University College Hospital Ibadan between December 1994 and April 1995 showed that 41.5% (78/188) of them were methicillin resistant (Okesola *et al*., 1999). Similarly, Adegoke and Komolafe (2009) reported 50% methicillin resistance amongst isolates of *Staphylococcus aureus* in Ile-Ife. Ghebremedhin *et al*. (2009) reported MRSA prevalence of 20.2% in Southwest Nigeria. In Benin, South-South Nigeria, Sani *et al*. (2011) reported 48.78% methicillin resistance out of 123 isolates of *S. aureus* from 2040 biological samples (pus, urine, sperm, genital, catheter and blood) of hospitalized and extra hospitalized patients.

Out of the 3 hospitals, Afikpo Medical Center had the highest MRSA incidence of 50% followed by Federal Teaching Hospital, Abakaliki with 33% and Onueke General Hospital with 17%.

Antibiotic resistance of the clinical isolates (Figure 4.5) was higher than those of the poultry isolates although lower than those reported elsewhere in Nigeria (Shittu *et al*., 2011). This work reports that there were no vancomycin resistant or intermediate resistant *S. aureus* in Ebonyi State; a finding at variance with earlier report in this environment (Orji *et al*., 2012) and reports elsewhere in Nigeria (Olayinka *et al*, 2010). However, it does agree with other findings elsewhere (White *et al*., 2003; Shittu *et al*., 2011). Vancomycin and daptomycin are the last line treatment drugs for MRSA and to date; only limited reports of resistance to them have been documented in Nigeria (Olayinka *et al*, 2010; Orji *et al*., 2012). The susceptibility of the isolates to vancomycin might be due to rare prescription of the drug in this environment. However, the complete susceptibility of all the isolates to quinupristin/dalfopristin, linezolid, rifampicin and vancomycin suggest that these antibiotics could hold great potential in the treatment of staphylococcal infection in Ebonyi State: a finding indicative of treatment options for staphylococcal infections in the area.

All the *S. aureus* in this study displayed varying percentages of resistance to each of the antibiotics used. Resistance recorded were augmenting, 3.3%; cefoxitin, 8.5%, cefuroxime, 6.7%; Chloramphenicol, 19.1%; doxycycline, 7.6%; erythromycin, 18.2%; gentamycin, 5.8%; levofloxacin 4.6%; tetracycline, 47.7%; trimethoprim/sulfamethoxazole 42.6%. The number of isolates resistant to one antibiotic was 63 while 65 isolates were resistant to 2 antibiotics. Furthermore, 46 isolates were resistant to 3 antibiotics and 29 resistant to 4 antibiotics and 13 and 4 isolates were resistant to 5 and 6 antibiotics respectively. These level of multi-antibiotic resistance was not surprising as the area where this study was carried out are cosmopolitan cities with lots of anthropogenic activities including waste water which is a known source of antibiotic resistance genes as earlier reported elsewhere (Lupo *et al*., 2012).

The high percentage (13.97%) of the isolates showing multi antibiotic resistance index (MARI) of 0.3 and above recorded in this study could suggest that the *S. aureus* with these

resistance patterns were from environment where these antibiotics were commonly used (Krumperman, (1983). The highest prevalent multidrug resistance pattern of 15.2% was a combined resistance to chloramphenicol, erythromycin, tetracycline and trimethoprim/sulfamethoxazole. This was followed by a combined resistance to doxycycline, erythromycin, tetracycline and trimethoprim/sulfamethoxazole (6.5%) and cefoxitin, chloramphenicol tetracycline and trimethoprim/sulfamethoxazole (6.5%).

Staphylococcus aureus with multi-drug resistance has been reported in poultry farms in China with prevalence that varied from farm to farm in relation to severity of use of antibiotics (Liu *et al.,* 2012). MRSA in this study exhibited higher multidrug resistance than the *S. aureus*. It has been documented that MRSA adaptability and propensity for acquiring resistance genes among species and from the environment by horizontal transfer (Lupo *et al*., 2012), accounts for its multidrug resistance.

In all the farms screened, most of the antibiotics used for susceptibility testing were commonly used as food supplements, prophylaxis and treatment of diseased birds and this could be an explanation for the resistance of these isolates to a good number of the antibiotics. Resistance to these drugs especially tetracycline and sulfamethoxazole has been reported in poultry (Persoons *et al*., 2009) and amongst meat sellers.

Molecular analysis of the isolates showed that all the *S. aureus* that were cefoxitin resistant, carried the *mecA* gene. *mecA* is a determinant of methicillin resistance and it not only confers resistance to penicillin but to all antibiotics carrying the β-lactam ring thereby making MRSA a multi resistant organism (Drawzand and Bonomo, 2010). SCC*mec* typing of all the MRSA isolates from poultry, hospitals and poultry farm worker showed that they were identical, presenting a possibility of horizontal transmission. The presence of *mecA* gene amongst chicken isolates of *S. aureus* in this study portends serious health risk because of reported cases of zoonosis elsewhere (Smith *et al*., 2009; Kock *et al*., 2009; Wulf *et al*., 2012; Kock *et*

al., 2013). The isolation of a *mecA* positive isolate from one of the poultry farm workers in this study was worrisome especially as the farm workers were mainly family members of the owners and chances of farmer-to-family spread is high, providing a possible reservoir for spread of the bacterium to the community. To the best of our knowledge, this was the first molecular characterization of *S. aureus* in poultry in Ebonyi state.

The percentage carriage of *mecA* amongst poultry and clinical isolates was 6% and 15% respectively. Carriage amongst poultry farm workers was 25%; a carriage regarded very high because of the intransigence and multidrug resistance capabilities of MRSA. This percentage carriage is too high as the bacterium might be transmitted from the farmers to their family members who in turn might become carriers and shedders of the bacterium and therefore source of community outbreaks. Nasal carriage of *S. aureus* has been demonstrated as significant risk factor in nosocomial and community acquired infections in several populations (Kumar *et al*., 2011; Kock *et al*., 2009). Exposure to livestock has been demonstrated as a serious risk factor also (Smith *et al*., 2009, Van Cleef *et al*., 2011).

Our data from *spa* PCR of 46 isolates showed that 4 *spa* types were circulating amongst poultry, poultry farm workers and in the clinics in Ebonyi State. The *spa* types were t002, t084, t2304, and t11469. Studies have shown that MRSA epidemiology vary from one geographic location to another (Breurec *et al*., 2011, Shittu *et al*., 2011; Mediaville *et al* 2012). The finding of t002 amongst the poultry isolates was consistent with earlier report of poultry adapted t002 of sequence type ST5 that originated from humans and spread to commercial poultry (Lowder *et al*., 2009; Kock *et al*., 2013).

spa type t002 were the most prevalent, constituting 48% of the *spa* types in this study. This was followed by t084 with 24%, t11469 with 15% and t2304 with 13%. *spa* types t002 and t11469 were found amongst the 3 groups of isolates (poultry, poultry farm workers and hospital). Prevalence of t002 belonging to ST5 in clinical samples has been reported (Torres-

Sangiao *et al*, 2012) which agrees with the findings of this study. Earlier studies had also described t002 strains in live animals (Khanna *et al*., 2008) and recent report show that humans transmitted ST5 (t002) to poultry several decades ago, where it later spread and has now become endemic (Lowder *et al*., 2009). The finding of t002 in poultry farm workers (am-26 and cl-8) in the same farm where the same *spa* type was found amongst poultry isolates (farm 1 and farm 8 respectively) as well as amongst hospital isolates, suggest human to animal or animal to human transmission; an indication of interspecies transmission and finding consistent with reports elsewhere (Voss *et al*., 2005; Witte *et al*., 2007; Waters *et al*., 2011) The exact route of transmission could not be determined. However, this finding further suggests that this *spa* type was circulating in this environment. As this *spa* type is a human strain involved in staphylococcal infection, chances are that it might dessiminate from poultry into the community and clinical settings where it could be involved in serious staphylococcal infections as reported earlier by Kock *et al*. (2009).

Furthermore, *spa* type t084 and t2304 were found amongst poultry and hospitals isolates; a result consistent with the report of Shittu *et al.* (2011), who reported *spa* type t2304 and t084 from nasal and clinical isolates in different parts of Nigeria including Lagos, Ogbomosho and Maiduguri (Shittu *et al*., 2011). This two *spa* types though recovered from nasal swabs of the poultry birds were not recovered from the nasal swabs of poultry farm workers. *spa* type t084 has been reported amongst hospital acquired infections (HAI) (Shittu *et al*., 2011; Torres-Sangiao *et al*, 2012). The finding of these *spa* types commonly found in humans in poultry is indicative of interspecies transmission and dissemination across species border. It is suggestive of reverse zoonosis and these birds might act as reserviours of this *spa* types and become sources of community and clinical outbreaks. To the best of our knowledge, this is the first report of this *spa* types in poultry birds in Ebonyi State.

A novel *spa* type t11469 was isolated from poultry birds (b-21, b-27 and b-93), poultry farm worker (b-13) and hospital isolates (amc 23 and amc-30) in Ebonyi State. Interestingly, this novel *spa* type was found in birds in a farm (farm 3) where one of the farm workers was carrying the same *spa* type. The same *spa* type was also found in a hospital servicing this environment. This was another evidence of transmission from human to animal or vice verse. To the best of our knowledge this was the first report of *spa* type t11469 in Nigeria or elsewhere. The finding of this novel *spa* type is of great concern since it had not been characterized as to determine its pathogenic potential. Whether this *spa* type might be more virulent and invasive is not known.

Our data showed that out of 54 isolates screened for PVL (*lukS-lukF*) gene associated with MRSA virulence, only 8 of them were PVL positive, representing a percentage prevalence of 14% (n=54).The number of MRSA that were PVL positive was one, representing a prevalence of 3.5% amongst MRSA in this study (n=28). This finding was similar to the report of Sani *et al*. (2011) who reported PVL gene prevalence of 3.25% amongst MRSA and 8.13% amongst MSSA from hospitalized and extra hospitalized patients in Nigeria. Several studies have reported PVL gene in many MSSA and MRSA isolates worldwide (Lina *et al*, 1999; Holmes *et al*., 2005; Melles *et al*., 2006; Bocchini *et al*., 2013). PVL has been reported in China not only in CA-MRSA but in HA-MRSA, which suggests that PVL is not an exclusive marker of CA-MRSA (Deurenberg *et al*., 2007; David and Daum, 2010).

All the PVL positive isolates in this study were from poultry. To the best of our knowledge, this was the first report of PVL positive isolates in poultry in Ebonyi State, Nigeria. Of the isolates that were PVL positive, 12.5% were MRSA while the remaining 87.5% were MSSA; a finding suggestive of the dominance of MSSA amongst PVL producing *S. aureus*. This finding was consistent with that of Okon *et al*. (2012) who had reported 52.1% PVL positive isolates amongst MSSA and none in MRSA in 6 States of Northeastern Nigeria. The finding

of PVL genes amongst poultry isolates in this study portend great risks to those working in close contact with poultry birds due to its association with necrosis and skin infections. Omuse *et al.* (2013) reported that PVL positive isolates appeared to be more successful pathogens in skin and soft tissue infections (SSTIs) and because of its high transmissibility, this strain could cause serious clinical conditions if it makes its way into the community and hospital setting just like MRSA USA300.

Furthermore, 47.7% (157/329) of the isolates of *S. aureus* were tetracycline resistant. Out of the isolates screened for tetracycline resistance gene, *tetM*, only one isolate was positive for *tetM*, representing a prevalence of 3.1% (n=32). The rest of the isolates were *tetM* negative, which suggests that other teracycline resistance genes might be responsible for the resistance observed in this study. The gene for tetracycline resistance is determined by several tetracyclines (*tet*) gene amongst which is *tetM* (Schmitz *et al*., 2001).

Two tetracycline genes *tetM* and *tetK* are common in poultry (Huys *et al*., 2004). The wide distribution of *tet* (K) and *tet* (M) among *S. aureus* strains has been associated to these genes being found on mobile genetic elements such as small plasmids and conjugative transposons respectively (Chopra and Roberts, 2001). While *tetM* is carried on conjugative transposons such as Tn*916* (Clewell *et al*., 1995) *tetK* is disseminated by a transmissible plasmid of the family pT181 (Guay *et al*., 1993).

The choice of *tetM* in this study was based on the fact that *tetM* genes were carried on conjugative transposons with wider host range compared to *tetK* and other tetracycline genes carried on plasmids which has limited host range as reported by Robert, (2012). Besides, the extensive and long-time application of chlortetracycline and oxytetracycline as growth promoters in poultry farming (Witte, 2000) has made tetracycline resistance one of the most frequently occurring resistance phenotypes among *S. aureus* from farming, processing, and

storage environments of poultry (Huys *et al*., 2004). In all the farms screened in this study, tetracycline was extensively used.

The fact that only one isolate in this study carried the *tetM* gene could suggest that the transposon carrying the *tetM* gene is not wide spread in poultry in Ebonyi State. This finding was consistent with that of Huys *et al*. (2004), who reported only one *tetM* isolate in poultry processing plant in South Africa. Tetracycline resistance genes *tet*K and *tet*M occur together in strains of *S. aureus* (Warsa *et al*., 1996). Our result, therefore, was limited by not screening for the *tetK* gene. As a consequence, it was not possible to establish whether *tetK* was responsible for the large tetracycline phenotypes (n=157) in this study or other tetracycline genes. Notwithstanding the finding of only one *tetM* positive isolate in this study, the fact that it was present is of great concern due to the ease with which they spread, usually carried on conjugative transposon with wide host range.

Multilocus sequencing of 9 *S. aureus* selected at random including the novel *spa* type t11469, showed that 3 (t002, t084 and t11469) of the 4 *spa* types identified in this study belong to 3 sequence types ST5, ST15 and ST121. Sequence type ST121, ST15 and ST5 have been reported amongst clinical isolates in Nigeria and elsewhere (Vorobieva *et al*., 2008; Breurec *et al*., 2011; Shittu *et al*., 2011). Sequence type ST15 had 2 *spa* types; t002 and t084 in this study. Breurec *et al*. (2011) had identified t084 as a common *spa* type in ST15. Sequence type ST5 had 2 *spa* types, t002 and a novel *spa* type t11469. *spa* type t002 has been reported as a major strain in ST5 (Lowder *et al*., 2009; Hasman *et al*., 2010). Sequence type ST121 had only one *spa* type t002. The occurrence of *spa* types t002 in all the sequence types ST5, ST15 and ST121 and t11489 in ST5 suggests genetic relatedness of the strains (t002 and t11469). It may also suggest a clonal relatedness of the 3 sequence types or high diversity of *S. aurous* strains and sequenece types; a finding collaborated by Vandenesch *et al.* (2003) who reported that strains with common features have arisen in geographically dispersed *S.*

aureus strains with unrelated genetic backgrounds, indicating multiple independent clonal origins. In this study, both sequence types ST5 and ST15 were found in MRSA and MSSA while ST121 was found in MSSA. This finding was consistent with that of Breurec *et al*. (2011) who reported that sequence type ST121 shared common features (common genetic background and prevalence) with ST15 and that MRSA was rarely documented in ST121. To the best of our Knowledge, this was the first report of these sequence types amongst poultry birds in Nigeria.

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing in combination with genotyping of the *Staphylococcus aureus* chromosome has become essential for defining and understanding molecular epidemiology in MRSA clones (Kondo *et al*., 2007; Derenberg *et al*., 2007). SCC*mec* typing of MRSA in this study showed that they belonged to SCC*mec* type V and therefore could be classified as community associated MRSA (CA-MRSA). SCC*mec* type IV and V have been reported as major clones in Africa and also in livestock associated MRSA (Breurec *et al*., 2011) and both types have been classified as CA-MRSA (IWG-SCC, 2009). The finding of SCC*mec* type V in all the clinical isolates in this study was in concordance with report of Shittu *et al*. (2011) who had reported MRSA with SCC*mec* type V in clinical settings in Nigeria. Our data also showed that all the MRSA-V isolates displayed relatively high resistance to erythromycin. This was consistent with earlier report by Adwan *et al*, (2012) who reported that SCC*mec* type V showed relatively high resistance to erythromycin. The fact that all these isolates were CA-MRSA as our data showed raises great health concern not only to the poultry farm workers and their families but to the general public who are constantly exposed to chicken due to the high adaptability of these strains to the ever changing environmental condition, including antibiotic environment.

There is evidence that CA-MRSA is displacing HA-MRSA in clinical setting (Chambers and Deleo, 2009) and this could be another reason for the presence of type V SCC*mec* amongst

the clinical isolates in this study. As these strains are becoming endemic in hospitals as reported elsewhere (Chambers and Deleo, 2009), it portends grave health hazard to the general public as they might disseminate from these birds into the community and the clinics where treatment options have become narrow and therefore difficult to combact due to its multi-antibiotic resistance propensities. This was another evidence of the changing epidemiology of MRSA from hospital to the community and the extreme versatility of the community strains as reported elsewhere (Mediavilla, 2012).

.

CHAPTER 6

6.0 SUMMARY

The following findings were made in this study;

a) *Staphylococcus aureus* were present in poultry farms across Ebonyi State, Nigeria. The percentage carriage of *S. aureus* in poultry in Ebonyi State was 14% (247/1800).

b) Cloaca was found to be a better site for recovery of *S. aureus* than nostril. A combination of the two sites was found to be better than a single site in the recovery of *S. aureus* from poultry farms in Ebonyi State.

c) The percentage carriage of *S. aureus* amongst poultry farm workers was 21% (4/19) while the percentage incidence of *S. aureus* amongst clinical isolates of staphylococci in hospitals was 52% (78/150).

d) Antibiotic resistance profile of *S. aureus* in this study, showed that 28 isolates were methicillin resistant, representing an MRSA carriage of 8.5% in the State. MRSA carriage in poultry was 6% (15/247) while MRSA carriage amongst clinical isolates was 15% (12/78) and 25% (1/4) amongst poultry farm workers. All the MRSA in this study were multidrug resistant. There was a correlation between cefoxitin resistance and detection of *mecA* gene. Multiplex polymerase chain reaction showed that all the 28 *S. aureus* isolates that were cefoxitin resistant carried the *mecA* gene.

e) Four *spa* types; t002, t084, t2304, and t11469 were circulating in Ebonyi State with *spa* type t002 as the predominant *spa* type. This study reports a novel *spa* type t11469 which to the best of our knowledge is the first report of this *spa* type in Nigeria or anywhere else.

f) Panton valentine leuckocidin (PVL) positive isolates of *S. aureus* were present in poultry in Ebonyi State; a finding with significant health implication. Percentage carriage of tetracycline resistance gene (*tetM*) was 3.1% despite the high tetracycline

resistance observed which suggests that resistance to tetracycline could have been as a result of other tetracycline genes for which many are known, or by other mechanisms that are tetracycline gene independent.

g) MLST analysis showed that 3 sequence types ST5, ST15 and ST121 were circulating in Ebonyi State.

h) All the MRSA isolates belonged to SCC*mec* type V. SCCmec type V is associated with CA-MRSA (IWG-SCC, 2009).

6.1 Conclusion

The high incidence of *Staphylococcus aureus* carriage among chicken in this study could mean an extensive distribution of this organism amongst poultry. It does indicate that poultry farm workers might be at high risk of infection and could be potential sources of community outbreaks. Although only one poultry farm worker carried MRSA in this study (n=4) representing 25% carriage, it portends serious health risk to the poultry farm workers and their families. The high resistance observed amongst poultry isolates of *S. aureus* against tetracycline 45.7% (113/247), trimethoprim/sulfamethoxazole 40.9% (101) and erythromycin 19.4% (48/247) calls for caution in the use of these antibiotics in poultry and animal husbandry in general.

Molecular epidemiological analysis of *S. aureus* reveals that 4 *spa* types (t002, t084, t2304 and a novel *spa* type t11469) were circulating in Ebonyi State. Some *spa* types present in poultry were also present in hospital and in poultry farm workers. The isolation of *spa* type t002, an MRSA of sequence type ST5 and a known poultry associated strain was an indication of the presence of livestock associated MRSA in poultry in Ebonyi State, Nigeria. This finding portends serious health risks to poultry farm workers, their families and the general public due to possible farmer-to-family transmission. The finding of the same *spa* types in poultry, poultry farm worker and hospital in Ebonyi State suggests possible zoonosis

though the actual route of transmission was not investigated. It was also suggestive of the involvement of poultry *S. aureus* in staphylococcal infection in Ebonyi state, Nigeria.

All the PVL positive isolates were from poultry. This pose serious health hazards to those in close contact with poultry birds as PVL is associated with tissue necrosis and other skin infections. In most homes in Ebonyi State, chicken live in and share facilities with their owners. Therefore, the presence of PVL carrying isolates of *S. aureus* in poultry in this environment constitutes a major health threat to the general public.

All the MRSA belonged to SCC*mec* type V suggesting that all the MRSA in this study were community associated.

This study therefore, concludes that although MRSA of clonal complex CC398 (ST398) were not detected probably due to geographic spread limitations of different sequence types, poultry endemic strain t002, a livestock indicator *spa* type belonging to ST5 was present in poultry in Ebonyi State. This finding was consistent with the 2008 report of European Food Safety Authority (EFSA, 2009) that non-ST398 can be found in pigs and other animal species and Kock *et al*. (2013) who classified t002 as livestock indicator *spa* type. Furthermore, the finding of the same *spa* types of *S. aureus* in poultry, poultry farm workers and amongst clinical isolates of *S. aureus* is suggestive of interspecies transmission, the presence of LA-MRSA and their involvement in staphylococcal infection in Ebonyi State.

6.2 RECOMMENDATIONS

a. Combination of modern bacteriological techniques and kits such as latex agglutination kits are recommended for proper phenotypic diagnosis of *S. aureus* and MRSA.

b. High hygiene standards in farms are recommended to avoid contamination and spread of MRSA. Good hand washing practices by staff and those exposed to poultry birds at present, remains the single most important infection control measure and if adhered to

strictly, would prevent the spread of MRSA and other infections to family members and the general public.

c. Restraints in the use of antibiotics as food supplements in animal feeds, prophylaxis and treatment and restraints of clinicians in the empirical treatment with antiobiotic are advocated to reduce the emergence and spread of MRSA.

d. Molecular survillance of *S. aureus* and MRSA on regular basis for molecular epidemiology of this ever changing organism is important to document emerging clones and *spa* types as to identify right treatment options.

e. Further studies are recommended to to identify tetracycline resistant genes responsible for the high rate of tetracycline resistance observed and the circulating *spa* and sequence types of *S. aureus* in Ebonyi State

REFERENCES

Aarestrup, F.M., Agerso, Y., Ahrens, P., Jorgensen, J.C.O., Madsen, M. and Jensen, L.B. (2000). Antimicrobial Susceptibility and Presence of Resistance Genes in Staphylococci from Poultry. *Vetenary Microbiology*. 74:353-364.

Adams, D.N. (2005). Shortcut Method for Extraction of *Staphylococcus aureus* DNA from Blood Cultures and Conventional Cultures for Use in Real-Time PCR Assays. *Journal of Clinical Microbiology*;43(6): 2932-2933

Adegoke, A.A., and Komolafe, A.O. (2009). Multidrug Resistant *Staphylococcus aureus* in Clinical cases in Ile-Ife, Southwest Nigeria. *International Journal of Medicine and Medical Science*; **1**(3): 68-72.

Adwan, K., Jarrar, N., Abu-Hijleh, A., Adwan, G., Awwad, E. and Salameh, Y.(2012). Molecular Analysis and Susceptibility Patterns of Methicillin-Resistant *Staphylococcus aureus* Strains Causing Community- and Health Care-Associated Infections in the Northern Region of Palestine. *American Journal of Infection Control* 41(3). 195-198.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davis, J., and Handelsman, J. (2010). Call of the Wild. Antibiotic Resistance Genes in Natural Environments. *Nature Review. Microbiology, 8(4):* 251-259.

Andiep, B., Carleton, H.A., Chang, R.F., Sensabaugh, G.F. and Perdreau-Remington, F. (2006). Roles of 34 Virulence Genes in the Evolution of Hospital- and Community-Associated Strains of Methicillin-Resistant *Staphylococcus aureus. Journal of Infectious Diseases,* 193(11):1405-1503.

Anwar, M.S., Ghazala, J., Bhatti, K.R., Tayyib, M., and Bokhari, S.R. (2004). Assesment of *Staphylococcus aureus* and MRSA Nasal Carriage in General Population. *Journal of College Physicians and Surgery of Pakistan*, 14:661–4

Atlas, R.M. and Snyder, J. W. (2006). Phenylathanol Agar. In: Hand Book of Media for Clinical Microbiology. 2nd Edn.CRC Taylor and Francis. London. Pg 345-346

Ayliffe, G.A. (1997). The Progressive Intercontinental Spread of Methicillin-Resistant *Staphylococcus aureus*. *Clinical Infectious Diseases,* 24 (1): 74-79.

Baba, T., Takeuchi, F., Kuroda, M., Yuzawa, H., Aoki, K., Oguchi, A., Nagai, Y., Iwama, N., Asano, K., Naimi, T., Kuroda, H., Cui L., Yamamoto, K. and Hiramatsu, K. (2002). Genome and Virulence Determinants of High Virulence Community-Acquired MRSA. *The Lancet*, 359:1819-1827.

Balazy, A., Toivola, M., Reponen, T., Podgorskia, A., Zimmer, A. and Grinshpun, S.A. (2006). Manikin-Based Performance Evaluation of N95 Filtering-Facepiece Respirators Challenged with Nanoparticles. *Annals of Occupational Hygiene,* 50(3):259-269.

Barrow, G.I. and Feltham, R.K. (1993). Characterization Tests. In: Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edn. Eds G. I. Barrow and R. K. A. Feltham. Cambridge University Press, Cambridge. Pp 219-238

Bartlett, A. H. and Hulten, K. G. (2010). *Staphylococcus aureus* Pathogenesis Systems, Adhesins and Invasins.*Pediatric Infectious Diseases Journal*, 29:860- 861.

Basset, P., Feil, E.J., Zanetti,G. and Blanc D.S. (2011). The Evolution and Dynamics of Methicillin-Resistant *Staphylococcus aureus*. In: Genetics and Evolution of Infectious Diseases Eds Michel Tibayrenc. Elservier London. Pg 669-676.

Basset, P., Hammer, N.B., Kuhn, G., Vogel, V., Sakwinska, O. and Blanc, D.S. (2009). *Staphylococcus aureus* clfB and *spa* Alleles of the Repeat Regions are Segregated into Major Phylogenetic Lineages. *Infection, Genetics and Evolution.* 9:941-947

Berger-Bachi, B. (1995). Factors Affecting Methicillin Resistance in *Staphylococcus aureus*. *International Journal of Antimicrobial Agents*, 6:13-21.

Bhalla, A., Aron, D.C. and Donskey, C.J (2007). *Staphylococcus aureus* Intestinal Colonization is Associated with Increased Frequency of *S. aureus* on Skin of Hospitalized Patients. *BMC Infectious Diseases*, 7:105:1-7.

Bien, J., Sokolova, O. and Bozko, P. (2011). Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That are Implicated in Activation of Airway Epithelial Proinflammatory Response. *Journal of Pathogens*: 2011:1-13

Bloemendaal, A.L.A., Brouwer, E.C. and Fluit, A.C. (2010). Methicillin Resistance Transfer from *Staphylocccus epidermidis* to Methicillin-Susceptible *Staphylococcus aureus* in a Patient during Antibiotic Therapy. *PLoS ONE,* **5** (7):1184

Bocchini, C.E., Hulten, K.G., Mason, E.O., Gonzalez, B.E., Hammerman, W.A. and Kaplan, S.L (2013). Panton-Valentine Leukocidin Genes are Associated With Enhanced Inflammatory Response and Local Disease in Acute Hematogenous *Staphylococcus aureus* Osteomyelitis in Children. *PEDIATRICS*, 117(2):433-440

Boerlin, P., Kuhnert, P., Hüssy, D. and Schaellibaum, M. (2003). Methods for Identification of *Staphylococcus aureus* Isolates in Cases of Bovine Mastitis. *Journal of Clinical Microbiology*, 41(2): 767–771.

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. and Thiberge, J.M. the Working Group on *Staphylococcus aureus* infections, Laurent, F. 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.

British Society for Antimicrobial Chemotherapy (2004). Antimicrobial Use in Pet Animal Veterinary Practice. *Journal of Antimicrobial Chemotherapy.* 54:321–332.

Bronner, S., Monteil, H. and Prevost, G. (2004). Regulation of Virulence Determinants in *Staphylococcus aureus*: Complexity and Applications. *FEMS Microbiology Reviews*, 28**(**2): 183-200

Brooks, G.F., Carroll K. C., Butel, J. S.and Morse, S. A. (2007). Jawetz, Melnicks and Adelberg's Medical Microbiology.24th ed. McGraw-Hill. New York. Pp. 224 - 227.

Brown, D.F.J., Edwards D.I., Hawkey P.M., Morrisons, D., Ridgeway, G.L., Towner, K.J. and Wrens M.W.D, (2005). Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Journal of Antimicrobial Chemotherapy*, 56: 1000–1018

Centers for Disease Control and Prevention (2005). Community Associated MRSA Information for Clinicians. Infection Control Topics. Centers for Disease Control and Prevention, Atlanta, G. A. http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_clinicians.html#4.

Chambers, H.F. (2001). The Changing Epidemiology of *Staphylococcus aureus*? 4th Decennial International Conference on Nosocomial and Healthcare Associated Infections:**7**(2).http://wwwnc.cdc.gov/eid/article/7/2/70-0178.htm

Chambers, H.F. and Deleo F.R. (2009). Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era. *Nature Reviews,* 7: 629-641.

Chambers, H.F. (2004). Origins of Community Strains of Methicillin-Resistant *Staphylococcus aureus. Clinical Infectious Diseases,* 39:47

Chan, C.X., Beiko, R.G. and Ragan, M.A. (2011). Lateral Transfer of Genes and Gene Fragments in *Staphylococcus* Extends beyond Mobile Elements. *Journal of Bacteriology,* 193(15):3964–3977

Charlebois, E.D., Perdreau-Remington, F., Kreiswirth, B., Bangsberg, D.R., Ciccarone, D., Diep, B.A., Ng, V.L., Chansky, K., Edlin, B. and Chambers, H.F. (2004). Origins of Community Strains of Methicillin-Resistant *Staphylococcus aureus. Clinical Infectious Diseases,* 39:47

Charlier, C., Cretenet, M., Even, S. and Le-Loir Y. (2009). Interactions between *Staphylococcus aureus* and Lactic Acid Bacteria: An Old Story with New Perspectives. *International Journal of Food Microbiology*, 131:30–39

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries Part II. Cambridge University Press, Cambridge UK. 65: 38-39.

Chen, L.F. (2012). The Changing Epidemiology of Methicillin-Resistant *Staphylococcus aureus*: 50 Years of a Superbug. *American Journal of Infection Control*, 41(2013): 448-51

Chongtrakool, P., Ito, T., Ma, X.X., Kondo, Y., Trakulsomboon, S., Tiensasitom, C., Jamklang, M., Chavalit, T., Song, J. H. and Hiramatsu, K. (2006). Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Typing of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated in 11 Asian Countries: A Proposal for a New Nomenclature for SCC*mec* Elements. *Antimicrobial Agents Chemotherapy,* 50(3):1001-1012

Chopra, I., Hodgson, J., Metcalf, B. and Poste, G. (1997). The Search for Antimicrobial Agents Effective against Bacteria Resistant to Multiple Antibiotics. *Antimicrobial Agents and Chemotherapy*, 41(3):497-503.

Chopra, I. and Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Review.* 65:232-260

Chung, M., de-Lencastre, H., Matthews, P., Tomasz, A., Adamsson I., de-Sousa, M A., Camou T., Cocuzza, C., Corso, A., Couto, I., Dominguez, A., Gniadkowski, M., Goering, R., Gomes, A., Kikuchi, K., Marchese, A., Mato, R., Melter, O., Oliveira, D., Palacio. R., Sá-Leão, R., Sanches, S.I., Song, J.H., Tassios, P.T. and Villari, P. Multilaboratory Project Collaborators. (2000). Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* by Pulsed-field Gel Electrophoresis: Comparison of Results Obtained in a Multilaboratory Effort Using Identical Protocols and MRSA Strains. *Microbial Drug Resistance,* 6(3):189-198

Clewell, D.B., Flannagan, S.E. and Jaworski D.D.(1995). Unconstrained Bacterial Promiscuity: The Tn916-Tn1545 Family of Conjugative Transposons. *Trends Microbiology.* 3:229-236.

Clinical and Laboratory Standards Institute (2008). Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. M100-S18, 28(1)

Coombs, G.W., Pearson, J.C., O'Brien, F.G., Murray, R.J., Grubb, W.B. and Christiansen, K.J. (2005). Methicillin-Resistant *Staphylococcus aureus* Clones in Western Australia. *Emerging Infectious Diseases;* 11(10):1584-1590.

Coombs, G.W., Goering, R.V., Chua, K.Y.L., Monecke, S., Howden, B.P., Stinear T.P., Ehricht R., O' Brien F.G. and Christiansen K.J. (2012). The Molecular Epidemiology of the Highly Virulent ST93 Australian Community *Staphylococcus aureus* Strain. *PLoS ONE,* **7**(8):1-12.

Crum-Cianflone, N.F., Marx, M., Kreiswirth, B., Ruane, P., Weiss, D. and Iyer, S. (2006).Understanding and Managing Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Among HIV-Infected Persons. *The PRN Notebook* 11(3) 1-21.

Crump, J.A., Griffin, P.M. and Angulo, F.J. (2002). Bacterial Contamination of Animal Feed and Its Relationship to Human Foodborne Illness. *Clinical Infectious Disease*, 35(7): 859-865

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nubel, 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(1):109–117

Dantas, G. and Sommer, M.O.A. (2012). Ecological and Clinical Consequences of Antibiotic Subsistence by Environmental Microbes. In: Antimicrobial Resistance in the Environment, 1st edn. (Ed) Keen P.L and Montforts. John Wiley and Sons Inc, New Jersey, USA. Pp. 7-14.

Datta, R. and Huang, S.S. (2008). Risk of Infection and Death due to Methicillin-Resistant *Staphylococcus aureus* in Long-Term Carriers. Clinical Infectious Diseases, 47: 176–81.

David, Z.M., Glikman, D., Crawford, S.E., Peng, I., King, K., Hostetler, M.A., Boyle-Vavra, S. and Daum, R. (2007). What Is Community-Associated Methicillin-Resistant *Staphylococcus aureus. Journal of Infectious Diseases,* 197(9): 1235-1243.

Davies, J. and Davies, D. (2010). Origins and Evolution of Antibiotic Resistance. *Microbiology. Molecular Biology Review,* 74(3):417-433.

David, M.Z. and Daum, R.S. (2010). Community-Associated Methicillin-Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic. *Clinical Microbioliology Review*, 23(3): 616–687

D'Costa, V.M., McGrann, K.M., Hughes, D.W. and Wright, G.D. (2006). Sampling the Antibiotic Resistome. *Science*, 311(5759):374-377.

De-Sousa, M.A. and de-Lencastre, H. (2004). Bridges from Hospitals to the Laboratory: Genetic Portraits of Methicillin-Resistant *Staphylococcus aureus* Clones. *FEMS Immunology and Medical Microbiology;* 40:101-111.

DeLeo, F.R., Otto, M., Kreiswirth, B.N. and Chambers, H.F. (2009). Community-Associated Meticillin-Resistant *Staphylococcus aureus*. *The Lancet*, 375(9725):1557-1568.

de Neeling, A.J., van den Broek, M.J., Salburg, E.C., van Santen-Verheuvel, W. D., Dam-Deisz, H. C., Boshuizen, A.W., van de Giessen, E., van Duijkeren, and Huijsdens X. W. (2007). High Resistance of Methicillin Resistant *Staphylococcus aureus* in Pigs. *Vetrinary Microbiology, 122*:366-372

Deurenberg, R.H., Kalenic, S., Friedrich, A.W., van Tiel, F.H. and Stobberingh, E.E. (2007). Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus*: Current Research, Technology and Education. Topics in Applied Microbiology and Microbial Technology. A. Mendez-Vilas (Ed). Pp.766-777

Devriese, L.A., Vancanneyt, M., Baele, M., Vaneechoutte, M., De Graef, E., Snauwaert, C., Cleenwrck, I., Dawyndt, P., Swings, J., Decostere, A. and Haesebrouck, F. (2005). *Staphylococcus pseudintermedius sp.* A Novel Coagulase-Positive Species from Animals. *International Journal of Systematic and Evolutionary Microbiology*; 55: 1569–1573

Donham, K.J (2010). Community and Occupational Health Concerns in Pork Production: A Review. *Journal of Animal Science;* 88(13):102-111.

Drawzand, S.M and Bonomo, R.A. (2010). Three Decades of β-Lactamase Inhibitors. *Clinical Microbiolology Review.*; 23(1): 160–201

Elston, J.W.T. and Barlow, G.D. (2009). Community-Associated MRSA in the United Kingdom. *Journal of Infection*, 59(3):149-155

Enright, M.C., Day, N.P.J., 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(3):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). *Proc.Natl Proceeding of National Academy of Science. U.S.A;* 99:7687-7692.

European Food Safety Authority (2009). Analysis of the Baseline Survey on the Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Holdings with Breeding Pigs, in the EU, 2008, Part A: MRSA Prevalence Estimates; On Request from the European Commission. *EFSA Journal*, **7**(11):1376.1458.

Faires, M.C., Kathy C., Tater K.C., and Weese, J. S, (2009). An Investigation of Methicillin-Resistant *Staphylococcus aureus* Colonization in People and Pets in the Same Household with an Infected Person or Infected pet. *Journal of American Vetrinary Medical Associaion,* 235(5): 540-543.

Fanoy, E., Helmhout, L.C., van der Vaart, W.L., Weijdema, K., van Santen-Verheuvel, M.G., Thijsen, S. F., de Neeling A.J., van Wamel W.J., Manaskova, S.H. and Kingma-Thijssen, J. L. (2009). An outbreak of non-typeable MRSA within a residential care facility. *Euro Surveillance*; 14(1):19080

Farzana, K., Rashid, Z., Akhtar, N., Sattar, A., Khan, J.A. and Nasir, B. (2008). Nasal Carriage of Staphylococci in Healthcare Workers: Antimicrobial Susceptibility Profile. *Pakistan Journal of Pharmaceutical Science;* 21(3):290-294.

Feng, Y., Chen, C., Su, L., Hu, S., Yu, J. and Chiu, C. (2008). Evolution and Pathogenesis of *Staphylococcus aureus*: Lessons Learned from Genotyping and Comparative Genomics. *FEMS Microbiology Review*; 32(1):23–37.

Ferry, T., Perpoint, T., Vandenesch, F. and Etienne, J. (2005).Virulence Determinants in Staphylococcus aureus and Their Involvement in Clinical Syndromes. *Current Infectious Disease Reports;* 7:420–428

Fey, P. D. Saïd-Salim, B. Rupp, M.E. Hinrichs, S.H. Boxrud, D.J. Davis, C.C., Kreiswirth, B.N. Schlievert, P.M. (2003). Comparative Molecular Analysis of Community- or Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy;* 47(1):196-203

Foster, T. (1996). Staphylococcus. In: Baron S, (ED). Medical Microbiology. 4th Edition. Galveston (TX): University of Texas Medical Branch at Galveston.

Foster, T.J. (2004). *Staphylococcus aureus* "Superbug". *Journal of Clinical Investigation*; 114(12):1963-1696.

Foster, T. J. (2005). Immune Evasion by Staphylococci. *Nature Reviews Microbiology*; 3:948-958

Freney, J. Kloos, W. E. Hajek, V. Webster, J. A. Bes, M., Brun, N Y. Vernozyrozand C. (1999) Recommended Minimal Standards for Description of New Staphylococcal Species. Subcommittee on the Taxonomy of Staphylococci and Streptococci of the International Committee on Systematic Bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, 49: 489-502

Friese, A. Schulz, J. Zimmermann, k. TenhageN, B-A. Fetsch, A. Hartung, J. Rosler, U. (2013). Occurrence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Turkey and Broiler Barns and Contamination of Air and Soil Surfaces in Their Vicinity *Appl. Environ. Microbiol.* 79 (8): 2759-2766.

García-Álvarez, L. Holden, M.T.G. Lindsay, H. Webb, C. R. Brown, D. F. J. Curran M. D. Walpole, E. Brooks, K. Derek, J. Pickard D. J, Teale C. Parkhill, J. Bentley, S. D. Edwards G. F. E Kirsty Girvan, E. K. Kearns, A. M. Pichon, B., Hill, R.L.R. Larsen, A. R. Skov, R.L. Peacock, S.J. Maskell D.J. Holmes, M.A. (2011). Methicillin-Resistant *Staphylococcus aureus* with a Novel *mecA* Homologue in Human and Bovine Populations in the UK and Denmark: A Descriptive Study. *The Lancet Infectious Diseases,*11**(**8): 595 – 603

Ghaznavi-Rad E. Shamsudin, M.N. Sekawi, Z. van Belkum, A. Neela, V. (2010). A Simplified Multiplex PCR Assay for Fast and Easy Discrination of Globally Distributed Staphylococcal Cassette Chromosome *mec* Types in Methicillin Resistant *Staphylococcus aureus*. *Journal of Medical Microbiology*, 59:1135-1139

Ghebremedhin, B. Olugbosi, M.O. Raji, A.M. Layer, F. Bakare, R.A. Kong, B. Konig, W. (2009). Emergence of a Community-Associated Methicillin Resistance *Stapylococcus aureus* Strain with a Unique Resistance Profile in South West Nigeria. *Journal of Clinical Microbiology*, 47(9):2975-2980

Golding, G R. Bryden, L. Levett, P.N. McDonald, R.R. Wong, A. Wylie, J. Graham, M.R. Tyler, S. Domselaar, G.V. Simor, A.E. Gravel, D. Mulvey, M. R. (2010). Livestock-Associated Methicillin Resistant *Staphylococcus aureus* Sequence Type 398 in Humans, Canada. *Emerging Infectious Diseases,* 16 (4):587-594.

Gómez-Sanz, E.C. Torres,C. Lozano, C. Zarazaga, M. (2013). High Diversity of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* Lineages and Toxigenic Traits in Healthy Pet-Owning Household Members. Under Estimating Normal Household Contact? *Comparative Immunology, Microbiology and Infectious Diseases*, 36(1):83-94

Gordon, R. J. Lowy, F. D. (2008). Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* Infection. *Infectious Diseases;* 46(5):350-359

Graveland, H. Wagenaar, J.A. Heesterbeek, H. Mevius, D. van Duijkeren E, Heederik D. (2010). Methicillin Resistant *Staphylococcus aureus* ST398 in Veal Calf Farming: Human MRSA Carriage Related with Animal Antimicrobial Usage and Farm Hygiene. *PLoS ONE,* **5**(6) 1-6.

Grundmann, H. Hori, S. Enright, M.C. Webster, C. Tami, A. Feil, E.J. Pitt T. (2002). Determining the Genetic Structure of the Natural Population of *Staphylococcus aureus*: A Comparison of Multilocus Sequence Typing with Pulsed-Field Gel Electrophoresis, Randomly Amplified Polymorphic DNA Analysis, and Phage Typing. *Journal of Clinical Microbiol*ogy, 40(12): 4544- 4546.

Guay, G.G. Khan, S.A. Rothstein D.M. (1993). The *tet(K)* Gene of Plasmid pT181 of *Staphylococcus aureus* Encodes an Efflux Protein that Contains 14 Transmembrane Helices. *Plasmid;* 30:163-166.

Haley, R.W. Hightower, A.W. Khabbaz, R. F. Thornsberry, C. Martone W. J. Allen, J. R. Hughes, J.M. (1982). The Emergence of Methicillin Resistant *Staphylococcus aureus* Infections in United States Hospitals. *Annals of Internal Medicine*, 97(3):297-308.

Hallin, M., A. Deplano, O. Denis, R. De Mendonc¸a, R. De Ryck, and M. J. Struelens (2007). Validation of Pulsed-field Gel Electrophoresis and *spa* Typing for Long-Term, Nationwide Epidemiological Surveillance Studies of S*taphylococcus aureus* Infections. J. Clin. Microbiol. 45:127–133

Hanson, B.M. Dressler, A.E. Harper, A.L. Scheibel, R.P. .Wardyn, S.E. Roberts, L.K. Kroeger, J.S. Smith, T.C (2011). Prevalence of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) on Retail Meat in Iowa. *Journal of Infection and Public Health*; 4:169—174

Hanssen, A.M. Sollid, J.U.E. (2007). Multiple Staphylococcal Cassette Chromosomes and Allelic Variants of Cassette Chromosome Recombinases in *Staphylococcus aureus* and Coaaulase-Negative Staphylococci from Norway. *Antimicrobial. Agents Chemotherapy,* 51:1671-1677.

Hasman, H. Moodley, A. Guardabass, L. Stegger, M. Skov, R. L, Aarestrup F. M (2010). *spa* Type Distribution in *Staphylococcus aureus* Originating from Pigs, Cattle and Poultry. *Veterinary Microbiology*; 141: 326–331

Henderson, K.D. (2006). Managing Methicillin-Resistant *Staphylococcus aureus*: A paradigm for Preventing Nosocomial Transmission of Resistant Organisms. *American Journal of Medicine,*119(6): 46-52

Higuchi, W. Teng, T.T. Yamamoto L. (2008). Structure and Specific Detection of Staphylococcal Cassette Chromosome *mec* Type VII. *Biochemical and Biophysical Communications;* 377(3):752-756.

Hiramatsu, K. Cui, L, Kuroda, M. Ito, T. (2001). The Emergence and Evolution of Methicillin-Resistant *Staphylococcus aureus*. *Trends Microbiology*; 10:486-493

Holden, M.T.G. Fell, E.J. Lindsay, J.A. Peacock, S.J. Day N.P.J Enright, M.C. Foster, T. J. Moore C.E. Hurst, L. Atkin, R. Barron, A. Bason, N. Bentley, S.D. Chillingworth C. Chillingworth T., Churcher C., Clark L., Corton C., Cronin A., Doggett J. Dowd, L. Feltwell, T. Hanca, Z. Harris, B. Hauser, H. Halroyd, S. Jagels, K. James, K.D. Lenard, N. Line, A. Mayes, R., Moule S. Mungall, K. Ormond, D. Quall, M A. Rabbinowitsch, E. Rutherford, Kim.Sanders, M. Sharp S. Simmonds, M. Stavens, K. Whitehead, S. Barrell, B.G. Spratt B. (2004). Complete Genomes of Two Clinical *Staphylococcus aureus* Strains: Evidence for the Rapid Evolution of Virulence and Drug Resistance. *PNAS;* 101(26):9786-9791.

Holmes, A. Ganner, M. McGuane, S. Pitt T.L. Cookson, B.D. Kearns, A.M. (2005). *Staphylococcus aureus* Isolates Carrying Panton-Valentine Leukocidin Genes in England and Wales: Frequency, Characterization, and Association with Clinical Disease. *Journal of Clinical Microbiology, 43*:2384–90.

Howden, B.P. Davies, J.K. Johnson, P.D.R. Stiner, T.P. (2010). Reduced Vancomycin Susceptibility in *Staphylococcus aureus,* Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clinical Implications. *Clinical Microbiology Review*; 23**(**1): 99-139

Howden, B.P. McEvoy, C.R.E, Allen, D.L. Chua, L, Gao, W. Harrison, P.F. Bell J. Coombs G. Bennett-Wood V. Porter, J.L Robins-Browne, R. Davies, J.K. Seemann T. Stinear T.P.(2011). Evolution of Multidrug Resistance during *Staphylococcus aureus* Infection Involves Mutation of the Essential Two Component Regulator WalKR. *PLoS Pathology,* **7**(11):1002359

Huang, Y. Chen, C. (2011). Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Children inTaiwan, 2000s. *International Journal of Antimicrobial Agents*,38: 2–8

Huijsdens, X.W. Bosch, T. van Santen-Verheuve, M.G. Spalburg, E. Pluister, G. N. van Luit, M. Heck, M.E.O.C. Haenen, A. de Neeling, A. J (2007). Molecular Characterization of PFGE Non-Typable Methicillin-Resistant *Staphylococcus aureus* in the Netherlands. *Euro Surveillance;* 14(38) *1-5.*

Humphreys, H. (2012). *Staphylococcus aureus*: The Enduring Pathogen in Surgery. *The Surgeon* 10(6): 357-360.

Hunter, P.A. Dawson, S. French, G.L. Goossens, H. Hawkey, P.M. Kuijper, E. J. Nathwani D. Taylor, D.J. Teale, C.J. Warren, R.E. Wilcox, M.H. Woodford N. Wulf, M. W. Piddock, L.J.V. (2011). Antimicrobial-Resistant Pathogens in Animals and Man: Prescribing, Practices and Policies. *Journal of Antimicrobial Chemotherapy,* 66:3-17.

Huys, G., D'Haene, K.J., Collard M. and Swings, J. (2004). Prevalence and Molecular Characterization of Tetracycline Resistance in Enterococcus Isolates from Food. *Applied Environmental Microbiology. 70:*1555-1562

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (2009). Classification of Staphylococcal Cassette Chromosome *mec* (SCC*mec*): Guidelines for Reporting Novel SCCmec Elements. *Antimicrobial Agents Chemotherapy;* 53:4961–4967.

International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) (2011). [Accessed on May 22, 2012].SCC*mec* up to date.http://www.sccmec.org/Pages/SCC_TypesEN.html.

Isenberg, H.D. (1998). Essential Procedures for Clinical Microbiology.*American Society for Microbiology*; Washington D.C. USA. Pp. 90 - 94

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C. and Hiramatsu, K. (2001). Structural Comparison of Three Types of Staphylococcal Cassette Chromosome mec Integrated in the Chromosome in Methicillin Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 45(5):1323-1336.

Ito, T., Katayama, Y. and Hiramatsu, K. (1999). Cloning and Nucleotide Sequence Determination of the Entire *mec* DNA of Pre-Methicillin Resistant *Staphylococcus aureus* N315. *Antimicrobial Agents Chemotherapy*; 43: 1449-1458

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H. and Hiramatsu, K. (2004). Novel Type V Staphylococcal Cassette Chromosome *mec* Driven by a Novel Cassette Chromosome Recombinase, *ccrC*. *Antimicrobial Agents and Chemotherapy;* 48:2637-2651.

Jaffe, R.I., Lane, D.J., Albury, S.V. and Niemeyer, D.M. (2000). Rapid Extraction from and Direct Identification in Clinical Samples of Methicillin-Resistant Staphylococci Using the PCR. *Journal of ClinicalMicrobiolog;* 38(9):3407-3412.

Jansen, M.D., Box, A.T.A. and Fluit, A.C. (2009). SCC*mec* Typing in Methicillin-Resistant *Staphylococcus aureus* Strains of Animal Origin. *Emerging Infectious Diseases.* 15:(136)1-2.

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, F., Forey, F., Nesme, X., Etienne, J. and Vandenesch, F. (2002*).* Relationship between *Staphylococcus aureus* Genetic Background, Virulent Factors, agr Group (Alleles) and Human Diseases. *Infection and Immunity*; 70(2):631-641.

Jensen, S.O. and Lyon, B.R. (2009). Genetics of Antimicrobial Resistance in *Staphylococcus aureus*. *Future Microbiology*; 4(5): 565-582.

Johnson, A. P. (2011). Methicillin-Resistant *Staphylococcus aureus*: the European Landscape. *Journal of Antimicrobial Chemotherapy* 66(4):43–48

Jongerius, I., Puister, M,. Wu, J., Ruyken, M., van Strijp, J.A.G. and Rooijkkers, S.H.M. (2010). Staphylococcal Complement Inhibitor Modulates Phagocyte Responses by Dimerization of Convertases. *The Journal of Immunology*; 184(1):420-425

Kaatz, G.W., McAleese, F. and Seo, S.M. (2005). Multidrug Resistance in *Staphylococcus aureus* Due to Over Expression of a Novel Multidrug and Toxin Extrusion (MATE) Transport Protein. *Antimicrobial Agents and Chemotherapy*; 49(5):1857-1864.

Kateete, D.P., Kimani, C.N., Katabazi, F.A., Okeng, A., Okee, M.S., Nanteza, A., Joloba, M.L. and Najjuka, F.C. (2010). Identification of *Staphylococcus aureus*: DNase and Mannitol Salt Agar Improve the Efficiency of the Tube Coagulase Test. *Annals of Clinical Microbiology and Antimicrobials* 9(23) 1-7.

Kayser, F.H. (1993). Evolution of Resistance in Microorganisms of Human Origin. *Vet Microbiol*.; 35(3-4):257-67.

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.

Kilonzo-Nthenge, A., Nahashon, S.N., Chen, F. and Adefope, N. (2008). Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in Chicken and Guinea Fowl. *Poultry Science,* 87:1841-1848.

Köck, R., Schaumburg, F., Mellmann, A., Köksal, M., Jurke, A., Becker, K., Alexander, W. 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

Köck, R., Harlizius, J., Bressan, N., Laerberg, R., Wieler, L.H., Witte, W., Deurenberg R.H., Voss, A., Becker, K. and Friedrich, A.W. (2009). Prevalence and Molecular Characteristics of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Pigs on German Farms and Import of Livestock-Related MRSA into Hospitals. Eur J Clin Microbiol Infect Dis 28: 1375–1382

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

Krumperman, P.H. (1983). Multiple Antibiotic Indexing of *Escherichia coli* to Identifying Risk Sources of Feacal Contamination of Foods. *Applied and Environmental Microbiology*; 46: 165-170.

Kumar, P., Shukla, I. and Varshney, S. (2011). Nasal Screening of Healthcare Workers for Nasal Carriage of Coagulase Positive MRSA and Prevalence of Nasal Colonization with *Staphylococcus aureus*. *Biology and Medicine*; 3(2):182-186.

Lambert, P.A. (1998). Mechanisms of Action of Antibiotics In: 'Pharmaceutical Microbiology'. 6th ed. Hugo W. B, Russell A. D (ED). Blackwell Science KK Tokyo Japan. Pp. 162-180.

Lamikanra, A., Paul, B.D., Akinwole, O.B. and Paul, M.O. (1985). Nasal Carriage of *Staphylococcus aureus* in a Population of Healthy Nigerian Students. *Journal of Medical Microbiology;* 19: 211-216.

Lewis, R.A. and Dyke, K.G.H. (2000). *MecI* Represses Synthesis from the β-lactamase Operon of *Staphylococcus aureus. Journal of Antimicrobial Chemotherapy;* 45:139-144.

Lewis, H.C., Mølbak, K., Reese, C., Aarestrup, F.M., Selchau, M., Sørum, M., and Skov, R.L. (2008). Pigs as Source of Methicillin-Resistant *Staphylococcus aureus* CC398 Infections in Humans in Denmark. *Emerging Infectious Diseases.* 14: 1383–1389.

Leung, Y., Lai, R.W., Chan, A.C., Lo, J.Y., Ho, P., Wong, M.M., Chuang, S. and Tsang T.H.(2012) Risk Factors for Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection in Hong Kong. *Journal of Infection* 64: 494-499

Lin, Y., Barker, E., Kislow, J., Kaldhone, P., Stemper, M.E., Pantrangi M., Moore, F.M., Hall, M., Fritsche, T.R., Novicki, T., Foley, S.L. and Shukla, S.K. (2010). Evidence of Multiple Virulence Subtypes in Nosocomial and Community-Associated Methicillin Resistant *Staphylococcus aureus* Genotypes in Companion Animals from the Upper Midwestern and Northeastern United States. *Clinical Medicine and Research*, 9(1):7-16.

Lina, G., Piemont, Y., Godial-Garmot, F., Bes, M., Peter M.O., Gauduchon, V., Vandenesch, F. and Ettiene, J. (1999). Involvement of Panton-Valentine Leukocidin Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia.*Clinical infectious Diseases*, 29**(**5):1128-1132.

Lindsay, J.A. and Foster, S.J. (1999). Interactive Regulatory Pathways Control Virulence Determinant Production and Stability in Response to Environmental Conditions in *Staphylococcus aureus. Molecular and General Genetics*, 262(2):323-331

Liu, D., Chai, T., Xia, X., Gao, Y., Cai, Y., Li, X., Miao, Z., Sun, L., Hao, H., Roesler ,U. and Wang, J. (2012). Formation and Transmission of *Staphylococcus aureus* (including MRSA) Aerosols Carrying Antibiotic-Resistant Genes in a Poultry Farming Environment. *Science of the Total Environment* 426: 139–145

Lowder,B.V., Guinane, C.M., Zakour, N.L., Weinert, L.A., Conway-Morris, A., Robyn A., Cartwright, R.A., Simpson A.J., Rambaut A., Nübel U. and Fitzgerald J.R (2009). Recent Human-to-Poultry Host Jump, Adaptation, and Pandemic Spread of *Staphylococcus aureus*. *Proceedings Nationall Academy of Science. USA 106*: 19545-19550

Lowy, F.D. (1998). *Staphylococcus aureus* Infections. *New England Journal of Medicine.* 339**:**520–32

Lowy, F.D. (2003). Antimicrobial Resistance: The Example of *Staphylococcus aureus*. *Journal of Clinical Investigation*. 111:1265–1273

Lozano, C., Aspiroz, C., Charlez, L., Go´mez-Sanz, E., Toledo, M., Zarazaga, M. and Torres, C. (2011). Skin Lesion by Methicillin-Resistant *Staphylococcus aureus* ST398-t1451 in a Spanish Pig Farmer: Possible Transmission from Animals to Humans. *Vector-borne and Zoonotic Diseases*, 11(6):605-7

LuczaK-Kadlubowska, A., Krzyston-Russjan, J. and Hryniewicz, W. (2006). Characteristics of *Staphylococcus aureus* Strains Isolated in Poland in 1996 to 2004 That Were Deficient in Species-Specific Proteins. *Journal of clinical Microbiology,*44(11):4018-4024.

Lupo, A., Coyne, S. and Berendonk, T. U. (2012). Origin and Evolution of Antibiotic Resistance: The Common Mechanisms of Emergence and Spread in Water Bodies. *Frontiers of Microbiology* 3:(18): 1-13.

Ma, X.X., Ito, T., Tiensasitorn, C., Jamklang, M., Chongtrakool, P., Boyle-Vavra, S., Daum, R.S. and Hiramatsu, K. (2002). Novel Type of Staphylococcal Cassette Chromosome *mec* Identified in Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Strains. *Antimicrobial Agents and Chemotherapy,* 46:1147-1152.

Maes, N., Magdalena, J., Rottiers, S., De Gheldre, Y. and Struelens M.J. (2002). Evaluation of a Triplex PCR Assay to Discriminate *Staphylococcus aureus* from Coagulase-Negative Staphylococci and Determine Methicillin Resistance from Blood Cultures. Journal of Clinical Microbiology, 40(4): 1514-1517.

Magiorakos ,A. P., Srinivasan, A., Carey, R.B., Carmeli Y., Falagas M. E., Giske, C.G., Harbarth S. Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B, Paterson, D.L. Rice, L.B., Stelling J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L. (2012). Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clincal Microbiology and Infection,* 18:268–281

Malachowa N. and DeLeo F. R. (2010) Mobile Genetic Elements of *Staphylococcus aureus*. *Cell. Mol. Life Sci.;*67 (2010):3057–3071

Martins, A., de Lourdes Ribeiro de Souza and da Cunha, M. (2012). Epidemiological Aspects of Oxacillin-Resistant Staphylococcus spp.: The Use of Molecular Tools with Emphasis on MLST, Epidemiology Insights, Dr. Maria De Lourdes Ribeiro De Souza Da Cunha (Ed.), ISBN: 978-953-51-0565-7, InTech, DOI: 10.5772/31615. Available from: http://www.intechopen.com/books/epidemiology-insights/epidemiological-aspects-of-oxacillin-resistant-staphylococcus-spp-the-use-of-molecular-tools-with

McCallum, N., Berger-Bachi, and Senn, B. (2010). Regulation of Antibiotic Resistance in *Staphylococcus aureus*. *International Journal of Medical Microbiology;* 300:118–129

McCarthy, A. J. and Lindsay, J. A (2010). Genetic Variation in *Staphylococcus aureus* Surface and Immune Evasion Genes is Lineage Associated: Implications for Vaccine Design and Host-Pathogen Interactions. *BMC* Microbiology,10:(173) 1-5

Mediavilla, J.R., Chen, L., Mathema, B. and Kreiswirth, B.N. (2012). Global Epidemiology of Community-Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA). *Current Opinion in Microbiology*, 15:588–595.

Mehndiratta, P. L. and Bhalla, P. (2012). Typing of Methicillin Resistant *Staphylococcus aureus*: A Technical Review. *Indian Journal of Med Microbiology,* 30:16-23

Melles, D.C., van Leeuwen, W.B., Boelens, H.A.M., Peeters, J. K., Henri, A., Verbrugh, H.A. and van Belkum, A. (2006). "Panton-Valentin Leukocidin in *Staphylococcus aureaus" Emerging Infectious Diseases:* 12 (7):1175-1175

Miao, V., Davis, D. and Davis, J. (2012). Path to resistance; In: Antimicrobial Resistance in the Environment, Keen P.L and Montforts (ED). Ist Edn. John Wiley and Sons Inc, New Jersey, USA. Pp. 7-14.

Milheirico C., Oliveira, D.C. and de Lencastre, H. (2007). Multiplex PCR Strategy for Subtyping the Staphylococcal Cassette Chromosome *mec* Type IV in Methicillin-Resistant *Staphylococcus aureus*: SCC*mec* IV Multiplex. *Journal of Antimicrobial Chemotherapy,* 60:42–48.

Mine, Y., Inakasone, I., Yamamoto, Y., Utani, Y., Yamane, N., Uezato, H. and Takahashi K. (2012). Dissemination of Panton–Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* in Okinawa, Japan. *Journal of Dermatology*; 39: 1–5

Monecke, S., Ruppelt A., Wendlandt, S., Schwarz, S., Slickers, P., Ehricht R. and de Ja¨ckel C.S. (2013). Genotyping of *Staphylococcus aureus* Isolates from Diseased Poultry. Veterinary Microbiology 162(2-4) 800-812.

Moran, G. J. Krishnadasan, A. Gorwitz, R.J.F. McDougal, L.K. Carey, R.B. Talan, D.A. (2006). Methicillin-Resistant *Staphylococcus aureus* Infections among Patients in the Emergency Department. *New England Journal of Medicine;* 355:666-674.

Mulders, M.N., Haenen, A.P.J., Gleenen, P.L., Vesseur, P.C., Poldervaart, E.S., Bosch, T., Huijsdens, X.W., Hengeveld, P.D., Dam-Deisz, W.D.C., Graat, E.A.M. and Mevius D. (2010). Prevalence of Livestock-Associated MRSA in Broiler Flocks and Risk Factors for Slaughterhouse Personnel in The Netherlands. Epidemiology of Infection. 138: 743–755

Murray, P.R., Rosenthal, K.S., Kobayashi, G.S. and Pfaller, M.A. (2002). Medical Microbiology. 4th Ed. Mosby Inc. Missouri. Pp. 202-216.

Muto, C.A., Jernigan, J.A., Ostrowsky, B.E., Richet, H.M., Jarvis W.R., Boyce J.M. and Farr, B.M. (2003). SHEA Guideline for Preventing Nosocomial Transmission of Multidrug-Resistant Strains of *Staphylococcus aureus* and *Enterococcus*. *Infection control and Epidemiology*, 362-386

Mwangi, M.M., Wu, S.W., Zhou, Y., Sieradzki, K., de Lencastre, H., Richardson, P., Bruce, D. Rubin E., Myers, E., Siggia, E.D. and Tomasz, A. (2007). Tracking the in Vivo Evolution of Multidrug Resistance in *Staphylococcus aureus* by Whole Genome Sequencing. *PNAS;* 104(22):9451-9456.

Nabera, C. K. (2009). *Staphylococcus aureus* Bacteremia: Epidemiology, Pathophysiology and Management Strategies. *Clinical Infectious Diseases*, 48(4):231-237

Ndi, O. Barton, M. (2012). Antibiotic Resistance in Animals-The Australian Perspective. In: Antimicrobial Resistance in the Environment, Keen P.L and Montforts Eds. 1st Edition. John Wiley and Sons Inc, New Jersey, USA. Pp. 265-289.

Nemati, M., Hermans, K. Lipinska, U., Denis, O., Deplano, A., Struelens, M., Devriese, L.A., Pasmans, F. and Haesebrouck, F. (2008). Antimicrobial Resistance of Old and Recent *Staphylococcus aureus* Isolates from Poultry: First Detection of Livestock-Associated Methicillin-Resistant Strain ST398. *Antimicrobial Agents and Chemotherapy;* 52(10) 3817- 3819.

Norman, A., Hansen, L.H. and Sorensen S.J. (2009). Conjugative Plasmids: Vessels of the Communal Gene Pool. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*. 364:2275–2289

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*

Nworie, A., Madubuko, E. F., Eze, U.A., Oti-Wilberforce R.O., Azi S.O., Ibiam G.A., Egwu I. H., Okereke E.C. and Obi I.A. (2013a). Incidence of *Staphylococcus aureus* in Clinical Specimens in Federal Teaching Hospital, Abakaliki, Ebonyi State. *Merit Research Journal of Medicine and Medical Sciences*, 1(3): 43-46.

Nworie, A., Azi, S. O., Ibiam, G.A., Egwu, I.H., Odoh I., Okereke E.C., Oti-Wilberforce R.O., Eze U.A. and Obi, I.A. (2013b). Nasal Carriage of Methicillin Resistant *Staphylococcus aureus* Amongst Meat Sellers in Abakaliki Metropolis, Ebonyi State, Nigeria. *Microbiology Research International.* 1(3):48-53

Nworie, A. and Umeh, N. (2010). Investigation of Urinary Tract Infection among High School Children in Abakaliki Metropolis. *Journal of Biomedical Sciences in Nigeria*, 8:1-2.

Obansa, S.A.J. and Maduekwe, I.M. (2013). Agriculture Financing And Economic Growth In Nigeria. *European Scientific Journal*. 9(1): 1857 – 7881

Ochei, J. and Kolhatkar, A. (2000). Medical Laboratory Science-Theory and Practice. Tata McGraw-Hill Publishing Company Ltd, New Delhi. Pp. 648-657

Okesola, A.O., Oni A.A. and and Bakare, R.A. (1999). Nosocomial Infections: Methicillin Resistant *Staphylococcus auerus* in Wound Infection in Ibadan, Nigeria. *African Journal of Medical Science,* 28(1-2) 55-7

Okon, K. O., Uba, A., Oyawoye, O.M., Yusuf, I.Z. and Adesina, O.O. (2012). Prevalence and Antibiotic Susceptibility Pattern of Panton-Valentine Leukocidin (PVL) Positive *Staphylococcus aureus* Strains from Clinical Specimens in Northeastern Nigeria. *Sierra Leone Journal of Biomedical Research,* 4(1): 43-52.

Okuma, K., Iwakawa, K., Turnidge, J.D., Grubb, W.B., Bell, J.M., O'Brien F.G., Coombs G. W., Pearman. J.W., Tenover, F.C., Kapi, M., Tiensasitorn, C. Ito, T., and Hiramatsu K. (2002). Dissemination of New Methicillin-Resistant *Staphylococcus aureus* Clones in the Community. *J Clin Microbiol*; 40(11): 4289–4294.

Olayinka, B.O., Bala, H.K., Ehinmidu, J.O. and Onaolapo, J.A. (2010). Multidrug Resistant *Staphylococcus aureus* Isolates from Poultry Farms in Zaria, Nigeria.14th *International Symposium on Staphylococci and Staphylococcal Infections,* Bath, UK, ISSSI2010.

Oliveira, D.C. and de Lencastre, H. (2002) Characterization of Structural Types and Variants of the *mec* Element in Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*; 46:2155-2161

Omuse, G., Shivachi, P., Karinki, S. and Revathi G. (2013). Prevalence of Panton Valentine Leukocidin Carriage and Infective Strains of *Staphylococcus aureus* at a Referral Hospital in Kenya. *Open journal of Medical Microbiology* 3:4-11.

Oliveira, D.C., Milheiriço, C. and de Lencastre, H. (2006). Redefining a Structural Variant of Staphylococcal Cassette Chromosome *mec*, SCC*mec* type VI. *Antimicrobial Agents and Chemotherapy;* 50:3457-3459.

Onanuga, A., Oyi, A.R., and Onaolapo, J.A. (2005). Prevalence and Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* Isolates Among Healthy Women in Zaria, Nigeria. *African Journal of Biotechnology*, 4(11):1321-1324.

Orji, I., Nworie, A., Eze, U.A., Abgerotimi, I.O., Okereke, E.C. and Azi, S.O. (2012). The Prevalence and Antimicrobial Susceptibility Profile of Methicillin-Resistant *S. aureus* Isolates from Clinical Specimens in a Tertiary Hospital, South East Nigeria. *Continental Journal of Phrmaceutical Sciences*, 6(1): 23-29.

Oscarsson, J., Harlos, C. and Arvidson, S. (2005). Regulatory Role of Proteins Binding to the *spa* (protein A) and sarS (staphylococcal accessory regulator) Promoter Regions in *Staphylococcus aureus* NTCC 8325-4. *International Journal of Medical Microbiology,* 295(4):253-66.

Otter, J.A. and French, G.L. (2011). Community Associated Methicilline Resistant *staphylococcus aureus* Strains as a Cause of Health Care Infection. *Journal of Hospital Infection*; 79: 189-193

Otto, M. (2010). Basis of Virulence in Community-Associated Methicillin-Resistant *Staphylococcus aureus*. *Annual Review of Microbiology,* 64:143–162

Palmqvist, N. Foster, T. Tarkowski, A. Josefsson E. (2002). Protein A is a Virulence Factor in *Staphylococcus aureus* Arthritis and Septic Death. *Microb Pathog;* 33 (5):239-249

Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., de Kruif, A., Haesebrouck, F. and Dewulf, J. (2009).Methicillin-Resistant *Staphylococcus aureus* in Poultry. *Emerging Infectious Diseases*; 15(3): 452–453

Pfaller, M.A. (2001). Molecular Approaches to Diagnosing and Managing Infectious Diseases: Practicality and Costs. *Emerging Infectious Diseases*; **7**(2):312-318

Piechowic, L., Garbacz, K., Wisniewska, K. and Da˛browska-Szponar, M. (2011). Screening of *Staphylococcus aureus* Nasal Strains Isolated from Medical Students for Toxingenes. *Folia Microbiol (Praha)*; 56:225-9.

Pletinckx, LJ., Verhegghe, M., Dewulf, J. Crombé, F., De Bleecker, Y., Rasschaert, G., Goddeeris, B.M. and De Man (2011). Screening of Poultry-Pig Farms for Methicillin-Resistant *Staphylococcus aureus* :Sampling Methodology and within Herd Prevalence in Broiler Flocks and Pigs. Infect Genet Evol. (8):2133-2137

Porrero, M.C., Wassenaar, T.M., Go´ mez-Barrero, S. M., Garcı´a, M., Ba´ rcena C. A,´ lvarez, J., Sa´ ez-Llorente, J.L., Bal, J.F.F., Moreno, M.A. and Domı´nguez, L. (2012). Detection of Methicillin-Resistant *Staphylococcus aureus* in Iberian Pigs. *Letters in Applied Microbiology*; 54: 280–285.

Prat, C., Haas, P., Bestebroer, J., de Haas, C.J.C., van Strijp, J.A.G. and van Kessel K.P.M. (2012). A Homolog of Formyl Peptide Receptor-Like 1 (FPRL1) Inhibitor from *Staphylococcus aureus* (FPRL1 Inhibitory Protein) That Inhibits FPRL1 and FPR. *Journal of Immunol*ogy 183: 6569-6578

Pratten, J., Foster, S.J., Chan, P.F., Wilson, M. and Nair, S.E (2001). *Staphylococcus aureus* Accessory Regulators: Expression within Biofilms and Effect on Adhesion. *Microbes and Infection*; 3:633−637.

Price, L.B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P.S.,Peason, T., Waters, A. E., Jeffrey, T., Foster, J.T., Schupp, J., Gillece J., Driebe, E., Liu, C.M., Springer, B. Zdovc I., Battisti, A., Franco, A., mudzki, J.Z., Schwarz, S. Butaye, P. Jouy, E. Pomba, C. Porrero, M.C. Ruimy, R. Smith, T.C. Robinson, D.A. Weese, J.S. Arriola, C.S. Yu, F. Laurent, F., Keim, P. Skov, R. and Aarestrupc F.,M. (2012). *Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. *Molecular Biology*.3(1): 1-6.

Pruden, A. and Arabi, M. (2012).Quantifying Anthropogenic Impacts on Environmenal Reservoirs of Antibiotic Resistance; In, Antimicrobial Resistance in the environment, Keen P.L and Montforts (ED).1st Edition. John Wiley and Sons Inc, New Jersey, USA. Pp. 173-201

Pu, S., Han, F. and Ge, B. (2009). Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* Strains from Louisiana Retail Meats. *Applied and Environmental Microbiology*, 75(1):265-267.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (2000). *Staphylococcus Species*. In: Clinical Veterinary Microbiology, Mosby, Edinburgh Pp.118–126.

Rafee, Y., Abde-Haq, N., Asmar, B., Salimnia, T., Pharm, V.C., Pharm, M.J.R. and Amjad, M. (2012). Increased Prevalence of Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization in Household Contacts of Children with Community Acquired Disease. *BMC Infectious Diseases* 12:45. 1-7

Reyes J., Rincon, S., Diaz L., Panesso, D., Contrera, G.A., Zurita, J., Carrillo, C., Rizzi, A. Guzman M., Adachi, J., Chowdhury, S., Murray, B.E. and Arias, C.A. (2009). Dissemination of Methicillin-Resistant *Staphylococcus aureus* USA300 Sequence Type 8 Lineage in Latin America. *Clinical Infectious Diseases*, 53(2):1861- 1877.

Roberts, M.C. (2012). Mechanisms of Bacterial Antibiotic Resistance and Lessons Learned from Environmental Tetracycline Resistant Bacteria; In: Antimicrobial Resistance in the Environment, Keen P.L and Montforts (ED). 1st Edition. John Wiley and Sons Inc, New Jersey, USA. Pp. 7-14

Robinson, D.A. and Enright, M.C. (2004). Multilocus Sequence Typing and the Evolution of Methicillin-Resistant Staphylococcus aureus. *Clinical Microbiology and Infection;*10(2): 92-97

Rodrı´guez-Noriega, E., Seas, C., Guzma´n-Blanco, M., Mejı´a, C., Alvarez. C., Bavestrello, L. Zurita, J., Labarca, J.M., Luna, C.M., Salles, M.J.C. and Gotuzzo, E. (2010). Evolution of Methicillin-Resistant *Staphylococcus aureus* Clones in Latin America. *International Journal of Infectious Diseases*. 14(7):560-566.

Russell, A.D. (1998). Types of Antibiotics and Synthetic Antimicrobial Agents. In: Pharmaceutical Microbiology. Hugo WB and Russell AD (ED).6th ed. Pg-91-129. Blackwell Science KK, Tokyo, Japan.

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(11): 3946–3951.

Salgado, C.D., Farr, B.M. and Calfee, D.P. (2003). Community-Acquired Methicillin-Resistant *Staphylococcus aureus:* A Meta-Analysis of Prevalence and Risk Factors. *Clinical infectious Diseases*; 36: 131-139.

Sani, H., Baba-Moussa, F., Ahoyo, T.A., Mousse, W., Anagonou, S., Gbenou, J.D., Prevost G., Kotochoni, S.O. and Baba-Moussa, L. (2011). Antibiotic Susceptibility and Toxins Production of *Staphylococcus aureus* Isolated from Clinical Samples from Benin. *African Journal of Microbiology Research;* 5(18):2797-2803.

Sapkota, A.R., Ojo. K.K., Roberts, M.C. and Schwab, K.J. (2006). Antibiotic Resistance Genes in Multidrug-Resistant Enterococcus spp. and Streptococcus spp. Recovered from the Indoor Air of a Large-Scale Swine-Feeding Operation. *Letters in Applied Microbiology.* 43(5):534-540.

Schijffelen M. J., Boel. C. H. E., van Strijp J. A. G. and Fluit A.C. (2010). Whole Genome Analysis of a Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* ST398 Isolate from a Case of Human Endocarditis. *BMC Genomics;* 11:(376): 1-10.

Schmitz, F., Maarten, R., Visserand, A.D. and Fluit, C. (2001).Molecular Detection of Antimicrobial Resistance. *Clinical Microbiology Reviews*, 14(4): 836–871.

Schouls, L.M., Spalburg, E.C., van Luit, M., Huijsdens, X.W. and Pluister, G.N. (2009). Multiple-Locus Variable Number Tandem Repeat Analysis of *Staphylococcus aureus:* Comparison with Pulsed-Field Gel Electrophoresis and *spa*-Typing. *PLoS ON*E 4(4): 5082

Schulthess, B., Bloes, D.A. and Berger-Bachi, (2012). Opposing roles of sB and sB-Controlled SpoVG in the Global Regulation of esxA in *Staphylococcus aureus. BMC Microbiology*; 12:(17): 1-12.

Schwartz, T. (2012). Strategies To Assess And Minimize The Biological Risk Of Antibiotic Resistance In: Antimicrobial resistance in the environment. Keen P.L and Montforts (ED).1st Edn. John Wiley and Sons Inc, New Jersey, USA. Pp. 251-264

Shaffer, R.K. (2013). The Challenge of Antibiotic-Resistant Staphylococcus: Lessons from Hospital Nurseries in the Mid-20th Century Yale. *J Biol Med.* 86(2): 261–270

Shittu, A.O., 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. *BioMed Central Microbiology.* 11(92):1-8.

Shopsin, B., Gomez, M., Montgomery, SO., Smith, D. H., Waddington, M., Dodge, E. E., Bost, A., Riehman, M., Naidich, S. and Kreiswirth, B.N. (1999). Evaluation of Protein A Gene Polymorphic Region DNA Sequencing for Typing of *Staphylococcus aureus* Strains. *Journal of Clinical Microbiology*,37(11): 3556-3563

Sievert, D.M., Rudrik, J. T., Patel, J. B., McDonald, L.C., Wilkins, M.J. and Hageman, J.C. (2008) Vancomycin-Resistant *Staphylococcus aureus* in the United States, 2002–2006. *Clinical Infectious Diseases*, 46:668–74

Skov, R., Christiansen, K., Dancer, S.J., Daumd R.S., Drydene M.Huang Y. and Lowy F.D (2012). Update on the Prevention and Control of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA). *International Journal of Antimicrobial Agents*; 39:193– 200

Skurray, R.A. and Firth, N. (1997). Molecular Evolution of Mult-Antibiotic-Resistant Staphylococci. *In:* D. J. Chadwick (ed.), Antibiotic Resistance: Origins, Evolution, Selection and Spread. Wiley, Chichester, United Kingdom. Pp. 167–191

Smith, M.A. (2005). Antibiotic Resistance. *Nursing Cinica of North America*; 40: 63–75

Smith T. C., Male M. J., Harper A .L., Kroeger J. S., Tinkler G. P., Moritz, E.D. Capuano, A.W., Herwaldf, L.A., and Diekema O.J. (2009). Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strain ST398 is Present in Midwestern U.S. Swine and Swine Workers. *PLoS ONE;* 4(1) 1-6.

Smith, T.C. and Pearson, N. (2011). The Emergence of *Staphylococcus aureus* ST398. *Vector-Borne and Zoonotic Diseases;* 11(4): 327-338

Smith, T.L. and Jarvis, W.R. (1999). Antimicrobial Resistance in *Staphylococcus aureus*. *Microbes and Infection*; 1(10): 795-805.

Soavi, L., Stellini, R., Signorini, L., Antonini, B., Pedroni, P., Zanetti, L., Milanesi B., Pantosti A. Matteelli, A., Pan, A. and Carosi, G. (2010). Methicillin-Resistant *Staphylococcus aureus* ST398, in Italy. *Emerging Infectious Diseases*,16 (2): 346–348..

Spanu, V., Spanu, C., Virdis, S., Cossu, F., Scarano, C. and de Santis, E.P. (2012). Virulence Factors and Genetic Variability of *Staphylococcus aureus* Strains Isolated from Raw Sheep's Milk Cheese. *International Journal of Food Microbiology*, 153:53-57.

Spence, M.R., Courser, S., Dammel, T. and Montana K. (2009). Polymerase Chain Reaction Screening for Methicillin-Resistant *Staphylococcus aureus* and Contact Isolation. *American Journal of Infection Control*, 37:601-602.

Spratt, B.G (2004). Exploring the Concept of Clonality in Bacteria. *Methods in Mol. Bio.266*. 323-352

Stefani, S., Chung, D.R., Lindsay, J.A., Friedrich, A.W., Kearns, A.M., Westh, H., Fiona, M. and MacKenzie, F.M. (2012). Meticillin-Resistant *Staphylococcus aureus* (MRSA): Global Epidemiology and Harmonisation of Typing Methods. *International Journal of Antimicrobial Agents*, 39: 273– 282

Stranden, A. Frei, R. Widmer, A.F. (2003). Molecular Typing of Methicillin- Resistant *Staphylococcus aureus*: Can PCR Replace Pulsed-Field Gel Electrophoresis? *Journal of Clinical Microbiology;* 41(7):3181–3186.

Struelens, M.I., Hawkey, P.M., French, G.L., Witte, W., and Tacconelli, E. (2009). Laboratory Tools and Strategies for Methicillin-Resistant *Staphylococcus aureus* Screening, Surveillance and Typing: State of the Art and Unmet Needs. *Clinical Microbiology and Infection.*15(2):112–119.

Szabo, I., Beck, B., Friese, A., Fetsch, A., Tenhage, B. and Roesle, U. (2012). Colonization Kinetics of Different Methicillin-Resistant *Staphylococcus aureus* Sequence Types in Pigs and Host Susceptibilities. *Appl. Environ*; 78(2):541-548

Tenover, F.C., Arbeit R.D. and Goering R.V. (1997). How to Select and Interpret Molecular Strain Typing Methods for Epidemiological Studies of Bacterial Infections: A Review for Healthcare Epidemiologists. *Infection Control and Hospital Epidemiology: SHEA Position Paper;* 18(6): 426-439.

Thorne, P. S., Perry, S.S., Saito, R., O'Shaughnessy, P. T., Mehaffy, J., Metwali, N. and Reynolds, S.J. (2010). Evaluation of the Limulus Amebocyte Lysate and Recombinant Factor C Assays for Assessment of Airborne Endotoxin. *Applied Environmental Microbiol*ogy, 76(15):4988-4995.

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(7):*2465-2472*

Torres-Sangiao, E. Pe´rez-Castro, S., Ferna´ndez-Natal, M.I., Cisterna-Cancer, R., Zapico-Gonza´lez, M., Ferna´ndez-Pe´rez B., Ojeda-Ferna´ndez, E. Nebreda T. Gozalo-Margu¨ello M. Fuster-Foz C., Roiz-Mesones M.P. Miguel-Martı´n M.D. Torroba L. Coira-Nieto A. Vasallo-Vidal F., Me´ndez-Lage S., Prieto-Rodriguez E., Eiros J.M., Torres J. and German,B.G. (2012). Identification of International Circulating Lineages of Methicillin-Resistant *Staphylococcus aureus* in the North of Spain and their Glycopeptide and Linezolid Susceptibility. *Journal of Medical Microbiology;* 61:305-307.

Uneke, C.J. and Ibeh, L.M. (2009). Impact of Deforestation on Malaria in Southeastern Nigeria: The Epidemiological, Socioeconomic and Ecological Implications. *The internet Journal of the Third world medicine* 8(1):3-9.

Umaru, G.A., Kabir, J., Adamu, N. and Umar, Y A. (2011). A Review of Emerging Methicillin-Resistant *Staphylococcus aureus* (MRSA): A growing Threat to Veterinarians. *Nigerian Veterinary Journal;* 32(3):174– 186.

Valsesia, G.M., Rossi, S., Bertschy, G. and Pfyffer, E.(2010). Emergence of SCC*mec* Type IV and SCC*mec* type V Methicillin-Resistant *Staphylococcus aurous* Containing the Panton-Valentine Leukocidin Genes in a Large Academic Teaching Hospital in Central Switzerland: External Invaders or Persisting Circulators? *Journal of Clinical. Microbiology*. 48:720-727.

van Cleef, B.A.G.L., Broens, E.M., Voss, A., Huijsdens, X.W., Zuchner, L., Van Benthem ,B.H.B., Kluytmans, J.A.J.W., Mulders, M.N. and Van De Giessen, A.W. (2010). High Prevalence of Nasal MRSA Carriage in Slaughterhouse Workers in Contact with Live Pigs in The Netherlands. *Epidemiology and Infection*; 138: 756-763

van Cleef, B.A.G.L., Graveland, H., Haenen, A.P.J., van de Giessen, A.W. Heederik, D., Wagenaar, J.A., and Kluytmans J.A.J.W. (2011). Persistence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Field Workers after Short-Term Occupational Exposure to Pigs and Veal Calves. *Journal of Clinical Micobiology;* 49(3): 1030-1033.

van Loo I., Huijsdens, X. and Tiemersma, E. (2007). Emergence of Methicillin Resistant *Staphylococcus aureus* of Animal Origin in Humans: *Emerging Infectious Diseases;* 13:1834-1839.

Vandenesch, F., Naimi, T., Enright, M.C., Lina, G., Nimmo, G.R., Heffernan, H., Liassine, N., Bes, M., Greenland, T., Reverdy, M. and Etienne, J. (2003). Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Carrying Panton-Valentine Leukocidin Genes: Worldwide Emergence. *Emerging Infectious Diseases*. 9(8):978–984

Vanderhaeghen, W., Cerpentier, T., Adriaensen, C., Vicca, J. Hermans, K. and Butaye, P. (2010). Methicillin-Resistant *Staphylococcus aureus* (MRSA) ST398 Associated with Clinical and Subclinical Mastitis in Belgian Cows. *Vetrinary Microbiology.* 144: 166–171

Vojtov, N., Ross, H.F. and Novick, R.P. (2002). Global Repression of Exotoxin Synthesis by Staphylococcal Superantigens. *PNAS; 99(15):*10102–10107,

Vorobieva, V., Bazhukova, T., Hanssen, A.M., Caugant, D.A., Semenova, N., Haldorsen B.C., Simosen G.S. and Sunsfjord, A. (2008). Clinical Isolates of *Staphylococcus aureus* from the Arkhangelsk region, Russia: Antimicrobial Susceptibility, Molecular Epidemiology, and Distribution of Panton-Valentine Leukocidin Genes. *APMIS;* 116: 877-887.

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

Warsa, U.M., Nonoyama, T., Ida, R., Okamoto, T., Okubo, C., Shimauchi, A., Kuga, and M. Inoue (1996). Detection of tet(K) and tet(M) in *Staphylococcus aureus* of Asian Countries by the Polymerase Chain Reaction. *Journal of Antibiotiotics (Tokyo)*; 49(11):1127-1132.

Waters, A.E., Contente-Cuomo, T., Buchhagen. J., Liu, CM., Watson. L., Pearce K Foster, JT., Bowers, J., Driebe, E.M., Engelthaler, D.M., Keirn, P.S. and Price, L.B. (2011). Multi-Drug Resistant *Staphylococcus aureus* in US Meat and Poultry. *Clin Infect Dis*; 53(2):1-4.

Weese, J.S. and VanDuijkeren, E. (2010). Methicillin-Resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in Veterinary Medicine. *Veterinary Microbiology*; 140 418–429

Weigel, L.M., Rodney, M.D., Sin, D.H., Jensen, B., Clark N.C., Mcdougal, L.K., Zhu, W., Musser, K.A., Thompson, J., Kohlerschmidt.Dumas, N., Limberger. R.J. and Patel, J.B. (2007). High-Level Vancomycin-Resistant *Staphylococcus aureus* Isolates Associated with a Polymicrobial Biofilm. *Antmicrobial Agents and Chemotherapy;* 5(1):231-238

Wertheim, H.F.L., Melles, D.C., Vos M.C., van Leeuwen, W., van Bekum A., Verbrugh H.A. and Nouwen, J. L. (2005). The Role of Nasal Carriage in *Staphylococcus aureus* Infections. *Lancet Infect Dis* 5: 751–62

Williams, R.E.O. (1963). Healthy carriage of *Staphylococcus aureus*: Its Prevalence and Importance. *Bacteriol Rev* 27**:** 56–71

White, D. G., Ayers, S J., Maurer, J.J., Thayer, S.G. and Hofacre. C (2003). Antimicrobial Susceptibilities of *Staphylococcus aureus* Isolated from Commercial Broilers in Northeastern Georgia. *Avian Dis*. 47:203-210

Witte, W. (2000). Selective Pressure by Antibiotic Use in Livestock. *Int. J. Antimicrob. Agents* 16:S19-S24.

Witte, W., Strommenger, B., Stanek C. and Cuny, C. (2007). Methicillin-Resistant *Staphylococcus aureus* ST398 in Humans and Animals, Central Europe. *Emerging Infectious Diseases.* 13(2): 255–258.

Wright, G.D (2012). Antibiotic Resistome: A framework Linking the Clinic and Environment. In: Antimicrobial Resistance in the Environment. Keen P.L and Montforts M.M.M. (ED). M. John Wiley and Sons Inc. New Jersey. USA. Pp. 15-2

Wu, D., Li, X., Yang, X., Zheng Y., Wang C., Deng, L., Liu, L., Li, C., Shang, Y., Zhao, C. ,Yu, S. and Shen X. (2010). Superantigen Gene Profiles and Presence of Exfoliative Toxin Genes in Community-Acquired Methicillin Resistant *Staphylococcus aureus* Isolated from Chinese Children**.** *Journal of Medical Microbiology*; 60(1):35-45.

Wulf, M.W., Verduin, C.M., van Nes, A., Huijsdens, X. and Voss, A (2012) Infection and Colonization with Methicillin Resistant *Staphylococcus aureus* ST398 versus other MRSA in an Area with a High Density of Pig Farms. *Eur J Clin Microbiol Infect Dis 31*: 61–65

Wulf, M.W., Sørum, M., Van Nes, A., Skov, R., Melchers. W.J. G., Klaassen, C.H.W. and Voss, A. (2008). Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study. *Clincal Microbiol Infect*; 14: 29–34.

Wulf, M., van Nes, A., Eikelenboom-Boskamp, A., de Vries, J., Melchers, W., Corné Klaassen, C. and Voss.A. (2006). Methicillin-Resistant *Staphylococcus aureus* in Veterinary Doctors and Students in the Netherlands (2006). *Emerging Infectious Diseases* 12:1939-1941

Yah, S.C., Enabulele I.O. and Eghafona, N.O. (2007). The Screening of Multi-Drug Resistance (MDR) Susceptibilities of *Staphylococcus aureus* and *Staphylococcus epidermidis* to Methicillin and Vancomycin in Teaching Hospitals in Nigeria. *Sudan Journal of Medical Science;* 2(4):257-262.

Zetola, N., Francis, J.S., Nuermberger, E., and Bishai W. (2005). Community-Acquired Meticillin-Resistant *Staphylococcus aureus*: An Emerging Threat. *The Lancet Infectious Diseases.* 5(5):275- 286.

Zhang, K., McClure, J.A., Elsayed, S. and Conly, J.M. (2009). Novel Staphylococcal Cassette Chromosome *mec* Type, Tentatively Designated Type VIII, Harboring Class A *mec* and Type 4 *ccr* Gene Complexes in a Canadian Epidemic Strain of Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy;* 53: 531-540.

APPENDIXES

Appendix 1a

Federal Teaching Hospital Abakaliki Ethical Clearance

FEDERAL TEACHING HOSPITAL ABAKALIKI

P.M.B. 102 ABAKALIKI EBONYI STATE, NIGERIA

TEL: 234-043-221628

DR. EZEONU OLISAEMEKA PAUL
M.B. BCH, FACH ARZT Für
Geburthilfe und gyäecology, Germany FICS.
Chief Medical Director

DR. NWIGWE, CHINEDU GREGORY
MB.BS, FMCS
Chairman Medical Advisory Committee

FETHA
LIVING FOR THE SICK

CHIEF C.C. OGBU JP, KSM
B.Sc. (Hons), MBA, MSc,
Fcai,Fnimn,Ahan,Mnim
Ag Director of Administration and Secretary to the Board.

Our Ref : _____
Your Ref : _____

Date: 26th February, 2013

Our Ref: FETHA/REC/VOL 1/2013/056

REC PROTOCOL NUMBER 10/12/2012 - 29/01/2013
REC APPROVAL NUMBER 10/12/2012 - 21/02/2013

Mr. Amos Nworie
Department of Medical Laboratory Sciences,
Ebonyi State University,
Abakliki

APPLICATION FOR ETHICAL APPROVAL

RE: MOLECULAR EVALUATION OF CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS FOR METHICILLIN RESISTANCE

I have the pleasure to inform you that the Research and Ethics Committee (REC) on 21st February, 2013 reviewed your research proposal on **"Molecular Evaluation of Clinical Isolates of Staphylococcus Aureus for Methicillin Resistance"** and has granted full approval for the study.

This approval dates from 21st February, 2013 to 20th February, 2014, if there is delay in starting the research, please inform the REC so that the dates of approval could be adjusted accordingly.

No changes are permitted in the research without prior approval by the committee. The committee reserves the right to conduct compliance visit to your research site without previous notification.

On completion of the study, a copy of the write-up must be made available to Research and Ethics Committee.

Yours Sincerely,

Assoc. Prof O. U. J. Umeora
Chairman, Research & Ethics Committee

APPENDIX 1b:

CDC Permit to Import or Transfer Etiological or Vectors of Human Disease

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL. 404-718-2077; FAX: 404-718-2093

CDC
SAFER · HEALTHIER · PEOPLE™

Permit to Import or Transfer Etiological Agents or Vectors of Human Disease

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in Item 1 below.

PHS PERMIT NO.	2012-03-016	
DATES	ISSUED: Friday, March 02, 2012	EXPIRES: Saturday, March 02, 2013
1. DESCRIPTION OF MATERIAL	ISOLATES OF STAPHYLOCOCCUS AUREUS, STREPTOCOCCUS PYOGENES AND STREPTOCOCCUS PNEUMONIAE.	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS)	TARA C. SMITH CEID, UNIVERSITY OF IOWA 2501 CROSSPARK RD., MTF B120 CORALVILLE, IA 52241	TEL: 319-335-4574 FAX: 319-335-4815
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	NIGERIA	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	☑ Multiple Importation into the US ☐ Single Transfer Within the US A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	
5. CONDITIONS OF ISSUANCE ITEMS APPLICABLE WHEN CHECKED	☐ A. Subsequent distribution, within the U.S., of the material described in this permit is prohibited without prior authorization by the Public Health Service. ☑ B. All material is for laboratory use only - Not for use in the production of biologics for humans or animals. ☑ C. All material is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. ☐ D. Additional Requirements: ☐ File APHIS/CDC Form 2 for select agents as defined in 42 CFR 73 ☐ IATA Packaged to preclude escape. ☐ USDA permit may be required (Telephone: 301-734-3277). ☑ E. Work with the agent(s) described shall be restricted to areas and conditions meeting requirements in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories." ☑ F. Packaging must conform to 49 CFR Sections 171-180. ☐ G. Select Agent. Receiving facility must be registered under 42 CFR Part 73.	
6. COPY SENT TO ☑ U.S. QUARANTINE STATION	7. Signature of issuing officer Robbin S. Weyant, PhD, RBP (ABSA) Captain, USPHS (Ret.) Etiologic Agent Import Permit Program	

CDC 0728 (F 13.40) REV. 2-91

42 CFR 71.54. Etiological agents, hosts, and vectors

(a) A person may not import into the United States, nor distribute after importation, any etiological agent or any arthropod or other animal host or vector of human disease, or any exotic living arthropod or other animal capable of being a host or vector of human disease unless accompanied by a permit issued by the Director.

(b) Any import coming within the provisions of the section will not be released from custody prior to receipt by the District Director of the U.S. Customs Service of a permit issued by the Director.

Note: Other permits may be required.

APPENDIX 2

Media preparation

Peptone water

Appropriate quantity of the premixed powder was dissolved in corresponding volume ofdeionized water and distributeded into tubes and flasks. They were autoclaved at 121^{o}C for 15 minutes.

Preparation of Manitol Salt Agar

Appropriate quantity of manitol salt agar powder containing 6.5% NaCl and phenol red was dissolved in the corresponding volume of distilled water, autoclaved at 121^{o}C for 15 minutes and allowed to cool and then dispensed aseptically into sterile Petri-dishes as described elsewhere (Kumar *et al*., 2011).

Preparation of Mueller-Hinton Agar

Appropriate quantity of Mueller-Hinton agar powder supplemented with 4% NaCl and another without NaCl was dissolved in corresponding volume of water according to the manufacturer's guideline and autoclaved at 121^{o}C for 15 minutes. This were allowed to cool and aseptically dispensed into sterile Petri-dishes for antibiotic sensitivity testing and for detection of MRSA as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008).

Preparation of Blood Agar

Appropriate quantity of nutrient agar powder was dissolved in corresponding volume of water and autoclaved at 121^{o}C for 15 minutes. The media was allowed to cool to 50^{o}C in a water bath. Sterile expired blood obtained from Federal Teaching Hospital Abakaliki was aseptically added and mixed properly. After this, the media was dispensed aseptically into sterile Petri-dishes and the surface flamed to remove air bubbles.

Appendix 3

Phenotypic Identification of isolates of *S. aureus* in hospital, poultry and poultry farm workers

	Hospital	Identification						
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	catalase	coag	latexaggl.
1.	amc-2	hemolysis	golden yellow	black col.wit clear zone	positve	positive	positive	positive
2.	amc-4	+	+	+	+	+	+	+
3.	amc-5	+	+	+	+	+	+	+
4.	amc-9	+	+	+	+	+	+	+
5.	amc-10	+	+	+	+	+	+	+
6.	amc-11	+	+	+	+	+	+	+
7.	amc-13	+	+	+	+	+	+	+
8.	amc-17	+	+	+	+	+	+	+
9.	amc-20	+	+	+	+	+	+	+
10.	amc-22	+	+	+	+	+	+	+
11.	amc-23	+	+	+	+	+	+	+
12.	amc-24	+	+	+	+	+	+	+
13.	amc-27	+	+	+	+	+	+	+
14.	amc-29	+	+	+	+	+	+	+
15.	amc-30	+	+	+	+	+	+	+
16.	amc-31	+	+	+	+	+	+	+
17.	amc-37	+	+	+	+	+	+	+
18.	amc-39	+	+	+	+	+	+	+
19.	amc-41	+	+	+	+	+	+	+
20.	amc-44	+	+	+	+	+	+	+
21.	amc-47	+	+	+	+	+	+	+
22.	amc-50	+	+	+	+	+	+	+
23.	amc-53	+	+	+	+	+	+	+
24.	amc-54	+	+	+	+	+	+	+
25.	amc-59	+	+	+	+	+	+	+
26.	amc-61	+	+	+	+	+	+	+
27.	amc-64	+	+	+	+	+	+	+
28.	amc-65	+	+	+	+	+	+	+
29.	amc-68	+	+	+	+	+	+	+
30.	amc-70	+	+	+	+	+	+	+
31.	amc-72	+	+	+	+	+	+	+
32.	amc-73	+	+	+	+	+	+	+
33.	amc-74	+	+	+	+	+	+	+
34.	amc-76	+	+	+	+	+	+	+
35.	amc-77	+	+	+	+	+	+	+
36.	amc-78	+	+	+	+	+	+	+
37.	amc-80	+	+	+	+	+	+	+
38.	amc-82	+	+	+	+	+	+	+
39.	amc-83	+	+	+	+	+	+	+

40.	amc-85	+	+	+	+	+	+	+
41.	amc-87	+	+	+	+	+	+	+
42.	amc-88	+	+	+	+	+	+	+
43.	amc-90	+	+	+	+	+	+	+
44.	amc-92	+	+	+	+	+	+	+
45.	amc-95	+	+	+	+	+	+	+
46.	amc-96	+	+	+	+	+	+	+
47	amc-98	+	+	+	+	+	+	+
48.	amc-101	+	+	+	+	+	+	+
49.	amc-103	+	+	+	+	+	+	+
50.	amc-104	+	+	+	+	+	+	+
51.	amc-106	+	+	+	+	+	+	+
52.	amc-108	+	+	+	+	+	+	+
53.	amc-110	+	+	+	+	+	+	+
54.	amc-111	+	+	+	+	+	+	+
55.	amc-112	+	+	+	+	+	+	+
56.	amc-114	+	+	+	+	+	+	+
57.	amc-116	+	+	+	+	+	+	+
58.	amc-118	+	+	+	+	+	+	+
59.	amc-119	+	+	+	+	+	+	+
60.	amc-120	+	+	+	+	+	+	+
61.	amc-122	+	+	+	+	+	+	+
62.	amc-123	+	+	+	+	+	+	+
63.	amc-125	+	+	+	+	+	+	+
64.	amc-128	+	+	+	+	+	+	+
65.	amc-129	+	+	+	+	+	+	+
66.	amc-131	+	+	+	+	+	+	+
67.	amc-133	+	+	+	+	+	+	+
68.	amc-135	+	+	+	+	+	+	+
69.	amc-137	+	+	+	+	+	+	+
70.	amc-139	+	+	+	+	+	+	+
71.	amc-140	+	+	+	+	+	+	+
72.	amc-142	+	+	+	+	+	+	+
73.	amc-143	+	+	+	+	+	+	+
74.	amc-145	+	+	+	+	+	+	+
75.	amc-147	+	+	+	+	+	+	+
76.	amc-148	+	+	+	+	+	+	+
77.	amc-149	+	+	+	+	+	+	+
78.	amc-150	+	+	+	+	+	+	+

	Farm-1			Identification				
	isolates	blood agar	Mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
79.	am-3	+	+	+	+	+	+	+
80.	am-6	+	+	+	+	+	+	+
81.	am-8	+	+	+	+	+	+	+
82.	am-11	+	+	+	+	+	+	+
83.	am-13	+	+	+	+	+	+	+

84.	am-17	+	+	+	+	+	+	+
85.	am-18	+	+	+	+	+	+	+
86.	am-27	+	+	+	+	+	+	+
87.	am-31	+	+	+	+	+	+	+
88.	am-46	+	+	+	+	+	+	+
89.	am-49	+	+	+	+	+	+	+
90.	am-53	+	+	+	+	+	+	+
91.	am-58	+	+	+	+	+	+	+
92.	am-67	+	+	+	+	+	+	+
93.	am-94	+	+	+	+	+	+	+
94.	am-114	+	+	+	+	+	+	+
95.	am-117	+	+	+	+	+	+	+
96.	am-118	+	+	+	+	+	+	+
97.	am-129	+	+	+	+	+	+	+
98.	am-132	+	+	+	+	+	+	+
99.	am-138	+	+	+	+	+	+	+
100.	am-140	+	+	+	+	+	+	+
101.	am-145	+	+	+	+	+	+	+
102.	am-157	+	+	+	+	+	+	+
103.	am-164	+	+	+	+	+	+	+
104.	am-169	+	+	+	+	+	+	+
105.	am-173	+	+	+	+	+	+	+
106.	am-174	+	+	+	+	+	+	+
107.	am-180	+	+	+	+	+	+	+
108.	am-191	+	+	+	+	+	+	+
109.	am-192	+	+	+	+	+	+	+
110.	am-196	+	+	+	+	+	+	+
111	am-197	+	+	+	+	+	+	+

	Farm 2	Identification						
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
112	ar-7	positve	positive	positive	positve	positive	positive	positive
113.	ar-10	+	+	+	+	+	+	+
114.	ar-13	+	+	+	+	+	+	+
115.	ar-15	+	+	+	+	+	+	+
116.	ar-21	+	+	+	+	+	+	+
117.	ar-37	+	+	+	+	+	+	+
118.	ar.42	+	+	+	+	+	+	+
119.	ar-49	+	+	+	+	+	+	+
120	ar-54	+	+	+	+	+	+	+
121.	ar-60	+	+	+	+	+	+	+
122.	ar-83	+	+	+	+	+	+	+
123.	ar-84	+	+	+	+	+	+	+
124.	ar-89	+	+	+	+	+	+	+
125.	ar-103	+	+	+	+	+	+	+
126.	ar-107	+	+	+	+	+	+	+
127.	ar-116	+	+	+	+	+	+	+

128.	ar-132	+	+	+	+	+	+	+
129.	ar-142	+	+	+	+	+	+	+
130.	ar-149	+	+	+	+	+	+	+
131.	ar-154	+	+	+	+	+	+	+
132.	ar-155	+	+	+	+	+	+	+
133.	ar-163	+	+	+	+	+	+	+
134.	ar-168	+	+	+	+	+	+	+
135.	ar-171	+	+	+	+	+	+	+
136.	ar-175	+	+	+	+	+	+	+
137.	ar-178	+	+	+	+	+	+	+
138.	ar-182	+	+	+	+	+	+	+
139.	ar-186	+	+	+	+	+	+	+
140.	ar-188	+	+	+	+	+	+	+
141.	ar-193	+	+	+	+	+	+	+
142.	ar-198	+	+	+	+	+	+	+
	Farm-3			Identification				
	isolates	blood agar	Mannitol salt agar	Baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
143.	b-3	positve	positive	positive	positve	positive	positive	positive
144.	b-8	+	+	+	+	+	+	+
145.	b-10	+	+	+	+	+	+	+
146.	b-11	+	+	+	+	+	+	+
147.	b-16	+	+	+	+	+	+	+
148.	b-17	+	+	+	+	+	+	+
149.	b-19	+	+	+	+	+	+	+
150.	b-20	+	+	+	+	+	+	+
151.	b-22	+	+	+	+	+	+	+
152.	b-27	+	+	+	+	+	+	+
153.	b-29	+	+	+	+	+	+	+
154.	b-37	+	+	+	+	+	+	+
155.	b-40	+	+	+	+	+	+	+
156.	b-45	+	+	+	+	+	+	+
157.	b-48	+	+	+	+	+	+	+
158.	b-53	+	+	+	+	+	+	+
159.	b-61	+	+	+	+	+	+	+
160.	b-72	+	+	+	+	+	+	+
161.	b-80	+	+	+	+	+	+	+
162.	b-93	+	+	+	+	+	+	+
163.	b-97	+	+	+	+	+	+	+
164.	b-103	+	+	+	+	+	+	+
165.	b-111	+	+	+	+	+	+	+
166.	b-117	+	+	+	+	+	+	+
167.	b-121	+	+	+	+	+	+	+
168.	b-136	+	+	+	+	+	+	+
169.	b-139	+	+	+	+		+	+
170.	b-140	+	+	+	+	+	+	+
171.	b-167	+	+	+	+			+

172.	b-175	+	+	+	+	+	+	+
	Farm 4			Identification				
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
173.	bp-1	+	+	+	+	+	+	+
174.	bp-4	+	+	+	+	+	+	+
175.	bp-11	+	+	+	+	+	+	+
176.	bp-13	+	+	+	+	+	+	+
177.	bp-16	+	+	+	+	+	+	+
178.	bp-19	+	+	+	+	+	+	+
179.	bp-24	+	+	+	+	+	+	+
180.	bp-32	+	+	+	+	+	+	+
181.	bp-39	+	+	+	+	+	+	+
182.	bp-47	+	+	+	+	+	+	+
183.	bp-49	+	+	+	+	+	+	+
184.	bp-63	+	+	+	+	+	+	+
185.	bp-66	+	+	+	+	+	+	+
186.	bp-71	+	+	+	+	+	+	+
187.	bp-80	+	+	+	+	+	+	+
188.	bp-88	+	+	+	+	+	+	+
189.	bp-92	+	+	+	+	+	+	+
190	bp-103	+	+	+	+	+	+	+
191.	bp-107	+	+	+	+	+	+	+
192.	bp-110	+	+	+	+	+	+	+
193.	bp-137	+	+	+	+	+	+	+
194.	bp-146	+	+	+	+	+	+	+
195.	bp-157	+	+	+	+	+	+	+
	Farm 5			Identification				
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
196.	l-8	positve	positive	positive	positve	positive	positive	positive
197.	l-10	+	+	+	+	+	+	+
198.	l-12	+	+	+	+	+	+	+
199.	l-17	+	+	+	+	+	+	+
200.	l-20	+	+	+	+	+	+	+
201.	l-27	+	+	+	+	+	+	+
202.	l-39	+	+	+	+	+	+	+
203.	l-40	+	+	+	+	+	+	+
204.	l-42	+	+	+	+	+	+	+
205.	l-60	+	+	+	+	+	+	+
206.	l-71	+	+	+	+	+	+	+
207.	l-79	+	+	+	+	+	+	+
208.	l-82	+	+	+	+	+	+	+
209.	l-95	+	+	+	+	+	+	+
210.	l-119	+	+	+	+	+	+	+
211.	l-132	+	+	+	+	+	+	+
212.	l-137	+	+	+	+	+	+	+

213.	l-144	+	+	+	+	+	+	+
214.	l-151	+	+	+	+	+	+	+
215.	l-177	+	+	+	+	+	+	+
216.	l-178	+	+	+	+	+	+	+
217.	l-180	+	+	+	+	+	+	+
218.	l-183	+	+	+	+	+	+	+
219.	l-187	+	+	+	+	+	+	+
220.	l-191	+	+	+	+	+	+	+
	Farm 6			Identification				
	isolates	blood agar	Mannitol salt agar	Baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
221.	ocl-5	positve	positive	positive	positve	Positive	positive	positive
222.	ocl-8	+	+	+	+	+	+	+
223.	ocl-15	+	+	+	+	+	+	+
224.	ocl-17	+	+	+	+	+	+	+
225.	ocl-22	+	+	+	+	+	+	+
226.	ocl-24	+	+	+	+	+	+	+
227.	ocl-33	+	+	+	+	+	+	+
228.	ocl-42	+	+	+	+	+	+	+
229.	ocl-48	+	+	+	+	+	+	+
230.	ocl-53	+	+	+	+	+	+	+
231.	ocl-59	+	+	+	+	+	+	+
232.	ocl-66	+	+	+	+	+	+	+
233.	ocl-83	+	+	+	+	+	+	+
234.	ocl-86	+	+	+	+	+	+	+
235.	ocl-89	+	+	+	+	+	+	+
236.	ocl-97	+	+	+	+	+	+	+
237.	ocl-109	+	+	+	+	+	+	+
238.	0cl-127	+	+	+	+	+	+	+
239.	ocl-130	+	+	+	+	+	+	+
240.	ocl-134	+	+	+	+	+	+	+
241.	ocl-147	+	+	+	+	+	+	+
242.	ocl-169	+	+	+	+	+	+	+
243.	ocl-182	+	+	+	+	+	+	+
	Farm 7			Identification				
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
244.	acl-1	positve	positive	positive	positve	positive	positive	positive
245.	acl-8	+	+	+	+	+	+	+
246.	acl-10	+	+	+	+	+	+	+
247.	acl-17	+	+	+	+	+	+	+
248.	acl-22	+	+	+	+	+	+	+
249.	acl-27	+	+	+	+	+	+	+
250.	acl-31	+	+	+	+	+	+	+
251.	acl-38	+	+	+	+	+	+	+
252.	acl-43	+	+	+	+	+	+	+
253.	acl-49	+	+	+	+	+	+	+

253.	acl-54	+	+	+	+	+	+	+
254.	acl-58	+	+	+	+	+	+	+
255.	acl-63	+	+	+	+	+	+	+
256.	acl-70	+	+	+	+	+	+	+
257.	acl-77	+	+	+	+	+	+	+
258.	acl-81	+	+	+	+	+	+	+
259.	acl-85	+	+	+	+	+	+	+
260.	acl-94	+	+	+	+	+	+	+
261.	acl-99	+	+	+	+	+	+	+
262.	acl-107	+	+	+	+	+	+	+
263.	acl-113	+	+	+	+	+	+	+
264.	acl-118	+	+	+	+	+	+	+
265.	acl-124	+	+	+	+	+	+	+
266.	acl-128	+	+	+	+	+	+	+
267.	acl-137	+	+	+	+	+	+	+
268.	acl-143	+	+	+	+	+	+	+
269.	acl-149	+	+	+	+	+	+	+
270.	acl-155	+	+	+	+	+	+	+
271.	acl-159	+	+	+	+	+	+	+
272.	acl-166	+	+	+	+	+	+	+
273.	acl-173	+	+	+	+	+	+	+
274.	acl-180	+	+	+	+	+	+	+
275.	acl-191	+	+	+	+	+	+	+
276.	acl-194	+	+	+	+	+	+	+
277.	acl-198	+	+	+	+	+	+	+

		Identification						
278.	Farm 8							
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
279.	cl-4	+	+	+	+	+	+	+
280.	cl-10	+	+	+	+	+	+	+
281.	cl-12	+	+	+	+	+	+	+
282.	cl-17	+	+	+	+	+	+	+
283.	cl-18	+	+	+	+	+	+	+
284.	c-l22	+	+	+	+	+	+	+
285.	cl-24	+	+	+	+	+	+	+
286.	cl-28	+	+	+	+	+	+	+
287.	cl-31	+	+	+	+	+	+	+
288.	cl-53	+	+	+	+	+	+	+
289.	cl-54	+	+	+	+	+	+	+
290.	cl-88	+	+	+	+	+	+	+
291.	cl-97	+	+	+	+	+	+	+
292.	cl-109	+	+	+	+	+	+	+
293.	cl-115	+	+	+	+	+	+	+
294.	cl-122	+	+	+	+	+	+	+
295.	cl-131	+	+	+	+	+	+	+
296.	cl-133	+	+	+	+	+	+	+
297.	cl-137	+	+	+	+	+	+	+

298.	cl-149	+	+	+	+	+	+	+
299.	cl-162	+	+	+	+	+	+	+
300.	cl-182	+	+	+	+	+	+	+
301.	Farm 9			Identification				
	isolates	blood agar	Mannitol salt agar	Baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
	sp-7	positve	positive	positive	positve	positive	positive	positive
302.	sp-11	+	+	+	+	+	+	+
303.	sp-18	+	+	+	+	+	+	+
304.	sp-19	+	+	+	+	+	+	+
305.	sp-29	+	+	+	+	+	+	+
306.	sp-40	+	+	+	+	+	+	+
307.	sp-52	+	+	+	+	+	+	+
308.	sp-60	+	+	+	+	+	+	+
309.	sp-68	+	+	+	+	+	+	+
310.	sp-70	+	+	+	+	+	+	+
311.	sp-77	+	+	+	+	+	+	+
312.	sp-94	+	+	+	+	+	+	+
313.	sp-99	+	+	+	+	+	+	+
314.	sp-106	+	+	+	+	+	+	+
315.	sp-111	+	+	+	+	+	+	+
316.	sp-118	+	+	+	+	+	+	+
317.	sp-119	+	+	+	+	+	+	+
318.	sp-138	+	+	+	+	+	+	+
319.	sp-151	+	+	+	+	+	+	+
320.	sp-153	+	+	+	+	+	+	+
321.	sp-154	+	+	+	+	+	+	+
322.	sp-167	+	+	+	+	+	+	+
323.	sp-173	+	+	+	+	+	+	+
324.	sp-180	+	+	+	+	+	+	+
325.	sp-186	+	+	+	+	+	+	+
326.	workers			Identification				
	isolates	blood agar	mannitol salt agar	baird parker agar	Gram rx	Catalase	Coag	Latex aggl.
	am-26	+	+	+	+	+	+	+
327.	b-13	+	+	+	+	+	+	+
328.	cl-8	+	+	+	+	+	+	+
329.	sp-3	+	+	+	+	+	+	+

Keys:

BAH = Blood agar hemolysis, MSAGY = Mannitol salt agar golden yellow, BPA = Baird parker agar, GS = Gram stain, CA = Catalase, CO = Coagulase, LA = Latex agglutination, + = positive reaction, - = negatice reaction.

Appendix 4

Isolation of *S. aureus* in both cloacae and nostril of poultry birds in Ebonyi State

Farm	Cloacae & Nostril	Cloacae	Nostril
Farm 1	17 (51.6%)	10 (30.3%)	6 (18.1%)
Farm 2	14 (45.2%)	9 (29.0%)	8 (25.8%)
Farm 3	14 (46.6%)	8 (26.7%)	8 (26.7%)
Farm 4	16 (69.6%)	5 (21.7%)	2 (8.7%)
Farm 5	14 (5.06%)	8 (32.0%)	3 (12.0%)
Farm 6	11 (48.0%)	7 (30.0%)	5 (22.0%)
Farm 7	22 (62.9%)	8 (22.0%)	5 (14.2%)
Farm 8	10 (45.4%)	6 (27.3%)	6 (27.3%)
Farm 9	11 (44.0%)	6 (24%)	8 (32.0%)

Appendix 5a

Table 4.2: *Staphylococcus aureus* recovered from broilers and layers in the poultry farms

Anova on staphylococcus aureus recovered from broilers and layers

ANOVA

S. aureus

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	.500	1	.500	.037	.850
Within Groups	215.111	16	13.444		
Total	215.611	17			

Significant at $P \leq 0.05$

Appendix 5b

Table 4.3: recovery of *S. aureus* from cloacae & nostrils, cloacae, nostrils)

Anova on birds with s.aureus in cloacae & nostril, cloacae and nostrils

ANOVA

S. aureus

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	254.889	2	127.444	9.351	.001
Within Groups	327.111	24	13.630		
Total	582.000	26			

Significant at P≤ 0.05

Appendix 5c

Table 4.9: Prevalence of MRSA in each of the farms screened.

Anova on the incidence of broilers and layers

ANOVA

MRSA

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	2.722	1	2.722	2.450	.137
Within Groups	17.778	16	1.111		
Total	20.500	17			

Significant at P≤ 0.05

Appendix 5d: Table 4.10: Anova on prevalence of MRSA amongst s.aureus in each of the hospitals screened

ANOVA

HOSPITAL

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.000	2	1.000	.818	.472
Within Groups	11.000	9	1.222		
Total	13.000	11			

Significant at P≤ 0.05

Appendix 6

***S. aureus* from isolates of staphylococci from 3 hospitals in Ebonyi State**

Hospital	Staphylococci	*S. aureus*	Total
Federal Teaching Hospital	22	28	50
Onueke General Hospital	31	19	50
Afikpo Medical Center	19	31	50
Total	**72**	**78**	**150**

Appendix 7

Antibiotics, Strength, Zone Of Inhibition and classes of antibiotics used

Antibiotic	Strength	Resistant zone diameter	Class of Antibiotics
Augmentin (Amox--clav)	30µg	≤ 19mm	β-lactamase inhibitor combination
Chloramphenicol	30µg	≤ 12mm	Phenolics
Cefoxitin	30µg	≤ 24 mm	Penicillins
Dalfopristin	15µg	≤ 15mm	Streptogramins
Deoxycycline	30µg	≤ 12mm	Tetracyclines
Erythromycin	15µg	≤ 13mm	Macrolides
Gentamycin	10µg	≤ 12mm	Aminoglycosides
Levofloxacin	5µg	≤ 15mm	Fluoroquinolones
Linezolid	30µg	≤ 20mm	Oxazolidinones
Rifampin	5µ	≤ 16mm	Ansamycins
Trim/Sulfamethoxazole	1.25/23.75µg	≤ 10mm	Folate Pathway Inhibitor
Tetracycline	30µg	≤ 14mm	Tetracyclines
Cefuroxime	30µg	≤ 14mm	Cephems
Vancomycin	30µg	≤ 14mm	Glycopeptide

Appendix 8

Antibiotic resistance profiles of the *S. aureus* isolates from hospital, poultry and poultry farm workers

	Hospital	Antibiotics profile													
	isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
1.	amc-2	R	R	S	S	S	S	R	S	R	S	S	R	R	S	6
2.	amc-4	S	R	R	R	S	S	S	R	S	S	S	R	R	S	6
3.	amc-5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
4.	amc-9	R	R	R	S	S	S	S	S	R	S	S	R	S	S	5
5.	amc-10	R	R	S	S	S	S	R	S	S	S	S	R	R	S	5
6.	amc-11	R	R	S	S	S	S	S	S	S	S	S	S	S	S	2
7.	amc-13	R	R	R	S	S	S	S	S	S	S	S	R	R	S	5
8.	amc-17	S	S	S	R	S	S	S	S	R	S	S	R	R	S	4
9.	amc-20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
10.	amc-22	R	R	S	R	S	S	R	S	S	S	S	R	S	S	5
11.	amc-23	R	R	S	R	S	S	S	S	S	S	S	S	R	S	4
12.	amc-24	R	R	R	S	S	R	S	S	R	S	S	R	S	S	6
13.	amc-27	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
14.	amc-29	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
15.	amc-30	R	R	S	R	S	S	R	S	S	S	S	R	R	S	6
16.	amc-31	S	R	R	S	S	S	S	R	S	S	S	R	S	S	4
17.	amc-37	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
18.	amc-39	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
19.	amc-41	S	S	S	R	S	S	S	R	R	S	S	S	S	S	3
20.	amc-44	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
21.	amc-47	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
22.	amc-50	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
23.	amc-53	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
24.	amc-54	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

25.	amc-59	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
26.	amc-61	S	S	R	S	S	S	S	S	S	S	S	R	S	S	2
27.	amc-64	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
28.	amc-65	S	S	S	R	S	S	R	S	S	S	S	R	S	S	3
29.	amc-68	S	S	S	R	S	S	R	S	S	S	S	S	S	S	2
30.	amc-70	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
31.	amc-72	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
32.	amc-73	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
33.	amc-74	S	S	S	R	S	S	R	S	R	S	S	R	R	S	5
34.	amc-76	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
35.	amc-77	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
36.	amc-78	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
37.	amc-80	S	R	R	R	S	S	S	S	S	S	S	S	R	S	4
38.	amc-82	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
39.	amc-83	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
40.	amc-85	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
41.	amc-87	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
42.	amc-88	S	S	S	S	S	R	S	R	S	S	R	R	R	S	5
43.	amc-90	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
44.	amc-92	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
45.	amc-95	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
46.	amc-96	S	S	R	R	S	S	R	S	S	S	S	S	R	S	4
47.	amc-98	S	S	R	R	S	S	R	S	S	S	S	R	R	S	5
48.	amc-101	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
49.	amc-103	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
50.	amc-104	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
51.	amc-106	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
52.	amc-108	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
53.	amc-110	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
54.	amc-111	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
55.	amc-112	S	S	S	R	S	R	S	S	S	S	S	S	S	S	2

56.	amc-114	S	S	R	R	S	S	S	R	S	S	S	R	S	S	4
57.	amc-116	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
58.	amc-118	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
59.	amc-119	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
60.	amc-120	S	S	R	R	S	R	S	S	S	S	S	S	R	S	4
61.	amc-122	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
62.	amc-123	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
63.	amc-125	S	S	S	R	S	S	S	S	S	S	S	S	S	S	1
64.	amc-128	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
65.	amc-129	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
66.	amc-131	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
67.	amc-133	S	S	R	R	S	S	S	S	S	S	S	R	R	S	4
68.	amc-135	S	S	S	S	S	R	S	S	S	S	S	S	S	S	1
69.	amc-137	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
70.	amc-139	S	S	S	R	S	R	S	S	S	S	S	R	S	S	3
71.	amc-140	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
72.	amc-142	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
73.	amc-143	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
74.	amc-145	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
75.	amc-147	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
76.	amc-148	S	S	R	R	S	R	S	S	S	S	S	R	R	S	5
77.	amc-149	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
78.	amc-150	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

	Farm-1	Antibiotic profile													
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
79.	am-1	R	S	R	S	S	R	S	R	S	S	S	R	R	S	6
80.	am-3	S	R	S	S	S	S	S	S	S	S	S	S	S	S	1
81.	am-6	S	R	S	R	S	S	S	S	S	S	S	S	R	S	3
82.	am-8	S	R	S	S	S	S	S	S	S	S	S	R	R	S	3

83.	am-11	S	R	R	S	S	S	S	R	S	S	S	S	R	S	4
84.	am-13	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
85.	am-17	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
86.	am-18	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
87.	am-27	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
88.	am-31	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
89.	am-46	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
90.	am-49	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
91.	am-53	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
92.	am-58	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
93.	am-67	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
94.	am-94	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
95.	am-114	S	S	R	S	S	S	S	S	S	S	S	S	S	S	1
96.	am-117	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
97.	am-118	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
98.	am-129	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
99.	am-132	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
100.	am-138	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
101.	am-140	S	S	R	R	S	R	S	S	S	S	S	R	R	S	5
102.	am-145	S	S	S	S	S	S	R	S	S	S	S	S	R	S	2
103.	am-157	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
104.	am-164	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
105.	am-169	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
106.	am-173	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
107.	am-174	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
108.	am-180	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
109.	am-191	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
110.	am-192	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
111.	am-196	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
	Farm 2	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	

112.	ar-7	S	S	R	S	S	S	S	R	S	S	S	R	S	S	3
113.	ar-10	S	S	S	S	S	R	R	S	S	S	S	R	R	S	4
114.	ar-13	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
115.	ar-15	S	S	S	R	S	S	R	S	S	S	S	S	R	S	3
116.	ar-21	R	R	R	S	S	S	S	R	S	S	S	R	S	S	5
117.	ar-37	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
118.	ar.42	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
119.	ar-49	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
120.	ar-54	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
121.	ar-60	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
122.	ar-83	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
123.	ar-84	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
124.	ar-89	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
125.	ar-103	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
126.	ar-107	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
127.	ar-116	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
128.	ar-132	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
129.	ar-142	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
130.	ar-149	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
131.	ar-154	S	S	S	S	S	R	S	S	S	S	S	S	R	S	2
132.	ar-155	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
133.	ar-163	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
134.	ar-168	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
135.	ar-171	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
136.	ar-175	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
137.	ar-178	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
138.	ar-182	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
139.	ar-186	S	S	S	S	S	S	R	S	S	S	S	S	R	S	2
140.	ar-188	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
141.	ar-193	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
142.	ar-198	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

	Farm-3	Antibiotic profile													
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sulf	Van	
143.	b-3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
144.	b-8	S	S	R	S	S	S	R	R	S	S	S	R	R	S	5
145.	b-10	S	S	S	S	S	R	R	S	S	S	S	R	R	S	4
146.	b-11	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
147.	b-16	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
148.	b-17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
149.	b-19	S	S	S	R	S	S	R	S	S	S	S	R	R	S	4
150.	b-20	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
151.	b-22	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
152.	b-27	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
153.	b-29	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
154.	b-37	S	S	S	S	S	R	R	S	S	S	S	R	R	S	4
155.	b-40	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
156.	b-45	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
157.	b-48	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
158.	b-53	S	S	S	S	S	S	S	R	S	S	S	S	R	S	2
159.	b-61	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
160.	b-72	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
161.	b-80	S	R	R	S	S	S	S	S	S	S	S	S	R	S	3
162.	b-93	S	S	S	S	S	S	S	R	S	S	S	R	R	S	3
163.	b-97	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
164.	b-103	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
165.	b-111	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
166.	b-117	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
167.	b-121	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
168.	b-136	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
169.	b-139	S	S	S	R	S	S	R	S	S	S	S	S	S	S	2
170.	b-140	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
171.	b-167	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

172.	b-175	S	S	S	S	S	S	R	R	S	S	S	R	R	S	4
	Farm 4	Antibiotic profile													
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
173.	bp-1	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
174.	bp-4	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
175.	bp-11	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
176.	bp-13	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
177.	bp-16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
178.	bp-19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
179.	bp-24	S	S	S	R	S	S	S	R	S	S	S	R	R	S	4
180.	bp-32	S	S	S	S	S	R	S	S	S	S	S	S	S	S	1
181.	bp-39	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
182.	bp-47	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
183.	bp-49	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
184.	bp-63	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
185.	bp-66	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
186.	bp-71	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
187.	bp-80	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
188.	bp-88	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
189.	bp-92	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
190.	bp-103	S	S	S	R	S	R	R	S	S	S	S	S	R	S	4
191.	bp-107	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
192.	bp-110	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
193.	bp-137	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
194.	bp-146	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
195.	bp-157	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
	Farm 5	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
196.	l-8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
197.	l-10	S	R	R	S	S	S	R	R	S	S	S	S	R	S	5
198.	l-12	S	S	S	S	S	R	S	S	S	S	S	S	S	S	0

199.	l-17	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
200.	l-20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
201.	l-27	S	R	S	S	S	R	S	R	S	S	S	R	R	S	5
202.	l-39	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
203.	l-40	S	S	S	R	S	S	R	S	S	S	S	S	R	S	3
204.	l-42	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
205.	l-60	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
206.	l-71	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
207.	l-79	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
208.	l-82	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
209.	l-95	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
210.	l-119	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
211.	l-132	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
212.	l-137	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
213.	l-144	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
214.	l-151	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
215.	l-177	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
216.	l-178	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
217.	l-180	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
218.	l-183	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
219.	l-187	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
220.	l-191	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
	Farm 6	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
221.	ocl-5	S	R	S	R	S	S	S	S	S	S	S	R	R	S	4
222.	ocl-8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
223.	ocl-15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
224.	ocl-17	S	R	S	R	S	S	S	S	S	S	S	R	R	S	4
225.	ocl-22	S	R	S	S	S	S	S	S	S	S	S	R	R	S	3
226.	ocl-24	S	R	S	S	S	R	S	R	R	S	S	S	S	S	4
227.	ocl-33	S	S	S	S	S	S	S	S	S	S	S	R	S	S	0

228.	ocl-42	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
229.	ocl-48	S	S	S	R	S	S	R	S	S	S	S	S	R	S	3
230.	ocl-53	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
231.	ocl-59	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
232.	ocl-66	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
233.	ocl-83	S	S	S	R	S	S	S	S	S	S	S	R	S	S	2
234.	ocl-86	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
235.	ocl-89	S	S	S	S	S	R	S	S	S	S	S	R	R	S	3
236.	ocl-97	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
237.	ocl-109	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
238.	0cl-127	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
239.	ocl-130	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
240.	ocl-134	S	S	S	S	S	R	R	S	S	S	S	R	S	S	3
241.	ocl-147	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
242.	ocl-169	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
243.	ocl-182	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
	Farm 7	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
244.	acl-1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
245.	acl-8	S	R	S	S	S	R	S	S	S	S	S	S	R	S	3
246.	acl-10	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
247.	acl-17	S	S	S	S	S	S	R	R	S	S	S	R	S	S	3
248.	acl-22	S	S	S	S	S	S	S	S	S	S	S	R	S	S	0
249.	acl-27	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
250.	acl-31	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
251.	acl-38	S	R	S	S	S	S	S	R	S	S	S	S	R	S	3
252.	acl-43	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
253.	acl-49	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
254.	acl-54	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
255.	acl-58	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
256.	acl-63	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

257.	acl-70	S	S	S	R	S	S	S	S	S	S	S	S	R	S	2
258.	acl-77	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
259.	acl-81	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
260.	acl-85	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
261.	acl-94	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
262.	acl-99	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
263.	acl-107	S	S	S	R	S	R	S	S	S	S	S	S	S	S	2
264.	acl-113	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
265.	acl-118	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
266.	acl-124	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
267.	acl-128	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
268.	acl-137	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
269.	acl-143	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
270.	acl-149	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
271.	acl-155	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
272.	acl-159	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
273.	acl-166	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
274.	acl-173	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
275.	acl-180	S	S	S	R	S	R	S	S	S	S	S	S	S	S	2
276.	acl-191	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
277.	acl-194	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
278.	acl-198	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
	Farm 8	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
279.	cl-4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
280.	cl-10	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
281.	cl-12	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
282.	cl-17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
283.	cl-18	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
284.	c-l22	S	S	S	R	S	S	R	S	S	S	S	S	R	S	S	3
285.	cl-24	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

286.	cl-28	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
287.	cl-31	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
288.	cl-53	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
289.	cl-54	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
290.	cl-88	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
291.	cl-97	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
292.	cl-109	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
293.	cl-115	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
294.	cl-122	S	S	S	S	S	R	S	S	S	S	S	S	S	S	1
295.	cl-131	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
296.	cl-133	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
297.	cl-137	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
298.	cl-149	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
299.	cl-162	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
300.	cl-182	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
	Farm 9	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
301.	sp-7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
302.	sp-11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
303.	sp-18	S	S	S	R	S	S	R	S	S	S	S	S	S	S	2
304.	sp-19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
305.	sp-29	S	S	S	S	S	S	R	S	S	S	S	R	S	S	2
306.	sp-40	S	S	S	S	S	R	R	S	S	S	S	R	S	S	3
307.	sp-52	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
308.	sp-60	S	S	S	S	S	S	R	S	S	S	S	R	R	S	3
309.	sp-68	S	S	S	S	S	S	R	S	R	S	S	R	S	S	3
310.	sp-70	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
311.	sp-77	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
312.	sp-94	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
313.	sp-99	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
314.	sp-106	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1

315.	sp-111	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
316.	sp-118	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
317.	sp-119	S	S	S	S	S	R	S	S	S	S	S	S	S	S	1
318.	sp-138	S	S	S	S	S	S	S	S	S	S	S	S	R	S	1
319.	sp-151	S	S	S	S	S	S	R	S	S	S	S	S	S	S	1
320.	sp-153	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
321.	sp-154	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
322.	sp-167	S	S	S	S	S	S	S	S	S	S	S	R	R	S	2
323.	sp-173	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
324.	sp-180	S	S	S	R	S	S	R	S	S	S	S	S	S	S	2
325.	sp-186	S	S	S	R	S	S	S	S	S	S	S	R	R	S	3
	Workers	Antibiotic profile														
	Isolates	Aug	Cef	Cefx	Chl	Dalf	Doxy	Ery	Genta	Levo	Line	Rif	Tetra	Trim/Sul	Van	
326.	am-26	R	R	S	R	S	S	S	S	S	S	S	R	S	S	4
327.	b-13	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
328.	cl-8	S	S	R	S	S	S	S	S	R	S	S	S	R	S	3
329.	sp-3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0

Keys:

Aug: Augmentin; Cef: Cefoxitin; Cefx: Cefruxime, Chl: Chlorophenicol; Quin/Dal: Quinqupristin/Dalfopristin; Doxy: Doxycycline; Ery: Erythromycin; Genta. Gentamycin: Lev: Levofloxazin; Line: Linezolid; Rif: Rifampin; Trim/Sul: Trimethoprim/Sulfamethaoxazole; Tetra: Tetracycline; Van: Vancomycin, AR: Antibiotics resistance, S: sensitive, R: Resistance

Appendix 9

No of *S. aureus* resistant to each of the antibiotics tested.

Antibiotics	Number of isolates resistant	% of isolates resistant
Augumentin	11	3.3
Chloramphenicol	63	19.1
Cefoxitin	28	8.5
Quinuspristin/Dalfopristin	0	0
Doxycycline	26	7.9
Erythromycin	60	18.2
Gentamycin	19	5.8
Levofloxacin	9	2.7
Linezolid	0	0
Rifampin	0	0
Trimethoprim/Sulfamethoxazole	140	42.6
Tetracycline	157	47.7
Cefuroxime	22	6.7
Vancomycin	0	0

Appendix 10

Antbiotic resistance patterns of all the isolates

Antibiotics Resistant Patterns	Number of Resistance	Percentage Prevalence (%)
Zero	109	33.13
Pattern 1		
Tetra	30	9.12
Trim/Sulf	16	4.86
Chl	1	0.30
Doxy	4	1.21
Ery	11	3.34
Cefx	1	0.30
Total	**63**	
Pattern 2		
Chl,Trim/Sulf	5	1.52
Chl, Ery	4	1.21
Tetra, Trim/Sulf	42	12.76
Ery, Trim/Sulf	2	0.60
Ery, Tetra	7	2.12
Chl, Tetra	1	0.30
Chl, Doxy	2	0.60
Levo, Trim/Sulf	1	0.30
Doxy, trim/Sulf	1	0.30
Total	**65**	

Pattern 3

Chl, Tetra, trim/Sulf	15	4.56
Chl, Genta, Levo	1	0.30
Chl, Ery, Tetra	2	0.60
Chl, Ery, Trim/Sulf	4	1.21
Chl, Doxy, Tetra	2	0.60
Ery, Tetra, Trim/Sulf	7	2.12
Cef, Chl, Trim/Sulf	1	0.30
Cef, Tetra, Trim/Sulf	1	0.30
Doxy, Tetra, Trim/Sulf	1	0.30
Cefx, Genta, Tetra	1	0.30
Cef, Cefx,Trim/Sulf	1	0.30
Genta, Tetra, Trim/Sulf	1	0.30
Doxy, Ery, Tetra	2	0.60
Cef, Doxy, Trim/Sulf	1	0.30
Ery, Genta, Tetra	1	0.30
Cef, Genta, Trim/Sulf	1	0.30
Cefx, Tetra, Trim/Sulf	3	0.91
Ery, Levo, Tetra	1	0.30
Total	**46**	

Pattern 4

Aug, Cef, Levo, Tetra	1	0.30
Aug, Cef, Cefx, Tetra	1	0.30
Aug, Cef, ,Chl, Trim/Sulf	1	0.30
Chl, Levo, Tetra, Trim/Sulf	1	0.30
Cef, Cefx, Genta, Tetra	1	0.30

Chl, Ery, Tetra, Trim/Sulf	7	2.12
Doxy, Genta, Tetra, Trim/Sulf	2	0.60
Cefx, Chl, Genta, Tetra	1	0.30
Cefx, Chl, Doxy, Trim/Sulf	1	0.30
Cefx, Chl, Tetra, Trim/Sulf	1	0.30
Doxy, Ery, Tetra, Trim/Sulf	3	0.91
Cef, Chl, Tetra, Trim/Sulf	3	0.91
Cef, Cefx, Genta, Trim/Sulf	1	0.30
Ery, Genta, Tetra, Trim/Sulf	1	0.30
Cef, Cefx, Tetra, Trim/Sulf	1	0.30
Chl, Genta, Tetra, Trim/Sulf	1	0.30
Chl, Doxy, Ery, Trim/Sulf	1	0.30
Cef, Doxy, Genta, Levo	1	0.30
Total	**29**	

Pattern 5

Aug, Cef, Ery, Tetra, Trim/Sulf	1	0.30
Aug, Cef, Cefx, Tetra, Trim/Sulf	1	0.30
Aug, Cef, Chl, Ery, Tetra	1	0.30
Chl, Ery, Levo, Tetra, Trim/Sulf	1	0.30
Cef, Cefx, Chl, Tetra, Trim/Sulf	1	0.30
Cefx, Chl, Doxy, Tetra, Trim/Sulf	2	0.60
Cef, Cefx, Doxy, Tetra, Trim/Sulf	1	0.30
Aug, Cef, Cefx, Genta, Tetra	1	0.30
Cefx, Ery, Genta, Tetra, Trim/Sulf	1	0.30
Cef, Cefx, Ery, Genta, Trim/Sulf	1	0.30
Cef, Doxy, Genta, Tetra, Trim/Sulf	1	0.30
Aug, Cef, Chl, Genta, Tetra	1	0.30

Total	**13**	
Pattern 6		0.30
Aug, Cef, Ery, Levo, Tetra, Trim/Sulf	1	0.30
Cef, Cefx, Chl, Genta, Tetra, Trim/Sulf	1	0.30
Aug, Cef, Cefx, Doxy, Levo, Tetra	1	0.30
Aug, Cef, Chl, Ery, Tetra, Trim/Sulf	1	0.30
Total	**4**	

Appendix 11

Reagents used in the molecular analysis

16S rRNA, *nuc* and *mecA* gene multiplex reagents		***S. aureus spa* typing**		**PVL (*lukF-lukS*) PCR of *S. aureus***	
Reagent	Quantity	Reagent	Quantity	Reagent	Quantity
Nuclease free water	17.8ul	Nuclease free water	39.7ul	Nuclease free water	39.7ul
HotStart 10x buffer	2.5ul	10X Buffer	5ul	10X Taq Buffer	5ul
10mM dNTPs	0.5ul	10uM dNTPs	1ul	10mM dNTPs	1ul
10uM Nuc F"	0.5ul	10uM spa F"	1ul	10uM F primer	1ul
10uM NucR"	0.5ul	10uM spa R"	1ul	10uM R primer	1ul
10uM mecA F"	0.5ul	Taq polymerase	0.3ul	Taq DNA polymerase	0.3ul
10uM mecA R"	0.5ul	DNA template	2ul	Sample DNA	2ul
10uM 16S F"	0.5ul				
10Um 16S"	0.5ul				
HotStartTaq	0.2ul				
DNA template	1ul				

Appendix 12

***spa* types of 46 isolates**

Hospital	
Isolate	spa type (t)
amc-2	t002
amc-9	t084
amc-10	t084
amc-11	t2304
amc-13	t002
amc-22	t2304
amc-23	t11469
amc-24	t2304
amc-30	t11469

Farm 1	
Isolate	spa type (t)
am-1	t2304
am-3	t002
am-8	t002

Farm 2	
Isolate	spa type (t)
ar-13	t002

Farm 3	
Isolate	spa type (t)
b-8	t002
b-10	t002
b-11	t11469
b-17	t084
b-19	t002
b-20	t084
b-21	t11469
b-27	t11469
b-45	t002
b-61	t002
b-71	t002
b-80	t084
b-93	t11469
b-97	t084

Farm 4	
Isolate	spa type (t)
bp-13	t084

Farm 5	
Isolate	spa type (t)
l-5	t2304
l-10	t002
l-40	t2304

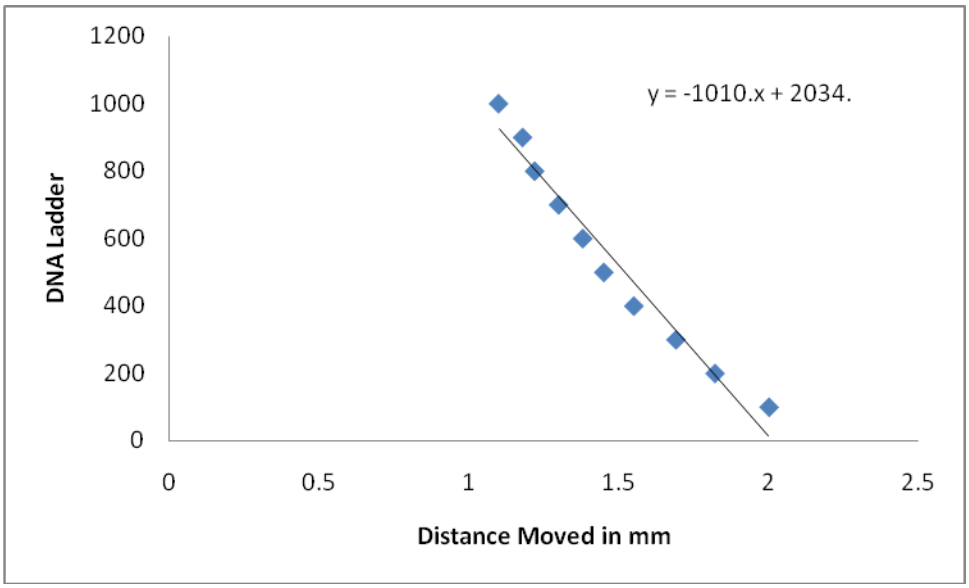
Farm 6	
Isolate	spa type (t)
ocl-10	t084
ocl-15	t002
ocl-17	t084
ocl-22	t002

Farm 7	
Isolate	spa type (t)
acl-8	t002
acl-10	t002
acl-17	t084
acl-38	t002

farm 8	
Isolate	spa type (t)
cl-10	t002
cl-24	t084
cl-53	t002

poultry farm workers	
Isolate	spa type (t)
am-26	t002
b-13	t11469
cl-8	t002
sp-3	t002

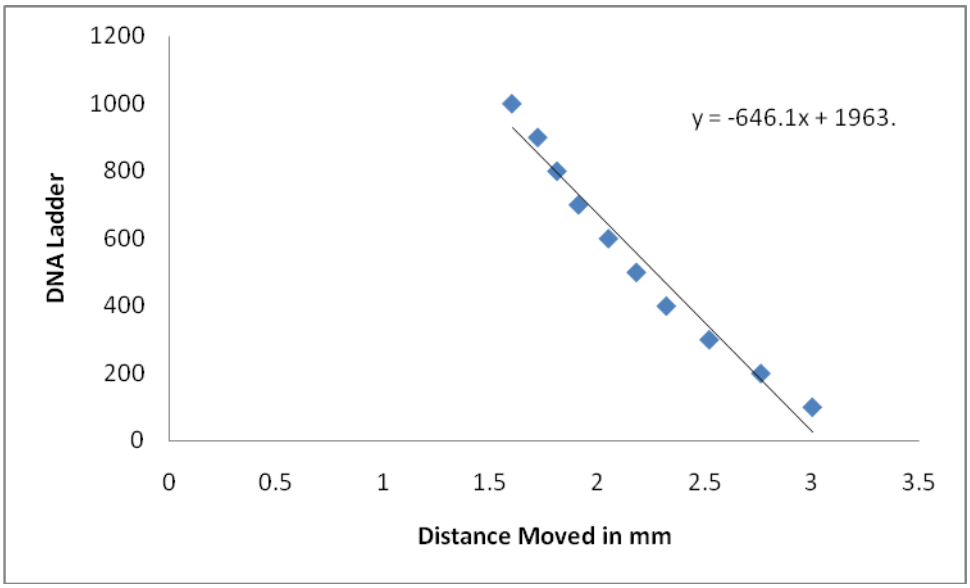
Appendix 13a



Calculation of the amplicon size of *S. aureus* 16SrRNA, *nuc* and *mecA* gene (Figure 4.12).

The sizes of the bands are 821.94, 518.7 and 216.9 respectively using the graphical equation y = - 1010.8 + 2034.9

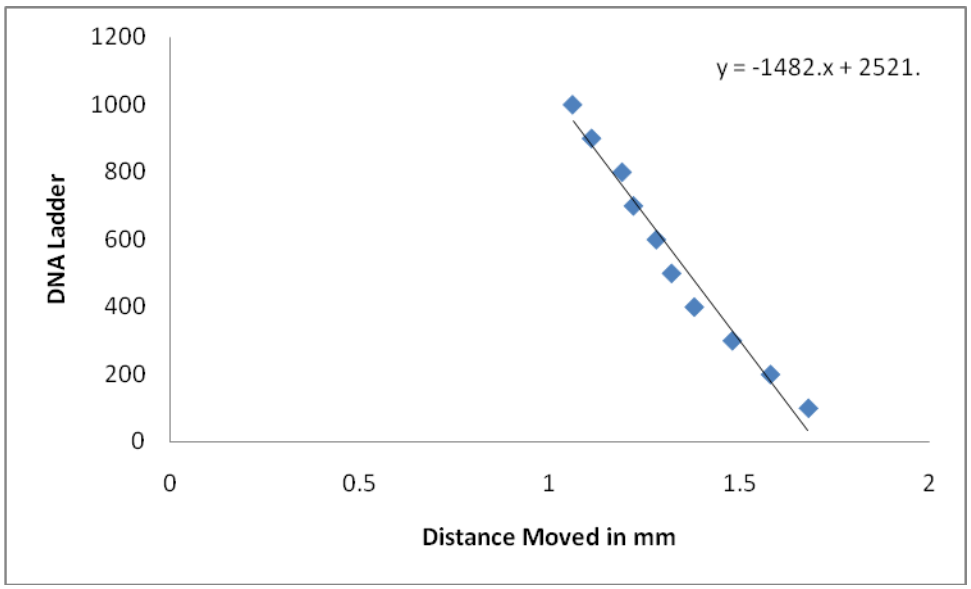
Appendix 13b



Calculation of the *spa* ampliconsize of 17 MRSA isolates (Figure 4.13)

The band size of the control isolate MRSA USA300 (lane 20) is 476.986 while the test isolates (lane2-18) have band size of 412.368bp

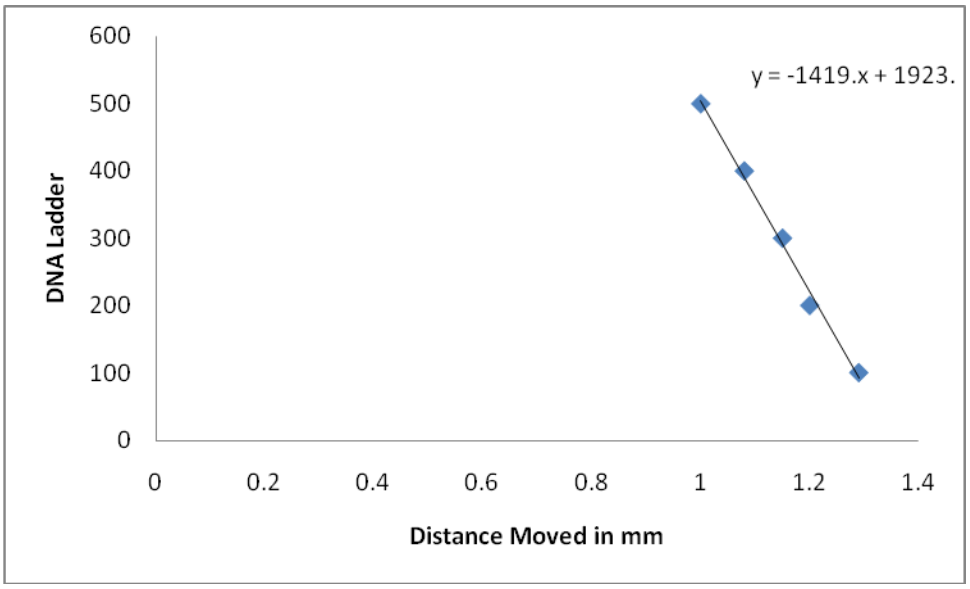
Appendix 13c



Calculation of the *spa*amplicon sizes of 26 *S. aureus* isolates from poultry (Figure 4.14).

Isolates b8 and USA300 in lanes 2 and 29 have band size of 505.5bp, isolates in lanes 11 to 25 have band size of 535.15bp, isolates in lanes 4, 26 and 27 have band size of 416.55bp, the isolate in lane 10 has a band size of 297.95bp.

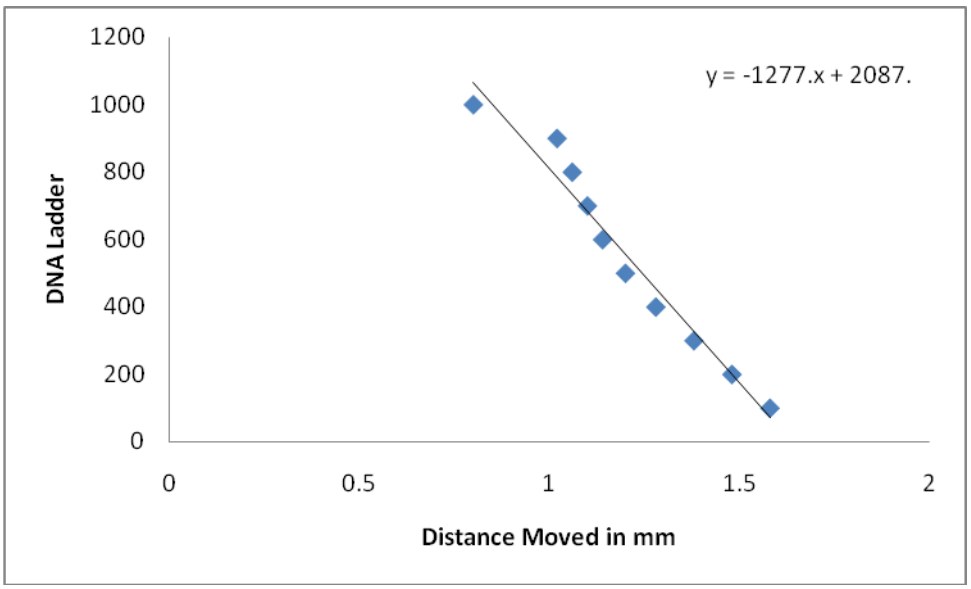
Appendix 13d



Calculation of the amplicon size of *lukS-lukF* gene in PVL positive isolates of *S. aureus* (Figure 4.15).

Test isolates in lanes 3, 10, 19, 21- 25 and the control isolate MRSA USA300 in lane 29 have band size of 424.25bp.

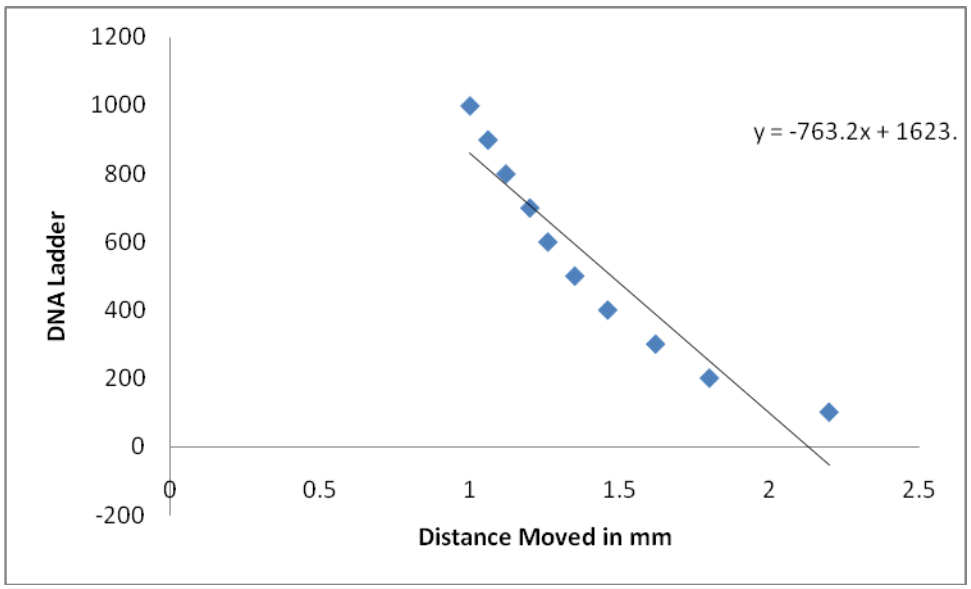
Appendix 13e



Calculation of the amplicon size of the *tetM* gene of a tetracycline resistant isolate (Figure 4.16)

Control isolate in lane 29 has a band size of 733.874bp and test isolate in lane 17 has bandsize of 733.874bp using the graphical equation y = -1277.1x + 2087.6.

Appendix 13f

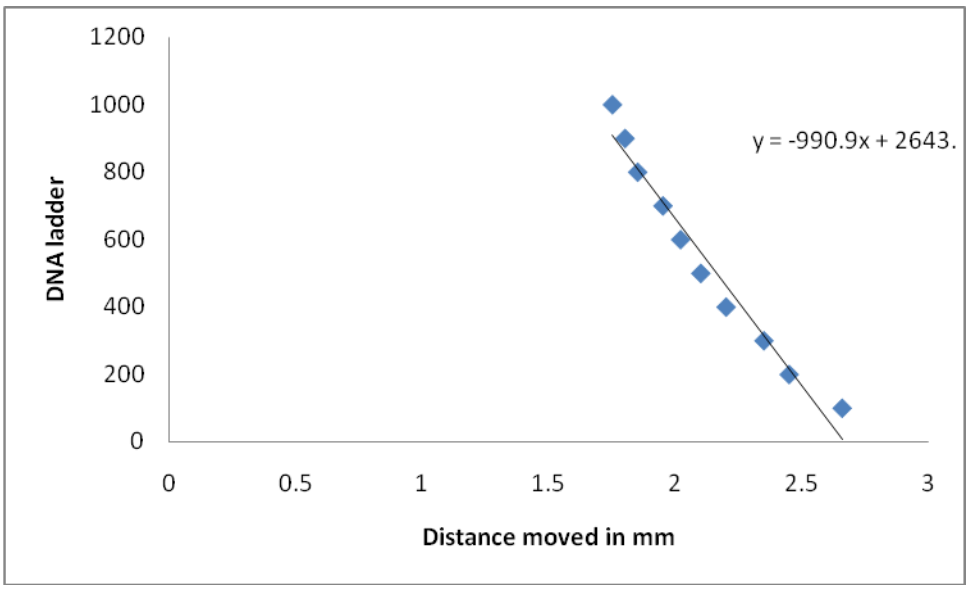


Calculation of the amplicon size of SCC*mec* of 11 *mecA* positive isolates

(Figure 4.17)

Lanes 2, 3, 4, 5, 7, 8, 9, 10, 11, 12 and 14 have SCC*mec* bands of 417.965bp, *mecA* band of 188.99bp and SAband of97.4bp, while lane 13 has an SCC*mec*band of 738.53 using the graphical equation y = - 763.25x + 1623.9.

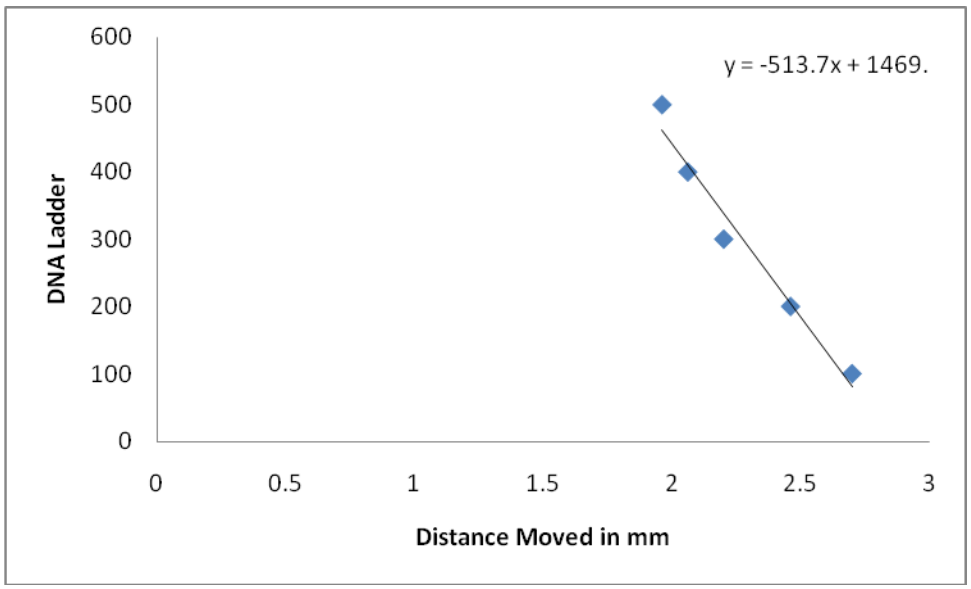
Appendix 13g



Calculation of amplicon size of the SCC*mec* of 5 *mecA* positive isolates (Figure 4.18).

Lanes 2, 3, 4, 5 and 7 have SCC*mec* band of 364.646 bp and *mecA* band of 166.45bp using the equation y = - 990.98 + 2643.9

Appendix 13h

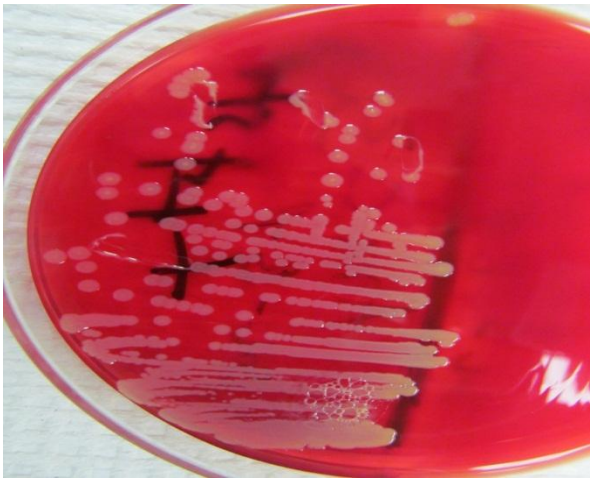


Calculation of the amplicon size of SCC*mec* of AMC 9, a *mecA* positive isolate (Figure 4.19)

Lane 4 has a base pair of 380.11 and lane 2 has a base pair of 359.557 using the graph equation y = -513.77x + 1469.3

Appendix 14

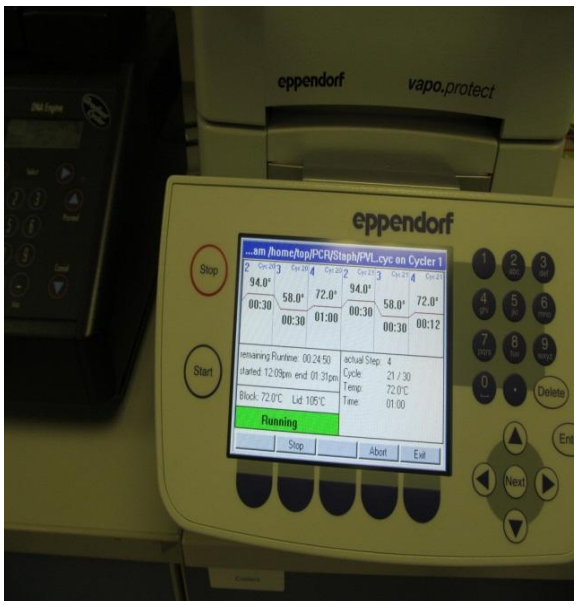
Picture of *S. aureus* on blood agar plate, equipment used and farms screened



S. aureus on blood agar plate



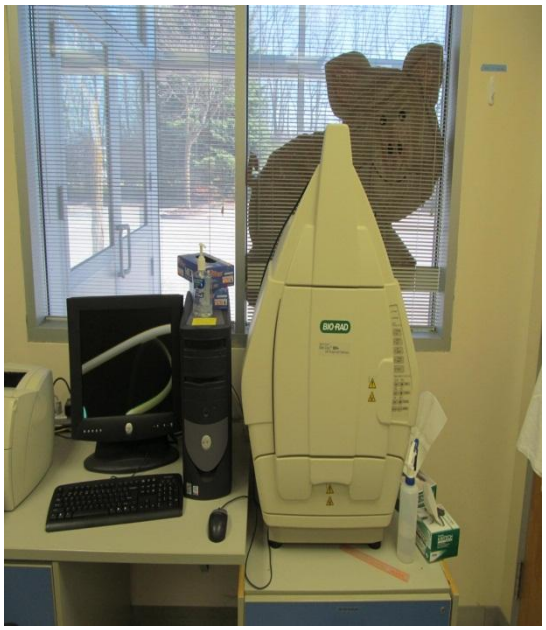
Thermocycler used for molecular analysis



Thermocycler for molecular analysis



loading of samples into a gel for electrophoresis



BIO-RAD transilluminating Gel doc used for viewing bands of the amplified PCR products



Structure of one of the poultry farms



One of the poultry farms sampled



One of the poultry farms sampled