ANTIBIOTIC SUSCEPTIBILITY AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM DOGS, PIGS AND THEIR HANDLERS IN ZARIA AND KADUNA, KADUNA STATE NIGERIA.

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Declaration

I declare that the work reported in this dissertation entitled "Antibiotic Susceptibility and Molecular characterization of Methicillin Resistant *Staphylococcus aureus* from Dogs, Pigs and their Handlers in Zaria and Kaduna, Kaduna State Nigeria'' was carried out by me in the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences. The information derived from the literature review has been duly acknowledged in the text and list of the reference provided. No part of this dissertation was previously presented for another degree at this or any other Institution.

Oyeronke Olamide OKENIYI _____ _____

Name of student Signature Date

Certification

The dissertation entitled **"**ANTIBIOTIC SUSCEPTIBILITY AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM DOGS, PIGS AND THEIR HANDLERS IN ZARIA AND KADUNA, KADUNA STATE NIGERIA'' by Oyeronke Olamide OKENIYI meets the regulations governing the award of Master of Science in Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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I would like to dedicate this dissertation to God Almighty, the Helper and Sustainer of men. To Him be glory and Honour, Power and Praise forever (Amen).

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Abstract

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a critically important human pathogen that is also an emerging concern in veterinary medicine and animal agriculture. It is present in a wide range of animal species, including dogs, cats, rabbits, horses, cattle, pigs, poultry, and exotic species, both as a cause of infection and in healthy carriers. Identification of MRSA in various species and in food has led to concerns about the roles of animals, both pets and livestock, in the epidemiology of MRSA infection and colonization in humans. In this study, a total of three hundred (300) samples were obtained from the Skin, anus and anterior-nares of pigs, dogs and hand swabs of their handlers in three (3) Communities; Buwaya Gonin-gora, Maraban-Rido, and Samaru, Zaria in Kaduna state. Nigeria. *S. aureus* were isolated and characterized using standard microbiological protocols. The isolates were further characterized phenotypically using the modified Kirby Bauer disc diffusion method to determine the antibiotic susceptibility. Also genotypically, the presence of multidrug resistance genes *mecA, tetK, tetL, tetM, tetO, blaZ,* and *staphA30* (16S rRNA).

Results revealed One hundred and sixty-five (165) of the isolates were presumptive *Staphylococcus* species. Ninety-five (95) out of the one hundred and sixty-five (165) were confirmed to be *Staphylococcus aureus* species after the coagulase test was carried out. Further identification of the Ninety-five (95) isolates using the Microgen Staph-ID kit results revealed that only 46 (48.4%) of the isolates were *S. aureus*, 49 (51.5%) were coagulase negative isolates, which consists of *S. xylosus* 28.4% (27), *S. chromogenes* 8.4 % (8), *S. hominis* 4.2% (4), *S. intermedius* 6.3% (6), (*S. hyicus* 4.2% (4). Forty-two (42) which amounted to Ninety-one percent (91%) of the isolates were phenotypically Methicillin-Resistant *Staphylococcus aureus* (MRSA). There were more MRSA isolates from the skin (n=20;43.5%) compared to nasal region (n=10;21.7%) and anus (n=9;19.6%) of the dogs and pigs compared to that from the hand swabs obtained from the handlers (n=7;15.2%).

All the *Staphylococcus aureus* isolates were susceptible to Chloramphenicol. Ninety-one percent (91%) of the isolates were resistant to cefoxitin, therefore phenotypically methicillin-resistant *Staphylococcus aureus* (MRSA). Resistance to commonly prescribed antibiotics (Linezolid, tetracycline, gentamicin, ciprofloxacin and amoxicillin-clavulanic) was between 30% to 74%.

Coagulase Negative isolates were susceptible to Chloramphenicol, Erythromycin and linezolid but they were resistant to (69.4%) Cefoxitin (40.8%), Ciprofloxacin (47%) Amoxicillin-clavulanic and (34.7%). Tetracycline There were more MRSA isolates from the (63.6%) handlers than from the (36.4%). dogs This reveals the need for further studies on the prevalence of MRSA in pets and their handlers because of the public health implications Molecular characterization of the MRSA isolates revealed that all tested isolates harbored *Staph A* 30 (16S rRNA) genes. Other resistance genes including *mecA, tetK, tetL, tetO,* and *blaZ* were isolated from 7, 7, 3, 10, and 5 isolates respectively. None of the isolates harbored *tetM* genes. This study demonstrated the presence of MRSA both phenotypically and genotypically in reared pigs and dogs in the Kaduna agricultural zone, Nigeria. This calls for public health concern because of the health risk associated with colonization of individuals with these MDR strains.

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List of Abbreviations

S. *aureus* -*Staphylococcus aureus*

MRSA- Methicillin Resistant *Staphylococcus aureus*

MSSA- Methicillin Susceptible *Staphylococcus aureus*

MLST- Multilocus Sequence Type

HA- MRSA- Hospital Acquired- Methicillin Resistant *Staphylococcus aureus*

CA-MRSA- Community Acquired Methicillin Resistant *Staphylococcus aureus*

LA-MRSA- Livestock Acquired Methicillin Resistant *Staphylococcus aureus*

DNA- Deoxyribonucleic Acid

MARI- Multiple antibiotic Resistance Index

EUCAST- European Committee on Antimicrobial Susceptibility Testing

MDR – Multidrug Resistance

XDR- Extended Drug Resistance

PCR- Polymerase chain reaction

DR- Drug Resistant

CONs- Coagulase Negative *Staphylococcus*

COPs- Coagulase Positive *Staphylococcus*

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Staphylococcus aureus is a Gram positive, facultative, anaerobic, non-motile and non-spore forming bacterium, that causes a wide spectrum of diseases that include bacteremia, endocarditis, osteomyelitis, nosocomial infection (Reddy *et al.*,2017; Taylor and Unakal, 2020). This pathogenic bacterium could also cause mild conditions such as skin and soft tissue infections, to life threatening debilitations like toxic shock syndrome. (Bukowski *et al.,* 2010; Underwood *et al.,* 2015). Staphylococci are important pathogens that reportedly cause healthcare-associated infections and were responsible for 21.3 % of all healthcare-associated infections in a UK survey in 2011 (Health Protection Agency, 2012). The success of *Staphylococcus aureus* as a pathogen is partly due to its ability to express a variety of virulence factors that mediate host colonization, tissue invasion and dissemination (Gordon and Lowy, 2008; Lozano *et al.,* 2014; Bertelloni *et al.,* 2021).

Endogenous spread of Staphylococci is a potential source of infection in surgical patients; surgical prophylaxis being administered to reduce the risk of post-operative surgical-site infection(Mcmurray *et al.,* 2015).

Staphylococcus aureus has persisted and is still reportedly resurging as an important hospital and community acquired pathogen and live-stock acquired pathogen (Bennimath *et al*. 2011; Chueahiran *et al.,* 2021). The development of resistance to a wide range of antibiotics *in S. aureus* is diversified, such as resistance in Methicillin that takes the account of *S. aureus* to most β-lactams, macrolides and aminoglycosides (Kaur and Chate 2015; Bal *et al.,* 2016).

Staphylococcus aureus has been reported to cause an array of infections in economically important livestock animals, particularly pigs (Chuang and Huang, 2015).

Humans are not the only reservoir for this organism because it can be isolated from companion animals, livestock, and wild animals (Schaumburg *et al.*,2013; Davies *et al.*,2014 Lozano *et al.*, 2016). Four Precent (4%) of dogs and some cats carry *S. aureus* at one or more body sites (e.g., abdomen), including MRSA strains (Davies *et al.*,2014; Ma *et al.*, 2020). Additionally, livestock, especially pigs but also chickens and cattle, carry strains of ST398 that have been the cause of human infections (Schaumburg *et al.*,2013; Larsen *et al.*,2015). Schuamburg *et al.*,(2013) reported that animals in wild populations e.g., chimpanzees harbor and shed *S. aureus.*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human pathogen that has also become an emerging concern in veterinary medicine and animal agriculture. It is present in a range of animal species, including dogs, cats, rabbits, horses, cattle, pigs, poultry, and exotic species, both as a cause of infection and in healthy carriers. MRSA sequence type (ST) 398 is the predominant clone in pigs from Europe and North America, whereas MRSA ST9 is predominant in Asia. Direct contact with MRSA-positive animals has been reported to be a risk factor for MRSA carriage in 5.6–37.8% farmers (Daley *et al.,* 2016). Livestock Associated-Methicillin Resistant *Staphylococcus aureus* strains are reportedly responsible for human infection (Kock *et al.*, 2014; Mishra *et al.*, 2019)

However, answers to the prevalence of Methicillin Resistance which is also known as multidrug resistance are still lacking in many of these areas and the studies are continuous. It is almost certain that animals are a source of human MRSA infection in some circumstances—but humans may also serve as sources of infection in animals. Changes in the epidemiology of MRSA in one species may be reflected in changes in other species (Verkade and Kluytmans 2014; Boswihi, Udo and Al-sweih 2016).

1.2 Statement of Research Problem

Staphylococcus aureus strains isolated from different clinical settings display significant genetic variations and it is this difference that dictates and is responsible for the strain variation in physiology and stress response (Sabirova *et al.,* 2014; Foster 2017). This includes the emergence of multiple drug-resistant *S. aureus* in hospitals and communities. These drug-resistant strains form the basis for Methicillin Resistant *S. aureus* (MRSA) or more specifically Hospital Acquired Methicillin Resistant *S. aureus* (HA-MRSA), Community Acquired Methicillin Resistant *S. aureus* (CA-MRSA) infections and Livestock acquired Methicillin Resistant *S. aureus* (Aires-de-Sousa 2017; Boswihi and Udo 2018).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human pathogen that is also an emerging concern in veterinary medicine and animal agriculture. It has been reportedly present in a wide range of animal species, including dogs, cats, rabbits, horses, cattle, pigs, poultry, and exotic species, both as a cause of infection and in healthy carriers (Reddy *et al.,* 2016). Isolation of MRSA in various species and in food has led to concerns about the roles of animals, both pets and livestock, in the epidemiology of MRSA infection and colonization in humans (Weese and Van Duijkeren 2010; Dweba *et al.,* 2018).

The presence of livestock associated MRSA (LA- MRSA) in farmers constitutes a major threat to public health care system (Angen *et al.,* 2017). Methicillin Resistant *Staphylococcus aureus* prevalence in humans has been reported to be strongly associated with prevalence in animals and intensity of contact with animals positive for methicillin resistant *Staphylococcus aureus* (Obaidat *et al.,* 2018; Pirolo *et al.,* 2019).

Methicillin Resistant *Staphylococcus aureus* has also been detected in several species and animal derived products (Anjum *et al.,* 2019). An emerging subtype of methicillin resistant *S. aureus,* a clonal Complex CC398, was found to be associated with animals particularly pigs (Armand *et al.,* 2005; Becker *et al.,* 2018; Pirolo *et al.,* 2019;). MRSA has been isolated from food of animal origin such as dairy products, beef, and chicken (Anjum *et al.,*2019; Wu *et al.,* 2019).

Methicillin resistance indicates resistance to all β-lactam antibiotics, including carbapenems. Methicillin resistant *Staphylococcus aureus* isolates are increasingly resistant to multiple non β-lactam antibiotics, with reports of strains not susceptible to Vancomycin creating a lot of problems in clinical settings for successful chemotherapy of infections. The leading role of MRSA in these infections is associated with its resistance to most commonly available antibiotics resulting in treatment failure (Ventola 2015; Lee *et al.,* 2018).

Although the mechanisms and other information about emergence of methicillin resistant *Staphylococcus aureus* in animal are rather poorly understood, the close contact between human and various animal species and antimicrobial use in animals presumably facilitate the emergence and spread of MRSA (Ugwu, *et al.,*2015; Fieri and Boutin 2017). Human colonization and infections with MRSA have been reported in several parts of Nigeria (IB *et al.,* 2014; Okunola and Ayandele 2015; Odetokun *et al.,* 2018).

MRSA has become a major public health concern because companion animals such as dogs and cats often are in close physical contact (touching, petting, and licking) with their owners exposing them to infection with pathogenic bacteria (Reddy *et al.,* 2016; Nworie *et al.,* 2017). Dogs are reported to usually colonized by MRSA strains from humans (Lin *et al.,* 2011; Mustapha *et al.,* 2014; Van Balen *et al.,* 2017).

1.3 Justification

The emergence and involvement of a distinct clone complex of MRSA (ST 398) associated with livestock in human disease in many countries suspected to have arisen from the increasing use of antibiotics in animal feeds, especially in pigs and also dogs give a good reason for the study since pig farming, and domestic dog breeding is common in some parts of this country (Smith *et al.,* 2013).

The occurrence of methicillin resistant *S. aureus* not only in livestock but also in food of animal origin might represent a relevant issue regarding human health and food safety for consumption. The MRSA isolated from human skin and soft tissue infections were mainly the MRSA (ST398) seen in cattle and poultry (Dullweber, 2010; Mulders *et al.,* 2010).

This study will provide compelling epidemiological and microbiological evidence that persons living and working with pigs, dogs and also the pigs and dogs may be at increased risk of being colonized or infected with live-stock associated Methicillin Resistant *Staphylococcus aureus* (LA-MRSA). It is important to identify the origin of the isolates and their dissemination on the farm and evaluate the potential health hazard.

Since MRSA (ST398) is implicated in human disease and has been reported in different parts of the world, they may also be present in dogs, pigs and pig farms in Nigeria and this study will ascertain the presence and prevalence of MRSA in pigs and dogs in some selected pig farms in Zaria and Kaduna metropolis of Kaduna state, Nigeria. Persons working on the farms or living with the dogs and pigs are at a high risk of contracting MRSA and transmitting it to their family members and community at large and may likely cause staphylococcal infection.

Among animals for food, pigs have been implicated as major source of potential infections to humans, especially farmers, slaughterhouse workers, and veterinarians who are in frequent contact with MRSA colonized pigs (Angoletti *et al.,* 2014; Cuny *et al.,* 2015; Aires-de-sousa 2017). It is an established fact that MRSA colonization of healthy pigs is prevalent in many countries like the Netherlands, Germany, and U.S.A. (Cuny *et al.,* 2015; Chon and Khan 2017). Also, pig farm workers and slaughter plant workers with live animal exposure are at elevated risk of colonization with LA-MRSA (Angoletti *et al.,* 2014; Momoh *et al.,* 2015; Dignard and Leibler 2019).

The presence of methicillin resistant *S. aureus* has been reported in Nigeria (Olayinka *et al.,* 2010) but there is no report of LA-MRSA of clonal complex ST398 in human infections in communities and hospital settings in this country. People working on the farms or with these animals have little or no knowledge about the MRSA and are at high risk of contacting the bacteria through handling, and cleaning of the farm and this could easily be transferred to the community. Thus, molecular analysis of isolates and a comprehensive analysis of their antimicrobial resistance properties would provide relevant information for health workers in the country and plan preventive and therapeutic measures in combating this emerging infection and minimizing risk factors.

However, there is a general paucity of information on *S. aureus* in livestock especially domesticated pigs and dogs especially in Nigeria. Therefore, there is need to obtain information on both Methicillin Susceptible *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) prevalence in the study population, as well as to determine the genetic background of the isolates in order to understand the dynamics of the emergence of

livestock associated *S. aureus*, their adaptation to humans and their potential impact on human health.

However, except for the report of Fall *et al.,* (2012) in Senegal, there are no relevant data available on MRSA/MSSA in pigs in Africa and Northern Nigeria in particular. The prevalence of MRSA/MSSA among pigs and farmers, as well as the genetic background of methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA strains and associated virulence factors in Nigeria is minimal.

In Nigeria, several studies conducted have shown that methicillin resistant *Staphylococcus aureus* is a common cause of hospital and community acquired infections (Sina *et al.*,2011; Ugwu *et al.,* 2015; Nworie *et al.,* 2017). A nasal carriage of *Staph. aureus* has also been reported (Abdulhadi *et al.,* 2008). Also, a study carried out by Okunola and Ayandele, (2015) also reported the prevalence of MRSA from pigs in selected farms in South West Nigeria.

However only a few studies have been carried out on the prevailing resistance of Methicillin Resistant *Staphylococcus aureus* from dogs, pigs and pig farms and dog handlers to commonly used antibiotics, especially in the northern part of the country particularly in Zaria and Kaduna. Therefore, it is the aim of this study to isolate and identify and to molecularly characterize *S. aureus* from pigs, dogs and owners/handlers in Zaria and Kaduna Metropolis.

1.4 Aim

To isolate and molecularly characterize MRSA from dogs, pigs, pig farm workers and dog owners/handlers in Buwaya community Gonin-gora, Maraban-rido, and Samaru-Zaria, Kaduna State, Nigeria.

1.5 Specific Objectives

The specific objectives are to;

i. isolate and identify *Staphylococcus aureus* in samples collected from the rectal region, anterior-nares and skin of pigs and dogs and hand swabs from the handlers of these animals.

ii. determine the antimicrobial susceptibility of the *S. aureus* isolates to some commonly used antibiotics using modified Kirby Bauer Disc Diffusion method.

iii. determine phenotypically isolates that are MRSA.

iv. molecularly confirm the presence of *mecA* gene in the identified MRSA using PCR.

v. To identify the genes (*mecA TetK, TetL, TetM, TetO, BlaZ,*) and *staphA30* (16sRNA) responsible for the antimicrobial resistance molecularly using PCR.

1.6 Hypothesis

1.6.1 Null Hypothesis (H_0):

There is no Methicillin Resistant *Staphylococcus aureus* (MRSA) in pigs, dogs, farm workers and their handlers in Kaduna State.

1.6.2 Alternate Hypothesis (H_1):

There is Methicillin Resistant *Staphylococcus aureus* (MRSA) in pigs, dogs, pig, farm workers and their handlers in Kaduna State.

1.7 Research Limitation

- This work is limited to pigs, dogs and farm workers/ handlers that are available within a period of three (3) to (6) months.
- The study will investigate only the prevailing *Staph. aureus* isolates obtained from the samples.

CHAPTER TWO
LITERATURE REVIEW

2.1 The Genus *Staphylococcus*

Staphylococcus is a genus of Gram- positive Bacteria. Under the Microscope, they appear round (Cocci) and form in grape-like clusters. However, the organism in clinical material may also appear as singles in pairs or short chains (Liu, 2011).

The *Staphylococcus* genus includes at least 40 Species, of these, nine have two subspecies, one has three subspecies and one has four subspecies (Jasim *et al.*, 2016)

2.2 Taxonomy

The Taxonomy is based on 16s rRNA Sequences (Ryan *et al.*, 2012) and most of the Staphylococcal species fall into 11 clusters:

1. *S.aureus* group: *S.aureus, S. simiae*
2. *S.auricularis* group: *S.auricularis*
3. *S. carnosus* group: *S.carnosus, S.condimenti, S.massiliensis, S.piscifermentans, S.simulans*
4. *S. epidermidis* group: *S.capitis, S.caprae, S.epidermidis, S.saccharolyticus*
5. *S. haemolyticus* group: *S. deveriesei, S. haemolyticus, S. hominis*
6. *S. hyicus- intermedius* group: *S. agnetis, S. chromogenes. S.felis, S.delphini, S.hyicus, S.intermedius, S.lutrae, S.microti, S.muscae, S. pseudintermedius, S.rostri, S.schleiferi*
7. *S. lugdunensis* group: *S. lugdunensis*
8. *S. saprophyticus* group: *S. arlettae, S. cohnii, S. equorum, S. gallinarum, S. kloosii, S. saprohyticus, S. xylosus.*
9. *S. sciuri* group: *S. fleuretti, S. lentus, S. sciuri, S. stepanovicii, S. vitulinus*

10. *S. simulans* group: *S. simulans*

11. *S. warneri* group: *S. pasteuri S. warneri*

Taxonomically, the genus *Staphylococcus* is in the bacterial family staphylococcaceae which include three lesser-known genera, *Gamell, Micrococcus* and *Salinicoccus* (Prax, *et al.*, 2013).

Traditionally, the genus *Staphylococcus* has been confused with the genus *Micrococcus* with the two genera being used interchangeably until definitive characteristics were established and modern evidence has confirmed that there is a distance in their relationship (Prax, *et al.*, 2013). There are consequently three levels of consideration when laboratory identification of an aerobic, catalase positive, coagulase positive, Gram positive, coccus is considered. These are:

1. Differentiation between *Staphylococcus* and *Micrococcus.*
2. Distinction between coagulase positive and Coagulase Negative species.
3. Speciation of the isolates.

The genus *Staphylococcus* can be defined into two sub groups on the basis of its ability to clot blood plasma by enzyme Coagulase:

- Coagulase –positive
- Coagulase-negative

Sub-group of Coagulase-negative species from human Staphylococci are rarely pathogenic and considered to be opportunistic pathogens of humans (Karsten *et al.*, 2014). They include:

- *Staph. epidermidis*
- *Staph. hominis*
- *Staph. xylosus*

- *Staph. haemolyticus*
- *Staph. Saprophyticus*
- *Staph. Simulans*

Staphylococcus can cause a wide variety of diseases in human and animals through toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, for they can be produced by bacteria growing in improperly stored food items. The most common sialadenitis is caused by Staphylococci, as bacterial infections (Chan *et al.*2011). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms.

2.3 *Staphylococcus aureus*

Staphylococci are Gram-positive bacteria, and are classified into two groups, coagulase-positive (CoPS) and coagulase-negative (CoNS), based on their ability to produce the enzyme coagulase which could either be free or bound coagulase (Bergeron *et al.,* 2011). *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are the most important species in the CoPS group as they are major pathogens for both humans and animals, especially *S. aureus*. Although CoNS are saprophytic and rarely pathogenic (Kloos and Bannerman, 1997; Chah *et al.,* 2014), multidrug-resistant (MDR) strains have been associated with severe cases of difficult to treat infections, especially in immunocompromised individuals (Arendrup and Patterson 2017).

Staph. aureus is perhaps the most notorious of all the bacterial pathogens associated with human infection. In 1942 the year penicillin G was introduced, some resistant strains of *Staph. aureus* was found (Lobanvoska and Pila 2017). *Staph. aureus* was the first bacterium to battle penicillin in 1967 because of its ability to produce β-lactamase. Enzyme resistant semi-synthetic penicillins

in the early 1960s provided temporary respite which ended with the emergence of methicillin resistant *Staph. aureus,* discovered shortly after methicillin became available for clinical use (Fluit *et al.*,2012).

Staphylococcus aureus, is a frequent cause of skin infections, such as boils and pimples. Since the late 70s the usual treatment for these infections has been penicillins and penicillinase resistant antibiotics such as Methicillin. In today's world however, this treatment is likely to fail due to the increasing drug resistance level. 60% of the *Staph.aureus* strains isolated in hospitals were resistant to this antibiotic (Castro *et al.,* 2016; Mohammed and Hafez 2017).

Staphylococci are natural residents on the skin and mucous membranes of a wide range of host species (Becker and von Eiff 2015; Sabaté Brescó *et al.,* 2017) Many of the bacterial species have a benign or symbiotic relationship with their host; however, the bacteria may become pathogenic if they gain entry into the host tissue through trauma of the cutaneous barrier (Belkaid and Hand 2014; Sangwan and Kumari 2018). *Staphylococcus aureus* is the most important species within this genus by virtue of its versatility as a pathogen in humans and animals (Osmann *et al.,* 2016; Lakhundi and Zhang 2018).

Staphylococcus aureus is a gram-positive coccus mostly arranged in irregular grape-like clusters. It is catalase positive, oxidase negative, Vogues-Proskauer positive, ferments glucose to produce acid and gas or acid only, ferments lactose to produce acid and gas or acid only, and ferments mannitol to produce acid. It is coagulase positive and DNase positive (Lamikanra *et al.,* 1985). *Staph.aureus* is a facultative anaerobe growing well under aerobic conditions within 24 hours (Safardoust-Hojaghan *et al.,* 2017). It is found in people and frequently colonizing the anterior-nares, the armpit, the perineum, skin fold and the vagina without giving rise to disease (Linhares *et al.,* 2015; Sakr *et al.,* 2018).

Pigs have been shown to be major reservoir of methicillin resistant *Staph. aureus* multilocus sequence type 398 (Neyra *et al.*,2014; Hau *et al.*, 2017). It has also shown potential for zoonotic transmission (Somayaji *et al.,* 2016). This clonal complex associated with disease in livestock has also been implicated in human infection (Aires-de-Sousa, 2017). It is known to cause diseases in poultry, feed and companion animals (Cuny *et al.,* 2015; Chon and Khan 2017).

Methicillin resistant *Staph. aureus* (MRSA) is becoming increasingly recognized among persons in the community without established risk factors (Calfee, 2017). MRSA in animal disease is considered a source of infection to humans, although transmission appears to be primarily between animals, undistinguished isolates have been found in their human contacts, particularly those with occupational exposure (Young *et al.*,2014; Friedman and Papodopolous 2015).

Swine have emerged as another reservoir of *S. aureus*, including MRSA. The majority of swine-associated *S. aureus* belong to the same multi locus sequence type (MLST) 398. This sequence type has also been referred to non-type able MRSA due to the inability of the restriction enzyme to cut DNA from these strains due to the presence of a unique methylase (Desai 2014). The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has become a serious challenge for human infection control and antibiotic therapy. Livestock-associated MRSA strains (LA-MRSA) have recently emerged in pig farming and are now prevalent in farms in industrialized countries (Chuang and Huang 2015).

2.4 Carriage of *Staphylococcus aureus*

Humans are the main source of *Staphylococcus* species with diverse clonality and toxigenicity (Gomez-Sanz *et al.,* 2013). Humans and animals have persistent, intermittent or transient nasal colonization from where *Staph.aureus* and MRSA can cause infection or be transmitted to another person (Murray *et al.,* 2002). *Staph.aureus* nasal carriage of 20-50% exists in the general population (Chuang and Huang 2015). But higher carriage rates have been observed in specific populations; injection drug users, persons with insulin dependent diabetes, patients with dermatological conditions, patients with long term in dwelling intravascular catheters and health care workers (Lee *et al.,* 2018). Almost 25% of healthcare workers are stable nasal carriers and 30 % to 50 % of them also carried the bacteria on their hands (Farzana *et al.,* 2008). Asymptomatic carriers of *Staph.aureus* are at high risk of infection and are presumed to be an important source of *Staph. aureus* strains that spread among individuals (Chambers and Deleo, 2009). In hospitals, the asymptomatic hosts can disseminate *Staph. aureus* to immune compromised patients (Martins *et al.,* 2012). In infants, colonization of the nasopharynx, perineum or skin (especially if the cutaneous barrier has been disrupted or damaged) occurs and shortly after birth and may recur any time thereafter (Bokaeian and Saeidi 2015).

Factors that enhance colonization by *Staph. aureus* are complex and are not fully understood. However, it does appear to involve the host's contact with other carriers, *Staph. aureus* ability to adhere to host cells and evade the immune response; and the antimicrobial properties of the nasal fluid which allows it to be transmitted among individuals in both healthcare and community settings (Kumar *et al.,* 2011; Kong *et al.,* 2016). Consequently, a combination of bacterial resistance mechanisms, defective nasal fluid and lack of alternative clearance mechanisms (host associated determinants) results in nasal colonization by *Staph.aureus* (Wertheim *et al.,* 2005).

2.5 Infections Caused by *Staphylococcus aureus*

2.5.1 Skin Infections

Skin Infections are the most common form of *Staph. aureus infection*. This can manifest in various ways including small benign boils, folliculitis, impetigo, cellulitis, and more severe, invasive soft-tissue infections (Ryan *et al.,* 2004). *Staph. aureus is* extremely prevalent in persons with atopic dermatitis, it is mostly found in fertile, active places, including the armpits, hair, and scalp. The presence of *Staph. aureus* in persons with atopic dermatitis is not an indication to treat with oral antibiotics, as evidence has not shown this to give benefit to the patient. The relationship between *Staph. aureus* and atopic dermatitis is unclear. (Rasmussen *et al.,* 2011).

2.5.2 Animal Infections

Staph. aureus can survive on dogs, cats, horses and can also cause bumble foot in chickens (Haenni *et al.,*2017). *Staph. aureus* is one of the causal agents of mastitis in dairy cows. Its large polysaccharide capsule protects the organism from recognition by the cow's immune defenses. (Rainard *et al.,*2018).

2.6 Virulence Factors in *Staphylococcus aureus*

Staph. aureus is a pathogen expressing multiple factors that mediate host colonization, invasion of damaged skin and mucous dissemination through the body and evasion of host defense mechanisms (Chanda *et al.,* 2010). The pathogenicity and virulence of *Staph.aureus* infections is associated to various bacterial surface components. Common examples are capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules e.g., Clumping factor (CLF) Fibronectin Binding Protein (FBN) and the extracurricular proteins like

coagulase, haemolysins, enterotoxins, toxic shock syndrome toxins, exfoliatins toxins and panton valentine leukocidin (Labandeira-Rey *et al.,* 2007).

Virulence factors can generally be separated into three; “antigens, enzymes and toxins” based on their function:

2.6.1 Antigens e.g; Adhesins: They are surface attached proteins that allow the bacteria to attach to a wide variety of human tissues. In *Staph.aureus* the adhesion genes which include clf and fnb that encode the fibrinogen and the fibronectin binding proteins respectively. Typical members of the family MSCRAMMs are the staphylococcal protein A(SpA), collagen binding protein, clumping factor and Fibronectin binding protein (FnBP) A and Fibronectin binding protein (FnBP) B encoded by the *finbA* and *FinbB* genes respectively, play prominent roles in *Staph.aureus* colonization and attachment of host tissues or implanted biomaterials (Greene *et al.,* 1995).

2.6.2 Enzymes: They are of exoproteins group that convert local host tissue into nutrients required for bacterial growth such as:

2.6.2.1 *Coagulase*

Staph. aureus strains possess two forms of coagulase; bound and free. Coagulase bound to the staphylococcal cell wall can directly convert fibrinogen to insoluble fibrin and cause the Staphylococci to clump. The cell free coagulase accomplishes the same result by reacting with a globulin plasma factor. The role of coagulase in the pathogenesis of disease is speculative but coagulase may cause the formation of fibrin layer around a staphylococcal abscess thus localizing the infection and protecting the organism from phagocytosis.

2.6.2.2 *Catalase*

All Staphylococci produce catalase which catalysis the conversion of toxic hydrogen peroxide to water and oxygen. Hydrogen peroxide can accumulate during bacterial metabolism or after phagocytosis.

2.6.2.3 *Hyaluronidase*

This enzyme hydrolyzes hyaluronic acid. This is the acidic mucopolysaccharides present in the cellular matrix of connective tissue. Hyaluronidase facilitates the spread of *Staph. aureus* in tissues. More than 90 *% of Staph. aureus* strains produce this enzyme.

2.6.2.4 *Fibrinolysin*

It is also called staphylokinase is produced by virtually all strains of *Staph. aureus* and can dissolve fibrin clot. Staphylokinase is distinct from the fibrinolytic enzyme produced by *streptococci*.

2.6.2.5 *Toxins*

They are secreted proteins that cause tissue damage and generate pus in abscesses which is believed to facilitate transmission between hosts. This includes

Enterotoxins are produced by 30 % to 50 % of the *Staph. aureus* strains. Eight serologically distinct enterotoxins (A-E, G-I) have been identified. Enterotoxin A is the most commonly associated with disease. Enterotoxin C and D are found in contaminated milk products and enterotoxin B causes staphylococcal pseudomembranous enterocolitis.

Toxic shock syndrome Toxin-1 (TSST-1 formerly called pyrogenic exotoxin C and enterotoxin F is a heat and proteolysis resistant chromosomally medicated exotoxin. The ability of TSST-1 to penetrate mucosal barriers even though the infection remains localized in the vagina or at the site of a wound is responsible for the systemic effect to TSS. Death in patient with TSS is due to

hypovolemic shock leading to multi-organ failure. Others toxins are exfoliative, α -toxins, β-toxinsα- toxins and PVL.

2.7 Antibiotic Resistance in *Staphylococcus aureus*

Antibiotic resistance in *Staph. aureus* was common when penicillin was first introduced in 1943. It was reported that, the original Petri dish on which Sir Alexander Flemming of Imperial College London observed the antibacterial activity of *Penicillium* fungus which was growing a culture of *Staph. aureus* and by 1950, 40% of hospital *Staph. aureus* isolates were penicillin resistant (Jevons, 1961).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a type of *Staph. aureus* that is resistant to antibiotic called beta-lactams. Beta-lactam antibiotics are a class of broad-spectrum antibiotic, consisting of all antibiotic agents that contain a beta-lactam ring in their molecular structures. This includes penicillin derivatives like methicillin and other more common antibiotic such as oxacillin, penicillin, and amoxicillin (penams), cephalosporins (cephems), monobactams, and carbapenems (Lima *et al.,* 2020). The majority of MRSA infections occur among patients in hospitals or other healthcare settings and they are referred to as Hospital-Acquired MRSA or HA-MRSA Infections. (Choo, 2017). However, *Staph. aureus* and MRSA can also cause illness in persons outside of hospitals and healthcare facilities. (Kim *et al.,* 2018).

Staph. aureus has been reported to exhibit resistance to antiseptics and disinfectants such as quartenary ammonium compounds (QACs) which may aid its survival in the hospital environment (Jennings *et al.,* 2015).

2.8 Biochemistry of Antibiotic Resistance

Understanding the mechanisms of resistance has become a significant biochemical issue over the years and nowadays there is a large pool of information about how bacteria can develop drug resistance (Brown and Wright, 2015).

Although the manner of acquisition of resistance may be different between bacterial species, resistance is created only by a few mechanisms;

a) Enzymatic Inactivation of The Antibiotic- direct inactivation of the active antibiotic molecule by enzymes (Brown and Wright, 2015).
b) Alteration of Target site: alteration of the sensitivity to the antibiotic by modification of the target (Blair, 2015).
c) Decreased Concentration of Drug at The Target site or Efflux pumps and outer Membrane (OM) permeability changes- reduction of the concentration of drug without modification of the compound itself (Douafer *et al.*, 2019)
d) Failure to Metabolize the Drugs: Some bacteria become refractory to specific antibiotics by bypassing the inactivation of particular or given enzyme. This mode of resistance is observed in many trimethoprim and sulfonamide-resistant bacteria. (Nas,2017).

2.9 Mechanism of Antibiotic Resistance

Drug resistant bacteria are now global health threat. Wounds, infection, septicemia and childhood ear infections are just a few diseases that have become hard to threat with antibiotics (Fymat, 2017). Microorganisms multiply very rapidly and have adapted to fill almost every available environmental niche (Shapiro, 2016). More than 70 percent of the bacteria that cause infections in hospitals are reportedly resistant to at least one of the drugs most commonly used

for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs (Nas, 2017).

2.9.1 Mechanism of Resistance to beta-lactam Antibiotic

Beta-lactams are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics like penicillins, carbapenems (ertapenem) and cephamycins, cephalosporins are relatively resistant to beta-lactamase, while cephalosporinase produced by Temoneira (TEM) and Sulfhydryl variable (SHV) genes are synthesized by bacteria against cephalosporins (Nas, 2017).

Staphylococcal resistance to penicillin is mediated by penicillase production: an enzyme that cleaves the Beta-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillin resistance such as methicillin nafcillin, oxacillin, are able to resist degradation by Staphylococcal penicillinase (Munita and Arias 2016)

Resistance to methicillin is mediated by the *mec* operon, part of the Staphylococcal cassette chromosome mec (SCC*mec*). Resistance is conferred by the *mecA* gene which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding beta lactams. This allows resistance to all beta-lactam antibiotics and obviates their clinical use during MRSA infections. As such, glycopeptides Vancomycin is often deployed against MRSA (Lakhundi and Zang 2018).

2.9.2 Mechanism of Resistance to Aminoglycoside Antibiotic

Aminoglycoside antibiotics such as Kanamycin. Streptomycin, Gentamicin etc.; were once effective against Staphylococcal infections until strains evolved mechanisms inhibits the

aminoglycosides' actions which occurs via protonated amine and or hydroxyl interactions with the ribosomal RNA of the bacterial 30s ribosomal subunit (Kibaba *et al.*, 2017).

Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a phosphate, nucleotide, or acetyl moiety to either the amine or the alcohol key functional group of the antibiotic. This changes the charge and hinders the antibiotics, decreasing its ribosomal binding affinity. In *Staph. aureus* the best characterized aminoglycoside-modifying enzyme is aminoglycoside adenyltranferase. This enzyme has been solved by X-ray crystallography (Garneau and Labby 2016). The enzyme is able to attach an adenyl moiety to the 4'hydroxyl group of many aminoglycosides including Kanamycin and Gentamicin.

2.9.3 Mechanism of Resistance to Glycopeptides

Glycopeptides comprises peptide antibiotics of clinical interest such as Vancomycin and teicoplanin. Their antimicrobial activity is due to binding to D-alanyl-D-alanine side chains of peptidoglycan or its precursors, thereby preventing cross-linking of the peptidoglycan chain. They are largely effective against Gram-positive microorganisms which poses a bigger layer of the peptidoglycan although not all gram-positive organisms are susceptible to these agents and because of their inability to penetrate the outer membrane of Gram-negative bacteria (Binda and Macrone 2014).

2.9.4 Mechanism of Resistance to Chloramphenicol

There are three mechanisms of resistance to Chloramphenicol:

i. Reduced membrane permeability

ii. Mutation of 50S ribosomal subunit and

iii. Elaboration of Chloramphenicol acetyltransferase (CAT).

It is easy to select for reduced membrane permeability to Chloramphenicol *in-vitro* by serial passage of bacteria and this is the most common mechanism of low-level Chloramphenicol resistance. High-level resistance is conferred by the CAT-gene; this gene codes for an enzyme called Chloramphenicol acetyltransferase (CAT), which inactivated Chloramphenicol by covalently linking one or two acetyl groups derived from acetyl-S- coenzyme A, to the hydroxyl groups on the Chloramphenicol molecule. The acetylation prevents Chloramphenicol from binding to the ribosome. Resistance-conferring mutations of the 50S ribosomal subunit are rare (Kapoor and Elongavan 2017).

Chloramphenicol resistance may be carried on a plasmid that also codes for resistance to other drugs. A single R-plasmid may code for resistance for up to 10 different antibiotics which mediates multiple-drug resistance (Roberts and Schwarz 2017).

2.9.5 Mechanism of Resistance to Macrolide, Lincosamide, and Streptogramin (MLS)

Bacteria resist Macrolide and Lincosamide antibiotics in three ways:

1. Through target site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target.
2. Through efflux of the antibiotics and
3. Drug inactivation.

These mechanisms have been found in the Macrolide and Lincosamide producers, which often combine several approaches to protect themselves against the antimicrobial that they produce. In pathogenic microorganisms, the impact of the three mechanisms is unequal in terms of incidence and of clinical implications. Modifications of the ribosomal target confer broad-spectrum

resistance to macrolides and lincosamides, whereas efflux and inactivation only affects some of these molecules (Schwarz *et al.*, 2016).

2.9.6 Mechanism of Resistance to Quinolones

Broad use of fluroquinolones has been followed by emergence of resistance, which have been due to chromosomal mutations in genes encoding the subunits of the drug's target enzymes; DNA gyrase and topoisomerase IV are genes that affect the expression of diffusion channels in the outer membrane using multidrug-resistance efflux systems (Brown and Wright 2015). The mechanisms of bacterial resistance of floroquinolone fall into two principal categories:

I. Alteration in drug target enzymes and

II. Alteration that limits permeation of drug to target.

Both result from chromosomal mutations. No specific resistance mechanisms of quinolones degradation or modification have been found. The target enzymes, DNA gyrase and topoisomerase IV are most commonly altered domains near enzymes active sites and in some cases, reduced drug affinity has been demonstrated. Drug permeation is altered by a mutation that increases expression of endogenous multidrug efflux pumps; alter outer membrane diffusion channels or both (Hooper and Jacoby 2015).

2.10 Methicillin-Resistant *Staphylococcus aureus*

After the presentation of penicillin, in 1945, most of *S. aureus* isolates had become resistant to penicillin through the production of beta-lactamase, an enzyme that breaks down penicillin. Be that as it may, after introduction, the first methicillin-resistant isolates of *S. aureus* were reported (Graveland *et al.*, 2011) Methicillin resistance is caused by the presence of the *mecA* gene. This gene codes for alternative penicillin-binding protein, called PBP2A, which has a low affinity for

beta-lactam antimicrobial agents (Vanderhaeghen *et al.,* 2010a). The *mecA gene* is part of a large genetic element, the staphylococcal cassette chromosome mec (SCCmec). SCCmec carries a set of cassette chromosome recombinase genes (*ccrA,ccrB* or c*crC*) for extraction and integration into the host chromosome. As indicated by the blends of the *mecA* and *ccr* gene complexes contained by the bacterial genome, molecular typing of MRSA strains has uncovered eleven noteworthy *SCCmec* types and a few subtypes and composites of at least two SCC components have risen around the world (IWG-SCC, 2009). Notwithstanding the distinction in these gene complexes, the various S*CCmec* elements differ from each other in the antibiotic resistance markers to antimicrobials other than beta-lactams (Grundmann *et al.,*2006).

MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents (Vidhani *et al.,* 2003). Multidrug resistant *Staph. aureus* evolved following acquisition of discrete preformed antimicrobial resistance genes by horizontal gene transfer and resistance determinants generated by chromosomal mutation which poses great challenges in treatment of staphylococcal infections (Jesen and Lyon, 2009).

Six SCC *mec* type (I-VI) were identified in *Staph. aureus* which are defined by combination of the *mec* gene complex class with the *ccr* allotype (Ito *et al.,* 2001; Oliveira *et al.,* 2006). SCC*mec* typing has been established as an important addendum to the characterization and identification of MRSA clones and is routinely used in many laboratories. Several strategies have been developed for SCC*mec* type (Okuma *et al.,* 2002) and their broad application has led to the detection of several variants or subtype of the major SCC type (Olivera and de Lancastre 2002; Ito *et al.,* 2003). Community acquired methicillin resistant *Staph. aureus* (CA-MRSA) has a

characteristic staphylococcal cassette chromosome type IV (SCCmec IV) gene, lacking in non β–lactam determinant and possessing distinct necrotizing toxin, Panton valentine leukocidin (PVL). Methicillin resistant *Staph. aureus* (MRSA) has likewise been found in companion animals such as Dogs, and Cats, but these strains generally vary from those in livestock. The reason thought for this is that the transmission route is assumed to be from humans to companion animals and therefore human epidemic clones are found in these animal species, i.e., MRSA in companion animals is primarily a humanosis (Verkarde and Klutymans 2014).

2.10.1 Methicillin Resistance *Staphylococcus aureus* in Companion Animals

In industrialized countries, companion animals have become an integral part of the household. More than 50% of households in the USA have pets and 25% of households in the United Kingdom have dogs (Chomel and Sun, 2011). In general, MRSA strains recovered from companion animals (cats, dogs, or horses) are different from those recovered from food animals. In the first case strains are usually similar to human HA-MRSA, while in the second case they appear to belong to specific animal-adapted clones, unrelated to the most common HA-MRSA.

Reports of MRSA isolated from pets were sporadic until the late 1990s and were mostly related to clinical infections. In addition, before the identification of *S. intermedius* and *S. pseudintermedius*, some misclassification may have occurred (Devries *et al.*, 2005). The emergence of CA-MRSA in the last decade and the importance of tracing antibiotic-resistant organisms also in the community, have prompted many studies on MRSA in pets and its possible transmission to pet owners.

According to studies performed in various countries, especially in the UK and Australia, MRSA colonization is rare in healthy pets. No MRSA was found in healthy cats in several studies

(Baptiste *et al.,* 2005; Loeffler *et al.,* 2005; Hanselman *et al.,*2009), although in a study 2.1% of cats presented to veterinary clinics in Greater London area were colonized by MRSA (Loeffler *et al.,*2011 Haenni *et al.,* 2017). In dogs in a household orated mission to a veterinary hospital, colonization rates varied from 0 to2.1% (Loeffler *et al.,*2011). In some particular settings, e.g., dogs in a rescue shelter or in a veterinary hospital (Harrison *et al.,* 2014), a high MRSA colonization rate, up to 9%, was found. Methicillin-resistant *S. aureus* infections in pets are mainly represented by skin and soft tissue infections and are sometimes associated with veterinary surgeries.

In studies, dogs appear to have more MRSA infections than cats (Mustapha *et al.,* 2014). The MRSA types recovered from cats and dogs are similar to those affecting humans, with a similar regional distribution (Chomel, 2014). Dogs have been found to be colonized by the livestock-associated (LA - MRSA clone characteristic of food animals and identified as ST398 (Aires-de-Sousa, 2017).

Transmission of bacterial strains between companion animals and their owners has been demonstrated in several instances. Molecular analyses have shown the presence of indistinguishable MRSA strains in pets and humans living in the same household. An important issue to be underlined at this regard is that both humans and animals are more often colonized than infected by MRSA. Such a colonization can be transient or lasting for different time depending on several aspects like exposure intensity or frequency. Determining the level of colonization in different and specific population is useful to know MRSA burden and the risk of transmission (Lee *et al.,* 2018)

2.10.2 Methicillin Resistance in Pigs

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important pathogen in human medicine, but can also colonize and cause infections in a variety of animal species. (Weese *et al* 2010; (Feßler *et al*. 2010) During recent years, MRSA isolates of the clonal lineage ST398 have gained particular attention as colonizers and more rarely causative agents of infections in pigs(van Duijkeren *et al* 2007; Schwarz *et al.*, 2007). Studies have shown that ST398 isolates are not restricted to pigs, but can also be isolated from humans, dogs and horses (Dennis *et al.*,2009; Aires-de-Sousa, 2017). Due to the low host specificity of MRSA ST398, transfer of such isolates between different animal species, but also between humans and animals, might occur in either direction (Feßler *et al*. 2010). People with close contact with swine, but also with other MRSA ST398-carryingand -shedding animals, are at risk of being colonized by these isolates (Weese *et al.*, 2005). Colonized people may play a role in the further spread of MRSA ST398 between different farms and different animal species (van Duijkeren *et al.*, 2008). To follow the dissemination of MRSA ST398 isolates within and beyond different animal and human populations, tools for detailed characterization are necessary. In a research carried out by (Feßler *et al.* 2010) it was found that Nine major ApaI PFGE patterns were detected. Three spa types (t011, t034 and t2576) and two SCCmec types (IV and V) were identified. Five different drug types were seen with dt11a being predominant. All isolates were negative for Panton–Valentine leucocidin, enterotoxin and exfoliative toxin genes. Ten resistance patterns were observed with 11 (40.7%) isolates being resistant to only β-lactam antibiotics and tetracyclines. Several resistance genes were detected: *blaZ* (penicillin resistance); *tet*(M), *tet(K)* and *tet(L)* (tetracycline resistance); *erm(A),erm(B),erm(C)* and *erm(T*) (macrolide/lincosamide/streptogramin B resistance); *aacA–aphD, aphA3, aadD and spc* (aminoglycoside or aminocyclitol resistance); *fexA* (phenicol resistance); *dfrK*(trimethoprim

resistance); and *vga(A)* and *vga(C)* (pleuromutilin/lincosamide/streptogramin A resistance). The two human isolates were indistinguishable in their genotypic and phenotypic characteristics from the mastitis isolates of the same farm. The research concluded that a uniform virulence gene pattern appeared to be conserved between ST398 isolates from both animal species.

2.11 Treatment Of Methicillin Resistant *Staphylococcus aureus* Infections

Methicillin-Resistant *Staphylococcus aureus* infections are reported to be fatal (Sganga *et al.*, 2016). The location and history related to the infection determines the treatment. The route of administration of antibiotics varies. Antibiotics effective against MRSA can be administered by Intravenous routes, Oral, or a Combination of both and depends the specific circumstances and patient characteristics (Lin *et al.,* 2011; Bassetti *et al.,* 2014).

Several newly discovered strains of MRSA show antibiotic resistance even to Vancomycin and teicoplanin (Kauer and Chate 2015). Linezolid, Quinupristin-Dalfoprisitin, daptomycin, ceftaroline and tigecycline are used to treat more severe infections that do not respond to glycopeptides (Yue *et al.*,2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Equipments

Incubator (National appliance: Aheinicke Company Portland, Oregon, U.S.A. Model-630 Serial No., 1-81-1550-1), Electronic weighing balance (Top balance digital, U.S.A. Ohaus, PA313-model), Hot-Air-Oven (Baird and Tatlock London limited), Autoclave (Portable 230V and 1850V Adelphi MFG Co Ltd, England), Microscope (Wild M11, Switzerland), Bunsen-burner, Micropipette (NAPCO Model 630 Portland, Oregon, U.S.A.), Refrigerator (Haier Thermocool: Model No. HRF-688-FF/A.), Polymerase Chain Reaction (PCR) Machine, Centrifuge.

Glasswares

Microscope glass slides and Petri dishes (Pyrex, England), Test tubes (Pyrex, England), Measuring Cylinder (Pyrex, England), Beakers (Pyrex, England), conical flask (Pyrex, England), Zymo-Spin Column, Eppendorf's Tube, Micropipettes, McCartney Bottles.

Laboratory Media and Reagents

Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England), Mannitol Salt Agar (Oxoid Ltd., Basingstoke, Hampshire, England), Nutrient Broth and Agar (Fluka, Bioche, Sigma-Aldrich). Crystal violet (May and Baker Ltd. Dagenham England), Ethanol, Carbolfuchsin, Starch Soluble (BDH Chemicals Ltd., England), Phosphate Buffer PH 8 Bromothyol Blue, Lugol's iodine (May and Baker Ltd. Dagenham England), Hydrogen peroxide (SKG Pharma Ltd. Ikeja Lagos, Nigeria), Sterile deionized water, Hydrochloric Acid, Ethanol-acetone, immersion oil (BDH), Serum Plasma, Sucrose, Normal Saline, 0.2M Monosodium Phosphate, Benzyl Penicillin potassium salt Deoxyribonuclease acid(Sigma Chemicals Company St Louis M.O. USA).

Antibiotic Susceptibility Discs

The antibiotic used were selected according to EUCAST 2019 and also by the Veterinary Doctor responsible for these animals.

Gentamicin (30µg), Amoxicillin-clavulanic acid (30µg), Cefoxitin (30µg), Chloramphenicol (30 µg), Erythromycin (15µg), Clindamycin(2µg) Tetracycline (30µg), Ciprofloxacin (5µg), Trimethoprim-sulfamethoxazole (1.25µg+23.75µg), Linezolid (15µg), Quinupristin-Dalfoprisitin (15µg) Vancomycin (30µg),

Other Materials

PCR kit, Microgen™ Staph-ID System, Microgen™ Staph-ID kit, Wire loop, DNA extraction kit (Zymogen®).

3.1.1 Genes and Primers Sequence and Base Pair

The primers used in the study with their genes, base pairs and sequences are shown in table 3.1

Table 3.1: Primer Sequence and Base Pair

Primers		**Genes**	**BP**	**Reference**
Forward	5′AAA ATC GAT GGT AAA GGT TGG C	*mecA*	533	Olowe *et al.,* 2007
Reverse	5′AGT TCT GCA GTA CCG GAT TTG C			
Forward	5’ ACT TCA ACA CCT GCT GCT TTC	*bla$_Z$*	173	Maliha *et al.,* 2018
Reverse	5’TGA CCA CTT TTA TCA GCA ACC 3’			
Forward	5’ACAGAAAGCTTATTATATAAC 3’	*tetM*	171	Muyzer *et al.*,1993;
Reverse	5’TGGCGTGTCTATGATGTTCAC 3’			
Forward	5’GTAGCGACAATAGGTAATAGT 3’	*tetK*	360	Maliha *et al.,* 2018
Reverse	5’GTAGTGACAATAAACCTCCTA 3’			
Forward	5’ATAAATTGTTTCGGGTCGGTAAT 3’	*tetL*	1077	Farhat *et al.,* 2012; Khoramrooz *et al*., 2017
Reverse	5’AACCAGCCAACTAATGACAATGAT 3’			
Forward	5’AACTTAGGCATTCTGGCTCAC 3’	*tetO*	514	Khoramrooz *et al.,* 2017
Reverse	5’TCCCACTGTTCCATATCGTCA 3’			
Forward	AATCTTTGTCGGTACACGATATTCACG	*staph.A30*	107	Martineau *et al*.,2001
Reverse	CGTAATGAGATTACAGTAGATAATACA ACA			

3.2 METHODS

3.2.1 Study Design

This involved a descriptive study which is limited to the occurrence of Methicillin Resistant *Staphylococcus aureus* isolated from selected pig farms and dogs and the owners/ handlers of the animals in Zaria and Buwaya Community Gonin Gora part of Kaduna State, Nigeria.

3.2.2 Study Areas

The study areas were available piggeries that allowed sample collection in Samaru, Zaria and also from Buwaya Community in Gonin-gora Kaduna state and the dogs, Maraban-rido area of Kaduna State, Nigeria. These areas were selected for the study based on the availability of the animals and the permission of the owners to allow for sample collection.

3.2.3 Sampling Technique

Convenience sampling technique was used and samples were collected from the nose, anus, and skin of the animals also hand and nose swabs were collected from the owners/ handlers of the pigs and dogs used in the research.

3.2.4 Sample Size

$N = \frac{Z^2P(1-P)}{E^2}$ (Kadam and Bhalerao, 2010)

Where N = Number of samples

Z = Standard normal deviation at 95% confidence limit = 1.96

P = Prevalence for pigs =9% (Okunola and Ayandele, 2015).

P= Prevalence for dogs = 4.91% (Habibullah *et al.*, 2017).

1-P = 1 – 0.09 = 0.91

1-P = 1 – 0.0491 = 0.950

L = Allowable error of 5% = 0.05

$$N = \frac{Z^2 P (1-P)}{E^2}$$

Sample size for Pig = $1.96^2 \times 0.09 \times 0.91 / 0.05^2$ = 125 pig samples

Sample size for Dogs= $1.96^2 \times 0.0491 \times 0.950 / 0.05^2$ = 72 Dog samples.

Approximately 197 samples were required but a total of 300 samples were collected.

3.2.5 Ethical approval.

Ethical Approval was obtained from the Ahmadu Bello University Committee on Animal use and Care (ABUCAUC) with the approval no: ABUCAUC/2019/15 as shown in Appendix I.

3.2.6 Collection of Samples

Samples were collected from nasal, rectal and skin swabs of the pigs and dogs and nasal and hand swab of workers and dog owners. Following aseptic technique, samples were collected using a sterile cotton swab and were taken to laboratory for bacteriological analysis in an ice pack. A total of Three Hundred (300) samples were collected from pigs, Dogs, farm workers and their Handlers.

3.2.7 Purification and Preliminary identification of Staphylococcal isolates

Samples were inoculated into sterile Nutrient agar (NA) and incubated at 37°C for 24 h. The overnight culture was then sub-cultured on the surface of sterile Mannitol Salt agar (MSA) by streaking and incubation at 37°C for 18-24 hours. Cultural characteristics of the resulting colonies were noted after which further confirmation of species were carried out on each isolate.

3.2.8 Isolation and Purification of *Staphylococcus aureus*

Simple Staining

Simple staining of suspected Staphylococci colonies was carried out as described by Cheesbrough (2006). Using sterile wire loop, smears were made from discrete colonies with desirable growth characteristics on clean glass slides. After heat fixing, smears were stained with methylene blue solution for one (1) minute and washed with distilled. Stained slides were observed under microscope. Colonies, whose smear appeared as cocci in characteristics clusters were picked into nutrient broth (NB) and incubated overnight at 37° C.

Gram Staining

Gram staining was carried out using the method described by Cheesbrough (2006). A smear of the culture of the test organism was made evenly on a clean glass slide and heat-fixed. The smear was stained with crystal violet for thirty (30) seconds to one (1) minute, flooded with Lugol's iodine for one (1) minute and decolorized rapidly with acetone for ten (10) seconds after which it was counter-stained for one (1) minute with dilute carbol fuchsin solution. The stained slides were microscopically examined under the oil immersion objective. Sample colonies that appeared as purple/violet cocci, predominantly in cluster were selected. Sample colonies were inoculated into sterile nutrient broth and incubated at 37°C for 18 hrs. Each sample was sub-cultured in duplicate on to slants and incubated at 37°C for 18 hrs. Slants were kept at 4°C until required.

Growth on Selective Media

A loopful of overnight NB culture of the isolates that were presumptively identified as probable Staphylococci (based on simple and Gram staining characteristics) were streaked on previously prepared Mannitol salt Agar (MSA) plates. Plates were incubated at 37°C for 24 hrs. Isolates that produced colonies exhibiting characteristic deep golden yellow coloration were selected and sub-

cultured into nutrient broth, overnight culture were sub cultured on to Nutrient Agar slants, after overnight incubation at 37°C, slant were stored in refrigerator at 4°C until required.

3.2.9 Biochemical and Confirmatory Tests

Catalase Test

The ability of the isolates to produce an enzyme catalase was demonstrated by the addition of 1ml of 3 % w/v hydrogen peroxide solution to 24h culture of the isolate from the plate. Rapid evolution of gas bubbles indicated the breakdown of hydrogen peroxide into oxygen and water by catalase peroxidase enzyme present a positive result and it was recorded. Absence of gas bubbles indicated negative result.

$$2H_2O_2 \rightarrow H_2O + O_2$$

Coagulase Test

Diluted rabbit plasma was prepared and 2ml of the plasma each was placed in sterile tubes. To these tubes, a loopful of the test organisms was added and then incubated at 37ºC for 4 hours. The tubes were observed after 1-, 2- and 3-hours intervals by tilting the tube through 90º angle. Presence of clumping in the tubes after incubation indicated a positive result. The tubes which did not clump were left overnight at room temperature and then re-examined. A negative result was recorded for tubes that did not show clumping.

Microgen Staph ID Test

The test was carried out according to the manufacturer's instructions. A single colony from an 18-24 hours culture was emulsified in the suspending medium supplied in the kit, and mixed thoroughly. Back of the adhesive tape sealing the microwell test strip(s) was carefully peeled. Using a sterile Pasteur pipette, 3-4 drops (approximately 100 ul) of the bacteria suspension were

added to each well of the strip(s). After inoculation, 10^{th} and 11^{th} wells were overlaid with 3-4 drops of mineral oil. The top of the microwell test strips was sealed with the adhesive tape removed earlier and incubated at 35-37°C. The microwell test strips were read after 18-24h incubation. Adhesive tape was removed and all positive reactions were recorded with the aid of the colour chart substrate reference table (appendix 4a and 4b). One drop of PYR reagent was added to well 12 and read after 10 minutes. Formation of a very deep pink/red colour indicates positive results. Nitrate reduction test was performed on well 9 after reading and recording the β-glucuronidase reaction, one drop of nitrate A reagent and one drop of nitrate B reagent was added to the well and read after 60 seconds. The development of a red colour indicated that nitrate has been reduced to nitrite. All reports were recorded on the form provided (appendix 5). Using the software provided specie was assigned to each bacterial isolate tested.

3.2.10 Determination of Antibiotic Susceptibility

The modified Kirby-Bauer disc diffusion technique was used to test for the antimicrobial susceptibility of the isolates (Bauer *et al.,* 1996). The antibiotic used were those prescribed according to EUCAST 2019 and also by the Veterinary Doctor responsible for the animals. (Cefoxitin (30µg), Gentamicin (10µg), Erythromycin (15µg), Clindamycin (2µg), Ciprofloxacin(10µg) Vancomycin (30µg), Linezolid (30µg), Quinupristin-Dalfoprisitin (15µg), Tetracycline (30µg), Amoxicillin/clavulanic acid (30µg) and Trimethoprim-sulfamethoxazole (1.25µg+23.75µg) Chloramphenicol(30µg).

An overnight culture of each isolate was prepared in a nutrient agar and incubated at 37°C for 18 hours. Distinct colony on the nutrient agar plate were emulsified in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard (1.5×10^{8} cfu/ml). Using sterile swab sticks, the surface of Mueller Hinton Agar in 90 mm- diameter plate was inoculated with the bacterial

suspension by streaking the surface of the agar in three directions, rotating the plate approximately to 60° to ensure even distribution. The inoculated plates were allowed to dry for 5 minutes after which the antibiotic discs were placed on the surface of the agar. The plates were left at room temperature for the pre-diffusion, inverted for 20 minutes and incubated aerobically at 37° C for 16-18 hrs. The diameter of the zones of growth inhibition were measured to the nearest millimeter and isolates classified as; sensitive, intermediate or resistant based on EUCAST interpretative chart zone size EUCAST, 2019 (Table 3.2). The isolates were reported as sensitive and resistant to the various antibiotics depending on the zones of inhibition measured. Isolates were also classified as multidrug resistance, extensively drug resistance and pandrug resistance according to the zone of inhibition (Magiorakos *et al.,* 2012). For Methicillin, inhibition zone diameter of 21mm was reported as methicillin resistant and ≥ 22mm was considered as methicillin sensitive.

3.2.11 Interpretative Chart for Susceptibility Testing

European Committee on Antimicrobial Susceptibility Testing (2019) Break point 9.0 used for the interpretation of the zones of inhibition during the Susceptibility testing is given in Table 3.2

Table 3.2: Interpretative Chart for Susceptibility Testing

ANTIBIOTIC	CODE	CONC.	SENSITIVE	INTERMEDIATE	RESISTANT
Linezolid	LZD	30 µg	≥ 21	-	≤ 21
Gentamicin	CN	10 µg	≥ 18	13-14	≤ 18
Tetracycline	TET	30 µg	≥ 22	15-18	≤ 19
Amoxicillin-clavulanic acid	AMC	30 µg	≥ 26	13-15	≤26
Ciprofloxacin	CIP	10µg	≥ 21	16-20	≤ 21
Trimethoprim-sulfamethoxazole	SXT	25 µg	≥ 17	-	≤ 14
Chloramphenicol	CH	30 µg	≥ 18	13-17	≤ 18
Cefoxitin	FOX	30 µg	≥ 22	-	≤ 22
Erythromycin	E	15 µg	≥ 21	-	≤ 18
Clindamycin	DA	2µg	≥ 22	-	≤ 19
Quinupristin-Dalfoprisitin	QD	15 µg	≥ 21	-	≤ 18

3.2.12 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Paul and Bezbarauh., 1997)

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

3.2.13 Determination of Methicillin (Oxacillin) Resistance Using Cefoxitin Disc

EUCAST recommends using cefoxitin instead of oxacillin when using disk diffusion method to determine methicillin resistance in *S. aureus* (EUCAST,2019). Cefoxitin result are easier to interpret and are thus more sensitive to *S. aureus* and enhance induction of PBP2a for the detection of *mecA* mediated resistance than oxacillin results.

A direct colony suspension of each *S. aureus* isolate was prepared equivalent to 0.5McFarland standard and plated on Mueller Hinton agar surface. Cefoxitin 30µg disc was placed on the inoculated plate. Plates was incubated at 35°C for 16- 18hours. The zones of inhibition were measured and compared to that of the EUCAST interpretative chart (EUCAST 2019). Inhibition zones diameter of 22mm was reported as methicillin resistant and ≥ 22mm was considered as methicillin sensitive.

3.2.14 Determination of Minimum Inhibitory Concentration of Vancomycin.

All isolates were tested with Vancomycin MIC Evaluator Strip (Oxoid) on Mueller Hinton agar plates. For each Isolate a bacterial suspension adjusted to 0.5 McFarland Standard (1.5 x 10^8cfu/ml) was used. The zones of inhibition were read after 18-24hours incubation at 37°C. Interpretation of results was carried out according to the manual instructions.

The strip was applied to the agar plates within 15 minutes of inoculation to avoid pre-growth of the organism. Using sterile forceps, the strip was removed from the sachet by handling the end with the logo and antibiotic code. The strip was placed with the scale facing upwards, and the antibiotic gradient downwards in contact with the agar. The strip was applied by putting the end with the lowest concentration onto the agar plate first and then carefully rolling the strip on to the agar to ensure good contact with the entire length of the Minimum Inhibitory Concentration Evaluator Strip. After applying strip onto the agar, the plates were incubated at inverted position at 37°C for 18-24hours after which zones of inhibition were measured.

3.2.15 Molecular Analysis

Molecular analysis of 12 isolates selected on the basis of their being Methicillin Resistant Phenotypically was carried out at the Nigerian Defence Academy Biotechnology Laboratory, Afaka, Mando, Kaduna. The isolates were analyzed for carriage of *mecA,* gene the genetic determinant of methicillin resistance, 16SrRNA, *tetK*, *tetL*, *tetM*, *tetO*, and *bla*$_Z$, genes respectively.

3.2.15.1 Bacterial Cell Preparation

The preparation of the bacteria cell was carried out using the method described by Dubey (2009); The isolates used were those more resistant to the tested antibiotics. Pure colonies from the culture on Muller Hinton Agar were inoculated into 5ml Luria and Bertani (LB) broth and incubated overnight at 37°C for 24h. The cells were harvested by centrifugation at 4°C, 8000 rpm (6800 ×g) in a micro-centrifuge for 2 minutes at room temperature in an Eppendorf's tube, the supernatant was discarded and cells harvested. The step was repeated for higher yield of cells (Lephoto and Gray, 2013).

3.2.15.2 DNA Extraction

Genomic DNA extraction was carried out using method described by Zymo Research Protocol (Lephoto and Gray, 2013). The harvested cell pellets were dislodged and 200µl of deionized water was added and mixed thoroughly by vortexing. Exactly 400µl of the lysis solution was added to the mixture and mixed thoroughly. The mixture was further incubated at 70^{o}C for 15 minutes until the cells were completely lysed and appearing viscous to prevent clogging of the zymo-spin column. Exactly 400µl supernatant was transferred to a zymo-spin™ IV spin filtered in collection tube and centrifuged at 7000 rpm for 1 minute. About 1200µl of DNA binding buffers was added to the filtrate in the collection tube from the preceding step. Exactly 800µl of the mixture from step the above was transferred to a zymo spin IIC Column in a new collection tube and centrifuged at 10000 ×g for 1 minute. The flow through in the collection tube was discarded and the step above repeated. A measure of 200µl DNA pre-wash buffer was added to zymo spin column in a new collection tube and centrifuged at 10000 ×g for 1 minute. About 500µl of DNA wash buffer was added to zymo spin column and centrifuged 10000 ×g for 1 minute. The zymo spin was transferred to a clean 1.5ml micro centrifuge tube and 100µl DNA elution buffer added directly to the column matrix and centrifuge at 10000 ×g for 1 minute to elude the DNA.

3.2.15.3 Detection of Antibiotic Resistance Gene

Detection of *mecA, tetM, tetK, tet*O, *tet*L, and *blaZ,* was carried out using PCR with the aid of their respective primers.

PCR was performed with the following thermal settings: 5 min at 94°C for initial enzyme activation followed by 40 cycles of amplification consisting of denaturing at 94°C for 30seconds for *mecA* and 1 minute for tetK. *tetL, tetM. tetO, bla$_{Z}$,* genes annealing at 57°C for 45seconds for

mecA, and at 55°C for 1 minute for *tetK. tetL, tetM. tetO, blaZ,* genes and extension at 72°C for 30seconds for *mecA* and 2minutes *for tetK. tetL, tetM. tetO, blaZ,* genes with a final extension at 72°C for 5minutes (DNeasy Blood and Tissue Handbook 2006)

3.2.16 Agarose Gel Electrophoresis and Polymerase Chain Reaction (PCR)Amplification of Genomic DNA

Amplification of antibiotic resistant genes was carried out using PCR after external optimization of the reaction to ensure a better amplification. The following process was carried out; The thin-walled PCR tubes were marked and the following components were added for each isolate for single reaction of 50µl Viz: 25µl of Dream Taq™ PCR master mix was added in the PCR tube, 1.0µl of forward primer, 1.0 of reverse primer, 7.0µl of temple DNA (genomic DNA), nuclease-free water (16µl) was added in the PCR tube to make up a total volume of 50µl. The samples were spun down as PCR is performed using the thermal cycling conditions as stated by Zymo Research UK.

CHAPTER FOUR

RESULTS

4.1 Identification and Purification of Swab Samples obtained from the various Farms and Sample sites

Three-hundred (300) swab samples were sub-cultured, one-hundred and ninety-eight (198) were Gram positive, they were further cultured on Mannitol Salt agar (MSA) plates, one hundred and sixty-five (165) showed characterized growth on the MSA plates. Further biochemical tests were carried out on the one hundred and sixty-five (165) positives on Mannitol salt agar plate all tested isolates were catalase positive. The coagulase test was carried out for further identification which showed a total of Ninety-five (95) were positive for coagulase.

Forty six (46) *S. aureus* were identified while Forty-nine (49) were coagulase negative broken into *S.xylosus* (27), *S.chromogenes* (8), *S.intermedius* (6), *S,hyicus* (4), and *S, hominis* was identified using the Microgen Staph-Identification Kit are shown in table 4.1.

Table 4.1: Identification and purification of *Staphylococcus* isolates (n= 300)

Test	**Observation**	**No. of Positive Isolates (%)**	**Inference**
Gram staining	Gram Positive cocci	198(66)	*Staphylococcus* spp/ *Micrococcus* spp/ *Streptococcus* spp
Culture on MSA	Distinct yellow growth	165(55)	*Staphylococcus* spp
Catalase	bubbles	165(55)	*Staphylococcus* spp
Coagulase	Clumping	95(57.5)	*Staphylococcus* spp
Microgen Staph^{ID}		46(48.4)	*Staphylococcus aureus*
		27(28.4)	*Staphylococcus xylosus*
		8(8.4)	*Staphylococcus chromogenes*
		6(6.3)	*Staphylococcus intermedius*
		4(4.2)	*Staphylococcus hominis*
		4(4.2)	*Staphylococcus hyicus*

Keys:

N= Total number of samples collected

4.2 Incidence of Coagulase Negative *Staphylococcus* (CoNS) among Humans, Pigs and Dogs according to Location

From the distribution of the CONs in the studied areas, a total of 49 different species of CONs were obtained, 22 from Buwaya Community (BGG), 14 from Maraban-rido (MR) and 13 from Samaru Zaria (SZ) respectively. Of the 49 CONs tested, 27 were S. *xylosus* (55.1%), *S. chromogens* 8(16.3%), *S. hominis* 4 (8.2 %), *S. hyicus* 4 (8.2 %) *S. intermedius* 6(12.2%). The distribution is as shown in table 4.2.

Table 4.2: Incidence of Coagulase Negative *Staphylococcus* (CONs) among Human, Pigs and Dogs according to Location

Staphylococcus **Species**	**BGG (%)** **N= 22**	**MR (%)** **N= 14**	**SZ (%)** **N= 13**	**Total** **N= 49**
S. xylosus	10(45.45)	8(57.14)	9(69.2)	27(55.1)
S. chromogens	3(13.63)	4(28.57)	1(7.69)	8(16.3)
S. intermedius	2(9.09)	2(14.28)	2(15.38)	6(12.2)
S. hominis	4(18.18)	0(0)	0(0)	4(8.2)
S. hyicus	3(13.63)	0(0)	1(7.69)	4(8.2)
Total CONs	22 (44.89%)	14 (28.57%)	13(25.58%)	49(100)

KEY:
BGG= Buwaya Gonin-Gora
MR= Maraban-rido,
SZ= Samaru Zaria
N= Total number of Coagulase Negative *Staphylococcus* spp from sampled locations

4.3 Distribution and occurrence of Coagulase negative *Staphylococcus* (CONs) isolates by source.

The sources of the CONs obtained in the study are shown in Table 4.3. Other isolates found to be Gram negative organisms were discarded.

Table 4.3: Distribution of Coagulase Negative Staphylococci (CONs) among Humans, Pigs and Dogs

	Humans (%)	**Pigs (%)**	**Dogs (%)**	**Total**
S. xylosus	4(30.76)	17(77.27)	6(42.85)	27(55.1)
S. chromogenes	3(23.07)	2(9.09)	3(21.42)	8(16.3)
S.intermedius	6(46.15)	0(0)	0(0)	6(12.2)
S. hominis	0(0)	0(0)	4(28.57)	4(8.2)
S.hyicus	0(0)	3(13.63)	1(7.14)	4(8.2)
Total	13(26.53%)	22 (44.89%)	14(28.57%)	49(100)

4.4 Occurrence of *Staphylococcus aureus* among the collected samples from the Swabs of the Animals in Zaria and Kaduna.

The occurrence and distribution of *Staphylococcus aureus* among the swabs samples collected from animals in Zaria and Kaduna are shown in table 4.4: Out of the total 46(100%) *Staph. aureus* samples obtained 20(43.5%) were obtained from Buwaya Gonin-Gora, 14(30.4%) from Maraban-rido while 12(26.1%) were obtained from Samaru Zaria (Table 4.4).

The skin of the animals had the highest occurrence 20(43.5%), the anterior nares and anus had occurrence of 10(21.7%) and 9(19.6%) respectively while the handlers palm had least occurrence of 7(15.2%) (Table 4.4).

Table 4.4:Occurrence and Distribution of *Staphylococcus aureus* from samples obtained from Animals and their Handlers in Zaria and Kaduna Metropolis

Location	Prevalence (%)	Source		Number (%)
Buwaya Gonin-Gora	20(43.5)	Skin	Pig	5(20)
			Dog	4(25)
		Nose	Pig	2(10)
			Dog	2(10)
		Anus	Pig	2(10)
			Dog	1(5)
		Handlers		4(20)
Maraban-rido	14(30.4)	Skin	Pig	3(21.43)
			Dog	3(21.43)
		Nose	Pig	1(7.14)
			Dog	2(14.28)
		Anus	Pig	1(7.14)
			Dog	2(14.28)
		Handlers		2(14.28)
Samaru Zaria	12(26.1)	Skin	Pig	4(33.33)
			Dog	1(8.33)
		Nose	Pig	3(25)
			Dog	-
		Anus	Pig	3(25)
			Dog	-
		Handlers		1(8.33)
Prevalence	46(100%)			

4.5 Antibiotic susceptibility

4.5.1 Antibiotic susceptibility profile of *Staphylococcus aureus* isolates

The antibiotic susceptibility of *Staphylococcus aureus* isolates are shown in Table 4.5. A high percentage of the isolates were resistant to Cefoxitin (91%), followed by Amoxicillin-clavulanic acid (74%), Gentamicin (67%), Tetracycline (65%), Ciprofloxacin (43%), Erythromycin (37%), Clindamycin (37%), Vancomycin (26%), Trimethoprim-sulfamethoxazole (20%), while the least resistance percentage was with Quinupristin-Dalfoprisitin (2%)

Table 4.5: Antibiotic Susceptibility Profile of *Stapylococcus aureus* isolates

S/NO	ANTIBIOTIC/Disc Potency (µg) N=46	SENSITIVE No(%)	RESISTANT No(%)
1	Linezolid(15µg)	32(70)	14(30)
2	Trimethoprim-sulfamethoxazole (1.25µg+23.75µg)	37(80)	9(20)
3	Tetracycline (30 µg)	16(35)	30(65)
4	Cefoxitin(30 µg)	4(9)	42(91)
5	Ciprofloxacin(5µg)	26(57)	20(43)
6	Gentamicin(30µg)	15(33)	31(67)
7	Erythromycin(15 µg)	29(63)	17(37)
8	Clindamycin(2 µg)	29(63)	17(37)
9	Chloramphenicol(30 µg)	42(91)	4(9)
10	Quinupristin-Dalfopristin (15µg)	45(98)	1(2)
11	Amoxicillin-clavulanic acid (30µg)	12(26)	34(74)
12	Vancomycin (30µg)	34(74)	12(26)

4.5.2 Antibiotic Resistance profile of *Staphylococcus* isolates

Generally, the coagulase negative *Staphylococcus* isolates were susceptible to chloramphenicol (97.9%), Quinupristin-Dalfoprisitin (97.9%), vancomycin (90%), erythromycin (71.4%), clindamycin (71.4%) and linezolid (69.4%) while they were least susceptible to cefoxitin (30.6%) (Table 4.6)

Table 4.6: Antibiotic Susceptibility profile of Coagulase Negative (CONs) *Staphylococcus* isolates

S/NO	ANTIBIOTIC/Disc Potency (µg) N=49	SENSITIVE No (%)	RESISTANT No (%)
1	Linezolid(15µg)	34(69.4)	15(30.6)
2	Trimethoprim-sulfamethoxazole (1.25µg+23.75µg)	35(71.4)	14(28.6)
3	Tetracycline (30 µg)	32(65.3)	17(34.7)
4	Cefoxitin (30 µg)	15(30.6)	34(69.4)
5	Ciprofloxacin(5µg)	29(59.2)	20(40.8)
6	Gentamicin (30µg)	47(96)	2(4)
7	Erythromycin (15 µg)	35(71.4)	14(28.6)
8	Clindamycin (2 µg)	35(71.4)	14(28.6)
9	Chloramphenicol (30 µg)	48(97.9)	1(2)
10	Quinupristin-Dalfoprisitin (15µg)	48(97.9)	1(2)
11	Amoxicillin-clavulanic acid (30µg)	26(53)	23(47)
12	Vancomycin (30µg)	44(90)	5(10)

4.5.3 Antibiotic Resistance profile of Coagulase Negative *Staphylococcus* (CONs) isolates

The antibiotic resistance profile of coagulase negative *Staphylococcus i*solates are presented in table 4.7. Out of the 12 antibiotic tested, the coagulase negative *Staphylococcus* were more resistance to Cefoxitin (63-100% resistance), Tetracycline (15-75%), Trimethoprim-sulfamethoxazole (15-41%), Linezolid (15-37%), Ciprofloxacin (15-52%) and Amoxicillin-clavulanic Acid (15-52%). Only *Staphylococcus xylosus* (7%) showed resistance to Gentamicin. Thirty-seven percent (37%) of *Staphylococcus xylosus* and *Staphylococcus chromogenes,* and 15% *Staphylococcus hyicus* were resistant to Erythromycin and Clindamycin. Among the organism tested, *Staphylococcus hominis* was the most susceptible with susceptibility to 7 out of the 12 antibiotics tested, followed by *Staphylococcus chromogenes* and *Staphylococcus intermedius* with susceptibility to 5 and 4 antibiotic respectively.

Table 4.7: Antibiotic Resistance Profile of Coagulase Negative *Staphylococcus* (CONs) isolates

***Staph* isolates**	**Antibiotic Resistance (%)**											
	LZD	**SXT**	**TE**	**FOX**	**CIP**	**CN**	**E**	**DA**	**CH**	**QD**	**AMC**	**VAN**
Staphylococcus xylosus **N=27**	30	41	48	63	52	7	37	37	2	0	52	14.8
Staphylococcus hyicus **N= 4**	15	0	75	100	15	0	15	15	0	23	50	0
Staphylococcus intermedius **N= 6**	33	17	33	67	15	0	0	0	0	17	50	17
Staphylococcus hominis **N= 4**	15	0	15	75	0	0	0	0	15	0	15	0
Staphylococcus chromogenes **N= 8**	37	15	0	75	37	0	37	37	0	0	37	0

Key: LZD= Linezolid SXT=Trimethoprim-sulfamethoxazole; TE= Tetracycline FOX= Cefoxitin CIP= Ciprofloxacin; CN=Gentamicin; E=Erythromycin; DA=Clindamycin; CH=Chloramphenicol; QD=Quinupristin-Dalfoprisitin ; AMC=Amoxicillin-clavulanic Acid; VAN=Vancomycin

4.6 Multiple Antibiotic Resistance (MAR) Index of Isolates

The multiple antibiotic resistant index for each of the 95 isolates were determined as:

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

As shown in Table 4.8

Table 4.8: Multiple Antibiotic Resistance (MAR) Index of Isolates

Number of Antibiotic to which resistant	MARI	No. of isolates (%)
1	0.08	5(5.26)
2	0.16	5(5.26)
3	0.25	20(21.05)
4	0.33	9(9.47)
5	0.42	27(28.42)
6	0.5	14(14.73)
7	0.58	6(6.31)
8	0.66	2(2.10)
9	0.75	1(1.05)
TOTAL	95	93.65

62.08

4.7 Phenotypic occurrence of MRSA among *Staphylococcus aureus* isolates

The distribution of Methicillin Resistant *Staphylococcus aureus* (MRSA) among *Staph. aureus* isolates are shown in table 4.9. An area was considered positive for MRSA if the isolates from the four types of samples (human palms, animal skin, nose, and anus) were resistant to cefoxitin antibiotic.

The farms used in the study were all positive for MRSA. A total of forty-two (42) isolates out of the 46 *Staphylococcus aureus* isolates were positive for MRSA (91.3%). MRSA-positive isolates in skin, nose, anus and human palms, were 47.6% (20/42), 23.8% (10/42), 19.1% (8/42) and 9.5% (4/42), respectively.

Table 4.9: Phenotypic occurrence of MRSA among *Staphylococcus aureus* Isolates

S/NO	Source	Number (%)
1	Skin	20(47.6)
2	Nose	10(23.8)
3	Anus	8(19.1)
4	Human palms	4(9.5)
	TOTAL	42(100)

4.8 Molecular Characterization of Antibiotic Resistance Genes in Methicillin Resistant *Staphylococcus aureus* and *Staphylococcus xylosus* isolates

Seven (7) genes including: *Staph A* 30 (16S RNA), *mecA, tetM tetK, tetO, tetL,* and, bla_Z, were detected from *S. aureus* and Five (5) including *Staph A* 30 (16S RNA), *tetK, tetO, tetL,* bla_Z were detected from *S. xylosus* isolates by polymerase chain reaction (Table 4.10).

Table 4.10: Molecular characterization of Antibiotic Resistance Genes in Methicillin Resistant *Staphylococcus aureus* and *Staphylococcus xylosus* isolates

	Isolate codes	*Staph A* 30 (16rRNA)	*mecA*	*tetM*	*tetK*	*tetO*	*tetL*	*blaZ*
S. *aureus*	PN17	+	+	-	+	+	-	+
	PA6	+	+	-	+	+	-	-
	PS40	+	+	-	-	-	-	-
	DS29	+	+	-	-	-	+	-
	PS30	+	+	-	-	+	-	-
	PA10	+	+	-	+	+	-	-
	HGN2	+	+	-	+	+	-	-
S.xylosus	PA12	+	-	-	_	+	-	-
	PN2	+	-	-	+	+	+	+
	PA18	+	-	-	+	+	-	+
	$PHF8_M$	+	-	-	-	+	+	+
	DS22	+	-	-	+	+	-	+

Key:

PS= Pig skin ; PN= Pig nose; PA= Pig Anus

DS= Dog skin; DN= Dog Nose.

PHF= Pig handler hand Female

HGN= Handler hand gonin-gora

4.9 Molecular Characterization of Antibiotic Resistance Genes in *Staphylococcus aureus*

All the isolates (S. *aureus* PN17; PA6; PS40; HGN2; DS29; PA10; and PS30, *S. xylosus* PN10; PA12; PA18; DS22; $PHF8_M$) tested harbored the *Staph* A30 genes. The *Staph A* 30 (16S RNA) was identified at 301bp confirming that all isolates are *Staphylococcus* spp (Plate I)

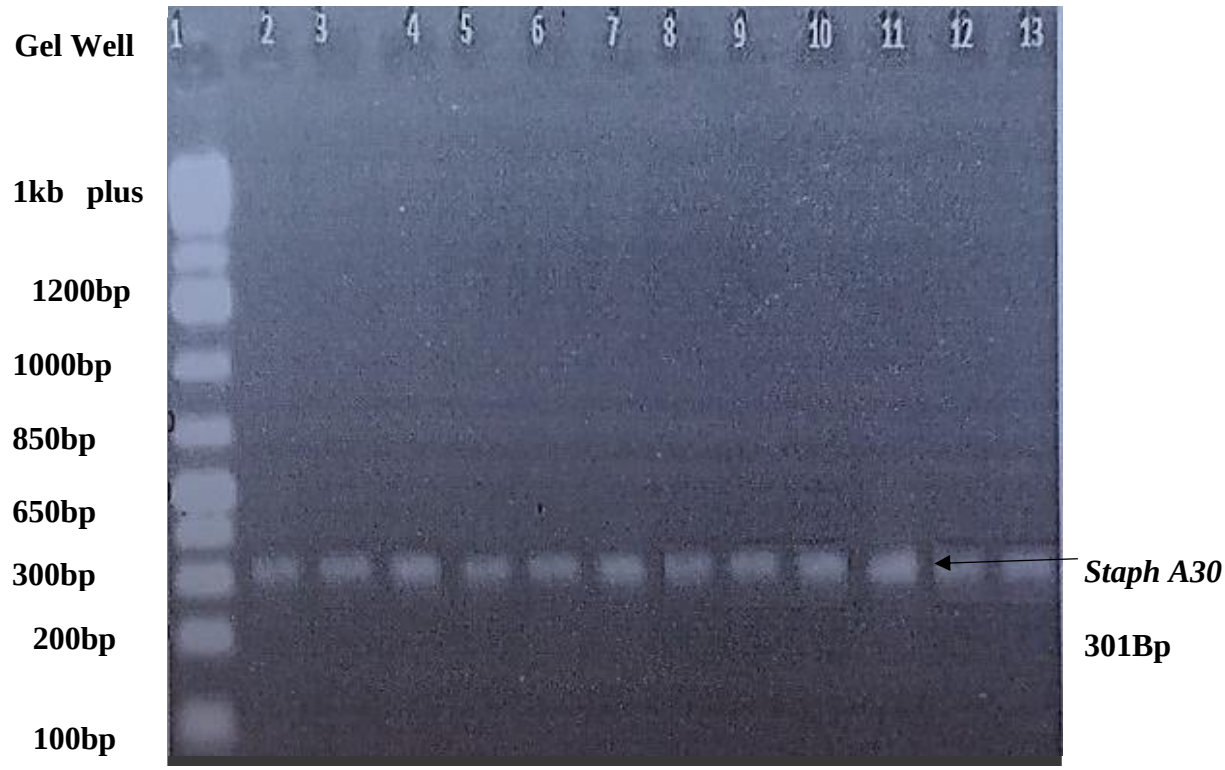

Plate I: Isolation of 16SRNa (301bp) amplified from *Staphylococcus* Isolates.

Keys:
Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6*S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.10 Molecular Characterization of mec*A* gene

Molecular detection of *mecA* gene revealed that only seven (7) out of twelve isolates showed carriage of mec*A* which was amplified at 532bp (Plate II). See Lanes 3, 4,9,10, 11,12, and 13.

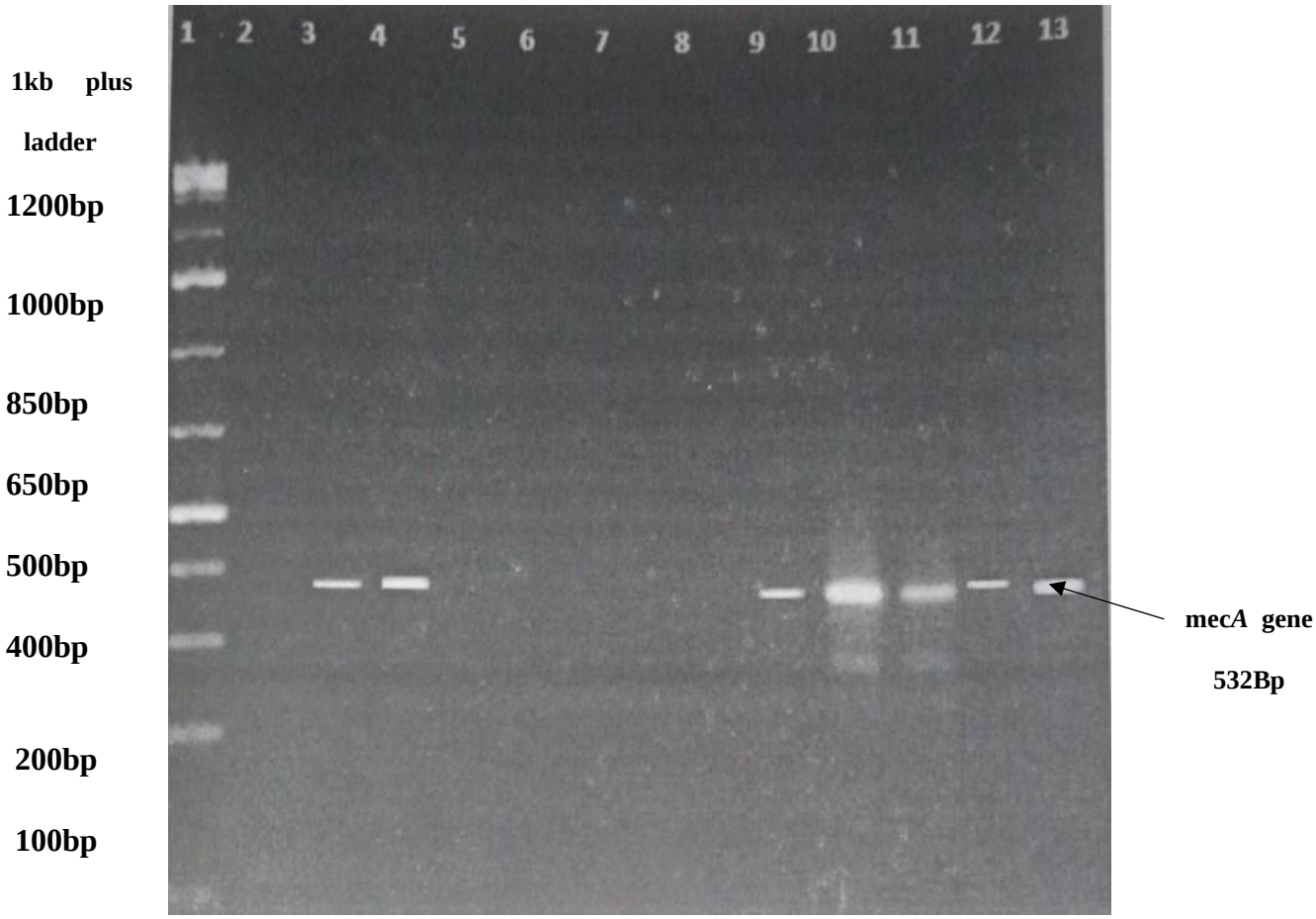

Plate II: Isolation and Amplification of *mecA* (532bp) from *Staphylococcus* Isolates

Keys:
Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.11 Molecular Characterization of *tetK* gene

Molecular Detection of *TetK* revealed that only seven (7) of the isolates amplified *tetK* gene (Plate III.) See lanes 3,4,5 6, 8, 9 and 10.

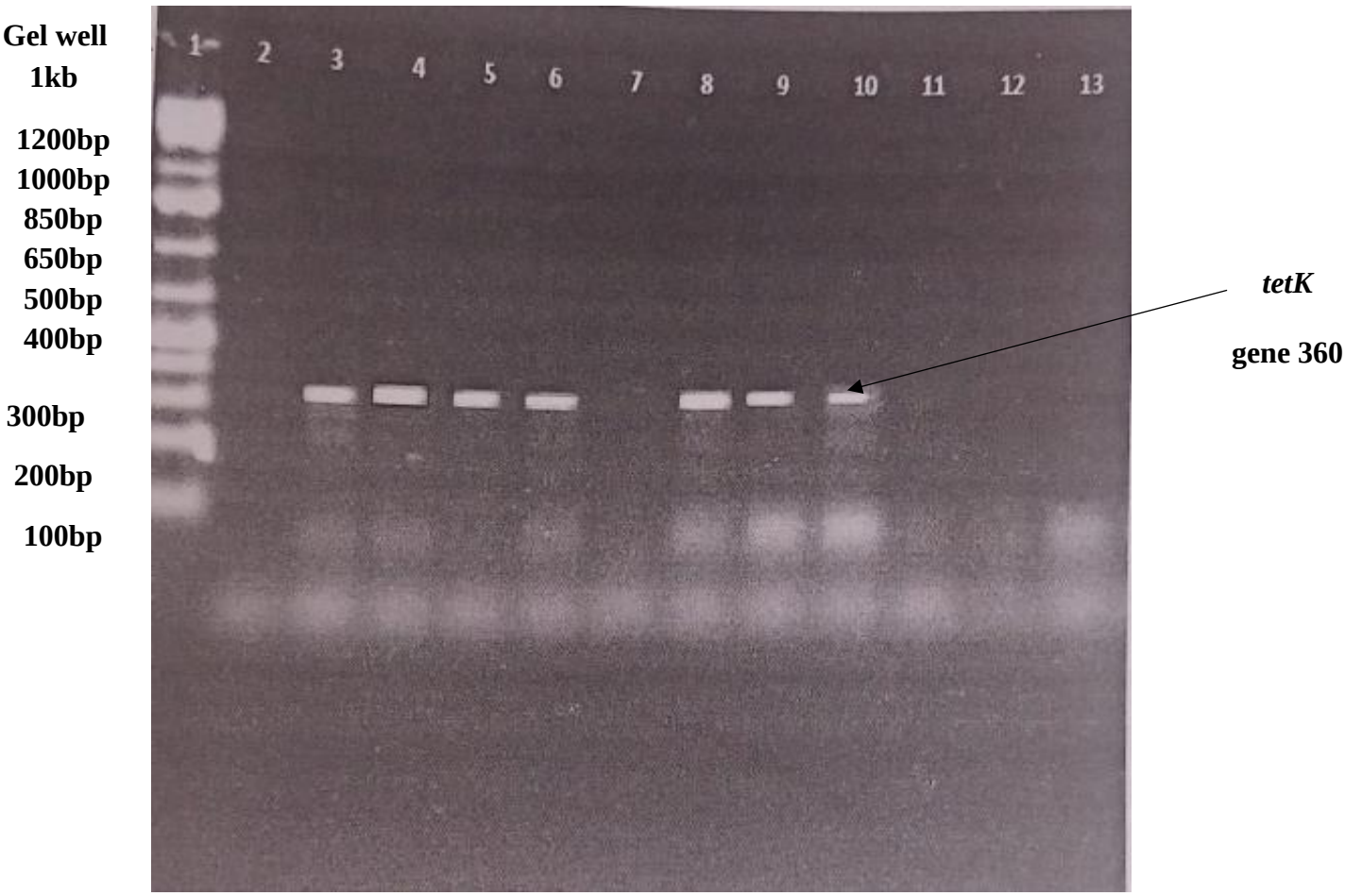

Plate III: Isolation and Amplification of *tetK* (360bp) from *Staphylococcus* isolates

Keys:

Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.12 Molecular Characterization of *tetL* Gene

Molecular detection of *tetL* gene showed that 3 of the isolates were positive for *tetL* gene (Plate IV) See Lanes 5,7, and 13.

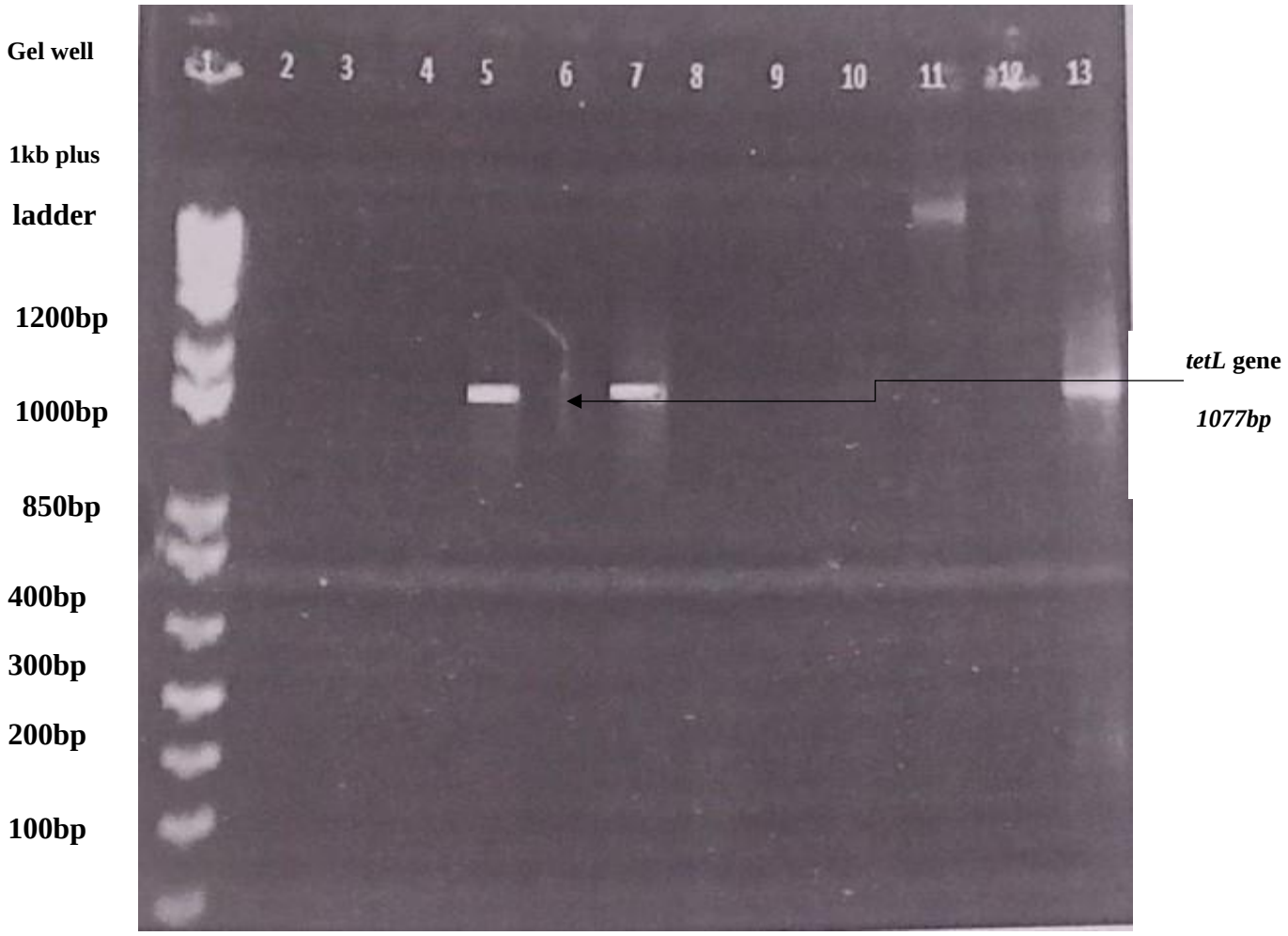

Plate IV: Isolation and Amplification of *tetL* (1077bp) gene from *Staphylococcus* Isolates

Keys:
Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.13 Molecular Characterization of *tetO* gene

Molecular detection of *TetO* gene showed that 10 of the isolates were positive for *tetO* gene (Plate V.) See Lanes 2,3,4, 5,6,7,8,9,10 and 12.

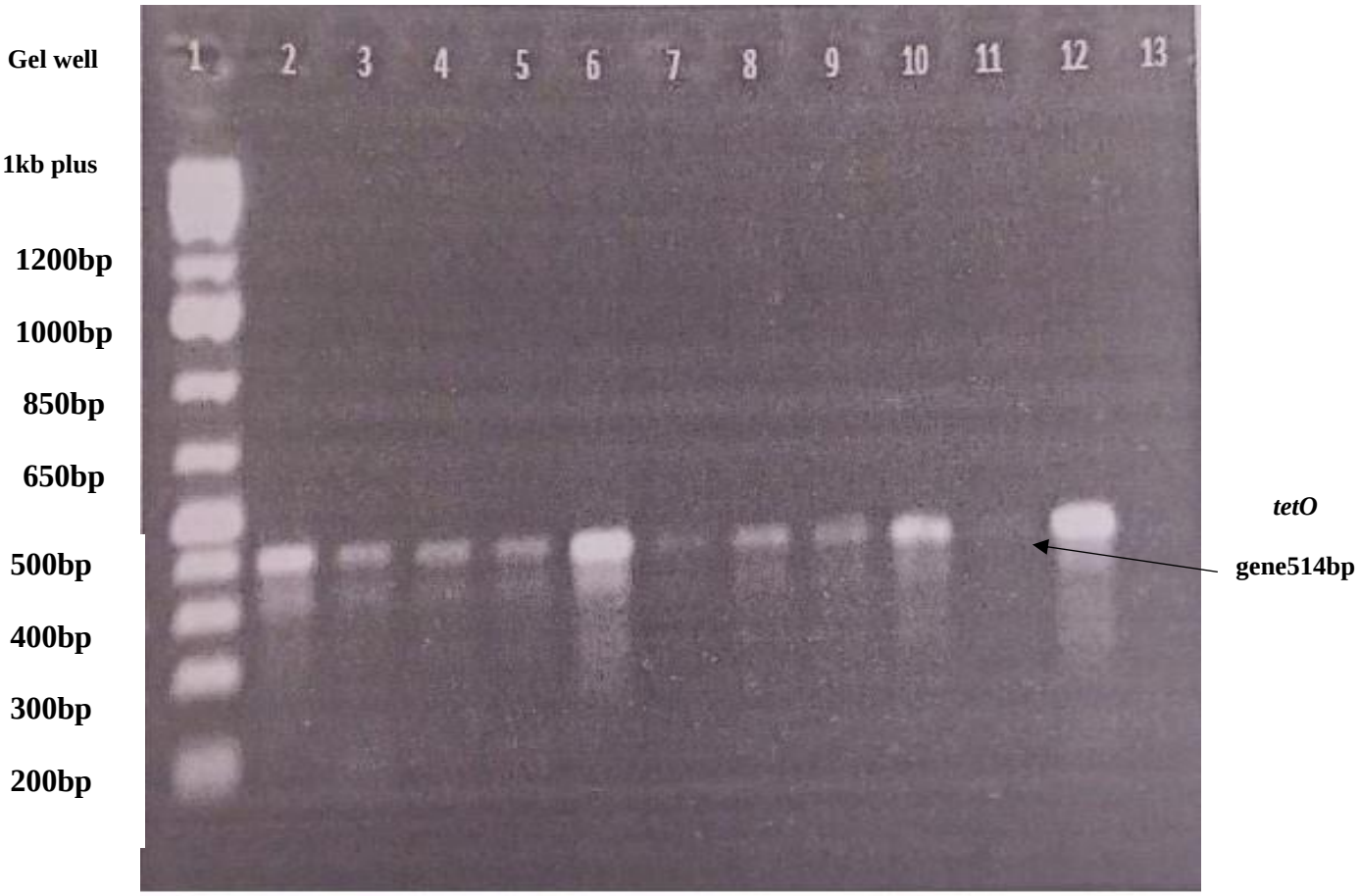

Plate V: Isolation and Amplification of *tetO* gene (514bp) from *Staphylococcus* Isolates

Keys:
Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.14 Molecular Characterization of *blaZ* gene

Molecular detection of *blaZ* gene showed that 5 of the isolates were positive for *blaZ* gene (Plate VI.) See Lanes4, 5,6,7 and 8.

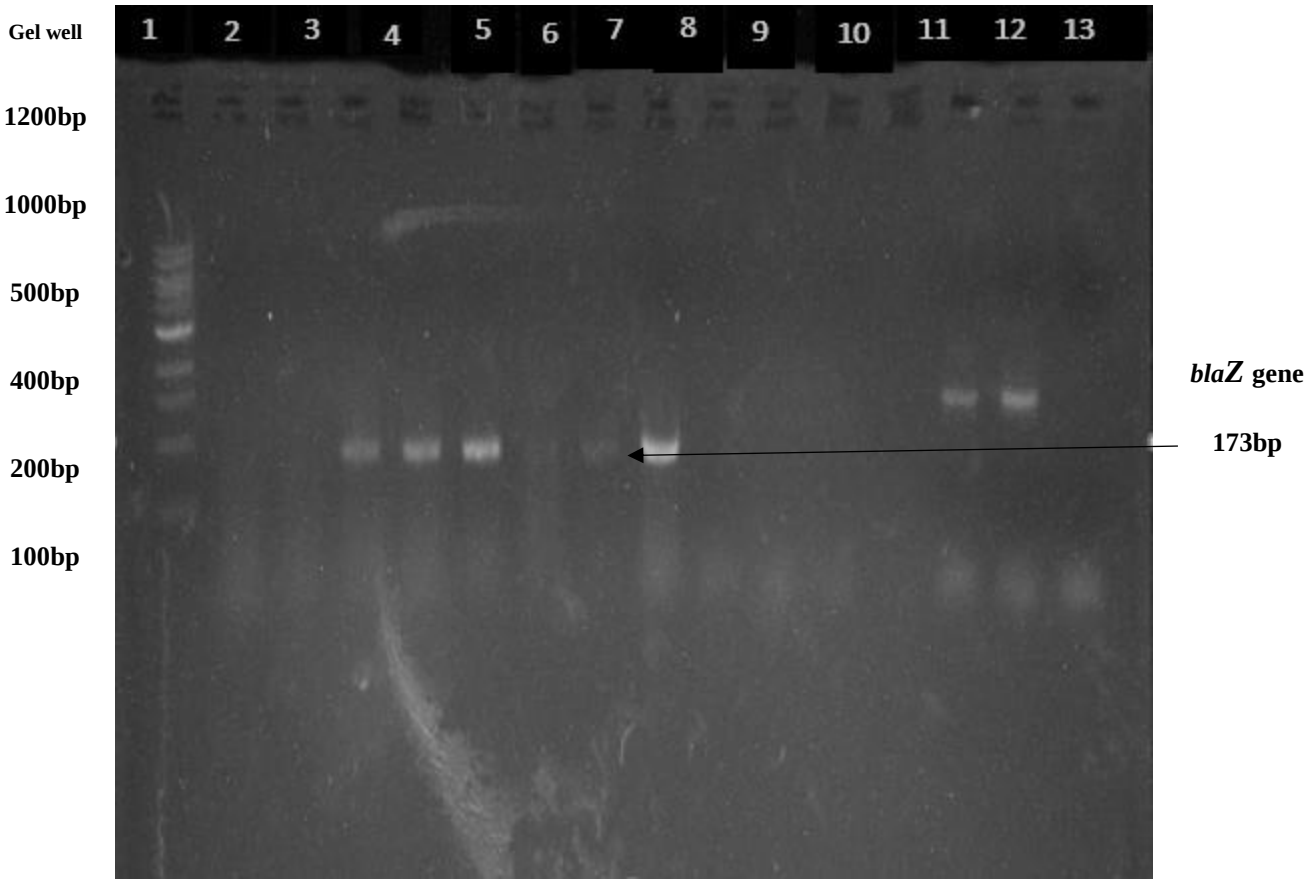

Plate VI: Isolation and Amplification of *blaZ* gene (173bp) from *Staphylococcus* Isolates

Keys:

Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

4.15 Molecular Characterization of *tetM* gene

Molecular detection of *TetM g*ene showed that none of the isolates were positive for tet*M* it goes to show that the resistance were only phenotypical gene (Plate VII)

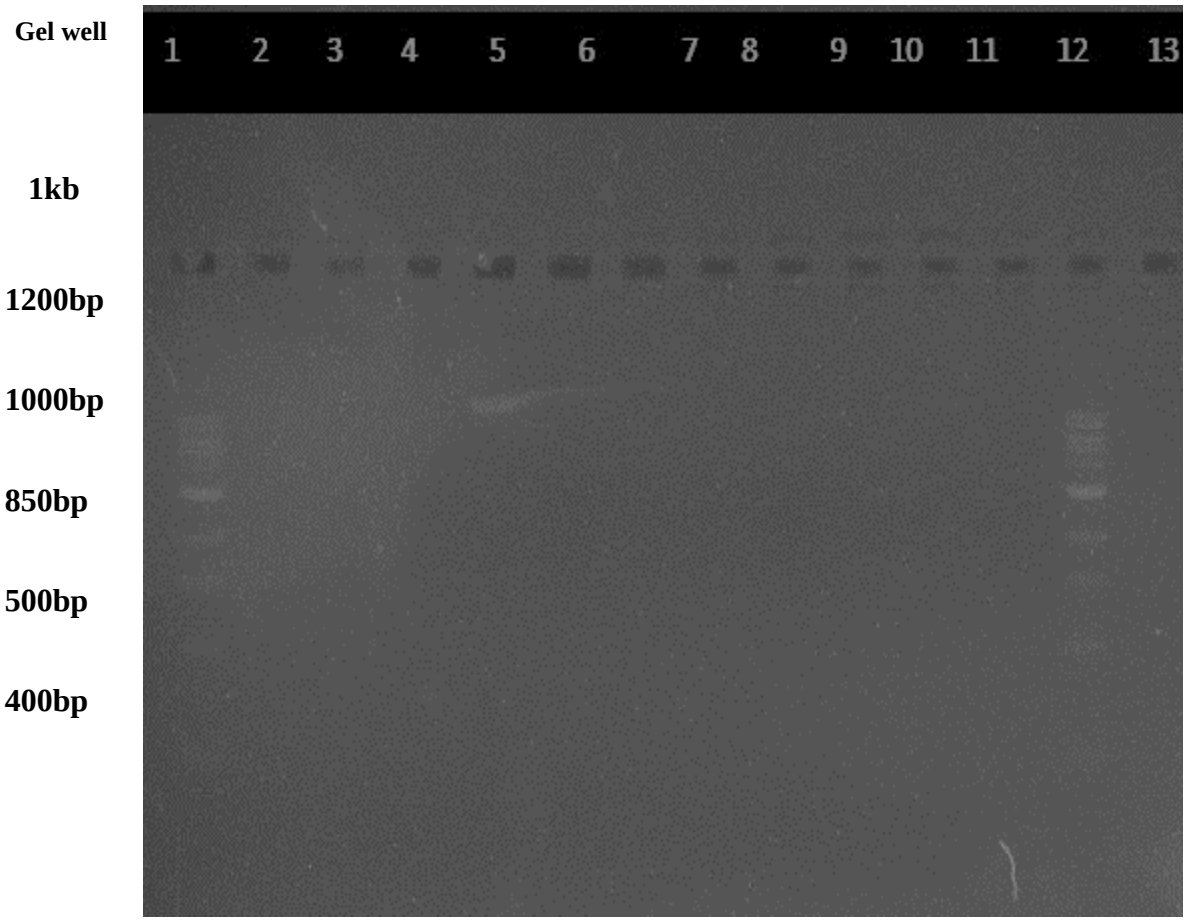

Plate VII: Isolation and Amplification of *tetM* gene(171bp) from *Staphylococcus Isolates*

Keys: Lane 1= 1kb DNA ladder,
Lane 2= PA12 *S.xylosus*
Lane 3= PA10 *S.aureus*
Lane 4= PN17 *S.aureus*
Lane 5= PN2 *S. xylosus*
Lane 6= PA18 *S.xylosus*
Lane 7= $PHF8_M$ *S.xylosus*
Lane 8= DS22 *S.xylosus*
Lane 9= PA6 *S. aureus*
Lane10= HGN2 *S.aureus*
Lane 11= PS40 *S.aureus*
Lane12= PS30 *S.aureus*
Lane13= DS29 *S.aureus*

CHAPTER FIVE

DISCUSSION

Staphylococcus aureus present in dogs, pigs and members of the household that own them in Buwaya, Gonin-gora, Maraban-rido area and Samaru Zaria all in Kaduna state, Nigeria was studied. This study was conducted in order to identify the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in pets i.e., dogs and farm animals i.e., pigs and its transmission to handlers.

The skin, nose, anus and palm swab samples were obtained from communities populated predominantly by farmers with little educational background and have the tendency of taking antibiotics without prescription. In all the households and pig farms where the study was conducted, the heads of the households admitted to self-medication with antibiotics.

This study reported species distribution, antimicrobial resistance (pheno-and genotypes) traits of *Staphylococcus* isolates isolated from the farm attendants, dogs and pigs in Kaduna, Nigeria. The epidemiology of *Staphylococcus* infections continued to evolve, with different characteristic patterns and associated clinical complications (Mai-siyama *et al.,* 2014). In this study, the *S. aureus* colonization rate was 48.4%(n=46) and 91.30%(n=42) MRSA isolates were phenotypically detected in dog and pig and also the palms of the farm attendants. The high MRSA colonization rate is different from the patterns reported in other studies by Alzohairy, (2011) and Gharsa *et al.,* (2012) who reported 57% (90) MRSA and 3% (5) MRSA colonization of their samples. This could be attributed to the fact that MRSA colonization rate differs with the animals sampled and geographical location. In study conducted in Saudi Arabia high MRSA colonization rate was recorded among camels (35.5%) and cattle (21.8%) while Maisi-yama *et al*. (2014) in Maiduguri reported the prevalence of 21.8% in cattle and 4.6% in sheep.

Furthermore, the MRSA prevalence rate of 44.8% were reported in France and 29% in Tunisia (Vautor *et al.,* 2005; Gharsa*et al,* 2012). The high level of contamination observed in this study could also be attributed to the poor and improper hygiene of the farm coupled with the unhygienic practices of the farmers.

Pigs and dogs constitute one of the important animals reared within the communities in the study area and an important source of animal proteins for the communities. Pigs were first reported as an animal reservoir of *S. aureus* in 2005 (Armand-Lefevre *et al.,* 2005). In Netherlands, 45% of Veterinarians attending pig farms were positive for MRSA (Cuny *et al.,* 2015) while In Belgium and Denmark, the prevalence of MRSA in Veterinarians was 9.5% and 1.4%, respectively (Garcia-Graells *et al.,* 2012). Apart from the fact that high MRSA colonization rate was recorded among the pigs and dogs in this study the prevalence of MRSA of 15.2% (7) *S. aureus* were isolated from the animals' handlers. This contamination occurs through the high level of contact with the animals through rearing and domestication. This is of public health concern because of the possible transmission and dissemination of the MRSA isolates within the community through nasal dropping during movement within the community and contamination of meat and milk products by colonized handlers (Mai-siyama *et al.,* 2014). Studies have reported that MRSA colonization of animals posed a potential risk of up to 60% transmission to the contact persons (Juhasz- Kaszanyitzky *et al.,* 2007; Lee, 2018; Bertelloni *et al.,* 2021)

The level of MRSA colonization rate among contact persons varied with geographic location, type of animals and culture methods employed in the studies (Vanderhaeghen *et al.,* 2010; Graveland *et al.*, 2011). In this study, the MRSA colonization rate among contact persons was 9.5% (4) this colonization rate is in comparison to reports in other studies; 3.4% reported by Maisi-yama *et al*. (2014), 7.1% reported in Asia by Chuang and Huang (2015). 6.5% reported

among Veterinary personnel by Haenni *et al.,* (2017), 16% among veterinarian handling large animals and 4.4% among those handling small animals (O'Mahony *et al.,* 2005;2000; Hanselman *et al.,* 2006; IB *et al.,* 2014).

The number of studies focused on the antibiotic resistance problem in the African Continent has grown in last decade and they suggest that in this continent, as in other parts of the world, this problem is increasing; however, its real extent is currently unknown since surveillance of drug resistance is only carried out in a few countries (WHO, 2015). The misuse of antibiotics due to poor control policies is promoting this resistance development (Kimang'a, 2012). Despite limited resources, during the last few in many of these countries for example Kenya, South Africa, Nigeria, there are important efforts to establish good control measures to avoid this worrisome situation (Bosco *et al.,* 2015). The present study showed the resistance of *S. aureus* to amoxicillin (74%), tetracycline (65%), and cefoxitin (91%) indicating increasing antimicrobial resistance which will necessitate the need for new discoveries and development of new antibiotic therapy and treatment. This is in accordance with the findings of Abebe *et al*. (2013) who reported resistance of *S. aureus* to tetracycline (73.8%) in farm animals around Addis Ababa, Reddy *et al.,* (2016) also reported resistance to Cefoxitin (100%), Amoxicillin (83.3%), Gentamicin (67%), from dogs in India.

The present study has also demonstrated the existence of alarming level of resistance of *S. aureus* to commonly used antimicrobials (cefoxitin, amoxicillin-clavulanic acid and tetracycline) in the study farms. The results are in similarity with reports from earlier studies in other countries in India by Yadav *et al.,* (2018), also in Thailand by Gentilini *et al* (2016) and Italy by Bertelloni *et al.,* (2021) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials.

Resistance to cefoxitin, amoxicillin-clavulanic acid and tetracycline are great of concern since these antibiotics represents the main antibiotic groups recommended for Staphylococcal infection. In pigs and poultry production in Nigeria, these drugs are commonly used for growth promotion as well as for disease prevention and control (Okorie-Kanu *et al.*, 2020).

The regular use of antibiotics for the treatment of farm animals may result in the spread of resistant strains. Antibiotic resistance is carried on plasmids and transposons which can pass from one bacteria species to another (Hulya *et al.,* 2006). The resistance of *S. aureus* to cefoxitin, amoxicillin-clavulanic acid and tetracycline may be attributed to the production of beta lactamase enzyme that inactivates the antibiotics. Similar suggestion was given by Jaims *et al*. (2016) that the development of antimicrobial resistance is nearly always as a result of repeated therapeutic and/or indiscriminate use of them.

The resistance of *S. aureus* isolates to beta-lactams such as cefoxitin, amoxicillin and tetracycline was evident. High percentage of *S. aureus* resistant to Cefoxitin (91%), Amoxicillin-clavulanic acid (74%), and Tetracycline (65%) in the present study is in line with the findings of Derese *et al*. (2012) who reported that high percentage of *S. aureus* isolates were resistant to penicillin G, amoxacilin, tetracycline, cefoxitin, and to some extent streptomycin. The high resistance to Amoxicillin-clavulanic acid (74%) next to amoxicillin is an indicator of MRSA (Daka *et al.,* 2012). This is also in line with the study of Clifford *et al.,* (2015) who reported that all (100%) cefoxitin resistant *S. aureus* isolates were also resistant to Oxacillin. In the present investigation it was proved that high resistance was observed in *S. aureus* isolates from the skin of the animals than in the nose and anus, suggesting that pig and dog skin are more contaminated with *S. aureus* than the animal's nose and anus because the skin is a normal microflora where

S.aureus is found and isolated. The pig and dog skin therefore constitute a higher risk in the transmission of *S. aureus* to the public than pig nose and anus.

Out of the 12 antibiotics tested, the coagulase negative *Staphylococcus* isolates were more resistance to cefoxitin (63-100% resistance), tetracycline (15-75%), trimethoprim-sulfamethoxazole (15-41%), linezolid (15-37%), ciprofloxacin (15-52%) and amoxicillin-clavulanic acid (15-52%).

There are several studies on molecular characterization of *S. aureus* isolated from pigs and raw pork, chickens and chicken meats, and human handlers (Mulders *et al.,* 2010; Stewart-Johnson *et al.,* 2019). In Nigeria, although live pigs, dog and poultry at slaughter houses or farms they are screened for MRSA (Abdulrahman *et al.,* 2018), and also phenotypic and genotypic detection of MRSA from these animals are used for identification. The Twelve *Staphylococcus* isolates tested for the presence *Staph A* 30 (16rRNA); All (100%) the isolates (S. *aureus* PN17; PA6; PS40; PS30; DS29; PA10; HGN2, *S.xylosus* PA12; PN2; $PHF8_M$; DS22 and PA18) harbored the *Staph* A30 genes. Similarly, most of the isolates also harbored Tetracycline, Gentamicin Ciprofloxacin and methicillin encoding resistance genes (*mecA, tetM, tetK, tetO, tetL, blaZ,*) in varying prevalence. These findings are in agreement with Ugwu *et al*., (2015); Yadav *et al.,* (2018); Okorie-kanu *et al.,* (2020); Chueahiran, *et al*.,(2021); Tanomsridachchai *et al.,* (2021) with the prevalence of multidrug resistance genes in S. *aureus* isolates, reports showed the presence of these genes obtained from pigs and companion animals in other countries, This study demonstrated both phenotypically and genetically the presence of MRSA in intensively reared pigs and dogs in the Kaduna agricultural zone, Nigeria. To the best of our knowledge this is the first joint report on MRSA in pigs and dog in North-west Nigeria. The study highlighted the fact that the *Staphylococcus* species isolated from pigs and dogs were MDR and, thus calls for public

health concern because of the health risk associated with colonization of individuals with these MDR strains.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

Approval for the Research was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) for the use of animals for this research. A total of three hundred (300) samples were collected from farms in the selected areas and were analyzed at the Postgraduate Pharmaceutical Microbiology Laboratory, Ahmadu Bello University Zaria, Nigeria.

A total of one hundred and sixty-five (165) were identified to be presumptive Staphylococci species after further analysis and identification using the Microgen Staph-ID kit. Ninety-five (95) were Staphylococci among which forty-six (46) were identified to be *Staphylococcus aureus*, forty-Nine (49) were identified to be other *Staphylococcus* species. Their exposure to antimicrobial susceptibility testing resulting in (90%) MDR and a total of (62.08%) isolates with MARI ≥0.3. The highest percentage for antibiotics resistance was found to be (91%) Cefoxitin for *Staphylococcus aureus* while the lowest was (2%). Quinupristin-Dalfoprisitin The highest percentage for other *Staphylococcus* species was also found to be Cefoxitin (69.4%) while the lowest was Chloramphenicol (2%).

Molecular characterization of identified *Staphylococcus aureus and Staphylococcus xylosus* showed amplification of *mecA* (58.3%) *tetK* (33%), *tetL* (8%), *tetO* (50%) and *BlaZ* (8.3%) for *S. aureus* isolates, amplification of *tetK* (25%), *tetL* (16.6%), *tetO* (50%), *blaZ* (33.3%) for *S. xylosus* while *tetM* did not amplify.

Conclusion

Conclusions made from this research are outlined below:

1. MRSA was isolated and identified from the skin, anterior-nares, rectal region of pigs dogs and palms of their handlers in Buwaya, Gonin-gora, Maraban-rido and Samaru-Zaria, Kaduna state Nigeria.
2. Antibiotic susceptibility and resistance ranging from 15% -70% susceptibility; 65-91% resistance was observed.
3. The presence of *mecA, tetK, tetL* and *tetO* genes were confirmed in the phenotypically resistant isolates.
4. The transmission is most likely animal-to-human as isolates from dogs and pigs' skin and anus are more compared to that of the handlers.
5. The occurrence of MRSA which are MDR and harboring some resistant genes identified in this study should be further investigated to understand their epidemiology in the population.

Recommendations

1. It is important to have a continuous antimicrobial resistance surveillance of antibiotics been dispensed to farmers pet owners to reduce and also to detect new strains and resistant genes.
2. A major control and surveillance program of antibiotic use in veterinary care is needed to reduce the presence of MRSA strains in livestock, as well as to control this significant multi-resistance increase with greater vigilance on the part of health authorities to comply with the strategies and plan of action established.

3. Awareness and prompt appropriate veterinary care seeking and intervention should be emphasized for prevention and management of drug resistance.
4. The use of phytochemicals in animal health care could be a better alternative to the use of antibiotics.

Contributions to Knowledge

1. The data and information generated from this study may serve as a baseline for future studies on *S. aureus* epidemiology among the study area and population.
2. The findings in this research can to the best of our knowledge also add to existing information and findings in other works to show that MRSA colonization is still prevailing and cannot be overemphasized.
3. The isolates obtained were found to be largely susceptible to Chloramphenicol, Vancomycin, Erythromycin, Gentamicin, Clindamycin and Linezolid which implies that, these drugs are still effective and should be used with caution in order to prevent more resistance in the study areas.
4. This study has also exposed the presence of methicillin resisant genes in the locality where methicillin antibiotics is rarely prescribed or administered.

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APPENDICES

Appendix I: Ethical Clearance

DIRECTORATE OF ACADEMIC PLANNING & MONITORING
AHMADU BELLO UNIVERSITY, ZARIA

Vice Chancellor *Prof. Ibrahim Garba*, B.Sc. (Hons) Geology, M.Sc. (Mineral Exploration) A.B.U., Ph.D Geology (London), D.I.C., FNMGS
Director: Prof. M.F. Ishiyaku, B.Sc. (Hons) Botany (ABU), M.Sc. Plant Breeding (UniJos), Ph.D Agriculture (University of Reading, U.K.), MASN, MBSN

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Appl No.: ABUCAUC/2019/ Pharmaceutics & Pharm. Microbiology /15 4th September, 2019
Approval No: ABUCAUC/2019/15

Prof. J.A. Onaolapo,
Department of Pharmaceutics & Pharm. Microbiology,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

APPROVAL OF RESEARCH STUDY 'MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS FROM DOGS, PIGS AND THEIR HANDLERS IN ZARIA METROPOLIS OF KADUNA STATE '

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Pharmaceutics and Pharmaceutical Microbiology. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

S.L. Usman
For: Chairman, ABUCAUC.

Cc. Director, DAPM
" Director, IC&ICT
" Head, Department of Pharmaceutics & Pharm. Microbiology
" Prof. C.A. Kudi, Chairman, ABUCAUC

Appendix II: EUCAST Interpretative Chart for Susceptibility Testing

ANTIBIOTIC	CODE	CONC.	SENSITIVE	INTERMEDIATE	RESISTANT
Linezolid	LZD	30 µg	≥ 21	-	≤ 21
Gentamicin	CN	10 µg	≥18	13-14	≤ 18
Tetracycline	TET	30 µg	≥ 22	15-18	≤ 19
Amoxicillin-clavulanic acid	AMC	30 µg	≥ 26	13-15	≤ 26
Ciprofloxacin	CIP	10µg	≥ 21	16-20	≤ 21
Trimethropin-sulfamethoxazole	SXT	25 µg	≥ 17	-	≤ 14
Chloramphenicol	CH	30 µg	≥ 18	13-17	≤ 18
Cefoxitin	FOX	30 µg	≥ 22	-	≤ 22
Erythromycin	E	15 µg	≥ 21	-	≤ 18
Climdamycin	DA	2µg	≥ 22	-	≤ 19
Quinupristin-dalfopristin	QD	15 µg	≥ 21	-	≤ 18

European Committee on Antimicrobial Susceptibility Testing (2019) Break point 9.0

Appendix III: McFARLAND Standard

1% V/V solution of Sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water. 1% w/v solution of barium chloride was prepared by distilling 0.5g of Dehydrated barium chloride in 50ml of distilled water 0.05ml of barium chloride and 9.95ml of Sulphuric acid was measured to make approximately a cell density of $1.5x10^8$ cfu/ml. (Cheesbrough 2002)

Appendix IV: PCR Cocktail Mix and Conditions

A: PCR COCKTAIL MIX

The DNA was subjected to the following cocktail mix and condition for PCR

10^X PCR buffer	1.0
25Mm $Mgcl^2$	1.0
5pMol forward primer	0.5
5pMol reverse primer	0.5
DMSO	1.0
2.5Mm DNTPs	0.8
Taq5u/ µl	0.1
10ng/µl DNA	2.0
H_2O	3.1
	10µl

B: PCR CONDITIONS

Initial Den	Den	Annnealing temp	Extension	No of Cycles	Final extension	Holding temperature
95°C	94°C	56°C	72°C	36	10°C	10°C
5min	30sec	30sec	45sec		7mins	∞

The amplicon from the reaction above was loaded on 1.5% agarose gel and gel picture is attached to as PCR. The ladder used is 1kbplus ladder from Invitrogen. The expected base pair of the amplicon is around 1500bp

APPENDIX V: Biochemical Characteristics of Presumptive *Staphylococcus* spp. Isolates

Isolate Code	Morphology on Agar		Gram Reaction	Catalase	Coagulase Production	Inference
	NA	MSA				
PA5	white	Yellow	+	+	+	*Staphylococcus xylosus*
PA6	cream	yellow	+	+	+	*Staphylococcus aureus*
PA7	Gold	Cream	+	+	-	*Staphylococcus aureus*
PA8	yellow	Cream	+	+	-	*Staphylococcus xylosus*
PA9	white	yellow	+	+	+	*Staphylococcus xylosus*
PA12	white	Gold	+	+	+	*Staphylococcus xylosus*
PA13	white	Cream	+	+	+	*Staphylococcus aureus*
PA14	Gold	yellow	+	+	+	*Staphylococcus xylosus*
PA16	white	Yellow	+	+	-	*Staphylococcus xylosus*
PA17	white	yellow	+	+	+	*Staphylococcus xylosus*
PA18	Gold	yellow	+	+	+	*Staphylococcus xylosus*
PA29	cream	yellow	+	+	-	*Staphylococcus hyicus*
PA32	white	yellow	+	+	+	*Staphylococcus aureus*
DA32	white	yellow	+	+	-	*Staphylococcus xylosus*
PA62	white	yellow	+	+	+	*Staphylococcus aureus*
PN1	white	yellow	+	+	+	*Staphylococcus aureus*
PN3	white	yellow	+	+	+	*Staphylococcus aureus*
FDA2	white	yellow	+	+	+	*Staphylococcus aureus*
PA4	white	yellow	+	+	+	*Staphylococcus aureus*
DA4	white	yellow	+	+	+	*Staphylococcus aureus*
PN20	cream	yellow	+	+	+	*Staphylococcus xylosus*
PN26	white	yellow	+	+	+	*Staphylococcus xylosus*

DN34	cream	yellow	+	+	+	*Staphylococcus aureus*
PN36	cream	Cream	+	+	+	*Staphylococcus aureus*
PN46	white	yellow	+	+	+	*Staphylococcus aureus*
DN4	white	yellow	+	+	+	*Staphylococcus aureus*
PN9	white	yellow	+	+	+	*Staphylococcus xylosus*
PN18	white	yellow	+	+	+	*Staphylococcus chromogenes*
PN11	white	yellow	+	+	+	*Staphylococcus xylosus*
PN12	white	yellow	+	+	+	*Staphylococcus xylosus*
PN13	white	yellow	+	+	-	*Staphylococcus xylosus*
PN15	white	yellow	+	+	+	*Staphylococcus aureus*
DN15	white	yellow	+	+	+	*Staphylococcus aureus*
PN17	white	yellow	+	+	+	*Staphylococcus aureus*
PN10	white	yellow	+	+	+	*Staphylococcus chromogenes*
HBN1	white	yellow	+	+	-	*Staphylococcus chromogenes*
HGN2	white	yellow	+	+	+	*Staphylococcus aureus*
PHF8m	white	yellow	+	+	+	*Staphylococcus xylosus*
PHF7	white	yellow	+	+	+	*Staphylococcus xylosus*
DHM9	white	yellow	+	+	+	*Staphylococcus aureus*
PHF9	white	yellow	+	+	+	*Staphylococcus xylosus*
DS9	white	yellow	+	+	+	*Staphylococcus aureus*
DS14	white	yellow	+	+	+	*Staphylococcus aureus*
DS22	white	yellow	+	+	+	*Staphylococcus xylosus*
DS29	white	yellow	+	+	+	*Staphylococcus aureus*
DN7	white	yellow	+	+	+	*Staphylococcus aureus*
PS34	white	yellow	+	+	+	*Staphylococcus aureus*
PS40	white	yellow	+	+	+	*Staphylococcus aureus*
PS39	white	yellow	+	+	+	*Staphylococcus aureus*

PS47	white	yellow	+	+	+	*Staphylococcus hominis*
PS48	white	yellow	+	+	+	*Staphylococcus aureus*
PS59	white	yellow	+	+	+	*Staphylococcus aureus*
PN2	white	yellow	+	+	+	*Staphylococcus xylosus*
PN3	white	yellow	+	+	+	*Staphylococcus xylosus*
PN4	white	yellow	+	+	+	*Staphylococcus xylosus*
PHF11	white	yellow	+	+	+	*Staphylococcus xylosus*
DS10	white	yellow	+	+	+	*Staphylococcus hominis*
10FD	white	yellow	+	+	+	*Staphylococcus aureus*
PN9	white	yellow	+	+	+	*Staphylococcus xylosus*
DS15	white	yellow	+	+	+	*Staphylococcus xylosus*
19GH	white	yellow	+	+	+	*Staphylococcus aureus*
21GD	white	yellow	+	+	+	*Staphylococcus aureus*
23GD	white	yellow	+	+	+	*Staphylococcus aureus*
24BH	white	yellow	+	+	+	*Staphylococcus aureus*
DN9	white	yellow	+	+	+	*Staphylococcus xylosus*
PN28	white	yellow	+	+	+	*Staphylococcus hyicus*
PS48	white	yellow	+	+	+	*Staphylococcus hominis*
DN19	white	yellow	+	+	+	*Staphylococcus chromogenes*
HBN3	white	yellow	+	+	+	*Staphylococcus chromogenes*
DHM19	white	yellow	+	+	+	*Staphylococcus intermedius*
DN23	white	yellow	+	+	+	*Staphylococcus chromogenes*
PS41	white	yellow	+	+	+	*Staphylococcus aureus*
DN26	white	yellow	+	+	+	*Staphylococcus aureus*
DA20	white	yellow	+	+	+	*Staphylococcus aureus*
DN16	white	yellow	+	+	+	*Staphylococcus hyicus*
DS34	white	yellow	+	+	+	*Staphylococcus aureus*

PHM11	white	yellow	+	+	+	*Staphylococcus xylosus*
28BH	white	yellow	+	+	+	*Staphylococcus aureus*
PA23	white	yellow	+	+	+	*Staphylococcus hyicus*
3CD	white	yellow	+	+	+	*Staphylococcus aureus*
5CD	white	yellow	+	+	+	*Staphylococcus aureus*
DHM23	white	yellow	+	+	+	*Staphylococcus intermedius*
PS30	white	yellow	+	+	+	*Staphylococcus aureus*
DHM14	white	yellow	+	+	+	*Staphylococcus intermedius*
27BH	white	yellow	+	+	+	*Staphylococcus aureus*
29BH	white	yellow	+	+	+	*Staphylococcus aureus*
DHM2	white	yellow	+	+	+	*Staphylococcus intermedius*
DS3	white	yellow	+	+	+	*Staphylococcus hominis*
17GH	white	yellow	+	+	+	*Staphylococcus aureus*
DN11	white	yellow	+	+	+	*Staphylococcus chromogenes*
33BH	white	yellow	+	+	+	*Staphylococcus aureus*
34BH	White	yellow	+	+	+	*Staphylococcus aureus*
PS33	White	yellow	+	+	+	*Staphylococcus aureus*
PS45	White	yellow	+	+	+	*Staphylococcus aureus*
HBN5	White	yellow	+	+	+	*Staphylococcus chromogenes*
PHM15	White	yellow	+	+	+	*Staphylococcus intermedius*
DHM26	White	yellow	+	+	+	*Staphylococcus intermedius*

Appendix VI: Antibiotics Susceptibility Profile

ISOLATE CODE	MARI	BACTERIA ISOLATES	LZD	SXT	TE	FOX	CIP	CN	E	DA	CH	QD	AMC	VAN
PA5	0.42	*Staphylococcus xylosus*	S	S	S	R	S	S	R	R	S	S	R	R
PA6	0.75	*Staphylococcus aureus*	R	S	R	R	R	R	R	R	S	S	R	R
PA7	0.5	*Staphylococcus aureus*	S	S	S	R	R	R	R	R	S	S	R	S
PA8	0.25	*Staphylococcus xylosus*	S	S	S	S	R	S	R	R	S	S	S	S
PA9	0.58	*Staphylococcus xylosus*	S	R	R	R	R	R	S	R	S	S	S	R
PA12	0.58	*Staphylococcus xylosus*	S	R	R	R	R	S	S	R	S	S	R	R
PA13	0.25	*Staphylococcus aureus*	S	S	R	R	S	S	S	R	S	S	S	S
PA14	0.33	*Staphylococcus xylosus*	S	S	S	R	S	S	S	R	R	S	R	S
PA16	0.58	*Staphylococcus xylosus*	S	R	R	R	R	S	R	R	S	S	R	S
PA17	0.16	*Staphylococcus xylosus*	S	S	S	R	R	S	S	S	S	S	S	S
PA18	0.5	*Staphylococcus xylosus*	S	S	R	R	R	S	S	R	S	S	R	R
PA29	0.25	*Staphylococcus hyicus*	S	S	R	R	R	S	S	S	S	S	S	S
PA32	0.42	*Staphylococcus aureus*	R	S	R	R	R	S	S	S	S	S	R	S
DA32	0.25	*Staphylococcus xylosus*	R	S	S	S	R	S	S	R	S	S	S	S
PA62	0.42	*Staphylococcus aureus*	S	R	R	R	R	S	S	S	S	S	R	S
PA1	0.5	*Staphylococcus aureus*	S	S	S	R	R	R	R	R	S	S	R	S
PA3	0.42	*Staphylococcus aureus*	S	R	R	R	R	S	S	S	S	S	R	S
FDA2	0.25	*Staphylococcus aureus*	R	S	R	S	S	S	S	S	S	S	S	R
PA4	0.42	*Staphylococcus aureus*	S	R	R	R	R	S	R	S	S	S	S	S
DA4	0.42	*Staphylococcus aureus*	R	S	R	R	R	S	S	S	S	S	R	S
PN20	0.42	*Staphylococcus xylosus*	S	R	R	R	S	S	R	S	S	S	R	S
PN26	0.08	*Staphylococcus xylosus*	S	S	S	R	S	S	S	S	S	S	S	S
DN34	0.25	*Staphylococcus aureus*	R	S	S	R	R	S	S	S	S	S	S	S

PN36	0.42	*Staphylococcus aureus*	R	S	R	R	R	R	S	S	S	S	S	S
PN46	0.5	*Staphylococcus aureus*	S	S	S	R	S	R	R	R	S	S	R	R
DN4	0.42	*Staphylococcus aureus*	S	S	R	R	S	R	R	R	S	S	S	S
PN9	0.33	*Staphylococcus xylosus*	S	R	S	R	R	S	S	S	S	S	R	S
PN10	0.5	*Staphylococcus chromogenes*	S	S	R	R	R	S	S	R	S	S	R	R
PN11	0.42	*Staphylococcus xylosus*	S	R	R	R	R	S	S	S	S	S	R	S
PN12	0.16	*Staphylococcus xylosus*	S	R	S	S	S	S	R	S	S	S	S	S
PN13	0.16	*Staphylococcus xylosus*	S	R	S	S	S	S	R	S	S	S	S	S
PN15	0.42	*Staphylococcus aureus*	S	S	R	R	S	S	R	R	S	S	R	S
DN15	0.25	*Staphylococcus aureus*	S	S	S	R	S	S	R	R	S	S	S	S
PN17	0.42	*Staphylococcus aureus*	S	S	R	R	R	S	S	R	S	S	R	S
PN18	0.5	*Staphylococcus chromogenes*	S	S	S	R	R	S	R	R	S	S	R	R
HBN1	0.42	*Staphylococcus chromogenes*	S	S	R	R	S	S	R	R	S	S	R	S
HGN2	0.58	*Staphylococcus aureus*	R	R	R	R	S	S	R	R	S	S	R	S
PHF8M	0.16	*Staphylococcus xylosus*	S	S	R	R	S	S	S	S	S	S	S	S
PHF7	0.16	*Staphylococcus xylosus*	S	S	R	R	S	S	S	S	S	S	S	S
DHM9	0.42	*Staphylococcus aureus*	R	S	S	R	R	S	R	R	S	S	S	S
PHF9	0.25	*Staphylococcus xylosus*	S	S	R	S	R	S	S	S	S	R	S	S
DS9	0.42	*Staphylococcus aureus*	S	S	S	R	S	S	R	R	S	S	R	R
DS14	0.33	*Staphylococcus aureus*	S	S	R	R	R	R	S	S	S	S	S	S
DS22	0.16	*Staphylococcus xylosus*	S	S	S	R	S	S	S	S	S	S	R	S
DS29	0.25	*Staphylococcus aureus*	S	S	S	R	R	S	S	S	S	S	S	R
DN 7	0.16	*Staphylococcus xylosus*	S	R	R	S	R	S	S	S	S	S	S	S
PS34	0.42	*Staphylococcus aureus*	S	S	S	R	S	S	R	R	S	S	R	R
PS40	0.66	*Staphylococcus aureus*	R	R	S	R	R	S	R	R	S	S	R	R
PS39	0.25	*Staphylococcus aureus*	S	S	R	R	S	S	S	S	S	S	S	R
PS47	0.33	*Staphylococcus hominis*	S	S	S	R	S	S	R	R	S	S	S	R
PS48	0.42	*Staphylococcus aureus*	R	S	S	R	R	S	R	R	S	S	S	S
PS59	0.5	*Staphylococcus aureus*	S	S	R	R	S	S	S	R	S	R	R	R

PN2	0.5	*Staphylococcus xylosus*	S	R	R	R	R	S	S	R	S	S	R	S
PN3	0.66	*Staphylococcus xylosus*	R	S	R	R	R	S	R	R	S	S	R	S
PN4	0.33	*Staphylococcus xylosus*	S	S	R	R	S	S	R	S	S	S	R	S
PHF11	0.08	*Staphylococcus xylosus*	S	S	S	R	S	S	S	S	S	S	S	S
DS 10	0.33	*Staphylococcus hominis*	R	S	S	R	R	S	S	S	R	S	S	S
10FD	0.25	*Staphylococcus aureus*	S	S	S	R	R	S	S	S	S	S	R	S
DS 15	0.25	*Staphylococcus xylosus*	R	S	S	R	R	S	S	S	S	S	S	S
19GH	0.42	*Staphylococcus aureus*	S	S	R	R	S	R	S	S	R	S	R	S
21GD	0.25	*Staphylococcus aureus*	S	R	S	R	S	S	S	S	S	S	R	S
23GD	0.58	*Staphylococcus aureus*	R	S	R	R	S	R	S	S	R	S	R	R
24BH	0.42	*Staphylococcus aureus*	S	R	R	R	S	S	S	S	S	S	R	R
DN 9	0.25	*Staphylococcus xylosus*	R	S	R	S	R	S	S	S	S	S	S	S
PN 28	0.16	*Staphylococcus hyicus*	R	S	S	R	S	S	S	S	S	S	S	S
PS 48	0.25	*Staphylococcus hominis*	S	S	S	R	R	S	S	S	S	S	R	S
DN 19	0	*Staphylococcus chromogenes*	S	S	S	S	S	S	S	S	S	S	S	S
HBN3	0.08	*Staphylococcus chromogenes*	R	S	S	S	S	S	S	S	S	S	S	S
DHM19	0	*Staphylococcus intermedius*	S	S	S	S	S	S	S	S	S	S	S	S
DN 23	0	*Staphylococcus chromogenes*	S	S	S	S	S	S	S	S	S	S	S	S
PS41	0.58	*Staphylococcus aureus*	R	S	R	R	S	R	S	S	R	S	R	R
DN26	0.42	*Staphylococcus aureus*	S	S	R	R	S	R	S	S	R	S	R	S
PA20	0.42	*Staphylococcus aureus*	S	S	R	R	S	R	S	S	R	S	R	S
DN16	0.16	*Staphylococcus hyicus*	R	S	R	S	S	S	S	S	S	S	S	S
DS34	0.25	*Staphylococcus aureus*	S	R	S	R	S	S	S	S	S	S	R	S
PHM11	0.08	*Staphylococcus xylosus*	R	S	S	S	S	S	S	S	S	S	S	S
28BH	0.33	*Staphylococcus aureus*	R	S	R	S	S	S	R	S	S	S	R	S
PA23	0.16	*Staphylococcus hyicus*	R	S	S	S	S	S	S	S	S	S	R	S
5CD	0.25	*Staphylococcus aureus*	S	S	S	R	S	S	R	S	S	S	R	S
DHM23	0.08	*Staphylococcus intermedius*	S	S	S	R	S	S	S	S	S	S	S	S
PS30	0.42	*Staphylococcus aureus*	R	S	S	R	S	S	R	R	S	S	R	S

DHM 14	0.42	*Staphylococcus intermedius*	R	R	S	R	S	S	R	R	S	S	R	S
27BH	0.16	*Staphylococcus aureus*	S	S	R	S	S	S	S	S	S	S	R	S
29BH	0.42	*Staphylococcus aureus*	S	S	R	R	R	R	S	S	S	S	R	S
DHM 2	0.42	*Staphylococcus intermedius*	S	R	S	R	S	S	R	R	S	S	R	S
DS 3	0.42	*Staphylococcus hominis*	R	S	S	R	S	S	R	R	S	S	R	S
17GH	0.25	*Staphylococcus aureus*	S	S	R	R	S	S	S	S	S	S	R	S
DN 11	0.25	*Staphylococcus chromogenes*	R	S	S	R	S	S	S	S	S	S	R	S
33BH	0.16	*Staphylococcus aureus*	S	S	R	S	S	S	S	S	S	S	R	S
34BH	0.33	*Staphylococcus aureus*	S	S	R	R	S	R	S	S	S	S	R	S
PS33	0.33	*Staphylococcus aureus*	S	S	R	R	S	R	S	S	S	S	R	S
PS45	0.42	*Staphylococcus aureus*	S	S	R	R	R	R	S	S	S	S	R	S
HBN5	0.25	*Staphylococcus chromogenes*	R	R	S	R	S	S	S	S	S	S	S	S
PHM15	0.16	*Staphylococcus intermedius*	S	R	S	R	S	S	S	S	S	S	S	S
DHM26	0.16	*Staphylococcus intermedius*	R	S	S	R	S	S	S	S	S	S	S	S

Key: LZD= Linezolid SXT=Trimethoprim-sulfamethoxazole; TE= Tetracycline FOX= Cefoxitin CIP= Ciprofloxacin; CN=Gentamicin; E=Erythromycin; DA=Clindamycin; CH=Chloramphenicol; QD=Quinupristindalfopristin ; AMC=Amoxicillin-clavulanic Acid; VAN=Vancomycin; MARI=Multiple Antibiotics Resistance Index

Appendix VII: Antibiotic Susceptibility Profile of *Staphylococcus* isolates

***Staph* isolates**	**Antibiotic Susceptibility (%)**											
	LZD	**SXT**	**TE**	**FOX**	**CIP**	**CN**	**E**	**DA**	**CH**	**QD**	**AMC**	**VAN**
S. xylosus	70	59	52	37	48	93	63	63	98	0	48	85.2
N=27												
S. hyicus	85	0	25	100	85	0	85	85	0	77	50	0
N= 4												
S. intermedius	67	83	67	67	85	0	0	0	0	83	50	87
N= 6												
S. hominis	85	0	85	25	0	0	0	0	85	0	85	0
N=4												
S. chromogenes	63	85	0	25	63	0	63	63	0	0	37	0
N=8												
S. aureus	70	80	35	9	57	33	63	37	9	2	26	74
N= 46												

Key: LZD= Linezolid SXT=Trimethoprim-sulfamethoxazole; TE= Tetracycline FOX= Cefoxitin CIP= Ciprofloxacin; CN=Gentamicin; E=Erythromycin; DA=Clindamycin; CH=Chloramphenicol; QD=Quinupristin-Dalfoprisitin ; AMC=Amoxicillin-clavulanic Acid; VAN=Vancomycin

Appendix VIII: Antibiotic Resistance Profile of *Staphylococcus* isolates

***Staph* isolates**	**Antibiotic Resistance (%)**											
	LZD	**SXT**	**TE**	**FOX**	**CIP**	**CN**	**E**	**DA**	**CH**	**QD**	**AMC**	**VAN**
S. xylosus	30	41	48	63	52	7	37	37	2	0	52	14.8
N=27												
S. hyicus	15	0	75	100	15	0	15	15	0	23	50	0
N= 4												
S. intermedius	33	17	33	67	15	0	0	0	0	17	50	17
N= 6												
S. hominis	15	0	15	75	0	0	0	0	15	0	15	0
N= 4												
S. chromogenes	37	15	0	75	37	0	37	37	0	0	37	0
N= 8												
S. aureus	30	20	65	91	43	67	37	37	9	2	74	26
N= 46												

Key: LZD= Linezolid SXT=Trimethoprim-sulfamethoxazole; TE= Tetracycline FOX= Cefoxitin CIP= Ciprofloxacin; CN=Gentamicin; E=Erythromycin; DA=Clindamycin; CH=Chloramphenicol; QD=Quinupristin-Dalfoprisitin ; AMC=Amoxicillin-clavulanic Acid; VAN=Vancomycin

Appendix IX: Classification of Multiple Antibiotics Resistance Index MARI

ISOLATE	ANTIBIOTICS RESISTANT TO	CLASS OF RESISTANCE
PA5	FOX, E, DA,AMC,VAN	MDR
PA6	LZD,TE,FOX,CIP,CN,E, DA,AMC,VAN	XDR
PA7	FOX,CIP,CN,E,DA,,AMC	MDR
PA8	CIP,E,DA	MDR
PA9	SXT,TE,FOX,CIP,CN,DA,VAN,	XDR
PA10	SXT,TE,FOX,CIP,DA,VAN	XDR
PA12	TE,FOX,DA,	MDR
PA14	FOX,DA,CH,AMC	MDR
PA16	SXT,TE,FOX,CIP, E, DA,AMC,	XDR
PA17	FOX,CIP,	DR
PA18	TE,FOX,CIP,DA,AMC,VAN	MDR
PA29	TE,FOX,CIP,	MDR
PA32	LZD,TE,FOX,CIP,AMC,	MDR
DA32	LZD,CIP,DA,	MDR
PA62	SXT,TE,FOX,CIP,AMC	MDR
PN1	FOX,CIP,CN,E,DA,AMC	MDR
PN2	SXT,TE,FOX,CIP,AMC,	MDR
FDA2	LZD,TE,VAN	MDR
PA4	LZD,TE,FOX,CIP,AMC	MDR
PN20	SXT,TE,FOX,DA,AMC,	MDR
PN26	FOX,	DR
DN34	LZD,FOX, CIP	MDR
PN36	LZD, TE, FOX,CIP,CN	MDR
PN46	FOX, CN,E,DA,,AMC VAN	MDR
DN4	TE, FOX, CN,E,DA	MDR
PN9	SXT, FOX,CIP,AMC	MDR
PN18	TE,FOX,CIP, DA, VAN	MDR
PN11	SXT,TE,FOX, CIP, AMC	MDR
PN12	SXT, E	DR
PN13	SXT,E,	DR
PN15	TE,FOX,E, DA, AMC	MDR
DN15	FOX,E,DA,	MDR
PN17	TE,FOX,CIP, DA,AMC	MDR
PN10	FOX,CIP, E, DA, AMC	MDR
HBN1	TE, FOX, E, DA, AMC	MDR
HGN2	LZD, SXT,TE,FOX, E, DA,AMC	XDR
PHF8m	TE, FOX	DR
PHF7	TE,FOX	DR
DHM9	LZD,FOX, CIP, E, DA	DR

PHF9	TE,CIP,QD	MDR
DS9	FOX, E, DA,AMC, VAN	MDR
DS14	TE,FOX, CIP, CN	MDR
DS22	FOX, AMC	MDR
DS29	FOX, TE	MDR
DN7	SXT TE	DR
PS34	FOX,E, DA, AMC, VAN	MDR
PS40	LZD, SXT, FOX, CIP, E,DA, AMC	MDR
PS39	TE, FOX, VAN	MDR
PS47	FOX,E,DA	MDR
PS48	LZD,FOX, CIP, E,DA,	MDR
PS59	TE, FOX,DA,QD,AMC, VAN	MDR
PN2	TE,FOX,CIP, DA,AMC,	MDR
PN3	LZD, TE, FOX,CIP, E, DA,AMC,	XDR
PN4	TE,FOX,E, AMC	MDR
PHF11	CIP	DR
DS10	LZD,FOX,CIP, CH	MDR
10FD	FOX,CIP,AMC	MDR
DS15	LZD, FOX,CIP,	MDR
19GH	TE,FOX,CN,CH,AMC	MDR
21GD	SXT,FOX,AMC	MDR
23GD	LZD,TE,FOX, CN, CH,AMC, VAN	XDR
24BH	SXT,TE,FOX,AMC, VAN	MDR
DN9	LZD,FOX, CN	MDR
PN28	LZD,FOX	DR
PS48	FOX, CIP,AMC	MDR
DN19	-	-
HBN3	LZD	DR
DHM19	-	-
DN23	-	-
PS41	LZD,TE,FOX, CN,CH, AMC, VAN	XDR
DN26	TE,FOX, CN, CH, AMC	MDR
DA20	TE, FOX CN,CH, AMC	MDR
DN16	LZD,TE,	DR
DS34	SXT,FOX, AMC	MDR
PHM11	LZD	DR
28BH	LZD, TE, E,AMC	MDR
PA23	LZD,AMC	DR
3CD	SXT,TE, FOX, CIP, AMC	MDR
5CD	FOX,E,AMC	MDR
DHM23	FOX,	DR
PS30	LZD,FOX, E, DA, AMC	MDR
DHM14	LZD, SXT, FOX, E, DA,AMC	MDR
27BH	TE, AMC	DR

29BH	TE, FOX,CIP,CN, AMC	MDR
DHM2	SXT, FOX,E,DA,AMC	MDR
DS3	LZD,FOX, E,DA, AMC	MDR
17GH	TE,FOX,AMC	MDR
DN11	LZD,FOX,AMC	MDR
33BH	TE,AMC	DR
34BH	TE,FOX,CN,AMC	MDR
PS33	TE,FOX,CN,AMC	MDR
PS45	TE,FOX,CIP,CN,AMC	MDR
HBN5	LZD, SXT,FOX,	MDR
PHM15	SXT,FOX	DR
DHM26	FOX	DR

Key:

LZD= Linezolid SXT=Trimethoprim-sulfamethoxazole; TE= Tetracycline FOX= Cefoxitin CIP= Ciprofloxacin; CN=Gentamicin; E=Erythromycin; DA=Clindamycin; CH=Chloramphenicol; QD=Quinupristindalfopristin ; AMC=Amoxicillin-clavulanic Acid; VAN=Vancomycin

DR= Drug Resistance
MDR= Multi-Drug Resistance
XDR= Extended drug Resistance

Appendix X: Pictorial presentation Antibiotics Susceptibility Testing