

**OCCURRENCE OF MASTITIS AND METHICILLIN RESISTANT  
*STAPHYLOCOCCUS AUREUS* IN GOATS IN ZARIA, NIGERIA**

**BY**

**Ekaette Constant UDOH, B.Sc. BIOLOGY (ABU) 2010**

**M.SC/VET.MED/5446/2011-2012**

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

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
AWARD OF A MASTER OF SCIENCE IN VETERINARY PUBLIC  
HEALTH AND PREVENTIVE MEDICINE.**

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

**MARCH, 2015**

## DECLARATION

I declare that the work in this thesis entitled “The occurrence of mastitis and methicillin resistant *Staphylococcus aureus* in goats in Zaria” has been carried by me in the Department of Veterinary Public Health and Preventive Medicine, under the supervision of Prof. J.K.P. Kwaga, Prof. M.A. Raji and Prof. V.J. Umoh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma in this or any other Institution.

Ekaette Constant UDOH\_\_\_\_\_

-----

-----

Name of Student

Signature

Date

## CERTIFICATION

This thesis entitled “THE OCCURRENCE OF MASTITIS AND METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN GOATS IN ZARIA” by Ekaette Constant UDOH meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. Jacob K.P. Kwaga .....

Chairman Supervisory Committee (Signature) Date

Prof. M.A. Raji .....

Member, Supervisory Committee (Signature) Date

Prof. V.J. Umoh .....

Member, Supervisory Committee (Signature) Date

Dr. E.C. Okolocha .....

Head of Department (Signature) Date

Prof. A.Z. Hassan .....

Dean, School of Postgraduate Studies (Signature) Date

## **DEDICATION**

This research work is dedicated to the Almighty and ever sufficient God who has been everything to me in life and my reason for existence.

## **ACKNOWLEDGEMENTS**

My utmost gratitude goes to the Almighty God for His grace and mercies upon my life and for always being there for me throughout the study period.

I am especially grateful to the Chairman of my Supervisory committee, Prof. J.K.P. Kwaga who was always available to make sure that my work was done well. I also appreciate the members of my Supervisory committee, Prof. M.A. Raji and Prof. V.J. Umoh for their support and meaningful contributions.

I am also indebted to the members of my family, my parents and siblings, uncles, aunties and friends for their love, encouragement and support.

I am grateful to Mr. M.B. Odoaba, Mallam Y.Y. Pala, Mallam SulaimanYahuza, Mr. Kevin Iwuanyawu, Mr. Martin Mgbeha, Mallam Mahmud of Veterinary Public health and Preventive Medicine Department and Dr. E.E. Ella of Microbiology Department, Faculty of Science, Ahmadu Bello University, Zaria.

## ABSTRACT

A cross sectional study was conducted to determine the occurrence of mastitis and methicillin resistant *Staphylococcus aureus* (MRSA) in milk samples obtained from lactating goats in Zaria, Kaduna state, Nigeria. The specific objectives of the study were to determine the occurrence of mastitis in goats in Zaria, determine the prevalence of *S. aureus* in goat milk, document the antimicrobial susceptibility profiles of *S. aureus* isolates to commonly used antibiotics and finally to detect *mecA* gene by PCR in phenotypically confirmed MRSA isolates. A total of 386 fresh milk samples from both right and left halves of 198 lactating goats from farms and households were examined for the occurrence of mastitis and MRSA. The observed prevalence of mastitis in goats, defined by positive ( $\geq +1$ ) California Mastitis Test (CMT) was 119 (60.1%). From a total of 386 fresh goat milk samples examined, 60 strains of staphylococci were isolated and identified using the conventional biochemical tests and 32 of which were identified as *Staphylococcus aureus* using the Microgen™STAPH-ID system. Furthermore, 12 of these 32 showed agglutination with the PBP-2a Latex agglutination test reagent. The susceptibilities of the isolates to 14 antimicrobial agents were evaluated using the Kirby-Bauer disc diffusion method and interpreted as sensitive, intermediate or resistant following the Clinical and Laboratory Standards Institute (CLSI, 2011). Out of the 32 isolates of *S. aureus*; 90.63% were resistant to cefoxitin, penicillin G (93.75%), ampicillin (100%), amoxicillin-clavulanic acid (34.38%), ceftriaxone (71.88%), vancomycin (31.25%), gentamicin (31.25%), kanamycin (46.88%), erythromycin (43.75%), tetracycline (40.63%), ciprofloxacin (3.13%), nitrofurantoin (15.63%), trimethoprim-sulfamethoxazole (25%) and chloramphenicol (15.63%). All the isolates were resistant to 2 or more antimicrobial agents, but none was simultaneously resistant to all. There were 30 antimicrobial resistant patterns in which resistance to ampicillin, penicillin G,

cefoxitin were the most frequent. Methicillin resistance was determined using cefoxitin discs as a surrogate marker of *mecA* gene as recommended by Clinical Laboratory Standards International (CLSI). A total of 19 *Staphylococcus aureus* isolates that showed phenotypic resistance to methicillin (cefoxitin) and showing multiple antibiotic resistance (resistance to 3 antibiotic classes apart from beta-lactams) were selected for molecular detection of *mecA* by Polymerase Chain Reaction (PCR). The results of the PCR showed that none of the 19 *S. aureus* harboured the methicillin resistance *mecA* gene. The findings of this study confirm the importance of *S. aureus* as a mastitis causing organism and the possible role of goats in the transmission of multiple drug resistant *S. aureus*, and it is recommended that microbiological examination of udder of lactating goats should be carried out at regular intervals to detect and treat mastitis and goat milk should be pasteurized before consumption.

## TABLE OF CONTENTS

<b>CONTENTS</b>	<b>Page</b>
Title page .....	i
Declaration .....	ii
Certification .....	iii
Dedication .....	iv
Acknowledgements .....	v
Abstract .....	vi
Table of Contents.....	viii
List of Tables .....	xvi
List of Figures .....	xvii
List of Appendices .....	xviii
Abbreviations.....	xix
<b>CHAPTER ONE.....</b>	<b>1</b>
<b>INTRODUCTION.....</b>	<b>1</b>
<b>1.1 Background of the Study.....</b>	<b>1</b>
<b>1.2 Statement of Research Problem.....</b>	<b>4</b>
<b>1.3 Justification of Research.....</b>	<b>6</b>
<b>1.4 Aim of Research.....</b>	<b>7</b>
<b>1.5 Objectives of Research.....</b>	<b>7</b>
<b>1.6 Research Questions.....</b>	<b>7</b>



<b>CHAPTER TWO.....</b>	<b>8</b>
<b>LITERATURE REVIEW.....</b>	<b>8</b>
<b>2.1 Goats.....</b>	<b>8</b>
2.1.1 Goat breeds in Nigeria.....	10
2.1.2 Sokoto red goats.....	10
2.1.3 Sahelian goats.....	10
2.1.4 West African dwarf goats.....	10
2.1.5 Goat management systems in Nigeria.....	11
<b>2.2Goat Milk.....</b>	<b>12</b>
<b>2.3 Mastitis in Goats.....</b>	<b>14</b>
2.3.1 Epidemiology of mastitis in dairy goats.....	15
2.3.2 Causes of mastitis in goats.....	16
2.3.3 Risk factors.....	16
<b>2.4 Nomenclature and General Characteristics of Genus <i>Staphylococcus</i>.....</b>	<b>18</b>
<b>2.5 Biochemical Identification of <i>Staphylococcus</i>.....</b>	<b>19</b>
2.5.1 Catalase test.....	19
2.5.2 Sugar fermentation test.....	20
2.5.3 Coagulase test.....	20
2.5.4 Novobiocin sensitivity.....	20
2.5.5 Urease test.....	20
2.5.6 DNase test.....	21
2.5.7 Microgen <sup>TM</sup> STAPH-ID testing of <i>Staphylococcus</i> .....	21
<b>2.6 <i>S. aureus</i> Infections in Man.....</b>	<b>22</b>

<b>2.7</b>	<b>Staphylococcal Mastitis.....</b>	<b>23</b>
<b>2.8</b>	<b>Discovery and History of Methicillin Resistant <i>S. aureus</i>.....</b>	<b>24</b>
<b>2.9</b>	<b>Nomenclature of MRSA Strains.....</b>	<b>26</b>
2.9.1	Hospital-acquired-MRSA.....	26
2.9.2	Community-associated-MRSA.....	27
2.9.3	Livestock-associated-MRSA.....	28
<b>2.10</b>	<b>Transmission of MRSA.....</b>	<b>29</b>
<b>2.11</b>	<b>Detection of MRSA.....</b>	<b>30</b>
2.11.1	Use of antibiotic discs.....	30
2.11.2	Detection of MRSA through detection of PBP 2a.....	30
<b>2.12</b>	<b>Typing of MRSA.....</b>	<b>31</b>
2.12.1	Phenotypic typing methods.....	31
2.12.2	Molecular typing of <i>S. aureus</i> .....	34
<b>2.13</b>	<b>Mechanism of Antibiotic Resistance by <i>S. aureus</i> .....</b>	<b>36</b>
<b>2.14</b>	<b>Occurrence of MRSA.....</b>	<b>37</b>
2.14.1	Prevalence of methicillin-resistant <i>Staphylococcus aureus</i> in milk and dairy products.....	38
2.14.2	MRSA and livestock production.....	39
<b>2.15</b>	<b>Clinical Findings in MRSA Infections.....</b>	<b>40</b>
<b>2.16</b>	<b>Treatment of Staphylococcal Infections .....</b>	<b>40</b>
<b>2.17</b>	<b>Control and Prevention of MRSA Infections .....</b>	<b>41</b>
<b>2.18</b>	<b>Genomics of <i>Staphylococcus aureus</i>.....</b>	<b>42</b>
<b>2.19</b>	<b>Staphylococcal Cassette Chromosome (SCC<i>mec</i>).....</b>	<b>43</b>

<b>CHAPTER THREE.....</b>	<b>45</b>
<b>MATERIALS AND METHODS.....</b>	<b>45</b>
<b>3.1 Study Area.....</b>	<b>45</b>
<b>3.2 Sample Size.....</b>	<b>45</b>
<b>3.3 Sample Collection and Transportation.....</b>	<b>46</b>
<b>3.4 Materials Used.....</b>	<b>46</b>
3.4.1 Bacteriological culture media.....	46
3.4.2 Other materials used.....	47
3.4.3 Sugars.....	47
3.4.4 Antimicrobial agents impregnated disks and their Concentrations.....	47
3.4.5 Biochemical test kits.....	47
3.4.6 Materials for molecular characterization.....	48
<b>3.5 Determination of the Occurrence of Mastitis.....</b>	<b>48</b>
<b>3.6 Isolation and Characterization of <i>Staphylococcus</i> Species from Goat Milk.....</b>	<b>48</b>
3.6.1 Isolation procedure.....	48
3.6.2 Identification of <i>Staphylococcus</i> Species.....	49
3.6.3 Phenotypic detection of methicillin resistance among isolates.....	53
3.6.4 Determination of the susceptibility of <i>S. aureus</i> to commonly used antimicrobial agents.....	53
3.6.5 Determination of multiple antibiotics resistance index (MAR index).....	54
<b>3.7 Molecular Characterization of <i>S. aureus</i> Isolates.....</b>	<b>54</b>
3.7.1 Protocol used for DNA extraction of <i>S. aureus</i> isolates.....	54
3.7.2 Determination of the presence of <i>Staphylococcus</i> cassette	

chromosomes <i>mecA</i> gene in <i>S. aureus</i> .....	55
3.7.3 Agarose gel electrophoresis.....	56
<b>3.8 Statistical Analysis.....</b>	<b>56</b>
<b>CHAPTER FOUR.....</b>	<b>57</b>
<b>RESULTS.....</b>	<b>57</b>
<b>4.1 Occurrence of Mastitis Based on the California Mastitis Test .....</b>	<b>57</b>
<b>4.2 Association between CMT Scores and Udder Halves of Goats in Zaria.....</b>	<b>58</b>
<b>4.3 Association between CMT Scores and Occurrence of <i>S. aureus</i> in Goat Milk.....</b>	<b>63</b>
<b>4.4 Association between CMT Scores and Udder-wise Isolation of <i>S. aureus</i>.....</b>	<b>63</b>
<b>4.5 Prevalence of <i>S. aureus</i> in Goat Milk .....</b>	<b>64</b>
<b>4.6 Antimicrobial Susceptibilities of Isolates.....</b>	<b>64</b>
<b>4.7 Antimicrobial Resistance Patterns of 32 <i>S. aureus</i> Isolates.....</b>	<b>69</b>
<b>4.8 Multiple Antibiotics Resistance Index (MARI).....</b>	<b>69</b>
<b>4.9 Detection of PBP-2a Among Isolates.....</b>	<b>69</b>
<b>4.10 Detection of <i>mecA</i> by PCR.....</b>	<b>69</b>
<b>CHAPTER FIVE.....</b>	<b>74</b>
<b>DISCUSSION.....</b>	<b>74</b>

<b>CHAPTER SIX.....</b>	<b>83</b>
<b>CONCLUSION AND RECOMMENDATIONS.....</b>	<b>83</b>
<b>6.1 Conclusion.....</b>	<b>83</b>
<b>6.2 Recommendations.....</b>	<b>83</b>
<b>REFERENCES.....</b>	<b>85</b>
<b>APPENDICES.....</b>	<b>103</b>

## LIST OF FIGURES

Figure 1: Prevalence of *Staphylococcus* species isolated from goat milk.

Figure 2: Percentage Distribution of resistance among *S. aureus* tested against 14 antimicrobial agents.

## LIST OF TABLES

Table 4.1	Occurrence of Mastitis in Goats in Zaria, Kaduna state, Nigeria.....	59
Table 4.2	Occurrence of SCM in four Breeds of Goats in Zaria, Kaduna state, Nigeria.....	60
Table 4.3	Occurrence of SCM in the different Age groups of Goats.....	61
Table 4.4	California Mastitis Test (CMT) Scores for subclinical mastitis (SCM) in Goats in Zaria based on System of Management.....	62
Table 4.5	Association between CMT Scores and Udder Halves of Goats in Zaria.....	65
Table 4.6	Association between CMT Scores and Occurrence of <i>S. aureus</i> .....	66
Table 4.7	Association between CMT Scores and Udder-wise Isolation of <i>S. aureus</i> .....	68
Table 4.8	Antimicrobial sensitivity Profile of <i>S. aureus</i> strains (n=32) Isolated from Goat Milk in Zaria, Kaduna state.....	70
Table 4.9	Antimicrobial Resistance Patterns of 32 <i>Staphylococcus aureus</i> Isolates.....	71
Table 4.10	MAR Index Analysis of the 32 <i>S. aureus</i> Isolates obtained from Goat Milk in Zaria.....	73

**LIST OF APPENDICES**

Appendix I: CMT scoring system and the corresponding somatic cell counts per ml.....103

Appendix II: Microgen 12S identification.....104

Appendix III: Antimicrobial sensitivity patterns of 32 *S. aureus* isolates  
to 14 antimicrobial agents tested and disk concentrations.....107

Appendix IV: Genomic DNA of MRSA isolates.....111



## ABBREVIATIONS

SE- Staphylococcal enterotoxins

MRSA- methicillin-resistant *Staphylococcus aureus*

NIRD- National institute for rural development

CNS- coagulase negative Staphylococcus

SCC- somatic cell count

DNA-Deoxyribonucleic acid

SCCmec- staphylococcal cassette chromosome mec

PBP2a-penicillin binding protein 2a

HA-MRSA-hospital-acquired methicillin-resistant *Staphylococcus aureus*

CA-MRSA-community-associated methicillin-resistant *Staphylococcus aureus*

LA-MRSA-livestock-associated methicillin-resistant *Staphylococcus aureus*

IDUS-injection drug users

EFSA- European food safety authority

PFGE- pulsed field gel electrophoresis

CLSI- clinical laboratory standard international

BORSA- borderline oxacillin-resistant *S. aureus*

NaCl- sodium chloride

PAGE- polyacrylamide gel electrophoresis

MLEE- multilocus enzyme electrophoresis

ET- electrophoretic type

REA- restriction enzyme analysis

RAPD- random amplified polymorphic DNA

SFP- staphylococcal food poisoning

NAPRI- National animal production research institute

NMC- National mastitis council

CMT- California mastitis test

HCl- hydrochloric acid

MSA- mannitol salt agar

MSSA methicillin-susceptible *Staphylococcus aureus*

PCR- polymerase chain reaction

MAR I- multiple antibiotic resistance index

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the Study

Milk is a very nutritious food that is rich in carbohydrate, proteins, fats, vitamins and minerals. However, health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens. Also, the quality of milk may be lowered by a number of factors such as adulteration, contamination during and after milking and the presence of udder infections (Esron *et al.*, 2005). The nutritional components that make milk an important part of human diet also act as a good medium for the growth of pathogenic microorganisms which may occur due to milk contamination or animal infections (Ayodha, 2013). The presence of microorganisms in milk can be demonstrated usually by microbial culture.

According to Jennes (1980), goats produce more milk per body weight compared to cows and other ruminants because of better feed utilization efficiency, higher lactation persistency, having mammary tissue comprising of greater proportion of the body weight and a more pronounced milk ejection reflex. Goats are used for customary ceremonies, religious purposes and for meat production in Nigeria (Odeyinka and Ajayi, 2004; Ozung *et al.*, 2011). Goat milk production is not popular in Nigeria; many households keep goats for other economic gains and not for milk whereas more people consume dairy products from goats than from any other animal in the developed countries (Ozung *et al.*, 2011). Goat milk greatly improves the diet of many rural families. It is traditionally valued for the elderly, the sick, babies, children who are allergic to

cow's milk, and patients with ulcers. It is even preferred for raising orphan foals and other young domestic animals (Ozung *et al.*, 2011).

Goat milk is richer than cow's milk in some important nutrients such as vitamin A, niacin, choline, and inositol; it is poorer in folic acid. However, the productivity of goats and their system of production has received little attention in research and development endeavours in Nigeria despite the huge number of goats that are reared by small holders and agro pastoralists. In Nigeria, cow milk consumption is common but its production is insufficient to meet consumption needs either as fresh or fermented milk (nono) (Joseph and Olafade, 1999; Ozung *et al.*, 2011). Goat meat and milk of the common breeds in Nigeria are important due to the rising demand for goat meat in recent years presumably due to the increased local consumption and the growing export market (Ozung *et al.*, 2011). With the provision of goat breeding and multiplication centres in Nigeria and increased general awareness and consumption of goat milk, the country could strive for self-sufficiency in meat and dairy production (Egwu *et al.*, 1995). In goats and sheep, *Staphylococcus* spp., *Escherichia coli*, *Corynebacterium* spp., *Streptococcus* spp and *Klebsiella* spp are the most frequently isolated microorganisms responsible for intramammary infection (Arlington *et al.*, 1990; Ayodha, 2013).

Mastitis is the inflammation of mammary glands resulting in physical, chemical and microbiological changes characterized by an increase in number of somatic cell counts, especially leukocytes in the milk and by the pathological changes in the mammary tissue. Generally, mastitis occurs in two forms which include clinical (overt) and subclinical (hidden). In clinical mastitis, all the five cardinal signs of udder inflammation (redness, heat, swelling,

pain and loss of milk production) are present, while the subclinical form is bereft of any obvious manifestation of inflammation (Sakar, 2011). Mastitis is a common problem in dairy animals and is also one of the most important threats affecting the world's dairy industry (Wallenberg *et al.*, 2002). This inflammatory condition of the mammary gland results in changes in the physical characteristics of the udder or milk (Nazifi *et al.*, 2011).

*Staphylococcus aureus* is a Gram-positive bacterium that is spherical in shape and occurs in microscopic clusters like grapes. They are non-motile, non-spore forming, catalase positive, facultative anaerobe which belongs to the family *Staphylococcaceae* and genus *Staphylococcus* (Kloos *et al.*, 1998). It is considered the world's third most important cause of food borne illnesses (Tirado and Schimdt, 2001). Most *Staphylococcus aureus* isolates are coagulase positive because they produce an enzyme that causes clotting of plasma. Beside the production of enterotoxins, *S. aureus* produces many other virulence factors such as exfoliative toxins, toxic shock syndrome toxin, and leukocidins. It is responsible for a variety of mild to severe skin and soft tissue infections and numerous serious infections, including endocarditis, endophthalmitis, osteomyelitis, meningitis, bacteraemia, pneumonia, and toxic shock syndrome (Larkin *et al.*, 2009). Although pasteurization kills *S. aureus* cells readily, thermostable *Staphylococcus* enterotoxins (SEs) generally retain their biological activity (Evenson *et al.*, 1988; Asao *et al.*, 2003), even after the death of the cells that produced them.

*S. aureus* has been reported as the causative agent of infections in many mammals as well as wild and domestic birds and also in some reptiles. Some animals are asymptomatic while others suffer respiratory, gastrointestinal, or skin and soft tissue infections. *S. aureus* is a significant

cause of mastitis in cows and small ruminants (Vanderhaeghen *et al.*, 2010). Molecular analyses of isolates from different animals have revealed that there are some strains that appear to be host-adapted to a particular animal species (horses, cattle, pigs, sheep, chickens, or humans) and other strains can colonize multiple species of animals (Cuny *et al.*, 2010).

Methicillin-resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* that have acquired genes conferring resistance to methicillin and other beta-lactam antibiotics. Methicillin resistance in *Staphylococcus aureus* may be caused by the acquisition of the *mecA* gene. This gene encodes an alternative penicillin-binding protein, called PBP2a, which has a low affinity for beta-lactam antibiotics (Vanderhaeghen *et al.*, 2010). Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen that has attracted public health interest worldwide because it is a major cause of nosocomial infection and colonization, which often result in morbidity and mortality (Okon *et al.*, 2011). Emerging disease reports have revealed that antibiotic-resistant pathogens are mostly responsible for the increased rate of hospital-acquired infections (Klevens *et al.*, 2007; Okon *et al.*, 2011).

## **1.2 Statement of Research Problem**

Mastitis is recognized worldwide as being among the most costly disease in the dairy industry (Alrawi *et al.*, 1979; Tanimomo *et al.*, 2012). It remains the most economically damaging disease in the dairy industry worldwide irrespective of the species of animal and despite the many years of research in this area (Owens *et al.*, 1997). *S. aureus* has been considered as the major causative agent of mastitis (NIRD, 1980; Blood *et al.*, 1989; Alawa *et al.*, 2000) especially in the Nigerian breeds of goats (Addo *et al.*, 1980; Chineme and Addo, 1984; Alawa *et al.*,

2000). These goats are rarely milked by people and their milk is rarely tested for quality and therefore, it is difficult to detect subclinically infected animals or early cases of mastitis with no swelling of the mammary glands (Addo *et al.*, 1980; Alawaet *et al.*, 2000).

Methicillin resistant *Staphylococcus aureus* (MRSA), like methicillin-susceptible *S. aureus* can cause infections ranging from relatively mild skin infections to life threatening invasive bloodstream infections, pneumonia, central nervous system infections, and pericarditis. MRSA has been a chronic problem in hospitals and long-term care facilities for over 40 years and has caused severe infections especially in patients in surgical wards and intensive care units (Rutland *et al.*, 2009). Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing worldwide and its colonization is posing a risk for humans because the transmission of MRSA in community has been shown to be as high as 60% (Matsumoto *et al.*, 2001; Stastkova *et al.*, 2009).

MRSA has in the last few years been found to be re-emerging in livestock (Kock *et al.*, 2010). Animals can act as reservoirs of MRSA, and the bacterium can be transmitted to humans in close contact with MRSA colonized animals. MRSA from this reservoir has been referred to as Livestock Associated-MRSA (LA-MRSA) (Smith and Pearson, 2010). Persons in direct contact with MRSA-positive animals have an increased risk of becoming MRSA positive. This has been documented for individuals working in companion animal and equine clinics, and livestock production environments (Morgan, 2008).

The emergence of pathogenic microorganisms resistant to commonly used antibiotics is a worldwide concern of the 21st century. *Staphylococcus aureus* is regarded as one of the most

important bacteria in this regard, especially the methicillin-resistant strains (Stastkova *et al.*, 2009).

### **1.3 Justification of Research**

*Staphylococcus aureus* is the most important pathogen of caprine mastitis worldwide. Intramammary infections caused by this pathogen necessitate special attention because *S. aureus* is responsible for both clinical and subclinical mastitis (Bergonier *et al.*, 2003; Contreras *et al.*, 2007).

*S. aureus* can be transmitted between humans and animals, and frequently infections in companion animals can be traced back to their human caretakers (Ruthland *et al.*, 2009). *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns (MAR). The detection of these MAR strains in raw milk, especially methicillin resistant *S. aureus* (MRSA) is regarded as an issue of great concern for their potential spread through the dairy food chain (Enright, 2002).

MRSA has attracted the attention of the medical research community, illustrating the urgent need to develop better ways to diagnose and treat bacterial infections. Information on MRSA in developing countries are still relatively limited which may be attributed to low level of awareness of MRSA infections and its clinical and societal consequences (Okon *et al.*, 2011). In Nigeria there is paucity of information in general regarding the occurrence of mastitis and MRSA in goats and in Zaria in particular despite an increase in the breeding of goats in the country (Aina, 2012). Therefore, it is necessary to conduct a study to address the information



gap. As pointed out by Wilson *et al.* (1997), Shpigel *et al.* (1998) and Reugg (2003) knowledge of the prevalence and causative agents of mastitis in an area is of importance to the dairy industry and veterinarians.

#### **1.4 Aim of Research**

The aim of this research was to determine the occurrence of mastitis and methicillin-resistant *Staphylococcus aureus* (MRSA) in goats in Zaria, Nigeria.

#### **1.5 Objectives of Research**

The objectives of the study were to:

1. Determine the occurrence of mastitis in goats in Zaria.
2. Determine the occurrence of *S. aureus* in goat milk in Zaria, Nigeria.
3. Document on the antimicrobial susceptibilities of *S. aureus* isolates to commonly used antibiotics.
4. Detect *mecA* gene in methicillin-resistant *S. aureus* strains isolated from goat milk in Zaria, Nigeria.

#### **1.6 Research Questions:**

1. Is mastitis prevalent among goats in Zaria?
2. Is *Staphylococcus aureus* associated with mastitis in goats in Zaria?
3. If yes, are the *S. aureus* strains resistant to methicillin?
4. Do the methicillin-resistant strains harbor *mecA* gene?

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Goats

Goats are considered to be the most widely distributed of the mammalian livestock because they are highly adapted to a wide range of geographic and climatic conditions (Assefa *et al.*, 2011). They are very important especially to the poor farmers because they reproduce fast and in multiples, consume a diversity of plant species and require low capital investment (Assefa *et al.*, 2011). Goat is regarded as a multipurpose animal, providing meat, milk, clothing, fertilizer, offering loyalty and companionship, alert, intelligent and socially inclined. They form an important economic and ecological niche in agricultural systems throughout the developing countries (Aina, 2012). About 30% of Africa's ruminant livestock is made up of goats and they contribute to about 17% of the total meat and 20% of the total milk production (Assefa *et al.*, 2011).

The role of goats and sheep in the food chain and livelihoods of rural households is very important and they are mostly owned by women and children (Lebbie, 2004; Ozung *et al.*, 2011). Goat meat is most widely consumed around the world and there are few, if any religious taboos limiting goat meat consumption. In terms of total numbers and production of total food and fibre products, sheep and goats are regarded as the principal domesticated small ruminants. This may be due to the fact that they require lower amount of feed because of their body size compared to cattle (Okunlola *et al.*, 2010; Ozung *et al.*, 2011). In Nigeria, the estimated contribution of goats to the meat supply is about 16% (Ozung *et al.*, 2011). They play a more important role in the north than in the south and are also more important in the rural than the urban areas (Adu and

Ngere, 1979). The world population of goats was estimated at 76 million (FAOSTAT, 2003), with 96% of these being kept in developing countries. The number of goats in Nigeria is about 21 million and this would improve the supply of animal requirements of consumers if goat production is increased (Ajayi *et al.*, 1987; Ameh and Tari, 1999). Goats are widely distributed in Nigeria (Ameh and Tari, 1999) and Nigeria is the 6<sup>th</sup> among the top 10 goat inventories and the top 10 goat meat producers in the world, but not among top 10 goat meat importers and top 10 goat meat exporters (Aina, 2012).

Goats are preferred dairy choice in many countries and the Nigerian Dwarf goat has been internationally recognized as dairy and companion animal since 1854 (William, 1854; Aina, 2012). In Nigeria, small ruminant production forms part of nearly all known farming systems. Goats have long association with nomadic pastoralism, mixed farming, shifting cultivation, small holder farms and even among non-farming communities where they are tethered and fed in backyards. Some charity organizations provide goats to impoverished people in poor countries, because goats are easier and cheaper to manage than cattle and have multiple uses.

Interest in goat production is increasing all over the world (VanNiekerk and Pimentel, 2004) due to the high ethnic demand for goat milk and meat products and its relative low cost of production in countries like U.S.A (Terill *et al.*, 2004); hence, it is regarded as a new option for farm income (Browning *et al.*, 2004; Aina, 2012).

There are about 570 breeds of goats all over the world out of which three breeds of goats are found in Nigeria (Aina, 2012).

### **2.1.1 Goat breeds in Nigeria**

The three distinct goat breeds recognized in Nigeria; Sokoto Red (Maradi), Sahel (Desert goat) and West African Dwarf are distributed in different ecological zones of the country (Jimmy, 2007).

### **2.1.2 Sokoto red goats**

Sokoto red goats are the most numerous and widely distributed breed of goats in Nigeria with some strains occurring as Kano Brown and Borno white (Jimmy, 2007). They are found in the northern part of the country around Sokoto extending to Niger Republic (Jimmy, 2007). They are horned, have horizontal ears, short hair coat and have a daily milk yield of 0.5-1.5kg and 100 days lactation period (Jimmy, 2007).

### **2.1.3 Sahelian goats**

Sahelian goats are found in the arid part of the country around Borno state. They are long legged goats weighing about 19-37kg and are about 51-68cm in height (Egwu *et al.*, 1995). They possess pendulous ears, short fine coat and are good milkers. They produce about 0.8-1.0kg milk daily and have a lactation length of about 120days (Gall, 1996).

### **2.1.4 West African dwarf goats**

West African dwarf goats are predominantly found in the southern part of the country, are well adapted to humid environment and are trypano-tolerant. They have short legs, are about 50cm in height and weigh 18kg-25kg. They are inferior milkers compared to other breeds of goats. Their daily milk yield is about 0.4kg and they have about 126 days lactation period (Gall, 1996).

## **2.1.5 Goat management systems in Nigeria**

Extensive or free range, intensive and semi intensive systems are the major management systems of goats and other small ruminants (Ozung *et al.*, 2011).

### **2.1.5.1 Extensive management system**

Under this system of management, goats are allowed to roam about to graze for themselves or scavenge food with no supplements being provided. It requires low input which results in low productivity. Housing and medical care are not provided for the animals but some form of ethno-veterinary care is provided in some places (Jimmy, 2007). The animals are prone to disease, risk of theft and infestation by parasites (Weaver, 2005; Ozung *et al.*, 2011).

### **2.1.5.2 Intensive management system**

Zero grazing is practiced under this system where the animals are not allowed to graze at all. They are completely confined in their houses where feed and water are provided for them (Jimmy, 2007). This system is used mainly by government establishments like the university farm centres or corporate farms which involve high capital and labour resulting in high productivity (Ozung *et al.*, 2011). Good health care which involves taking preventive measures by vaccination, control of endoparasites and ectoparasites and therapeutic treatment are provided for the animals. Proper record keeping is also maintained for breeding and planning purposes (Jimmy,2007). Good quality fodder such as hay, silage, and soilage supplemented with concentrates are often fed to the animals (Ozung *et al.*, 2011).

### **2.1.5.3 Semi intensive management system**

This system combines the features of both the extensive and intensive systems of management. Here, animals are not completely confined but allowed to graze on improved fenced pastures. Health care and other management practices are also provided (Lakpini, 2002; Jimmy, 2007). Animals are housed and often released for grazing (Ozung *et al.*, 2011).

## **2.2 Goat Milk**

Goat milk contributes about 40% of the world's total production (7.2 million tonnes) in both the tropics and subtropics and the milk has been accepted as an alternative to cow milk in most developed countries (Ozung *et al.*, 2011). Goat milk has been reported to contain a higher proportion of short and medium chain fatty acids with smaller globules than the cow milk and hence it is easily digestible and good in relieving stress and constipation (Ozung *et al.*, 2011). The goat milk casein and fat are more digestible than those in cow milk. Goat milk is valued for the elderly, the sick, babies, children with new milk allergies, patients with ulcers and even preferred for raising orphan foals or puppies. It is higher in vitamin A, niacin, choline and inositol than cow milk, but it is lower in vitamins B<sub>6</sub>, B<sub>12</sub>, C and carotenoids. It has been established that total cholesterol level can be reduced by the consumption of goat milk because of the higher presence of medium chain triglycerides (MCT) which is 36% in goat milk compared to 21% in cow milk and this higher level of MCT is said to decrease the synthesis of endogenous cholesterol (Alferez *et al.*, 2001; Midau, 2012).

Some goats are bred for milk which can be consumed fresh but pasteurization is recommended to reduce the naturally occurring bacteria such as *S. aureus* and *E. coli* (Ekici, 2008; Aina, 2012).

Goat milk is usually processed into cheese, butter, ice cream, “cajeta” and other products. It can replace cow’s milk in diets of those who are allergic to cow’s milk (Wikipedia, 2008; Aina 2012). Goat milk is usually homogenized which means the cream remains suspended in the milk, instead of rising to the top as in raw cow’s milk. In several countries, including Nigeria, Kenya, Rwanda and Ghana there is an increase in the consumption of goat milk in the growing urban areas. Goat keepers indirectly enhance national economic stability through the reduction of foreign currency expenditure on importation of these products to meet domestic demands. Goat milk is superior to cow milk in that tuberculosis (TB) bacteria are absent or rare in goat milk (Aina, 2012). Its protein resembles that of humans, it is used in treatment of gastrointestinal disorders, liver diseases as well as infantile pyloric stenosis. It has also been recommended for the treatment of ulcers since it is alkaline in nature (Fajeminsin, 1991).

The production of milk from dairy goat requires a lower capital investments such as land, labour, technical, financial and managerial skills and capacity unlike dairy cows. The maintenance requirements for goats are lower compared to the cow and this makes it an ideal animal for milk production by small scale farmers and rural households (Vander Nest, 1997; Norris *et al.*, 2011). A number of studies have been carried out on goat milk around the world. Norris *et al.* (2011) considered milk production of three exotic dairy goat genotypes in Limpopo province of South Africa while Egwu *et al.* (1995) worked on improved productivity of goats and utilization of goat milk in Nigeria. Ozung *et al.* (2011) reviewed the potentials of small ruminant production in Cross river rain forest of Nigeria, while Sarker and Samad, (2011) in Bangladesh worked on udder-half-wise comparative prevalence of clinical and subclinical mastitis in lactating goats with their bacterial pathogens and antibiotic sensitivity patterns. However, very few studies have

addressed the occurrence of mastitis and methicillin resistant *Staphylococcus aureus* (MRSA) in goats especially in Zaria, Nigeria.

### **2.3 Mastitis in Goats**

Mastitis is economically important in the goat industry due to the high rate of mortality of kids born to goats with mastitis (Addo *et al.*, 1980; Tanimomo *et al.*, 2012). The result of mastitis can be categorized as economic loss which includes reduction in milk production, kid mortality, culling, cost of treatment, reduction in quality of milk and zoonotic potential (Radostits *et al.*, 2007; Sori *et al.*, 2011). Losses due to the cost of treatment, culling, death and decreased milk production and constituent milk quality which are usually associated with clinical and subclinical mastitis is difficult to estimate (Bradley, 2002; Tanimomo *et al.*, 2012). Total elimination of mastitis from goat herd is difficult but the incidence can be reduced to the barest minimum by the application of control strategies, sound husbandry practices, sanitation, appropriate treatment of infected animals, dehorning, regular foot care practice, isolation and elimination of goats with open draining abscesses from the herd before subsequent treatment (Tanimomo *et al.*, 2012).

Some studies have been carried out on mastitis in small ruminants in Nigeria. Kawu *et al.* (1992) reviewed the prevalence and seasonal variation in the occurrence of clinical mastitis in small ruminants in Zaria. Ameh and Tari (1999) reviewed the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. The results of their study revealed that dairy goats at various ages were susceptible to mastitis and the prevalence was higher in does aged 2-5 years than in goats between 1 and 2 years. Also, *S. aureus* and *E. coli* were the most commonly isolated microorganisms from the study. Ajuwape *et al.* (2005) reviewed the bacteriological and



haematological studies of clinical mastitis in goats in Ibadan, Oyo state while Alayande *et al.* (2003) studied the prevalence of clinical mastitis in scavenging Sokoto red goats in Sokoto, Nigeria. Most recently, Tanimomo *et al.* (2012) reviewed the prevalence of mastitis in goat herds in some northwestern villages in Nigeria and had a 15.5% prevalence of mastitis from samples collected from normal sized udder based on rapid field catalase test (RFCT).

Problems associated with mastitis in goats are not taken seriously by small scale farmers and commercial dairy goat production is non-existent in the country (Addo *et al.*, 1980; Ameh and Tari, 1999). Reugg *et al.* (2011) reported that subclinical mastitis may occur in up to 15-30% of animals while less than 5% of the lactating does and ewes may have clinical mastitis. The interpretation of somatic cell count (SCC) values in lactating goats is difficult due to the presence of cytoplasmic particles in milk. For a successful management of dairy farms, the understanding of mastitis and prevention of mastitis is essential and veterinarians play an important role for small ruminant dairy producers (Reugg, 2011).

### **2.3.1 Epidemiology of mastitis in dairy goats**

The incidence rate of <5% is generally reported for clinical mastitis in does per year (Bergonier *et al.*, 2003; Reugg, 2011). Koop *et al.* (2009) surveyed about 90% (300 farms) of all dairy goat farms in Holland and reported an annual incidence of clinical mastitis of 2%. They also reported that two-thirds of the farms culled majority of their affected does rather than treating them (Reugg, 2011). Sanchez *et al.* (2007) linked the incidence of clinical mastitis to selenium deficiency in a study carried out in Spain. The incidence rate of clinical mastitis was 3.8% for

does that consumed a deficient diet and 15.4% for does that had been treated with slow release barium selenite or were enrolled in a non-supplemented control group.

### **2.3.2 Causes of mastitis in goats**

Coagulase negative *Staphylococcus* (CNS) has been consistently reported by researchers to be responsible for the greatest proportion of subclinical mastitis infections occurring in goats (White and Hinckley, 1999; McDougall *et al.*, 2002; Bergonier *et al.*, 2003; Reugg, 2011). Infections by CNS are especially prevalent during parturition and about 17% of CNS has been recovered from goats (McDougall *et al.*, 2002, Reugg *et al.*, 2002). Other pathogens that are frequently recovered from goats with subclinical mastitis include corynebacterium, streptococci or miscellaneous pathogens such as yeast.

Milk samples obtained from infected udder halves generally exhibit somatic cell count (SCC) values > 500,000 cells/ml and > 1,000,000 cells/ml at later stages of lactation. Several researchers have reported that SCC values vary by breed but they have not been able to explain the exact reason for this effect. It is suggested that this may be related to their physiological differences or differences in resistance to mastitis. A relationship between estrus and increased SCC has been postulated because many goat producers have indicated that SCC values increased after does are exposed to bucks (Reugg *et al.*, 2011).

### **2.3.3 Risk factors**

The risk factors often associated with mastitis in goats includes; breed, age, parity, stage of lactation, housing system, management practices, season and geographical location.

Poor housing system is found to increase the risk of mastitis. This is explained by the fact that dirty, wet bedding tend to harbor a variety of preservation media for microorganisms (Ndegwa *et al.*, 2000). Goats reared in houses with concrete floors tend to have little contact with manure and wet bedding than those kept in pens with earthen floors. Heavy faecal contamination may enhance the buildup of mastitis pathogen infection and transmission. Bedding materials have been reported to have crucial role in the transmission of infection from animal to animal (Swai *et al.*, 2008).

Suckling does were found to be at increased risk of intramammary infections than milking does. This could be that suckling does are rarely examined and with increasing milk production, the kids may not empty the glands fully. Infectious agents may also gain access through injuries inflicted by suckling kids (Ndegwa *et al.*, 2000). The Sokoto red and Kano brown goats and their crosses are relatively high milk yielders and may yield 0.46-0.55kg per day (Akinsoyinu *et al.*, 1982; Ehoche and Buvanendran, 1983; Alawa *et al.*, 2000). Since they are high yielders, they may not be completely stripped out; hence there is an increased likelihood of frequent lactiferous sinuses which are prone to blockage and may lead to the damming of milk in the udder during lactation. Such accumulation of milk encourages bacterial proliferation especially in the presence of residual infection which has been reported to be the main source of infection in goats (Blood *et al.*, 1989; Alawa *et al.*, 2000).

Higher parity, late lactation stage and lower milk yield are shown to be significantly associated with higher probability of infection in a study carried out by Koop *et al.* (2009). Several other authors have reported higher prevalence of intramammary infection for higher parity goats

(Ndegwa *et al.*, 2000; Moroni *et al.*, 2005). Moroni *et al.* (2005) hypothesized that the greater prevalence of intramammary infections in higher parity animals was partially caused by chronic infections from the previous lactation that were not eliminated in the dry period. Nigerian breeds of goats are rarely milked, hence, their milk are rarely tested for quality and other physical characteristics. It is therefore difficult to detect early cases of mastitis or subclinically infected animals; without the swelling of the mammary glands (Addo *et al.*, 1980; Alawa *et al.*, 2000).

A peak occurrence of clinical mastitis in does examined over a 5 year period was linked with hot dry to early humid season (may-september), a period noted for high kidding rate and reduced hygienic status of the environment (Kawu *et al.*, 1992; Alawa *et al.*, 2011).

Herds that are experiencing mastitis problems caused by *S. aureus* should focus on reducing the prevalence of infected animals and identifying and segregating the infected animals. When CNS is the prevalent cause of mastitis, control procedures should be focused on pre-milking hygiene, use of best management practices for milking and maintaining healthy teat ends.

#### **2.4 Nomenclature and General Characteristics of Genus *Staphylococcus***

The genus *Staphylococcus* is made up of Gram-positive bacteria that are spherical in shape and occur in microscopic clusters like grapes. The genus belongs to the family *Staphylococcaceae* and the genus *Bacillus* is its nearest phylogenetic relative. They are facultative anaerobes that grow by aerobic respiration or by fermentation that yields lactic acid principally (Todar, 2004).

Staphylococci are perfectly spherical cells with a diameter of about 1micrometer. They grow in clusters due to the cells that divide in three perpendicular planes successively with the sister cells remaining attached to one another after each successive division. This helps in distinguishing it from the *Streptococcus* that grows in chains and divide in one plane only like *Bacillus*.They are non-motile, non-spore forming, and usually catalase positive, facultative anaerobes with exception of *S. saccharolyticus* and *S. aureus* subspecies *Anaerobius* which are catalase negative and grow more rapidly under anaerobic conditions (Kloos *et al.*, 1998).

They are widespread in nature, although they are mainly found living on the skin, skin glands and mucus membranes of mammals and birds. They can also be found in the mouth, blood, mammary glands, intestine, genitourinary and upper respiratory tracts of their host.

The pathogenic members of this genus include *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S.lugdunensis*,*S. warneri* and *S. saprophyticus* which are commonly associated with human infections, while *S. intermedius* and *S. hyicus* are species of special veterinary interest.

## **2.5 Biochemical Identification of *Staphylococcus***

### **2.5.1 Catalase test**

This test is used to differentiate those bacteria that produce catalase, such as staphylococci from non-catalase producing bacteria such as streptococci. Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen. An organism from a 24 hour culture is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released by organisms that produce catalase (Cheesbrough, 2000).

### **2.5.2 Sugar fermentation test**

Sugar fermentation tests determine for the ability of staphylococci strains to break down certain sugars, such as mannitol and trehalose, into alcohol. The test organism is inoculated into a tube containing 1percent sugar. The tubes are incubated at 37°C for 24 hours. If the tube turns yellow, the bacterium is said to ferment that particular sugar with or without gas production.

### **2.5.3 Coagulase test**

This test is used to identify *S. aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase, free and bound coagulase (clumping factor) are produced by most strains of *S. aureus* (Cheesbrough, 2000).

### **2.5.4 Novobiocin sensitivity**

Sensitivity to the antibiotic novobiocin is used to distinguish between different species of *Staphylococcus*. A small portion of the bacterial colony isolated from the patient is placed in growth medium plates with the antibiotic novobiocin, and presence of growth after incubation at 37°C for 24hours indicates the presence of *Staphylococcus saprophyticus*.

### **2.5.5 Urease test**

Urease broth is a growth medium that tests the ability of bacteria to produce an enzyme, called urease, which breaks down urea into ammonia and carbon dioxide. The broth contains phenol red that turns pink in the presence of ammonia, thereby indicating a positive reaction. Urease test is used to distinguish between different species and subspecies of staphylococci such as *S.*

*cohnii* subsp. *Cohnii*, *S. cohnii* subsp. *ureolyticus*, and *S. epidermidis* isolates that have atypical results for other tests.

### **2.5.6 DNase test**

This test is used to identify *S. aureus* which produces deoxyribonuclease (DNase) enzymes. It is particularly useful when plasma is not available to perform a coagulase test or when results of coagulase test are difficult to interpret. The principle of the test is that Deoxyribonuclease hydrolyses deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA and incubated at 37°C for 24 hours. Colonies are tested for Dnase production by flooding the plate with a weak hydrochloric acid solution. Dnase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2000).

### **2.5.7 Microgen™ STAPH-ID testing of *Staphylococcus***

The Microgen™ STAPH-ID system is an identification system for staphylococci of clinical, animal health and environmental importance. It employs 12 standardized biochemical substrates (Sucrose, Trehalose, Mannitol, N-Acetyl Glucosamine, Mannose, Turanose, Alkaline phosphatase, Glucosidase, Glucuronidase, Urease, Arginine, Pyrrolidonyl arylamidase (PYR) in micro wells to identify medically important members of the genus *Staphylococcus*. The inoculation and incubation of the test organism is usually carried out as described by the manufacturer, Microgen Bioproducts Ltd, Camberly, United Kingdom. Organism identification is based on colour change and substrate utilization. The substrates are organized into triplets (sets of 3 reactions) with each substrate assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet forms a single digit of the octal code that is used to determine the

identity of the isolate. The reactions are recorded in a report form with 5 blocks of reactions with four blocks containing the biochemical substrates and a single block for recording latex agglutination, colony pigmentation production and nitrate utilization by the organism. Each block of three reactions is converted into a numeric value to obtain a five-digit of microgen code (octal code). The octal code is entered into the Microgen Identification System Software (MID-60) which generates a report of the five most likely organisms in the selected data base. The software also provides identification based on probability, percentage probability and likelihood with an analysis of the quality of differentiation.

## **2.6 *S. aureus* Infection in Man**

*Staphylococcus aureus* is a Gram-positive bacterium that colonizes epithelial surfaces and causes infections in humans (Stefani *et al.*, 2012). Most *Staphylococcus aureus* isolates are coagulase positive because they produce an enzyme that causes clotting of blood plasma and are well documented as human opportunistic pathogen. *S. aureus* is considered the world's third most important cause of food borne illnesses (Tirado and Schimdt, 2001) because they produce *Staphylococcus* enterotoxins (SEs) which generally retain their biological activity even though *S. aureus* cells are killed by pasteurization (Evenson *et al.*, 1988; Asao *et al.*, 2003). *S. aureus* produces many other virulence factors such as exfoliative toxins, toxic shock syndrome toxin, and leukocidins besides its enterotoxin production. It is responsible for a variety of mild to severe skin and soft tissue infections and numerous serious infections including endocarditis, endophthalmitis, osteomyelitis, meningitis, bacteremia, pneumonia, and toxic shock syndrome (Larkin *et al.*, 2009).



## 2.7 Staphylococcal Mastitis

Staphylococcal mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. The chief reservoir of this bacterium is an infected udder. The organism is well adapted to survive in the udder and usually establishes mild subclinical infection of long duration. Bacteria are shed into milk from infected quarters (Abera *et al.*, 2010). Transmission occurs mainly at milking time through contaminated milking machines, clothes and hands of milkers or machine operators (Radostits *et al.*, 1994).

Efforts have only been concentrated on the treatment of clinical cases of mastitis owing to the heavy financial implications involved and the inevitable existence of latent infection. Mastitis is obviously an important factor that limits dairy production. Hence, the disease should be studied as it causes financial losses as a result of reduced milk yield, discarded milk following antibiotic therapy, veterinary expense and culling of infected animals (Abera *et al.*, 2010).

Mastitis in goats can be caused by a number of pathogens, but the most important bacterial genus is *Staphylococcus*, usually divided into *Staphylococcus aureus* and coagulase-negative staphylococci (CNS). *S. aureus* is a major pathogen that causes clinical and also subclinical cases of mastitis in goats (Bergonier *et al.*, 2003; Contreras *et al.*, 2003; Koop *et al.*, 2009). It also causes acute clinical or gangrenous mastitis. Gangrenous mastitis is a peracute form of mastitis, characterized by necrosis of the udder tissue, caused by alpha-toxins (Smith and Sherman, 2009). The severity and painfulness of this disease makes *S. aureus* mastitis a serious threat for animal welfare. *S. aureus* is also an economically important pathogen and a public health hazard because of the possible shedding of this bacterium and its toxins into milk (Koop *et al.*, 2009).

Coagulase-negative staphylococci (CNS) have been reported to be responsible for the majority of subclinical cases of mastitis (Contreras *et al.*, 2003), but clinical mastitis caused by these pathogens has occasionally been described. CNS are also regarded as major pathogens in mastitis, given their potential to significantly increase somatic cell counts and decrease milk yield. Although the increase in somatic cell counts (SCC) caused by CNS may be statistically significant, it is not nearly as strong as the effect of *S.aureus* (Koop *et al.*, 2009).

## **2.8 Discovery and History of Methicillin-resistant *Staphylococcus aureus***

In the late 1940s and throughout 1950s, *S. aureus* developed resistance to penicillin. Methicillin was the most common type of antibiotics used to treat *S. aureus* infections, but in 1961, British scientists identified the first strain of *S. aureus* bacteria that was resistant to methicillin and was called methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a major cause of hospital-acquired infections that are becoming increasingly difficult to cure because of their resistance to almost all the current antibiotic classes (Enright *et al.*, 2002). The methicillin resistance (*mecA*) gene encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species (Hiramatsu *et al.*, 2001). The *mecA* gene is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), of which the various forms have been described to differ in size and genetic composition (Hiramatsu *et al.*, 1997). Many MRSA isolates are multiple resistant and are susceptible only to glycopeptide antibiotics such as vancomycin. MRSA isolates that have decreased susceptibility to glycopeptides (glycopeptide intermediately susceptible *S. aureus*, GISA) have been reported as a cause of great public health concern (Hiramatsu *et al.*, 1997).

The origins of the major MRSA clones are still poorly understood. Kreiswirth *et al.* (1993) reported that all MRSAs were descended from a single ancestral *S. aureus* strain that acquired *mecA*, but other studies (Musser and Kapur, 1992; Fitzgerald *et al.*, 2001) show that some MRSAs are very divergent, implying that *mecA* has been transferred between *S. aureus* lineages. The first reported case of MRSA in human in United States came in 1968. Subsequently, new strains of bacteria have developed that can now resist previous effective drugs e.g. methicillin and most related antibiotics. MRSA is resistant to an entire class of penicillin-like antibiotics called beta-lactams which include penicillin, amoxicillin, oxacillin, methicillin and others. *S. aureus* is evolving even more and has begun to show resistance to additional antibiotics (National Institute of Allergy and Infectious Diseases, 2008). MRSA has evolved from a controllable nuisance into a serious public health concern. About one-third of people in the world have *S. aureus* bacteria in their bodies at any given time especially in the nose and on the skin.

*Staphylococcus aureus* is a major pathogen both within hospitals and in the community (Stapleton and Taylor, 2002). In the pre-antibiotic era, the rate of mortality from invasive *S. aureus* disease was high and the introduction of penicillin had a dramatic impact on treatment (Grubb, 1998). The semi-synthetic penicillin, methicillin was introduced in 1959 to overcome the problems that arose from the increasing prevalence of penicillinase-producing isolates of *Staphylococcus aureus* resistant to penicillin G and penicillin V (Enright *et al.*, 2002).

The whole world contends with methicillin-resistant *Staphylococcus aureus* (MRSA) as a major clinical problem (Lowy, 1998) with many strains resistant to most other classes of antimicrobial agents (Panlilio *et al.*, 1992; Speller *et al.*, 1997). The mechanism of methicillin resistance is the possession of an altered penicillin binding protein (PBP2a), that is encoded by the *mecA* gene

carried on a mobile DNA element, the Staphylococcal cassette chromosome *mec* (*SCC<sub>mec</sub>*) (Katayama *et al.*, 2000; Lowy, 2003). The *mecA* gene-product, PBP2a is an inducible, 76-78 kDa penicillin binding protein which in MRSA strains substitutes the other PBPs and its low affinity for all  $\beta$ -lactam antibiotics enables the organism to survive exposure to high concentrations of this class of antimicrobial agents (Chambers, 1997; Chambers, 2001; Lowy, 2003).

In recent years, *S. aureus* has been increasingly associated with antibiotic resistance. Methicillin-resistant *S. aureus* (MRSA) includes those strains that have acquired genes conferring resistance to methicillin and essentially all other beta-lactam antibiotics. MRSA was initially reported as a nosocomial pathogen in human hospitals (or hospital-associated MRSA, HA-MRSA). Since the 1990s, community-acquired or community-associated MRSA (CA-MRSA) infections have also been reported to affect people having no epidemiological connection with hospitals.

## **2.9 Nomenclature of MRSA Strains**

Methicillin-resistant *Staphylococcus aureus* (MRSA) can be categorized according to where the infection was acquired into: hospital-acquired MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA) and Livestock-associated MRSA (LA-MRSA).

### **2.9.1 Hospital-acquired MRSA (HA-MRSA)**

Hospital acquired-MRSA is acquired in the hospital setting and is one of many hospital-acquired infections exhibiting increased antimicrobial resistance. HA-MRSA has increased during the past decade due to a number of factors including an increased number of immune-compromised and elderly patients; an increase in the number of invasive procedures, e.g., advanced surgical

operations and life support treatments; and failures in infection control measures such as hand washing prior to patient contact and removal of non-essential catheters (Enright *et al.*, 2002).

The majority of HA-MRSA strains isolated in European countries have emerged from the introduction of the staphylococcal cassette chromosome *mec* (SCC*mec*) harbouring the methicillin-resistance gene *mecA*, and are classified into five *S. aureus* clonal complexes (CC), as defined by multilocus sequence typing (MLST): CC5, CC8, CC22, CC30 and CC45 (Deurenberg *et al.*, 2007).

### **2.9.2 Community-associated MRSA (CA-MRSA)**

Community acquired-MRSA is caused by newly emerging strains unlike those responsible for HA-MRSA and can cause infections in otherwise healthy persons with no links to healthcare systems. CA-MRSA is occurring with increasing frequency around the world. Risk factors for the development of CA-MRSA infection include close contact with other people with CA-MRSA, e.g. having a family member from a country with a high prevalence of CA-MRSA, living in crowded facilities, poor hygiene, sharing of personal items and performing contact sports (Ellington *et al.*, 2009; Diederer *et al.*, 2006). CA-MRSA causes mainly skin- and soft-tissue infections ranging in severity from furuncles to necrotizing fasciitis and other life threatening infections (Diederer *et al.*, 2006). Moreover, the description of serious invasive CA-MRSA infections, such as necrotizing pneumonia, is cause for concern, because these infections are associated with a mortality of up to 75% (Gillet *et al.*, 2002).

The early 2000s saw the emergence of community-associated MRSA (CA-MRSA), which in contrast to hospital-associated MRSA (HA-MRSA), spreads and causes disease in the general population, outside of the healthcare setting and often in people without typical risk factors (Zetola *et al.*, 2005). Although colonization with CA-MRSA is widespread around the world, the incidence of disease is greater in homeless people and injection drug users (IDUs) compared to the general population. This is likely the result of a combination of factors including compromised health, crowding in shelters, poor skin integrity, and injecting in unhygienic environments (Kerr *et al.*, 2005).

### **2.9.3 Livestock-associated MRSA**

The burden of MRSA colonisation and infection has been found to also involve animals, particularly livestock. In Europe, a survey published by the European Food Safety Authority (EFSA) identified MRSA in pig holdings of 17 EU Member States (EFSA, 2008). The MRSA clone, which was isolated from the vast majority of pigs, was non-typeable by PFGE (EFSA, 2008). Besides swine, MRSA CC398 strains have also been detected in other animals such as cattle and poultry (Moneck *et al.*, 2007; Nemati *et al.*, 2008). Latest Dutch figures indicate that 40% of pigs, 13% of calves and a high proportion of chickens carry MRSA. All people living on pig and cattle farms are now considered to be at high risk of being MRSA carriers (Voss *et al.*, 2005). Two outbreaks of human disease have been related to the consumption of MRSA-contaminated meat, one as classical food intoxication (Jones *et al.*, 2002) and the other with contaminated food as the source of nosocomial transmission (Kluytmans *et al.*, 1995).

## 2.10 Transmission of MRSA

Methicillin-resistant *Staphylococcus aureus* is transmitted by having direct contact with someone who has an active infection, someone who is a carrier of the infection, or a contaminated object. The main route of transmission of MRSA between humans, humans and animals, animal to animal and between animals is through contaminated materials, surfaces, food or through dust (Lee, 2003). This is also the route of transmission of HA-MRSA and CA-MRSA. Conditions such as overcrowding and understaffing in hospitals can contribute to high colonization pressure and increase the transmission risk (Clements *et al.*, 2008). An active infection with MRSA can develop when a person is colonized and the bacteria enter an opening, such as a cut or scrape in the skin. A person can become colonized with MRSA in a couple of different ways, such as:

- Touching the skin of another individual who is colonized with MRSA, or who has an active MRSA infection, breathing the tiny droplets that are expelled during breathing, coughing, or sneezing.
- Touching a contaminated surface.

Once colonized with MRSA, a person can remain a carrier of the bacteria from a few days or weeks, up to several years. During this time period, people colonized with MRSA are not only at an increased risk of infecting others, but also themselves. The process of transmission that occurs with colonized individuals is the same that occurs with direct contact with an active infection. (Lee, 2003).

## **2.11 Detection of MRSA**

### **2.11.1 Use of antibiotic discs**

Oxacillin disc diffusion method was the earliest method of detection of methicillin resistance in *Staphylococcus aureus* but because of its low sensitivity and specificity, cefoxitin disc diffusion was introduced as a better surrogate marker of *mecA* gene detection by Clinical Laboratory Standard International (CLSI, 2011). Cefoxitin is regarded as a good marker (Skov, 2003).

### **2.11.2 Detection of MRSA through detection of PBP 2a**

Conventional methods for MRSA identification, such as disc susceptibility testing, are not always reliable since phenotypic expression of methicillin resistance is known to be heterogeneous, depending on such factors as incubation time, temperature, NaCl concentration and other environmental factors. Difficulties in the differentiation of MRSA from borderline oxacillin-resistant *S. aureus* (BORSA), for example, may also occur. Various studies have shown that in the identification of MRSA, it is more accurate to either directly detect the gene encoding the methicillin resistance determinant (*mecA*) or its product, penicillin-binding protein 2' (2a), or PBP2' (PBP2a), which is found in the cell membrane of MRSA. However, as nucleic acid hybridization and DNA amplification techniques such as PCR for detecting the *mecA* gene are expensive and technically demanding, simple and more inexpensive techniques are required for routine use. MRSA detection kits consist of a latex reagent sensitized with monoclonal antibody against PBP2' together with reagents to rapidly extract PBP2' from the bacterial membranes of MRSA. Extracts are prepared by boiling a suspension of *S. aureus* cells under alkaline conditions, followed by neutralization and a centrifugation step. The supernatant is then mixed



with the latex reagent on a test card and visible clumping or agglutination within three minutes indicates the presumptive presence of PBP2' (Mehndiratta and Bhalla, 2012).

## **2.12 Typing of MRSA**

Methicillin-resistant *Staphylococcus aureus* is a world-wide concern and the incidence continues to rise (Witte *et al.*, 1997). Due to the spread of MRSA to all parts of the world, effective control measures which are dependent on the thorough knowledge of the organism's epidemiology through typing techniques which can be universally applied is necessary (Weller, 2000). MRSA typing is an essential component of an effective surveillance system to describe epidemiological trends and infection control strategies. Current challenges for MRSA typing are focused on selecting the most appropriate technique in terms of efficiency, reliability, ease of performance and cost involved. Without a thorough knowledge of the factors which affect the acquisition of MRSA and the transmissibility of different strains, there will be no clear measures to stop it (Aber and Mackel, 1981). The different methods used in *S. aureus* typing include; phenotypic and molecular typing methods.

### **2.12.1 Phenotypic typing methods**

#### **2.12.1.1 *Antibiogram***

MRSA isolates are compared on the basis of their susceptibility to a range of antibiotics. The technique is easy to perform, gives rapid results, cheap, readily available in routine microbiological laboratory but has poor discriminatory ability and lack reproducibility. Antibiotic patterns are often influenced by the local environment, thus, the same antibiogram may be produced by unrelated strains as a consequence of similar selective pressure upon them.

It is also possible that the antibiogram of two isolates from the same clone may differ due to acquisition or loss of plasmids carrying resistance genes. In most circumstances, the antibiogram cannot be used as the sole typing method for MRSA (Weller, 2000).

#### **2.13.1.2      *Phage typing***

The concept of typing staphylococci with bacteriophages was first developed in the 1940s. It was noticed that some *S. aureus* contained temperate phages which lysed other bacteria of the same species. Those with a narrow host range could be used in groups to produce a pattern of lyses characteristic for individual strains. For many years, phage typing was the method of choice for the investigation of MRSA epidemiology. Several outbreaks have been defined with this technique and its discriminatory power is demonstrably greater than phenotypic tests such as capsular typing and zymotyping (Kerr *et al.*, 1990). It is a time consuming and technically demanding procedure which is most efficiently done on large batches. This and the necessity for keeping stocks of phages and the propagating strains, has confined it to larger laboratories and reference facilities (Weller, 2000).

#### **2.13.1.3      *Serotyping***

Serotyping has never been used extensively for *S. aureus*. Tests have been developed, particularly to detect differences in the capsular polysaccharide and the antigenic properties of coagulase. There are a total of 11 *S. aureus* capsular types but 85-90% of clinical isolates belong to just two of them. Serological surveys have shown that it is rare to find an MRSA which is not capsular type 5 or 8, with the former being more common (Schlichting *et al.*, 1993). Eight

coagulase serotypes have been described and used in Japan with the predominance of two types (Kobayashi *et al.*, 1995).

#### **2.13.1.4 Protein electrophoresis**

##### **Whole cell protein:**

Analysis of cellular proteins, produced by lysostaphin degradation, can be performed using polyacrylamide gel electrophoresis (PAGE). The result is a reproducible pattern consisting of approximately 50 bands. Despite the large number of fragments, differences between unrelated isolates of MRSA are small. Interpretation of protein profiles is often hindered by the lack of correlation to typing results obtained with phages and gel-to-gel variation caused by minor changes in the running conditions. This leads to poor discrimination, only slightly enhanced by computer analysis of band intensity as well as size (Costas *et al.*, 1989).

##### **Multilocus enzyme electrophoresis:**

Typing by multilocus enzyme electrophoresis (MLEE) involves extraction of enzymes from bacterial cell, separation by electrophoresis and examination by selective staining. The rate of enzyme migration depends on its amino acid composition and over 80% of single substitutions can be detected by a change in its electrophoretic properties (Selander *et al.*, 1986). Enzyme variation is used as a surrogate marker for differences in the genetic loci from which they originate. Bacteria can be assigned to an electrophoretic type (ET) on the basis of similarities between the enzymes and the degree of relatedness between two isolates can be assessed by the proportion of loci which show differences (Musser and Kapur, 1992).

### **Zymotyping:**

Zymotyping is a variation of MLEE based solely on differences in the electrophoretic properties of the bacterial esterase enzymes. *S. aureus* possesses three esterases, designated A, B and C in order of decreasing affinity for the anode. Each has been defined by its relative activity on five synthetic substrates (a- and b-naphthyl acetates, indoxyl acetate and a- and b-naphthyl butyrates) and its resistance to di-isopropyl fluorophosphates (Branger and Goulet, 1987). Zymotyping is not discriminatory enough to be a useful typing method for MRSA on its own and is too lengthy a procedure to be employed as a preliminary screening test (Weller, 2000).

### **2.13.2 Molecular typing of *S. aureus***

The key for identifying outbreaks and new strains of *S. aureus* is the diagnostic microbiology laboratory and reference laboratories. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real time. These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics. These techniques include real-time PCR and quantitative PCR and are increasingly being employed in clinical laboratories ([www.mrsaresources](http://www.mrsaresources)).

#### **2.13.2.1 *Plasmid analysis***

The first molecular technique used for epidemiological investigation of MRSA was plasmid analysis. This differentiates isolates according to the number and size of plasmids, measured by electrophoresis. The method is easy to perform and simple to interpret, but it also has some features that are less than ideal. Although more MRSA than MSSA possess plasmids, they are still not present in every isolate leaving many organisms non-typable (Tenover *et al.*, 1994). In

addition, the plasmid DNA may exist in more than one form (supercoiled, nicked or linear) all of which have different electrophoretic properties. Thus, bands seemingly of different sizes may, in fact, represent the same plasmid and the presence of only one or two plasmids in many *S. aureus* leads to poor strain differentiation (Melo and Torres, 1989).

### **2.13.2.2 Chromosomal DNA**

#### **Restriction enzyme analysis (REA):**

Chromosomal DNA is too big to analyse as a whole and must, therefore, be cut into smaller pieces. This can be done with restriction enzymes which recognize and cleave specific sequences. Enzymes, such as *BglII* and *EcoR1*, which bind to a site found frequently on the staphylococcal chromosome, produce multiple small fragments (Hartstein *et al.*, 1995). These are then separated by constant voltage electrophoresis and the patterns produced can be compared with those of other isolates. All MRSAs are typable by this method and it has been used successfully to distinguish epidemic isolates from sporadic cases (Hartstein *et al.*, 1995; Weller, 2000).

#### **Pulsed-field gel electrophoresis:**

Interpretation of restriction endonuclease patterns can be aided by the use of enzymes which produce fewer and larger fragments. These 'rare cutters' have longer recognition sequences which naturally occur less often within the chromosome. Thus, instead of hundreds of fragments ranging from 0.5 to 50 kb in length, a simpler pattern consisting of between 10 and 30 fragments, 10–800 kb in length is produced. Consequently PFGE allows comparison of the entire chromosome without the complicated patterns produced by frequent cutting restriction enzymes. PFGE has been used to investigate MRSA and compared to other techniques in numerous studies (Struelens *et*

*al.*, 1992). Although a variety of restriction enzymes have been used, none has been found to be better than *Sma*I. All isolates are typable and the pattern is reproducible even after many subcultures (Tenover *et al.*, 1994). Discriminatory ability is high and has been shown to be superior to bacteriophage typing, antibiograms, Random amplified polymorphic DNA (RAPD), ribotyping and zymotyping. Other genotypic typing methods for *S. aureus* include; southern hybridization which include ribotyping, insertion sequences, *mecA*: Tn 554 probe typing and binary typing.

### **2.13 Mechanism of Antibiotic Resistance by *S. aureus***

Staphylococcal resistance to penicillin is mediated by penicillinase (a form of beta-lactamase) production: an enzyme which breaks down the beta-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase. The mechanism of resistance to methicillin is mediated via the *mec* operon, part of the staphylococcal cassette chromosome *mec* (SSC*mec*). Resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP 2a or PBP2') that has a lower affinity for binding beta lactams (penicillins, cephalosprins and carbapenems). This allows for resistance to all beta-lactam antibiotics and obviates their clinical use during MRSA infections. Aminoglycosides such as kanamycin, gentamicin, streptomycin etc. were once effective against staphylococcal infections until the organism evolved mechanisms to alter the aminoglycosides action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosome (Carter *et al.*, 2000).

### **2.14 Occurrence of MRSA**

The first report of MRSA in livestock was a case of bovine mastitis in Belgium (Devriese *et al.*, 1972). Biotyping suggested a human origin of this isolate since molecular typing methods were not available. Two decades later, investigations found cattle to be colonized by LA-MRSA, with 88% positive farms among Dutch veal calves rearing units studied (Graveland *et al.*, 2008). A study in Belgium showed that up to 15% of lactating cows in herds with a previous history of MRSA were positive (Vicca *et al.*, 2008). In general, the occurrence of MRSA among bovine mastitis isolates is well studied and the prevalence seems to be low (Hendriksen *et al.*, 2008). In 2005, a high prevalence of LA-MRSA was found in Dutch pigs in slaughterhouses (de Neeling *et al.*, 2007). Other reports have confirmed these findings in countries like Denmark, Germany, Canada and Belgium and the predominant *spa* types found were t108, t034, and t011, which are all relatives of CC398. In poultry, a Belgian study revealed a new *spa* type t1456 within CC398 in poultry farms (14.3%) (Persoons *et al.*, 2009). The occupational hazard for LA-MRSA colonization through pig contact has also been confirmed in Belgian farmers (Denis *et al.*, 2008). Infections in humans with LA-MRSA have been described since 2004 (Vos *et al.*, 2005), with an increasing frequency in the Netherlands and Denmark.

In horses, the first report on the isolation of MRSA was published in 1996 in Japan. Weese *et al.* (2006) found that 5.3% of horses at a Canadian veterinary teaching hospital and 4.7% of horses on farms in Canada and USA were colonized with MRSA. Cuny *et al.* (2006) reported an infection rate of 4.8% MRSA cases in Austria. In equine clinic in Belgium, MRSA CC398 *spa* type t011 were isolated from various infections of 13 hospitalized horses (Hermans *et al.*, 2008). Occupational

or recreational exposure to horses has been incriminated as a risk factor for human colonization (Weese *et al.*, 2006).

The first companion-animal related outbreak of MRSA was reported by Scott *et al.* (1988) in a rehabilitation geriatric ward where the ward cat was colonized.

Since then, the number of reports on infections and colonization with MRSA from companion animals has increased (Leonard and Markey, 2008).

The transmission of a *apl* positive MRSA strain between humans and a dog has been reported (van Duijkere *et al.*, 2003). The available evidence suggests that humans are the source of infection of colonization of companion animals but animals can act as carriers and pass the infecting strain to humans in contact (Strommenger *et al.*, 2006). Several studies in veterinary hospitals in United Kingdom have shown that veterinary staff and their pets have a higher prevalence of MRSA although they are mostly asymptomatic carriers (Hanselman *et al.*, 2003). A study by Moodley *et al.* (2008) showed that MRSA carriage was significantly higher among the veterinary practitioners (3.9%) than among the participants not professionally exposed to animals (0.7%). MRSA infections in owners with involvement of their companion animals like dogs and cats have been suggested for many years and evidence of this hazard has increased (Leonard and Markey, 2008).

#### **2.14.1 Prevalence of methicillin-resistant *Staphylococcus aureus* in milk and dairy products**

*Staphylococcus aureus* is an opportunistic gram positive pathogen and the causative agent of many human and animal diseases. It is also an important human food borne pathogen. Certain strains of *S. aureus* can produce staphylococcal enterotoxins (SEs) in foods and cause



staphylococcal food poisonings (SFP). More recently, MRSA has been isolated from most food-producing animals and foods of animal origin, raising public health concerns. MRSA strains have been isolated from cows' or small ruminants' milk and various dairy products in many countries. The MRSA prevalence in milk and dairy products recorded in different countries or even regions of the same country differs significantly. High MRSA prevalence have been recorded in milk produced in most African countries, for instance as high as 60.3% in Ethiopia. The MRSA prevalence in Asian countries varies from high e.g. 28.3% in Iran to low (e.g. in Korea and Japan). In most European countries, the MRSA prevalence in milk and dairy products has been generally found to be low. In the US and Canada, zero to low MRSA prevalence estimates have been reported. The investigation of MRSA prevalence in milk may serve as a tool for assessing both the sanitary conditions employed in dairy herds and the health risks that humans may encounter when infected with antibiotic-resistant strains (Pexara *et al.*, 2012).

#### **2.14.2 MRSA and livestock production**

Several years ago scientists from the Netherlands published a study that identified MRSA in both swine and individuals who worked with swine. In the study, individuals who worked with pigs were more often carriers of MRSA than individuals who did not work with pigs. Since then, other groups have confirmed that MRSA can be isolated from pigs, while others have isolated MRSA from cattle and wildlife, indicating that the organism is present throughout all of nature spreading into different environments or communities. The strain of MRSA that is regularly isolated from livestock is often referred to as ST398. The ST398 strain is different from MRSA strains responsible for HA-MRSA infections and MRSA strains that cause CA-MRSA infections. The ST393 strain isolated from livestock appears to be a third, distinct type of MRSA. As

mentioned above, HA-MRSA strains are usually resistant to several antibiotics while CA-MRSA strains are usually susceptible to most antibiotics. The antibiotic resistance profiles of ST398 isolates seem to be less consistent where some are multi-resistant while others are not. There may be other genetic differences between the three MRSA strains, giving rise to the theory that they developed independently and not from one strain. It has been shown that MRSA ST398 has limited host specificity; it is able to colonize and to cause infections in various hosts. So far, the mechanisms of host adaptation are poorly understood (Cuny *et al.*, 2010). However, incidentally reported so far, MRSA ST398 can cause serious (invasive) infections and outbreaks in humans (Kluytmans, 2010).

### **2.15 Clinical Findings in MRSA Infections**

Colonization with *Staphylococcus aureus* or MRSA is similar to being colonized with other naturally-occurring bacteria and refers to the asymptomatic carriage of MRSA on the skin or in the nose. Most people with MRSA on their skin or in their nose are unaware they are colonized, and never develop a MRSA infection. When *Staphylococcus aureus* or MRSA enters through a broken skin, it can cause infections that may look like a pimple or boil and can be red, swollen, painful, or have pus or other drainage. More serious *Staphylococcus aureus* or MRSA infections include pneumonia, blood stream infections, or severe skin or wound infections (Larkin *et al.*, 2009).

### **2.16 Treatment of Staphylococcal Infections**

Vancomycin and teicoplanin are glycopeptide antibiotics used to treat MRSA infections. Vancomycin is still the preferred drug for the treatment of serious MRSA infections. However,

its effectiveness is limited by prolonged, persistent or recurrent bacteraemia during therapy, high rates of microbiological and clinical failures, nephrotoxicity and the increasing prevalence of non-susceptible strains (Lodise *et al.*, 2008). Teicoplanin is a structural congener of vancomycin that has a similar activity but a longer half-life. Linezolid, quinupristin/dalfopristin, daptomycin and tetracycline are used to treat more severe infections that do not respond to glycopeptides such as vancomycin (Mongkolrattanothai *et al.*, 2003). MRSA infections can also be treated with oral agents including linezolid, rifampicin-fusidic acid, rifampicin+ fluoroquinolone, pristinamycin, co-trimoxazole (trimethoprim-sulfamethoxazole), doxycycline or minocycline and clindamycin (Birmingham *et al.*, 2003).

## **2.17 Control and Prevention of MRSA Infections**

A variety of procedures have been developed by infectious disease specialists for the control and prevention of MRSA. These procedures involve options from preventive measures such as decolonization and isolation of MRSA-confirmed patients to simpler measures such as hand washing, use of gloves and reducing time in hospitals. There are expanded efforts towards more direct intervention, such as the use of anti-MRSA agents and vaccines, in an attempt to reduce the overall burden of MRSA (Wang and Barret, 2007). Surface sanitization with alcohol and quaternary ammonium of health care environments is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures (Leonard and Markey, 2008).

Prevention of MRSA strains among livestock involves;

- 1.Reduction in the dissemination of MRSA from farm to farm would be to improve biosecurity between herds and during transport. Prevention of trade from MRSA-positive to MRSA-negative herds should also be considered.

2. Sanitary control measures should be implemented.
3. Identification and isolation of animals to minimize the risk of zoonotic transmission.
4. Use of contact precautions such as protective outwear like overalls, aprons or coats, gloves, masks, boots and overshoes.
5. Protective outwears and all items handled during the treatment of an MRSA positive animal (e.g. boards to drive livestock) should be regarded as potentially contaminated.
6. Hand hygiene e.g. through alcohol gel pouches is essential but need to be performed correctly.
7. There is need for educational programs for veterinarians to be organized by competent authorities.
8. Hygiene measures such as hand disinfection and adequate wound management are essential.

### **2.18 Genomics of *Staphylococcus aureus***

*Staphylococcus aureus* is phylogenetically classified in the *Bacillus/ Staphylococcus* group. They typically contain genes involved in essential functions of the vegetative life of the bacteria such as DNA replication, protein synthesis and carbohydrate metabolism. The eight *S. aureus* sequenced genomes range in size from 2.820mb to 2.903mb and is composed of core genes which are associated with central metabolism and other housekeeping functions and auxiliary genes. Approximately 75% of *Staphylococcus aureus* genome comprises a component of genes present in all of the strains. Other genes within the core genome that are not essential for growth and survival include virulence genes that are not carried by other staphylococcal species, surface binding proteins, toxins, exoenzymes and capsule biosynthetic cluster (Shittuet *al.*, 2007). The accessory genome of the *S. aureus* genome encodes a diverse range of non-essential

functions ranging from virulence, drug and metal resistance to substrate utilization and miscellaneous metabolism. They usually consist of mobile genetic elements (MGEs) that transfer horizontally between strains. These elements include bacteriophages, pathogenicity islands, chromosomal cassettes, genomic islands and transposons. The identification and characterization of these elements has provided valuable information into how *S. aureus* causes diseases and its relative diversity (Shittuet *al.*, 2007).

### **2.19 Staphylococcal Cassette Chromosomes (SCC $mec$ )**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be resistant to virtually all available beta-lactam antibiotics. The *mecA* gene is carried with a larger family of DNA sequences called SCC $mec$  which can also encode other antibiotic resistance genes and inserts into a specific site on the *S. aureus* chromosome called *orfX* (Ito *et al.*, 1999). SCC $mec$  regions vary in size and the genes they carry but are relatively stable in the genome and have originated in other staphylococcal species (Katayama *et al.*, 2003). The *S. aureus* genome can also carry multiple mobile genetic elements (MGE) that encode a wide range of virulence and resistance determinants which are capable of horizontal transmission between strains (Lindsay and Holden, 2004).

Staphylococcal cassette chromosome (SCC) elements are mobile genetic elements that integrate at the unique site (*attB $sc$* ) in the chromosome of MRSA (Adebayo *et al.*, 2007). It is located near the replication origin of *S. aureus* which is 10kb downstream of *purA* and 66kb to 89 upstream of *spa* gene depending on the size of the integrated copy of SCC $mec$ . Each SCC $mec$  type is further classified into subtypes on the basis of the J-region sequence (Shittuet *al.*, 2007). The J-regions

contain various genes or pseudo genes whose presence does not appear essential or useful for the bacterial cell; except resistance genes for non beta-lactam antibiotics or heavy metals, some which are derived from plasmids or transposons.

Six major types of SCC*mec* have been found; SCC*mec* types I to VI. SCC*mec* types I-III are the most common in HA-MRSA (de Lencastre *et al.*, 2007). These strains often carry additional plasmids or transposons enhancing the spread of resistance to 2 or more unrelated classes of antimicrobials. CA-MRSA typically harbour the smaller and possibly more mobile SCC*mec* type IV and also type V, while multiresistance is less common. These strains often carry an exotoxin-panton valentine leukocidin toxin (PVL) (Appelbaum, 2007) while LA-MRSA carry SCC*mec* types III, IV or V. The Type II SCC*mec* of N315, MU50 and MRSA 252 contain an integrated copy of plasmid pUB110 with bleomycin and kanamycin resistance genes and transposon Tn554 carrying erythromycin and spectinomycin resistance in the J-region (Shittu *et al.*, 2007).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study Area

The study area spanned across three L.G.As which constituted Zaria and environs. Zaria is located in Kaduna state, within latitudes  $11^{\circ} 7' N$  and longitudes  $07^{\circ} 41' E$ . It is administratively divided into Zaria and Sabon gari Local Government Areas (Ministry of Economic Development, 1996). Giwa LGA is sandwiched between Sabon Gari and the neighbouring Katsina State. Samples were collected from households in Kongo, Saye, Jos road, Zaria city in Zaria L.G.A. and Kwangila, Palladan, Samaru, Bomo in Sabon gari L.G.A and also from the National Animal Production Research Institute (NAPRI) in Shika, Giwa L.G.A of Kaduna State.

A preliminary survey of the areas was conducted together with local informants to determine goat rearing households in the areas. Goats in the consenting households and goat units at the National Animal Production Research Institute (NAPRI) were included in the study.

#### 3.2 Sample Size:

The sample size used for the isolation of *Staphylococcus aureus* was calculated as described by Thrushfield (2007) at 95% confidence level.

$$N = \frac{Z^2 pq}{d^2}$$

Where,

N = sample size

q = 1 - p

p = anticipated prevalence for *S. aureus* in goat milk = 14.7 % (Stastkova *et al.*, 2009).

d = level of significance (0.05)

Z = 1.96

Therefore, 
$$N = \frac{1.96^2 \times (0.147 \times 0.853)}{(0.05)^2} = 192.68$$

Fresh milk samples were collected from 198 lactating goats using convenience sampling method. Three hundred and eighty six (386) milk samples were collected from both right and left halves of 198 goats based on availability of lactating goats and acceptance by the owners. Background information on the age, breed, location and system of management of the goats were recorded.

### **3.3 Sample Collection and Transportation**

Three millimetre of milk sample was aseptically collected in a sterile sample bottle following the standard procedures described by National Mastitis Council (NMC, 1990). Each teat was disinfected with a cotton wool soaked in 70% ethyl alcohol and the first squirt of milk was discarded before the collection of the milk samples. The samples were properly labeled and transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria in Coleman box with ice packs. The samples were immediately subjected to California mastitis test (CMT) and enrichment. Samples were collected between May and July 2013.

### **3.4 Materials Used**

#### **3.4.1 Bacteriological culture media**

Modified tryptone soy broth, sodium chloride, Baird Parker agar, Egg yolk tellurite emulsion, Nutrient agar, Mannitol salt agar, Mueller Hinton agar, Blood agar base, Andrade peptone water,



Sheep blood, Dnase agar. All the media used were products of Oxoid<sup>®</sup>Ltd, Basingstoke, UK, except otherwise stated and they were prepared according to the manufacturer's instructions.

### **3.4.2 Other materials used**

California mastitis test reagent, human plasma, 3% Hydrogen peroxide, 0.1N Hydrochloric acid, normal saline, CMT paddle, grease-free slides, bijou bottles, sample bottles, test tubes, foil papers, masking tapes, permanent markers, disposable petri dishes, conical flasks.

### **3.4.3 Sugars**

Glucose, lactose, maltose, mannitol, sucrose, xylose, arabinose and raffinose.

### **3.4.4 Antimicrobial impregnated disks and their concentrations**

Penicillin G (10units), sulphamethoxazole trimethoprim (25ug), gentamicin (10ug), ciprofloxacin (5ug), erythromycin (15ug), amoxicillin/clavulanic acid (30ug), nitrofurantoin (300ug), chloramphenicol (30ug), tetracycline (30ug), kanamycin (30ug), ampicillin (10ug), vancomycin (30ug), ceftriaxone (30ug) and ceftioxin (30ug) (CLSI, 2011).

### **3.4.5 Biochemical test kits**

Microgen STAPH-ID (Microgen Ltd, UK), PBP2a latex agglutination kit (Oxoid).

### **3.4.6 Materials for molecular characterization**

DNA extraction kit (Qiagen sciences, Maryland, USA), Primers, Taq polymerase (Fermentas® Master Mix), Agarose.

### **3.5 Determination of the Occurrence of Mastitis**

The California mastitis test was conducted to detect the presence of subclinical mastitis according to procedures given by Quinn *et al.* (1994). Two millilitre of each milk sample were mixed in 2ml of the CMT reagent (Alkyl aryl sulphuric acid) (Kruuse, Denmark) in each well of the test plate (CMT paddle). A gentle circular motion was made with the plate for 10 seconds to mix the reagent and milk; formation of milk clots upon addition of the reagent was recorded based on changes in the milk (colour change, viscosity and gel formation). Positive samples showed change in viscosity or gel formation within a few seconds. Results were read immediately according to the manufacturer's recommendation and were scored as 0 (negative), ± (trace), 1+ (weak positive), 2+ (distinct positive), 3+ (strong positive) depending on the degree of viscosity and of gel formed as described by Schalm *et al.* (1971), Ikram (1997) and Swai *et al.* (2008). In this study, the CMT scores of 0 and trace (±) were considered as negative and 1+, 2+ and 3+ as positive. A goat was considered positive if at least one half of the udder was positive by CMT with or without the isolation of microorganisms.

### **3.6 Isolation and Characterization of *Staphylococcus* species from Goat Milk**

#### **3.6.1 Isolation procedure**

One ml of goat milk sample was aseptically added to 9mls of Modified typtone soy broth (MTSB) supplemented with 6.5% NaCl and incubated at 37°C for 24 hours. After the incubation,

a loopful of the broth culture was streaked onto the surface of Baird Parker Agar (BPA) (Oxoid®) plates and incubated at 37°C for 24 hours. Presumptive colonies of *Staphylococcus aureus* species appearing as black shiny colonies on BPA were picked, sub-cultured on nutrient agar slants and grown at 37°C for 24 hours. The isolates were stored in the refrigerator at 4°C for further characterization.

### **3.6.2 Identification of *Staphylococcus* species**

Presumptive colonies were subsequently subjected to Gram-staining, biochemical tests and Microgen STAPH-ID system.

#### **3.6.2.1 Gram staining**

This was performed following the procedures described by Cheesbrough (2000). A colony from a 24 hour culture was emulsified in sterile distilled water and a thin smear was made on a grease free slide and was fixed by gently passing it through a flame. It was covered with crystal violet stain for 30-60 seconds and then the stain was washed off with slow running tap water and covered with Lugol's iodine for 30-60 seconds. The iodine was washed off with clean water and acetone-alcohol was used to decolorize the smear for few seconds before washing with clean water. The smear was covered with safranin for 2 minutes, rinsed and air dried. The prepared slides were examined microscopically at 100x (oil immersion) objective. Gram positive cocci (0.5 to 1.5µm in diameter) that occurred singly and in pairs, tetrads, short chains and irregular grape-like clusters were suggestive of *Staphylococcus* species.

### **3.6.2.2 Conventional biochemical tests for *Staphylococcus* species**

The following biochemical tests were carried out for the presumptive identification of *Staphylococcus aureus*; catalase, coagulase, sugar fermentation (glucose, lactose, maltose, sucrose, xylose, arabinose and raffinose), haemolysis on 5% sheep blood agar, pigmentation on mannitol salt agar and DNase activity.

#### **Catalase test:**

Suspected *Staphylococcus* colonies were tested for their ability to produce catalase enzyme. A drop of 3% hydrogen peroxide was put on a clean slide and a colony of the suspected isolate was picked with a sterile wire loop and suspended in the hydrogen peroxide. The formation of bubbles indicating the production of oxygen gas and water was indicated as a positive reaction.

#### **Sugar fermentation test:**

Isolates were subjected to fermentation test using the following sugars: glucose, mannitol, maltose, xylose, arabinose, lactose, sucrose and raffinose. To 4.2g of Andrade peptone water, 2.8g of each sugar was added and dissolved in 280mls of distilled water and heated gently to dissolve completely. Three milliliters each were dispensed into clean sample bottles and sterilized in an autoclave at 110°C for 10 minutes and left to cool. A colony of the suspected *Staphylococcus* isolate sub-cultured on Baird Parker plate was inoculated into the prepared sugar solution and incubated for 24 hours at 37°C. A change in colour to pink was indicative of fermentation.

**Pigmentation on mannitol salt agar (MSA):**

Isolates were streaked on mannitol salt agar plates to determine the ability of their cell wall to ferment mannitol. The agar was prepared according to the manufacturer's instruction and the suspected isolates were streaked and incubated at 37°C for 24 hours. Isolates that produced yellowish colonies were suggestive of being *S. aureus*.

**Hemolysis on sheep blood agar:**

The isolates suspected to be *Staphylococcus* species from pigmentation test on MSA were grown on 5% sheep blood agar to obtain discrete colonies and a clear zone of beta hemolysis around the colonies upon incubation at 37°C for 18-24 hours.

**Coagulase test:**

Isolates suspected to be *S. aureus* from hemolysis test were tested for coagulase activity using human plasma by tube method as described by Cruickshank (1975). A 1:10 of the plasma was prepared with normal saline and dispensed into sterile test tubes and each strain was inoculated into each tube by adding 0.1 ml of an 18-24 hour culture. The inoculated tubes were incubated at 37°C and checked at an interval of 1, 3 and 6 hours for coagulation. Test samples that failed to react after 6 hours were left over night at room temperature and re-examined. A positive reaction was indicated by definite clot formation.

**DNase test:**

Suspected *S. aureus* isolates from coagulase test were streaked on DNase agar plates and incubated at 37°C for 24 hours. The plates were flooded with a weak hydrochloric acid and the

appearance of clear zones around the colonies indicated a positive test while a negative test was seen as opaque zones (Cheesbrough, 2000).

### **3.6.2.3 *Microgen Staph-ID testing of Staphylococcus***

This test was carried out following the manufacturer's instructions. A single colony from an 18-24 hour culture was emulsified in the suspending medium supplied in the kit and mixed thoroughly. The adhesive tape sealing the microwell test strips was carefully peeled back. A sterile Pasteur pipette was used to add 3 drops (100ul) of the bacterial suspension to each well of the strips. After inoculation, wells 10 and 11 were overlaid with 3 drops of mineral oil. The top of the microwell test strip was sealed with the adhesive tape earlier removed and the strip was incubated at 37°C for 24 hours. After 24 hours, the adhesive tape was removed and initial readings of positive reactions were recorded with the aid of the colour chart and substrate reference table included in the kit. One drop of PYR reagent was added to well 12 and the result was read after 10 minutes. Formation of very deep pink colour indicated a positive result. Nitrate reduction test was carried out on well 9 by adding 1 drop of nitrate A and 1 drop of nitrate B reagent to the well and the result was read after 60 seconds. Results were recorded on the microgen Staph-ID forms to produce a five digit code which was then entered into the Microgen Identification System Software (MID-60) and the organism was identified based on probability, percentage probability and likelihood with the analysis of quality of differentiation.

### **3.6.3 Phenotypic detection of methicillin resistance among isolates**

#### **3.6.5.1 Penicillin- Binding Protein (PBP 2a) latex agglutination test**

The ability of the *S. aureus* isolates to produce PBP-2a was detected using the Oxoid Penicillin Binding Protein (PBP-2a) Latex Agglutination Test kit following the manufacturer's instructions (Oxoid® Ltd, Basingstoke, United Kingdom). Four drops of extraction reagent 1 was added in a micro centrifuge tube and 3-5 colonies of the test culture were suspended into the tube to obtain a turbid suspension. The tubes were placed in a water bath and heated at 95°C for 3 minutes and then allowed to cool to room temperature. One drop of the extraction reagent 2 was added into the tube and the content was thoroughly mixed and then centrifuged at 3000G for 5 minutes. The test cards were properly labeled as directed by the manufacturer and one drop each of the test and control latex was added to each of the test and control labeled circles on the cards. 50ul of the supernatant was carefully placed in each of the test and control circles, mixed thoroughly with a mixing stick and the card was rocked for about 3 minutes. Positive isolates showed agglutination with the test latex and not with the control within 3 minutes under normal lighting conditions.

#### **3.6.4 Determination of the susceptibility of *S. aureus* to commonly used antimicrobial agents**

The susceptibilities of 32 *S. aureus* isolates to 14 antimicrobial agents were determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Bauer *et al.*, 1966), and the results were interpreted according to the recommendations of Clinical Laboratory Standard Institute (CLSI, 2011). The antimicrobial disks used were; Penicillin G (10units), sulphamethoxazole trimethoprim (25ug), gentamicin (10ug), ciprofloxacin (5ug), erythromycin (15ug), amoxicillin/clavulanic acid (30ug), nitrofurantoin (300ug), chloramphenicol (30ug), tetracycline

(30ug), kanamycin (30ug), ampicillin (10ug), vancomycin (30ug), ceftriaxone (30ug) and cefoxitin (30ug).

A sterile wire loop was used to pick 3-5 well isolated colonies of the test organism and they were emulsified in 3ml of normal saline. The turbidity of the suspension was matched to the turbidity of 0.5 McFarland standards. A sterile swab was used to inoculate the test organism on Muller Hinton agar plates. The swab was evenly streaked over the surface of the plate and left covered for about 3-5 minutes for the surface of the agar to dry. A multidisc dispenser (Oxoid®) was used to distribute 7 discs of the antimicrobial agents per inoculated plate (20ml petridish). The plates were inverted and incubated aerobically at 37°C for 18-24 hours. After incubation, the diameter of zones of complete inhibition was measured and interpreted using the guidelines of CLSI (2011).

### **3.6.5 Determination of multiple antibiotics resistance index (MAR index)**

The MAR index was determined using the formula;  $MAR = a/b$ , where 'a' was the total number of antibiotics to which the test isolate was resistant and 'b' is the total number of antibiotics to which the test isolate has been evaluated for sensitivity (Akinjogunla and Enabulele, 2010; Tula *et al.*, 2013).

## **3.7 Molecular Characterization of *S. aureus* Isolates**

### **3.7.1 Protocol used for DNA extraction of *S. aureus* isolates**

The *S. aureus* isolates that were resistant to cefoxitin (used as a surrogate for oxacillin) (CLSI, 2011) were grown on Baird Parker media and incubated at 37°C for 24 hours. Three colonies of



each isolate were picked, inoculated into 5ml of Luria Bertani (LB) medium and incubated at 37°C for 24 hours. 1.5ml of the broth culture of each isolate was dispensed into a sterile eppendorf tube and centrifuged at 8000G for 2 minutes to obtain the bacterial cells. The supernatant was decanted, 400µl of lysis buffer was added to the tube and the content was thoroughly mixed and incubated at 70°C for 30minutes. The tube and its content were centrifuged for 2 minutes at 8000G. The supernatant was transferred to a spin-column and centrifuged after which 300µl of wash buffer II (Qaigen) was added and the tube was centrifuged at 8000G for 2 minutes. Subsequently, 300µl of wash buffer III was added and the tube was centrifuged at 8000G for 2 minutes. The flow through from the collection tube was discarded and this step was repeated. The spin column was centrifuged empty to remove excess buffer. The spin-column was then transferred to a clean eppendorf tube, 50µl of T.E elution buffer was then added directly to the column matrix, and this was allowed to stand for 1minute and centrifuged at 8000G for 2 minutes. This step was repeated with the addition of 30µl of the elution buffer and the pure DNA was collected in the eppendorf tube.

### **3.7.2 Determination of the presence of *Staphylococcus casstte* chromosomes *mecA* gene in *S. aureus***

The DNA preparations of the following isolates were used: L160, L49, R161, R89, L150, L127, L175, L105, R105, L94, R156, L13, R160, L156, L137, L50and R13.

Conventional PCR was used for the amplification of the *mecA* gene using the primers;MR1 5' GTC GAA TTG GCC AAT ACA GG-3' (forward) and MR2 5' TGA GTT CTG CAG TAC CGG AT-3' (reverse), expected to yield a PCR product of 1399bp. The PCR was performed in a 25µl volume containing 5µl Q solution, 5µl buffer, 1µl dNTPs, 7µl template DNA, 1µl *mecA* forward primer, 1µl *mecA* reverse primer, 4µl nuclease free water, 1µl enzyme. Amplification

was carried out using 35 cycles at 94°C for 30 seconds, 54°C for 1 minute and 72°C for seconds with an initial denaturation temperature of 95°C for 5 minutes and final extension temperature of 72°C for 7 minutes. This was carried out in a thermal cycler (Applied Biosystems GeneAmp PCR system 9700 version 3.05).

### **3.7.3 Agarose gel electrophoresis**

A 1.5% agarose gel was prepared by suspending 1.5g of agarose powder in 100mls of buffer. The mixture was heated to boil after which it was allowed to cool to 50°C and 0.6ul of ethidium bromide was added. It was then poured into the agarose gel chamber with the comb and allowed to solidify. Fifteen microlitre (15µl) of each PCR product was mixed with a drop of loading dye which was then loaded into wells of the gel. The circuit was connected to power source and the amplicons were allowed to migrate for 45 minutes at 100 volts. A 100bp DNA ladder (Finnzymes, Espoo, Finland) was used and a UV transilluminator (UVtec, Sigma, Germany) was used to view the bands of the PCR products. Bioprofile gel documentation system (Mitsubishi, Tokyo, Japan) was used to generate a final print of the gel picture.

### **3.8 Statistical Analysis**

Chi-square test and odds ratio was used to determine the level of association between the samples and the age, breed, system of management and the location from which the isolates were obtained. P values < 0.05 and odds ratio > 1 were considered significant.

## CHAPTER FOUR

### RESULTS

#### 4.1 Occurrence of Mastitis Based on the California Mastitis Test (CMT)

A cross sectional study on subclinical mastitis (SCM) was carried out on 198 lactating goats in Zaria, Kaduna State. Milk samples were collected from 386 udder halves (194 right udder halves and 192 left udder halves) while 10 udder halves had blind ends. Of these, 101 right udder halves and 100 left udder halves had CMT values  $\geq +1$ . The overall prevalence of mastitis based on CMT was 60.1%. Goats sampled from Sabon-gari L.G.A had the highest prevalence (64.40%) of subclinical mastitis (SCM) while the goats sampled from NAPRI and Zaria L.G.A had prevalence rates of 57.84% and 59.46% respectively (Table 4.1). There was no significant association ( $p=0.569$ ) between the occurrence of mastitis and location.

The prevalence of subclinical mastitis with respect to the breed of goats sampled is shown on Table 4.2. Kano brown had the highest prevalence of 79.4%, while Sahelian, Sokoto red and West African Dwarf had prevalence rates of 54.9%, 55.8% and 66.7% respectively (Table 4.2). There was a significant association ( $p=0.048$ ) between the breeds of goats and the occurrence of mastitis.

Based on age distribution of the goats, does aged 7 years and above had the highest prevalence of subclinical mastitis, 66.67%, while the goats aged between 1-3 years and 4-6 years had prevalence rates of 59.8% and 59.8% respectively. There was no significant association ( $p=0.89$ ) between the occurrence of SCM and the age of goats.

The prevalence of subclinical mastitis with respect to the management system under which the goats were kept is shown on Table 4.4. The results showed that goats kept under intensive system of management had a lower prevalence of subclinical mastitis (57.8%) compared to the goats kept under the extensive system of management (62.5%). There was no significant association ( $p=0.438$ ) between the occurrence of SCM and the system of management.

#### **4.2 Association between CMT Scores and Udder Halves of Goats in Zaria**

The association between CMT scores and the udder halves of goats in Zaria is shown on Table 4.5. Out of a total of 194 right udder halves sampled, 93 showed negative CMT score (CMT score=0 and trace), 47 weak positive, 26 distinct positive and 28 strong positive CMT scores. From a total of 192 left udder halves of goats sampled, 92 milk samples were scored as CMT negative, 53 weak positive, 28 distinct positive, and 19 as strong positive results. From the results obtained, 101 right udder halves were CMT-positive while 100 left udder halves were CMT-positive. From the odds ratio calculated, there was no association between CMT results and the udder halves of goats in Zaria ( $p>0.05$ ).

**Table 4.1: Occurrence of Mastitis in Goats in Zaria, Kaduna state, Nigeria**

Location	Total No. Sampled	No. Positive (%)*
NAPRI	102	59 (57.84)
Zaria L.G.A	37	22 (59.46)
Sabon gari L.G.A	59	38 (64.40)
Total	198	119 (60.1)

Chi Square=1.126, p=0.569

\*= % of row total

**Table 4.2: Occurrence of SCM in four Breeds of Goats in Zaria, Kaduna state, Nigeria**

Breed	Total No. Ssampled	No.Positive (%)*
Sokoto Red	104	58 (55.77)
Sahelian	51	28 (54.90)
Kano Brown	34	27 (79.4)
West African Dwarf	9	6 (66.66)
Total	198	119 (60.1)

Chi Square=7.884, p=0.048

\*= % of row total

**Table 4.3: Occurrence of SCM in the different Age groups of Goats**

---

Age (Years)	Total No. Sampled	No.Positive (%)*
1-3	82	49 (59.8)
4-6	107	64 (59.8)
7 and above	9	6 (66.67)
Total	198	119 (60.1)

---

Chi Square=0.232, p=0.89

\*= % of row total

**Table 4.4: California Mastitis Test (CMT) results for Subclinical Mastitis (SCM) in Goats in Zaria based on System of Management**

System of Management	Total no. sampled	No. positive (%)*	No. negative (%)
Intensive	102	59 (57.8)	43 (42.15)
Semi-intensive	96	60 (62.5)	36 (37.5)
Total	198	119 (60.1)	79 (39.9)

Chi-square = 0.601, p = 0.438

\*= % of row total



### **4.3 Association between CMT Scores and Occurrence of *S. aureus* in Goat Milk**

The association between CMT scores and the occurrence of *S. aureus* in goat milk is shown in Table 4.6. Out of a total of 386 milk samples tested, 32 *S. aureus* were isolated. Nine *S. aureus* were isolated from milk samples with CMT negative score while 13, 6, 3 and 1 *S. aureus* isolates were observed from trace, weak, distinct and strong positive CMT scores, respectively. From the odds ratio calculated, *S. aureus* was more likely to be isolated from milk samples that showed trace CMT score than from those with negative, weak, distinct and strong positive CMT scores.

Of a total of 386 milk samples tested by CMT, 201 samples were CMT-positive while 185 milk samples were CMT negative (CMT score 0 or trace ( $\pm$ )). Of the 185 milk samples taken as CMT-negative, 22 (11.9%) yielded *Staphylococcus aureus*, while only 10 (5%) out of the CMT-positive samples yielded *S. aureus* (Table 4.6).

### **4.4 Association between CMT Scores and Udder-Wise Isolation of *S. aureus***

The association between CMT results and the frequency of isolation of *S. aureus* from the udder halves of goats is shown on Table 4.7. The result shows that a higher prevalence of *S. aureus*, (62.5%) was isolated from the left udder halves in comparison to the right udder half (37.5%). The odds ratio calculation showed that *S. aureus* were more likely to be isolated from milk samples with CMT trace scores than the negative, weak positive, distinct and strong positive CMT scores.

#### **4.5 Prevalence of *S. aureus* in Goat Milk**

The results of this study showed the overall prevalence of *S. aureus* isolated from goats in Zaria to be 8.3%. From a total of 386 goat milk samples collected, 60 (15.5%) milk samples contained *Staphylococcus* species while 32 (8.3%) were confirmed as *S. aureus* by conventional biochemical testing and microgen Staph-ID system. The other *Staphylococcus* species identified in this study were *S. chromogenes*, 4(1.04%), 8 (2.1%) *S. hominis*, 5 (1.3%) *S. caprae* and 11 (2.85%) *S. haemolyticus* (Fig. 1).

#### **4.6 Antimicrobial Susceptibilities of Isolates**

The results of the antimicrobial testing (Table 4.8, fig. 2) showed high percentage of resistance by the isolates to ampicillin (100%), penicillin G (93.8%), cefoxitin (90.6%) and ceftriaxone (71.9%). The isolates were moderately resistant to kanamycin (46.9%), tetracycline (40.6%), erythromycin (43.8%), gentamicin (31.3%), amoxicillin/clavulanic acid (34.4%), vancomycin (31.3%) and trimethoprim-sulfamthozole (25%) and relatively low frequency of resistance was observed to chloramphenicol (15.6%), nitrofurantoin (15.6%) and ciprofloxacin (3.1%).

**Table 4.5: Association between CMT Scores and Udder Halves of Goats in Zaria**

CMT score	R	L	OR	95% C.I on OR
0	58	63	1.0	
Trace	35	29	1.31	0.71, 2.41
1+	47	53	0.96	0.57, 1.64
2+	26	28	1.01	0.53, 1.92
3+	28	19	1.60	0.81, 3.17
	194	192		

OR= odds ratio

R= left udder half

L= right udder half

**Table 4.6: Association between CMT Scores and Occurrence of *S. aureus***

CMT score	<i>S.aureus</i>		SR (%)	OR	95% CI on OR
	+	-			
0	9	112	7.4	1	
Trace	13	51	20.3	3.17	1.27, 7.90 *
1+	6	94	6	0.79	0.27, 2.31
2+	3	51	5.5	0.73	0.19, 2.82
3+	1	46	2.1	0.27	0.33, 2.20
Total	32	354			

\* Significant at  $P < 0.05$

SR= specific rate

OR=3.17, 95% C.I on OR=1.27, 7.90

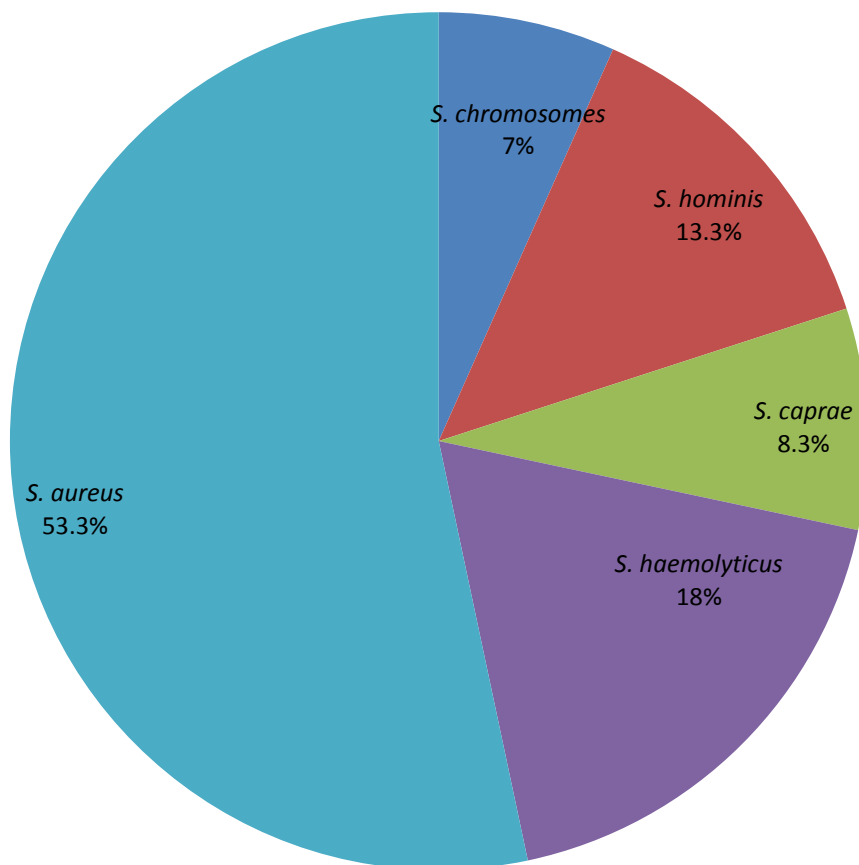


Figure 1: Prevalence of *Staphylococcus* species isolated from goat milk

**Table 4.7: Association between CMT Scores and Udder-wise Isolation of *S. aureus***

CMT score	<i>S. aureus</i>				OR	95% CI
	L		R			
	+	-	+	-		
0	2	61	7	51	0.239	0.048, 1.20
Trace ( $\pm$ )	10	19	3	32	5.614	1.371, 23.00*
1+	5	48	1	46	4.792	0.539, 42.59
$\geq 2+$	3	44	1	53	3.614	0.363, 35.98
Total	20	172	12	182		

\*Significant at  $< 0.05$

OR=5.61, 95% C.I on OR=1.37, 23.00

#### **4.7 Antimicrobial Resistance Patterns of 32 *Staphylococcus aureus* Isolates**

All the 32 *S. aureus* strains tested exhibited multiple antibiotic resistance patterns (Table 4.9) to the 14 antibiotics tested. Thirty antimicrobial resistance patterns were observed against the tested antimicrobials. The antimicrobial agents most commonly encountered among the patterns were ampicillin, penicillin G, ceftiofloxacin and ceftriaxone, AMP-CRO-P-FOX-CN and AMP-CRO-K-P-FOX-CN were the most occurring patterns that appeared twice, while all the others once.

#### **4.8 Multiple Antibiotics Resistance Index (MARI)**

The results of the multiple antibiotics resistance index showed that 96.9% of the *S. aureus* isolates tested were multidrug resistant. A total of 31 isolates were resistant to three or more antimicrobial agents tested while only one isolate showed resistance to two antimicrobial agents. None of the test isolates was simultaneously resistant to all the antimicrobial agents. The MAR index for the *S. aureus* isolates tested ranged from 0.14 to 0.86 where 40.6% of the isolates were resistant to at least 7 antimicrobial agents (Table 4.10). Also, 29 (90.6%) of the isolates had MAR index greater than 0.2.

#### **4.9 Detection of PBP 2a Among Isolates**

Twelve out of 32 isolates were PBP 2a positive.

#### **4.10 Detection of *mecA* by PCR**

All the isolates tested were negative for *mecA* gene by PCR using primers selected.

**Table 4.8: Antimicrobial sensitivity profile of *S. aureus* strains (n=32) Isolated from Goat Milk in Zaria, Kaduna state**

Antimicrobial agent	Disc content	No. (%) Susceptible	No. (%) Resistant
Ampicillin	10µg	0 (0)	32 (100)
Penicillin G	1.u	2 (6.25)	30(93.75)
Cefoxitin	30µg	3 (9.38)	29 (90.63)
Ceftriaxone	30µg	9 (28.13)	23 (71.88)
Kanamycin	30µg	17 (53.13)	15 (46.88)
Erythromycin	15µg	18 (56.25)	14 (43.75)
Tetracycline	30µg	19 (59.38)	13 (40.63)
Amoxicillin-clavulanic acid	30µg	21 (65.63)	11 (34.38)
Vancomycin	30µg	22 (68.75)	10 (31.25)
Gentamicin	10µg	22 (68.75)	10 (31.25)
Trimethoprim Sulphamethoxazole	25µg	24 (75.0)	8 (25.0)
Chloramphenicol	30µg	27 (84.38)	5 (15.63)
Nitrofurantoin	300µg	27 (84.38)	5 (15.63)
Ciprofloxacin	5µg	31 (96.88)	1 (3.13)



**Table 4.9: Antimicrobial Resistance Patterns of 32 *Staphylococcus aureus* Isolates**

---

S/N	Pattern	Frequency
1	AMP, CRO	1
2	AMP, CRO, P	1
3	AMP,FOX,P	1
4	AMP, FOX, E, CN	1
5	AMP, SXT, FOX, P	1
6	AMP, CRO, K, P	1
7	AMP, FOX, CRO, P	1
8	AMP, FOX, AMC, K, P	1
9	AMP, FOX, E, CRO, P	1
10	AMP,FOX, CRO, K, P	1
11	AMP, FOX, CN, TE, CRO	1
12	AMP, FOX, CN, CRO, K, P	2
13	AMP, FOX, CN, CRO, P	2
14	AMP,FOX, E,TE,C, CRO, P	1
15	AMP, FOX, E, VA, CRO, P	1
16	AMP, FOX, E, CRO, K, P	1
17	AMP, FOX, TE, CRO, K, P	1
18	AMP, SXT, FOX, AMC, F, P	1
19	AMP, FOX, TE, VA, CRO, K, P	1
20	AMP, FOX, E, AMC, TE, VA, K, P	1
21	AMP, FOX, E, TE, VA, CRO, K, P	1
22	AMP, FOX, E, CN, VA, CRO, K, P	1
23	AMP, SXT, FOX, AMC, F, CRO, P	1
24	AMP, SXT, FOX, E, AMC, TE, C, K, P	1
25	AMP, FOX, E, AMC, TE,VA , CRO, P	1
26	AMP, CIP, FOX, AMC, TE, C, CRO, K, P	1
27	AMP, SXT, FOX, E, CN, AMC, TE,VA, C, F, P	1
28	AMP, SXT, FOX, E, AMC, TE, VA, F, CRO, P	1
29	AMP, SXT, FOX, E, CN, AMC, TE, VA, C, K, P,	1
30	AMP, SXT, FOX, E, CN, AMC, TE, VA, F, CRO, K, P	1

---

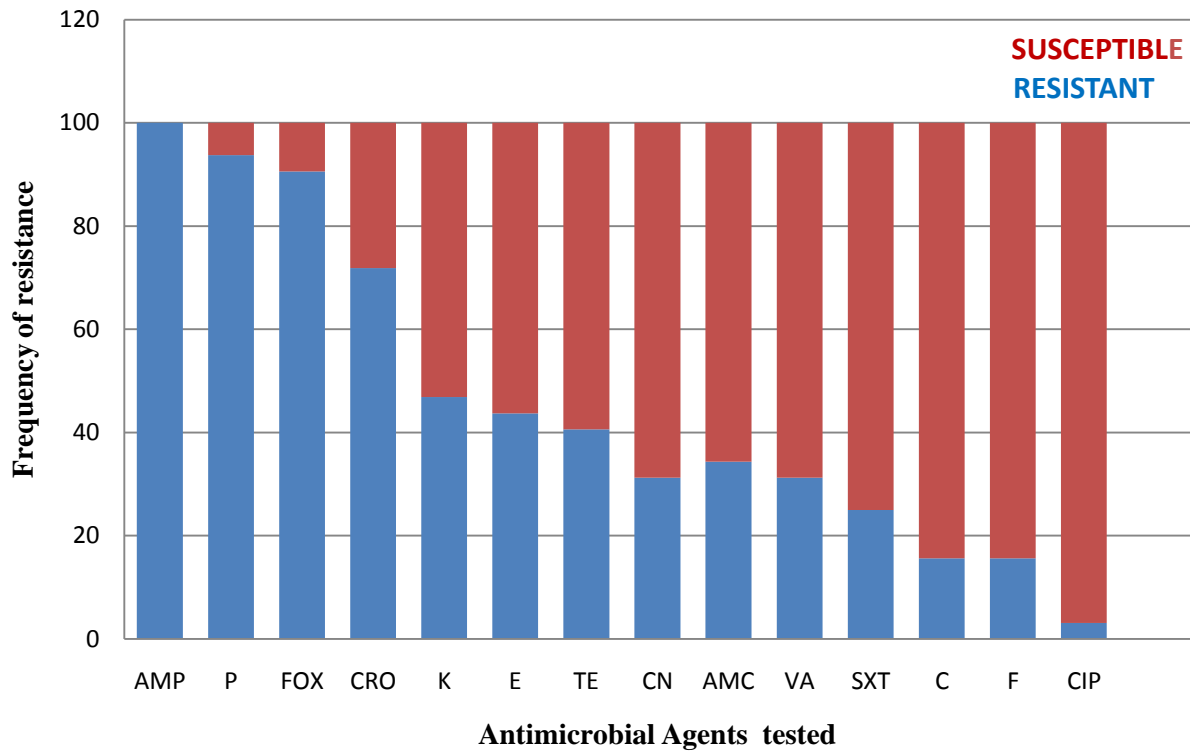


Figure 2: Percentage Distribution of resistance among *S. aureus* tested against 14 antimicrobial agents.

KEY

- TE - Tetracycline
- C - Chloramphenicol
- F - Nitrofurantoin
- CRO - Ceftriaxone
- K - Kanamycin
- P - Penicillin G
- AMP - Ampicillin
- CIP - Ciprofloxacin
- SXT - Trimetoprim-sulphamethoxazole
- AMC - Amoxicillin/clavulanic acid
- VA - Vancomycin
- CN - Gentamicin
- E - Erythromycin
- FOX - Cefoxitin

**Table 4.10: MAR Index Analysis of the 32 *S. aureus* isolates obtained from Goat Milk in Zaria**

S/N	Isolate	No. of antibiotics to which the isolate was resistant(a)	MAR Index (a/b)
1	L134	12	0.86
2	L127	11	0.79
3	L97	11	0.79
4	L156	10	0.71
5	R147	9	0.64
6	L151	9	0.64
7	R161	8	0.57
8	L160	8	0.57
9	L137	8	0.57
10	L150	8	0.57
11	L175	7	0.5
12	R159	7	0.5
13	L157	7	0.5
14	R136	6	0.43
15	L148	6	0.43
16	R110	6	0.43
17	L161	6	0.43
18	L111	6	0.43
19	L13	6	0.43
20	L105	6	0.43
21	L136	5	0.36
22	L19	5	0.36
23	L50	5	0.36
24	R105	5	0.36
25	R160	5	0.36
26	R114	4	0.29
27	R13	4	0.29
28	R184	4	0.29
29	L94	4	0.29
30	R89	3	0.21
31	R94	3	0.21
32	L49	2	0.14

b=Total no. of antibiotics used; 14

## CHAPTER FIVE

### DISCUSSION

Mastitis is one of the most important and frequent diseases of goats (Najeeb *et al.*, 2013). California Mastitis Test (CMT) was conducted on 386 milk samples collected from 198 lactating goats in Zaria to detect the presence of subclinical mastitis. A total of 201 (52.1%) milk samples from both the right and left udder halves were CMT positive (CMT  $\geq$ +1) while 185 (47.9%) were negative CMT result (CMT=0, trace;  $\pm$ ) (Table 4.6). Out of the 198 lactating goats sampled, 119 (60.1%) had subclinical mastitis in that a positive CMT result was detected in at least one of the udder halves, while 79 (39.9%) showed no evidence of subclinical mastitis from the CMT test (Table 4.1).

In this study, the prevalence of subclinical mastitis (SCM) by CMT was 60.1% which is within the range of 6.5%-67.0% reported in previous studies carried out by Contreras *et al.* (2003) in dairy goat farms. The finding of this study was similar to the 61% reported in Tanzania (Mbindyo *et al.*, 2014) and 52% in Palestine (Adwan *et al.*, 2005). This result is also comparable with the findings of Anyam and Adekeye (1995) in Nigeria and Moshi *et al.* (1998) from Tanzania who reported prevalences of 56% and 72.8% of SCM in goats, respectively. However, the 60.1% obtained in this study is higher than the 28.7% recorded in Kenya (Ndegwa *et al.*, 2000), 18% in Ethiopia (Gebrewahid *et al.*, 2012) and 15.5% in South Ethiopia (Megersa *et al.*, 2009). It is however lower than the 76.7% prevalence recorded in Tanzania (Mibilu *et al.*, 2007). The high prevalence of SCM observed in this study could be due to the period when the goats were sampled (May- July). Kawu *et al.* (1992) reported peak occurrence of clinical mastitis in does examined over a 5-year period to coincide with the hot dry to early humid season (May-

September), a period noted for high kidding rate and reduced hygienic status of the environment. In general, the variability in the prevalence of caprine mastitis between reports could be attributed to the difference in management systems, breeds of goat or technical knowledge of the investigators (Islam *et al.*, 2011).

In this study, goats that were sampled from Sabon-gari L.G.A had a higher prevalence of subclinical mastitis (64.4%) compared with goats sampled from Zaria L.G.A and NAPRI with prevalences of 59.46% and 57.84%, respectively. This may be due to the lower hygienic standards of the environment where the goats were kept and other management practices, since all the goats sampled from Sabon-gari L.G.A. were kept under the semi-intensive management system. Goats sampled from NAPRI were kept under intensive system of management where the hygienic standard of the environment is higher compared with the other locations from where the goats were sampled. Also, this could be due to the availability of technical expertise in the management of goats in NAPRI which is absent in the other locations.

Goats that were aged 7 years and above had higher prevalence (66.67%) of subclinical mastitis compared with goats aged between 1-3 years and 4-6 years with occurrence rates of 59.8% and 59.8%, respectively. There was no significant association ( $p=0.89$ ) between CMT scores and age of goats. The increase in occurrence of mastitis with age may be due to the fact that older goats are long term nannies that have stayed longer in the herd and hence are at higher risk to come in contact with mastitis causing organisms than the younger does. Older age and multiple parturitions have been reported to produce stress on animals and as a result, such animals become hosts of infectious agents due to low immunity level (Togun *et al.*, 2003). This in

agreement with the findings of Kerro and Tareke (2003) who found that the risk of clinical and subclinical mastitis increased significantly with advancing age of the cow. Also, Islam *et al.* (2011) reported a higher prevalence of SCM in older goats aged 5 years and above compared to younger does. Similarly, Zeng *et al.* (1999) also found the prevalence of SCM to increase with increasing age and lactation.

A higher prevalence of subclinical mastitis was found in goats raised under the semi-intensive system of management (62.5%) compared with goats kept under the intensive management system (57.8%). The reason for this difference in prevalence may be that goats kept under the semi-intensive management system are allowed to graze outside their pens during day time and their udders could be exposed to trauma from hard objects like tree branches, grasses, thorns and rusty metals in their scavenging environment, which makes them susceptible to mastitis causing organisms. Also, these animals have greater chances of mixing with other animals that may harbour mastitis-causing organisms and could get infected as a result of this exposure. This is similar to the findings of Togun *et al.* (2013) who recorded a high prevalence of mastitis in West African dwarf goats kept under extensive system in Ogbomosho, Nigeria.

The CMT-positive milk samples which yielded no bacterial growth on culture could be due to other bacteria that did not induce detectable levels of somatic cell counts. It could also be partly explained by the fact that the udder could be injured and is recovering from infection or the infection was not caused by bacterial pathogens (Menzie and Ramanan, 1993; Assefa *et al.*, 2006) or bacterial pathogens other than *S. aureus* would have been responsible for the infection since a selective medium was used for the bacterial isolation. It could also be due to

organisms such as mycoplasmas, which require a special media and cannot be identified by the routine bacterial isolation techniques (Menzies and Ramanoon, 2001). Also, it has been reported that a CMT score of 1+ or higher is a good indicator of mastitis in goats which corresponds to a somatic cell count of greater than  $0.8 \times 10^6$  cells/mL (Upadhyaya and Rao, 1993; Assefa *et al.*, 2006). Similarly, Assefa *et al.* (2006) reported that CMT negative and trace results could be culture positive (false negative) and a number of samples with CMT scores of  $\geq +1$  could also be culture negative (false positive; 20-40%). This result is in agreement with the reports of National Mastitis Council which states that 25-40% of milk samples of animals with clinical mastitis do not yield a bacterial isolate. Also, an infected quarter with a bacterial concentration below 100 cfu/ml could cause the bacteria to be shed intermittently; white blood cells in the milk may also have engulfed and sequestered bacteria preventing isolation, or some other mastitis causing pathogens such as viruses and fungi may have accounted for the high number of culture negative samples (NMC, 1987; Wallenberg *et al.*, 2002). The result of this study is also comparable with the findings of Kwanashie *et al.* (2012) who isolated bacteria from 27.75% of CMT negative milk samples in Zaria and also with the reports of Ndegwa *et al.* (2000), Wamukoya *et al.* (2006) and Mbindyo *et al.* (2014) who indicated that these bacteria may cause latent infection or they do not stimulate detectable increase in somatic cell counts. Also, Assefa *et al.* (2006) and Ndegwa *et al.* (2001) isolated bacteria from 32.8% and 22.5% of CMT negative samples respectively.

Previous studies on subclinical mastitis (SCM) in dairy goats revealed that Coagulase Negative Staphylococci (CNS) make up 44.7% to 95.9% of the isolated pathogens from milk samples, and *S. aureus*, which is usually considered to have the greater pathogenicity, accounts from 4.1% to

18.0% of SCM agents (Contreras *et al.*, 2007). This could be linked to the wide distribution of *S. aureus* inside the mammary glands and the skin of teats and udders (Jones *et al.*, 1998) and the fact that the organism has adapted to survive in the udder to establish chronic and subclinical infections (Salvatore *et al.*, 2010).

The prevalence of *S. aureus* in this study is similar to the 8% prevalence of *S. aureus* reported by Witaya (2011) in small holder dairy farms in Chiang Mai province of Thailand and 6.3% prevalence obtained from goat milk sampled from Iran (Alian *et al.*, 2012). It is however lower than the 17.3% prevalence reported by Kwanashie *et al.* (2012) in milk samples presented/ submitted to the microbiology laboratory, A.B.U, Zaria from 1980-1991 and 1999-2009 and also the 12.8% prevalence reported by Assefa *et al.* (2006) from goats in the southern rift valley region of Ethiopia.

The high prevalence of *S. aureus* intramammary infection can be of veterinary and public health concern because it is an important zoonotic bacterial pathogen which can also be transmitted to humans through raw milk and cause food borne intoxication associated with enterotoxin production.

In the present study, the prevalence of SCM was higher in milk samples collected from the right udder halves compared to the left udder halves, which was not found to be significant ( $P < 0.05$ ). This is in agreement with the reports of Swai *et al.* (2008) in Tanzania who reported 32.2% prevalence for the right udder halves and 25.2% for the left udder halves. This was thought to be due to the greater milk production capacity of the right udder halves (Swai *et al.*, 2008).



Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and possible contamination of food and water (Normanno *et al.*, 2007; Alian *et al.*, 2012). *S. aureus* has been known to show multiple antimicrobial resistance patterns. The widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus*, which as a result of the acquisition of multiple resistance genes has become able to survive almost all antibiotic families (Stefani and Gonglio, 2010).

In this study, resistance of *Staphylococcus aureus* isolates to ampicillin was higher compared to cefoxitin, penicillin G and ceftriaxone. The susceptibility of the isolates to ampicillin was within the range reported by other authors like Adegoke and Ojo (1982) and Ebrahimi *et al.*, (2007). The resistance observed for ampicillin (100%) is also similar to the reports by Onanuga *et al.* (2005) who observed a resistivity of 100% to ampilcillin in a study carried out in Zaria. The resistance of the isolates to ampicillin in this study may reflect the excessive use of the drug in treatment in the study area.

The resistance by *S. aureus* isolates to chloramphenicol observed in this study is comparable with the findings of Assefa *et al.* (2006) who reported resistance to chloramphenicol to be 18.7%. The result is similar to the report of Bhujbal *et al.* (1999) who reported resistance to chloramphenicol to be 12.2%, gentamicin (21.3%) and kanamycin (44%). The high proportion of susceptibility by the isolates to nitrofurantoin (84.38%), ciprofloxacin (78.13%) and chloramphenicol (78.13%) is suggestive of the potential efficacy of these drugs in treating MRSA infections and may reflect the fact that they may be less abused in the environment. Also,

the resistance to sulphamethozole/trimethoprim (25%) was significantly lower than that reported by Ghebremedin *et al.* (2009) in Ibadan (97%).

The evaluation of the antimicrobial susceptibility of *Staphylococcus* species isolated from goats with subclinical mastitis is of interest for clinical purposes in order to decide which antibiotics should be administered, as well as, for monitoring the spread of multiple resistant strains on farms (Salvatore *et al.*, 2010). This is due to the fact that in the literature, antibiotic resistance patterns for staphylococci isolated from subclinical mastitis refers mainly to cattle and little is known about dairy goats (Bochev and Russenova, 2005).

The multiple antibiotic resistance index (MAR) showed that 90.6% of isolates had MAR index greater than 0.25. This suggests that the isolates have originated from a high risk source of contamination where antibiotics are often used and probably abused. This may be due to the fact that most goat owners prefer administering drugs to their animals themselves without employing the services of veterinarians. The presence of multidrug resistant (MDR) *Staphylococcus aureus* in goat milk suggests the risk of the transfer of this bacterium via milk to humans.

The gold standard for detecting MRSA is by Polymerase Chain Reaction (PCR) detection of *mecA* gene (Bereger and Roher, 2002; Olayinka *et al.*, 2009) or alternatively detecting the *mecA* gene product PBP2a by latex agglutination test. Twenty nine isolates showed resistance to methicillin by disk diffusion, 12 isolates showed agglutination with PBP-2a Latex agglutination test kit (Oxoid) but none was shown to harbour the *mecA* gene by PCR. The discrepancy observed in this study between phenotypic expression of methicillin resistance by disc diffusion

(7.2%) and by latex agglutination test (3.1%) and the failure of the detection of the *mecA* gene in the presumed MRSA positive isolates, may be due to a number of factors such as culture conditions, pH of the medium, NaCl concentration in the medium, all of which could influence the phenotypic expression of the methicillin resistance giving rise to variable results (Choi *et al.*, 2003). Also, the isolates may not actually harbor the *mecA* gene but could contain genes encoding beta-lactamase which gradually hydrolyse methicillin (Alborzi *et al.*, 2000). Also, hyperproducers of beta-lactamase can be confused with true MRSA (Cookson and Philip, 1990).

The findings of *mecA* negative isolates which were phenotypically resistant to  $\beta$ -lactam antimicrobial agents could also be related to a less common type of resistance due to either over production of  $\beta$ -lactamase or the presence of altered penicillin binding protein (PBP) not related to 2a or 2' (Georgopapadakou, 1993). This is also in agreement with reports of Adesida *et al.* (2005) who reported 9.4% prevalence of MRSA based on zone of inhibition diameter but only 1.4% carried the *mecA* gene product, PBP2a using MRSA-screen latex agglutination test.

The stability of SCC*mec* in *S. aureus* is also influenced by environmental factors e.g. the spontaneous *mecA* deletion has been observed during long term storage in drug free medium (Hiramatsu *et al.*, 1990) and in cultures that have been aged/ starved, grown at high temperature, or given small doses of UV radiation (Annear and Grub, 1976; Inglis *et al.*, 1990).

The *mecA* gene was not detected by PCR in any of the strains tested. This indicates that alternative genes other than *mecA* that code for methicillin resistance would have been present in the isolates. It could also be due to alternative mechanism of methicillin resistance. The absence

of *mecA* gene in MRSA isolates in this study is in agreement with the findings of Olayinka *et al.*(2009) in Nigeria who found *mecA* gene to be absent in MRSA isolates from clinical specimens in Zaria. It also agrees with results of previous studies carried out on methicillin-resistant *S. aureus* strains isolated from sheep with SCM (De Santis *et al.*, 2005) and in goats with SCM from the island of Sardinia (Italy) (Salvatore, 2010) where *mecA* gene was absent in the MRSA isolates.

The results of the present study confirm that the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) is low in goats as previously reported in small ruminants (Alves *et al.*, 2009; Salvatore *et al.*, 2010).

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

The prevalence of subclinical caprine mastitis by California Mastitis Test (CMT) in Zaria was found to be 60.1% while the prevalence of *S. aureus* in goat milk in Zaria was found to be 8.3%. The prevalence of MRSA detected phenotypically was found to be 7.5%. The percentage resistance of the isolates to various antimicrobial agents ranged from 3.13% to 100%. Thirty one (96.88%) isolates showed multiple antibiotic resistance which is suggestive of the extent of misuse of antibiotics in veterinary practice in the study area and the possible transfer of these multidrug resistant *S. aureus* via goat milk to humans. Routine milking of lactating does and testing for mastitis would lead to early detection and treatment of subclinical cases of mastitis while culling of advanced and chronic cases would reduce the prevalence and spread of mastitis.

#### 6.2 Recommendations

The following recommendations are made based on the key findings of this study:

1. Microbiological examination of udder of lactating goats at regular intervals should be routinely carried out in order to detect subclinical mastitis.
2. There is need for prudent use of antimicrobial agents in the treatment of mastitis in goats to prevent drug abuse.
3. Appropriate guidelines should be drawn up for surveillance of MRSA in Nigeria in order to develop control and prevention strategies.

4. The isolates should be screened for variant forms of *mecA* so as to understand the genotypes circulating in the study area.
5. Goat milk should be pasteurized before consumption.
6. Further studies are necessary for assessing the importance of intra-mammary infections caused by other bacterial pathogens in goats.

## REFERENCES

- Aber, R.C. and Mackel, D.C. (1981).Epidemiologic typing of nosocomial microorganisms. *American Journal of Medicine*, 70:899-905.
- Aberal, M., Demie, B., Aragaw, K., Regassa, F., and Regassa, A. (2010).Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia.*Journal of Veterinary Medicine and Animal Health*, 2: 29-34.
- Adegoke, G.O. and Ojo, M.O. (1982). Biochemical characterization of staphylococci isolated from goats. *Veterinary Microbiology*, 7: 463-470.
- Adesida, S., Boelens, H., Babajide, B., Kehinde, A., Snijders, S., van Leeuwen, W., Verbrugh, H. and van Belkum, A. (2005). Major epidemic clones of *Staphylococcus aureus* in Nigeria. *Microbial Drug Resistance*, 11: 115-121.
- Addo, P. B., Chineme, C. N. and Eid, F. I. A. (1980).Incidence and importance of chronic mastitis in Nigeria goats.*Bulletin of Animal Health and Production in Africa*, 28: 225-231.
- Adu, I.F., and Ngere, L.O. (1979).The indigeneous sheep of Nigeria.*World Review of Animal Production*, 15: 51-62.
- Adwan, G., Abusafieh, D., Aref, R. and Omar, A. (2005). Prevalence of microorganisms associated with intramammary infection in cows and small ruminants in the north of Palestine. *Journal of the Islamic University of Gaza*, 13: 165-173.
- Aina, A.B.J. (2012). Goat (*Capra hircus*): A misunderstood animal. 35<sup>th</sup>Inaugural Lecture, Federal University of Agriculture, Abeokuta.
- Ajayi, S. A., Shuaib, Y., Abu, F.D., Asagba, M.A. and Lamorde, A.G. (1987).Sheep and Goat production and health management in Nigeria.*Nigeria Livestock Farmers*, 7: 18-21.
- Ajuwape, A.T.P., Roberts, A.A., Solarin, O.O. and Adetosoye, A.I. (2005).Bacteriological and haematological studies of clinical mastitis in goats in Ibadan, Oyo state, Nigeria.*Small Ruminant Research*, 60: 307-310.
- Akinjogunla O.J. and Enabulele, I.O. (2010). Virulence factors, plasmid profiling and curing analysis of multidrug resistant *S. aureus* and coagulase-negative *Staphylococcus* spp. isolated from patients with acute otitis. *Journal of American Science*, 6: 1022-1033.
- Alawa, J.P., Negele, M.B. and Ogwu, D. (2000). Chronic caprine mastitis in Nigerian goat breeds: microbiological flora and histopathological findings. *Small Ruminant Research*, 35: 203-207.
- Alayande, M. O. Hassan, W. A. and Kikisagbe, M.K. (2003). Prevalence of clinical mastitis in

- scavenging Red Sokoto goats in Sokoto, Nigeria. Proceedings of 28<sup>th</sup> Annual Conference. NSAP, Ibadan, pp 8-9.
- Alborzi, A., Pourabbas, B.A., Salehi, H., Pourabbas, H., Oboodi, B. and Panjeshashin, M.R. (2000). Prevalence and patterns of antibiotic sensitivity of methicillin-sensitive and methicillin-resistant *S. aureus* in Shiraz. *Iranian Journal Medical science*, 25: 1-8.
- Alferez, M.J.M., Barrionuevo, M., Lopez, A.I., Sanzsampelayo, M.R., Lisbona, F., Robles, J.C. and Campos, M.S. (2001). Digestive utilization of goat and cow milk fat in mal-absorption syndrome. *Journal of Dairy Resources*, 68: 451-561.
- Alian, F., Rahimi, E., Shakerian, A., Momtaz, H., Riahi, M. and Monemi, M. (2012). Antimicrobial resistance of *S. aureus* isolated from bovine, sheep and raw goat milk. *Global Veterinaria*, 8: 111-114.
- Alrawi, A.A., Pollak, E.J. and Laben, R.C. (1979). Genetic analysis of California mastitis test records. *Journal of Dairy Science*, 62: 1115-1124.
- Alves, P.D.D., McCulloch, J.A. and Even, S. (2009). Molecular characterisation of *Staphylococcus aureus* strains isolated from small and large ruminants reveals a host rather than tissue specificity. *Veterinary Microbiology*, 137: 190-195.
- Ameh, J.A. and Tari, I.S. (1999). Observations on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. *Small Ruminant Research*, 35: 1-5.
- Annear, D.I. and Grubb, W.B. (1976). Methicillin-sensitive variants in ageing broth cultures of methicillin-resistant *S. aureus*. *Pathology*, 8: 69-72.
- Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K., Nakazawa, H., and Kozyki, S. (2003). An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology and Infection*, 130: 33-40.
- Anyam, A.A. and Adekeye, J.O. (1995). Bacterial flora associated with mastitis in goats and sheep in Zaria (Nigeria) area. *Bulletin of Animal Health Production in Africa*, 43: 163-166.
- Akinsoyinu, A.O., Tewe, O.O., Ngere, L.O. and Mba, A.U. (1982). Milk composition and yield of the Red Sokoto (Maradi) goats in Nigeria. *Dairy Science*, 43: 83-84.
- Appelbaum, P.C. (2007). Microbiology of Antibiotics resistance in *Staphylococcus aureus*. *Clinical Infectious Diseases*, 45: 165-170.
- Arlington, V.A., Carter, G.R. and Cole, J.R.J. (1990). Diagnostic procedures in veterinary bacteriology and mycology. In: *Essentials of veterinary bacteriology and mycology*. 5th Edition, Academic press, USA.



- Assefa, W., Molla, B., Belihu, K., Kleer, J. and Hildebrandt, G. (2006). A cross-sectional study on the prevalence, antimicrobial susceptibility patterns, and associated bacterial pathogens of goat mastitis. *International Journal of Applied Research Veterinary Medicine*, 4: 169-176.
- Assefa, E., Abebe, G., Tegegne, A., Yosef, T. and Berhanu, G. (2011). Goat production system and opportunities for market orientation. *Tropentag*, 1: 1-4.
- Ayodhya, S. (2013). Isolation, identification and therapeutic management of bacterial mastitis in Jamunapari goat in Hyderabad. *Global Advanced Research Journal of Microbiology*, 2: 127-130.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C., and Turk, M. (1966). Cited by Kwaga J.K.P. and Adesiyun, A.A. (1984). Antibigram of *Staphylococcus aureus* isolates in some ready-to-eat products. *Journal of Food Protection*, 47: 865-867.
- Bergonier, D. and Berthelot, X. (2003). New advances in Epizootiology and control of ewe mastitis. *Livestock Production Science*, 79: 1-16.
- Bergonier, D., Crémouxb, R.D., Rupp, R., Lagriffould, G. and Berthelota, X. (2003). Mastitis of dairy small ruminants. *Veterinary Research*, 34: 689-716.
- Bhujbal, J., Deshumukh, V., Abdul, A., Gujar, M. and Aziz, A. (1999). Goat mastitis microflora and antibiogram. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 20: 44-46.
- Birmingham, M.C., Rayner, C.R., Meagher, A.K. Flavin, S.M., Batts, D.H. and Schentag, J. (2003). Linezolid for the treatment of multidrug resistant, gram-positive infections: Experience from a compassionate-use program. *Clinical Infectious Diseases*, 36: 159-168.
- Blood, D.C., Radostits, O.M. and Henderson, J.A. (1989). *Veterinary Medicine*, 7th edition. ELBS Bailliere Tindall, East England, pp.501-512, 1502.
- Bochev, I. and Russenova, N. (2005). Resistance of *Staphylococcus* spp. strains isolated from goat with sub-clinical mastitis. *Bulgarian Journal of Veterinary Medicine*, 8: 109-118.
- Bradley, A.J. (2002). Bovine mastitis: an evolving disease. *Veterinary Journal*, 164: 116-128.
- Branger, C. and Goulet, P. (1987). Esterase electrophoretic polymorphism of methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. *Journal of Medical Microbiology*, 24: 275-281.
- Browning, R., Kebe, S. H., and Byars, M. (2004). Preliminary assessment of Boer and Kiko does as maternal lines for kid performance under humid-sub-tropical conditions. *South African Journal of Animal Science*, 34: 1-3.

- Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T. and Ramakrishnan, V. (2000). "Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics". *Nature*, 407: 340-348.
- Chambers, H.F. (1997). Methicillin resistance in Staphylococci: molecular and biochemical basis and clinical implications. *Clinical Microbiology*, 10: 781-791.
- Chambers, H.F. (2001). The Changing epidemiology of *Staphylococcus aureus*. *Emerging Infectious Diseases*, 7: 178-182.
- Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press. UK. pp.136-142.
- Choi, S.M., Kim, S., Kim, H., Lee, D., Choi, J., Yoo, J., Kang, J., Shin, W., and Kang, M. (2003). Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among *Staphylococcus* species. *Journal of Korean Medical science*, 18: 631-636.
- Chigbu, C.O. and Ezeronye, O.U. (2003). Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria. *African Journal of Biotechnology*, 2: 374-378.
- Chineme, C.N. and Addo, P. B. (1984). Chronic caprine mastitis; Clinical, Microbiological and pathological findings in spontaneously occurring case in Nigerian goats. *International Goat and Sheep Research*, 2: 266-273.
- Clements, A., Halton, K., Graves, N., Pettit, A., Morton, A., Looke, D., and Whithy, M. (2008). Overcrowding and understaffing in modern health-care systems: key determinants in methicillin-resistant *S. aureus* transmission. *Lancet Infectious Diseases*, 8: 427-434.
- CLSI (2011). Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI Document M100- S1. Wayne, PA 19087, USA. pp.68-76.
- Contreras, A., Luengo, C., Sanchez, A. and Corrales, J.C. (2003). The role of intramammary pathogens in dairy goats. *Livestock Production Science*, 79: 273-283.
- Contreras, A., Sierra, D., Sanchez, A., Corrales, J., Marcoc, J., Paape, M. and Gonzalo, C. (2007). Mastitis in small ruminants. *Small Ruminant Research*, 68: 145-153.
- Cookson B. and Phillips, I. (1990). Methicillin-resistant staphylococci. *Journal of Applied Bacteriology*, 19: 552-570.
- Costas, M., Cookson, B.D., Talsania, H. and Owen, R. (1989). Numerical analysis of electrophoretic protein patterns of methicillin-resistant strains of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 27:2574-2581.

- Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975). *Medical Microbiology*. Vol. II. 12th edn., Churchill Livingstone, New York.
- Cuny, C., Kuemmerle, J., Stanek, C., Willey, B., Strommenger, B. and Witte, B. (2006). Emergence of MRSA infections in horses in veterinary hospital: strain characterization and comparison with MRSA from humans. *European Surveillance*, 11: 4477.
- Cuny, C., Friedrich, S., Kozytska, F., Layer, U. Nubel, K. Ohlsen, B., Strommenger, B., Walther, L. Wieler and Witte, W. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *International Journal of Medicinal Microbiology*, 300: 109-117.
- de Lencastre, H., Oliveira, D. and Tomasz, A. (2007). Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. *Current Opinion in Microbiology*, 10: 428-435.
- de Neeling, A.J., van den Broek, M.J., Spalburg, E.C., Santen-Verheuevel, M.G., Dam-Deisz, W.D., Boshuizen, H.C., van de Giessen, A.W., van Duijkeren, E. and Huijsdens, X.W. (2007). High prevalence of methicillin-resistant *Staphylococcus aureus* in pigs. *Veterinary Microbiology*, 122: 366-372.
- Denis, O., Suelens, C., Hallin, M., Ramboer, I., Catry, B., Gordts, B., Butaye, P. and Strulens, M.J. (2008). High prevalence of "Livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 in swine and pig farms in Belgium. 18<sup>th</sup> European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, Spain.
- De Santis, E.P.L., Mureddu, A., Mazzette, R., Scarano, C. and Bes, M. (2005). Detection of enterotoxins and TSST-1 genes in *S. aureus* isolates from sheep subclinical mastitis. In: Proceedings of the 4th IDF International Mastitis Conference: Maastricht, The Netherlands. pp. 410-504.
- Deurenberg, R.H., Vink, C., Kalenic, S., Friedrich, A.W., Bruggeman, C.A. and Stobberingh, E.E. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology Infection*, 13: 222-235.
- Devriese, L.A., Van Damme, L.R. and Fameree, L. (1972). Methicillin (cloxacillin)-resistant *S. aureus* strains isolated from bovine mastitis cases. *Zentralbl Veterinarmed B*, 19: 598-605.
- Diederer, B.M. and Kluytmans, J.A. (2006). The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. *Journal of Infectious Diseases*, 52: 157-168.
- Ebrahim R., Forough A., Amir S., Hassan M., Majid R. and Manochehr, M.Y. (2012). Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Bovine, Sheep and Goat Raw Milk. *Global Veterinaria*, 8: 111-114.

- Ebrahimi, A., Lotfalian, S. and Karimi, S. (2007). Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in Shahrekord district. *Iranian Journal of Veterinary Research*, 8: 76-79.
- Egwu, G. O., Onyeyili, P.A., Chibuzo, G.A. and Ameh, J.A. (1995). Improved productivity of goats and utilization of goat milk in Nigeria. *Small Ruminant Research*, 16: 195-201.
- Ehoche, O.W. and Buvanendran (1983). The yield and composition of milk and reweaning growth rate of Red sokoto goats in Nigeria. *World Review of Animal Production*, 19: 19-24.
- Ellington, M.J., Perry, C., Ganner, M., Warner, M., McCormick, S. and Hill, R.L. (2009). Clinical and molecular epidemiology of ciprofloxacin-susceptible MRSA encoding PVL in England and Wales. *European Journal of Clinical Microbiology and Infectious Diseases*, 28: 1113-1121.
- Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H. and Spratt B.G. (2002). The Evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of National Academy of Sciences. U.S.A.* 99: 7687-7692.
- Esrn, D., Karimuebo, E., Lughano, T., Kusiluka, R.H., Melegela, Aglowisye, M., Kapaa, M. and Calvin, S. (2005). Study on Mastitis, milk quality and health risk associated with consumption of milk from pastoral herds in Dodoma and Morgora region, Tanzania. *Journal of Veterinary Science*, 6: 213-221.
- European Food Safety Authority (EFSA) (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. *EFSA Journal*, 7: 1376.
- Evenson, M.L., Hinds, M.W., Bernstein, R.S. and Bergdoll, M.S. (1988). Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. *International Journal of Food Microbiology*, 7: 311-316.
- Fajemisin, B.A. (1991). Goat milk production. In: Proceedings of National Animal Production Research Institute, Institute seminar, pp.1-13.
- FAOSTAT (2003). <http://www.apps.fao.org/page/collections?Subset+Agriculture>.
- Fitzgerald, J.R., Monday, S.R., Foster, T.J., Bohach, G.A., Hartigan, P.J., Meaney, W.J. and Smyth, C.J. (2001). Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *Journal of Bacteriology*, 183: 63-70.
- Fthenakis, G.C. (1994). Prevalence and aetiology of subclinical mastitis in ewes of Southern Greece. *Small Ruminant Research*, 13: 293-300.

- Gall, C. (1996). *Goat breeds of the world*. Margraf publishers, Weikersheim, Germany. pp 186.
- Gebrewahid, T. T., Abera, B. H. and Menghistu, H. T. (2012). Prevalence and Etiology of Subclinical Mastitis in Small Ruminants of Tigray Regional State. *North Ethiopia Veterinary World*, 5: 103-109.
- Ghebremedhin, B., Olugbosi, M.O., Raji, M.A., Layer, F., Bakare, R.A., Konig, B. and Konig, W. (2009). Emergence of a community-associated methicillin-resistant *Staphylococcus aureus* with unique resistance profile in Southwest of Nigeria. *Journal of Clinical Microbiology*, 47: 2975-2980.
- Georgopapadakou, N.H. (1993). Penicillin-binding proteins and bacterial resistance to  $\beta$ -lactams. *Antimicrobial Agents and Chemotherapy*, 37: 2045-2053.
- Gillet, Y., Issartel, B., Vanhems, P., Fournet, J.C., Lina, G. and Bes M. (2002). Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotizing pneumonia in young immune-competent patients. *Lancet*, 359: 753-759.
- Graveland, H., Wagenaar, J.A., Broekhuizen-stins, I.M., Oosting-schothorst, J., Schoormans, A., van Duijkerenn, E., Huijsclens, X.W., Mevius, D. and Heederick, D. (2008). Methicillin-resistant *Staphylococcus aureus* strains (MRSA) in veal calf farmers and veal calves in the Netherlands. American Society for Microbiology on: Antimicrobial resistance in zoonotic bacteria and food borne pathogens. Copenhagen, Denmark.
- Grubb, W.B. (1998). Genetics of MRSA. *Reviews in Medical Microbiology*, 9: 153-162.
- Hanselman, B.A., Kruth, S. and Weese, J.S. (2008). Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital: *Veterinary Microbiology*, 126: 277-281.
- Hartstein, A.I., Phelps, C.L., Kwok, R.Y.Y. and Mulligan, M.E. (1995). In vivo stability and discriminatory power of methicillin-resistant *Staphylococcus aureus* typing by restriction endonuclease analysis of plasmid DNA compared with those of other molecular methods. *Journal of Clinical Microbiology*, 33:2022-2026.
- Hendricksen, R.S., Mevius, D.J., Schroeter, A., Teale, C., Meunier, D., Butaye, P., Franco, A., Utinane, A., Amado, M., Moreno, M., Greko, C., Stark, K., Berghold, C., Myllyneni, A.L., Wasyl, D., Sunde, M. and Aarestrup, F.M. (2008). Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002-2004. *Acta Veterinaria Scandinavica*, 50:28.
- Hermans, K., Lipinska, U., Denis, O., Deplano, A., Struelens, M., Nemati, M., Pasmans, F., Butaye, P., Martens, A., Deprez, P. and Haesebrouck, F. (2008). MRSA clone ST38-SCCmec IV as a cause of infections in an equine clinic. *Vlaams Diergeneeskundig Tijdschrift*, 77: 429-433.

- Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T. and Tenover, F. C. (1997).Methicillin resistant *Staphylococcus*.*Journal of Antimicrobial Chemotherapy*, 40:135-136.
- Hiramatsu, K., Ciu, L., Kuroda, M. and Ito, T. (2001).The Emergence and evolution of methicillin resistant *Staphylococcus aureus*. *Trends Microbiology*,9: 486-493.
- Ikram, M. (1997).Diagnostic Microbiology. In: Paul, W. and Pratt, V.M.D. (ed.), *Laboratory Procedures for Veterinary Technicians*. Donnelley, R.R. and Sons Company, St. Louis, Missouri.pp. 159-160.
- Inglis, B., Matthews, P.R., and Stewart, P.R. (1990).Induced deletions within a cluster of resistance genes in *mec* region of chromosome of *Staphylococcus aureus*.*Journal of GeneralMicrobiology*, 136: 2231-2239.
- Islam, M.R., Ahamed, M.S., Alam, M.S., Rahman, M.M., Sultana, T., Roh, Y.S. and Kim, B. (2011).Identification and antibiotic sensitivity of the causative organisms of sub-clinical mastitis in sheep and goats.*Pakistan Veterinary Journal*, 32: 179-182.
- Jennes, R. (1980). Composition and characteristics of goat milk: review 1968-1979. *Journal of Dairy Science*, 63: 1605-1630.
- Jimmy, L.M. (2007). Factors affecting goat and sheep milk consumption in Minna metropolis. *Bachelor Thesis*.Federal University of Technology, Minna.
- Jones, T.F., Kellum, M.E., Porter, S.S., Bell.M. and Schaffner, W. (2002).An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*.*Emerging Infectious Diseases*. 8: 82-84.
- Joseph, J.K. and Olafade, A.A. (1999).Consumer acceptability, preference and quality attribute of fresh milk, ‘nono’ (fermented milk) and West African soft cheese from cow and goat Milk. *Tropical Journal of Animal Science*, 2: 97-103.
- Katayama, Y., Ito, T. and Hiramatsu, K. (2000). A new class of genetic element *Staphylococcus* cassette chromosome *mec* encodes methicillin resistance in *S. aureus*. *Antimicrobial Agents Chemotherapy*,44: 1549-1555.
- Katayama, Y., Takeuchi, F., Ito, T., Max, X., Ui-Mizutani, Y., Kobayashi, I. and Hiramatsu, K. (2003). Identification in methicillin-susceptible susceptible *S. hominis* of an active primordial mobile genetic element for staphylococcal cassette chromosome *mec* of methicillin-resistant *S.aureus*.*Journal of Bacteriology*, 185: 2711-2722.
- Kawu, M.U., Umoh, J.U., Adekeye, J.O. and Kwanashie, C. (1992).Prevalence and seasonal variation in the occurrence of clinical mastitis in small ruminants in Zaria, Nigeria. Paper presented at the 29th Annual Conference of the Nigerian Veterinary Medical Association, Durbar Hotel, Kaduna, pp. 27-30.

- Kerr, S., Kerr, G.E., Mackintosh, C.A. and Marples, R.R. (1990). A survey of methicillin-resistant *Staphylococcus aureus* affecting patients in England and Wales. *Journal of Hospital Infection*, 16: 35-48.
- Kerr T., Wood, E., Grafstein, E., Ishida, T. and Shannon K. (2005). High rates of primary care and emergency department use among injection drug users in Vancouver. *Journal of Public Health*, 27: 62-66.
- Kerro, D. and Tareke, F. (2003). Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health Production*, 35: 197-205.
- Klevens, R.M., Edwards, J.R., Tenover, F.C., Mc Donald, L.C., Horan, T., Gaynes, R. and National Nosocomial infections surveillance system (2007). Changes in the epidemiology of methicillin resistant *S. aureus* in intensive care unit in US hospitals, 1992-2003. *Clinical Infectious Diseases*, 42: 389-391.
- Kloos, W.E., Ballard, D.N., George, C.G., Webster, J.A., Hubner, R.J, Ludwig, W., Schleifer, K.H., Fiedler, F. and Schubert, K. (1998). Delimiting the genus *Staphylococcus* through description of *Macrococcus caseolyticus* gen. nov., comb. nov., and the new species *Macrococcus carouselicus* sp. nov., *Macrococcus bovicus* sp. nov., *International Journal of Systematic Bacteriology*, 48: 859-877.
- Kluytmans (2010). Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? *Clinical Microbiology*, 16:11-15.
- Kluytmans, J., van Leeuwen, W., Goessens, W., Hollis, R., Messer, S. and Herwaldt, L. (1995). Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. *Journal of Clinical Microbiology*, 33: 1121-1128.
- Kobayashi, N., Taniguchi, K. and Kojima, K. (1995). Analysis of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* by a molecular typing method based on coagulase gene polymorphisms. *Epidemiology and Infection*, 115: 419-426.
- Kock, R., Becker, K., Cookson, B., Van Gemert-Pijnen, J.E., Harbarth, S., Kluytmans, J., Peters, G., Skov, R.L., Struelens, M.J., Tacconelli, E., Navarro Torne, A., Witte, W., and Friedrich, A.W. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *European Surveillance*, 15: 19688.
- Koop, G., Nielen, M. and Werven, T. (2009). Bulk milk somatic cell counts in dairy goats. *Journal of Dairy Science*, 92: 4355-4364.
- Kreiswirth, B., Kornblum, J., Arbeit, R. D., Eisner, W., Maslow, J. N., McGeer, A., Low, D. E. and Novick, R. P. (1993). Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. *Science*, 259:227-230.

- Kwanashie, C.N., Oputeh, K., Ngbede, E.O. (2012). Retrospective studies of the trends of occurrence of ruminant mastitis pathogens in a veterinary teaching hospital in North West Nigeria. *Scientific Journal of Animal science*, 1: 75-80.
- Lakpini, C.A.M. (2002). Management of sheep and goats. In: *Manual of small ruminants products in Nigeria*, pp. 12-16.
- Larkin, E.A., Carman, R.J., Krakauer, T., and Stiles, B.G. (2009). *Staphylococcus aureus*: the toxic presence of a pathogen extraordinaire. *Current Medical Chemistry*, 16: 4003-4019.
- Lebbie, S.H.B. (2004). Goats under household conditions. *Small Ruminant Research*, 51: 131-136.
- Leach, K.A., Green, M.J., Breen, J.E., Huxley, J.N., Macaulay, R., Newton, H.T. and Bradley, A.J., (2008). Use of domestic detergents in the California mastitis test for high somatic cell counts in milk. *Veterinary Record*, 163: 566-570.
- Leonard, F.C. and Markey, B.K. (2008). Methicillin-resistant *Staphylococcus aureus* in animals: A review. *Veterinary Journal*, 175: 27-36.
- Lindsay, J.A. and Holden, M.T. (2004). *Staphylococcus aureus* superbug, super genome? *Trends in Microbiology*, 12: 378-385.
- Lodise, T.P., Lomaestro, B., Graves, J. and Drusano, G.L. (2008). Larger vancomycin doses (at least four grams per day) are associated with an increased incidence of nephrotoxicity. *Antimicrobial Agents and Chemotherapy*, 52: 1330-1336.
- Lowy, F.D. (1998). *Staphylococcus aureus* infections. *New England Journal of Medicine*, 339: 520-532.
- Lowy, F.D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *Journal of Clinical Investigation*, 9: 1265-1273.
- Matsumoto, K., Hohashi, N. and Sugishita, C. (2001). A study on the transmission of MRSA among the family members including clients of visiting nurse and related infection control. *Nippon Koshu Eisei Zasshi*, 48: 190-199.
- Mbindyo, C. M., Gitao, C.G. and Bebora, L. (2014). A cross-sectional study on the prevalence of subclinical mastitis and antimicrobial susceptibility patterns of the bacterial isolates in milk samples of smallholder dairy goats in Kenya. *American Journal of Research Communication*, 2: 30-51.
- McDougall, S., Pankey, W., Delaney, C., Barlow, J., Murdough, P.A. and Scruton, D. (2002). Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont, USA. *Small Ruminant Research*, 46: 115-121.



- Megersa, B., Tadesse, C., Abunna, F., Mkibib, B., and Debela, E. (2009). Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. *Tropical Animal Health Production International. Journal of Applied Research Veterinary Medicine*, 42: 1249-1255.
- Mehndiratta, P.L. and Bhalla, P. (2012). Typing of methicillin resistant *Staphylococcus aureus*: a technical review. *Indian Journal of Medical Microbiology*, 30: 16-23.
- Melo, J.A.G. and Torres P. A. (1989). Plasmid analysis of 219 methicillin-resistant *Staphylococcus aureus* strains with uncommon profiles isolated in Lisbon. *Journal of Hospital Infection*, 13:133-141.
- Menzies, P.I. and Ramanoon, S.Z. (2001). Mastitis of sheep and goats. *Veterinary Clinics of North American Food Animal Practice*, 17: 332-352.
- Mibilu T. J. N. K. (2007). Status of mastitis in lactating goats at Sokoine University of Agriculture and neighboring small holder farms in Morogoro Municipality, Tanzania. *Livestock Rural Development*, 19: 54-60.
- Midau, A. (2012). Goat milk a possible alternative source of protein and minerals to the rural poor populace in Nigeria: A case study of Mubi area of Adamawa state, Nigeria. *International Journal of Dairy Science*, 7: 29-33.
- Ministry of Economic Development (1996). Kaduna State Statistical year book, Kaduna, Nigeria, Ministry of Economic Development, Statistical Division.
- Monecke, S., Kuhnert, P., Hotzel, H., Slickers, P. and Ehricht, R. (2007). Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Veterinary Microbiology*, 125: 128-40.
- Mongkolrattanothai, K., Boyle, S., Kahana, M.D. and Daum, R.S. (2003). Severe *Staphylococcus aureus* infections caused by clonally related community-associated methicillin-sensitive *Staphylococcus aureus* isolates. *Clinical Infectious Diseases*, 38: 1050-1058.
- Moodley, A., Nighting, E.C., Stegger, M., Nielsen, S.S., Skov, R.L. and Guardabassi, L. (2008). High risk of nasal carriage of methicillin-resistant *S. aureus* among Danish veterinary practitioners. *Scandinavian Journal of Work, Environment and Health*, 3: 151-157.
- Morgan, M. (2008). Methicillin-resistant *Staphylococcus aureus* and animals: Zoonosis or humanosis? *Journal of Antimicrobial Chemotherapy*, 62: 1181-1187.
- Moshi, N.G., Kitaro, G.C. and Minga, U.M. (1998). Prevalence of mastitis in dairy goats in some selected farms in Morogoro and Arusha, Tanzania. *Tanzania Journal of Agricultural Sciences*, 1: 173-180.

- Musser, J.M. and Kapur, V. (1992). Clonal analysis of methicillin-resistant *Staphylococcus aureus* strains from intercontinental sources: association of the *mec* gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *Journal of Clinical Microbiology*, 30:2058-2063.
- Nazifi, S.M., Haghkhal, Z., Asadi, M., Ansari, Lari, M.R., Tabandeh, Z. Esmailnezhad and Aghamiri, M. (2011). Evaluation of sialic acid and acute phase proteins (haptoglobin and serum amyloid A) in clinical and subclinical bovine mastitis. *Pakistan Veterinary Journal*, 31: 55-59.
- Ndegwa, E.N., Mulei, C.M. and Munyua, S.J.M. (2000). Risk factors associated with subclinical subacute mastitis in Kenyan dairy goats. *Israel Journal of Veterinary Medicine*, 56:
- Ndegwa, E.N, Mulei, C.M. and Munyua, S.J.M. (2001). Prevalence of microorganisms associated with udder infections in dairy goats on small-scale farms in Kenya. *Journal of South African Veterinary Association*, 72: 97-98.
- Nemati, M., Hermans, K., Lipinska, U., Denis, O., Deplano, A. and Struelens, M. (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: 3817-3819.
- NIRD (1980). In: Bramley, A.J., Dodd, F.H. and Griffin, T.K. (Eds.). Mastitis Control and Herd Management. *Technical Bulletin*, 4. NIRD, Reading, England and Hannah Research.
- NMC (1987). *Laboratory and Field Handbook on Bovine Mastitis*. National Mastitis Council, Madison, WI, USA.
- NMC (1990). Microbiological procedures for the diagnosis of udder infection. 3rd ed. Arlington, VA: National Mastitis Council Inc.
- Normanno, T.G., La Salandra, G., Dambrosio, A., Quaglia, N.C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., E. Crisetti, E. and Celano, G.V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, 115: 290-296.
- Norris, D. Ngambi, J.W. Benyi, K. and Mbajiorgu, C.A. (2011). Milk production of three exotic dairy goat genotypes in Limpopo province, South Africa. *Asian Journal of Animal and Veterinary Advances*, 6: 274-281.
- Odeyinka, S.M. and Ajayi, D.A. (2004). A Survey of Feed stuffs for Goats in Osun State. *Tropical Journal of Animal Science*, 7: 161-168.

- Okon, K.O., Basset, A., Uba, P., Oyawoye, O.M., Yusuf, I.Z., Shittu, A.O. and Blanc, J. (2011). Epidemiology and characteristic pattern of Methicillin resistant *Staphylococcus aureus* recovered from tertiary hospitals in northwestern Nigeria. *International Journal of Tropical Medicine*, 6: 16-112.
- Okunlola, O.O., Amuda, A.J. and Ayanwamide, F.M. (2010). Farmers Perception of Livestock Farming in Oyo State: A case study of small ruminant farmers. Proceedings of 35<sup>th</sup> Conference, Nigeria Society for Animal Production. 14 - 17 March, University of Ibadan, Nigeria.
- Olayinka, B.O, Olayinka, A.T., Obajuluwa, A.F., Onaolapo, J.A. and P.F. Olurinola (2009). Absence of *mecA* gene in methicillin-resistant *Staphylococcus aureus* isolates. *African journal of Infectious Diseases*, 3: 49-56.
- Onanuga, A.O., Oyi, A.H. 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: 1321-1324.
- Owens, W.E., Ray, E.H., Watts, J.L. and Yancey, R.T. (1997). Comparison of success and results of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *Journal of Dairy Science*, 80: 313-317.
- Ozung, P.O., Nsa, E.E., Ebegbulem, V.N. and Ubuja, J.A. (2011). The potentials of small ruminant production in Cross river rain forest zone of Nigeria. *Continental Journal of Animal and Veterinary Research*, 3: 33-37.
- Panlilio, A.L., Culver, D.H., Gaynes, R.P., Banerjee, S., Henderson, T.S., Tolson, J.S., and Martone, W.J. (1992). Methicillin-resistant *Staphylococcus aureus* in United States hospitals, 1975-1991. *Infection Control and Hospital Epidemiology*, 13: 582-586.
- Persons, D., Hoorebeke, S., Hermans, K., Butaye, P., de Kruif, A. and Haesebrouk, F. (2009). Methicillin-resistant *S. aureus* in poultry. *Emerging Infectious Diseases*, 11: 1942-1944.
- Pexara A., Solomakos, N., Sergellidis, D. and Govaris, A. (2012). Fate of enterotoxigenic *Staphylococcus aureus* and Staphylococcal enterotoxins in Feta and Galotyri cheeses. *Journal of Dairy Resources*, 79: 405-413.
- Quinn, P.J, Carter, M.E., Markey, B. and Carter, G.R. (1994). *Clinical Veterinary Microbiology*, Wilfe Publishing, London pp. 95-101.
- Rabello, R. F., Moreira, B. M., Lopes, R. M., Teixeira, L. M., Riley, L.W. and Castro, A. C. (2007). Multilocus sequence typing of *Staphylococcus aureus* isolates recovered from cows with mastitis in Brazilian dairy herds. *Journal of Medical Microbiology*, 56: 1505-1511.

- Radostits, O. M., Leslie, K. E., and Fetrow, J. (1994). *Herd health: Food, animal production medicine* (2nd ed.) W.B. Saunders.
- Radostits, O.R., Blood, D.C. and Gay, C.C. (2007). *Mastitis. Veterinary medicine: In a textbook of Diseases of cattle, sheep, goats and horses* (9th ed., pp.563-614). BaillierTindall, London.
- Ruegg, P.L. (2003). Investigation of mastitis problems on farms. *Veterinary Clinics of North American Food Animal Practice*, 19: 47-73.
- Ruegg, P. L. (2011). Mastitis in small ruminants. 44th Annual Conference of the American Association of Bovine Practitioners, Small Ruminant Session: September, 22-25, St. Louis M.O. pp.1-26.
- Rutland, B.E., Weese, J.S., Bolin, C., A, and Malani, A.N. (2009). Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerging Infectious Diseases*, 15: 1328-1330.
- Sanchez, J., Montes, P., Jimenez, A. and Andres, S. (2007). Prevention of clinical mastitis with barium selenate in dairy goats from a selenium-deficient area. *Journal of Dairy Science*, 90: 2350-2354.
- Sarker, H. and Samad, M.A. (2011). Udder-half wise comparative prevalence of clinical and subclinical mastitis in lactating goats with their bacterial pathogens and antibiotic sensitivity patterns in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 9: 137-143.
- Schalm, O.W., Carroll, E.J. and Jain, N.C. (1971). Bovine mastitis. In: *Physical and chemical tests for detection of mastitis* (pp. 128-157). Philadelphia, Lea and Febiger.
- Schlichting, C., Branger, C. and Fournier, J.M. (1993). Typing of *Staphylococcus aureus* by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. *Journal of Clinical Microbiology*, 31:227-232.
- Schnellmann, C., Gerber, V. and Rossano, A.(2006). Presence of new *mecA* and *mph(C)* variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *Journal of Clinical Microbiology*. 44: 4444-54.
- Scott, G.M., Thompson, R., Malone-Lee, J. and Ridgway, G.L. (1988). Cross-infection between animals and man: possible feline transmission of *S. aureus* infection in humans. *Journal of Hospital Infection*, 12: 29-34.
- Selander, R.K., Caugant, D.A., Ochman, H., Musser, J.M., Gilmour, M.N. and Whittam, T.S. (1986). Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Applied and Environmental Microbiology*, 51: 873-884.

- Shpigel, N.Y., Winkler, M., Ziv, G. and Saran, A. (1998). Clinical, bacteriological and epidemiology aspect of clinical mastitis in Israeli dairy herds. *Preventive Veterinary Medicine*, 35: 1-9.
- Shittu, A.O., Udo, E.E. and Lin, J. (2007). Insights on virulence and Antibiotics resistance: A review of the accessory genome of *S. aureus*. *Wounds*, 19: 237-244.
- Skov, R., Smyth, R. Clausen, M., Larsen, A.R., Frimodt-Moller, N., Oslo-Liljequist, B. and Kahlmeter, G. (2003). Evaluation of a cefoxitin 30idisc in iso-sentest agar for detection of methicillin-resistant *S. aureus*. *Journal of Antimicrobial Chemotherapy*, 52: 204-207.
- Smith, M. C. and Sherman, D.M. (2009). Mammary gland and milk production: *In Goat Medicine* (2nd ed., pp.647-689). Wiley-Blackwell, Ames, Iowa.
- Smith, T.C. and Pearson, N. (2010). The emergence of *Staphylococcus aureus* ST398. *Vector Borne Zoonotic Diseases*, 11: 327-339.
- Speller, D.C, Johnson, A.P., James, D., Marples, R..R., Charlett, A. and George, R.C. (1997). Resistance to methicillin and other antibiotics in isolates of *Staphylococcus aureus* from blood and cerebrospinal fluid, England and Wales, 1989-1995. *Lancet*, 250: 323-325.
- Stapleton, P.D and Taylor, P.W. (2002). Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Science Progress*, 85: 57-72.
- Stefani, S. and Gonglio, A. (2010). Methicillin-resistant *Staphylococcus aureus*: related infections and antibiotic resistance. *International Journal of Infectious Diseases*, 14: 519-522.
- Stastkova, Z., Karpiskova, S. and Karpiskova, R. (2009). "Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at goat breeding farm". *Veterinary Medicine*, 54: 419-442.
- Stefani, S., Chung, D.R., Lindsay, J.A., Friedrich, A.W. and Kearns, A.M, (2012). Methicillin-resistant *Staphylococcus aureus* (MRSA): Global epidemiology and harmonisation of typing methods. *International Journal of Antimicrobial Agents*, 39: 273-282.
- Strommenger, B., Kehrenberg, C., Kettlitz, C., Cuny, C., Verspohl, J., Witte, W. and Schwarz, S. (2006). Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *Journal of Antimicrobial Chemotherapy*, 57: 461-465.
- Struelens, M.J., Deplano, A., Godard, C., Maes, N. and Serruys, E. (1992). Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*, 30: 2599-2605.

- Sori, T., Hussein, J. and Bitew, M. (2011). Prevalence and susceptibility assay of *S. aureus* isolated from bovine mastitis in dairy farms of Jimma town, south west Ethiopia. *Journal of Animal and Veterinary Advances*, 10: 745-749.
- Swai E. S., Mobise, E. and Mtui, P. F. (2008). Occurrence and factors associated with udder infections in small holder lactating dairy goats in Arumeru district, Tanzania, Veterinary Investigation center. *Livestock Research Rural Development*, 12: 1-12.
- Tanimomo, B., Hena, S.A., Ngbede, E.O., Tarhyel, R. and Owoleke, O.E. (2012). Prevalence of mastitis in goat herds in some Northwestern villages in Nigeria. *Scientific Journal of Veterinary Advances*, 1: 52-56.
- Tenover, F.C., Arbeit, R. and Archer, G. (1994). Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 32: 407-415.
- Thrushfield, M. (2007). *Veterinary Epidemiology*, 3<sup>rd</sup> edition. Blackwell Scientific publications, Oxford, pp. 434-440.
- Tirado, C. and Schimdt, K. (2001). WHO surveillance programme for control of food-borne infections and intoxications: preliminary results and trends across greater Europe. *Journal of Infection*, 43: 80-84.
- Todar, K. (2004). Web Review of Todar's Online *Textbook of Bacteriology*. June 4, 2004-vol 304, pp.121.
- Togun, V.A., Amao, O.A., Oyebode, .A. and Ajibade, E.A. (2003). Occurrence of mastitis cases in West African dwarf goats under extensive system of management in Ogbomso. *Asset*, 3: 121-125.
- Tula, M.Y., Azih, A.V. and Okojie, R.O. (2013). Antimicrobial susceptibility pattern and plasmid-mediated antibacterial resistance in *S. aureus* and coagulase-negative Staphylococci (CoNs). *American Journal of Research Communication*, 1: 149-166.
- Upadhyaya, T.N. and Rao, A.T. (1993). Diagnosis and threshold values of subclinical mastitis in goats. *Small Ruminant Research*, 12: 201-210.
- Van Niekerk, W.A. and Pimentel, P.L. (2004). Goat production in the small-holder Section in the Boane District in Southern Mozambique. *South African Journal of Animal Science*, 34: 123-125.
- Vanderhaeghen, W., Hermans, K., Haesebrouck, F., and Butaye, P. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiology and Infections*, 138: 606-625.

- Vander Nest, M., (1997). Accelerated breeding techniques in boer and indigenous goats. M.Sc. Dissertation, University of the Orange Free State, Bloemfontein, South Africa.
- Vicca, J., Cerpentier, T. and Butaye, P. (2008). Prevalence at herd-level of MRSA in milk samples of dairy herds. International Conference on mastitis control, from science to practice, the Hague, The Netherlands.
- 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.
- Wamukoya, A., Molla, B., Belihu, K., Kleer, J., and Hildebrandt, G. (2006). A Cross-Sectional Study on the Prevalence, Antimicrobial Susceptibility Patterns, and Associated Bacterial Pathogens of Goat Mastitis. *International Journal of Applied Research in Veterinary Medicine*, 4: 169-176.
- Wallenberg, G.J., Vanderpoel H.M. and Vanior, J.T. (2002). Viral infection and bovine mastitis. *Journal of Veterinary Microbiology*, 88: 27-45.
- Wang, L. and Barret, J.F. (2007). Control and prevention of MRSA infections. *Methods in Molecular Biology*, 31: 209-225.
- Weaver, S. (2005). Sheep: Small Scale Sheep Keeping for Pleasure and Profit. Burroughs Irvine. Hobby Farm Press. pp. 121-234.
- Weese, J.S., Caldwell, F., Willey, B.M., Kreiswirth, B.N., McGeer, A., Rousseau, J. and Low, D.E. (2006). An outbreak of methicillin-resistant *S. aureus* skin infections resulting from horse to human transmission in a veterinary hospital. *Veterinary Microbiology*, 11: 160-164.
- Weller, T.M.A. (2000). Methicillin-resistant *Staphylococcus aureus* typing methods: which should be the international standard? *Journal of Hospital Infection*, 44: 160-172.
- White, E.C. and Hinckley, L.S. (1999). Prevalence of mastitis pathogens in goat milk. *Small Ruminant Research*, 33: 117-121.
- Wikipedia (2008). The World's Healthiest Foods, "Goat".
- William, H.H. (1854). The scapegoat. In: Goat-Wikipedia. pp.11.
- Wilson, D.J., Gonzalez, R.N. and Das, H.H. (1997). Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *Journal of Dairy Science*, 80: 2592-2598.

- Witaya, S. (2011). Epidemiology of subclinical mastitis and their antibacterial susceptibility in small holder dairy farms, Chiang Mai province, Thailand. *Journal of Animal and Veterinary Advances*, 10: 316-321.
- Witte, W., Kresken, M., Braulke, C. and Cuny, C. (1997). Increasing incidence and widespread dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in central Europe, with special reference to German hospitals. *Clinical Microbiology and Infection*, 3: 414-422.
- Zeng, S.S., Escobar, E.N., Hart, S.P., Hincley, L., Baulthaus, M., Robinson, G.T. and Jahane, G. (1999). Comprehensive study of the effect of testing laboratory, counting method, storage and shipment on somatic cell count in goat milk. *Small Ruminant Research*, 31: 253-260.
- Zetola, N., Francis, J.S, Nuermberger, E.L. and Bishai, W.R. (2005). Community-acquired methicillin-resistant *Staphylococcus aureus*: An emerging threat. *Lancet Infectious Diseases*, 5: 275-286.



## APPENDICES

### Appendix I

CMT scoring system and the corresponding somatic cell count per ml

<b>CMT (Symbol)</b>	<b>score</b>	<b>Interpretation</b>	<b>Reaction and corresponding (Mean no. neutrophils per ml)</b>
-VE		Negative	No reaction ( 68,000)
T		Trace	Slight slime, tends to disappear with continued swirling (268,000)
+1		Weak positive	Distinct slime but without gel (800,000)
+2		Distinct positive	Immediate gel formation; moves as a mass during swirling(2,560,000)
+3		Strong positive	Gel develops a convex surface and adheres to the bottom of the cup ( > 10,000,000)

## Appendix II

### MICROGEN 12S IDENTIFICATION

S/N	SAMPLE NO	<i>Staphylococci</i> species	% Probability
1	L 134	<i>S. aureus</i> subspecies <i>aureus</i>	97.93%
2	L 151	<i>S. aureus</i> subspecies <i>aureus</i>	97.93%
3	L 50	<i>S aureus</i> subspecies <i>aureus</i>	98.45%
4	L 157	<i>S. aureus</i> subspecies <i>aureus</i>	83.66%
5	R 147	<i>S. aureus</i> subspecies <i>aureus</i>	68.66%
6	R 94	<i>S. aureus</i> subspecies <i>aureus</i>	93.68%
7	R 114	<i>S. aureus</i> subspecies <i>aureus</i>	98.45%
8	L 19	<i>S. aureus</i> subspecies <i>aureus</i>	98.45%
9	L 175	<i>S. aureus</i> subspecies <i>aureus</i>	88.42%
10	L 94	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
11	L 161	<i>S aureus</i> subspecies <i>aureus</i>	99.99%
12	L 88	<i>S. hominis</i> subspecies <i>hominis</i>	69.29%
13	L 148	<i>S. aureus</i> subspecies <i>aureus</i>	99.99%
14	L 53	<i>S. hominis</i> subspecies <i>hominis</i>	69.29%
15	R 184	<i>S. aureus</i> subspecies <i>aureus</i>	96.37%
16	R 13	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
17	R 110	<i>S. aureus</i> subspecies <i>aureus</i>	91.35%
18	R 136	<i>S. aureus</i> subspecies <i>aureus</i>	100%

19	L 49	<i>S. aureus</i> subspecies <i>aureus</i>	89.58%
20	R 160	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
21	R 150	<i>S. haemolyticus</i>	54.74%
22	R 146	<i>S. caprae</i>	48.65%
23	R 139	<i>S. haemolyticus</i>	67.1%
24	R 149	<i>S. chromogenes</i>	93.5%
25	L 136	<i>S. aureus</i> subspecies <i>aureus</i>	99.99%
26	L 13	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
27	L 111	<i>S. aureus</i> subspecies <i>aureus</i>	98.45%
28	R 156	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
29	R 105	<i>S. aureus</i> subspecies <i>aureus</i>	98.25%
30	R 103	<i>S. haemolyticus</i>	93.2%
31	R 100	<i>S. haemolyticus</i>	96.65%
32	R 89	<i>S. aureus</i> subspecies <i>aureus</i>	52.49%
33	L 11	<i>S. caprae</i>	48.6%
34	L 98	<i>S. haemolyticus</i>	92.16%
35	L 125	<i>S. haemolyticus</i>	92.16%
36	L 108	<i>S. haemolyticus</i>	92.16%
37	L 112	<i>S. haemolyticus</i>	92.16%
38	L 141	<i>S. chromogenes</i>	73.22%
39	R 154	<i>S. haemolyticus</i>	92.16%
40	R 158	<i>S. hominis</i> subspecies <i>hominis</i>	74.74%
41	R 79	<i>S. hominis</i> subspecies <i>hominis</i>	74.74%

42	L 150	<i>S.aureus</i> subspecies <i>aureus</i>	99.64%
43	L 101	<i>S. caprae</i>	48.6%
44	L 97	<i>S.aureus</i> subspecies <i>aureus</i>	100%
45	L 130	<i>S. hominis</i> subspecies <i>hominis</i>	69.29%
46	L 156	<i>S.aureus</i> subspecies <i>aureus</i>	100%
47	R 16	<i>S. caprae</i>	69.29%
48	L 137	<i>S.aureus</i> subspecies <i>aureus</i>	100%
49	L 149	<i>S. haemolyticus</i>	86.86%
50	L 105	<i>S.aureus</i> subspecies <i>aureus</i>	100%
51	L 161	<i>S.aureus</i> subspecies <i>aureus</i>	99.96%
52	L 160	<i>S.aureus</i> subspecies <i>aureus</i>	100%
53	L 127	<i>S.aureus</i> subspecies <i>aureus</i>	97.17%
54	R 152	<i>S. hominis</i> subspecies <i>hominis</i>	69.29%
55	R 120	<i>S. haemolyticus</i>	73.41%
56	L 153	<i>S. hominis</i> subspecies <i>hominis</i>	69.29%
57	L 104	<i>S. caprae</i>	69.29%
58	L 115	<i>S. haemolyticus</i>	73.41%
59	L 142	<i>S. hominis</i> subspecies <i>hominis</i>	74.74%
60	L 138	<i>S. chromogenes</i>	73.22%

**APPENDIX III**

**ANTIMICROBIAL SENSITIVITY PATTERNS OF 32 *S. AUREUS* ISOLATES TO 14**

**ANTIMICROBIAL AGENTS AND DISK CONCENTRATIONS**

SAMPLE NO	TE 30µg	VA 30µg	C 30µg	F 300µg	CRO 30µg	K 30µg	P 10u	AMP 10µg	CIP 5µg	SXT 25µg	FOX 30µg	E 15µg	CN 10µg	AMC 30µg
L134	R 0	R 11	S 27	R 0	R 0	R 0	R 12	R 12	I 20	R 0	R 0	R 0	R 9	R 0
R114	S 36	S 20	S 31	S 32	S 22	I 16	S 30	R 13	S 26	S 36	R 13	R 0	R 20	S 30
L111	S 32	S 19	S 30	R 14	I 14	I 14	R 25	R 9	S 36	R 0	R 0	S 24	S 14	R 16
R184	S 30	S 19	S 18	S 24	I 20	S 27	R 24	R 13	S 29	R 0	R 0	S 30	S 22	S 40
L157	S 26	S 17	S 20	R 12	R 12	I 15	R 20	R 12	I 20	R 0	R 18	I 19	S 13	R 11
R147	R 0	S 19	R 0	S 22	R 0	R 0	R 15	R 10	R 15	S 23	R 0	I 20	S 15	R 12
L50	S 34	S 18	I 13	S 26	I 15	R 13	R 25	R 16	S 25	S 17	R 0	S 25	S 15	R 16
R94	S 28	S 17	S 27	S 26	I 16	I 14	R 20	R 11	S 22	S 16	R 0	S 23	S 14	S 34

L151	R	S	R	S	I	R	R	R	I	R	R	R	S	R
	0	18	7	24	19	12	23	14	20	0	0	0	15	17
L148	S	S	S	S	R	R	R	R	S	S	R	S	R	S
	26	18	24	24	0	0	25	15	23	32	8	24	12	32
L175	R	S	R	S	R	S	R	R	S	S	R	R	S	S
	9	18	0	18	10	19	18	13	27	25	12	0	17	24
R13	S	S	S	S	R	I	R	R	S	S	R	S	S	S
	30	19	24	26	0	14	24	14	24	32	0	26	15	32
L13	R	S	S	S	R	S	R	R	S	S	R	S	R	S
	10	21	28	21	0	24	12	15	23	30	0	26	12	30
L156	R	R	S	R	R	S	R	R	S	R	R	R	S	R
	0	0	16	12	0	21	0	0	40	0	8	0	21	10
L19	S	S	S	S	R	R	R	R	S	S	R	S	I	S
	30	18	25	26	0	10	25	11	22	34	12	24	13	27
R136	R	S	S	S	R	R	R	R	S	S	R	S	S	S
	12	18	30	28	0	13	25	12	26	30	14	30	16	28
L136	S	S	S	S	R	I	R	R	S	S	R	S	R	S
	26	18	26	25	0	14	25	14	23	34	0	24	12	30
R110	S	S	S	S	R	R	R	R	S	S	R	S	R	S
	30	18	24	25	0	12	25	12	21	32	0	25	12	30

L94	S 30	S 20	S 30	S 28	R 0	R 13	R 26	R 10	S 24	S 38	S 34	S 24	S 16	S 32
R89	I 16	S 16	S 30	S 22	R 10	S 22	R 20	R 17	I 20	S 32	S 10	S 26	I 14	S 24
R161	I 16	R 13	S 22	S 25	R 0	R 0	R 0	R 14	S 22	S 28	R 0	R 0	R 11	S 21
L161	S 25	S 18	S 26	S 28	R 0	R 0	R 21	R 10	S 21	S 34	R 0	R 10	I 14	S 30
R160	I 15	S 18	S 25	S 24	R 0	I 14	R 21	R 8	S 23	S 30	R 0	S 22	R 12	S 30
L160	R 0	R 0	I 13	S 22	R 0	S 21	R 0	R 0	S 25	S 28	R 10	R 7	S 18	R 0
R105	I 15	S 20	S 30	S 32	R 0	I 15	R 25	R 10	S 27	S 40	R 15	R 0	S 19	S 28
L105	I 17	R 8	S 20	S 22	R 0	I 14	R 0	R 18	S 22	S 25	R 0	R 0	I 13	S 24
L49	S 20	S 22	S 34	S 24	R 0	S 28	S 34	R 20	S 32	S 28	S 38	S 40	S 30	S 36
L150	R 0	R 0	S 34	S 21	I 20	R 13	R 0	R 0	S 46	S 26	R 14	R 0	S 26	R 18

R159	R	R	S	S	R	R	R	R	I	S	R	S	I	S
	0	12	21	26	0	0	0	12	20	20	0	18	11	21
L97	R	R	R	R	S	I	R	R	S	R	R	R	R	R
	0	0	0	6	24	17	0	0	42	0	17	0	0	8
L137	R	R	S	S	R	R	R	R	S	S	R	R	S	S
	14	0	22	24	0	12	0	15	24	22	0	0	15	22
L127	R	R	R	S	I	R	R	R	I	R	R	R	R	R
	0	15	10	16	19	0	0	0	17	0	17	0	0	16

**KEY**

TE- Tetracycline

VA- Vancomycin

C- Chloramphenicol

F- Nitrofurantoin

CRO- Ceftriaxone

K- Kanamycin

P- Penicillin G

AMP- Ampicillin

CIP- Ciprofloxacin

SXT- Sulfamethxazole/Trimethoprim

FOX- Cefoxitine

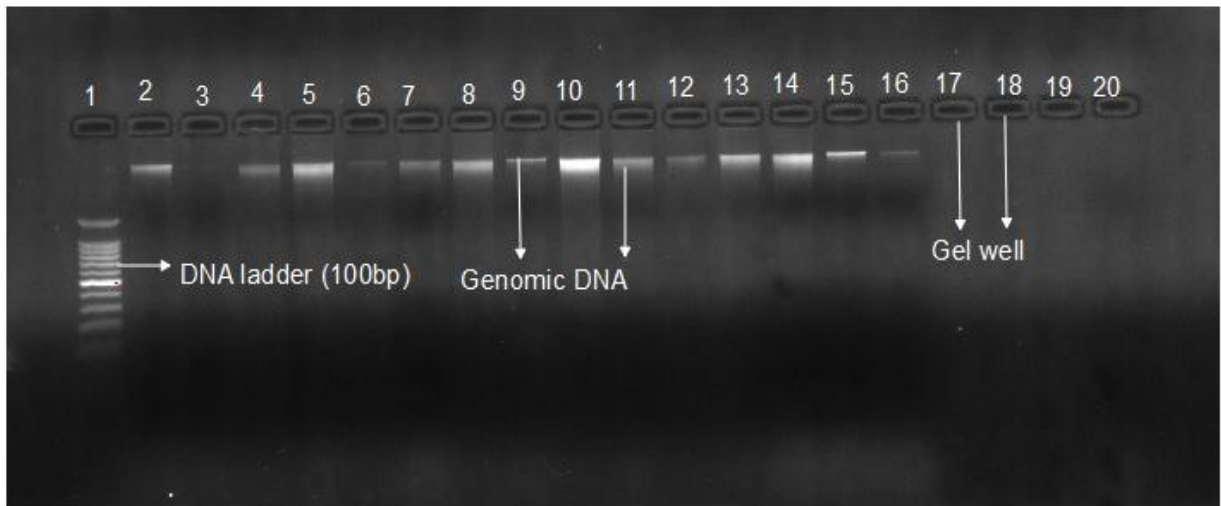
E- Erythromycin

CN- Gentamicin

AMC- Amoxicillin-clavulanic acid



## Appendix IV



Genomic DNA of MRSA isolates