# A CROSS-SECTIONAL STUDY ON THE PREVALENCE OF METHICILLIN-RESISTANT *Staphylococcus aureus* AMONG OUTPATIENTS PRESENT AT EVBUOTUBU PRIMARY HEALTH CENTRE IN BENIN-CITY, EDO STATE, NIGERIA.

**BY:**

**Rejoice Aisosa JOHN (Miss)**

**LSC1605453**

**A PROJECT SUBMMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENECES, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF BSc (HONS) IN MICROBIOLOGY**

**JUNE, 2021.**

## CERTIFICATION

This is to certify that this work was carried out by in the **Rejoice Aisosa JOHN (Miss)** with the matriculation number **LSC1605453** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

_____ _____

**MR A. DUNKWU-OKAFOR** Date

(Project Supervisor)

## APPROVAL

This project work was carried out by **Rejoice Aisosa JOHN (Miss)** in partial fulfillment of the award of a Bachelor of Science, (B.Sc) degree in the Department of Microbiology, University of Benin, Benin City.

_____ _____

**Prof. S.E. OMONIGHO** Date

(Head of department)

## DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

## ACKNOWLEDGEMENT

This research project is a complex entity and emerges from the contributions of many people.

I would like to express my deep gratitude to my parents, Mr. and Mrs. Osayande. They were very supportive throughout the period I carried out this research work and their advices were very crucial in carrying out this research.

My appreciation also goes to my lecturer, seminar as well as project supervisor, Mr. A. Dunkwu-Okafor for his guidance and enlightenments before, during and after my research work.

**TABLE OF CONTENTS** **PAGES**

## LIST OF TABLES

## LIST OF APPENDIX

## ABSTRACT

Antimicrobial resistance is mainly caused by the overuse, underuse or misuse of antimicrobials. Antimicrobial resistance (AMR) is defined as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism The present study was designed to assess the prevalence of MRSA among outpatients presenting Evboutubu primary health center, Egor LGA Benin city, Nigeria. A total of 11 urine and swab samples were studied between February 2021 to April 2021. This study was extrapolated from a wider study of MDRE and MRSA prevalence among patients in health centers in Benin-city, Nigeria. Microbial enumeration and identification were carried out using cultural and biochemical methods. Microbial counts were carried out using cultural characterization, Gram staining techniques and biochemical tests including; oxidase, indole, sugar fermentation, citrate, motility, methyl red, catalase, urease and antimicrobial susceptibility test (using Kirby-Bauer disc diffusion technique). Most of the samples tested were positive for *S. aureus* and MRSA. From the study, it was observed that, patients between the ages of 18-24 and 25-34 had the highest percentage of MRSA (45.5%), while females had a higher percentage (54.6%) than males, other works had the highest frequency of 36.4% and Patients receiving between 11,000-99,999 had the highest percentage of 77.8%. Findings from this study revealed that both *S. aureus* and MRSA had high prevalence in the distribution by participants' social demographic characteristics. Therefore, MRSA poses a potential public health risk and Antimicrobial sensitivity and resistance must ultimately be understood at the population, organism, cellular, and molecular levels if antimicrobial resistance is to be reduced.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Overview

Antimicrobials are substances of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host. All agents that work against bacteria (antibacterial), viruses (antiviral), fungi (antifungal), and protozoa (antiprotozoan) are referred to as "antimicrobials" (Bhaskar and Sanjukta, 2017).

Antimicrobials have revolutionized man's approach to the care, control, and deterrence of human and animal infectious diseases since their introduction. As catastrophic disease outbreaks were managed and previously lethal infections became clinically manageable, the modern antibiotic period significantly improved survival rates and longevity. Overall, these changes greatly improved the quality of human life and animal welfare (Onofrey and Bruce, 2017).

The emergence and spread of antimicrobial resistance, on the other hand, has become a major issue. This global epidemic has posed the frightening prospect of future generations returning to a time before antibiotics, when common diseases were frequently fatal due to a lack of effective treatments. The prevalence of antibiotic-resistant bacteria and genes increases in response to the selective pressure provided by antibiotic use, according to medical history and science (Onofrey and Bruce, 2017).

The World Health Organization defines antimicrobial resistance (AMR) as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism (W.H.O*., 2014*). AMR is the formation (by a disease-causing microbe) of the ability to survive exposure to an antimicrobial agent that was previously an effective treatment by mutation or gene transfer. Antimicrobial resistance is just one of the many adaptive traits that resilient bacterial subpopulations may possess or acquire, enabling them to out-compete and out-survive their microbial neighbors and overcome host strategies aimed against them.

WHO report released April 2014 stated, "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect

anyone, of any age, in any country. Antibiotic resistance—when bacteria change so antibiotics no longer work in people who need them to treat infections—is now a major threat to public health." (W.H.O., 2014). In 2018, WHO considered antibiotic resistance to be one of the biggest threats to global health, food security and development (W.H.O., 2020).

After several decades in which it appeared that human ingenuity had outwitted the pathogens, multidrug-resistant "superbugs" have become a global challenge, aided and abetted by the use, misuse, and overuse of once highly effective anti-infective drugs. (Lederberg, 2000).

Antimicrobial resistance (AMR) develops when, over time, bacteria, viruses, fungi and parasites alter and no longer respond to drugs that make infections more difficult to treat and increase the risk of spread of disease, serious disease and death. (W.H.O., 2020)

Our ability to treat common infections continues to be challenged by the advent and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance. The rapid global spread of multi-and pan-resistant bacteria that cause infections that are not treatable with current antimicrobial drugs, such as antibiotics, is particularly concerning.

Antimicrobial resistance is mainly caused by the overuse, underuse or misuse of antimicrobials. This leads to microbes either evolving a defense against drugs used to treat them, or certain strains of microbes that have a natural resistance to antimicrobials becoming much more prevalent than the ones that are easily defeated with medication. Possibility remains that much of the issue stems from the unsafe and wasteful use of antimicrobials, and that practicing cautious and judicious antimicrobial use is one of the most effective countermeasures. While antimicrobial resistance does occur naturally over time, the use of antimicrobial agents in a variety of settings both within the healthcare industry and outside of has led to antimicrobial resistance becoming increasingly more prevalent (*Holmes et al., 2016*).

## 1.2 AIMS AND OBJECTIVES

The purpose of this project is to provide a detailed cross-sectional study on the prevalence of methicillin resistant *Staphylococcus aureus* among outpatients presenting to Evbuotubu primary health centre in Benin City, Nigeria. This project would explicitly discuss the following goals:

- To describe the prevalence of MRSA carriage in patients.
- To describe the occurrence of simultaneous patient carriage with MRSA.
- To identify risk factors for MRSA and carriage among patients under investigation.
- Based on the identified risk factors, to develop screening tools for identification of both MRSA carriage in the study setting.

## 1.3 JUSTIFICATION OF STUDY

AMR poses a huge challenge on national budgets and healthcare systems. The antibiotic discovery meant that previously lethal infections could be treated and surgical procedures made safer, enabling modern medicine to be developed. We are now, with the rise of AMR, Moving into a 'post-antibiotic age' where it could again be lethal for common infections.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 HISTORY

Many ancient cultures used molds, soil, and plants to treat bacterial infections. In Ancient Serbia, China and Greece, old moldy bread was pressed against wounds to prevent infection. In Egypt, crusts of moldy wheaten bread were applied on pustular scalp infections and “medicinal earth” was dispensed for its curative properties (Keyes *et al.,* 2003).

Egyptians were known to use a concoction of honey, lard, and lint to treat wounds around 1550 BC. We already know that honey contains significant quantities of hydrogen peroxide, which is capable of killing bacteria. The spirits or gods responsible for sickness and pain is thought to be influenced by these remedies. We now know that the active metabolites and chemicals found in these concoctions were responsible for the early treatments' sporadic efficacy (Keyes *et al.,* 2003).

People living before the antibiotic period depended on a number of relatively untested treatments to manage their illnesses, owing to the devastating effects of infectious diseases and the absence of any other successful cures. These treatments had a wide range of effectiveness and protection, and often had little effect on the cure or relief of disease symptoms, but they were patented and used by desperate people who had no other options (T.E.C. Jr., 1970).

Godfrey’s Cordial (also called Mother’s Friend) and Dalby’s Carminative were among the most widely used patent medicines given to infants and children in England and the United States during the latter years of the 18th and early part of the 19th centuries. Both preparations were used-almost always without a physician’s advice for a wide variety of symptoms ranging from run of-the mill fretfulness and colic, to the severest forms of dehydration caused by explosive, bloody diarrhea. Despite their innocuous names, they were sinister preparations because of their opium content; Godfrey’s Cordial contained one grain of opium in each two ounces; Dalby’s Carminative contained 3ı grain of opium in the same amount. As a result many infants died of opium poisoning during this time (T.E.C. Jr., 1970).

Weapons against bacterial diseases improved just before the turn of the 20th century. The advent of the germ theory of disease, which proposed that microorganisms are the causes of many diseases, caused a revolutionary change in the understanding of the vital role of microbes in infectious diseases. Specific microbial pathogens were identified as the causative agents of many diseases, and a race immediately began to find effective means to kill these implicated microbes (Levy, 2002).

The first recorded microbial by-product shown to have antimicrobial activity was the blue pigment from Bacillus pyocyaneus (now Pseudomonas aeruginosa) which stopped the growth of some kinds of bacteria in the test tube. This was serendipitously observed by E. de Freudenreich (Germany) in 1888. Rudolf Emmerich and Oscar Loew (Germany), who later named the substance "pyocyanase", performed clinical trials in 1889 showing some effectiveness against many of the infectious diseases of that time. This understandably raised excitement in the scientific community, however, this compound's instability and inherent toxicity in patients later made it clear that pyocyanase had no real clinical application, and thus its popularity eventually declined (Levy, 2002).

Another German physician, named Paul Ehrlich, tirelessly searched for a "magic bullet" that could selectively kill microorganisms. After several failures, in 1910 he finally came up with an arsphenamine chemical dye they referred to as compound 606 and later named Salvarsan - the first chemical compound shown to cure a human disease, syphilis. (Schwartz *et al.*, 2004).

Alexander Fleming, more notable for his discovery of penicllin in the later years, reported in 1920 of a naturally occurring antibacterial substance in human tears that causes lysis in some bacterial cells. He later called this lysozyme (Hare, 1983). Unfortunately, this too did not realize clinical application because of its limited effect on mostly non-pathogenic bacteria, and because it could not be produced in quantities large enough for further trials (Levy, 2002).

In 1928, Fleming's major medical breakthrough came about as he serendipitously discovered penicillin, later to be claimed as the miracle drug of the 20th century. However, the impact of this discovery was not realized until the 1940s, when its applicability as a therapeutic agent was made possible by Florey and Chain. Blamed for this delay was the lack of biochemical and microbiological expertise at that time, as well as the lack of interest and support from the

scientific community brought about by previous experience with the failure of pyocyanase and the toxicity of Salvarsan (Hare, 1983)

In 1935, a breakthrough that ushered the era of antibacterials was made by the German biochemist Gerhard Domagk at the Bayer Laboratories of the I.G. Farben company in Germany. He discovered and developed the first sulfonamide, a synthetic red dye more popularly known by its trade name of Prontosil, the first commercially available antibacterial (Otten, 1986).

Inspired by the groundbreaking work of Domagk (with sulfa) and Fleming, Florey and Chain (with Penicllin), a number of subsequent antimicrobial discoveries quickly followed. To this day, newer antimicrobial compounds continue to be discovered and introduced, although the rate has slowed considerably (Otten, 1986).

Antimicrobial resistance warning signals were discovered only a few years after the golden age of antimicrobials. Antimicrobial resistance is being countered by microorganisms at an unprecedented rate, according to new research.

Based on his laboratory observations, the famed penicillin discoverer Alexander Fleming had predicted in 1945 that misuse of this discovery could lead to the election and propagation of mutant forms of bacteria resistant to the drug. He warned that too small doses that fail to completely clear the infection would breed microbes trained to resist the drug, which could then eventually be passed on to other susceptible individuals. Against this warning, penicillin was eventually made freely available to the public, driven by the public clamor for this “miracle drug” and the business opportunities that came along with this medical breakthrough. Various preparations of salves, lozenges, nasal ointments and even cosmetic creams were sold over-the-counter. And true enough, as Fleming correctly foretold, bacterial resistance to penicillin slowly but steadily built up over the years, to the point that by 1955, most countries restricted the use of penicillin as “by prescription” only. However, the uncontrolled usage was already widespread, and so is the observed resistance in several bacterial pathogens, particularly staphylococci (Aarestrup *et al.,* 2001).

A concerted effort was exerted by pharmaceutical companies to thwart this resistance, which eventually led to the discovery and introduction in the early 1960s of a semisynthetic penicillin, called methicillin, which was insensitive to the bacterial enzymes that degrade penicillin.

Although this seemed to have initially controlled penicillin resistance in the years that followed, the subsequent emergence of resistance to methicillin, such as that seen in methicillin resistant Staphylococcus aureus (MRSA), is now a current problem faced in hospitals worldwide (Bennett, 2015).

This resistance phenomenon is not restricted to penicillin alone. The same was observed for the other antibiotics which were subsequently discovered and made commercially available to the public in the latter half of the 20th century. In the recent years, this was made even more complicated by the fact that the observed development of antimicrobial resistance has superseded the pace at which discoveries and development of better antibiotic treatments are made. It is feared that if this is not addressed properly in time, the world will be back to the pre-antibiotic era when currently treatable infectious conditions such as pneumonia, diarrhea, or even wound infections, will eventually be considered as life-threatening due to the lack of available effective treatment in the medical arsenal. This has become one of the major medical issues of concern in the 21st century. What is most alarming today is the rate at which antibiotic resistance often develops and how quickly it spreads across the globe and among different species of bacteria (MacGowan, 2008).

## 2.2 BACTERIA

Bacteria are an integral part of the world and are ubiquitous to every habitat on Earth, adapting readily to shifts in environmental parameters by means of a short generation period, from minutes to hours. These adaptive capabilities, in fact, account for the ease with which microorganisms respond to culture conditions in the laboratory, which are often radically different from the natural habitat of the organism. Most of these microorganisms are harmless. Some are symbiotic and actually protect the host from even more harmful bacteria. However, the emergence of bacterial pathogens that are resistant to medically important antimicrobial drugs is recognized as a significant public health concern (Tadesse *et al.,* 2017). Infections that were once cured by the introduction of an antibiotic are now more difficult to combat because of resistance. Antimicrobial resistance develops as a natural consequence of the bacterial

population's ability to adapt. Bacteria's continued exposure to antibiotics has resulted in this inevitable resistance to individual and multiple antimicrobial agents in many types of bacteria.

## 2.3 CLASSIFICATION OF ANTIBACTERIAL AGENTS

Antimicrobials are classified in several ways, including:

1. Spectrum of activity

2. Effect on bacteria

3. Mode of action

### 2.3.1 CLASSIFICATION ACCORDING TO SPECTRUM OF ACTIVITY.

Depending on the range of bacterial species susceptible to these agents, antibacterial are classified as:

- Broad-spectrum antibacterial
- Narrow- spectrum antibacterial

**BROAD-SPECTRUM ANTIBACTERIAL** are active against the two major bacterial groups, Gram-positive and Gram-negative *(Ory and Yow, 1963)* or any antibiotic that acts against a wide range of disease-causing bacteria *(Clayton and Thomas, 1993)*. Examples include: tetracyclines, phenicols, fluoroquinolones, "third-generation" and "fourth-generation" cephalosporins.

**NARROW-SPECTRUM ANTIBACTERIAL** are limited in activity and are primarily only useful against particular species of microorganisms. For example, glycopeptides and bacitracin are only effective against Gram-positive bacteria, whereas polymixins are usually only effective

against Gram negative bacteria. Aminoglycosides and sulfonamides are only effective against aerobic organisms, while nitroimidazoles are generally only effective for anaerobes (Denyer *et al.,* 2011)

### 2.3.2 EFFECTS ON BACTERIA

Due to variations in the mechanisms by which antibiotics affect bacteria, clinical application of antibiotics can have very different effects on bacterial agents, resulting in either bacterial inactivation or death.

**BACTERIOCIDAL DRUGS** are those that kill target organisms. Examples of bactericidal drugs include aminoglycosides, cephalosporins, penicillins, and quinolones.

**BACTERIOSTATIC DRUGS** inhibit or delay bacterial growth and replication. Examples of such include tetracyclines, sulfonamides, and macrolides.

Depending on the dosage, length of exposure and condition of the invading bacteria, certain antibiotics may be both bacteriostatic and bactericidal. For example, aminoglycosides, fluoroquinolones, and metronidazole exert concentration-dependent killing characteristics; their rate of killing increases as the drug concentration increases (Denyer *et al.,* 2011).

### 2.3.3 MODE OF ACTION

Because of the nature of their structure and degree of affinity for specific target sites within bacterial cells, different antibiotics have different modes of action.

1. Inhibitors of cell wall synthesis. While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacterial species. A drug that targets cell walls

can therefore selectively kill or inhibit bacterial organisms. Examples: penicllins, cephalosporins, bacitracin and vancomycin.

2. Inhibitors of cell membrane function. Cell membranes are important barriers that segregate and regulate the intra- and extracellular flow of substances. A disruption or damage to this structure could result in leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can often be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymixin B and colistin.

3. Inhibitors of protein synthesis. Enzymes and cellular structures are primarily made of proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication. Examples: Aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines.

4. Inhibitors of nucleic acid synthesis. DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin.

5. Inhibitors of other metabolic processes. Other antibiotics act on selected cellular processes essential for the survival of the bacterial pathogens. For example, both sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind to dihydropteroate synthase, trimethophrim inhibit dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans.

(Tripathi, 1994).

## 2.4 BACTERIAL RESISTANCE STRATEGIES

To survive in the presence of an antibiotic, bacterial organisms must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial agent. The intended modes of action of antibiotics may be counter-acted by bacterial organisms via several different means. This may involve preventing antibiotic access into the bacterial cell or perhaps removal or even degradation of the active component of the antimicrobial agent. No single mechanism of resistance is considered responsible for the observed resistance in a bacterial organism. In fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent. Four major bacterial resistance strategies:

1. By prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell
2. By expulsion of the antimicrobial agents from the cell via general or specific efflux pumps
3. By inactivation of antimicrobial agents via modification or degradation
4. By modification of the antimicrobial target within the bacteria

(Watts, 2006)

**2.4.1 By prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell:** Antimicrobial compounds almost always require access into the bacterial cell to reach their target site where they can interfere with the normal function of the bacterial organism. Porin channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls.

This strategy has been observed in:

- Pseudomonas aeruginosa against imipenem (a beta-lactam antibiotic)
- Enterobacter aerogenes and Klebsiella spp. against imipenem
- Vancomycin intermediate-resistant S. aureus

**2.4.2 By expulsion of the antimicrobial agents from the cell via general or specific efflux pumps:** for this to be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria possess membrane proteins that act as an export or efflux pump for certain antimicrobials, extruding the antibiotic out of the cell as fast as it can enter. This results in low intracellular concentrations that are insufficient to elicit an effect.

This strategy has been observed in:

- E.coli and other Enterobacteriaceae against tetracyclines
- Enterobacteriaceae against chloramphenicol
- Staphylococci against macrolides and streptogramins
- Staphylococcus aureus and Streptococcus pneumoniae against fluoroquinolones

These efflux pumps are variants of membrane pumps possessed by all bacteria, both pathogenic and non-pathogenic, to move lipophilic or amphipathic molecules in and out of the cells. Some are used by antibiotic producers to pump antibiotics out of the cells as fast as they are made, and so constitute an immunity protective mechanism for the bacteria to prevent being killed by their own chemical weapons.

.

**2.4.3 By inactivation of antimicrobial agents via modification or degradation:** Another means by which bacteria preserve themselves is by destroying the active component of the antimicrobial agent. A classic example is the hydrolytic deactivation of the beta-lactam ring in penicillins and cephalosporins by the bacterial enzyme called beta lactamase. The inactivated penicilloic acid will then be ineffective in binding to PBPs (penicllin binding proteins), thereby protecting the process of cell wall synthesis.

This strategy has also been observed in:

- Enterobacteriaceae against chloramphenicol (acetylation)
- Gram negative and Gram positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation)

Less than 10 years after the clinical introduction of penicillins, penicillin-resistant Staphylococcus aureus was observed in a majority of Gram-positive infections in people. The initial response by the pharmaceutical industry was to develop beta-lactam antibiotics that were unaffected by the specific beta-lactamases secreted by S. aureus. However, as a result, bacterial strains producing beta-lactamases with different properties began to emerge, as well as those with other resistance mechanisms. This cycle of resistance counteracting resistance continues even today (Bush, 1988).

**2.4.4 By modification of the antimicrobial target within the bacteria:** Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, in spite of the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place.

This strategy has been observed in:

- Staphylococci against methicillin and other beta-lactams (Changes or acquisition of different PBPs that do not sufficiently bind betalactams to inhibit cell wall synthesis.)
- Enterococci against vancomycin (alteration in cell wall precursor components to decrease binding of vancomycin)
- Mycobacterium spp. against streptomycin (modification of ribosomal proteins or of 16s rRNA)
- Mutations in RNA polymerase resulting in resistance to the rifamycins
- Mutations in DNA gyrase resulting in resistance to quinolones

(Watts, 2006)

## 2.5 MECHANISMS OF RESISTANCE

The abilities of bacterial organisms to utilize the various strategies to resist antimicrobial compounds are all genetically encoded. Intrinsic resistance is that type of resistance which is naturally coded and expressed by all (or almost all) strains of that particular bacterial species. An

example of intrinsic resistance is the natural resistance of anaerobes to aminoglycosides and Gramnegative bacteria against vancomycin. (Tang *et al.,* 2017).

Changes in bacterial genome through mutation or horizontal gene acquisition, on the other hand, may consequently lead to a change in the nature of proteins expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic. This is referred to as acquired resistance, which is limited to selected isolates of that particular species or group of microorganisms.

**2.5.1 INTRINSIC RESISTANCE** is the innate ability of a bacterial species to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics, which allow tolerance of a particular drug or antimicrobial class. This can also be called "insensitivity" since it occurs in organisms that have never been susceptible to that particular drug. Such natural insensitivity can be due to: lack of affinity of the drug for the bacterial target inaccessibility of the drug into the bacterial cell extrusion of the drug by chromosomally encoded active exporters innate production of enzymes that inactivate the drug. (Tripathi, 1994).

**2.5.1 ACQUIRED RESISTANCE** is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. This can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes or from a combination of these two mechanisms. Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of each particular bacterial species. Laboratory methods are therefore needed to detect acquired resistance in bacterial species that are not intrinsically resistant. These same methods are used for monitoring rates of acquired resistance as a means of combating the emergence and spread of acquired resistance traits in pathogenic and non-pathogenic bacterial species. Acquired resistance results from successful gene change and/or exchange that may involve: mutation or horizontal gene transfer via transformation, transduction or conjugation. (Wall *et al.,* 2016).

**MUTATION** is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait which it codes for. Any change in a single base pair may lead to a corresponding change in one or more of the amino acids for which it codes, which can then change the enzyme or cell structure that consequently changes the affinity or effective activity of the targeted antimicrobials. In prokaryotic genomes, mutations frequently occur due to base changes caused by exogenous agents, DNA polymerase errors, deletions, insertions and duplications. For prokaryotes, there is a constant rate of spontaneous mutation of about 0.0033 mutations per DNA replication that is relatively uniform for a diverse spectrum of organisms. The mutation rate for individual genes varies significantly among and within genes (Drlica, 2003).

**HORIZONTAL GENE TRANSFER** or the process of swapping genetic material between neighboring "contemporary" bacteria, is another means by which resistance can be acquired. Many of the antibiotic resistance genes are carried on plasmids, transposons or integrons that can act as vectors that transfer these genes to other members of the same bacterial species, as well as to bacteria in another genus or species. Horizontal gene transfer may occur via three main mechanisms: transformation, transduction or conjugation.

- **Transformation** involves uptake of short fragments of naked DNA by naturally transformable bacteria.
- **Transduction** involves transfer of DNA from one bacterium into another via bacteriophages.
- **Conjugation** involves transfer of DNA via sexual pilus and requires cell –to-cell contact. DNA fragments that contain resistance genes from resistant donors can then make previously susceptible bacteria express resistance as coded by these newly acquired resistance genes.

  Conjugation was first described in 1946 by Lederberg and Tatum, based on studies showing that the intestinal bacteria E.coli uses a process resembling sex to exchange circular, extrachromosomal elements, now known as plasmids.

  (Keyes *et al.,* 2003).

## 2.6 BIOLOGICAL VERSUS CLINICAL RESISTANCE

Biological resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent than has been previously observed. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is then said to have achieved clinical resistance. It is important to note that often, biologic resistance and clinical resistance do not necessarily coincide. From a clinical laboratory and public health perspective it is important to realize that biologic development of antimicrobial resistance is an ongoing process, while clinical resistance is dependent on current laboratory methods and established cut-offs. Our inability to reliably detect all these processes with current laboratory procedures and criteria should not be perceived as evidence that they are not occurring. (*Holmes et al.,* 2016).

## 2.7 DETECTING ANTIMICROBIAL RESISTANCE

Historically, veterinary practitioners prescribed antibiotics based on expected mode of action, spectrum of activity and clinical experience. With the emergence and spread of antimicrobial resistance, treatment of bacterial infections has become increasingly difficult and is no longer as straightforward as it was many years prior. Practitioners now need to consider that the particular pathogen they wish to treat may be resistant to some or all of the available antibiotics, thus making antimicrobial susceptibility testing a standard procedure. Antimicrobial susceptibility testing methods are in vitro procedures used to detect antimicrobial resistance in individual bacterial isolates. Because these laboratory detection methods can determine resistance or susceptibility of an isolate against an array of possible therapeutic candidates, antimicrobial susceptibility testing results can be a useful clinical guideline in selecting the best antibiotic treatment option for each particular patient. These same methods can also be used for monitoring the emergence and spread of resistant microorganisms in the population. Clinical Breakpoints are threshold values established for each pathogen-antibiotic (i.e., bug-drug) combination indicating at what level of antibiotic the isolate should be considered to be sensitive, intermediate or resistant. The interpretative criteria for these are based on extensive studies that correlate laboratory resistance data with serum achievable levels for each antimicrobial agent and a history

of successful and unsuccessful therapeutic outcomes. Although veterinary laboratories originally based interpretations on standards established using human pathogens, it became apparent by the early 1980s that such an approach did not reliably predict clinical outcomes when applied to veterinary practrice. Subsequently, groups within organizations that set standards were created for the purpose of developing veterinary-specific standards (Watts and Lindeman, 2006).

### 2.8 Lab approaches and strategies

When deciding whether or not to conduct antimicrobial susceptibility testing, the following points should be considered:

- Clinical relevance of the isolate
- Purity of the isolate
- Logical panel of antimicrobial agents to be tested (i.e., do not include antibiotics to which the isolate is known to have intrinsic resistance)
- Availability of test methodology, resources, and trained personnel
- Standardization of testing
- Valid interpretation of results
- Cost efficiency
- Effective means to communicate results and interpretation to end-users

Most often, interpretation is reduced to whether the isolate is classified as susceptible, intermediately susceptible, or resistant to a particular antibiotic. It should, however, be remembered that these in vitro procedures are only approximations of in vivo conditions which can be very different depending on the nature of the drug, the nature of the host and the conditions surrounding the interaction between the antibiotic and the target pathogen. One critical aspect is following standardized procedures that can generate reproducible results, i.e. quality control. Aspects of quality control include:

- Standardized bacterial inoculum size
- Culture conditions (growth medium, pH, cation concentration blood and serum supplements and thymidine content)

- Incubation conditions (atmosphere, temperature, duration)
- Concentration of antimicrobials for testing.

Because of the required culture time, antimicrobial susceptibility testing may take several days, which is not ideal particularly in critical clinical cases demanding urgency. Often, practitioners may utilize locally established antibiograms as guideline for therapy. An antibiogram is a compiled susceptibility report or table of commonly isolated organisms in a particular hospital, farm, or geographic area, which can serve as a useful guideline in therapy before actual culture and susceptibility data becomes available for reference.

### 2.9 Test Methods in Detecting Antimicrobial Resistance

There are several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotic treatment. This data may be utilized as guidelines for chemotherapy, or at the population level as indicators of emergence and spread of resistance based on passive or active surveillance. Some examples of antibiotic sensitivity testing methods are:

- Dilution method (broth and agar dilution method)
- Disk-diffusion method
- E-test
- Automated methods
- Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test
- Genotypic methods such as PCR and DNA hybridization methods

Selection of the appropriate method will depend on the intended degree of accuracy, convenience, urgency, availability of resources, availability of technical expertise and cost.. Interpretation should be based on veterinary standards whenever possible, rather than on human medical standards, which may not always be applicable. Among these available tests, the two most

commonly used methods in veterinary laboratories are the agar disk-diffusion method and the broth micro dilution method.

### 2.9.1 DILUTION METHODS

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC. The MIC is thus the minimum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth. A procedure similar to broth dilution is agar dilution. Agar dilution method follows the principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited. (Bernabé *et al.,* 2017).

### 2.9.2 DISK DIFFUSION METHOD

Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinics. A growth medium, usually Mueller-Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 108 colony forming units per ml). Commercially prepared disks, each of which are pre-impregnated with a standard concentration of a particular antibiotic are then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of “no growth” will be observed

around that particular disk. The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate or resistant. (Bockstael and Aerschot,2009).

### 2.9.3 E-TEST

E-test (AB Biodisk, Solna, Sweden) is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein. This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.

### 2.9.4 AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY TESTING SYSTEMS

Several commercial systems have been developed that provide conveniently prepared and formatted microdilution panels as well as instrumentation and automated reading of plates. These methods are intended to reduce technical errors and lengthy preparation times. Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading and interpretation. These systems have the advantage of being rapid (some results can be generated within hours) and convenient, but one major limitation for most laboratories is the cost entailed in initial purchase, operation and maintenance of the machinery. Some examples of these include: Vitek System (bioMerieux, France), Walk-Away System (Dade International, Sacramento, Calif.), Sensititre ARIS (Trek Diagnostic Systems, East Grinstead, UK), Avantage Test System (Abbott Laboratories, Irving, Texas), Micronaut (Merlin, Bornheim-Hesel, Germany), Phoenix (BD Biosciences, Maryland) and many more. (Cantón and Morosini, 2011)

### 2.9.5 MECHANISM-SPECIFIC TESTS

Resistance may also be established through tests that directly detect the presence of a particular resistance mechanism. For example, beta lactamase detection can be accomplished using an assay such as the chromogenic cephalosporinase test (Cefinase disk by BD Microbiology Systems, Cockeysville, MD and BBL DrySlide Nitrocefin, Becton Dickinson, Sparks, MD) and detection for chloramphenicol modifying enzyme chloramphenicol acetyltransferase (CAT) may utilize commercial colorimetric assays such as a CAT reagent kit (Remel, Lenexa, Kansas). (MacGowan, 2008)

### 2.9.6 GENOTYPIC METHODS

Since resistance traits are genetically encoded, we can sometimes test for the specific genes that confer antibiotic resistance. However, although nucleic acid-based detections systems are generally rapid and sensitive, it is important to remember that the presence of a resistance gene does not necessarily equate to treatment failure, because resistance is also dependent on the mode and level of expression of these genes (Fluit *et al.*, 2001).

Some of the most common molecular techniques utilized for antimicrobial resistance detection includes:

- Polymerase chain reaction (PCR) is one of the most commonly used molecular techniques for detecting certain DNA sequences of interest. This involves several cycles of denaturation of sample DNA, annealing of specific primers to the target sequence (if present), and the extension of this sequence as facilitated by a thermo stable polymerase leading to replication of a duplicate DNA sequence, in an exponential manner, to a point which will be visibly detectable by gel electrophoresis with the aid of a DNA-intercalating chemical which fluoresces under UV light. (Rodloff *et al.,* 2008).
- DNA hybridization. This is based on the fact that the DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine; or uracil for RNA). Therefore, a labeled probe with a known specific sequence can pair up with opened or

denatured DNA from the test sample, as long as their sequences complement each other. If this “hybridization” occurs, the probe labels this with a detectable radioactive isotope, antigenic substrate, enzyme or chemiluminescent compound. Whereas if no target sequence is present or the isolate does not have the specific gene of interest, no attachment of probes will occur, and therefore no signals will be detected. (Call *et al.,* 2011)

- Modifications of PCR and DNA hybridization. With these basic principles, several modifications have been introduced which further improve the sensitivity and specificity of these standard procedures. Examples of such development were the use of 5’-fluorescence-labeled oligonucleotides, the development of molecular beacons, development of DNA arrays and DNA chips. (Cantón *et al.,* 2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY LOCATION

This study was conducted in Benin City, capital of Edo State, which is one of the 36 states in Nigeria. Administratively, Benin City comprises of three Local Government Areas (LGAs) including Oredo, Egor and Ikpoba-Okha. In terms of healthcare system, the city populace and those from neighbouring towns and states are serviced by several primary health care facilities, two government-owned secondary hospitals (Stella Obasanjo Specialist Hospital and Central Hospital). For this study, however, sample collection was at Evbuotubu primary health centre in Egor Local Government in Benin-city, Edo State, Nigeria.

## 3.2 Eligibility criteria

### *3.2.1* Inclusion criteria

- Persons presenting at one of the participating health facilities at least 4 hours prior to enrolment.
- Mentally competent and capable of understanding and providing informed consent.

### *3.2.2* Exclusion criteria

- Patients who have been admitted for more than 16 hours before enrolment into the study.
- Patients where swabbing in the nose or throat is not possible due to anatomic or surgical reasons.

## 3.3 Outcome variables

The primary outcome variables for this study are the presence of MRSA carriage in persons who presented in Evbuotubu health centre, defined as the proportion of persons having these bacteria among all the study participants. The secondary outcome variables include the risk factors for carriage of antibiotic resistance, defined as the proportion and odds ratios between patients with and without MRSA.

### 3.4 Potential risk factors for MRSA carriage

As well as the outcome variables, we will collect information from each study participant on the following variables: gender (male or female); age (in years); ethnicity; occupation (e.g. work/stay at hospital, nursing home, schools, prison, military, hostel, day-care); average income per month (for those working); religion (Christian or Muslim); self-medication practice for perceived malaria (yes or no); belief for buying medication over the counter without prescription (it is fine or it is bad); frequency of buying medication from pharmacy or chemist stores (for those who responded in the affirmative to the previous question); signs of infections; shared sports equipment; tattoos/piercings; contact with farm produce (poultry, live pigs, or cattle); hospitalized for the past six months; invasive surgery performed in the last six months; indication of any drainage tubes, indwelling catheters, nutrition tubes, ulcers, abscesses, eczema, chronic skin disorders or IV drug abuse within the last six months; antibiotic treatment within the last 6 months; location within a health facility (emergency vs non-emergency); patient type (inpatients vs outpatients); Type of toilet at home; contact with farm/market for >10years; and history of diarrhoea in last three months.

### 3.5 Data collection methods

#### *3.5.1* Interview

Information on sociodemographic and potential risk factors for carrier status will be obtained using an interviewer-administered questionnaire (to be pre-tested for ambiguity and clarity prior to data collection).

#### *3.5.2* Collection of specimen for microbiological analyses

First, a study participant was swabbed in the nose using a standard swab stick. Nasal sample was obtained by inserting a swab into the anterior nares and rotating it along the mucous membrane. It is worth noting that since COVID-19 pandemic, nasal sample collection has become a routine practice in Nigeria as part of COVID19 diagnosis. The swab sample container as labelled containing information on study health facility and participant unique identification number. Additionally, urine sample was collected using sterile, wide mouthed container with screw cap tops as per conventional procedures. Again, the urine sample was labelled as before. All samples

was collected by qualified staff of the centre (**NURSES AND MEDICAL LABORATORY SCIENTIST**) present in the centre. All samples was analysed at the Department of Microbiology, University of Benin, with a view to minimising bias from variations in microbiological techniques.

### 3.6 Microbiological analysis

#### 3.6.1 Media Preparation

The media employed in this research work were MacConkey Agar, Cled Agar and Mueller Hinton Agar.

The various media was prepared with strict adherence to the manufacturers' instruction. They were then sterilized by autoclaving at 121$^{o}$C for 15 minutes and were poured into disposable petri dishes after cooling and allowed to set and solidify before use.

All of these were done observing strict microbiological aseptic conditions.

#### 3.6.2 Gram's Staining

These tests were done according to Chesebrough,2000. The Gram stain is by far the most widely used procedure for staining bacteria and separating it into two major groups Gram (+) positive and Gram (-) negative. A thin film of the specimen was spread over a clean grease free slide and allowed to air dry. The specimen was then fixed by passing it over a Bunsen flame thrice. The film was flooded with crystal violet and left for 60 seconds. The slide was then being washed and flooded with lugol's iodine and (mordant) and left for 60 seconds. Subsequently, iodine was washed off the slide and decolourized with acetone for a seconds and washed off. Then Slide was finally air dried and examined with oil immersion and observed under the microscope.

### 3.7 Biochemical Tests

#### 3.7.1 Catalase Tests

The test was performed by dropping a loopful of the isolates mix with hydrogen peroxide on the slide. The production of gas bubbles ($O_2$) from the mixture which occur almost immediately is positive reaction.

#### 3.7.2 Oxidase Test

The test was done by dropping 2-5 drops of freshly prepared oxidase (p-aminodimethylanine) reagent on a filter paper, the suspected organisms is picked using a sterile wire loop and mix with the oxidase reagent. A change from the normal colour to deep purple means a positive result, while no change means negative.

#### 3.7.3 Coagulase Test

This is a test to differentiate Staphylococcus aureus and other Staphylococcus species 2-3 drops of normal saline was added on a grease free slide to the normal saline. The suspected organism was mixed and 1-2 drops of plasma will be added and rocked, the presence of agglutination indicate a positive result while no agglutination means a negative result.

#### 3.7.4 Indole test

Indole test helps to determine the ability of bacterial species to convert tryptophan into Indole. Pure isolated bacterial colony was smeared on a Whatman paper, five drops of Kovac's Indole reagent was added to the Whatman paper. A positive result was indicated by the presence of red or red-violet coloration after 10 sec.

### 3.7.5 Citrate utilization test

This test detects the ability of an organism to use citrate as the sole source of carbon and energy. Isolates was picked up using a sterilized wire loop and inoculated onto Simmons citrate agar (SCA) and incubated overnight at 37°C, a citrate positive organism changes colour from green to blue.

### 3.7.6 Urease production test

It was used to determine the ability of a bacterium to produce urease, an enzyme that breaks down urea to release ammonia, the test will be done with the bacteria been inoculated onto urea agar base (UAB) supplemented with urea in slants and incubated for 24h at 37$^{o}$C. A colour change of the colourless agar to pink indicates a positive reaction.

### 3.7.7 Sugar fermentation test

This test was used in identification of bacteria isolates that are able to grow in the presence of glucose, sucrose and lactose. The bacteria isolates were inoculated into test tubes with triple sugar iron agar (TSI) and incubated at 37°C and the observation was taken 24h after inoculation.

### 3.7.8 Methyl Red Test

This test is used to detect the production of sufficient acid during sugar fermentation and the maintenance of conditions such as the pH of an old culture of the methyl red indicator added after incubating the culture at 37°C for 48 hours. Positive results was shown by a bright red coloration while a yellow coloration indicated a negative result.

### 3.8 Antibiotics Susceptibility Test

The antibiotic resistance pattern of the various S. aureus isolates after the coagulase test screening was determined against ten commercially available antibiotics with each disc impregnated with a potency Gentamycin, Ceffriazone, Ofloxacin, Augumentin, Ceffaziame, Cefurozorine, Cloxacillin, Erythromycin hours before testing. The turbidity of the actively growing culture was adjusted to correspond to with that of barium sulphate (0.5 Mac Farland) standard. Subsequently 0.1ml of the nutrient culture was inoculated into Mueller Hinton agar plates (Disposable petri dishes) and spread over the surface with sterile cotton swabs. Antimicrobial discs were then placed on the surface of each plate using a forceps' and incubated at $37^{O}C$ for 18-24 hours. Inhibition zone diameters were measured using a transparent ruler and interpretative break points for resistance determined by comparing zone diameters according to the clinical laboratory standards institute.

# CHAPTER 4

## RESULTS

Table 4.1 shows the socio-demographic characteristics consisting of age, sex, ethnicity, occupation, religion and income per month of the study participants with age frequency showing the highest age range; age 18-24 (44.6%) and the lowest 8 (3.4%). It also shows the percentage of the gender of the participants, with females (67.4%) participating more than male (32.6%). Table 4.1 also shows the frequency of the participants' ethnicity, the highest being others (e.g Yoruba, Igbo, Urhobo) (43.8%) and lowest being Afemai (2.2%).

This result encompasses a larger study done in the Department of Microbiology, University of Benin.

**Table 4.1: Socio-Demographic Characteristics of the Study Participants**

| Variable | Frequency (%) |
|---|---|
| **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 (42k.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) |

Table 4.2 shows the frequency of the health facility where samples were collected from participants. It shows 46.8% of samples were collected from a primary health facility. It also shows that 15.5% of the samples collected were from evbuotubu primary health centre.

**Table 4.2: Health Facility Characteristics**

| Variable | Frequency (%) |
|---|---|
| **Name of health facility** | |
| Evbuotubu primary health centre | 36 (15.5) |
| **Health facility type** | |
| Primary | 109 (46.8) |

Table 4.3 shows the microbiological assessment which includes the sample type with both nasal swab and urine being the highest (44.6%). Table 4.3 also shows positive detection of *S. aureus* is highest (39.1%) and samples that were not applicable are lowest (29.2%). In the totality of samples that tested positive for S. *aureus*, 35.6% of those samples tested positive for MRSA.

**Table 4.3: Microbiological Assessment**

| Variable | Frequency (%) |
|---|---|
| **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) |

Table 4.4 shows the potential risk factors of AMR consisting of tattoo/piercing on body with (92.3%) for no and (7.7%) for yes, contact with farm produce (e.g., poultry) for the past one month with (71.7%) for no and (28.3%) for yes, hospitalized [at least one night] in the last six months with (91.0%) as no and (9.0%) as yes, received an invasive surgery in the last six months with (97.9%) as no and (2.1%) as yes, recipient of drainage tube during hospitalization with (98.7%) as no and (1.3%) as yes, recipient of nutrition tube during hospitalization with the response no at (95.7%) and yes at (4.3%) and ulcer with the highest percentage of (79.8%) as yes and (20.2%) as no. It shows the potential risk factors for AMR consisting of abscesses at (94.4%) with a negative response and (5.6%) with a positive response, contact with farm market for more than 10 years with (70.0%) as yes and (30.0%) as no, history of diarrhea in the last three months with (73.4%) response as no and (26.6%) response as yes, eczema\chronic skin disorder with (83.7%) as no and (16.3%) as yes, taken antibiotics within the last six months with (94.0%) as a positive response and (6.0%) as a negative one, self-medicated for common illness (e.g. Malaria) with (94.0%) as yes and (6.0%) as no, frequency of self-medication in a year with the highest frequency of (34.3%) as yes and (7.3%) as no and toilet type with water system at (97.4%) and pit latrine at (2.6%).

**Table 4.4: Potential Risk Factors for AMR**

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

AMR=Antimicrobial resistance

Table 4.5 shows the distribution of *S. aureus* in participants' urine and nasal swab by participants' socio-demographic characteristics. Patients of ages 18-24 having the highest frequency (55.6%) and the lowest frequency with patients between ages of 25-34 (44.4%), sex with females having the highest frequency of (66.7%) and lowest being males at (33.3.%), occupation with business and factory worker being the highest frequency (22.2%) each and lowest being student (11.1%) and self-reported income per month (₦) as 11,000-90,999 being the highest at (75.0%) and the lowest as ≤10,000 (25.0%).

**Table 4.5: Distribution of *S. aureus* by Participants' Socio-demographic Characteristics**

| Variable | *S .aureus [N=11]* | |
|---|---|---|
| | **Urine [n=2]** | **Nasal swab [n=9]** |
| | **No [n (%)]** | **Yes [n (%)]** |
| **Age category ( year)** | | |
| 6-17 | 1 (50.0) | - |
| 18-24 | - | 5 (55.6) |
| 25-34 | 1 (50.0) | 4 (44.4) |
| 35-49 | - | - |
| ≥50 | - | - |
| **Sex** | | |
| Female | 0 (0.0) | 6 (66.7) |
| Male | 2 (100.0) | 3 (33.3) |
| **Occupation** | | |
| Student | 1 (50.0) | 1 (11.1) |
| Business | - | 2 (22.2) |
| Housewife | - | - |
| Teacher | - | - |
| Driver | - | - |
| Caterer | - | - |
| Factory worker | 1 (50.0) | (22.2) |
| Farmer | - | - |
| Other works (e.g., mechanic etc.) | - | - |
| Not working yet | - | - |
| **Self-reported income per month, Naira** | | |
| ≤10,000 | - | 2 (25.0) |
| 11,000-90,999 | 1 (100.0) | 6 (75.0) |
| ≥100,000 | - | - |
| Missing/did not respond to Question | - | - |

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

Table 4.6 shows the distribution of methicillin resistant *S. aureus* by participants' socio-demographic characteristics consisting of age with the highest frequency (45.5%) for ages between 18-24 and 25-34 and the lowest between ages 6-17 at (9.1%), sex with females having the highest frequency of (54.6%) and males at (45.4%), occupation with other works (e.g., mechanic etc.) having the highest frequency (36.4%) and the lowest being student and business (18.2%) each and self-reported income per month(₦) with 11,000-90,999 being the highest frequency at (77.8%) and the lowest of less than 10,000 at (22.2%).

**Table 4.6: Distribution Of Methicillin Resistant *Staphylococcus aureus* by Participants' Socio-Demographic Characteristics**

| Variable | Methicillin resistant *S. aureus* | | p-value |
|---|---|---|---|
| | **No [n=0; 0.0%]** | **Yes [n=11; 100.0%]** | |
| **Age category(year)** | | | |
| 6-17 | | 1 (9.1) | |
| 18-24 | | 5 (45.5) | |
| 25-34 | | 5 (45.5) | |
| 35-49 | | - | |
| ≥50 | | - | |
| **Sex** | | | |
| Female | | 6 (54.6) | |
| Male | | 5 (45.4) | |
| **Occupation** | | | |
| Student | | 2 (18.2) | |
| Business | | 2 (18.2) | |
| Housewife | | - | |
| Teacher | | - | |
| Driver | | - | |
| Caterer | | - | |
| Factory worker | | 3 (27.3) | |
| Farmer | | - | |
| Other works (e.g., mechanic etc.) | | 4 (36.4) | |
| Not working yet | | - | |
| **Self-reported income per month, Naira** | | | |
| ≤10,000 | | 2 (22.2) | |
| 11,000-90,999 | | 7 (77.8) | |
| ≥100,000 | | - | |
| Missing/did not respond to question | | - | |

# CHAPTER 5

## DISCUSSIONS

According to a study carried out in Ebonyi State, Nigeria by Afiukwa *et al* in 2015, a total of 200 nasal swab samples were collected from apparently healthy students, 76 (38.0 %) isolates were isolated. MRSA positive bacteria were only detected in 33 (43.4 %) of the S. aureus isolates.

The present study shows that 100% of the samples collected tested positive for MRSA, these variations may be attributed to the characteristics of the population under study. A population that is on antibiotics as at the time of sampling may yield a much lower prevalence of *S. aureus* while a population from hospital settings may yield a much higher prevalence because of the high prevalence of infectious patients in that environment.

According to the present study, participants of ages 18-24 and 25-34 were more likely to be colonized by MRSA than any other age groups, these age groups adopt self-medication at any slight evidence of an infection and due to the misuse of these antimicrobial drugs the organism becomes resistant and causes more damage. Females were also more likely to be affected by MRSA. The females involved in this study were recruited at a primary health facility in the course of vaccination attendance. So, the over representation of women as compared to men could simply be a function of higher chances of being tested or recruited into the study, rather than being at a high risk of being infected with *S. aureus* and indeed harboring MRSA. Although, they were shown to have lesser knowledge about the use of these drugs and the effect of its misuse. Females usually traders, hair dressers, tailors etc. were shown to self-medicate during an infection.

The occupation of participants highly affected with MRSA were those with manual jobs like mechanic, tailors, hair dressers etc., whom are likely to seek the nearest pharmacy for antimicrobial drugs without being prescribed or those who get these drugs at any slight symptom of an infection. This study also shows that the income of the participants with the highest MRSA were those being paid an amount of 11,000-90,999, these set of participants were shown to be able to afford these antimicrobials at any giving time of an infection.

This study showed that all the *Staphylococcus aureus* isolates obtained were resistant to two or more antibiotics with 81.8% of them identified as MRSA. This observation indicates that there is high degree of resistance found in *Staphylococcus aureus* isolated and is a source of public health concern. The high degree of resistance found in *Staphylococcus aureus* could be attributed to the exposure to potential risk factors like; indiscriminate use of antibiotics, over-prescription, poor quality and cheaper medicines that are being delivered to patients over the counter. The methicillin resistant *Staphylococcus aureus* remains a reservoir of drug resistance and may also be responsible for the resistance pattern observed.

The cross-sectional study of the socio-demographic characteristic of the participants showed the prevalence and distribution of MRSA among outpatients and gives a general overview on how it affects the population as a whole and how further research could establish a permanent solution to the problem of MRSA. The concerted strategies at monitoring and prescribing habits of clinicians, the diagnostic efficiency of laboratory scientist, the dispensing habits of pharmacists, the inappropriate use of antibiotics, as well as encouraging good hygiene measures could help curtail possible transmission of MRSA infections within and outside the hospital environment. This finding also indicates that the MRSA recovered in this study expressed high level of resistance to antimicrobials that are commonly used in clinical medicine. This could contribute to the spread and persistence of antimicrobial resistant bacteria and resistance determinants in humans and the environments.

Population-based studies of the true impact of antimicrobial resistance would require large multicenter study groups and would be valuable to help address the different perspectives. Relevant studies will require sufficient size to describe baseline antimicrobial-drug resistance deal with limits of random variations, and control for variables. Multicenter study groups will likely have to be assembled to provide enough observations, as well as sufficient resources. Only when this is done can there be adequate exploration of the true magnitude of the economic impact of antimicrobial-drug resistance.

*S. aureus* is a well-known pathogen with an alarmingly increasing level of developing resistance to many available antimicrobial agents. Nasal *S. aureus* has been implicated in community associated infections like soft tissue infection and hospital infections like bacteremia. The

adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction. The emergence of multidrug-resistant MRSA strains with unique properties has raised public health concern. Acquisition of MRSA pathogens presents complications in the antibiotic management of such infections, with the attendant increase in hospital stay, cost of treatment and the morbidity and mortality of patients.

## CONCLUSION

The discovery of antibimicrobials transformed the treatment of infectious diseases. Consequently, ordinary diseases were treatable, and infectious disease outbreaks were easily controlled. However, it was premature to declare victory over bacterial pathogens. Antimicrobial resistance emerged swiftly, reducing the clinical use of each new drug. The high prevalence of MRSA among patients of this study has the potential to increase with possible resistance spreading to other infectious agents accompanying antimicrobial failure. Antimicrobial sensitivity and resistance must ultimately be understood at the population, organism, cellular, and molecular levels if antimicrobial resistance is to be reduced.

## REFERENCES


Aarestrup, F.M., Seyfarth, A.M., Emborg, H.D., Pedersen, K., Hendriksen, R.S., and Bager, F. (2001). Effect of Abolishment of the Use of Antimicrobial Agents for Growth Promotion on Occurrence of Antimicrobial Resistance in Fecal Enterococci from Food Animals in Denmark. *Antimicrobial Agents and Chemotherapy*. **45**(7):2054-2059.

Alonso, C.A., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama K., and Torres, C. (2017) Antibiotic resistance in Escherichia coli in husbandry animals: the African perspective. *Let Applied Microbiology.* **64**:318–34.

Ampaire, L., Muhindo, A., Orikiriza, P., Mwanga-Amumpaire, J., Bebell, L., and Boum, Y. (2016): A review of antmicrobial resistance in East Africa. *African Journal of Laby Med.* **5**(1):432.

Ashley, A.E., Havumaki, J., Ongarello, S., Tadesse, T.B., Wijegoonewardena, M., González, J.I., and Dittrich, S. (2017): Antimicrobial resistance in Africa: a systematic review. *BMC Infectious Disease* **17**:(616)12713-12871.

Bambeke, Balzi. and Tulkens. (2000): Antibiotic Efflux Pumps. *Biochemical Pharmacology*, **60**:457–470.

Bbosa, Mwebaza, Odda, Kyegombe, and Ntale. (2014): Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance. *Health* **6**(5)410-425.

Bennett, J.W. (2015). What is an Antibiotic? In: Sánchez S., Demain A.L., editors. *Antibiotics: Current Innovations and Future Trends.* Caister Academic Press; Poole, UK: **pp**. 1–18.

Bennett. (2008): Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*. **153**:347–357.

Bernabé, K.J., Langendorf, C., Ford, N., Ronat, J.B., and Murphy, A.R. (2017): Antimicrobial resistance in West Africa: a systematic review and meta-analysis. *International Journal Antimicrobial Agents*. **50**:629–39.

Bhaskar Das, and Sanjukta Patra, (2017): Nanostructures for Antimicrobial Therapy.

Bockstael, and Aerschot. (2009): Antimicrobial resistance in bacteria. *Central European Journal of Medicine* **4**(2):141-155.

Bougnom, P.B., and Piddock, J.L. (2017): Wastewater for urban agriculture. A significant factor in dissemination of antibiotic resistance. *Environmental Science & Technology.* **51**:5863–4.

Bush, (1988). Beta-Lactamase Inhibitors from Laboratory to Clinic. *Clinical Microbiology Reviews*. **1**(1):109-123.

Busz, H.W., Inglis, G.D., McAllister, T.A., Morck, D.W., Olson, M.E., Read, R.R. and Yanke, L.J., (2005): Effects of Subtherapeutic Administration of Antimicrobial Agents to Beef Cattle on the Prevalence of Antimicrobial resistance in Campylobacter jejuni and Campylobacter hyointestinalis. *Applied and Environmental Microbiology*. **71**(7):3872-3881.

Call, Mitchell, Ullman, and Subbiah. (2011): β-Lactams and Florfenicol Antibiotics Remain Bioactive in Soils while Ciprofloxacin, Neomycin, and Tetracycline Are Neutralized. *Applied and Environmental Microbiology*, **pp**. 7255–7260.

*Clayton, L., and Thomas,* E.D*. (1993). Taber's Cyclopedic Medical Dictionary (17th ed.). F. A. Davis Co.* 978:8036-8313*.*

Cantón, and Morosini. (2011): Emergence and spread of antibiotic resistance following exposure to antibiotics. *FEMS Microbiological Revue* **35**:977–991.

Cantón, Horcajada, Oliver, Garbajosa, and Vila. (2013): Inappropriate use of antibiotics in hospitals: The complex relationship between antibiotic use and antimicrobial resistance. *Enferm Infecc Microbiol Clinic*. **31**(4):3-11.

Cars and Olofsson. (2007): Optimizing Drug Exposure to Minimize Selection of Antibiotic Resistance. *Clinical Infectious Diseases.* **45**:129–36.

Denyer, S.P, Hodges, N., Gorman,S.P and Gilmore B.F. (2011): Hugo and Russell's pharmaceutical microbiology. *John Wiley and sons, limited*. **8**:169-193.

Donlan. (2001): Biofilm Formation: A Clinically Relevant Microbiological Process Clinical Infectious Diseases. **33**:1387–92.

Drlica. (2003): The mutant selection window and antimicrobial resistance. *Journal of Antimicrobial Chemotherapy*. **52**:11–17.

Essack, S.Y., Desta, A.T., Abotsi, R.E., and Agoba, E.E. (2016): Antimicrobial resistance in the WHO African region: current status and roadmap for action. *Journal of Public Health*. **39**:38–13.

Fluit, A.D.C., Visser, M.R., and Schmitz, F.J. (2001). Molecular Detection of Antimicrobial resistance. *Clinical Microbiology Reviews*, **14**(4):836-871.

*Gurusamy, K.S., Koti, R., Toon, C.D., Wilson P, and Davidson BR (2013). "Antibiotic therapy for the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in surgical wounds". The Cochrane Database of Systematic Reviews* ***(8):****CD009726*

Hare, R. (1983). The Scientific Activities of Alexander Fleming, Other Than the Discovery of Penicillin. *Medical History*. **27**:347-372.

Hillerton, Irvine, Bryan, Scott, and Merchant. (2016): Use of antimicrobials for animals in New Zealand, and in comparison with other countries. *New Zealand Veterinary Journal.*

*Holmes, A.H., Moore, L.S., Sundsfjord, A., Steinbakk, M., Regmi, S., and Karkey, A. (2016). "Understanding the mechanisms and drivers of antimicrobial resistance". Lancet.* ***387****:176–87.*

Jr., T.E.C. (1970): "What Were Godfrey's Cordial and Dalby's Carminative?" *Pediatrics* **45**:1011

Keyes, K., Lee, M.D., and Maurer, J.J. (2003). Antibiotics: Mode of Action, Mechanisms of Resistance and Transfer. Microbial Food Safety in Animal Agriculture Current Topics. ME Torrence and RE Isaacson, eds. Iowa State Press, Ames, Iowa, USA.

Lederberg, J. (2000): Infectious history. Science. **288**(5464):287–93.

Levy, S.B. (2002). *The Antibiotic Paradox*, 2nd edition. Perseus Publishing, USA.

Luangtongkum, T., Morishita, T.Y., Ison, A.J., Huang, S., McDermott, P.F., and Zhang, Q. 2006. Effect of Conventional and Organic Production Practices on the Prevalence and Antimicrobial resistance of Campylobacter spp. In Poultry. *Applied and Environmental Microbiology* **72**(5):3600-3607.

MacGowan. (2008): Clinical implications of antimicrobial resistance for therapy. *Journal of Antimicrobial Chemotherapy*. **62**(2):105–114.

McEwen, and Fedorka-Cray. (2002): Antimicrobial Use and Resistance in Animals. *Clinical Infectious Diseases*. **34**(3):93–106.

Mellon, M., Benbrook, C., and Benbrook, K.L. (2001). Hogging It! Estimates of Antimicrobial Abuse in Livestock. Union of Concerned Scientists, Cambridge.

Michigan State University Antimicrobials: An introduction. (2011). [(accessed on 27 November 2015)]. Antimicrobial Resistance Learning Site. Pharmacology.

Obasuyi, O., McClure, J., Oronsaye, F.E., Akerele, J.O., Conly, J., Zhang, K. (2020): Molecular Characterization and Pathogenicity of *Staphylococcus aureus* Isolated from Benin-City, Nigeria. *Microorganisms*. **8**(6):912.

Onofrey, Bruce. (2017) "How antibiotics work and why they sometimes don't: before reaching for the Rx pad, know the drug, the patient and the disease." *Review of Optometry*, 154, (4): **p**. 30.

*Ory, E.M., and Yow, E.M. (1963). "The use and abuse of the broad spectrum antibiotics". JAMA. **185** (4): 273–9.*

Otten, H. (1986). Domagk and the Development of the Sulphonamides. *Journal of Antimicrobial Chemotherapy*. **17**:689-696.

Phillips, P.L., Wolcott, R.D., Fletcher, J., Schultz, G.S. (2010): Biofilms Made Easy. *Wounds International*. **1**(3).

Rodloff, Bauer, Ewig, Kujath, and Müller. (2008): Susceptible, Intermediate, and Resistant – The Intensity of Antibiotic Action. *Deutsches Ärzteblatt International Dtsch Arztebl International*.**105**(39): 657–62.

Schwartz, R.S. (2004). Paul Ehrlich's Magic Bullets. *New England Journal of Medicine* **350**(11):1079-1080.

Soto. (2013): Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virulence *Landes Bioscience* **4**(3):223–229.

Tang, L.K., Caffrey, P.N., Nóbrega, B.D., Cork, C.S., Ronksley, E.P., Barkema, W.H., Polachek, J.A., Ganshorn, H., Sharma, N., Kellner, D.J. (2017): Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Planet Health.* **1**:316–27.

Thoburn, A.L. (1983). Paul ehrlich: Pioneer of chemotherapy and cure by Arsenic. *Nritish Journal of Venereal Diseases.* **59**:404-405.

Tripathi, K.D. (1994): *Essentials of medical pharmacology.* Jaypee brothers medical publishers limited. **3**:614-716.

United States General Accounting Office. (2004). Antibiotic Resistance: Federal Agencies Need to Better Focus Efforts to Address Risk to Humans from Antibiotic Use in Animals. Report to Congressional Requesters. Accessed Online June, 2008.

Wainwright, W. (1989). Moulds in ancient and more recent medicine. *Mycologist.* **3**(1):21-23

Wall, Mateus, Marshall, Pfeiffer, Lubroth, Ormel, Otto, and Patriarchi. (2016): Drivers, Dynamics and Epidemiology of Antimicrobial Resistance in Animal Production. FAO.

Watts, J.L., and Lindeman, C.J. (2006). Chapter 3: Antimicrobial Susceptibility Testing of Bacteria of Veterinary Origin. *Antimicrobial Resistance in Bacteria of Animal Origin, FM Aarestrup*, ed. ASM Press, Washington DC, USA.

Webber, and Piddock. (2003): The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy*. 51:9–11.

WHO. (2020): Antimicrobial resistance Bull. World Health Org. **61**.

WHO. (2017): WHO guidelines on use of medically important antimicrobials in food-producing animals. Geneva: World Health Organization; **p**. 88.

WHO's (2014): WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health" .

Winau, F., Westphal, O., and Winau, R. (2004). Paul Ehrlich in search of the Magic Bullet. *Microbes and Infection*. **6**:786-789.

# APPENDIX

## Appendix 1: *Health facility characteristics*

| | |
|---|---|
| **Name of health facility** | |
| Central hospital | 124(26.2) |
| 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) |

## Appendix 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) |
| **Detection of *E. coli* in sample** | |
| No | 83(35.6) |
| Yes | 89(38.2) |
| Not applicable | 61(26.2) |

| | |
|---|---|
| **Detection of MDRE in sample** | |
| No | 90(38.6) |
| Yes | 82(35.2) |
| Not applicable | 61(26.2) |