ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *STAPHYLOCOCCUS* SPECIES ISOLATED FROM STAIRCASE RAILINGS IN CALEB UNIVERSITY

BY

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18/4797

A PROJECT REPORT SUBMITTED TO THE

DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY

COLLEGE OF PURE AND APPLIED SCIENCES

CALEB UNIVERSITY, IMOTA, LAGOS STATE, NIGERIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN MICROBIOLOGY AND BIOTECHNOLOGY FROM THE kDEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY

CALEB UNIVERSITY, IMOTA, LAGOS STATE, NIGERIA

JULY, 2022

DECLARATION

I, OGWEH JEMIMAH, do hereby declare that this project is entirely my and composition. The work embodied in this project has not been submitted in candidature for any degree and is not concurrently being submitted for any other degree. All references made to works of other persons have been dully acknowledged.

__________________________ __________________________

Signature Date

CERTIFICATION

This is to certify that this project titled Antibiotics susceptibility profile of *Staphylococcus* sp. Isolated from Staircase Railings in Caleb University was carried out by Ogweh Jemimah with Matric number 18/4797 in the Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Lagos, Nigeria.

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(EXTERNAL EXAMINER) DATE

DEDICATION

This research is dedicated to Almighty God who makes all things possible, to my mother Mrs. Abigail Ogweh and my father Dr. Peter Ogweh.

ACKNOWLEDGMENTS

My greatest thanks goes to Jehovah God for giving me the strength, knowledge and understanding to complete this project. I'm thankful to my mum and dad Mrs Abigail Ogweh and Dr Peter Ogweh for their consistent encouragement, enthusiasm and financial support. To my siblings Jeremiah, Jephthah, Jennifer, Jediah and Jeriel who have been a source of inspiration towards my academic pursuit I thank you all.

My profound gratitude goes to my wonderful supervisor, Dr. (Mrs.) T. C. Bayo-Olajide for her invaluable support, patience and time and guidance in seeing me to the completion of this research work. I also extend gratitude and appreciation to my lecturers in the Department of Biological Sciences and Biotechnology who have taught me at one point or the other. May God continue to bless, protect and guide you all.

Finally, I wish to acknowledge the consistent encouragement and emotional support of my best friend Mr. Timeyin Annomuogharan, to my wonderful coursemates Natasha, Mariam, Elvis, Chidera, Michael and Afeez I want to say a very big thank you for your contributions during our interactions and relationship which contributed enormously to the completion of this exploration work. God bless you all.

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ABSTRACT

Surfaces frequently touched with hands in school environment harbour potential pathogens and may act as source of infectious agents. The aim of this research was to determine the antibiotic susceptibility profile of bacteria present on staircase railings in Caleb University. A total of ten (10) samples were collected from the three (3) female hostels in Caleb University using sterile swab-sticks. Nutrient Agar and Mannitol Salt Agar was used to isolate and count *Staphylococci* isolates. Biochemical tests such as the Gram staining reaction, Catalase, Sugar Fermentation Test, Citrate Utilization Test and Motility Tests were used to identify the isolates. *Staphylococcus aureus* accounting for 70% of the total isolates and *Staphylococcus epidermidis* accounting for 30% of the isolates. All the isolates were subjected to antibiotic susceptibility tests by Kirby Bauer disc diffusion. Diameters of the zone of inhibition on Mueller Hinton agar were measured and interpretation was made based on CLSI guidelines. The percentage resistance of the isolates were Ofloxacin (30%), Cefotaxime (100%), Ceftriaxone (40%), Cefixime (100%), Levofloxacin (30%), Ciprofloxacin (20%), Azithromycin (30%), Cefuroxime (80%), Amoxicillin (100%), Erythromycin (30%), Imipenem (90%), Gentamicin (40%) and Oxacillin (70%). Some of the isolates were susceptible to Ofloxacin (70%), Ceftriaxone (60%), levofloxacin (70%), Ciprofloxacin (80%), Azithromycin (70%), cefuroxime (20%), Erythromycin (20%), Imepinem (10%), Oxacillin (30%) and Gentamicin (50%). This research work showed that *Staphylococcus* spp. contamination is common on the staircase surfaces in Caleb University. The antimicrobial susceptibility profile of the isolates suggests the presence of multi-drug resistant organisms, which could potentially be passed from person to person via the staircase railings surfaces in the University. This calls for proper and effective cleaning and sanitization of dry surfaces.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The spread of antibiotic resistance genes among pathogenic bacteria has lately become a focus of scientific investigation due to its strong link to the prevalence and severity of infection affecting human health. The current rise in antimicrobial resistance has been attributed to high levels of antimicrobial use in medicinal settings. Antibiotic resistance is generated by widespread inappropriate antibiotic prescriptions and non-adherence to prescription instructions (both of which are examples of poor antimicrobial use) (Wassmer *et al*., 2006). While most antimicrobials used in agriculture are different from those used in human medicine, some antimicrobials (such as bacitracin, tetracyclines, and sulfonamides) are used in both. Antibiotic-resistant bacteria such as *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Enterococcus* spp. have been found in agriculture and have harmed humans (Conly, 20012).

Recently, antimicrobial agents were revealed to have a new application. They have been used in a number of consumer cleaning products, including hand soaps, toothpastes, cosmetics, and fabrics, to kill bacteria (Gilbert and McBain, 20013). Developing consumer things that can kill large numbers of germs looks to be a wonderful idea at first glance, but there are numerous important factors to consider when trying to kill bacteria. Continued exposure to the same antimicrobial agent will only promote antimicrobial resistance through natural selection if an antibiotic agent does not entirely destroy a bacterial population (Martinez and Baquero, 2002; Depardieu *et al*., 2007).

Bacteria's techniques for avoiding antimicrobial agents' bactericidal effects vary significantly from one bacterium to the next. Bacterial resistance mechanisms are usually passed down via the generations. Antibiotics resistance can be produced by chromosomal alterations that cause an antibacterial drug's target to change. Resistance to erythromycin, a ribosome inhibitor, occurs when an adenosine in the bacterium's

rRNA gets methylated. This produces a modification in the erythromycin binding site on the bacteria's ribosome (Depardieu *et al.,* 2007). (Depardieu *et al.,* 2007). Bacteria can also acquire genes, such as the -lactamase gene, that code for antimicrobial agent dismantling enzymes. A β-lactam ring is the backbone structure of several antibiotics, including penicillins and cephalosporins. The enzyme -lactamase hydrolyzes the ring structure of -lactam antibiotics, rendering them inactive (Depardieu *et al*., 2007). Other bacteria acquire genes for drug efflux pumps. Drug efflux pumps, which are membrane problems, pump antimicrobial agents and other harmful chemicals out of the bacterial cell (Fan *et al*., 2002; Ono *et al.*, 2005).

Antibiotic resistance does not simply affect individual microbes. In order to become more antibiotic resistant, microorganisms can share genetic information. Bacteria have been proven to be capable of spreading resistance genes via a process known as conjugation in recent studies. In the process of conjugation, bacterial cells transfer genetic information by making direct cell-to-cell contact through hollow tubes called pili (Brunsima *et al*., 2003; Depardieu *et al*., 2007). As a result, preventing bacteria from being exposed to antimicrobial medications that would allow them to flourish and, in certain situations, propagate resistance genes could be one strategy to reduce resistance levels (Munita and Arias, 2015).

1.2 Statement of Problem

Studies have shown that frequently touched surfaces in public settings are colonized by viable organisms (Peng *et al*., 2015; Lakhundi and Zhang, 2018) and methicillin-resistant *Staphylococci* have been found on staircase railings as shown in a study done in a teaching hospital in Zambia (Mulango *et al*., 2018) with *Staphylococcus aureus* amounting to 65% of the total isolates, also in parks there was a high rate of methicillin resistance (56.2%) among *S. aureus* strains and most of them (91.4%) were multi-drug resistant (Thapaliya *et al*., 2017). However, data on the epidemiology and the Antibiotic susceptibility profile of

bacteria present on staircase railings in Universities is limited in Nigeria and this could pose a serious issue being that there could be an even higher percentage of resistant strains of *Staphylococci* present. Hence, this study aims to elucidate the Antibiotic susceptibility profile of bacteria present on staircase railings in Caleb University.

1.3 Aim and Objectives

The aim of this study is to determine the Antibiotic susceptibility profile of *Staphylococcus* spp. present on staircase railings in Caleb University.

Objectives

i. To isolate *Staphylococcus* spp. from staircase railings in female hostels in Caleb University.

ii. To determine the antibiotic susceptibility profile of the isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antibiotics Resistance

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 (Norland, 2018). 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 (Dufrene and Persat, 2020). 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 (Peterson and Kaur, 2018).

When a Dutch scientist named Anton van Leeuwenhoek discovered bacteria in 1674, he was credited as the first microbiologist. Louis Pasteur, the father of modern microbiology, continued Leeuwenhoek's work in 1859. (Fleming, 1946). Bacterial pathogens were discovered to be the cause of several infectious diseases during the dawn of microbiology, and they were discovered to be able to quickly adapt to new antibiotics. Alexander Fleming, a Scottish bacteriologist, discovered the antibacterial chemical generated by Penicillin notatum by accident in 1928, when it prevented the growth of the bacteria *Staphylococcus aureus* (Fleming, 1946). In 1945, Fleming was awarded the Nobel Prize for his discovery, which was the first modern antibiotic.

Antibiotics, which are microorganism-produced chemicals that kill or inhibit the growth of bacteria (Fleming, 1946), have proven vital in the fight against illnesses for more than fifty years. However, owing of antibiotic resistance, diseases that were once healed by the administration of an antibiotic are now more difficult to combat. Antimicrobial resistance arises as a result of bacteria's ability to adapt. Bacteria have

developed an inexorable resistance to single and multiple antimicrobial agents as a result of their continued exposure to antibiotics (Schramm *et al*., 2020).

The emergence and spread of antimicrobial resistant microorganisms are essential to public health. Microorganisms that have evolved antimicrobial treatment resistance play an important role in the progression and persistence of illness. Once antimicrobials labelled as second or third line therapies are no longer able to inhibit or kill their intended targets, pathogens will gain an advantage and be able to infect a large number of hosts without being inhibited. Resistance is a hazard to public health, especially for persons with impaired immune systems who rely on antimicrobials as their only protection against infection. While the majority of the population is not immune-compromised all of the time, when individuals are subjected to heightened stress or sickness, they can easily become immune-compromised and susceptible to opportunistic infection (as is often the case in closed environments such as a university). It is vital to educate the public on how resistant organisms evolve and transfer from person to person in order to limit the amount of antimicrobial resistance seen. It has been reported that, bacteria can survive for variable duration on surfaces including staircase hand railings (Schmidt *et al*., 2012; Shiferaw *et al.,* 2013).

Gram positive bacteria such as *Staphylococcus* spp. have been reported to survive up to months on dry inanimate surfaces in the school environment (Finley *et al*., 2013). The use of soap and water, as well as thorough rubbing of the hands, aids in the removal of organisms from the hands. Although it has been demonstrated that conventional soap and water are sufficient for pathogen eradication, many companies encourage the use of antimicrobial ingredients in soaps to completely eradicate bacteria from the hands (Kampf and Kramer, 2003; Sickbert-Bennett *et al.,* 2005). The use of only water and soap is recommended since it eliminates the risk of bacterial antibiotic resistance. Plain soap lowers the surface tension of any transient bacteria on the hands, allowing water to effectively rinse them away instead of killing them.

Hand washing for at least 15 seconds has been found to be an effective means of preventing the spread of most illnesses (Martinez and Baquero, 2002).

2.2 Origin and Evolution of Antibiotics Resistance

Antibiotics are a class of chemicals capable of killing or inhibiting the growth of bacteria. They have been widely used to treat many bacterial infectious diseases in humans and animals since their discovery, saving millions of lives and allowing us to avoid and manage infectious outbreaks, epidemics, and pandemics that would otherwise have wiped out human populations (Yahara *et al.,* 2018). However, the usage of antibiotics and the emergence of resistance are inextricably linked, and resistance to every new antibiotic discovered or created is unavoidable. Antibiotic resistance develops when bacteria use metabolic or other defenses to live and proliferate in the presence of antibiotics. Antibiotic resistance has been exacerbated by the release of antibiotic residues into the environment, as well as overuse and non-therapeutic use of antibiotics, resulting in a global public health crisis (Wang *et al.,* 2019).

Antibiotic resistance and antibiotic resistance genes (ARGs) are old (Zhang *et al*., 2021) and pervasive in the environment, particularly in soils, where varied bacteria and fungi create antimicrobials to protect themselves from other microorganisms, creating a selection pressure for the transmission and acquisition of ARGs (Benjak *et al.,* 2018). As a result, antibiotic-producing microbes must also survive their own attack, thus they frequently carry ARGs that enable them to survive in the presence of these toxicants (Florez-Cuadrado *et al*., 2018). Because resistance genes are an inherent and natural mechanism in many bacteria, it is normal to detect resistant bacteria even in areas where antibiotic usage/residues are low (Lomazzi *et al.,* 2019). Once inside ecosystems, ARGs are easily maintained, with the possibility for horizontal gene transfer (HGT) between non-pathogenic and pathogenic bacteria of the same or nearly related species (Christaki *et al*., 2020), highlighting the importance of this phenomenon for infectious diseases in humans. Antibiotic resistance can be developed in bacteria through mutations in target proteins

and regulatory genes that can be passed down to daughter cells in a vertical manner. Antibiotic resistance can also be acquired through horizontal transfer of antibiotic resistance determinants across similar and dissimilar bacteria using mobile elements like plasmids, integrons, and transposons (Brinkac *et al*., 2019). HGT is widely acknowledged as one of the most essential and critical vehicles for ARG mobilization and dissemination (Florez-Cuadrado *et al*., 2018). Processes such as a) conjugation by bacterial plasmids and conjugative transposons, b) transformation by acquiring free DNA from the environment, and c) transduction by bacteriophages are all used to mobilize genetic elements encoding ARGs (Florez-Cuadrado *et al*., 2018). The incidence of these HGT mechanisms is influenced by a variety of limitations and rate parameters, with bacterial plasmid conjugation and transfer taking precedence (Wang *et al*., 2019). Furthermore, co-resistance mechanisms (coselection of several resistance genes inside a mobile genetic element (MGE) and/or cross-resistance mechanisms (existence of resistance genes with a broad substrate range) have created multi-drug resistance in bacteria (Florez-Cuadrado *et a*l., 2018; Wang *et al.,* 2019).

2.3 Antibiotics Mechanisms of Action

Antibiotics are designed to attack certain bacterial properties such as; the cell wall, cell membrane, and activities like nucleic acid and protein synthesis. Antibiotics that target cell wall production include the big beta-lactam group, which includes antibiotics like penicillin and ampicillin that have been around for decades, as well as more modern derivate like cephalosporins, carbapenems, and monobactams. β-lactam bind to penicillin binding proteins (PBPs) in the cell wall and prevent fresh peptidoglycan production, resulting in bacterial lysis. Vancomycin and other glycopeptides suppress cell wall formation by interfering with the binding of peptidoglycan precursors to penicillin-binding proteins (PBPs) (Boolchandani *et al.,* 2019).

Tetracyclines, aminoglycosides, macrolides, and chloramphenicol, among other antibiotics, interact with the ribosome, the bacterial machinery for protein synthesis. They attack the ribosome's big (50S) or small (30S) subunits, inhibiting protein synthesis by binding with t-RNA, interrupting translation, and releasing incomplete peptide chains in bacterial cells. Quinolones are able to disrupt and interrupt DNA replication by binding to the subunit A of DNA gyrase and also to topoisomerase IV in bacteria. These two essential enzymes are required for DNA replication in bacteria to cut and release supercoiling of DNA during replication and re-join the DNA strands to continue the process. Finally, sulfonamides and trimethoprim block folic acid metabolism in bacteria by inhibiting the enzymes dihydropteroate synthase and dihydrofolatereductase, respectively (Käppeli *et al*., 2019).

2.4 Antibiotic Resistance in the Environment

The discharge of antibiotic residues and antibiotic resistant bacteria (ARB) from municipal, agricultural, and pharmaceutical sectors into the environment, particularly watersheds and soils, has aided the evolution of antibiotic resistance. ARB could potentially be spread from the environment to humans and animals by polluted water or food (Peterson and Kaur, 2018). Antibiotics released at sub-lethal concentrations into the environment have been proven in numerous investigations to facilitate the transfer and spread of antibiotic resistance determinants (Schramm *et al.,* 2020). Furthermore, metals and bioides are released into the environment at sublethal quantities, posing a danger of antibiotic resistance selection and transmission via co- and cross-resistance mechanisms (Schramm *et al*., 2020). Various environmental compartments, such as urban watersheds and sewage systems, agricultural soils, and heavy metal contaminated habitats, have been identified as major reservoirs of ARB and ARGs. However, in order to fully comprehend the role of the environment in the emergence of antibiotic resistance in clinical and community settings, a greater understanding of the evolution, mobilization, transfer, and diffusion of ARB and ARGs in the environment is required (Schramm *et al*., 2020). To reduce the development and spread

of antibiotic resistance, it is critical to prevent the construction of settings that encourage the selection and dissemination of ARB and ARGs, as well as the transmission of ARB and ARGs to human and animal microbiota (Dadgostar, 2019).

2.5 Economic and Medical Concern of Antibiotic Resistant Bacteria

Infectious diseases claim the lives of about 17 million people every year around the world. More than 70% of bacteria that cause hospital-acquired illnesses are resistant to at least one of the medicines most routinely used to treat them, and bacteria resistant to at least one antibiotic are responsible for more than 60% of deaths (De Oliveira *et al*., 2020). The financial burden on the health-care system is immense. In the United States alone, resistant bacterial infections are expected to cost $4 billion a year in health care expenses (Norland, 2018). Infections caused by resistant bacteria are more difficult to treat, necessitating the use of medications that are less commonly available, more expensive, and more hazardous (Norland, 2018). Bacteria that cause pneumonia, ear infections, and meningitis (e.g., *Streptococcus pneumoniae*), skin, bone, lung, and bloodstream infections (e.g., *Staphylococcus aureus*), urinary tract infections (e.g., *Escherichia coli*), food-borne infections (e.g., *Salmonella*), and infections transmitted in health care settings (e.g., *Enterococci* and *Klebsiella* spp. (Norland, 2018; Dadgostar *et al*., 2019; De Oliveira *et al.,* 2020; Zhang *et al.,* 2021). In the United States, nearly all strains of *Staphylococcus aureus* have developed penicillin resistance, (Twomey, 2010). In the United States, around 40,000 cases of Salmonella are reported each year, with high rates of drug resistance. According to the CDC (1999), about 11% of *Streptococcus pneumoniae* strains are resistant to third-generation cephalosporin medicines and are developing resistance to newer fluoroquinolones. Many strains are becoming multi-drug resistant, according to reports (MDR).

It's unclear how much antimicrobial antibiotic use in humans and agriculture contributes to resistance. According to the Animal Health Institute's (AHI) most current survey, 24.9 million pounds of antibiotics

were used in 1999 (of which 88.3 percent was for therapeutic use). The total usage had reduced to 21.7 million pounds by 2004 (of which 95 percent was for therapeutic use) (De Oliveira *et al*., 2020; Dadgostar *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

A total of 10 samples were collected from the three (3) female hostels in Caleb University, all in Lagos state. The samples were collected using sterile swab-sticks. Prior to swabbing the staircase rails, the swab sticks were briefly soaked in normal saline solution, and the samples were promptly transferred to Caleb University's microbiology laboratory for analyses.

3.2 Laboratory Procedures

3.2.1 Isolation

The specimen was inoculated onto Nutrient agar for enumeration and Mannitol salt Agar for isolation of total *Staphylococci* and incubated for 18 hours at 37°C. Reference numbers or coding was used to appropriately label the samples. Standard laboratory methods such as colony morphology, Gram staining, catalase test, and coagulase test were used to validate the isolates' identities.

3.3 Biochemical Tests and Identification of the Isolates

Biochemical tests such as the Gram staining reaction, Catalase test, Sugar Fermentation Test, Citrate Utilization Test, Motility Test, and Urease test were used to identify the isolates.

3.3.1 Gram Staining Reaction

On a sterile, grease-free slide, a thin smear film of the organism (18-24 hours old bacteria culture) was made, air dried, and heat fixed. After staining the dried smear with crystal violet for a minute and then rinsing it with water, the iodine was added for one minute and then rinsed off. After that, a decolourizer (70% ethanol) was poured for 5 seconds before being washed off. Finally, safranin was added for a minute

before being washed off. The slide was allowed to dry before being viewed using an X40 objective lens and an X100 oil immersion objective lens.

3.3.2 Catalase Test

Colonies of the isolates was aseptically picked with an inoculating loop and placed on a clean grease free slide. A drop of 6% hydrogen peroxide was dropped on the colonies. Evolution of gas bubbles caused by free oxygen indicated presence of catalase enzymes which shows positive result, while absence of bubbles indicates a negative reaction.

3.3.3 Sugar Fermentation Test

For each sugar, Peptone water was prepared in a beaker and the sugar (maltose, glucose, sucrose, and galactose was added to each beaker and labelled accordingly. Phenol red was added and the mixture dispensed into test tubes with inverted Durham tubes placed in them. They were sterilized and inoculated with colonies of the isolates and was incubated at 37^0C for 48 hours. Colour change from red to yellow indicated positive result while negative result has no colour change. In glucose, colour change from red to yellow indicated positive result for presence of acid while formation of bubbles in the Durham tube indicated positive result for gas production.

3.3.4 Citrate Utilization Test

The Citrate test was done in order to determine the ability of the isolates to utilize citrate as their sole source of carbon and ammonia as their sole source of nitrogen. Simmons citrate was prepared and homogenized in a water bath. It was put in test tubes and autoclaved. After sterilization, it was slanted and allowed to solidify. It was inoculated with the isolates and incubated at 37^0C for 48 hours. Colour change from green to deep blue indicated positive results while no colour change indicated negative result.

3.3.5 Motility test

Half strength of Nutrient agar was prepared and homogenized in a water bath. It was put in test tubes and sterilized. The agar was allowed to partially solidify and the isolates was stabbed into the agar and incubated at 37^0C for 48 hours. Motility is indicated by the spreading of the organisms outside the line of stab.

3.3.6 Urease Test

Urea agar base and urea solution was prepared. They were autoclaved in conical flasks together with empty test tubes. After sterilization, the urea agar base was mixed with the urea solution and dispensed into the test tubes and slanted. After it solidified, the isolates were aseptically taken with inoculating loops and inoculated on the slants. The tubes were incubated at 37^0C for 72hours. Colour change from yellow to pink indicated positive result while no colour change indicated negative result.

3.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using Kirby Bauer disk diffusion method following Clinical Laboratory Standards Institute (CLSI) 2011 guidelines. Five colonies of the 24hrs old culture of the microbe were emulsified in 5mls of sterile normal saline and mixed well; the turbidity was compared to 0.5 Mac Farland standard. A sterile cotton swab was used to inoculate the sample into Mueller-Hinton agar plates ensuring even distribution of the culture and allowed to dry. The antibiotics used included 5µg oxacillin, 30µg amoxicillin/clavulanic acid, 10µg gentamycin, 30µg Cefuroxime, 25µg cefotaxime, 5µg Levofloxacin, 5µg Cefixime, 45µg ceftriaxone sulbactam, 15ug Azithromycin, 10/10ug Imipenem, 5µg Ofloxacin, 5µg ciprofloxacin and 15µg erythromycin. The antibiotic ring containing the disc were aseptically placed on the plate using sterilized forceps. The plates were incubated for 18-24 hours at 37°C.

Zones of inhibitions were determined by measuring the size of clear zones around the discs and recorded in millimetres which was then compared to the CLSI standard.

CHAPTER FOUR

RESULTS

Ten bacteria were isolated from **S**taircase Railings in Caleb University. They were identified on the basis of morphological tests; microscopic and macroscopic examination, and biochemical tests. The bacteria identified were *Staphylococcus aureus* and *Staphylococcus epidermidis*.

The colony forming units of the bacteria isolates on Mannitol Salt Agar plate are shown in the (Table 4.1). The bacteria isolates appeared small in size with colour ranging from white to yellow, elevation ranging from convex to elevated, texture ranging from moist, mucoid, to dry, and all were round and opaque. Some isolates were whole, while others have curled margins (Table 4.2). All the bacteria isolates are gram positive and clustered cocci (Table 4.3).

All the bacteria isolates were catalase and urease positive. All bacteria isolates test negative to motility test (Table 4.4). *Staphylococcus aureus* and *Staphylococcus epidermidis* were both positive for glucose, sucrose, maltose and galactose fermentation (Table 4.5). Antibiotic Sensitivity Test of the bacteria isolates (Table 4.6). Antibiotic profile of the bacteria isolates (Table 4.7). Frequency of the Susceptibility of the bacteria isolates to antibiotics (Table 4.8).

In this study, *Staphylococcus aureus* had the highest occurrence of 70% while *Staphylococcus epidermidis* had the least occurrence of 30% (Figure 4.1).

Some of the bacteria isolates were resistant to OFX (30%), GN (40%), AZN (30%), LBC (30%), and CIP (20%), while all the bacteria isolates were resistance to ZEM (100%), CXM (80%), AUG (100%), CTX (100%) and IMP (90%) (Figure 4.2).

Table 4.1: Colony Forming Units of bacteria isolated from Staircase Railings in Caleb University on Mannitol Salt Agar plate

Sample Code	Isolates A ($x10^1$)	Isolates B ($x10^1$)
DR	4.7	0
DL	4.1	0
RR	7.2	0
RL	5.2	3.1
RL	2.5	0.8
ML	3.0	0
CAR	5.9	3.1
CAR	6.0	2.2
CAL	7.5	0
CAL	7.9	0

Table 4.2: Colonial Morphology of Bacteria Isolated from Staircase Railings in Caleb University

Sample code	Form	Size	Opacity	Elevation	Texture	Colour	Margin
ML	Round	Small	Opaque	convex	Moist	yellow	Even
RR	Round	Small	Opaque	convex	Moist	yellow	Even
RL[-a]	Round	Small	Opaque	convex	Moist	yellow	Even
RL[-b]	Round	Small	Opaque	convex	Moist	Pink	Even
DR	Round	Small	Opaque	convex	Moist	yellow	Even
DL	Round	Small	Opaque	convex	Moist	yellow	Even
CAR[-a]	Round	Small	Opaque	convex	Moist	yellow	Even
CAR[-b]	Round	Small	Opaque	convex	Moist	pink	Even
CAL[-a]	Round	Small	Opaque	Raised	Moist	yellow	Even
CAL[-b]	Round	Small	Opaque	Raised	Moist	Pink	Even

Table 4.3: Microscopy of Bacteria Isolated from Staircase Railings in Caleb University

S/N	Isolate Code	Gram stain	Shape
1.	DR	+	Cocci, clustered
2.	DL	+	Cocci, clustered
3.	RR	+	Cocci, clustered
4.	Rly	+	Cocci, clustered
5.	RLP	+	Cocci, clustered
6.	ML	+	Cocci, clustered
7.	CARP	+	Cocci, clustered
8.	CARY	+	Cocci, clustered
9.	CALP	+	Cocci, clustered
10.	CALY	+	Cocci, clustered

Table 4.4: Biochemical Test of Bacteria Isolated from Staircase Railings in Caleb University

S/N	Isolate code	Catalase	Urease	Citrate	Motility
1.	DR	+	+	+	-
2.	DL	+	+	+	-
3.	RR	+	+	+	-
4.	Rly	+	+	+	-
5.	RLP	+	+	-	-
6.	ML	+	+	+	-
7.	CARP	+	+	-	-
8.	CARY	+	+	+	-
9.	CALP	+	+	-	-
10.	CALY	+	+	+	-

Table 4.5: Sugar Fermentation Tests of Bacteria Isolated from Staircase Railings in Caleb University

S/N	Isolate code	Sucrose	Galactose	Maltose	Glucose	Probable organisms
1.	DR	+	+	+	+	*Staphylococcus aureus*
2.	DL	+	+	+	+	*Staphylococcus aureus*
3.	RR	+	+	+	+	*Staphylococcus aureus*
4.	Rly	+	+	+	+	*Staphylococcus aureus*
5.	RLP	+	+	+	+	*Staphylococcus epidermidis*
6.	ML	+	+	+	+	*Staphylococcus aureus*
7.	CARP	+	+	+	+	*Staphylococcus epidermidis*
8.	CARY	+	+	+	+	*Staphylococcus aureus*
9.	CALP	+	+	+	+	*Staphylococcus epidermidis*
10.	CALY	+	+	+	+	*Staphylococcus aureus*

Table 4.6: Antibiotic Sensitivity Test of Bacteria Isolated from Staircase Railings in Caleb University

Isolate code	OX	IMP	CXM	OFX	ERY	GN	AZN	AUG	CTX	CRO	ZEM	LBC	CIP
DR	28 (S)	24 (S)	20 (S)	62 (S)	14 (I)	12 (R)	20 (S)	10 (R)	6 (R)	28 (S)	6 (R)	20 (S)	30 (S)
DL	6 (R)	2 (R)	30 (S)	22 (S)	26 (S)	24 (S)	22 (S)	6 (R)	6 (R)	20 (R)	6 (R)	30 (S)	6 (R)
RR	23 (S)	10 (R)	6 (R)	12 (R)	10 (R)	14 (I)	10 (R)	6 (R)	6 (R)	36 (S)	6 (R)	38 (S)	20 (S)
Rly	6 (R)	16 (R)	6 (R)	30 (S)	16 (I)	8 (R)	20 (S)	8 (R)	6 (R)	8 (R)	6 (R)	36 (S)	4 (S)
RLP	13 (R)	10 (R)	6 (R)	20 (S)	12 (R)	12 (R)	14 (R)	6 (R)	6 (R)	16 (R)	12 (R)	14 (R)	20 (S)
ML	15 (R)	6 (R)	6 (R)	18 (S)	10 (R)	32 (S)	28 (S)	6 (R)	6 (R)	8 (R)	6 (R)	10 (R)	6 (S)
CARP	6 (R)	6 (R)	6 (R)	12 (R)	14 (I)	22 (S)	38 (S)	6 (R)	6 (R)	34 (S)	6 (R)	10 (R)	22 (S)
CARY	23 (S)	10 (R)	6 (R)	28 (S)	20 (I)	24 (S)	20 (S)	6 (R)	6 (R)	30 (S)	10 (R)	40 (S)	28 (S)
CALP	12 (R)	6 (R)	6 (R)	26 (S)	20 (I)	12 (R)	14 (R)	10 (R)	6 (R)	40 (S)	6 (R)	22 (S)	6 (R)
CALY	20 (R)	6 (R)	6 (R)	10 (R)	28 (S)	20 (S)	22 (S)	10 (R)	6 (R)	22 (S)	6 (R)	20 (S)	30 (S)

Table 4.7: Antibiotic Profile of Bacteria Isolated from Staircase Railings in Caleb University

Isolate code	OX	IMP	CXM	OFX	ERY	GN	AZN	AUG	CTX	CRO	ZEM	LBC	CIP
DR	S	S	S	S	I	R	S	R	R	S	R	S	S
DL	R	R	S	S	S	S	S	R	R	R	R	S	R
RR	S	R	R	R	R	I	R	R	R	S	R	S	S
Rly	R	R	R	S	I	R	S	R	R	R	R	S	S
RLP	R	R	R	S	R	R	R	R	R	R	R	R	S
ML	R	R	R	S	R	S	S	R	R	R	R	R	S
CARP	R	R	R	R	I	S	S	R	R	S	R	R	S
CARY	S	R	R	S	I	S	S	R	R	S	R	S	S
CALP	R	R	R	S	I	R	R	R	R	S	R	S	R
CALY	R	R	R	R	S	S	S	R	R	S	R	S	S

N.B: R = Resistance, S = Susceptible, I = Intermediate.

Table 4.8: Frequency of the Susceptibility of bacteria isolated from Staircase Railings in Caleb University

	NUMBER			PERCENTAGE		
ANTIBIOTICS	**R**	**I**	**S**	**%R**	**% I**	**% S**
OX	7	-	3	70	-	30
IMP	9	-	1	90	-	10
CXM	8	-	2	80	-	20
ERY	3	5	2	30	50	20
OFX	3	-	7	30	-	70
GN	4	1	5	40	10	50
AZN	3	-	7	30	-	70
AUG	10	-	-	100	-	-
CTX	10	-	-	100	-	-
CRO	4	-	6	40	-	60
ZEM	10	-	-	100	-	-
LBC	3	-	7	30	-	70
CIP	2	-	8	20	-	80

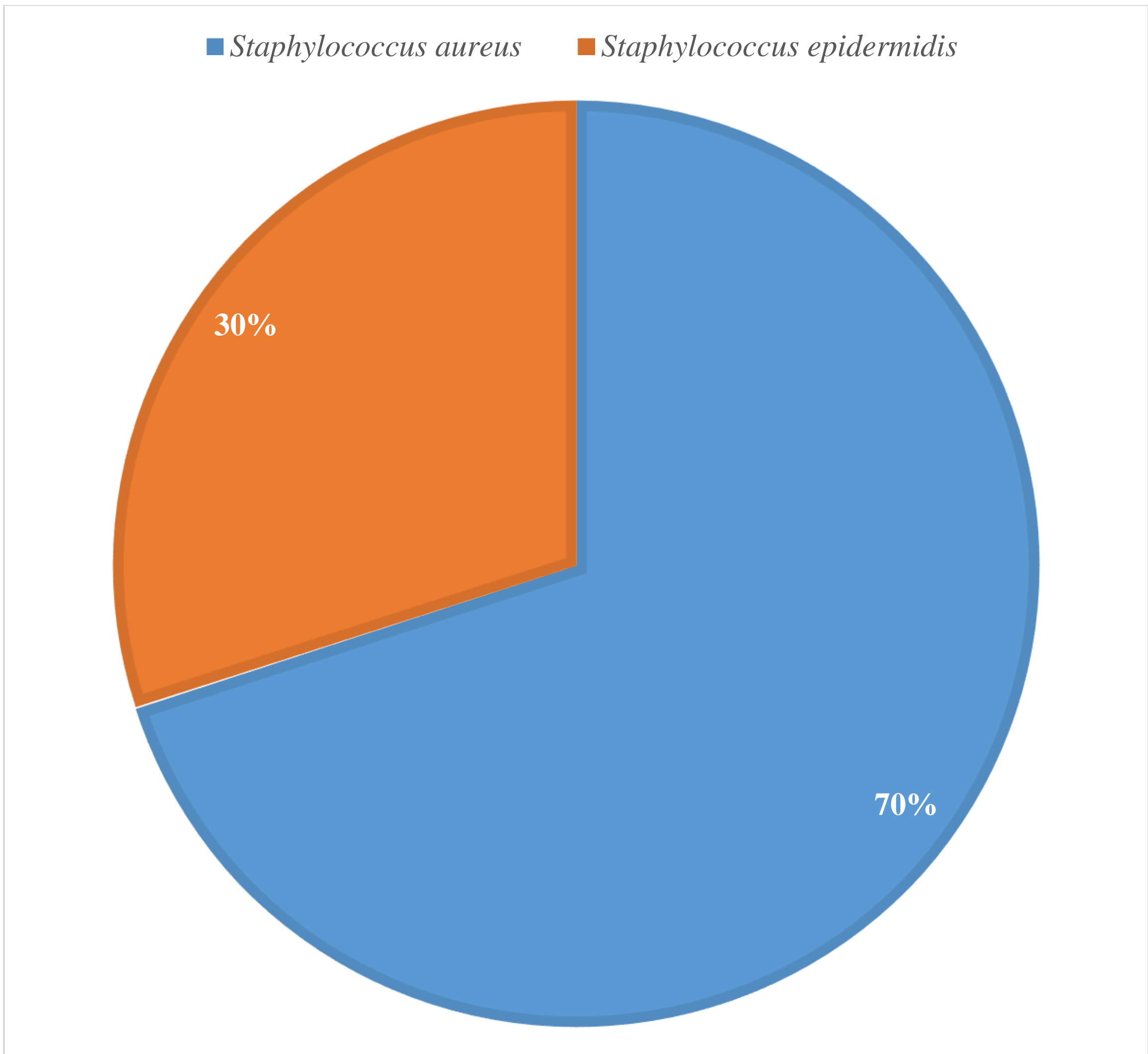
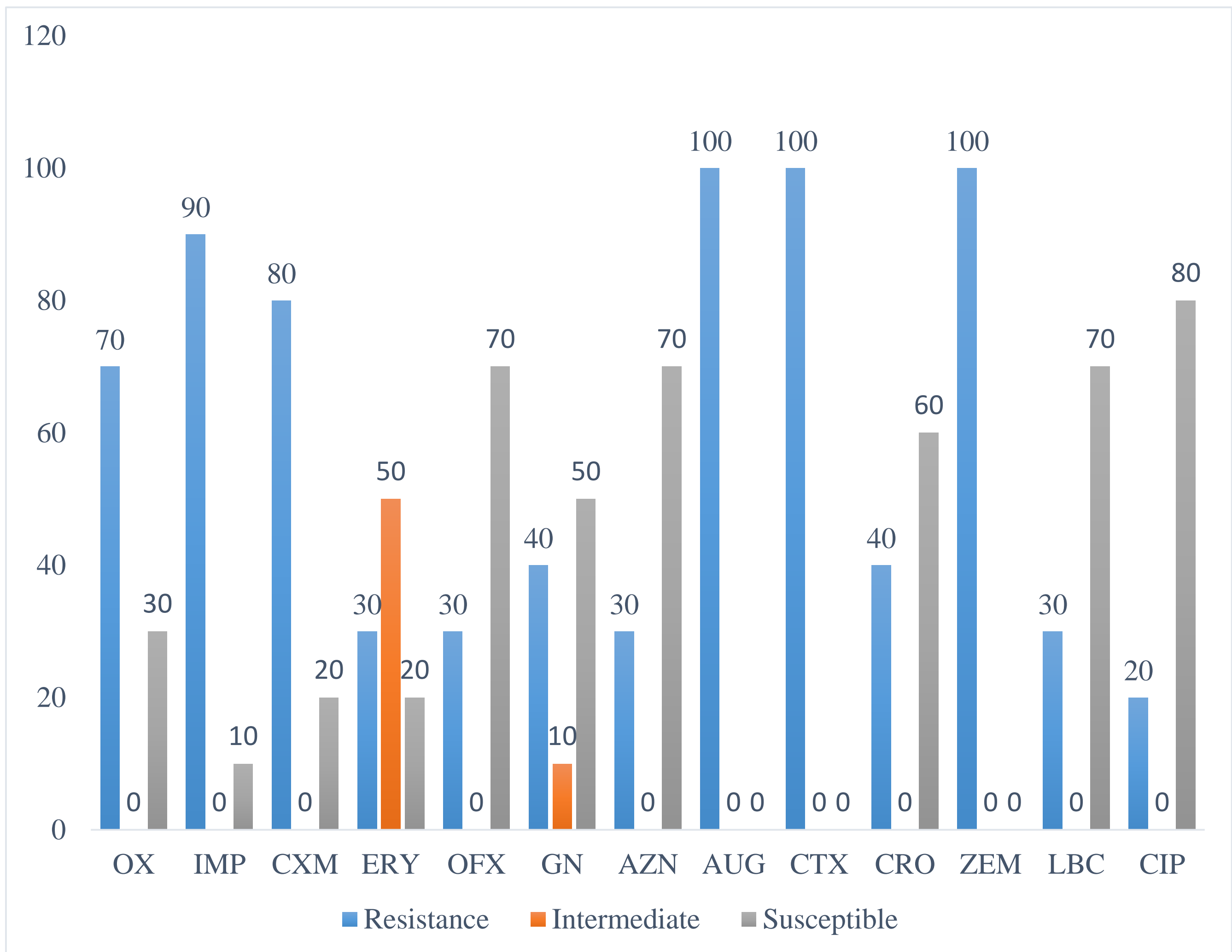


Figure 4.1: Frequency of Bacteria isolated from Staircase Railings in Caleb University



Key: R-resistance, S- Susceptible, I- Intermediate, OFX- Ofloxacin, CTX- Cefotaxime, CRO-Ceftriaxone, ZEM- Cefixime, LBC- Levofloxacin, CIP- Ciprofloxacin, AZN- Azithromycin, CXM- Cefuroxime, AUG- Amoxicillin, ERY- Erythromycin, IMP- Imepinem, GN- Gentamicin, OX- Oxacillin.

Figure 4.2: Percentage Susceptibility of Bacteria isolated from Staircase Railings in Caleb University

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The results of the current study reveals that solid surfaces are colonized by viable organisms. These findings are in agreement with the results obtained in previous studies (Pumipuntu *et al*., 2019; Dopcea *et al*., 2020) where they isolated pathogenic bacteria from staircase railing and concluded that staircase railing are actually contaminated with potential pathogens, a study based on *Staphylococcus* spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand.

From this study, 70% of the total isolates was *Staphylococcus aureus* with *Staphylococcus epidermidis* accounting for 30%. Thus, the prevalence of *Staphylococcus aureus* among the samples in this study is higher than in previous studies which 38.3% in a study conducted on the bacterial contamination, occurrence and Antimicrobial Susceptibility of *Staphylococcus aureus* on commercial banks automated teller machines (ATMs) in Kaduna Metropolis, Kaduna State, Northwestern Nigeria by Abdulaziz (2019) Another study also conducted at a large university in the United States reported 22.4% of *Staphylococcus aureus* (Thapaliya *et al*., 2017). These findings could be as a result of poor cleaning and disinfecting of the handrails in Caleb University. The difference in this study and previous studies can be due to the location and sample size.

All the isolates were subjected to antibiotic susceptibility tests by Kirby Bauer disc diffusion. Diameters of the zone of inhibition on Mueller-Hinton agar were measured and interpretation was made based on CLSI guidelines. In this study, the trends of zone of inhibitions for Resistant isolates (%) are; Ofloxacin (30%), Cefotaxime (100%), CRO-Ceftriaxone (40%), cefixime (100%), levofloxacin (30%), Ciprofloxacin (20%), Azithromycin (30%), cefuroxime (80%), amoxicillin (100%), Erythromycin (30%), Imipenem (90%), Gentamicin (40%) and oxacillin (70%). Some of the isolates were Susceptible to

Ofloxacin (70%), Ceftriaxone (60%), levofloxacin (70%), Ciprofloxacin (80%), Azithromycin (70%), cefuroxime (20%), Erythromycin (20%), Imipenem (10%), Gentamicin (50%) and oxacillin (30%). The high level of resistance of the isolates to Augmentin and cefuroxime is similar to a study carried out by Azuonwu *et al*; (2019) on antibiotics resistance pattern of bacteria from abattoirs in Port-Harcourt city, Nigeria.

It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Rahman *et al*., 2007). This therefore demands the need for periodic screening for common bacterial pathogens for their antibiotic susceptibility profiles in different communities (Rahman *et al*., 2010). Occurrence of resistance in pathogens may reduce the effectiveness of previously useful antibiotics (Toroglu and Dincer, 2018).

The high percentage susceptibility of the isolates to Ofloxacin is also in agreement with the report by Azuonwu *et al.* (2019) on antibiotics resistance pattern of bacteria from abattoirs in Port-Harcourt city, Nigeria.

This study is similar to the study of Issmat *et al*. (2007) who isolated multi-drug resistant bacteria from public computer surfaces in Ohio, USA. The results might be because of variation in geographical locations, environmental conditions and genetic background of the organisms.

Generally, the *Staphylococcus* spp. isolates in this study were more susceptible to antibiotics, but regular surveillance exercises should still be used to control the emergence of *Staphylococcus* spp. and other pathogens in school settings. Resistance rates were fairly high for most of the antibiotics tested, although the pattern of resistance to Cefotaxime, amoxicillin and cefixime in *Staphylococcus* spp. isolates mean that these antibiotics cannot be recommended for the treatment of *Staphylococcus* spp. infections if antibiotics used in this study are the only available ones for treatment.

5.2 Conclusion

This study shows that *Staphylococcus* spp. contamination is common on the staircase surfaces in Caleb University. The antimicrobial resistance profile of the isolates suggests the presence of multi-drug resistant organisms, which could potentially be passed from person to person via the staircase railings surfaces.

5.3 Recommendation

Cleaning and sanitizing of staircase handrails should be done regularly as this is often neglected. Washing and sanitizing of hands frequently should be done. Antibiotics should be used only when prescribed by a certified health professional as antibiotic resistance is accelerated by the misuse of antibiotics. Future research could include increasing the sampling size and area, as well as molecular characterization of the isolates.

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APPENDIX

APPENDIX I

Zone diameter interpretive standards chart for the determination of antibiotic sensitivity and resistance status by Disk Diffusion method (CLSI Standard)

S/N	Clases	Antibiotics	Susceptible	Intermediate	Resistant
1	Fluoroquinoles	Ofloxacin	>18	15 – 17	<14
		Ciprofloxacin	>21	16 – 20	<15
		Levofloxacine	>19	16 – 18	<15
2	Cephalosporins	Cefuroxime	>20	17 – 19	<16
		Ceftraxine	>26	-	-
		Cefotaxine	>26	-	-
		Cefixime	>21	-	-
3	Penicillin	Amoxilin clauvulamate	>20	-	<19
		Oxacillin	>25	-	<24
4	Macrolide	Azithromycin	>18	14 – 17	<13
		Erythromicin	>23	14 – 22	<13
5	Aminoglycoside	Gentamicin	>15	13 – 14	<12
6	Carbapenem	Imipenem	>16	14 -15	<13