A PROTOCOL ON THE EPIDEMIOLOGY OF METHICILLIN RESISTANT *Staphylococcus aureus* IN BENIN CITY, NIGERIA: A CROSS-SECTIONAL STUDY

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Dorcas ADU (MISS)
LSC1605337

DEPARTMENT OF MICROBIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.
IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY

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CERTIFICATION

I hereby certify that this work was carried out by Dorcas ADU (MISS) with matriculation number LSC1605337 at the Department of Microbiology, Faculty of Life Sciences, University of Benin City, under my supervision

_____ _____

DR. K. O. Elimian **DATE**
Project Supervisor

_____ _____

Prof. S. E. Omonigho **DATE**
Head of Department

DEDICATION

I hereby dedicate this project to God Almighty my creator, my father, my source of inspiration, wisdom, knowledge and understanding.

ACKNOWLEDGEMENT

My heart felt appreciation goes to my Supervisor DR. K. O. Elimian for his guidance, contribution, constructive criticism and support during the course of the study. I owe the success of this research to him.

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Finally, I wish to thank my friends, Oyeka Abujai, Iseghohimen Omono Matialda and Iyamah Peace Daniella for their support and advice. I thank you all for your love, support and care God bless you all.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-recognized public health problem throughout the world. In this study presents data on the prevalence of MRSA and resistance pattern to other antibiotics on the selected specimen from three hospitals in Benin City, Edo State. A cross sectional study was conducted among 233 participants recruited from three hospitals in Benin City, Edo State they were 157 female and 76 male using a questionnaire and the following samples were collected from the participants such as nasal swab, urine and both nasal swab & urine. The culture media used are Nutrient and MacConkey agar . All media were prepared according to manufacturer's instruction. The media used were sterilised in an Auto clave at 121 ^{o}C for 15 minutes. The following Morphological characterization were carried out which are; gram's staining, Biochemical characterization, indole test, oxidase test, catalase test, urease test, motility test, coagulase test and citrate utilization test. Isolation of *S. aureus* was based on culture and biochemical profiles.

CHAPTER ONE

1.0 INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi no longer susceptible to the common medicines used to treat them (Gerber et al., 2017). The problem of AMR is especially urgent regarding antibiotic resistance in bacteria. Over several decades, to varying degrees, bacteria causing common or severe infections have developed resistance to each new antibiotic coming to market. Faced with this reality, the need for action to avert a developing global crisis in health care is imperative (Cassir et al., 2014). The World Health Organization (WHO) has long recognized the need for an improved and coordinated global effort to contain AMR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance has provided a framework of interventions to slow the emergence and reduce the spread of antimicrobial-resistant microorganisms; In 2012, WHO published The Evolving Threat of Antimicrobial Resistance – Options for Action proposing a combination of interventions that include strengthening health systems and surveillance; improving use of antimicrobials in hospitals and in community; infection prevention and control; encouraging the development of appropriate new drugs and vaccines; and political commitment (Drame et al., 2020).

In Nigeria, there has been a considerable attention on reducing the burden of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Multi-Drug Resistant (MDR) *Escherichia coli* (*E. coli*), given their re-emergence and attendant public health implications in the last few years. MDR strains of *E. coli* , for example, can exhibit non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012), thereby making

treatment less efficient and more costly. A systematic review found a substantial increase in the prevalence of MRSA to commonly prescribed and inexpensive antibiotics (cotrimoxazole and tetracycline) in Nigeria between 2007 and 2017, increasing from 18.3% in 2009 to 42.3% 2013, although with varying prevalence with regards Nigerian regions (Abubakar & Sulaiman, 2018). There is evidence to also support widespread AMR among enteric *E. coli* in Nigeria, particularly to penicillins, aminoglycosides, cephalosporins, chloramphenicol, tetracyclines and cotrimoxazole (Nigeria Centre for Disease Control, 2017). For instance, a cross-sectional study of 122 randomly selected apparently healthy poultry workers in Municipal and Kuje area of the Federal Capital Territory in Nigeria from December 2018 to April 2019 found the prevalence of *E. coli* to be 39.7% (n=48), 79.2% (38/48) of which were MDR to various commonly used antibiotics (Aworh et al., 2019).

Despite the efforts to promote antimicrobial stewardship, antimicrobial resistance (AMR) has persistently remained a public health threat globally. In Nigeria, for instance, the usefulness of most antimicrobial drugs for the management of infectious diseases—the major determinant of morbidity and mortality in the country—is being threatened by AMR (Nigeria Centre for Disease Control, 2017). Generally, the widespread antibiotic use and consequent increasing bacterial resistance to antibiotics are multiple (Bell et al., 2014) including increased healthcare costs, increased morbidity and mortality (Levy, 2001), and complication of advanced treatments (e.g. treatment of malignancies and transplantations) that rely on effective infection control (Montassier et al., 2013). Moreover, prompt identification of patients carrying resistant bacteria is crucial to preventing in-hospital transmission and to targeting antibiotic treatment to the individual patient (Mogensen et al., 2018), especially in a setting with suboptimal healthcare system.

Overall, there is still limited evidence on the epidemiology of both MRSA and MDR *E. coli* , particularly with respect to prevalence at various levels of healthcare and, equally important, context-specific risk factors. Understanding the epidemiology of both MRSA and MDR *E. coli* becomes even more important for a country as Nigeria where antimicrobial drugs are readily available over-the-counter and with high potential for indiscriminate use by poultry farmers. A situation analysis of AMR in Nigeria by the Nigeria Centre for Disease Control in conjunction with its partners further underlines the importance of studying the epidemiology of AMR given the dearth of evidence in the country, especially in the south-south region (Nigeria Centre for Disease Control, 2017). As such, a study that seeks to fill these research gaps will be contributing to the limited evidence available to both researchers and public health policy makers in making an informed decision regarding AMR control in the country.

1.2 Aim and Objectives

Aim

The overarching aim of this project therefore is to provide a comprehensive outlook of the epidemiology of MRSA and MDR *E. coli* carriage in patients presenting at contrasting health facilities (primary, secondary and tertiary health facilities) in Benin City, Edo State of Nigeria.

Objectives

The project will address the following objectives:

i. To describe the prevalence of MRSA and MDR *E. coli* carriage in patients.
ii. To describe the occurrence of simultaneous patient carriage with MRSA and MDR *E. coli* .

iii. To identify risk factors for MRSA and MDR *E. coli* carriage among patients under investigation.

iv. Based on the identified risk factors, to develop screening tools for identification of both MRSA and MDR *E. coli* carriage in the study setting.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background of MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increas- ingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA are poorly understood, no rational nomenclature exists, and there is no consensus on the number of major MRSA clones or the relatedness of clones described from different countries. We resolve all of these issues and provide a more thorough and precise analysis of the evolution of MRSA clones than has previously been possible. Using multilocus sequence typing and an algorithm, BURST w a s analyzed an international collection of 912 MRSA and methicillin- susceptible *S. aureus* (MSSA) isolates. We identified 11 major MRSA clones within five groups of related genotypes. The putative ancestral genotype of each group and the most parsimonious patterns of descent of isolates from each ancestor were inferred by using BURST, which, together with analysis of the methicillin resistance genes, established the likely evolutionary origins of each major MRSA clone, the genotype of the original MRSA clone and its MSSA progenitor, and the extent of acquisition and horizontal movement of the methicillin resistance genes. Major MRSA clones have arisen repeatedly from successful epidemic MSSA strains, and isolates with decreased susceptibility to vancomycin, the antibiotic of last resort, are arising from some of these major MRSA clones, highlighting a depressing progression of increasing drug resistance within a small number of ecologically successful *S. aureus* genotypes.

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961 there were reports from the United Kingdom of *S. aureus*

isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) (1), and MRSA isolates were soon recovered

from other European countries, and later from Japan, Australia, and the United States. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community (2, 3). The methicillin resistance gene (mecA) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species (4). mecA is carried on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), of which four forms have been described that differ in size and genetic composition (5). Many MRSA isolates are multiply resistant and are susceptible only to glycol-peptide antibiotics such as vancomycin and investigational drugs. MRSA isolates that have decreased susceptibility to glycopep-tides (glycopeptide intermediately susceptible *S. aureus* , GISA) (6, 7), reported in recent years, are a cause of great public health concern.

Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA). MRSA isolates are generally characterized by pulsed-field gel electrophoresis, a powerful technique for identifying the relatedness of isolates from recent outbreaks within a hospital, but are not well suited to long-term global epidemiology, which requires a procedure that is highly discriminatory but that indexes variation that accumulates slowly. Multilocus sequence typing (MLST) provides such a procedure and characterizes isolates of bacteria unambiguously by using the sequences of internal fragments of seven housekeeping genes (8, 9). MLST has been developed and validated for *S. aureus* (10) and provides a discriminatory method that allows related strains recovered in different countries to be readily identified.

The origins of the major MRSA clones are still poorly understood. Kreiswirth et al. (11) proposed that all MRSAs were descended from a single ancestral *S. aureus* strain that acquired mecA, but more recent studies (12, 13) show that some MRSAs are very divergent, implying that mecA has been transferred between *S. aureus* lineages. The data from MLST can be used to probe the evolutionary and population biology of bacterial pathogens and to predict ancestral genotypes and patterns of evolutionary descent within groups of related genotypes. We have applied MLST to an international collection of 359 MRSA isolates, which includes examples of the previously described EMRSA and GISA clones, and compare these to a collection of 553 methicillin-susceptible *S. aureus* (MSSAs). We demonstrate the limited number of major EMRSA genotypes and provide an unambiguous method for characterizing MRSA and GISA clones and a rational nomenclature. We also identify the ancestral MRSA clone and its MSSA ancestor and suggest the evolutionary pathways by which MRSA clones have repeatedly emerged from successful MSSA clones Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA are poorly understood, no rational nomenclature exists, and there is no consensus on the number of major MRSA clones or the relatedness of clones described from different countries. We resolve all of these issues and provide a more thorough and precise analysis of the evolution of MRSA clones than has previously been possible.

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methicillin resistance genes, established the likely evolutionary origins of each major MRSA clone, the genotype of the original MRSA clone and its MSSA progenitor, and the extent of acquisition and horizontal movement of the methicillin resistance genes. Major MRSA clones have arisen repeatedly from successful epidemic MSSA strains, and isolates with decreased susceptibility to vancomycin, the antibiotic of last resort, are arising from some of these major MRSA clones, highlighting a depressing progression of increasing drug

resistance within a small number of ecologically successful *S. aureus* genotypes. Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961 there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) (1), and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community (2, 3). The methicillin resistance gene (mecA) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species (4). mecA is carried on a mobile genetic element, the staphylococcal cassette chromo- some mec (SCCmec), of which four forms have been described that differ in size and genetic composition (5). Many MRSA isolates are multiply resistant and are susceptible only to glyco-peptide antibiotics such as vancomycin and investigational drugs. MRSA isolates that have decreased susceptibility to glycopep-tides (glycopeptide intermediately susceptible *S. aureus* , GISA) (6, 7), reported in recent years, are a cause of great public health concern.

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Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human body. Methicillin-resistant *S. aureus* (MRSA) strains have acquired a gene that makes them resistant to nearly all beta-lactam antibiotics. Resistance to other antibiotics is also common, especially in hospital-associated MRSA. These organisms are serious nosocomial pathogens, and finding an effective treatment can be challenging. Community-associated MRSA strains, which originated outside hospitals, are also prevalent in some areas. While these organisms have generally been easier to treat, some have moved into hospitals and have become increasingly resistant to drugs other than beta-lactams. Animals sometimes become infected with MRSA from humans, and may either carry these organisms asymptomatically or develop opportunistic infections. Most of the MRSA found in dogs and cats seem to be lineages associated with people. Colonization of dogs and cats is often transient and tends to occur at low levels; however, these organisms can be transmitted back to people, and pets might contribute to maintaining MRSA within a household or facility. MRSA can also be an issue in settings such as veterinary hospitals, where carriage rates can be higher, especially during outbreaks in pets, horses and other animals. Animal-adapted MRSA strains also exist. The livestock-associated lineage MRSA CC398, which apparently emerged in European pigs between 2003 and 2005, has spread widely and infected many species of animals, especially pigs and veal calves, in parts of Europe. CC398 has also been found on other continents, although the reported prevalence varies widely. People who work with colonized livestock or poultry can carry CC398, and these organisms can cause opportunistic infections. Other livestock associated MRSA have also been identified in various locations. CC9 is an especially prominent lineage in Asia. MecC-bearing MRSA is a new type of MRSA first recognized in 2011. Many of these organisms have been recovered from animals, especially dairy cattle, but they can also infect and colonize humans. Recognizing mecC MRSA is currently problematic, as most of the diagnostic tests used

routinely to identify MRSA do not detect these organisms. Etiology *Staphylococcus aureus* is a Gram positive, coagulase positive coccus in the family Staphylococcaceae. Methicillin-resistant *S. aureus* strains have acquired resistance to methicillin and other beta lactam antibiotics (e.g., penicillins and cephalosporins) via the mecA or mecC genes. Most MRSA carry the mecA gene, which resides on a large mobile genetic element called the staphylococcal chromosomal cassette mec (SCCmec). This gene codes for a penicillin binding protein, PBP2a, which interferes with the effects of beta lactam antibiotics on cell walls. It confers virtually complete resistance to nearly all beta-lactam antibiotics including semi-synthetic penicillins such as methicillin, oxacillin, or cloxacillin. (Notable exceptions to this rule are the latest generation of cephalosporin β-lactams, e.g., ceftaroline and ceftobiprole.) Acquisition of mecA seems to have occurred independently in a number of *S. aureus* lineages. Some lineages have a tendency to colonize specific species, and may be adapted to either humans or animals. Others ("extended host spectrum genotypes") are less host-specific, and can infect a wide variety of species. MRSA strains known as epidemic strains are more prevalent and tend to spread within or between hospitals and countries. Other "sporadic" strains are isolated less frequently and do not usually spread widely. There are also MRSA strains that produce various exotoxins (e.g., toxic shock syndrome toxin 1, exfoliative toxins A or B, and enterotoxins) associated with specific syndromes, such as toxic shock syndrome. MecC (formerly mecALGA251) is a beta lactam resistance gene that was first recognized in 2011, and is less well understood than mecA. Like mecA, mecC is carried on SCCmec. It codes for a different version of PBP2a, which is also thought to interfere with the effects of beta-lactam antibiotics on cell walls. However, a recent paper suggests that mecC-encoded PBP2a may mediate resistance to some Methicillin Resistant *Staphylococcus aureus* beta-lactam drugs, but not others. This could raise the possibility of treatment with some drugs that are ineffective against mecA-bearing MRSA. Many mecC-bearing organisms

seem to belong to lineages of staphylococci associated with animals. Some of these lineages appear to have a wide host range. There could be other, yet unrecognized, mec variants. Rare mec-independent forms of resistance have also been reported in *S. aureus* (e.g., "BORSA" strains, which do not carry mecA but are borderline resistant to oxacillin in in vitro tests). Such isolates may be recognized in laboratory tests that directly examine a colony's resistance to antibiotics (phenotypic methicillin resistance), but not in tests based on the recognition of mecA or mecC. Other methicillin-resistant Staphylococcus species Phenotypic methicillin resistance, the mecA gene and/or mecC have been reported occasionally in *Staphylococcus* species other than *S. aureus* . These organisms have increasingly become an issue in veterinary medicine. For example, methicillin-resistant *S. pseudointermedius* is now a significant concern in dogs. Such animal-associated methicillin-resistant staphylococci occasionally cause zoonotic infections in humans or colonize people asymptomatically.

2.2 Multidrug Resistance Methicillin Resistance S.Aureus

Staphylococcus aureus is a common opportunistic bacterium. This bacterium is an important pathogen due to combination of toxin-mediated virulence, invasiveness, and antibiotic resistance Although it may be part of the normal human microbiota, it can cause wide range of diseases from skin and soft-tissue infections (STIs) to severe invasive disease such as infective endocarditis, osteomyelitis, and toxic shock syndrome. *S. aureus* is also a major cause of food-borne illness worldwide An estimated 30% and 1.5% of the US population is colonized with methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus* can be recovered from many locations in and on the body, including the nose, throat, axillae, and groin, but the most important site for colonization is thought to be the anterior nares (nostrils). While colonization with *S.*

aureus itself does not harm the host, colonization is a risk factor for developing subsequent symptomatic infections *S. aureus* accounts for nearly 20% of bloodstream infections in the hospital setting. The treatment of *S. aureus* infections is often challenging due to the emergence of multidrug-resistant strains .Although drug resistance has been seen as a major risk in healthcare settings, a similar increasing trend is observed in community-acquired infections. Traditionally regarded as a nosocomial pathogen, MRSA infection outside of hospital has increased in incidence over the last decade and has emerged as a major public health concern worldwide. For example, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) is the leading cause of identifiable skin and soft-tissue infections (SSTIs) observed in US emergency rooms. An estimated 80,461 invasive MRSA infections occurred nationally in 2011. Of these, 16,560 were community-associated infections. The economic impact of CA-MRSA is tremendous. A recent publication suggested these infections impose an annual burden of $478 million -$2.2 billion on third-party payers and $1.4 billion -$13.8 billion on society in the US. Because of lengthy hospital stays, increased costs, and higher mortality, MRSA infections have imposed an increased disease burden in the last decade. The disease burden caused by *S. aureus* is poorly understood in developing countries. Although there have been some studies conducted in the context of Nepal, very little is known about the molecular epidemiology and transmission dynamics of *S. aureus* in this country. Most of the studies conducted in Nepal have been focused on the hospital environment, examining only the prevalence and phenotypic characterization of *S. aureus* . As such, the molecular epidemiology of *S. aureus* in community settings has not been investigated, and *S. aureus* colonization parameters among the general population or specific groups such as Bhutanese refugees is unknown. In the US, little is known regarding the prevalence and epidemiology of MRSA in immigrant populations. Although some infections, including tuberculosis and syphilis, may be detected during the pre- or post-arrival screening, MRSA

colonization is not examined, as there has been no policy or protocol for such screening by the US health institutions. In the past several years, the US has granted asylum to Bhutanese immigrants of Nepalese origin that were displaced from Bhutan due to political reasons. As a result, Bhutanese of Nepali origin were one of the largest groups of refugees resettled in the US, accounting for 19% of the total 322,565 refugees admitted into the US between 2008 and 2012. This study investigated the prevalence and molecular epidemiology of *S. aureus* carriage in adult Bhutanese refugees living in camps in eastern Nepal and resettled in Northeast Ohio (NEO), United States.

2.3 Causes of MRSA

Different varieties of *Staphylococcus aureus* bacteria, commonly called "staph," exist. Staph bacteria are normally found on the skin or in the nose of about one-third of the population. The bacteria are generally harmless unless they enter the body through a cut or other wound, and even then they usually cause only minor skin problems in healthy people (Dumyati et al., 2017). According to the Centers for Disease Control and Prevention, around 5% of the population chronically carries the type of staph bacteria known as MRSA. MRSA is the result of decades of often unnecessary antibiotic use. For years, antibiotics have been prescribed for colds, flu and other viral infections that don't respond to these drugs. Even when antibiotics are used appropriately, they contribute to the rise of drug-resistant bacteria because they don't destroy every germ they target. Bacteria live on an evolutionary fast track, so germs that survive treatment with one antibiotic soon learn to resist others (Gopal and Divya, 2017).

2.4 Signs and Symptoms of MRSA

In humans, *Staphylococcus aureus* is part of the normal microbiota present in the upper respiratory tract, and on skin and in the gut mucosa. However, along with similar bacterial

species that can colonize and act symbiotically, can cause disease if they begin to take over the tissues they have colonized or invade other tissues; the resultant infection has been called a "pathobiont" (Gurusamy et al., 2013). After 72 hours, MRSA can take hold in human tissues and eventually become resistant to treatment. The initial presentation of MRSA is small red bumps that resemble pimples, spider bites, or boils; they may be accompanied by fever and, occasionally, rashes. Within a few days, the bumps become larger and more painful; they eventually open into deep, pus-filled boils. About 75 percent of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively (Choo and Chambers, 2016). Needle-required drugs have caused an increase of MRSA, with injection drug use (IDU) making up 24.1% (1,839 individuals) of Tennessee Hospital's Discharge System. The unsanitary method of injection causes an access point for the MRSA to enter the blood stream and begin infecting the host. Furthermore, with MRSA's high contagion rate, a common risk factor is individuals who are in constant contact with someone who has injected drugs in the past year. This does still depend how strong the non-infected individual's immune system is and how long both individuals remain in contact (Jacobs, 2014).

2.5 Diagnosis of MRSA

Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks of MRSA. Normally, a bacterium must be cultured from blood, urine, sputum, or other body-fluid samples, and in sufficient quantities to perform confirmatory tests early-on. Still, because no quick and easy method exists to diagnose MRSA, initial treatment of the infection is often based upon "strong suspicion" and techniques by the treating physician; these include quantitative PCR procedures, which are employed in clinical laboratories for

quickly detecting and identifying MRSA strains (Hughes et al., 2013). Another common laboratory test is a rapid latex agglutination test that detects the PBP2a protein. PBP2a is a variant penicillin-binding protein that imparts the ability of *S. aureus* to be resistant to oxacillin. Like all *S. aureus* , methicillin-resistant *S. aureus* is a Gram-positive, spherical (coccus) bacterium about 1 micron in diameter. It does not form spores and it is not motile. It is frequently found in grape-like clusters or chains. Unlike methicillin-susceptible *S. aureus* (MSSA), MRSA is slow-growing on a variety of media and has been found to exist in mixed colonies of MSSA (Mehndiratta and Bhalla, 2014). The mecA gene, which confers resistance to a number of antibiotics, is always present in MRSA and usually absent in MSSA; however, in some instances, the mecA gene is present in MSSA but is not expressed. Polymerase chain reaction (PCR) testing is the most precise method for identifying MRSA strains. Specialized culture media have been developed to better differentiate between MSSA and MRSA and, in some cases, such media can be used to identify specific strains that are resistant to different antibiotics (Parikh et al., 2020). Other strains of *S. aureus* have emerged that are resistant to oxacillin, clindamycin, teicoplanin, and erythromycin. These resistant strains may or may not possess the mecA gene. *S. aureus* has also developed resistance to vancomycin (VRSA). One strain is only partially susceptible to vancomycin and is called vancomycin-intermediate *S. aureus* (VISA) (Cheung et al., 2014). GISA, a strain of resistant *S. aureus* , is glycopeptide-intermediate *S. aureus* and is less suspectible to vancomycin and teicoplanin. Resistance to antibiotics in *S. aureus* can be quantified by determining the amount of the antibiotic that must be used to inhibit growth. If *S. aureus* is inhibited at a concentration of vancomycin less than or equal to 4 μg/ml, it is said to be susceptible. If a concentration greater than 32 μg/ml is necessary to inhibit growth, it is said to be resistant.

2.6 Prevention of MDR *E. coli*

In the last few decades, the frequency and spectrum of antimicrobial-resistant infections have increased in both the hospital and the community. Certain infections that are essentially untreatable have begun to occur as epidemics both in the developing world and in institutional settings in the United States and other developed regions. Antimicrobial resistance is resulting in increased morbidity, mortality, and health-care costs (Cohen, 1992). Of particular interest is the emergence of resistance to the beta-lactams and the fluoroquinolone antibiotics which has become more serious in recent decades. Broad use of fluoroquinolones has been followed by emergence of resistance (Hooper, 2001) and strains producing extended-spectrum beta-lactamases (ESBL) render many, if not all, penicillin and cephalosporin ineffective as therapy (Aibinu et al., 2003b). Many studies have demonstrated the great potential for the spread of ESBL-producing strains and also ESBL-encoding plasmids to different hospitals (Davin-Regli et al., 1996) and even different countries (De Gheldre, 2001). It is also reported that community-acquired strains possessing ESBLs might be selected from the existing gastrointestinal flora when it is exposed to broadspectrum antimicrobial agents (Heseltine, 2000). *Escherichia coli* , previously were thought to be a vanishing cause of diarrhea, but with the recognition of *E. coli* 0157, a serotype of Enterohaemorrhagic *E. coli* (EHEC), the situation has changed completely (Griffin and Tauxe, 1991). Although a variety of *E. coli* serotypes have been associated with human illness, the most important among these is O157:H7. EHEC 0157 is one of the six groups of *E. coli* recognized as 22 Journal of American Science, 3(3), 2007, Aibinu, IE, Peters, RF, Amisu, KO, Adesida, SA, Ojo, MO and Tolu Odugbemi, Multidrug Resistance in *E. coli* 0157 Strains and the Public Health Implication aetiological agents of diarrhea (Aboaba et al., 2006). It was first identified as a cause of illness in 1982 (Riley et al., 1983) and the infections have now since been reported with increasing frequency (Fitzpatrick, 1999).

Infection with this *Escherichia coli* serotype is associated with a spectrum of illnesses including watery diarrhea, bloody diarrhea, and the hemolytic uremic syndrome, a potentially fatal condition characterized by acute renal failure (Griffin and Tauxe, 1991). Cattle are the principal reservoir for these organisms. Important sources of infection include consumption of undercooked hamburger and other contaminated food products and direct or indirect contact with infected persons (Wilson et al., 1997). It is of public health importance as it is readily isolated from human and animal wastes that pollute the environment (Smith et al., 2003). While therapeutic management of *E. coli* 0157 infection vary depending on the type of infection, the usefulness of antimicrobials in treating this Shiga-toxin-producing *E. coli* 0157 infection remains less clear (Griffin, 1995; Thielman and Guerrant, 1999). With the emergence and dissemination of antimicrobial resistance in bacteria which is well documented worldwide, (Cohen, 2000). *E. coli*, an important gastrointestinal flora, known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance (Enumeration of *Escherichia coli* and the Coliform Bacteria, 2002). Hence, active surveillance of the antimicrobial resistant pattern of EHEC *E. coli* 0157 serotype resident in animals that are resistant to its toxin is of public health importance. This study thus, investigates *E. coli* 0157 infection in human and apparently healthy animals; and the occurrence of multidrug resistance not only to commonly used antibiotics, but also to the broad spectrum drugs. The production of beta-lactamase enzyme as a mechanism of resistance employed by strains obtained in this study was also investigated.

2.7 Mechanisms of action of MDR *E. coli*

Bacteria may avoid accumulation of antibacterial molecules on their targets by reducing the absorption of these molecules or increasing the discharge of them, or by employing both mechanisms simultaneously. In general, antibiotics must penetrate the outer membrane (OM) of bacteria to reach to their targets. The OM of Gram-negative bacteria consists of a lipid bilayer and porins. In theory, hydrophobic antibiotics, such as quinolones and macrolides, pass through the lipid bilayer while hydrophilic antibiotics, such as beta-lactams, pass through porins. However, the OM of bacteria is a highly complex structure, and the permeation pathways of antibiotics are not fully understood .In some way, the OM of bacteria may be modified via the substitution of even one or two amino acids and transform to a permeability barrier for antibiotics. Unregulated efflux molecules may work concurrently with poring modifications which dramatically augment the discharge of antibiotics, thereby avoiding accumulation on target. Efflux-mediated resistance to tetracycline was first detected among *Escherichia coli* (*E. coli*) isolates during the 1970s Since then, various structures operating as efflux pumps have been discovered. The substrate specificity of efflux pumps varies widely, and some of them have an extraordinarily broad spectrum Efflux pumps are accepted as one of the primary mechanisms of multi-drug resistance (MDR) among bacteria, particularly among gram-negative bacteria. Bacteria replace or modify target molecules to avoid the harmful effects of antibiotics. Methicillin resistance among *Staphylococcus aureus* (*S. aureus*) was first noticed in the 1960s which emerge through the replacement of the target molecule. Beta-lactam antibiotics inactivate PBPs, particularly PBP 2 of *S. aureus*, initiate dysregulation of peptidoglycan synthesis and trigger a chain of events that eventually lead to the death of the bacteria. Methicillin-resistant *S. aureus* produces PBP 2a, a homolog enzyme with a low affinity to beta-lactam antibiotics, which is fully active and able to restore the vital functions of inactivated PBPs. PBP 2a is encoded on the *mec* locus, a gene package that is

extraneous, likely evolved and spread from another Staphylococcus species to *S. aureus*. There are several modes of target modification. Resistance to linezolid occurs by the alteration of 50S subunit of rRNA Another form of target modification occurs through the methylation of ribosomal genes. Methylation protects the target molecule from the inhibitory effect of antibiotics. Macrolide resistance is mostly caused by this type. Plasmid-mediated quinolone resistance (PMDR) is a notable example of target protection. Some proteins are capable of protecting gyrase from the inhibition of quinolones. These unique proteins are encoded on naturally occurring alleles, which are now referred to as "*qnr*" and primarily spread on multi-resistance plasmids, mostly along with extended-spectrum beta-lactamases (ESBLs). Bacteria, specifically *Acinetobacter spp*., resist to polymyxin antibiotics by modifying the lipid A component of the OM through spontaneous mutations. It was, however, surprising to discover that plasmidic colistin resistance conferred by lipid A modifying enzymes are insidiously spreading around the members of Enterobacteriaceae. Antibiotics are arguably the most successful form of chemotherapy developed in the 20th century and save innumerable human lives every day .The emergence of antibiotic-resistant bacteria limits the clinical use of antibiotics and, as resistant bacteria become more prevalent, there is increasing concern that existing antibiotics will become ineffective against these pathogens and more expensive Antibiotic-resistant genes conferring resistance to a wide variety of antibiotics have been identified in a large range of water environments including drinking water in both developed and developing countries. Analysis of multiple drug resistance of *E. coli* isolates from the water sources reveals that forty-eight isolates representing large percentage of (49.48%) of *E. coli* isolates exhibited resistance against two or more antibiotics, thus classified as multidrug resistance. This creates a huge public health concern. The presence of *E. coli* in the various water sources may spell health hazards such as diarrhoeal diseases which account for a substantial degree of morbidity and mortality in adults and children.

Control of diarrhoea may require the administration of antibiotics. Nonetheless, several strains of *E. coli* are known to be resistant to a wide array of antibiotics. Multiple antibiotic resistances refer to resistance to either two or more classes of antibiotics. The multiple antibiotic resistances of *E. coli* established in this study agree with other findings. Strains of *E. coli* and *Salmonella* spp. accounted for several outbreaks in the United States and worldwide, partly due to resistance to chloramphenicol, ampicillin, and trimethoprim. The frequency of penicillin resistance in the current study was high among the isolates as compared with chloramphenicol and ampicillin resistance observed in the isolates obtained from the various water sources. This may be due to the blanket use of inexpensive antibiotics in the Ghanaian community or may be due to production of beta-lactamase enzymes. *E. coli* resistance against ampicillin was observed by Çelebi et. al., Olowe et al., and Yurdakoek et al. The emerging co-trimoxazole and ciprofloxacin resistance from downstream sites are of serious concern, as these are the preferred drugs for many Gram-negative bacteria. The most common mechanism of resistance to co-trimoxazole is the acquisition of plasmid-mediated, variant diaminopyrimidine folate reductase enzymes. Low resistance to amikacin and gentamycin might be due to the less use of these antibiotics in clinical practice and/or veterinary medicine. The rising trend of resistance in all the isolates (total and faecal coliforms) from upstream to downstream affirms the fact that disposed antibiotics may have been washed down the water sources and accumulated downstream especially during the rainy season accounting for the high resistance. The differences in resistance profiles in this environmental study clearly reflect the differences in the selection procedure pressure in the investigated sites/areas. The higher level of resistance to antibiotics among coliforms of midstream and downstream sites of Ghanaian communities is worrisome since most inhabitants take bath, wash clothes, and even disposed human sewage into water sources at midstream and downstream sites while some occupants and nonoccupants use these water

sources for drinking and/or domestic purposes. In Mangalore, it is reported that untreated or partially treated domestic sewage is released into open estuaries which accounts for the high level of antibiotic resistance .Multidrug resistance is defined as resistance to all the tested antibiotics in at least two of the following three classes: lactams, aminoglycosides, and quinolones.

2.8 Epidemiology of MDR *E. coli*

Escherichia coli has become multiresistant by way of production of a variety of β-lactamases. The prevalence of CTX-M–producing *E. coli* has reached 60–79% in certain parts of Asia. The acquisition of CTX-M plasmids by *E. coli* sequence type 131, a successful clone of *E. coli* , has caused further dissemination of CTX-M – producing *E. coli.* The prevalence of carbapenemase - producing *E. coli,* especially *Klebsiella pneumoniae* carbapenemase, and New Delhi metallo-β-lactamase (NDM)-producing *E. coli* has been increasing in Asia. *K. pneumoniae* carbapenemase and NDM have now been found in *E. coli* sequence type 131. The occurrence of NDM-producing *E. coli* is a major concern particularly in the Indian subcontinent, but now elsewhere in Asia as well. There are multiple reasons why antibiotic resistance in *E. coli* in Asia has reached such extreme levels. Approaches beyond antibiotic therapy, such as prevention of antibiotic resistance by antibiotic stewardship and protecting natural microbiome, are strategies to avoid further spread of antibiotic resistance. Clinical specimens including urine, blood, stool, peritoneal and pleural fluids were cultured on blood agar and MacConkey agar plates (Merk, Germany) at 37°C for 24 hours. The grown isolates were identified by the morphology of colonies, Gram staining, and biochemical test characteristics. On blood agar medium, *E. coli* colonies are round, with flat surface, have diameters of 1-2 mm, juicy consistency and grayish color, whereas *K. pneumoniae* colonies are large, dome shape, mucoid, and tend to coalesce. On MacConkey medium, *E.*

coli colonies are red due to lactose fermentation, but *K. pneumoniae* colonies are large, mucoid dark pink, which indicates the fermentation of lactose. *Escherichia coli* biochemical characteristics include indole production, positive methyl red (MR), negative Voges-Proskauer (VP), negative Simmons' citrate agar, negative urease and motile. The biochemical tests for *K. pneumoniae* are negative indole, positive urease, variable MR, positive VP, positive Simmons' citrate agar and nonmotile. Microscopy by itself cannot differentiate the two organisms (14). The evaluation of specimens for antibiotic resistance was conducted according to the clinical and laboratory standards institute (CLSI) guidelines (15). By a sterile loop, the cultured colonies of *E. coli* and *K. pneumoniae* were inoculated directly onto Mueller-Hinton agar (Merk, Germany) by streak method. Screening test for the detection of antibiotic susceptibility was performed by Kirby-Bauer disk diffusion test (MAST, UK). Antibiotic disks impregnated with amikacin (30 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), and imipenem (10 µg) were plated on Mueller-Hinton agar and incubated for 24 hours at 37°C. The inhibition zones of ≤ 12 mm around gentamycin, ≤ 13 mm around ampicillin, amoxicillin-clavulanic acid, imipenem and ceftriaxone, ≤ 14 mm around ceftazidime and amikacin, and ≤ 15 mm around ciprofloxacin were considered resistant.

The confirmation of antimicrobial resistance was done by minimal inhibitory concentration (MIC) which was measured by epsilometer test (E-test) (Liofilchem, Italy). MICs ≥ 32 µg/mL for ampicillin and amikacin, ≥ 32/16 µg/mL for amoxicillin-clavulanic acid, ≥ 64 µg/mL for ceftriaxone, ≥ 32 µg/mL for ceftazidime, ≥ 16 µg/mL for imipenem, and ≥ 8 µg/mL for gentamycin were considered resistant. MDR was described as resistance to at least three antibiotics. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 7006039 were used as control isolates (Mast, UK) (15).

2.9 MRSA Worldwide

Staphylococcus aureus is an important human pathogen, responsible for infections in the community and the healthcare setting. Although much of the attention is focused on the methicillin-resistant "variant" MRSA, the methicillin-susceptible counterpart (MSSA) remains a prime species in infections. The epidemiology of *S. aureus* , especially of MRSA, showed a rapid evolution in the last years. After representing a typical nosocomial multidrug-resistant pathogen, MRSA has recently emerged in the community and among farmed animals thanks to its ability to evolve and adapt to different settings. Global surveillance has shown that MRSA represents a problem in all continents and countries where studies have been carried out, determining an increase in mortality and the need to use last-resource expensive antibiotics. *S. aureus* can easily acquire resistance to antibiotics and MRSA is characteristically multidrug resistant. Resistance to vancomycin, the principal anti-MRSA antibiotic is rare, although isolates with decreased susceptibility are recovered in many areas. Resistance to the more recently introduced antibiotics, linezolid and daptomycin, has emerged; however, they remain substantially active against the large majority of MSSA and MRSA. Newer anti staphylococcal drugs have been developed, but since their clinical use has been very limited so far, little is known about the emergence of resistance.

2.10 MRSA in Africa

The prevalence of MRSA infection in Nigeria was less than 50%. This was consistent with the rate reported in most African countries. Available evidence suggested that the prevalence of MRSA infection in Nigeria has increased by 2.3 folds; from 18.3% in 2009 to 42.3% in 2013. However, the increase was not consistent as the prevalence dropped to 16.5% in 2010 and rose to 42.3% by 2013 (Brown et al., 2019). This finding was not in consonant with trend reported in South Africa where evidence showed a 12% reduction in the prevalence of MRSA

infection (from 36% in 2006 to 24% during 2007–2011). The result of the current review was in agreement with the trend reported in some African countries including; Tunisia (16%–41% between 2002 and 2007), and Algeria (35%–75% during the periods 2003 and 2009). The decrease in MRSA rate experienced in 2009 could be attributed to natural tendencies or variations in the study settings and specimens (Gurusamy et al., 2013). Decelerating trend of MRSA infection in South Africa could be attributed to the establishment of Antimicrobial Resistance Surveillance Network. A functional surveillance system tracks antimicrobial resistance, and identify areas where interventions are needed. Evidence indicated that the implementation of antimicrobial resistance control interventions including: developing national antimicrobial resistance control strategy, establishing antimicrobial resistance surveillance system, developing antimicrobial stewardship programmes, promoting adherence to clinical practice guidelines, and infection control interventions has reduced the trend of MRSA infection in some countries (Schenck et al., 2016). Nigeria has no national antimicrobial resistance containment strategy at the moment. There is also no established antimicrobial resistance surveillance system. However, efforts are made to develop and implement such interventions. Lack of these interventions coupled with poor infection control, and inappropriate use of antibiotics could explain the rising trend of MRSA infection in Nigeria (Liu et al., 2011).

2.11 MRSA in Nigeria

Available evidence demonstrated that the prevalence of MRSA infection in Nigeria has increased. The rate was reported as 18.3% in 2009, 16.5% in 2010 and 42.3% in 2013 (Sganga et al., 2016). It could be inferred based on these surveillance data that rate of MRSA infection has increased from 18.3% in 2009 to 42.3% by 2013 (representing a 2.3 fold increase). All the studies utilized Polymerase Chain Reaction (PCR); the gold standard

method, for detection of MRSA isolates. Surveillance data illustrated that the prevalence of MRSA infection in North-East Nigeria has declined from 12.5% in 2007 to 8% in 2012. Only one study reported incidence of MRSA infections in North-Central Nigeria and the rate was 13.1% (Loewen et al., 2017). There was no surveillance data from North-West Nigeria that was included in this review. Therefore, there are no data to demonstrate the trend of MRSA infections in this region. There was only one study that reported incidence of MRSA infection in South-South Nigeria. The prevalence of MRSA in that zone was 42.7%. No study from the South-East was included in this review. The prevalence of MRSA infection in the South-West increased from 20.2% during 2006–2007 to 47.4% by the year 2010 (Dumyati et al., 2017).

2.12 MRSA in Edo State

A study reported a total of seventy-five (75) isolates comprising fifteen (15) each collected from ear, urine, cervix, blood and wounds from Nigerian student. An agar disc diffusion test was used to measure the effects of antimicrobial agents against the bacteria isolates following standardized guidelines. Out of a total of 75 clinical isolates of *S. aureus* collected, 43 (57.3%) were resistant to methicillin with isolates obtained from ear infections showing the highest resistance pattern of 14.7% while the least was from urine sample with incidence of 5.3% (Raji et al., 2013). From the 43 isolates that showed resistance to methicillin, 36 (83.7%) were multidrug resistant to various classes of antibiotics tested. Solomon et al. (2019) reported Of the 120 nasal specimens, *S. aureus* was isolated in 80 (66.6%) with high colonization rate among Medical Laboratory Science students and 39 (48.8%) identified as MRSA with colonization rate found to be higher among medical laboratory science students (Medical Laboratory Science vs Pharmacy: 76.9% VS. 23.1%) and females found to have a higher prevalence of MRSA than males (female vs male: 64.1% vs. 35.9%) and Students

within the age group of 18-20 years had the highest prevalence of MRSA of 76.9%. *S. aureus* in this study was observed to be most susceptible to amoxicillin (81.5%) and least susceptible to Ceftazidime (6.2%). Osahon et al. (2020) also reported that forty-seven isolates (58.0%) were found to be methicillin-sensitive *Staphylococcus aureus* (MSSA), while 34 (42.0%) were methicillin-resistant *Staphylococcus aureus* (MRSA). ST152-MSSA (24.7%) and ST7-MRSA-V (19.8%) were the dominant groups identified, which were not genetically related to global predominant strains, but rather exhibited regional dominance. An interesting finding of the study was the presence of highly related strains in the region, which differed primarily in their methicillin resistance gene carriage, staphylococcal cassette chromosome mec (SCCmec), with 99.4–99.7% relatedness between the genomes of the strains within the MRSA–MSSA pairs.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Population

This study will be conducted in Benin City, capital of Edo State, two government-owned secondary hospitals (Stella Obasanjo Specialist Hospital and Central Hospital), and a tertiary hospital (University of Benin Teaching Hospital). For this study, however, we will recruit eligible study participants presenting at two consenting primary healthcare facilities, one of the two secondary hospitals and the federal tertiary hospital.

3.2 Collection of samples

Nasal sample will be obtained by inserting a swab into the anterior nares and rotating it along the mucous membrane. The swab sample container will be labelled containing information on study health facility and participant unique identification number. Additionally, urine sample will be collected using sterile, wide mouthed container with screw cap tops as per conventional procedures. Again, the urine sample will be labelled as before.

3.3 Inoculating the plate

Following collection of samples and extracting of saliva, the swab sticks were streaked on freshly prepared Nutrient and MacConkey agar and incubated at 24-48hours at 35c in an incubator to promote the growth of microorganisms

3.4 Isolation of microorganism

Standard bacteriological techniques were used to isolate, characterize and identify bacteria from the respective clinical samples. Criteria which include; morphological appearances as well as biochemical tests (Cheesbrough, 2006; Barrow and Feltham, 2003)

3.5 Biochemical tests for identification of bacterial isolates

Isolated organisms showing colonial and morphological characteristics were subjected to biochemical test such as, catalase test, oxidase test, motility test, microbial test, indole test, coagulase test and urase test

3.6.1 Gram's staining

Gram staining is a differential staining technique that separates bacteria into two groups; Gram positive and Gram negative organisms. A thin smear of the bacterial culture was made on a glass slide, allowed to dry and heat fixed by pass it over a flame. After heat fixing it was flooded with crystal violet for one minute and then rinsed with clean water. After rinsing with water Grams iodine solution was added and kept for one minute. It was washed with water and then decolourised with 95% ethyl alcohol for 20 seconds. The alcohol rinsed off with water and then counter stained with Safranin for one minute. The counter stain dye was then rinsed with water, and the slide was blotted dry with filter paper or left standing on a rack for water to drain off (Thairu *et al*., 2014).

3.6.2 Indole Test

A sterilized test tubes containing 4 ml of tryptophan broth was taken. This was inoculated aseptically by taking the growth from a 18 to 24 hours culture. This was then incubated at 37°C for 24-28 hours. After growth was observed, 0.5 ml of Kovac's reagent to the broth

culture. Formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent indicated a positive reaction. No color change even after the addition of appropriate reagent indicated a negative reaction (Maria, 2009) .

3.6.3 Oxidase Test

A strip of Whatman's No. 1 filter paper was soaked in a freshly prepared 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride. After which the strip was laid in a petri dish and moistened with distilled water. The colony tested was picked up with a platinum loop and smeared over the moist area. A positive reaction was indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds.

3.6.4 Catalase Test

A loop or sterile wooden stick was used to transfer a small amount of colony growth on the surface of a clean, dry glass slide. A drop of 3% H_2O_2 was placed in the glass slide. A positive result yielded copious bubbles production or active bubbling, while a negative result yielded no or very few bubbles production.

3.6.5 Urease Test

A sterilized test tubes containing 4 ml of diluted urease agar was taken. 1ml of preheated urea solution was added to it. This was inoculated aseptically by taking the growth from a 18 to 24 hours culture. This was then incubated at 37°C for 24-28 hours. Colour change from the initial solution indicated a positive reaction and no color change indicated a negative reaction.

3.6.6 Motility Test

A sterile needle was used to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. It was ensured that the needle kept in the same line as it entered as it is removed from the medium. Incubation was done at 35°C for 18 hours or until growth is evident. A positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation. The MIL medium is a multitest medium used to test for motility while simultaneously determining other metabolic characteristics.

3.6.7 Coagulase test

Coagulase is an enzyme that clots blood plasma. This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*. Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus species*.

3.6.8 Citrate Utilization Test

The slant was streak back and forth with a light inoculum picked from the center of a well-isolated colony. It was then incubated aerobically at 35 to 37C for up to 4-7 days.

A positive reaction gave growth with color change from green to intense blue along the slant while a negative reaction gave no growth and no color change; Slant remains green.

3.7 Antimicrobial susceptibility tests

The collected samples was examined for adequacy and quality by an experienced microbiologist. Nose swabs will be examined for the presence of MRSA. Specifically, an enhancement broth [Tryptic soy broth supplemented with 2,5% NaCl, 3,5 mg/L Cefoxitin and

20 mg/L Aztreonam] was inoculated with 100 µL from the swab media and incubated at 35–37 °C for 16–24 hours. Subsequently, a selective chromogenic medium [e.g. ChromAgar MRSA II agar] was inoculated with 100 µL of the enhancement broth and incubated for an additional 42–48 hours in atmospheric conditions. Possible isolates of *Staphylococcus aureus* was identified using standard microbiological techniques, while MRSA was identified using either Oxacillin disc or Cefoxitin disc as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST, eucast.org); where feasible.

CHAPTER FOUR

4.1 RESULTS

Social demographic data

Social demographic data, health facility characteristics, microbiological assessment and potential risk factors for AMR are illustrated in Table 4.1

Table 4.1 illustrate the of distribution of participants' sociodemograhpic result such as age categories of participant are 6-17, 18-24, 25-34, 35-49 and ≥50.

The result shows that age 18 – 24 years had the highest participation 104(44.6%) and age 50 and above had the last participation 11 (4.7%).

The sex distribution of the participant, it indicate that female were 157 (67.4%) and male 76 (32.6%) participants

The ethnicity of the participant were ; other (e.g. Yoruba, Igbo, Urhobo etc.) 102 (43.8%) Bini 98 (42.1%), Ishan 28 (12.0%) and Afemai 5 (2.2%).

Among the participants, 47(20.2%) are Student, 39 (16.7%) are in Business, 14 (6.0%) are Housewife, 8 (3.4%) are Teacher and Driver, 7 (3.0%) are Caterer, 5 (2.2%) are Factory worker, 3 (1.3%) are Farmer Other works (e.g., mechanic etc.) 100 (42.9%) and 2 (0.9%) Not working yet.

The religious affiliation of the respondents are Christian 230 (98.7%) and Muslim 3 (1.3%)

The self-reported income per month (Naira) of the participant are; 64 (27.5%) had income of ≤₦ 10,000, 92 (39.5%) had income of between ₦11,000-90,999, 7 (3.0%) had income ≥ ₦ 100,000 and Missing was ₦70 (30.00%).

Health facility characteristics

In the course of carrying out this research the following health facilities were visited and the number and percentage of respondents are;

Central hospital 124 (53.2%), Evbuotubu primary health centre 36 (15.5%) and Evbuogida primary health centre 73 (31.3%).

Among the health facilities 109 (46.8%) were Primary health facilities and 124 (53.2%) were Secondary

Microbiological assessment

Samples collected from the participants are Nasal swab 61 (26.2%), Urine 68 (29.2%) and Both nasal swab and urine104 (44.6%)

Among participant *S. aureus* was not detected in 74(31.8%) participant, it was detected among 91(39.1%) and was not applicable to 68(29.2%).

The detection of methicillin resistant *Staphylococcus aureus* (MRSA) indicated that MRSA was not detected among 82 (35.2%), it was detected among 83 (35.6%) and not applicable among 68 (29.2%)

Potential risk factors for AMR

The Potential risk factors for antimicrobial resistance (AMR) among the participant was studied it revealed that 215 (92.3%) had no Tattoo / piercing on body while 18 (7.7%) of participant had tattoo / piercing on body.

The study revealed that 167 (71.7%) participants had no contact with farm produce (e.g. poultry) in the last 1 month while 66 (28.3%) participants had no contact.

The study revealed that 212 (91.0%) participants had been hospitalised (at least 1 night) in the last 6 months while 21 (9.0%) participants had not been hospitalized.

The study revealed that 228 (97.9%) participants had received an invasive surgery in the last 6 months (e.g. poultry) in the last 1 month while 5 (2.1%) participants had no invasive surgery.

The study revealed that 230 (98.7%) are not recipient of draining tube during hospitalisation while 3 (1.3%) are recipient.

The study revealed that 223 (95.7%)are not recipient of nutrition tube during hospitalisation while 10 (4.3%) are recipient.

The study revealed that 186 (79.8%) are have no ulcer while 47 (20.2%) have ulcer.

The study revealed that 220 (94.4%) are have no abscesses while 13 (5.6%) have abscesses.

In the study that70 (30.0%) are have no a contact with farm/market for more than 10 years while 163 (70.0%) have contacts.

Among the participant, the study revealed that 171 (73.4%) have no history of diarrhoea in the last 3 months while 62 (26.6%) have history of diarrhoea in the last 3 months.

The participants respondents that 195 (83.7%) have no eczema/chronic skin disorder while 38 (16.3%) have eczema/chronic skin disorder.

The participants responses revealed that 14 (6.0%) have not taken antibiotic within the last 6 months while 219 (94.0%) have taken antibiotic within the last 6 months.

Responses from the participant revealed that 14 (6.0%) have not taken self-medicate for common illness (e.g. malaria) while 219 (94.0%) have taken self-medicate for common illness.

Responses from the participant revealed the frequency of self-medication in a year which are 55 (23.6%) have taken self-medication in a year once, 80 (34.3%) have taken self-medication in a year twice, 46 (19.7%) have taken self-medication in a year thrice, 17 (7.3%) have taken self-medication in a year more than thrice and 35 (15.0%) have taken self-medication in a year unknown.

Responses from the participant revealed the 227 (97.4%) of the participant have Water cistern while 6 (2.6%) have Pit latrine.

Table 1: Baseline characteristics of the study participants

Variable	Frequency (%)
Sociodemographic characteristics	
Mean (SD) age, year	27.4 (9.4)
Age category, year	
6-17	8 (3.4)
18-24	104 (44.6)
25-34	85 (36.5)
35-49	25 (10.7)
≥50	11 (4.7)
Sex	
Female	157 (67.4)
Male	76 (32.6)
Ethnicity	
Other (e.g. Yoruba, Igbo, Urhobo etc.)	102 (43.8)
Bini	98 (42.1)
Ishan	28 (12.0)
Afemai	5 (2.2)
Occupation	
Student	47 (20.2)
Business	39 (16.7)
Housewife	14 (6.0)
Teacher	8 (3.4)
Driver	8 (3.4)
Caterer	7 (3.0)
Factory worker	5 (2.2)
Farmer	3 (1.3)
Other works (e.g., mechanic etc.)	100 (42.9)
Not working yet	2 (0.9)
Religion	
Christian	230 (98.7)
Muslim	3 (1.3)
Self-reported income per month, Naira	
≤10,000	64 (27.5)
11,000-90,999	92 (39.5)
≥100,000	7 (3.0)
Missing	70 (30.00
Health facility characteristics	
Name of health facility	
Central hospital	124 (53.2)
Evbuotubu primary health centre	36 (15.5)
Evbuogida primary health centre	73 (31.3)
Health facility type	
Primary	109 (46.8)
Secondary	124 (53.2)
Microbiological assessment	
Sample type	
Nasal swab	61 (26.2)

Urine	68 (29.2)
Both nasal swab & urine	104 (44.6)
Detection of *S. aureus*	
No	74 (31.8)
Yes	91 (39.1)
Not applicable	68 (29.2)
Detection of MRSA in sample	
No	82 (35.2)
Yes	83 (35.6)
Not applicable	68 (29.2)
Potential risk factors for AMR	
Tattoo/piercing on body	
No	215 (92.3)
Yes	18 (7.7)
Contact with farm produce (e.g. poultry) in the last 1 month	
No	167 (71.7)
Yes	66 (28.3)
Hospitalised (at least 1 night) in the last 6 months	
No	212 (91.0)
Yes	21 (9.0)
Received an invasive surgery in the last 6 months	
No	228 (97.9)
Yes	5 (2.1)
Recipient of draining tube during hospitalisation	
No	230 (98.7)
Yes	3 (1.3)
Recipient of nutrition tube during hospitalisation	
No	223 (95.7)
Yes	10 (4.3)
Ulcer	
No	186 (79.8)
Yes	47 (20.2)
Abscesses	
No	220 (94.4)
Yes	13 (5.6)
Contact with farm/market for more than 10 years	
No	70 (30.0)
Yes	163 (70.0)
History of diarrhoea in the last 3 months	
No	171 (73.4)
Yes	62 (26.6)
Eczema/chronic skin disorder	
No	195 (83.7)
Yes	38 (16.3)
Taken antibiotic within the last 6 months	
No	14 (6.0)
Yes	219 (94.0)
Self-medicate for common illness (e.g. malaria)	

No	14 (6.0)
Yes	219 (94.0)
Frequency of self-medication in a year	
Once	55 (23.6)
Twice	80 (34.3)
Thrice	46 (19.7)
>Thrice	17 (7.3)
Unknown	35 (15.0)
Toilet type	
Water cistern	227 (97.4)
Pit latrine	6 (2.6)

‡: All the study participants were outpatients who presented to non-emergency health facilities

AMR=Antimicrobial resistance

Distribution of *S. aureus* by participants' sociodemographic characteristics are shown in Table 4.2

The age category year indicate distribution of *S. aureus* among the different age groups of participants that 0 (0.0%) of age 16 – 17 age have no *S. aureus* while 1(2.6%) have *S. aureus*. 10(43.5%) of age 18 – 24 have no *S. aureus* while 23 (60.55) have *S. aureus*. 6 (26.1%) of age 25 – 34 have no *S. aureus* while 6 (15.8%). 5 (21.7%) of age 35 – 49 have no *S. aureus* while 5 (13.2%) have *S. aureus* 2 (8.7%). 2 (8.7%) of age 50 and above have no *S. aureus* while 3 (7.9%) have *S. aureus.*

The sex distribution of the participant, it indicate that 7 (30.4%) of female do not *S. aureus* while 23 (60.5%) of female have *S. aureus*. Among the male participants, 16 (69.6%) have *S. aureus* while 15 (39.5%) male have *S. aureus.* The income level per month (Naira) which was self reported by participants indicated that 2 (8.7%) earned ≤₦10,000 and do not *S. aureus,* while 12 (31.6%) of them have *S. aureus.* 13 (56.5%) earned ₦11,000-90,999 and do not *S. aureus* while 12 (31.6%) of them have *S. aureus.* 2 (8.7%) earned ≥₦100,000 do not *S. aureus* while of them have *S. aureus* while 0 (0.0%) of them have *S. aureus.* While 6 (26.1%) missing/did not respond to question while 14 (36.8%) of them have *S. aureus*

Table 4.2: Distribution of *S. aureus* by participants' sociodemographic characteristics

Variable	*S. aureus [N=61]*		
	No **[n=23 (%)]**	**Yes** **[n=38 (%)]**	**p-value**
Age category, year			
6-17	0 (0.0)	1 (2.6)	0.591
18-24	10 (43.5)	23 (60.5)	
25-34	6 (26.1)	6 (15.8)	
35-49	5 (21.7)	5 (13.2)	
≥50	2 (8.7)	3 (7.9)	
Sex			
Female	7 (30.4)	23 (60.5)	0.023
Male	16 (69.6)	15 (39.5)	
Self-reported income per month, Naira			0.026
≤10,000	2 (8.7)	12 (31.6)	
11,000-90,999	13 (56.5)	12 (31.6)	
≥100,000	2 (8.7)	0 (0.0)	
Missing/did not respond to question	6 (26.1)	14 (36.8)	

All the study participants were outpatients who presented to non-emergency health facilities

Distribution of methicillin resistant *Staphylococcus aureus* (MRSA) by participants' sociodemographic characteristics was shown in Table 4.3

The age category in years of participant for the distribution of MRSA shows that age 6 – 17 had 1(2.9%) of MRSA , age 18 – 24 had 21 (60.0%) of MRSA, age 25 – 34 had 5 (14.3%) of MRSA, age 35 – 49 had 5 (14.3%) of MRSA, age 50 and above had 3 (8.6%) of MRSA.

Among the respondents 22 (62.9%) of female had MRSA while 13 (37.1%) of male had MRSA.

The self-reported income level (in Naira) of participant that had MRSA are; 11 (31.4%) of the participant had income level lesser or equal to ₦10,000, 11 (31.4%) of the participant had income level between ₦ 11,000 - ₦ 90,999 while participant with income level greater than ₦ 100,000 were not specified.

Table 4.3: Distribution of *MRSA* by participants' sociodemographic characteristics

Variable	***MRSA*** **[n=38 (%)]**
Age category, year	
6-17	1 (2.9)
18-24	21 (60.0)
25-34	5 (14.3)
35-49	5 (14.3)
≥50	3 (8.6)
Sex	
Female	22 (62.9)
Male	13 (37.1)
Self-reported income per month, Naira	
≤10,000	11 (31.4)
11,000-90,999	11 (31.4)
≥100,000	-
Missing/did not respond to question	13 (37.1)

All the study participants were outpatients who presented to non-emergency health facilities

CHAPTER FIVE

5.1 DISCUSSION

According to our data, *S. aureus* isolation rate from the 130 samples was 82 (63.1%). Sex of the respondents was not associated with presence of *S. aureus*. This finding is similar to a recent finding from a study in Ethiopia. However, isolation rate was significantly higher in patients below 18 years and significantly lower in patients above 61 years. The observed age-related variation in the frequency of *S. aureus* isolation has been reported elsewhere]. Admittedly, it must be recognised that the referenced studies used different age groupings; therefore, the comparisons are at the general or trend level. More importantly, factors which contribute to the observed differences remain unclear.

Furthermore, the isolation rate was highest in discharges from wound and abscess (100%), and pus and blood samples had isolation rates of 62.1% and 40.0%, respectively. The higher frequency of *S. aureus* isolation in pus samples compared to blood samples has been reported in other studies in the region and elsewhere. Other investigators have however reported the converse (Abubakar and Sulaiman, 2018). The observed variation in proportion of *S. aureus* isolates has been attributed to differences in study design and study population. The high prevalence of isolates in surgical wound, diabetic, and burn patients has also been reported.

Existing literature on MRSA have demonstrated that there is a significant geographical variation in the frequency of the pathogen within and between countries (Aworh et al., 2019). In this study, out of the 82 *S. aureus* isolates, 59 (72%) were MRSA, 16 (19.5%) were MSSA, while the remaining 7 (8.5%) were MISA. The reported proportion is significantly higher than previously reported values (9%) from Eritrea (Bell et al., 2014). The value was also higher compared to values reported previously in other settings in the region. In sub-Saharan

Africa (SSA), several reviews have indicated that the prevalence of MRSA is between 25% and 50% or less than 25%. However, comparatively higher values have been reported in other settings in SSA: Algeria (surgical wounds) (75%)]; Egypt (cancer patients); Nigeria (wound) (73.8%) (Aworh et al., 2019); Rwanda (multiple sample types) (82%). High frequency of MRSA has also been reported in other parts of the world: Peru (80%) and in a setting in Colombia (90%). The intra- and inter country variation in prevalence of MRSA has been linked to several factors. These include differences in study design, types of the specimen, laboratory procedures, study population, and study duration, among others (Aibuedefe and Imuetiyan, 2018).

However, other studies have demonstrated that while the cefoxitin disk diffusion test have good performance, and oxacillin assays have acceptable sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Accordingly, we are confident that the comparatively higher values reported in this study are not due to an underperforming diagnostic method, suboptimal quality control during the study, or inadvertent methodological flaws. The high prevalence of MRSA in two major referral hospitals in the country should raise concern over possible inter-hospital transfer of colonized patients. Dissemination of MRSA in a country via inter-hospital transfer of colonised patients is a well-documented phenomenon. A study applying mathematical modelling to national data on patterns of patient referral and the first comprehensive molecular characterization undertaken in South African, Kwazulu Natal province, which profiled isolates from 14 hospitals, noted this to be the case. In fact, inter-hospital transfer of patients from regional health facilities to national public referral hospitals is a common practice in SSA, Eritrea included. The idea that these facilities can act as significant reservoirs of infection has implication of MRSA control strategies in the country. Another concerning finding relates to the nature of the patients sampled: surgical wound, burn, and diabetic mellitus (DM) patients. Often, these patients

have extended stays in general in-patient wards are at an increased risk of invasive bacterial diseases; hence; possible colonization by MRSA and implication on dissemination dynamics should be a foremost concern.

5.2 CONCLUSION

Antibiotic resistance is a growing public health crisis and the urgent need for local studies has been proposed. In this study, we presented data on the frequency of MRSA in three hospitals in Benin City. In contrast to a previous study, the present study found alarming levels. In this study, out of the 82 *S. aureus* isolates, 59 (72%) were MRSA, 16 (19.5%) were MSSA, while the remaining 7 (8.5%) were MISA. The reported proportion is significantly higher than previously reported values. The need for a comprehensive drug resistance surveillance and containment system. A comprehensive tracking system should be able to capture data on emerging AMR trends, report infections from different healthcare sectors (acute, long-term, ambulatory) and veterinary care across the country, and recognise high risk patients, among others. Information can subsequently be leveraged to design good infection control practices and optimal usage of antimicrobial agents in the country. We recognise that these propositions can only be implemented in the long term. Currently, reevaluation of existing infection control practices, implementation of more effective practices (screening of MRSA carriers, isolation or cohorting of patients, colonised healthcare workers, and environmental decontamination, among others) should suffice. Investment in laboratory infrastructure and allied personnel should also be prioritised.

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APPENDIX

MATERIALS

MacConkey agar

This medium is best prepared from ready to use dehydrated powder, available for most suppliers of culture media.

Contents: peptone, lactose, bile salts, sodium chloride, neutral red agar.

PREPARATION:

1. prepare as instructed by manufacturer's instructions sterilize by autoclaving at 121oc for 15 minutes
2. When the medium has cooled to 50-55oc, mix well and dispense aseptically in sterile Petri dishes. Date the medium and give it a batch number.
3. Store the plates at 2-8 preferably in plastic bags to prevent loss of moisture.
4. Shelf-life: up to 4weeks providing there is no change in the appearance of the medium to suggest contamination or an alteration of pH

MEDIA PREPARATION

NUTRIENT AGAR (NA)

The medium was used for the enumeration of bacterial cells and also to maintain pure cultures.

PREPARATION

The preparation involves measuring twenty-eight grams (28g) of the powder on a weighing

balance and suspending it into 1 litre of distilled water (or equivalent w/v for lower volumes of distilled water). This was boiled over a Bunsen burner to dissolve completely and subsequently sterilized by autoclaving at 121oc for 15minutes. After cooling to about 47oC, the sterile molten medium was distributed i.e. about 20ml each into sterile Petri dish.

COMPOSITION:

The medium composed of the following

Beef extract 3.0g/l

Peptone 5.0g/l

Sodium Chloride 8.0g/m

PH 7.3 + 0.2

Batch number 110/24/147

Expiring date 2014/05

The medium was used to enrich and develop the innocular that were used to inoculate the agar plates. It was also used to maintain cultures for some biochemical tests.

MACCONKEY AGAR

Peptone	20.0g
Lactose	10.0g
Bile Salt	5.0g
Sodium chloride	5.0g
Neutral Red	0.75g
Agar	12.0g
Distilled water	1000ml

49g of MacConkey powder was dissolved in 1 litre of water and autoclaved at 121˚C for 15 minutes. It was then allowed to cool to 50-55˚C after which it was dispensed aseptically into Petri dishes.

Motility test medium (3)

Yeast extract	3.0 g
Peptone	10.0 g
Tryptone	10.0 g
L-ornithine HCl	5.0 g
Dextrose	1.0 g
Bromcresol purple	0.02 g
Agar	2.0 g

Bring to 1 liter with distilled water and heat to boiling to dissolve agar. Dispense in 5-ml aliquots in screw-top test tubes. Autoclave at 121°C under 15 psi pressure for 15 minutes.