PREVALENCE OF ANTIBIOTIC RESISTANT Enterobacteria IN SELECTED PUBLIC HEALTHCARE CENTRES IN IMO STATE

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THESIS SUBMITTED TO
POSTGRADUATE SCHOOL

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M. Sc.) DEGREE IN BIOTECHNOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

NOVEMBER 2023

CERTIFICATION

This is to certify that this research study on "prevalence of antibiotic resistant Enterobacteria in selected public healthcare centres in Imo State" is the original work of Emmanuel Ibe with registration number 20184139428 in partial fulfilment for the awards of M. Sc. Degree in Biotechnology under the supervisor of Dr. I. Emeka-Nwabunnia and Dr. R. N. Okechi of the Department of Biotechnology, School of Biological Sciences (SOBS), Federal University of Technology, Owerri (FUTO)

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DEDICATION

This research work is dedicated to God Almighty

ACKNOWLEDGEMENT

My deepest gratitude goes to my supervisors Dr. (Mrs.) I. Emeka-Nwabunnia and Dr. R.N. Okechi, for their immeasurable intellectual assistance, encouragement, and guidance through the course of this research. I immensely appreciate the efforts of the Head of Department Prof J.N. Okereke for his contribution towards the progress of this academic pursuit by being tenaciously positive and inspiring. I am also grateful to Dr. (Mrs.) T.E Ogbulie for her intellectual counsel and advice. I wish to acknowledge the massive initial efforts of my previous supervisor Late Prof. N.C.D Ukwandu. My sincere appreciation goes to all the lecturers in the Department of Biotechnology: Prof. (Mrs.) H.C Nwigwe (retired), Prof. A.I Okwujiako (retired), Prof. T.I.N Ezejiofor, Prof. I.C Mgbemena, Dr (Mrs.) S.O Anyadoh-Nwadike, Dr E.U Ezeji, Dr I.O Onyeocha, Dr E.A Anyalogu, Dr C.A Nsofor for their immerse impact of knowledge through the lectures delivered during this Masters' programme.

I am most grateful to my parents Mr. & Mrs. Francis Ibe, my siblings Miss Victory Ibe and Mr. Joshua Ibe, for their unceasing prayers, encouragement, love, and financial support. I also acknowledge the contributions of Miss Jocelyn Effe, Miss Damilola Anjolaiya, and Miss Bisola Anjolaiya.

The efforts and encouragement of my course mates Miss C. Ukachukwu, Mrs. C.A Unegbu, Mrs. Justina Uche, Mr. A. Moses, and Mr. A. Joshua can only be rewarded by God Almighty.

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ABSTRACT

The greatest threat on the utilization of antibiotic medications in the treatment of bacterial nosocomial infection is the emergence and distribution of resistance in the pathogen. Most of the prevalent nosocomial bacteria have been reported to exhibit multidrug resistance which is postulated to be either acquired or natural. The present study was targeted at investigating the prevalence of antibiotic resistant Enterobacteria from some public healthcare centres in Imo State. Using randomized complete block sample design, four public healthcare centres [Imo State University Teaching Hospital (ISUTH) Orlu, Federal Medical Centre Owerri (FMC) Owerri municipal, Aboh-Mbaise General Hospital (AMGH) Mbaise, and Imo State Specialist Hospital (ISSH) Umuguma] were selected at random. Swab samples were collected, isolated, and purified using standard procedures. Kirby-Bauer disc diffusion method was employed to determine the antibiotic sensitivity of the isolates. The resistant genes were determined using forward and reverse primers specific for the following resistant genes *bla*$_{TEM}$, *qnr*A, and aac(3)-1. The result showed 64.17 % growth of Enterobacteria. The highest Enterobacteria isolate was in materials from ISUTH (20.67±1.45), while the materials from FMC showed the lowest Enterobacteria isolates (18.00±1.15). *Escherichia coli* was the most abundant Enterobacteria (29.00 %) and *Shigella spp*. was the least abundant Enterobacteria (19.05 %). The isolates showed multiple resistance to some of the antibiotics tested (which included Pefloxacin, Ciproflox, Tarivid, and Augmentin). All the isolates were susceptible to Gentamicin, while area of technical uncertainty was noticed for Septrin, Cefalexin, and Streptomycin treatment on some of the isolates. The molecular characterization showed the presence of the three genes (*bla*$_{TEM}$, *qnr*A, and aac(3)-1) investigated. The *bla*$_{TEM}$ gene-band was the most prevalent (80.0 %) while the aac(3)-1 gene-band was the lowest prevalent resistant gene (60.0 %). These resistant genes were most abundant in *Klebsiella pneumoniae* (93.33 % of the isolates) and least abundant in *Shigella sp*. (33.34 % of the isolates). It was concluded that the genes: *bla*$_{TEM}$, *qnr*A, and aac(3)-1 were present in Enterobacteria species (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and *Shigella sp.*) isolated from public healthcare centres in Imo State. Antibiotics stewardship should be practiced in healthcare settings. The application of antibiotic on surfaces is recommended when cleaning invasive devices and certain types of hospital equipment.

Keywords: Antibiotics, Enterobacteria, sensitivity, prevalence, Resistant genes

CHAPTER ONE

1.1 BACKGROUND OF THE STUDY

Infections that are acquired in hospitals have been in existence since the inception of healthcare facilities and has been a significant health challenge even in this modern era (Bereket *et al*., 2012). According to Baka *et al*. (2014), hospital-acquired infections are usually identified from 48-72 hours following the patient's admission in the health care facility. Hospital-acquired infection refers to infections that emerge in patients during their stay in any healthcare facility. Thus, these infections were not present in the patient during the time of admission (Bereket *et al.*, 2012).

In a broad sense, aerobic Gram-negative rod-shaped bacteria are the most common pathogens of hospital-acquired infection (Mehrad, Clark, Zhanel & Lynch, 2015). A subset of these Gram-negative bacilli, referred to as Enterobacteriaceae, which commonly exist as commensal flora in the gut of humans have been associated to most of the hospital-acquired infection (Mehrad *et al*., 2015). Enterobacteriaceae asymptomatically colonize the upper aerodigestive tract and the skin of hospitalized patients; they cause infection from micro-aspiration or introduction into sterile sites. The most common types of HCAI are surgical wound infection, urinary tract infections (UTIs), bloodstream infections (BSIs), and pneumonia (Baka *et al*., 2014).

Organisms associated with hospital acquired infections, usually exhibit these features; they are established medically significant pathogens and they can adapt to the severity of hospital environment (Baka *et al.*, 2014). According to Baka *et al.* (2014), bacterial pathogens that are commonly associated with hospital-acquired infections include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Enterococcus* spp., *Enterbacter* spp. *and Acinetobacter* spp. Enterobacteria possess various modes of resisting

antibiotics and can proficiently transfer resistant genes horizontally between species. This have contributed to the dramatic increase in antibiotic resistant Enterobacteriaceae (Centers for Disease Control and Prevention CDC, 2013).

The greatest threat on the utilization of antibiotic medications in the treatment of bacterial infections is the emergence and widespread of resistance in the pathogens (Bolaji, Akande, Iromini, Adewoye & Opasola, 2011). Antibiotic resistance is the capability of bacteria to resist the action of antibiotic medication that was previously effective against the bacteria (CDC, 2017). The four main mechanisms by which bacteria exhibit resistance are drug inactivation or modification, alteration of target or binding site, alteration of metabolic pathway and reduced drug accumulation (Jose & Cesar, 2016). Most of the prevalent nosocomial bacteria are multidrug resistant that are either acquired (e.g., extended-spectrum β-lactamase producers and methicillin resistant *Staphylococcus aureus*) or are naturally resistant (Bereket *et al*., 2012).

In Nigeria, there is inadequate knowledge on the prevalence and characterization of Enterobacteria associated with hospital-acquired infection. Most research focus on the types of diseases caused by nosocomial pathogens and are usually a questionnaire survey study. For proper prevention of hospital-acquired infections, it is necessary to identify the sources and causal organisms of the infection, in order to strategize and implement preventive practices.

1.2 STATEMENT OF THE PROBLEM

The widespread of antibiotic resistance can be partly attributed to selection pressure that results from antibiotic medication which incites mutations that conferred antibiotic resistance to bacteria (Nessme, Cecillon, Delmont, Monier, Vogel & Simonet, 2014). Antibiotic resistant bacteria have found a niche in hospital environment where there is high density of susceptible hosts, intense

antibiotic resistance selection pressure, and numerous opportunities for infection transmission (Mehrad *et al*., 2015). In the recent years there have been explosive rise in antibiotic resistance resulting from clonal spread of resistant organisms over geographically distant regions (Petty, Ben Zakour, Stanton-Cook, Skippington, Totsika, & Forde, 2014). This global spread is increased by the poor hygiene of healthcare facilities and common use of over-the-counter antibiotics in developing countries.

About 1.4 million people suffer from hospital acquired infections (Saka, Saka & Adebara, 2011). Uneke and Ijeoma (2010), estimated 25 - 40% hospital-acquired infection prevalence in poor resource settings. That is to say, the prevalence of nosocomial infection is two to three folds higher in developing countries (Naidu, Nabose, Ram, Viney, Stephen, Graham *et al*., 2014). About 45.8% nosocomial infection prevalence have been recorded in Nigeria, with an incidence density of 26.7 infections per 1000 patients (Azeez-Akande, 2012). Non-negligible degree of deaths has been associated with hospital acquired infections. Death rate of 0.7 – 2.5% have been reported in Nigeria by Azeez-Akande (2012). Ige, Adesanmi, and Asuzu (2011), observed an increase in the prevalence of nosocomial infections in the southwestern region of Nigeria.

Currently, healthcare acquired infections is one of Africa's great challenges, and the emergence of antimicrobial resistant strains of nosocomial infection pathogens has made it more threatening. This is because, the efficacy of available therapy is reduced and the death cases are increasing (Akingbode, Ojo, Okerentugba, Adejuwon & Okonko, 2013). This issue has been aggravated by inefficient social and healthcare systems, overcrowding of hospitals, inadequate staffing, poor infection control policies, and lack of trained professionals (Nejad, Allegranzi, Syed, Benjamin & Pittet, 2011). The age of the patient and most especially the length of hospital stay adds considerably to the extent of the problem (Mbim, Mboto & Agbo, 2016).

Unlike other related research, this present research isolated Enterobacteria from healthcare outfits and compared the prevalence of these pathogens among the various healthcare facilities. The study also investigated the gene bands of some common resistant genes responsible for multi-drug resistance after investigating the antimicrobial sensitivity of the Enterobacteria species isolated from the healthcare facilities.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to investigate the prevalence of antibiotic resistant Enterobacteria in selected public healthcare outfits in Imo state.

In specific terms, the objectives of the study are:

i. To isolate and purify Enterobacteria from gloves, ward floor, bed pans, and bed covers utilized in healthcare centres.
ii. To identify and characterize the isolates using microscopic, cultural, and biochemical assays.
iii. To investigate the antibiotic resistance potential to some commercially available antimicrobials on some of the bacterial isolates.
iv. To determine the gene(s) responsible for resistance through molecular assay.
v. To compare the prevalence pattern of the isolates from the healthcare centres.

1.4 JUSTIFICATION OF THE STUDY

Almost any organism can cause nosocomial infection, but only a few numbers of organisms are consistently responsible for hospital acquired diseases (Bereket *et al*., 2012). In order for antibiotic resistant Gram-negative bacilli infections to be efficiently prevented and controlled a proper knowledge on the pathogens responsible for the infections is required (Manikandan & Amsath, 2013). Therefore, it is necessary to identify the various species isolated from the samples obtained from the hospitals and other healthcare facilities.

It has become evident that most of the serious Gram-negative bacilli infections are caused by bacteria that are resistant to common antibiotics, making the treatment of these infections to be more challenging and expensive (Baka *et al*., 2014). This resistance could be natural or acquired. Studies have identified some Gram-negative pathogenic bacteria that are resistance to ceftazidime and β-lactam antibiotics (Baka *et al*., 2014). According to Bereket *et al*. (2012), most of the commonly identified healthcare-associated bacteria are multidrug resistant, and as such the present study investigates the antibiotic resistance potential of the bacterial isolates.

Generally, studies have shown that Gram-negative bacilli infections are a global issue. There have been reports of higher occurrences of hospital acquired infections in developing and under-developed countries than developed countries (Uneke & Ijeoma, 2010). A high rate of hospital acquired infection prevalence have also been recorded in Nigeria compared to other sub-Saharan countries, with mortality rate of 0.7 to 2.5% (Azeez-Akande, 2012). Nosocomial infection prevalence increases annually in the southwest region of Nigeria (Ige *et al*., 2011). It is therefore important to determine the prevalence of antibiotic resistant Enterobacteria associated with hospital environment.

1.5 SCOPE OF THE STUDY

A Large group of microorganisms can be associated to healthcare environment; this study focuses on the isolation and characterization of Enterobacteria associated with healthcare environment. The phenotypic characterization to be applied encompasses microscopy, and biochemical test. Molecular assay (which involves; DNA extraction and PCR amplification, using specific primers that code for various resistivity) will be employed to investigate multi-drug resistivity. The prevalence of antibiotic resistant Enterobacteria was determined in some public healthcare centres in some parts of Imo State.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF HOSPITAL ACQUIRED INFECTION

2.1.1 Concept of Hospital Acquired Infection

Nosocomial infection or "healthcare associated infection (HCAI)" refers to infections that occur in a patient receiving medical care in a health care facility or hospital; this infection wasn't in the patient at the time of admission (Hassan, Fatima & Riffat, 2017). According to Hassan *et al*. (2017), these infections may occur during treatment for other illness or after the patients are discharged. Also, they can be responsible for occupational infections among medical staff (WHO, 2016). Mbim *et al*. (2016) defined nosocomial infections as infections that occur in patients during their stay in hospitals and other types of medical facilities which weren't present in the patients at the time of their admission. Amoran, Sogebi & Fatugase (2013), opined that acquired infections by visitors or staffs in hospitals or any other health care facility can also be identified as nosocomial.

An infection occurs when a foreign organism (that is capable of utilizing the host's body to sustain itself, reproduce, and colonize) enters the body of a host and cause harm (Qyli, 2017). These infectious organisms are known as pathogens. According to Mbim *et al*. (2016), pathogenic microorganism usually occurs in body fluids or body sites that generally are believed to be sterile (such as blood or cerebrospinal fluid), and this may be seen as infection. Bereket *et al*. (2012) stated that, if an infection is already present in a patient on admission it is not considered nosocomial. However, when there is a change in symptoms or pathogenic organisms, indicating the acquisition of a new infection, such infections can be called nosocomial (Mbim *et al*., 2016).

The increase of nosocomial infections has caused an increase in antimicrobial resistance, prolonged hospital stays, long term disability in patients, and increased mortality rate (Hassan *et al*., 2017). According to Nejad *et al*. (2011), the care of patients is provided in facilities ranging from frontline units (with basic facilities) to university teaching hospitals and clinics (with well-equipped facilities) which have been implicated in nosocomial infections from superficial infections to necrotizing soft tissue infections.

2.1.2 Origin of Hospital Acquired Infections

Based on research conducted by Jain, Agarwal, Verma, Awasthi & Singh (2011); and Sherifa and Moataz (2011), nosocomial pathogens often come from patient's own endogenous flora while occasionally these pathogens may come from cross contamination (contact with staff), environment, and contaminated needles. In practice, virtually all organisms have the ability to initiate infection in hospitalized patients (Nejad *et al*., 2011). This may be because the patient is more susceptible to infection due to diminished immunity. Generally, fungi, bacteria, viruses, and other parasites are often responsible for most nosocomial infections (Bereket *et al*., 2012; Sikka, Mann, Vashist, Chaudhary & Antriksh, 2012). The diseases primarily associated with these organisms include urinary tract infections (Jombo, Emanghe, Amefule & Damen, 2011), nosocomial respiratory site infections (Mbim *et al*., 2016), surgical site infection, meningitis (van-de Beek, Drake & Tunkel, 2010), gastroenteritis (Polage, Solnick & Cohen, 2012), and blood stream infections (Afshari, Schrenzel, Ieven & Harbarth, 2012).

2.1.3 Types of Nosocomial Infections

The most frequent types of nosocomial infections are outlined as follows:

1. **Central line-associated bloodstream infections (CLABSI):** According to Hassan *et al*. (2017), "CLABSIs are deadly infections with death incidence rate of 12% - 25%. Catheters are inserted in central line in order to provide medicines and fluids, but its prolonged use may cause bloodstream infections that compromises health and increase care cost (WHO, 2016). This type of nosocomial infection occurs principally among seriously ill patients (Elliot & Justiz-Vaillant, 2018). Based on research studies, in 85% prevalence of bloodstream infection in patients, *Enterococcus* and *Klebsiella* species were the most common pathogens; and patients with these infections had higher comorbidity. Bloodstream infection was observed to occur more in males that are critically ill, under immunosuppression and also a central venous catheter (Ulrich, Santhosh & Mogle, 2017). There are also reports suspecting the idea that certain medical or surgical procedures can increase the occurrence chances of CLABSI (Kutlesa, Santini & Krajinovic, 2017; Wu, Chang & Huang, 2017). The most common pathogen responsible for nosocomial bloodstream infection is *Staphylococcus aureus* which accounts for 34.6% of infection cases (of which $^{2}/_{3}$ were methicillin resistant) (Elliot & Justiz-Vaillant, 2018).
2. **Catheter associated urinary tract infections (CAUTI):** Globally, CAUTI is the majority type of nosocomial infection. According to acute care hospital statistics in 2011, more than 12% of reported nosocomial infections are UTIs (CDC, 2015). The endogenous native microflora of patients is the cause of CAUTIs. Catheters serve as a conduct for bacteria entry while the imperfect drainage of urine from the catheter retains some urine in the bladder providing stability to bacterial resistance. The following complications can result from CAUTI; epididymitis, orchitis, and prostatitis in males, and cystitis, pyelonephritis, and meningitis in all patients (CDC, 2016).

3. **Surgical Site Infections (SSI):** 2% - 5% of patients subjected to surgery contracts nosocomial SSIs. It is the second most common nosocomial infection caused mainly by *Staphylococcus aureus* which results to prolonged hospitalization and death risk (Anderson, 2011). The causative organisms of SSI originate from endogenous microflora of the patient. The incidence of SSI can be as high as 20% based on the procedure and surveillance utilized (Hassan *et al*., 2017).
4. **Ventilator associated pneumonia (VAP):** VAP, a nosocomial pneumonia, occurs in 9-27% of patients using mechanically assisted ventilator. It often occurs within 48 hours after the tracheal incubation (Hunter, 2012). Ventilation is associated with 86% of nosocomial pneumonia (Hassan *et al*., 2017). Leucopenia, fever, and bronchial sounds are symptoms of VAP (Hjatmarson, 2010).
5. **Nosocomial Skin and Soft Tissue Infection (SSTI):** these infections include edema, warmth, presentation of pain, erythema, cutaneous blood loss, violaceous bullae, skin anesthesia, skin sloughing, gas in the tissue, and rapid evolution (Stevens, Bisno & Chambers, 2014). Skin and soft tissue infections arise as a result of skin invasion and is mainly due to surgery or trauma. According to Ramakrishman, Duse, Wattal, Zaidi, Wertheim, Sumpradit *et al*. (2015), "SSTIs can be classified as simple necrotizing, or suppurative". SSTIs have a general prevalence estimate of 7% to 10% (Elliot & Justiz-Vaillant, 2018). SSTIs occurs frequently among male patients and the pathogens associated with SSTIs include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus* spp.

2.1.4 Nosocomial pathogens

Pathogens that are responsible for nosocomial infections are viruses, bacteria, and fungal parasites (Hassan *et al*., 2017). These pathogenic microorganisms may vary depending on medical facilities, patient populations, and environment in which healthcare is given (Hassan *et al*., 2017).

1. **Viruses:** Frequent monitoring showed that 5% of nosocomial infections are caused by viruses. Their transmission can be through respiratory route, hand-mouth, and fecal-oral route. Delivery of healthcare services can transmit hepatitis viruses to both patients and workers. Commonly, hepatitis B and C are transmitted through unsafe injection practices (CDC, 2016). Other viruses commonly associated with nosocomial infections include Human Immune Virus, Influenza, Herpes-simplex virus, and rotavirus (Hassan *et al*., 2017).
2. **Bacteria:** The most common pathogens responsible for nosocomial infections are bacteria. Some are among the natural microflora of the patient and causes infection when the patient's immune system becomes compromised and thus susceptible to infections. *Acinetobacter*, a kind of pathogenic bacteria, is responsible for infections that occur in intensive care units (ICUs) and accounts for 80% of reported infections (Suresh & Joshi, 2013). Also, *Bacteroides fragilis* which is a commensal in intestinal tract and colon, can cause infection when combined with other bacteria. *Clostridium difficile* which causes colon inflammation can be transferred from infected patients to others through improperly cleansed hands of healthcare staff. Other pathogenic bacteria that are associated with nosocomial infections include Enterobacteriaceae which constitute of *Klebsiella* species and *Escherichia coli*, that possess high resistance to carbapenem (CDC, 2016). Methicillin-resistance *Staphylococcus aureus* (MRSA) can be transmitted through open wound, direct

contact, and contaminated hands. It causes pneumonia, SSI, and sepsis by travelling to organs or bloodstream (CDC, 2016).

3. **Fungal parasites:** Fungal parasites cause nosocomial infections in immune-compromised patients, acting as opportunistic pathogens. *Aspergillus* spp. cause infections via environmental contamination. *Cryptococcus neoformans* and *Candida albicans* also cause nosocomial infections during hospital stay. *Aspergillus* infections are caused by inhalation of fungal spores from contaminated air while *Candida* infections develops from the patient's endogenous microflora (Emily & Sydnor, 2011).

2.2 FACTORS RESPONSIBLE FOR HOSPITAL ACQUIRED INFECTION AND TRANSMISSION

The development of nosocomial infection can be characterized by series of events and often can be influenced by the microbe (virulence and inoculums size), microorganisms, environment, transmission route, and the patient's immune system (Samuel, Kayode, Musa, Nwigwe, Aboderin, Salami *et al*., 2010). The major factors that determine nosocomial infection include the following:

Environment: pathogens live in healthcare environment (e.g., food, water, equipment) and can be a source of nosocomial infection. The presence of nosocomial pathogens can be due to poor hygienic conditions and improper waste disposal system of healthcare centres (Hassan *et al*., 2017).

Susceptibility: according to Hassan *et al*. (2017), "susceptibility refers to immunosuppression in the patients, which can be attributed to prolonged stay of patients in intensive care unit, and prolonged use of antibiotics".

Unawareness: nosocomial infection can be facilitated by poor knowledge of basic infection control measures, improper use of injection techniques, lack of control policies, and inappropriate use of invasive devices (catheters) (Chand, 2014). In under-developed, and developing countries, the factors responsible for nosocomial infection can also be attributed to understaffed healthcare settings, poverty, lack of equipment, and lack of financial support (Allegranzi, 2011).

Microflora of patient: bacteria in the patient's endogenous microflora can cause infection when transferred to surgical site or tissue wound. Studies have shown that endogenous Gram-negative bacteria in the digestive tract can cause SSI after abdominal surgery (Hassan *et al*., 2017).

Nosocomial transmission

According to Manikandan and Amsath (2013), microorganisms' major entry points are compromised skin, and mucosal surfaces (such as gastrointestinal tract, urinary tract, and respiratory tract). The primary routes of transmission include airborne, direct or indirect contact, droplets, equipment, food, medication, as well as devices (Bereket *et al*., 2012). Nosocomial infection can be transmitted through the following means:

Transmission from environment: unhygienic environment is the best medium for the growth and development of pathogenic organisms. Contaminated water, air, and food can serve as means of pathogen transmission to patients under healthcare setting.

Transmission from staff: Nosocomial pathogens can be transferred to patients from healthcare staffs. This type of transmission can be attributed to lack of personal hygiene by the staffs (e.g., improper hand disinfection after contact with infected patient), unsafe injection practices, and un-sterilization of equipment (Hassan *et al*., 2017).

Studies have shown that patients admitted into hospitals are usually at high risk of infections. Akingbode *et al*. (2013), reported that openings and the skin of patients can be colonized by nosocomial pathogens. He proposed that this colonization is enhanced by intrinsic and extrinsic factors. The intrinsic factors include underlying disease, age, disrupter of natural barriers (such as injury/lesion), while the extrinsic factors include factors within the healthcare environment or staff. Bereket *et al*. (2012), also reported that conditions and practices of the entire healthcare facility or hospital can significantly enhance nosocomial pathogen colonization. Samuel *et al*. (2010), listed some prevailing conditions that enhance nosocomial pathogen transmission, which include air conditioning system, water systems, and resistance to antimicrobial agents. In addition, they also reported that patients previously colonized by nosocomial pathogens on admission are frequently at a high risk of infection especially when under invasive routines (use of invasive devices) (Samuel *et al*., 2010).

According to Mbim *et al*. (2016), the length of hospital stay is the most significant factor for the acquisition of nosocomial infections. Newborns admitted into neonatal intensive care unit are at disadvantage. They may possess a variety of host factors that doesn't only make them susceptible to infections but also increases their risk of contracting more severe illnesses (Samuel *et al.,* 2010*;* Polin *et al*., 2013).

2.3 EPIDEMIOLOGY AND DISTRIBUTION OF HOSPITAL ACQUIRED INFECTION IN AFRICA

According to WHO (as cited by Mbim *et al*., 2016), health care acquired infections are among the major infectious diseases with huge economic consequences worldwide. These infections have been reported to affect about two million people annually, in which 5 to 15% of the affected population requires hospitalization (Apanga, Adda, Issahaku, Amofa, Mawufemor & Bugr, 2014).

Also, some studies have reported disparity in nosocomial infection due to income status. WHO (2011) reported that the occurrence of healthcare patients that contracted nosocomial infections varied from 3.5% - 12% in high income countries. The prevalence of nosocomial infection in developing countries varied from 5.7% - 19% with a pooled occurrence of 10.1 per 100 patients (WHO, 2011).

In Africa, major reports on drug resistance and infectious diseases are limited to problems associated with Tuberculosis, Human Immune Virus (HIV), Malaria, and other emerging resistance pathogen (Bello, Asiedu, Adegoke, Quartey, Appiah-Kubi & Bertha, 2011). However, Azeez-Akande (2012), reported that healthcare associated infection rate ranges from 2% - 49%. He further stated that various locations showed high figures of 21.1% to 35.6% (nosocomial infection) which occurred primarily in intensive care unit. Generally, research data have suggested that healthcare associated infections are prevalent in sub-Saharan Africa with surgical site infections being the most common nosocomial infection. The prevalence rate of nosocomial infection in some selected countries in Africa is represented in the Table 1 below.

However, studies have shown that disparity also exist in variation of nosocomial infections within Nigeria (Mbim *et al*., 2016). According to Samuel *et al*. (2010), the prevalence rate of 2.7%, 3.8%, and 4.2% were recorded from Ife, Lagos, and Ilorin respectively. The Figure 1 below illustrates the percentage distribution of nosocomial infections in the sub-Saharan regions of Africa.

Table 2.1: Prevalence of Nosocomial Infection in Some Sub-Saharan Africa countries

S/N	% Prevalence of nosocomial infection per population	Countries	Sources
1	2.5% to 14.8%	Burkina Faso, United Republic of Tanzania and Senegal	Nejad *et al*., 2011; WHO, 2011
2	6.7%	Ghana	WHO, 2011
3	9.6% to 18.7%	Mali	WHO, 2011
4	10.9%	Senegal	Nejad *et al*., 2011; WHO, 2011
5	2.1%	Cameroun	Kensah *et al*., 2013
6	1.6% -11%	Gabon	Kareem *et al*., 2014
7	1.7%	Democratic Republic of Congo	Chu *et al*., 2014
8	28%	Uganda	Greco and Magombe, 2011
9	5.7%– 45.8%	Nigeria and Ethiopia	Azeez-Akande, 2012; Mulu *et al*., 2012

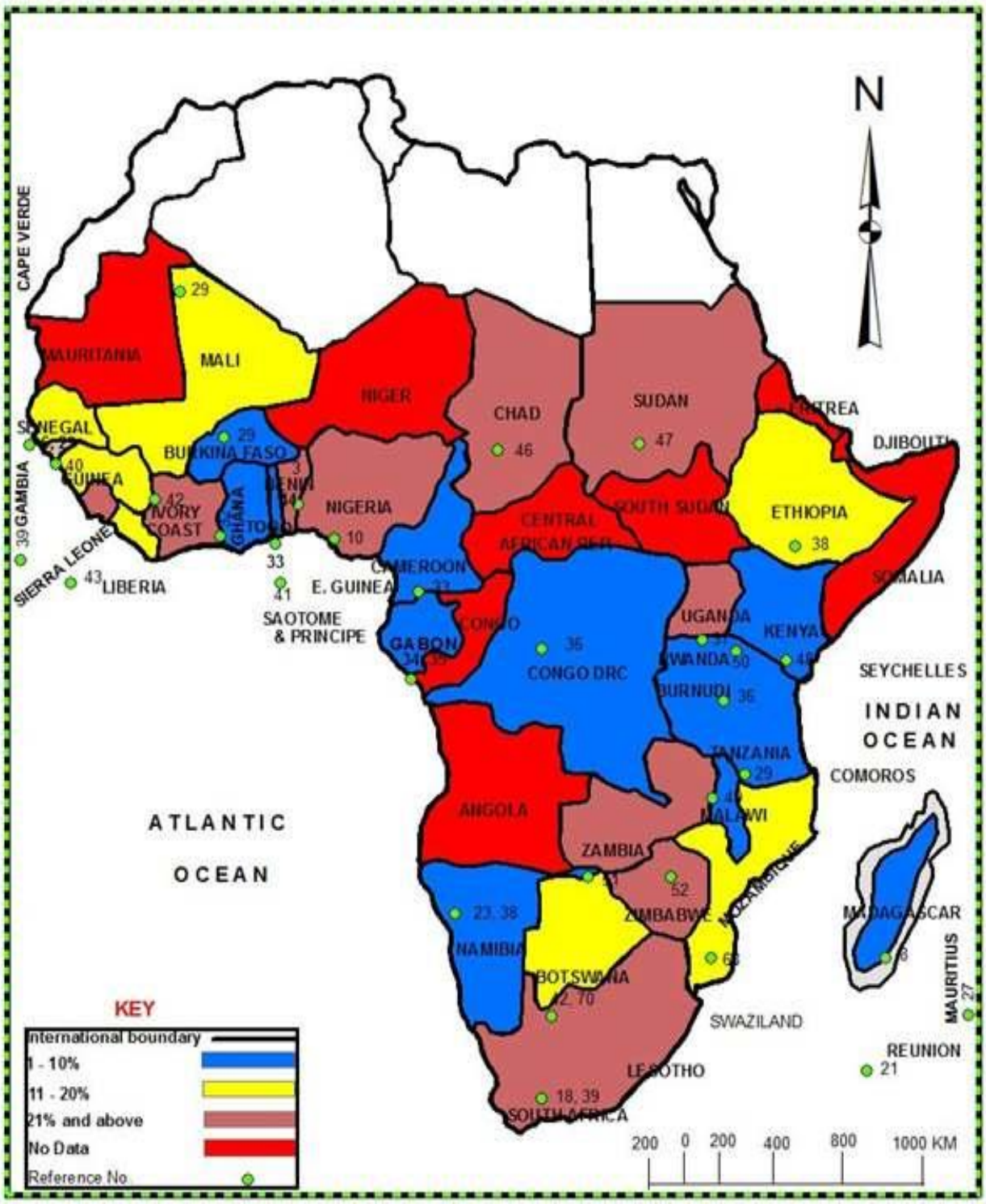


Figure 2.1: Map of Africa showing the percentage distribution of nosocomial infection in the sub-Sahara

Source: Mbim *et al*. (2016).

A study conducted by Ige, Adesanmi & Asuzu (2011), on reviewing the records of nosocomial infection from tertiary hospitals in southwest Nigeria for the years 2005-2009 showed a gradual increase in the prevalence of nosocomial infection. The record was obtained from the infection control unit of the hospital. This review showed an increase in nosocomial infection from 2.4% in 2005 to 3.1% in 2008, although a decline was noted in 2009 with a prevalence rate of 2.3% (Figure 2.2) (Ige *et al*., 2011). The study also highlighted the distribution of healthcare acquired infections in each ward. Over 48.3% of all nosocomial infections were from the surgical wards, 20.5% from medical, 16.1% from obstetrics and gynecology, and 15.1% from pediatric ward. Although as the years progressed, there were disparities in the prevalence rate among the wards, but surgical ward was consistently the ward with the highest nosocomial infection rate (Figure 2.3). The isolates from the study were more of Gram-negative bacteria (about 78.3% of all the pathogens associated with HCAI) (Ige *et al*., 2011). The isolates identified in the study are listed in Table 2.2.

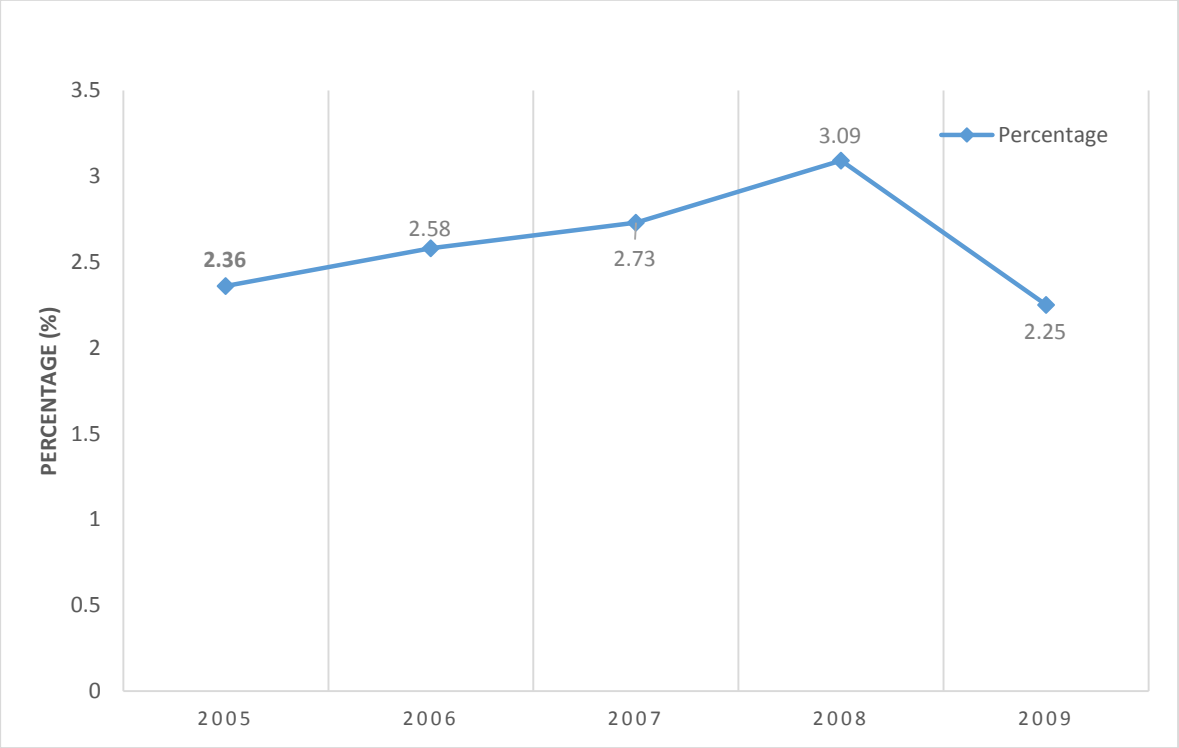
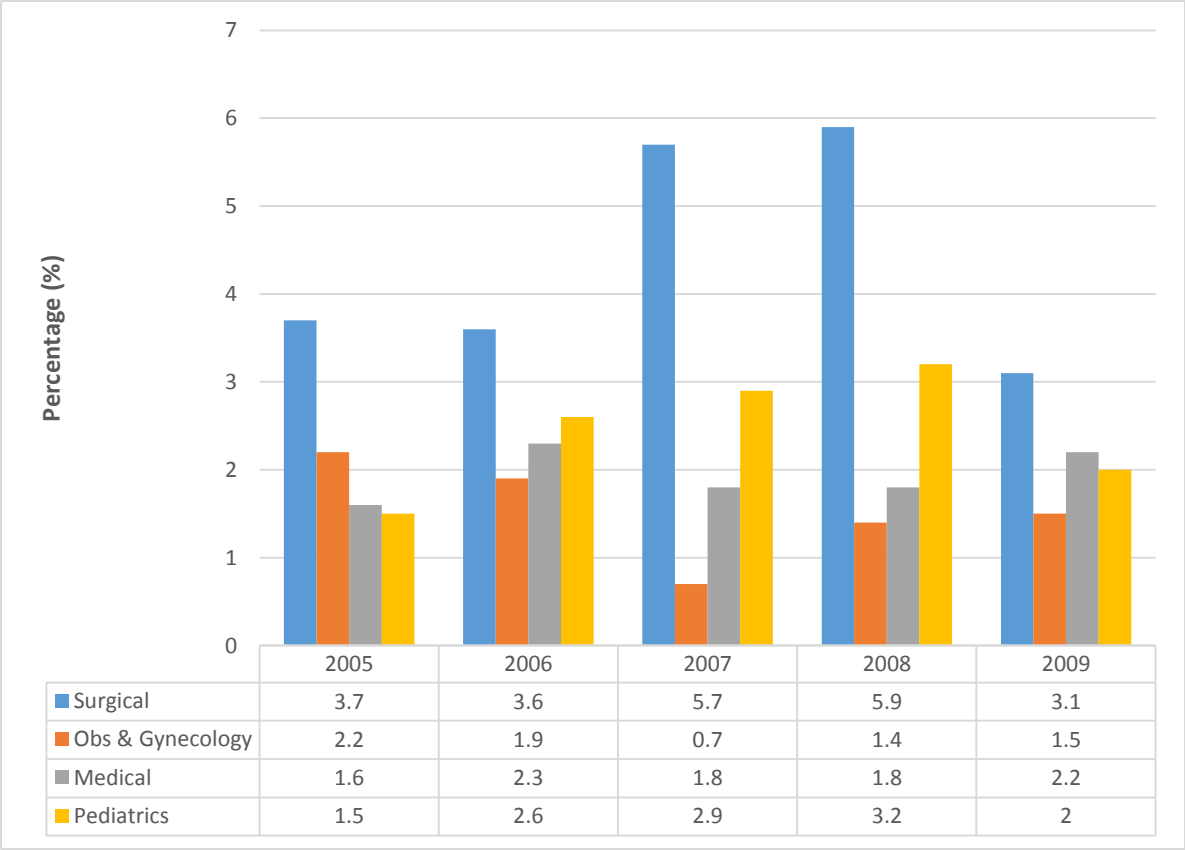


Figure 2.2: Prevalence of HCAI in UCH Ibadan from 2005-2009.

Source: Ige *et al*. (2011)



	2005	2006	2007	2008	2009
Surgical	3.7	3.6	5.7	5.9	3.1
Obs & Gynecology	2.2	1.9	0.7	1.4	1.5
Medical	1.6	2.3	1.8	1.8	2.2
Pediatrics	1.5	2.6	2.9	3.2	2

Figure 2.3: HCAI in each ward in UCH Ibadan for 2005-2009.

Source: Ige *et al*. (2011)

Table 2.2: Isolates from HCAI in UCH Ibadan

Pathogen	N (%)
Klebsiella spp.	242 (34.28)
Staphylococcus aureus	142 (20.11)
Escherichia coli	138 (19.55)
Pseudomonas aeruginosa	86 (12.18)
Proteus mirabilis	32 (4.53)
Pseudomonas spp.	31 (4.39)
Proteus spp.	24 (3.4)
Enterococcus faecalis	6 (0.85)
Streptococcus spp.	5 (0.71)

Key: HCAI – Healthcare acquired infections, UCH – University College Hospital

Source: Ige *et al*. (2011)

2.4 Enterobacteria AND ANTIBIOTIC RESISTANCE MECHANNISMS

Enterobacteria are the most common causes of hospital-acquire Gram-negative infection (Mehran *et al*., 2015). They are capable of growth in the presence of bile salts, utilizing lactose as energy source on MacConkey's agar. Some other Gram-negative bacilli that are capable of growth on MacConkey agar but do not utilize lactose include *Pseudomonas* and *Acinetobacter* species (Sun, Fujitani, Quintiliani & Yu, 2011). The bacteria family Enterobacteria contain multiple genera (which include *Klebsiella, Proteus, Salmonella, Enterobacter, Yersinia, Escherichia coli, Citrobacter, Shigella, Serratia, Morganella, Providencia,* and *Pseudomonas*) which are capable of existing as part of the microbiota of the mammalian intestinal tract. As a common response to systemic illness, these organisms colonize the upper aerodigestive tract and as such can be transmitted through hand carriage and fomites (Mehran *et al*., 2015). The inhalation or introduction of colonizing Enterobacteria into the lower airways/lungs can result in nosocomial pneumonia. Furthermore, introduction of these Enterobacteria species into sterile sites can result to infections of surgical sites, urinary tract, and venous catheters (Piednoir, Thibon & Borderan, 2011).

According to Tankeshwar (2013), the common characteristics of Enterobacteria include the following:

1. They are short rod-shaped, Gram-negative bacteria.
2. They are non-sporulating, facultative anaerobes.
3. Species that are motile, move by means of peritrichous (lateral) flagella. *Shigella* and *Klebsiella* species are non-motile.
4. They are catalase positive.

5. The cells of these group of organisms contain a characteristic antigen called the enterobacterial common antigen.
6. They have simple nutritional requirements and can be easily isolated and differentiated using MacConkey agar. They form pink-coloured colonies for lactose fermenter, and pale-coloured colonies for non-lactose fermenter.

2.4.1 Antibiotic resistant Gram-negative bacilli pathogens

Antimicrobial resistance can be defined as the capability of microorganisms to resist the action of antimicrobial medication that was once effective in treating the microorganism (CDC, 2017). The term "antibiotic resistance" can be seen as a subset of antimicrobial resistance, in that it applies only to bacteria that have become resistant to antibiotic medication. Antibiotic resistant pathogens can be more challenging to treat, sometimes requiring higher dosages of antibiotics or alternative medications which can lead to more toxicity, or expenses or both (CDC, 2013). Some microbes are resistant to multiple antimicrobials and are referred to as multidrug resistant (MDR) microbes (CDC, 2013). Resistance arises via the following mechanisms: genetic mutation, natural resistance in certain bacteria species, or acquisition from another resistant species. Antimicrobial resistance can arise from any of the classes of microorganisms; virus develop antiviral resistance, fungi develop antifungal resistance, bacteria develop antibiotic resistance, and protozoa develop antiprotozoal resistance. Bacteria resistance to antibiotics predates the medical use of antibiotics by man (Caldwell & Lindberg, 2011). But the widespread of antibiotic use made more bacteria to be resistant through evolutionary pressure process. According to Laxminarayan *et al*. (2013), the reasons for the widespread antibiotic use by humans include.

- Increased global availability over time since its discovery in the 1950s

- Uncontrolled sale, especially in low- or middle-income countries, where antibiotics can be obtained over the counter without a prescription.

Other causes of bacterial antibiotics resistance can be attributed to the following: use of antibiotics in livestock feed as growth promotion, large discharge of antibiotics into the environment as pharmaceutical waste, and the use of antiseptics (CDC, 2018).

2.4.2 Mechanisms of antibiotics resistance

The four main mechanisms by which bacteria exhibit resistance are:

2.4.2.1 Drug inactivation or modification: an example of this mechanism of resistance is penicillin G deactivation in some penicillin-resistant bacteria via the production of the enzyme β-lactamases by the bacteria. This mechanism generally involves the addition of an acetyl or phosphate group to specific site of the antibiotic through the activity of enzymes synthesized by the bacterial cell. This action reduces the ability of the antibiotics to bind to the target site of the bacterial cell (Jose & Cesar, 2016).

2.4.2.2 Alteration of target or binding site: an example of this mechanism of resistance is the alteration of penicillin binding protein (PBP), which is the binding site of penicillin in methicillin-resistant *Staphylococcus aureus* (MRSA) and other penicillin-resistant bacteria. Alteration of target can be exemplified in some bacteria species that synthesize ribosomal protective protein, which binds to the bacteria ribosome changing its conformational shape. This permits the synthesis of protein by the ribosome but prevents antibiotics from binding to the ribosome to inhibit protein synthesis (Jose & Cesar, 2016).

2.4.2.3 Alteration of metabolic pathway: an example of this mechanism of resistance can be observed in sulfonamide-resistant bacteria which use preformed folic acid rather than

utilizing para-aminobenzoic acid (PABA) to synthesize folic acid (Chopra & Robert, 2011).

2.4.2.4 Reduced drug accumulation: by increasing the active efflux (pumping out) of the antibiotics across the bacterial cell surface or by decreasing drug permeability. Efflux pumps are located within the cell membrane of some bacterial species and are used to pump out antibiotics before they exert their action. These efflux pumps are often activated by specific substrate associated with antibiotics (Jose & Cesar, 2016). This mechanism of resistance can be observed in fluoroquinolone resistance.

Plasmid-mediated resistance genes, as observed in Gram negative bacteria, produces proteins that is capable of binding to DNA gyrase, protecting the enzyme from the action of quinolones. Also, mutation at key sites in Topoisomerase IV or DNA gyrase can decrease their binding affinity to quinolones (Haggins *et al*., 2013).

Most of the prevalent nosocomial pathogens are multidrug resistant (MDR) that are either acquired [e.g., extended-spectrum β-lactamase (ESBL) producers and methicillin-resistant *Staphylococcus aureus* (MRSA)] or natural resistance (e.g., *Clostridium difficile*) (Bereket *et al*., 2012). Alarming increase in the occurrence of resistant antimicrobial pathogens in serious nosocomial infections have been observed in the past few decades (Bereket *et al*., 2012). The indiscriminately use of antibiotics has been identified to be an important factor in the uprising of antibiotic resistance in both Gram negative and Gram-positive bacteria (Bereket *et al*., 2012). Although, these pathogens exhibit different mechanisms of antibiotics resistance, some of the resistant nosocomial pathogens and their resistance mechanisms are highlighted below.

2.4.3 Resistance mechanisms of Enterobacteria to some Antibiotics

Enterobacteria adapts to the severity of the environment by sharing genetic material; resistance is through mobile resistance genes (Partridge, 2015). They possess different mobile genetic elements for capturing and transporting these genes between DNA molecules; the transfer of these genes can be horizontally (if contained in the plasmid) or vertically (during cell division) (Partridge, 2015). Multiple drug resistance (MDR) is possible when several resistance genes are carried on the same plasmid. The major mobile genetic elements which are responsible for capturing resistance genes and moving them between DNA molecules are transposons, insertion sequence (IS), and the gene cassette/integron system (Partridge, 2015).

Resistance to Beta-lactam antibiotics: the main method Enterobacteria utilizes in resisting antibiotic β-lactam drugs is through the production of beta-lactamase (Ruppe, Armad-Levefre & Estellat, 2015). Beta-lactam antibiotics (such as penicillins, cephalosporins, carbapenems, and monobactams) exert their effect through the formation of an irreversible bond with penicillin binding protein (PBP) of bacterial cell wall. These PBP are acquired for final transpeptidation (cross-linking) in the production of peptidoglycan during bacterial cell wall formation (Partridge, 2015). Beta-lactamase enzymes confer resistance by hydrolyzing the beta-lactam ring of the antibiotics, preventing penicillin-binding protein inhibition (Ruppe *et al*., 2015).

Resistance to Aminoglycoside antibiotics: aminoglycosides exert their antibacterial action by binding to the ribosome of the bacteria; interfering with bacterial protein translation. Thus, disrupting the bacterial metabolism (Shrestha, Fosso, & Garneau-Tsodikova, 2015). Generally, Enterobacteria resist the effects of aminoglycoside through efflux pumps or the acquisition of genes that code for aminoglycoside-modifying enzymes (AMEs). The AMEs contain three

subclasses: aminoglycoside *N*-acetyltransferases (AACs), aminoglycoside *O*-nucleotidyltransferases (ANTs), and aminoglycoside *O*-phosphotransferases (APHs), based on the chemical modification they apply to the aminoglycoside substrates (Ramirez & Tolmasky, 2010).

Resistance to Fluoroquinolones: fluoroquinolones are bactericidal antimicrobials that exert their antibacterial effect by targeting DNA gyrase and topoisomerase IV disabling bacterial DNA replication. Enterobacteria resists fluoroquinolone antibiotics by accumulation of mutations in the target site (primarily DNA gyrase (GyrA) and topoisomerase IV) and efflux pump (Mitra, Mukherjee, Chattopadhyay, Dutta & Basu, 2019).

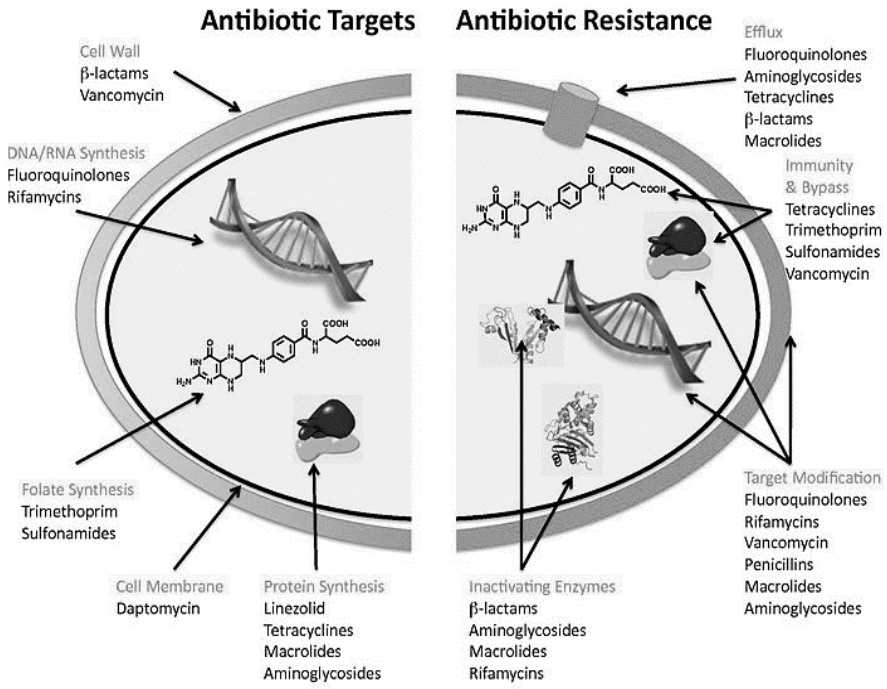


Figure 2.4: Mode of action of antibiotics and bacteria resistance mechanisms.

Source: Wright (2010)

2.5 CHARACTERIZATION OF GRAM-NEGATIVE BACILLI PATHOGENS

Based on the nature and type of sample, the diagnosis of nosocomial infections varies significantly. Effective method should be employed in the identification of the aetiological agents or organisms of healthcare acquired infections. These methods should enhance the proper isolation, identification, and characterization of HCAI agents (Mbim *et al*., 2016). According to Samuel *et al*. (2010), phenotypic characterization of nosocomial pathogens which include culture, serotyping, and biochemical techniques have been vital in the isolation and characterization of pathogens. They however are limited, in that they cannot be employed for high discriminatory identification of microorganisms, lack standardization and have poor reproducibility. According to Afshari, Schrenzel, Ieven, & Harbarth (2012), "molecular characterization of isolates includes plasmid analysis, Amplified Fragment Length Polymorphism (AFLP), Southern blot analysis, Polymerase Chain Reaction (PCR), Ribotyping, and Pulse-Field Gel Electrophoresis (PFGE) which generally involve lysis, extraction and purification of nucleic acid, nucleic acid amplification by PCR and identification methods". Generally, incorporating molecular typing into surveillance and phenotypic methods has been observed to be effective in the diagnosis of nosocomial infections (Afshari *et al*., 2012).

2.5.1 Phenotypic characterization of Gram-Negative Bacilli

Biochemical tests are commonly applied in the phenotypic identification of bacteria due to the following reasons: they are inexpensive, easily applied in all laboratories, and are reliable (Talaiekhozani, Alaee & Mohanadoss, 2015). As an initial step in bacterial identification, two tests (Gram and morphological characteristics) should be done. This initial step helps categorize bacteria into smaller groups before applying other tests for specific categories until final

identification (Talaiekhozani *et al*., 2015). Generally, bacteria can morphologically be grouped as bacillus (rod shaped) or coccus (spherical). Although some other morphological groups such as spirochetes and vibrio exist. The morphological characteristics of unknown bacteria can be determined through examination by light microscope (Talaiekhozani *et al*., 2015).

Gram test enables the categorization of bacteria into two categories, Gram-negative and Gram-positive, based on the chemical and physical properties of bacterial cell walls. Prior to Gram test, the bacteria should be purified, then a small quantity of bacterial colonies is transferred onto a slide. After fixing the bacteria onto the slide by gentle flame, the fixed bacteria are first washed with crystal violet dye, then iodine solution. When viewed under light microscope, Gram-negative bacteria will appear as red or pink while Gram-positive bacteria will appear blue in colour (Talaiekhozani *et al*., 2015). After these first steps (Gram stain and morphology observation), bacteria can be divided as: Gram-positive coccus, Gram-positive bacillus, Gram-negative coccus, and Gram-negative bacillus.

2.5.1.1 Gram-negative Rod Bacteria

Using oxidase test, Gram-negative rod bacteria can be broadly grouped into two; oxidase positive (*Ameromonas, Pseudomonas, Vibrio* families) and oxidase negative (Enterobacteriaceae family). The GFA test is used to distinguish *Pseudomonas* (GFA negative) from *Aeromonas* and *Vibrio* families (GFA positive). *Pseudomonas* P agar medium is used to categorize *Pseudomonas* spp. family. A positive result is observed by the appearance of blue colonies on the medium.

2.6 BACTERIOLOGY OF COMMONLY ISOLATED GRAM-NEGATIVE BACILLI PATHOGENS

A large variety of microorganisms are responsible for hospital-acquired infections, and any microorganism may have the capability of causing infection in hospitalized patients. Studies have shown that ninety percent of hospital acquired infections are caused by bacteria, whereas viral, mycobacterial, fungal, or protozoal pathogens are less commonly associated (Bereket *et al*., 2012). The bacteria that are commonly associated with hospital-acquired infections include *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and members of Enterobacteriaceae such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella* spp., *Serratia marcescens* (Bereket *et al*., 2012).

2.6.1 *Escherichia coli*

This is a facultative anaerobic, Gram-negative, oxidase negative bacterium. It is one of the common microorganisms involved in Gram negative endotoxin-induced shock and sepsis. *E. coli* is responsible for some nosocomial infections such as pneumonia in immune-compromised patients, urinary tract and wound infections, meningitis in neonates, and gastroenteritis. *E. coli* have a wide range of virulence factors which include endotoxin, antigenic phase variation, capsule sequestration of growth factors, antimicrobial resistant, and resistant to serum killing (Bereket *et al*., 2012).

2.6.2 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative bacterium, typically arranged in pair, and possess a mucoid polysaccharide capsule. The identification of *P. aeruginosa* is based on some simple biochemical

test and colonial characteristics. This bacterial species can continuously colonize the gastrointestinal and respiratory tracts of healthcare patients, especially patients treated with broad spectrum antibiotics, prolonged hospital stays, or patients exposed to respiratory therapy equipment (Bereket *et al*., 2012). Pathogenesis by *P. aeruginosa* is usually initiated when the patient's normal defense system is impaired. *P. aeruginosa* attaches to and colonizes the skin or mucus membrane, invades, and produce systemic disease (Bereket *et al*., 2012). According to Bereket *et al*. (2012), the processes of *P. aeruginosa* establishment are facilitated by various virulent factors such as enzymes (phospholipase C, elastases, proteases), pili, and toxins (endotoxin A). the infection spectrum caused by *P. aeruginosa* includes meningitis, blue-green pus producing wound infections, necrotizing pneumonia, and urinary tract infection (Bereket *et al*., 2012).

2.6.3 *Enterococcus* spp.

These are Gram positive cocci bacteria typically arranged in pairs and short chains. They grow at an optimum temperature of 35 ^{o}C on a complex media containing vitamin B, carbon source (e.g., glucose), and nucleic acid base. Also, they are facultative anaerobes. Enriched sheep blood agar favours the growth of *Enterococcus* which can be observed as large, white colonies. Despite the fact that *Enterococci* are considered as part of the gastrointestinal and genitourinary tracts normal flora of humans, it has emerged as one of the most significant nosocomial pathogens. Two species of *Enterococcus*; *E. faecalis*, and *E. faecium* are the most common pathogens of human enterococcal infections (Bereket *et al*., 2012). These two pathogens are the third and fourth (respectively) most prevalent nosocomial pathogens worldwide. The infections caused by these pathogens are urinary tract infection, intra-abdominal and pelvic infection. They are also

associated with bacteremia, surgical wound infection, neonatal sepsis, endocarditis, and rarely meningitis (Bereket *et al*., 2012).

2.7 PREVENTION AND CONTROL OF NOSOCOMIAL INFECTION

According to Samuel *et al*. (2010), "control measures are aimed at protecting possible infection sites, interrupting transmission routes, boosting defenses and discouraging selection of hospital strains of organisms". Azeez-Akande (2012), opined that effective prevention and control of nosocomial infection requires scientific, technical, professional, and administrative leadership. It is generally accepted that the prevention of nosocomial infection is the responsibility of everyone; healthcare givers and patients inclusive (Nejad, Allegranzi, Syed, Benjamin & Pittet, 2011; Sikka *et al*., 2012). This can be achieved through stratifying risk, proper management, provision of essential materials and products, and training of health workers (Nejad *et al*., 2011). Samuel *et al*. (2010), opined that for an effective infection control programme in hospitals to be attainable, the first step is to set up a committee with the authority to establish infection control policies. These policies should encompass surveillance and prevention activities, implementation, and consistent training of staffs (Nejad *et al*., 2011).

Generally, preventive measures against nosocomial infections should be geared towards identifying the reservoirs of the pathogens responsible for nosocomial infections (Jose, Sandra & de OcaRaul, 2014). Bereket *et al*. (2012), observed that infection control principles are based on the application of practices that are safe and that minimizes the chances of transmission of infections from a source to a susceptible host. These principles therefore range from washing of hands to good personal and hospital hygiene which involves ensuring aseptic techniques in hospital theatres, use of protective clothing, good specimen handling, proper waste disposal, effective use

of antiseptics or disinfectants during sterilization processes, daily surveillance of infection within the hospital, and efficient management of soiled linen (Uwaezuoke & Obu, 2013; Mbim *et al*., 2016).

CHAPTER THREE

MATERIALS AND METHOD

3.1 AREA OF STUDY

The study was carried out in Imo State, which is in the Southeastern geopolitical zone of Nigeria. It is bordered by Anambra State to the north, Rivers State to the east and south, and Abia State to the east. The State occupies a land area of 5,530 km^2, having an estimated population of 4,927,563 (NPC, 2017). Despite being the third smallest State in Nigeria, it ranks among the most populous. The State economy is highly dependent on agricultural production and some minor crude oil extraction and natural gas industries (Opiah, 2022). The annual rainfall experienced in the State vary from 1,500 mm to 2,200 mm, having an average annual temperature above 20 ^oC. The dry season are usually two to four months from late December to March.

3.2 SAMPLING TECHNIQUE

The randomized complete block sampling design was adopted for the study. Using Cochran formula to determine the sample size when the population size is large but unknown. A total of 360 samples were collected for the study. To avoid nuisance variation, four public health care centres (Imo State University Teaching Hospital (ISUTH), Federal Medical Centre Owerri (FMC), Aboh-Mbaise General Hospital (AMGH), and Imo State Specialist Hospital (ISSH)) within the State were selected at random. The sources of variation in the study include the hospital wards and the hospital materials. The wards used in the study were maternity wards, surgical theatres, and intensive care units (ICU). The materials from which samples were collected include bed cover, bed pan, staff gloves, and ward floor.

3.3 SAMPLE COLLECTION

Swab samples were aseptically collected from various equipment in the wards and staff apparels. The items from which the samples were collected include floor, bed cover, bed pan, and hospital staff apparel (protective gown and hand gloves) within maternity ward, surgical theatres, and intensive care unit of each hospital.

Sterile swab tubes (appropriately labelled) were taken to the hospitals. Swabbing of the surface of each item was made using sterile swab soaked with saline water. Many portions as possible of each item were swabbed and more than one swab stick was used for each subject. The swabs were immediately conveyed to the laboratory where they were inoculated unto MacConkey agar plates for cultivation (Muhammed *et al*., 2013).

3.4 PREPARATION OF MacConkey AGAR PLATES AND CULTURING OF THE SAMPLES

MacConkey Agar Preparation: according to the manufacturer's instruction, fifty plates of MacConkey were prepared by dissolving 49.53 g of MacConkey agar into 1000 mL of sterile distilled water in a conical flask. The solution was heated to boiling for complete dissolution. The conical flasks were tightly sealed using cotton wool and aluminum foil and autoclaved at 15 Ibs (121 ^{o}C) for 15 minutes. After autoclaving, the mixture was allowed to cool to about 45 to 50 ^{o}C. It was then transferred into the petri-dishes to gel under a biosafety cabinet.

Isolation of Enterobacteria: Using sterile cotton swabs, the samples were streaked on the surfaces of the sterile solid MacConkey plates. Each sterile cotton swab was used for a sample and

discarded after inoculation. The agar plates were inoculated in duplicates then incubated in an incubator at 37 ^{o}C for 36 h. Plates were then observed for growth and colonies recorded.

3.5 PHENOTYPIC CHARACTERIZATION OF ISOLATES

3.5.1 Morphological characterization: The morphological identification of the bacterial culture was done following the methods of Aneja (2003).

3.5.1.1 Gram staining: thin smear of bacterial culture was made on clean glass slide, air dried and heat fixed. The smear was covered with crystal violet for 30 seconds. The slide was then washed with distilled water and the smear was covered with Gram iodine solution for 60 seconds., washed with distilled water and blot dried, air dried and observed under microscope. The shape and colour of the organisms were observed. Purple colour depicts Gram-positive bacteria while pink colour shows Gram-negative bacteria.

3.5.1.2 Motility test: Clean cavity slide was taken and placed on a table with depletion upper side. A cover slip was taken, and wax was applied on its four corners. A loop-full of culture was transferred exactly at the center of the cover slip and the cavity slide was placed on the cover slip and pressed gently. The preparation was lifted gently so that the culture drop is suspended in the form of hanging drop. The edge was observed under a microscope.

3.5.2 Cultural characterization of isolates

3.5.2.1 Cetrimide Agar Preparation: according to the manufacturer's instruction, 45.3 g of cetrimide agar was dissolved into 1000 mL of sterile distilled water. Then 10 mL of glycerol was added to the mixture and boiled to dissolve completely. The media was autoclaved at 15 Ibs (121 ^{o}C) for 15 minutes. After autoclaving, the mixture was allowed to cool to about 50 ^{o}C. It was then

transferred into the petri-dishes to gel under a biosafety cabinet. Cetrimide inhibits the growth of bacteria by acting as a detergent, it is used to select for *P. aeruginosa* as this bacterium is capable of producing soluble iron chelators. Thus, resisting the germicidal effect of Cetrimide.

Isolation of pure *P. aeruginosa*: Using sterile wire loop, distinct colonies from the MacConkey plates were collected and inoculated onto the cetrimide agar gel in duplicates. The inoculated plates were incubated at 37 ^{o}C for 48 h in an incubator. The inoculation was carried out under a flame inoculating cabinet. The isolates were preserved in aseptic condition for further analysis.

3.5.2.3 *Salmonella-Shigella* Agar Preparation: according to the manufacturer's instruction, 60g of *Salmonella-Shigella* medium was measured into 1000 mL of distilled water. The mixture was agitated to mix thoroughly. It was then heated to boil with frequent agitation. The solution was allowed to cool and transferred into petri-dishes to gel under a biosafety cabinet.

Isolation of pure *Shigella* spp.: Using sterile wire loop, distinct colonies from the MacConkey plates were collected and inoculated onto the *Salmonella-Shigella* agar gel. The inoculated plates were incubated at 37 ^{o}C for 48 h in an incubator. The inoculation was carried out under a flame inoculating cabinet.

3.5.3 Biochemical test for Bacterial Isolates

3.5.3.1 Oxidase test: the test was carried out according to the protocols stated by Li *et al.* (2015). 1% (wt/vol) solution of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) was prepared in dimethylsulfoxide (DMSO). A piece of filter paper was divided into three equal sections and labelled. A loop full of culture was rubbed on the moistened filter paper (moistened with 1% Kovacs oxidase reagent (TMPD-DMSO)) using a sterile loop. The colour of the smear was

checked exactly 28 seconds after rubbing the cells on the reagent moistened filter paper. A deep blue colour indicated positive reaction. Light violet or purple colour developed within 10 seconds was recorded as negative.

3.5.3.2 Carbohydrate fermentation test: fermentation medium was prepared with specific carbohydrate (glucose, sucrose, fructose, maltose). 10 g trypticase or peptone, 5 g carbohydrate, 15 g NaCl, 0.015 g phenol red was dissolved in 1000 ml distilled water. The media was sterilized using autoclave at 15 Ibs pressure for 15 minutes. Each of specified fermentation tubes of media was labelled with the name of the organism to be inoculated. Four types of sugars fermentation broth were inoculated with test organism for 36 h. at 30 ^{o}C and one un-inoculated tube with each fermentation broth was kept as comparative control. The tubes were observed for the change in colour (due to production of acid) or change in colour and appearance of bubbles (due to production of acid and gas) (Aneja, 2003).

3.5.3.3 IMVIC Test

Indole production test: one percent (1%) tryptone broth was prepared (dissolving 10 g peptone or tryptone in 1000 ml distilled water) and sterilized using autoclave at 151 bs for 15 minutes. The tryptone broth was inoculated with test organism and an un-inoculated tube was kept as control. The tubes were incubated at 35^{o}C for 48 hours; 1ml of Kovac's reagent (dimethyl aminobenzaldehyde) was added 48 h. of incubation. The tubes were shaken gently after intervals of 10 to 15 minutes. The tubes were allowed to stand for few minutes to permit the reagent to come to the top. Then it was observed for cherry red layers in the top layer (Aneja, 2003).

Methyl-Red and Voges-Proskauer test: according to Aneja (2003), MRVP broth was prepared (by dissolving 7 g peptone, 5 g dextrose, 5 g potassium phosphate in 1000 ml distilled water) and

sterilized using autoclave. 5 ml of the broth was poured into each tube. The tubes were inoculated with test organism. All the tubes were incubated at 25°C for 48 h. 5 drops of methyl red indicator was added to the tubes of each set. The change in color of methyl red was observed for MR test. 12 drops of VP Reagent-I and 3 drops of VP reagent-II was added to the other set of tubes. The tubes were gently shaken for 30 seconds with the caps off to expose the media to oxygen. The tubes were kept aside for 24 minutes and observed for change in colour for the VP test. Development of deep red colour in the inoculated tubes 15 minutes after addition of Barrit's reagent was the indication of positive the absence of red colouration indicates negative.

3.6 ANTIBIOTIC SENSITIVITY TEST (ANTIBIOGRAM)

Antibacterial susceptibility testing was carried out on all the isolates using the Kirby-Bauer disc diffusion method as recommended by the [Clinical and Laboratory Standards Institute (CLSI), 2010]. Sterile petri-dishes of Mueller Hinton agar were prepared according to manufacturer's specification. This was done by dissolving 38.0 g of Mueller Hinton agar into 1000 mL of sterile distilled water. The mixture was heated with frequent agitation for complete dissolution. After which it was autoclaved at 121 ^{o}C for 15 minutes, allowed to cool and poured into sterile petri-dishes. Colonies of an overnight culture were suspended in sterile water and vortexed. The microbial solution was brought to the McFarland standard by comparing its turbidity using black stripes on a sheet of paper. A sterile cotton wool swab was inserted into each test tube containing the standardized inoculums suspension, rotated with firm pressure on the side wall of the test tube to remove excess fluid and used to inoculate the entire surface of the Mueller Hinton agar plate.

Antibiotic disks containing Tarivid 10 µg, Pefloxacin 10 µg, Ciproflox 10 µg, Augmentin 30 µg, Gentamicin 30 µg, Streptomycin 30 µg, Cefalexin 10 µg, Nalidixic acid 30 µg, Septrin 30 µg,

Ampicillin 30 µg was placed on each inoculated plate in duplicates. All plates were incubated at 37 ^{o}C for twenty-four hours and the diameter of the zones of inhibition were measured to the nearest millimetre using a transparent metre rule (CLSI, 2010). The bacterial isolates were designated Sensitive (S), Intermediate (I) or Resistant (R).

3.7 MOLECULAR ANALYSIS

3.7.1 DNA extraction and PCR amplification: The bacterial genomic DNA extraction from bacterial cells was carried out using DNA purification kit (QIAGEN) according to manufacturer's instructions (QIAGEN, 2006). One and half milliliter (1.5 ml) sample tubes were labelled for each bacterial colony. A 1.75 ml portion of the bacterial culture broth was transferred into the labelled tube and centrifuged at 20,000 rpm for 5 minutes, then the liquid was decanted. 180 µl of enzymatic lysis buffer was added to the tube and vortex for 15 s, it was then incubated at 37 ^{o}C for 30 minutes. Twenty-five microliters (25 µl) of proteinase K and 200 µl of buffer AL was added to the tube, and then vortex briefly. It was incubated at 56 ^{o}C for 30 minutes, and 200 µl of 100% ethanol was added, then the mixture was briefly vortexed. Using a micropipette, the entire content (~600 µl) was transferred to a labeled spin column; centrifuged at 10,000 rpm for 1 minute then the column was removed from the collection tube and placed in a new collection tube. Five hundred microliter (500 µl) of buffer AW1 was added to the column and centrifuged at 10,000 rpm for 1 minute. The column was removed from the collection tube and placed in a new collection tube. 500 µl of buffer AW2 was added to the column and centrifuged at 20,000 rpm for 3 minutes. The tube was carefully removed from the centrifuge, the column was transferred to a 1.5 ml tube and 200 µl of buffer AE was added to the column. Then it was allowed to stand at room temperature for 1 minute. Centrifuged at 10,000 rpm for 1 minute. The column was discarded, and the DNA appropriately stored.

Three Primers (Table 3.1), corresponding to the polymorphic region of *bla*$_{TEM,}$ *gnr*A, and aac(3)-1 genes were used to amplify the extracted DNA. Briefly, 1 µl of the forward and reverse primers was added to 2.5 µl Taq polymerase buffer 10x containing a final concentration of 1 mM $MgCl_2$, 0.2 mM dNTPs and 0.2 µl Taq polymerase (5 U/µl) in a final reaction volume of 25 µl. PCR reaction conditions were initial denaturation at 95 °C for 5 min, 34 cycles at 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. Final extension at 72 °C for 10 min was done. The results were visualized on 1.5 % agarose gel stained by Cyber green and photographed using gel documentation system (Olowe *et al*., 2015).

Table 3.1: The primers used for polymerase chain reaction (PCR)

Gene		Sequence (5' 3')
bla$_{TEM}$	F	ATGAGTATTCAACATTTCCG
	R	GTCACAGTTACCAATGCTTA
*qnr*A	F	AGAGGATTTCTCACGCCAGG
	R	TGCCAGGCACAGATCTTGAC
aac(3)-1	F	TTCATCGCGCTTGCTGCYTTYGA
	R	GCCACTGCGGGATCGTCRCCRTA

3.8 STATISTICAL ANALYSIS

The data acquired from the research were analyzed and represented in charts and tables. The mean and standard error values were obtained. Two-way ANOVA was employed to determine the significant difference in the prevalence of bacteria isolated from the healthcare samples; and to check for interaction between the medical wards and healthcare equipment (at 0.5 level of significance). The following formulas were employed in the study.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained from the study are represented using the following charts and tables to explain the observations in line with the study objectives.

4.1.1 Isolation of Enterobacteria species from materials utilized in healthcare centres

The Enterobacteria species were isolated from the studied material using MacConkey agar, the result as represented in Figure 4.1 showed that out of the 360 MacConkey agar plates inoculated, growth was observed in 231 (64.17 %) while the remaining 129 (35.83 %) plates showed no observable growth.

Enterobacteria species were isolated from materials utilized in four healthcare centres; Imo State University Teaching Hospital (ISUTH), Federal Medical Centre (FMC), Aboh-Mbaise General Hospital (AMGH), and Imo State Specialist Hospital (ISSH). It was observed that the highest number of Enterobacteria species were observed in materials collected from ISUTH (20.67 ± 1.45), followed by the materials from ISSH (19.67 ± 0.33). Materials collected from FMC (18.00 ± 1.15) showed the lowest number of Enterobacteria species (Fig 4.2). The number of Enterobacteria species observed in material from AMGH (18.67 ± 1.20) was slightly lower than the observation from ISSH. From the error bars in Figure 4.2, it could be observed that there is a less disparity in the observed number of Enterobacteria species in materials from ISSH, compared to the other healthcare centres.

The number of Enterobacteria species isolated from these healthcare centres were further analysed in respect to the various wards. It was observed that materials from surgical theatre showed the highest number of Enterobacteria species (20.25 ± 6.55). While materials from the ICU recorded the lowest number of Enterobacteria species (17.75 ± 5.63). The number of Enterobacteria species observed in materials from the maternity wards (19.75 ± 4.59) were higher than that observed from ICU materials (17.75 ± 5.63) but below the observation made from materials collected from surgical theatre (20.25 ± 6.55) (Figure 4.3).

Analysis of the number of Enterobacteria species isolation based on the healthcare equipment and surroundings (as represented in Figure 4.4) showed that the floors of the wards recorded highest number of Enterobacteria species observed [Maternity ward floor n = 30, ICU floor n = 28 and Surgical theatre ward floor n = 32]. This was followed by the samples from the wards' bed pan [Maternity (n = 25), ICU (n = 27), and surgical theatre (n = 31)]. Equal number of isolates were observed in samples collected from bed covers and gloves for both maternity wards and ICUs (Maternity (n =12 for bed cover and gloves), ICU (n = 8 for bed cover and gloves). From the surgical wards' bed covers and gloves, the number of Enterobacteria species observed (n = 11) were higher than those observed for gloves samples (n = 7). This is summarized in Figure 4.4.

The values for these figures are recorded in Tables in Appendix I

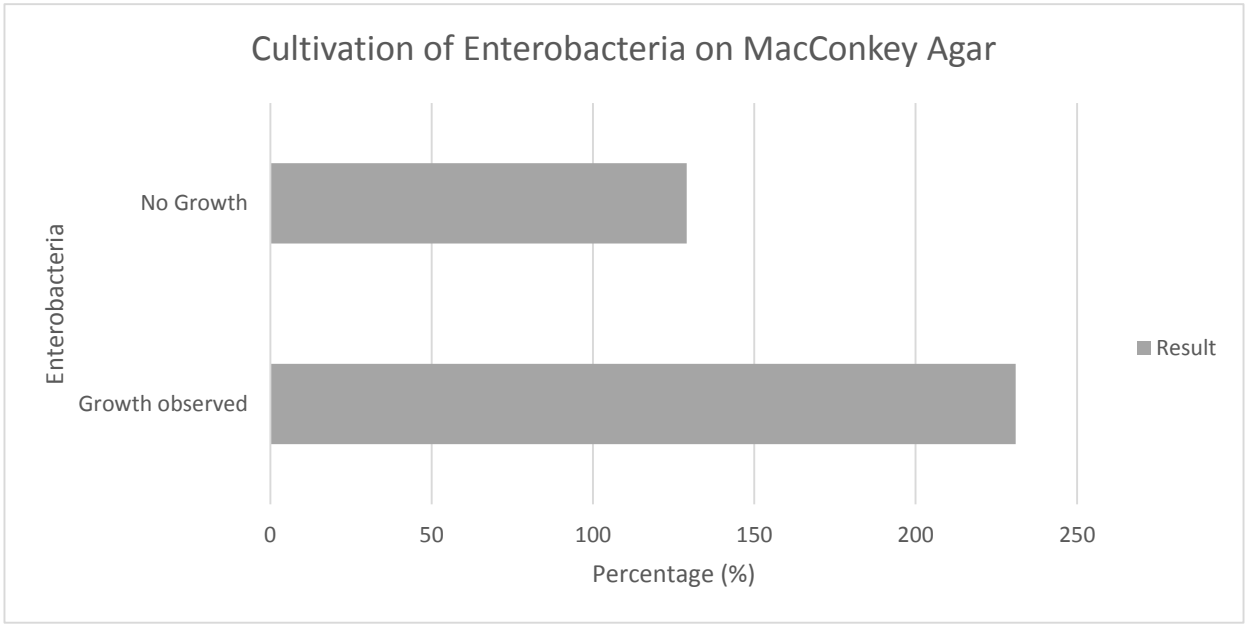


Fig 4.1: Percentage of Enterobacteria isolated from hospital samples using MacConkey agar

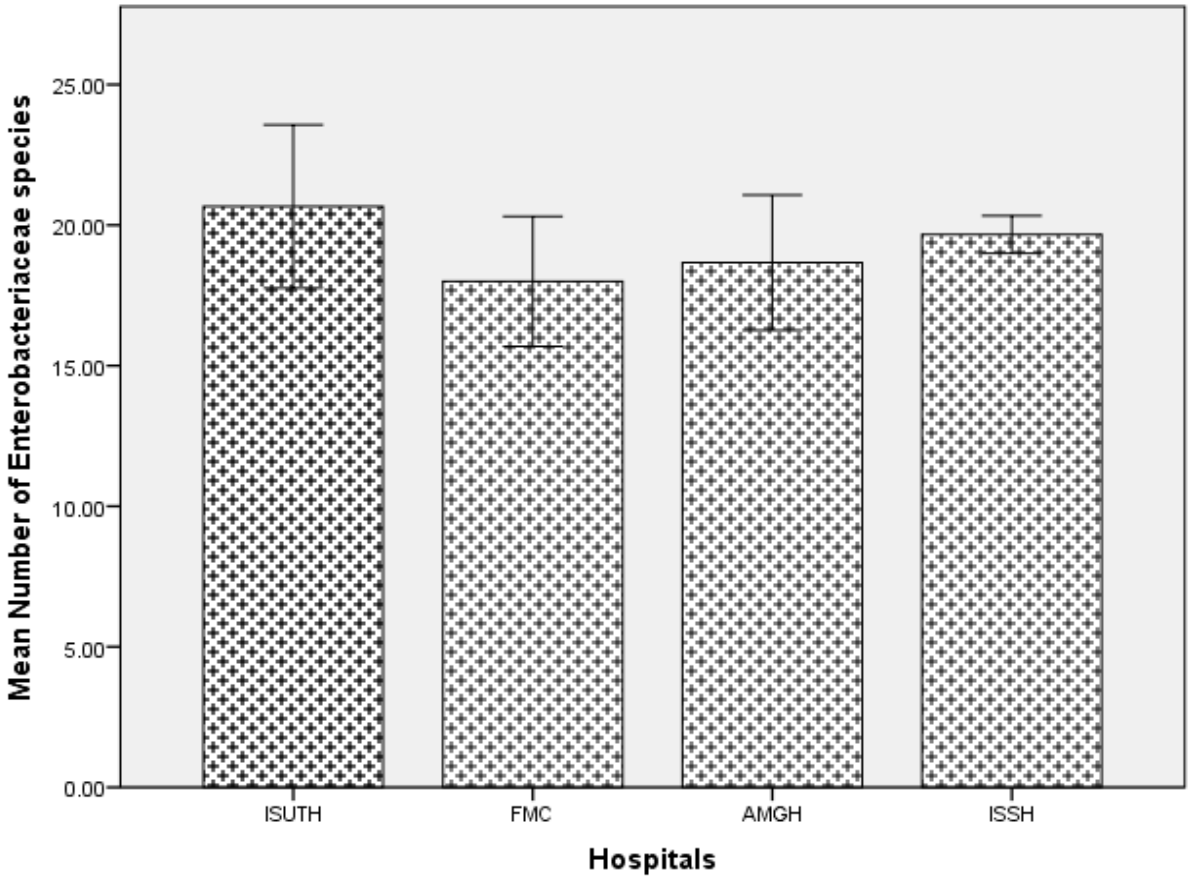


Fig 4.2: Number of Enterobacteria species isolated from four healthcare centres

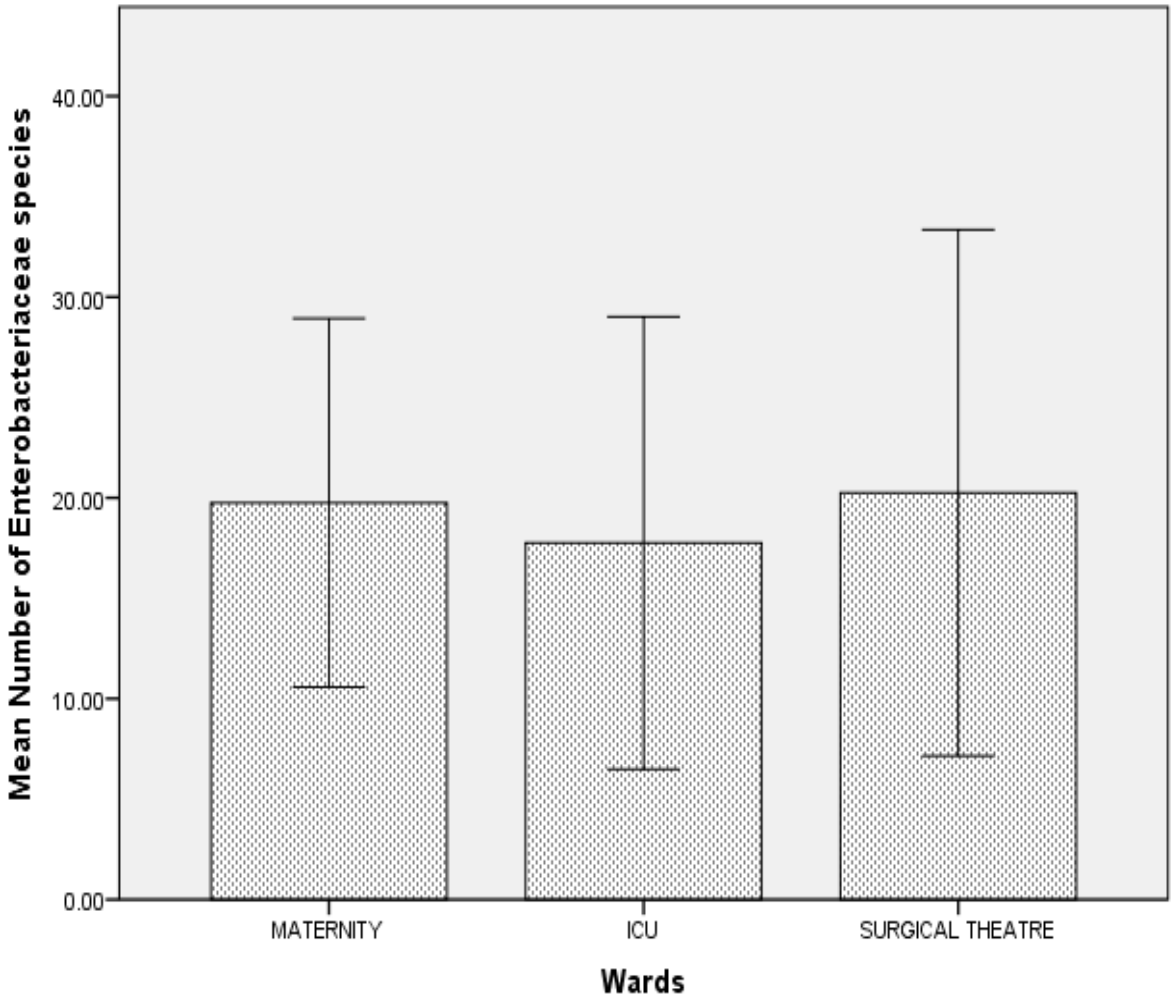


Fig 4.3: Number of Enterobacteria species isolated from the various wards in the healthcare centre

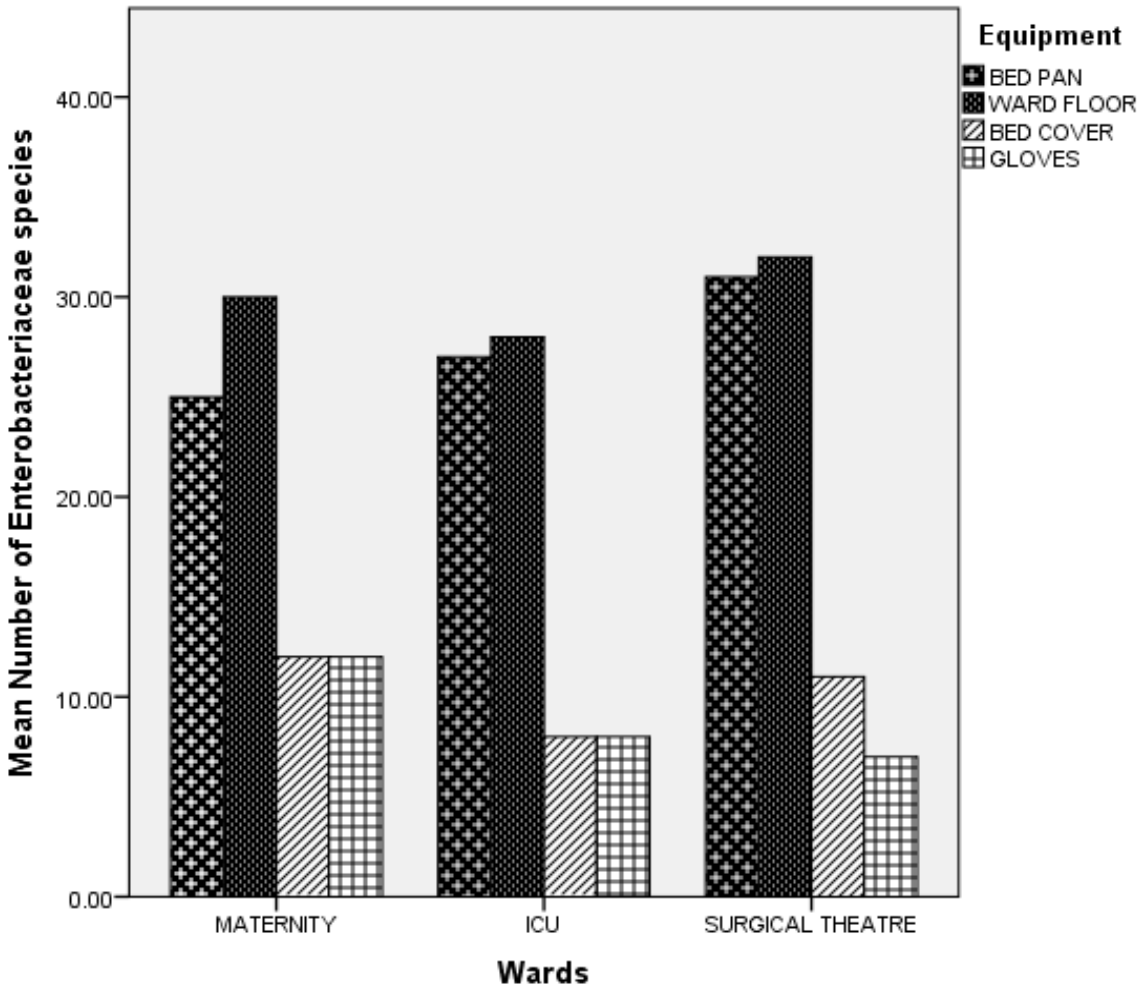


Fig 4.4: Number of Enterobacteria species isolated from healthcare equipment and surrounding.

4.1.2 Identification and Characterization of Enterobacteria isolates using microscopic, cultural, and biochemical assay

The isolates cultured from the hospital samples were characterized using microscopic, cultural, and biochemical assay. The results are represented in Table 4.1.

The result for the microscopic and cultural assay are represented in Table 4.1. It was observed that the isolates when viewed under a microscope were pink to reddish rod-shaped organisms (Appendix II). Isolates growth on MacConkey agar produced brownish colonies for *Pseudomonas aeruginosa*; pink colonies was observed for *Escherichia coli* while *Klebsiella pneumoniae* produced pinkish-red colonies; and transparent colonies were observed for *Shigella spp.* (Appendix II). Isolates on Salmonella-Shigella agar showed inhibited growth for *P. aeruginosa*; reduced pinkish colonies for *E. coli*; inhibited growth for *K. pneumoniae,* and colourless colonies for *Shigella spp*. Table 4.1. Culturing of isolates on Cetrimide Agar showed inhibited growth for *E. coli, K. pneumoniae* and *Shigella spp. P. aeruginosa* showed growth which gave a green, fluorescent radiation when viewed under UV rays.

The results from the biochemical assay as represented in Table 4.1, showed that *P. aeruginosa* tested positive for oxidase test and lactose fermentation test, but negative for indole, methyl red and VP test. *E. coli* tested positive for indole, methyl red and lactose fermenting test; but a negative result was observed for oxidase and VP test. *K. pneumoniae* tested positive for only VP and lactose fermenting test; a negative result was observed for the other tests (oxidase, methyl red, and indole test). For *Shigella spp.* a positive result was observed only for indole test, all other tests yielded negative results (Table 4.1).

The percentages of the Enterobacteria species isolated from the samples was represented in Figure 4.5. It was observed that the species *E. coli* showed the highest percentage (29.00 %) of growth on nutrient media after inoculation. *P. aeruginosa* showed the second highest growth percentage (26.84 %), *K. pneumoniae* showed 25.11 % growth while *Shigella spp*. showed the lowest percentage growth (19.05 %).

Table 4.1: Microscopic, cultural, and biochemical assay of Enterobacteria isolates

Isolate ID	Morphology		Gram Stain	Growth on MacConkey	Growth Salmonella-Shigella agar	Growth on Cetrimide agar	Biochemical test					Probable organism
	Shape	Motility					Oxidase	Indole	Methyl red	VP	Lactose	
MW1	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
MW2	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW3	Rod	-	-	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
MW4	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW5	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
MW6	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW7	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
MW8	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC1	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC2	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
IC3	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC4	Rod	-	-	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
IC5	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
IC6	Rod	-	-	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
IC7	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC8	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST1	Rod	-	-	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
ST2	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST3	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST4	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST5	Rod	+	-	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST6	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
ST7	Rod	-	-	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST8	Rod	+	-	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*

+ = Positive result; - = Negative result

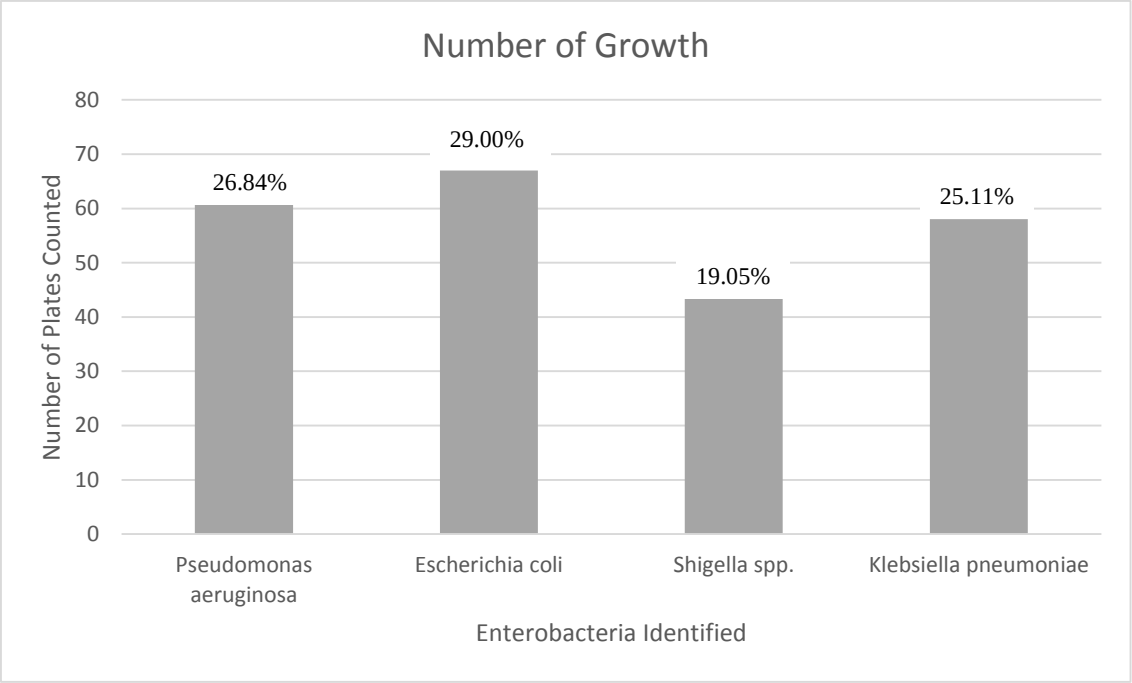


Fig 4.5: Growth percentages of the various Enterobacteria identified from the study.

4.1.3 The antibiotic resistance potential of bacterial isolates to some commercially available antibiotics

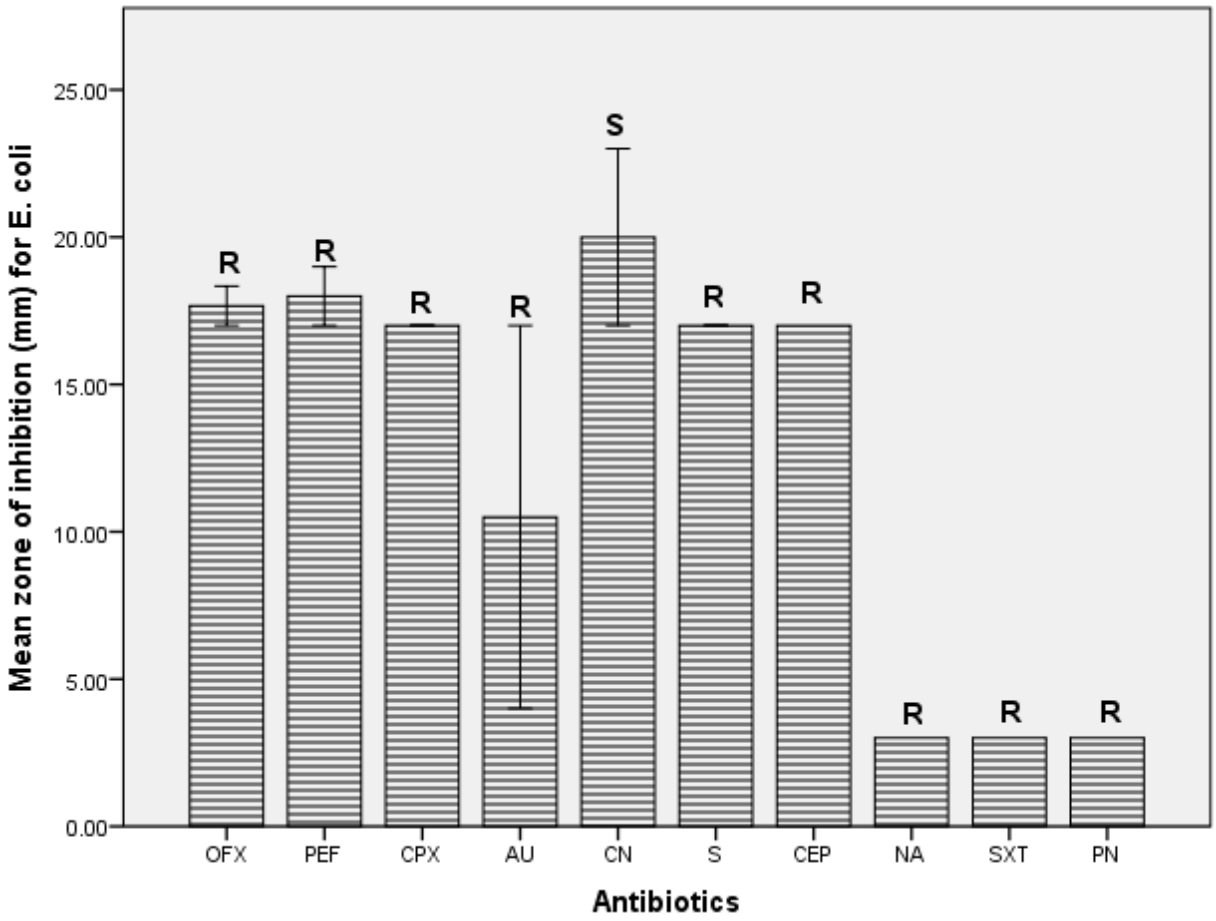
The antibiotic resistivity of the bacterial isolates to multiple antibiotics is represented using Figures 4.6 to 4.9. It was observed that the isolates were resistant to majority of the antibiotics investigated. From the Figures, the isolates (*P. aeruginosa, E. coli, K. pneumoniae,* and *Shigella spp.*) were all resistant to Pefloxacin (PEF) and Ciproflox (CPX). *E. coli* and *K. pneumoniae* were observed to be resistant to Tarivid (OFX) (Fig 4.6 and Fig 4.7 respectively), while *P. aeruginosa* and *Shigella spp*. were susceptible to Tarivid (Fig 4.8 and Fig 4.9 respectively). *Pseudomonas aeruginosa* and *E. coli* were resistant to Augmentin (AU), *Shigella spp*. was observed to be susceptible to Augmentin while *K. pneumoniae* showed area of technical uncertainty towards Augmentin antibiotics. Gentamicin (CN) was observed to be effective against all the bacterial isolates as the isolates were susceptible to Gentamicin. *Pseudomonas aeruginosa, K. pneumoniae* and *Shigella spp*. were resistant to Septrin (S); while an area of technical uncertainty was also observed for Septrin effect on *E. coli*. *Pseudomonas aeruginosa* and *E. coli* were resistant to Cefalexin (CEF); there was area of technical uncertainty for the zone of inhibition observed for Cefalexin effect on *K. pneumoniae* and *Shigella spp*. Majority of the isolates (*P. aeruginosa, E. coli* and *K. pneumoniae*) were observed to be resistant to Nalidixic acid (NA). *Shigella spp.* was the only isolate that was susceptible to Nalidixic acid.

The bacterial isolates: *Pseudomonas aeruginosa* and *E. coli* were observed to be resistant towards the antibiotics, Streptomycin (SXT) and Ampicillin (PN). An area of technical uncertainty was observed for *K. pneumonia* and *Shigella spp*. isolates, investigated against Streptomycin. *Klebsiella pneumonia* and *Shigella spp*. were susceptible to Ampicillin antibiotics. These results

are represented using bar charts. The measurements for the zones of inhibition are represented in Appendix III.

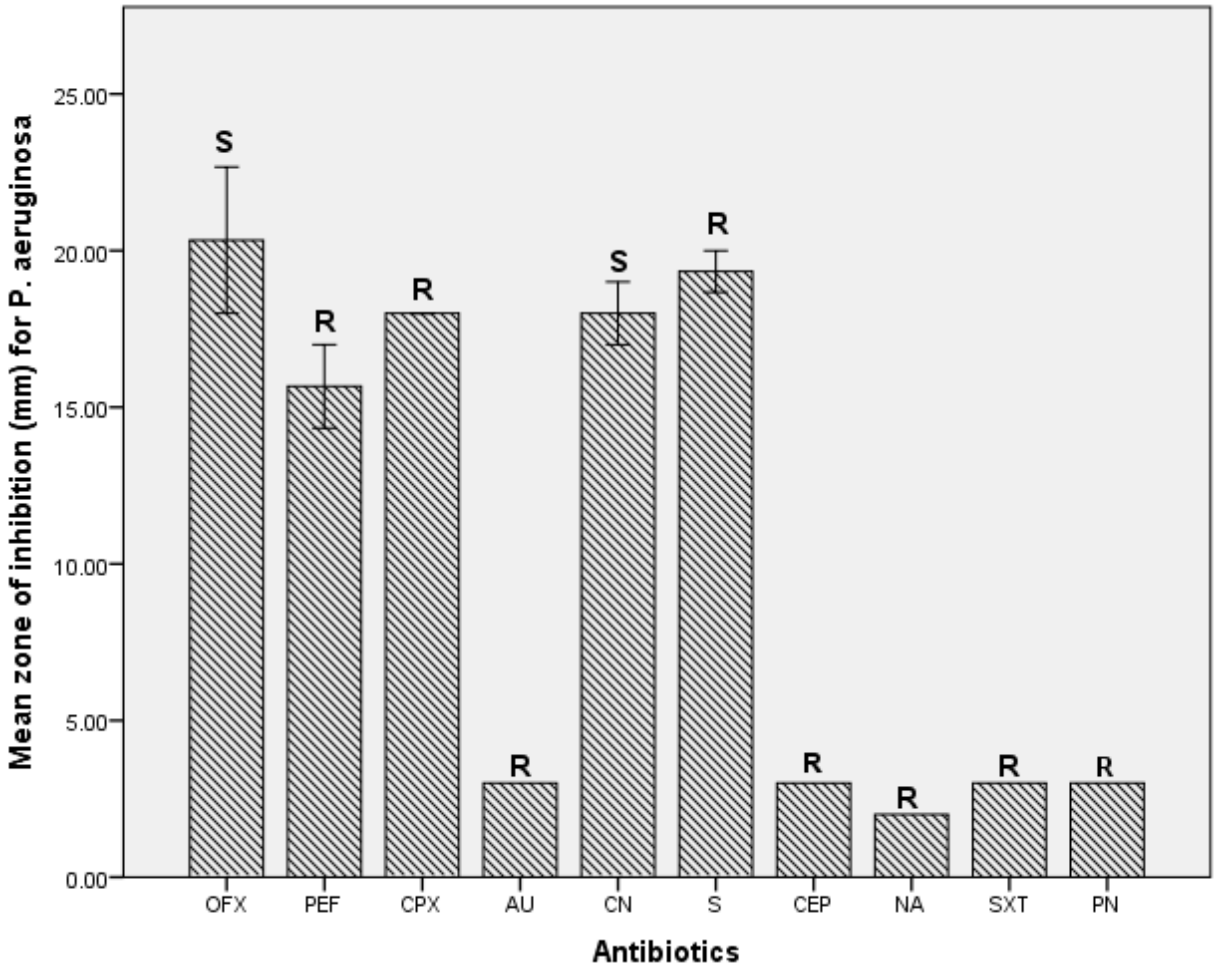
Escherichia coli showed resistance to all the antibiotics except gentamicin (CN) (Fig.4.6). *Pseudomonas aeruginosa* was susceptible to travid (OFX) and gentamicin (CN) but resistant to the other antibiotics (Fig. 4.7). Some of the antibiotics like AU, S, CEP, and SXT activity on *K. pneumoniae* showed area of technical uncertainty (ATU: when it is neither susceptible nor resistant); gentamicin and ampicillin were effective against *K. pneumoniae* (it was observed to be susceptible). *Klebsiella pneumoniae* was resistant OFX, PEF, CPX and NA (Fig. 4.8). The result for *Shigella spp*. showed susceptibility for OFX, AU, CN, NA, and PN; but was resistant to PEF, CPX, S (Fig. 4.9). Treatment with CEP and SXT on *Shigella spp*. showed area of technical uncertainty (Fig. 4.9).

Figure 4.10 illustrated a comparison of the antibiotic resistivity of the bacterial isolate; it was observed that *E. coli* showed the highest resistivity towards the antibiotics employed in the study (38 %). This was followed by *P aeruginosa*, which showed the second highest resistivity towards the antibiotics tested (32 %). *Klebsiella pneumoniae* showed a resistivity of 16 %, while *Shigella spp.* showed the least resistivity of all the bacterial isolate (14 %) (Fig 4.10).



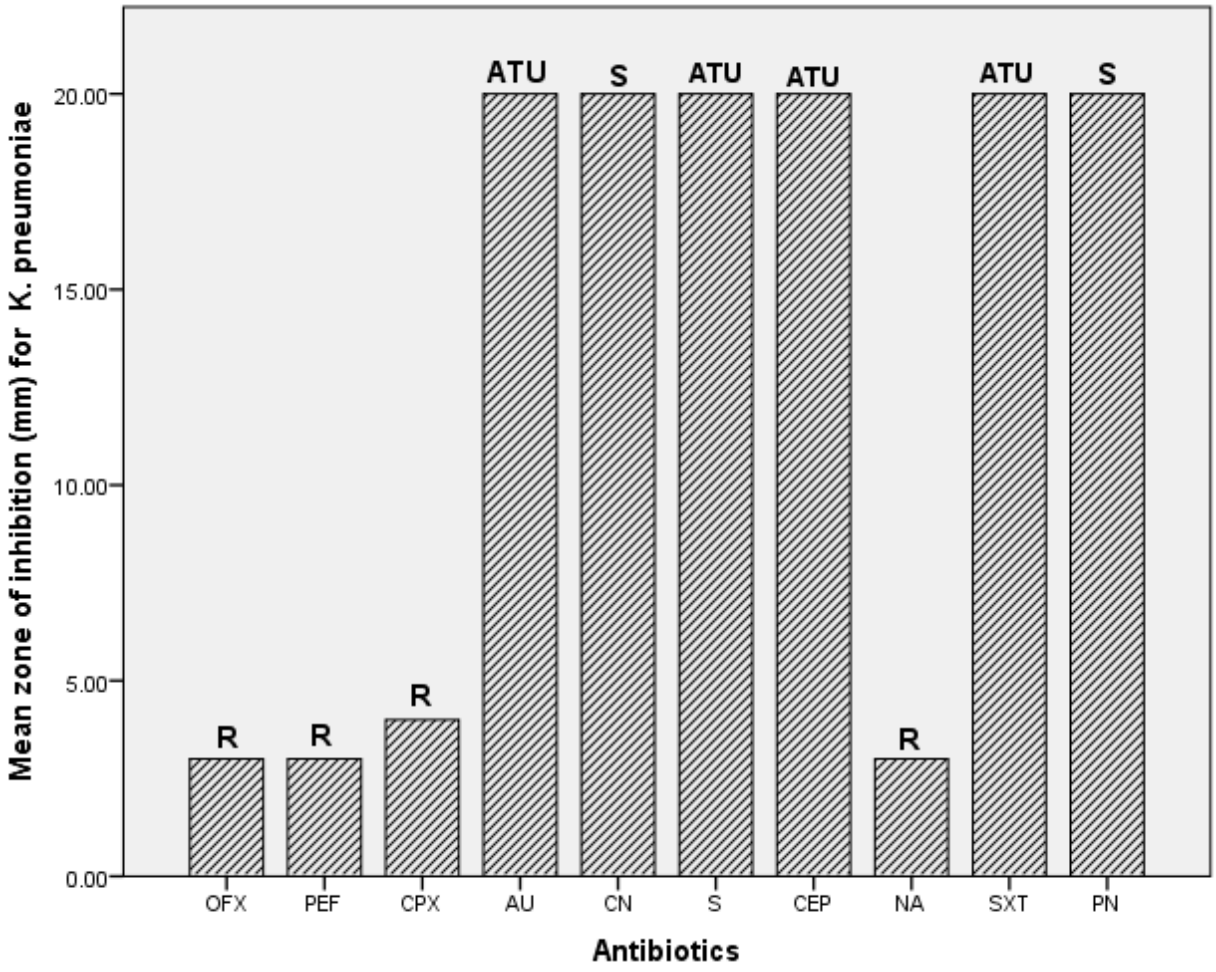
R – Resistance, S – susceptible, ATU – area of technical uncertainty

Fig 4.6: Antibiotic resistivity of *E. coli* to multiple disc antibiotics.



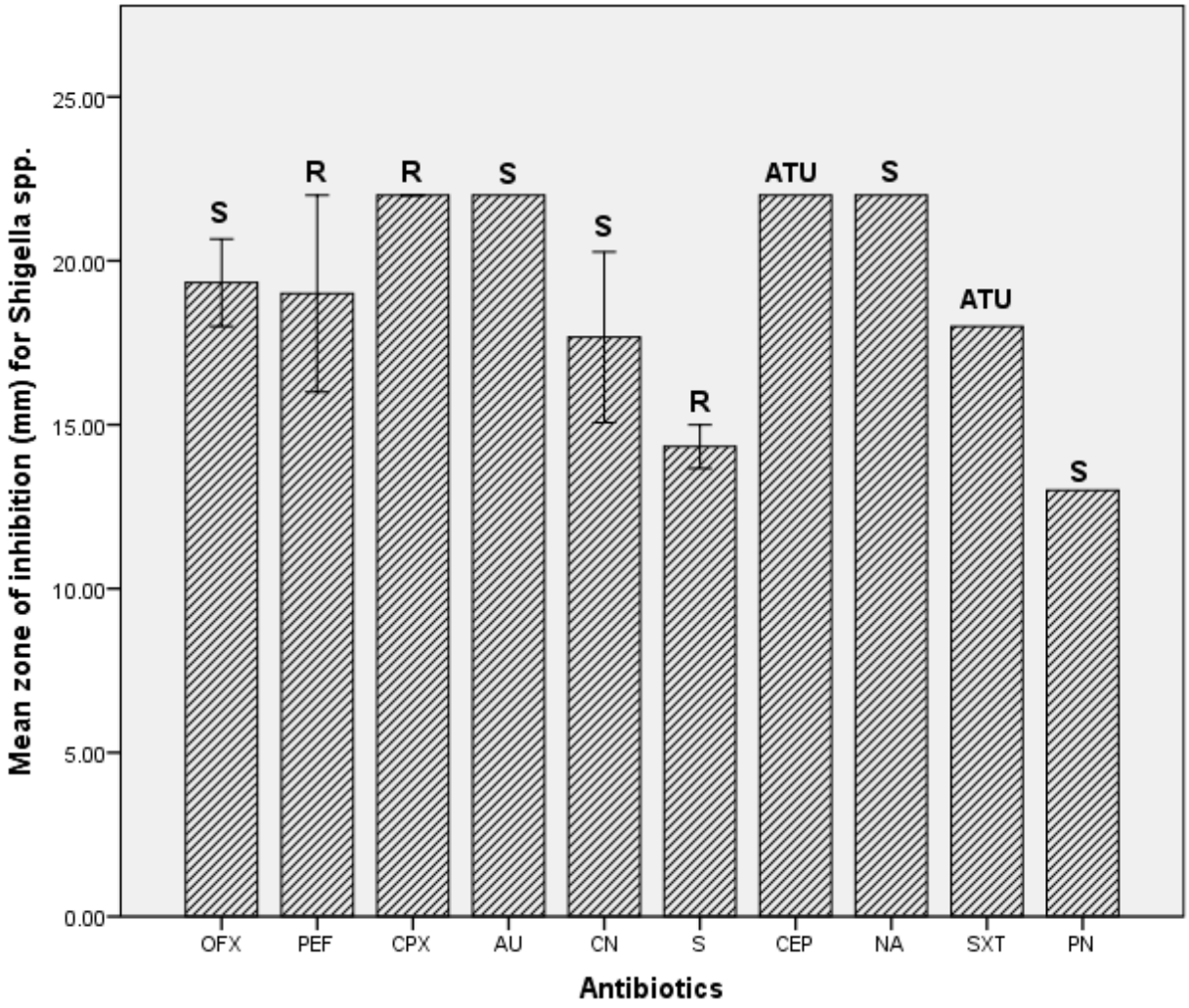
R – Resistance, S – susceptible, ATU – area of technical uncertainty

Fig 4.7: Antibiotic resistivity of *P. aeruginosa* to multiple disc antibiotics.



R – Resistance, S – susceptible, ATU – area of technical uncertainty

Fig 4.8: Antibiotic resistivity of *K. pneumoniae* to multiple disc antibiotics.



R – Resistance, S – susceptible, ATU – area of technical uncertainty

Fig 4.9 Antibiotic resistivity of *Shigella* spp. to multiple disc antibiotics.

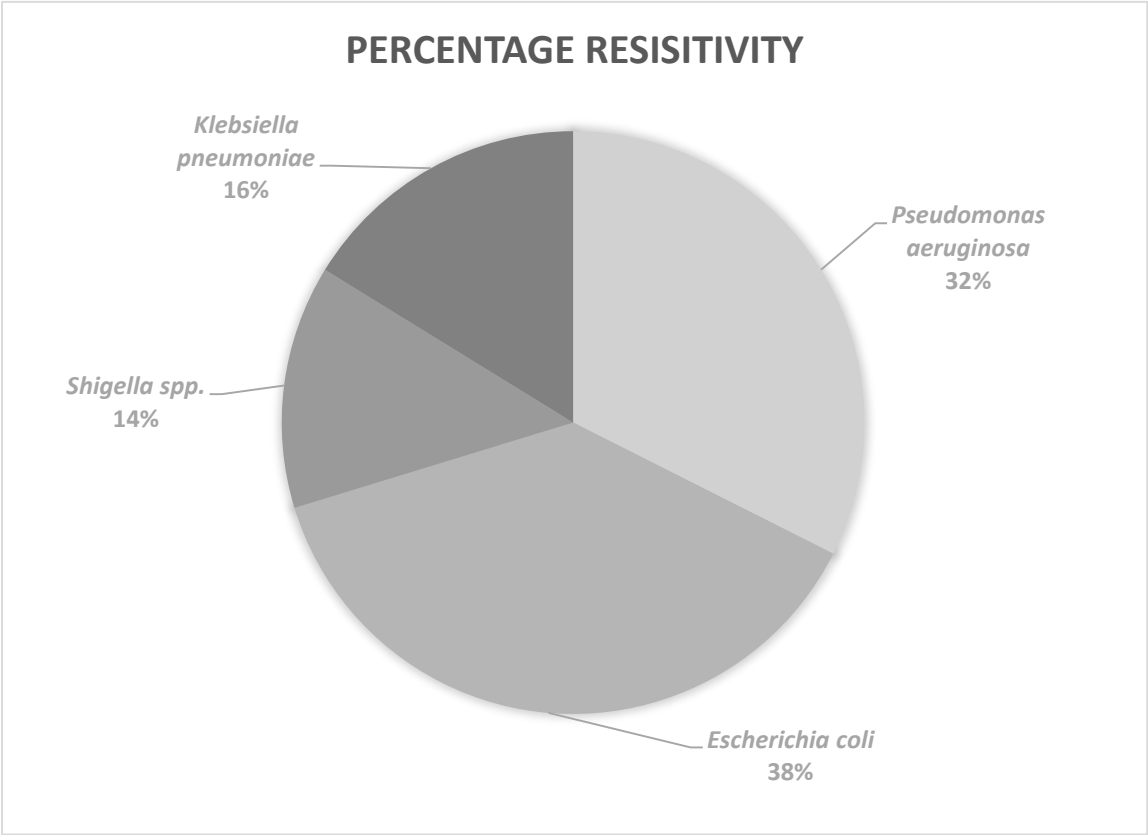
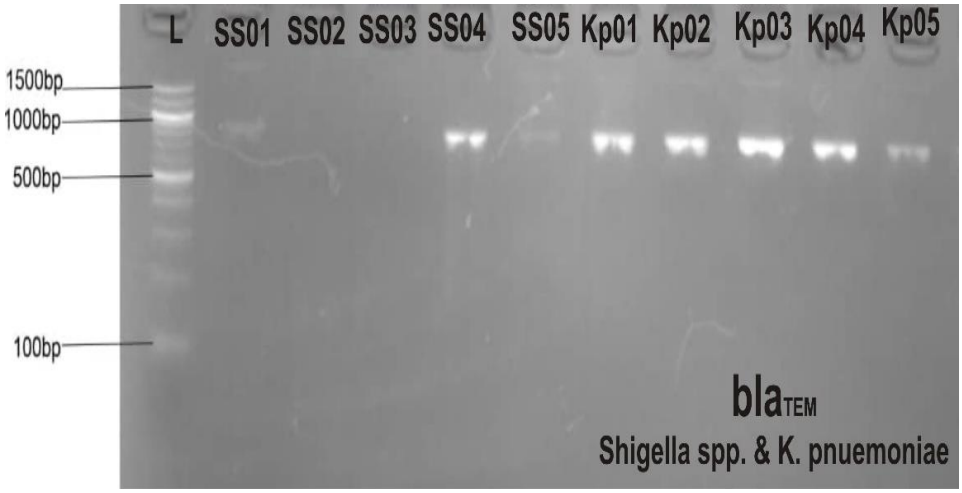


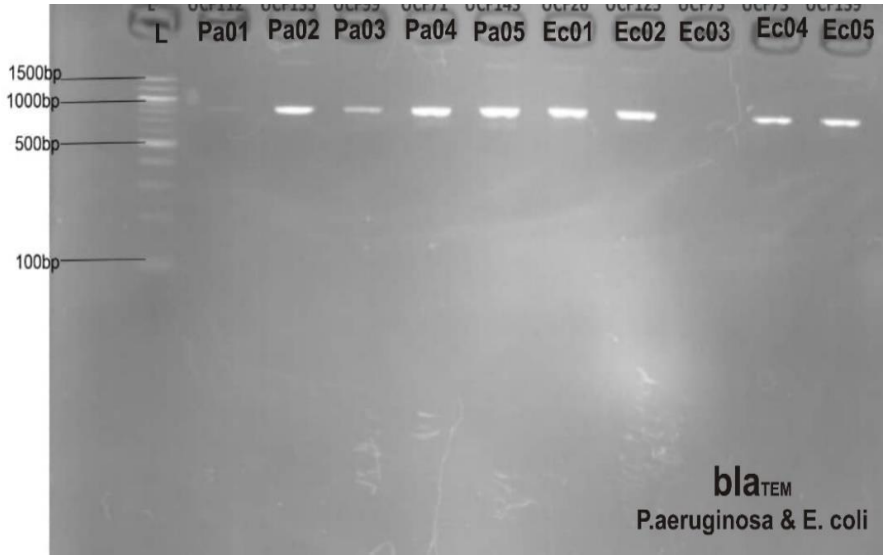
Fig 4.10: Antibiotic resistivity comparison of the Bacterial isolates.

4.1.4 Molecular Characterization of genes responsible for multi-drug resistivity

Three antibiotic resistant genes (*bla*$_{TEM}$, *qnr*A, and aac(3)-1) were investigated from the DNA extracted from the bacteria under study (*P. aeruginosa, E. coli, K. pnuemoniae*, and *Shigella spp.*). It was observed that the *bla*$_{TEM}$ gene-band was the most prevalent (80.00 %) antibiotic resistant gene-band identified in the study (Table 4.2). The *bla*$_{TEM}$ gene-bands were observed at 1000 bp of the DNA ladder (Figure 4.11). The antibiotic resistant gene-band that codes for *qnr*A was observed to be the second most prevalent gene (70.00 %) identified in the study; while the aac(3)-1 antibiotic resistant gene-band was the least prevalent gene (60.00 %) identified in the study (Table 4.2). The *qnr*A gene-bands were observed at 300 bp of the DNA ladder; the aac(3)-1 gene-bands were observed between 600 and 500 bp of the DNA ladder (Figure 4.12 and Figure 4.13 respectively). Among the four bacteria identified, the three antibiotic resistant genes were more abundant in *K. pneumoniae* (93.33 %), followed by *E. coli* (80.0) %). *Pseudomonas aeruginosa* showed percentage (73.34 %) higher than that of *Shigella spp.* (33.34 %) but lower than the percentages observed for *K. pneumoniae* and *E. coli* (Figure 4.14). *Shigella spp*. showed the lowest percentage of the three antibiotic resistant gene-band amongst all the bacteria species (Figure 4.14).



PCR band for *Bla*$_{TEM}$-gene for *Shigella spp*. and *K. pneumoniae*



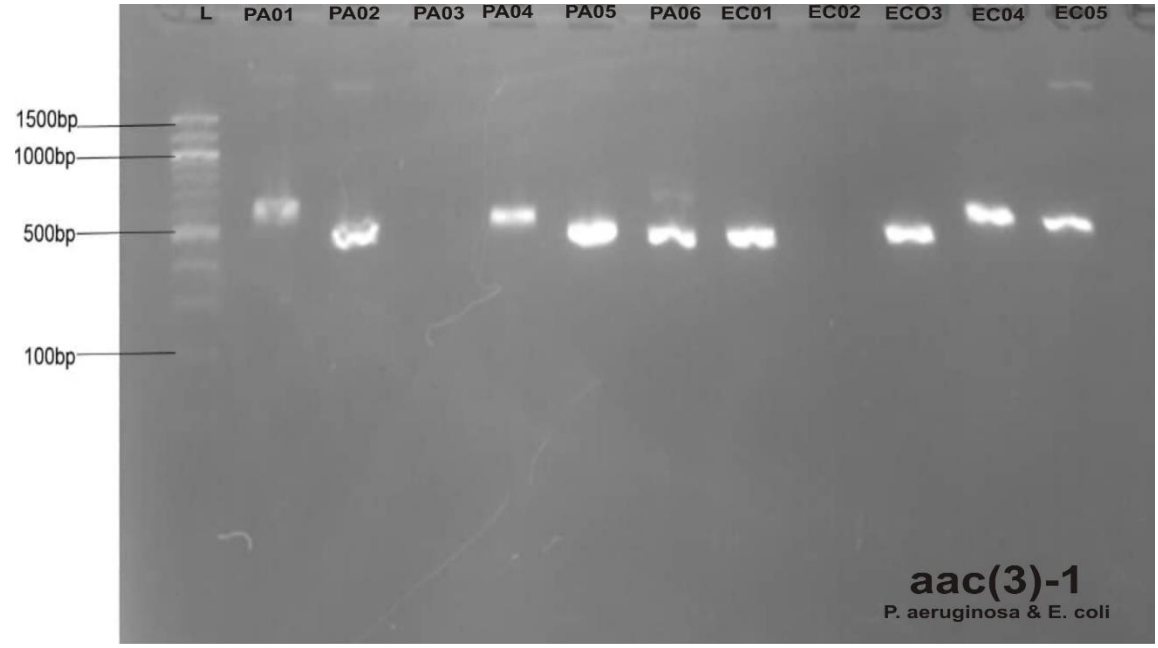
PCR band for *qnr*A-gene for *P. aeruginosa* and *E. coli*

Figure 4.11: PCR Band for *Bla*$_{TEM}$ gene for the Enterobacteria Identified

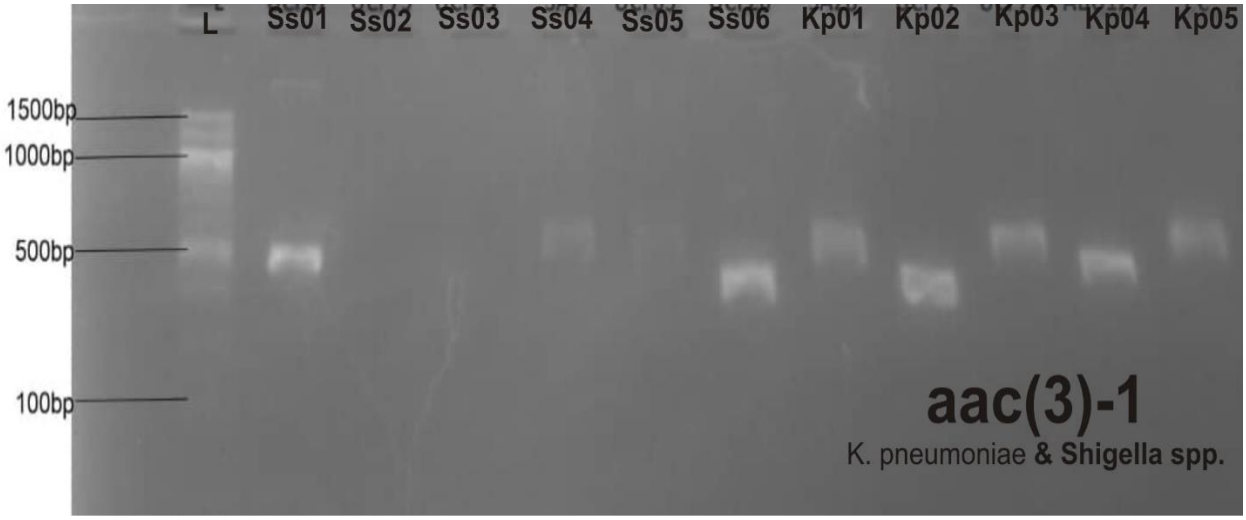
PCR band for *qnr*A-gene for *P. aeruginosa* and *E. coli*

PCR band for *qnr*A-gene for *Shigella spp*. and *K. pneumoniae*

Figure 4.12: PCR band for qnrA-gene for the Enterobacteria Identified



PCR band for aac(3)-1-gene for *P. aeruginosa* and *E. coli*



PCR band for aac(3)-1-gene for *Shigella spp*. and *K. pneumoniae*

Figure 4.13: PCR band for aac(3)-1 gene for the Enterobacteria Identified

Table 4.2: Multi-drug resistant band detection from the bacteria isolates DNA

Organisms	Resistivity genes			Total (%)
	***bla*$_{TEM}$**	***qnr*A**	**aac(3)-1**	
P. aeruginosa				
Pa01	-	+	-	**6.67**
Pa02	+	+	+	**20.00**
Pa03	+	-	-	**6.67**
Pa04	+	+	+	**20.00**
Pa05	+	+	+	**20.00**
Total (%)				**73.34**
E. coli				
Ec01	+	+	+	**20.00**
Ec02	+	+	+	**20.00**
Ec03	-	-	-	**0.00**
Ec04	+	+	+	**20.00**
Ec05	+	+	+	**20.00**
Total (%)				**80.00**
K. pneumoniae				
Kp01	+	+	+	**20.00**
Kp02	+	+	+	**20.00**
Kp03	+	+	+	**20.00**
Kp04	+	+	+	**20.00**
Kp05	+	+	-	**13.33**
Total (%)				**93.33**
Shigella spp.				
SS01	+	+	+	**20.00**
SS02	-	-	-	**0.00**
SS03	-	-	-	**0.00**
SS04	+	-	-	**6.67**
SS05	+	-	-	**6.67**
Total (%)				**33.34**
Total	**16**	**14**	**12**	
Percentage (%)	**80.0**	**70.0**	**60.0**	

+ present -absent

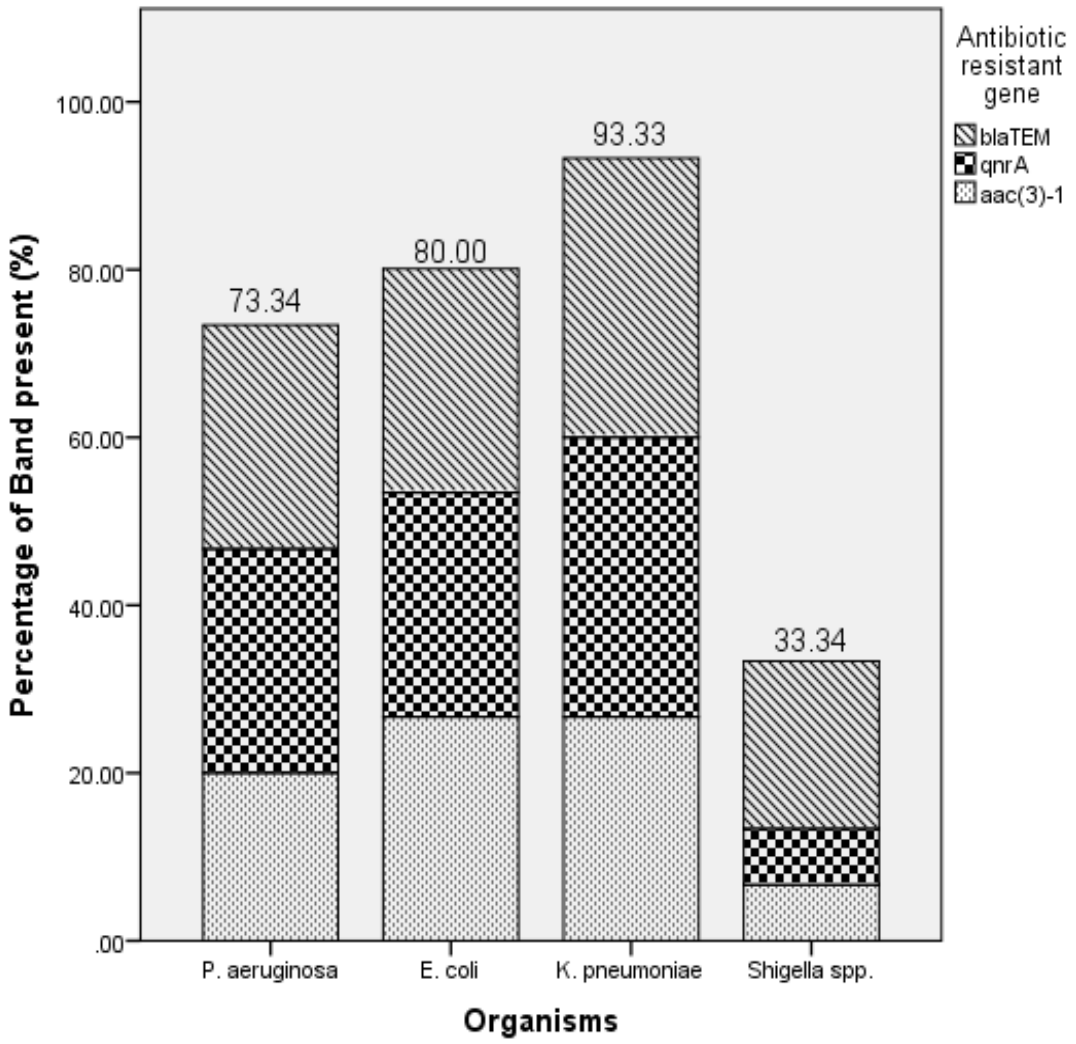


Fig 4.14: Percentage of gene-band for *bla*$_{TEM}$, *qnr*A, and aac(3)-1 for the DNA isolated from the study bacteria species

4.1.5 Comparison of the prevalence pattern of the Isolates from the Healthcare centres using inferential statistics

The prevalence pattern of the Enterobacteria species isolated from the various healthcare centres was investigated by comparing the mean number of isolates (Table 4.3).

Table 4.3: ANOVA: Two-Factor with Replication

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Equipment	278.2292	3	92.74306	28.11579	1.52E-09	2.866266
Wards	3.5	2	1.75	0.530526	0.592824	3.259446
Interaction	8.833333	6	1.472222	0.446316	0.842712	2.363751
Within	118.75	36	3.298611			
Total	409.3125	47				

4.2 DISCUSSION

This study was conducted with the primary aim of investigating the prevalence of antibiotic resistant Enterobacteria from some public healthcare outfits in Imo state. In the course of the investigation, Enterobacteria species were isolated from healthcare outfits (which include bed cover, bed pan, staff gloves, and ward floor) in various wards (maternity, ICU and surgical theatre) in four public healthcare centres (Imo State University Teaching Hospital (ISUTH), Federal Medical Centre Owerri (FMC), Aboh-Mbaise General Hospital (AMGH), and Imo State Specialist Hospital (ISSH) within Imo State. From the present study, Enterobacteria species were isolated from healthcare materials utilised in all the four healthcare centres. It was observed that materials collected from Imo State University Teaching Hospital showed the highest number of Enterobacteria species while materials collected from Federal Medical Center, Owerri, showed the least number of Enterobacteria species (Fig 4.2). This implies that there are disparities in the prevalence of Enterobacteria species isolated from the various healthcare centres. The finding lends support to Azeez-Akande (2012), which reported disparities in the prevalence of nosocomial infection according to different locations. He further noted that higher prevalence rate was observed in University Teaching Hospital (Azeez-Akande, 2012). WHO (2011) also reported disparities in the prevalence rate of hospital acquired infection based on the income level of the locations.

Disparities were also observed based on the different wards where these materials were collected. Materials from Surgical theatre was observed to contain the highest number of Enterobacteria isolate, while materials from ICU contained the least Enterobacteria isolated (Fig 4.3). This is in contrast to Azeez-Akande (2012) report, which reported higher numbers of isolates from ICU. In line with the findings, Tolera *et al*. (2018) reported disparities in Enterobacteria isolated from

various wards; but in contrast to the study, higher number of Enterobacteria was observed in materials from Obstetrics/Gynaecology ward. Ige *et al.* (2011) research on hospital acquired infection from tertiary healthcare centres in Southwestern Nigeria, recorded higher prevalence (48.3 %) for surgical wards compared to the other wards investigated. The reason for such disparities recorded in this research and by other researchers might be according to Hassan *et al.* (2017) postulation, that poor hygienic condition of the hospital environment is a determinant factor for increase in nosocomial pathogens. According to WHO (2011), the financial status of the healthcare centres also plays a role in the prevalence rate of nosocomial infection. This research indicated that the isolates (Enterobacteria species) were higher in bed pan samples and least in glove samples (Figure 4.4). Lending support to Bereket *et al*. (2012), that extrinsic factors (such as staff hygiene and hospital practices) are high risk factors of hospital acquired infection.

The phenotypic and biochemical characterisation of the Enterobacteria isolated revealed that the species isolated were *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *Shigella spp*. In line with this finding, Ige *et al*. (2011), reported that majority of the isolates (78.3 %) collected from tertiary hospitals in Ibadan were Gram-negative bacteria; of which most were Enterobacteria species (*Klebsiella spp.*, *E. coli*, *P. aeruginosa*, *Pseudomonas spp.,* and *Proteus spp.*). According to Bereket *et al*. (2012), pathogens responsible for nosocomial infections are mostly medically established Gram-negative bacteria; of which the most frequently reported are *P. aeruginosa*, *E. coli*, and *Enterococcus.* To ascertain the presence of multiple drug resistance (MDR) genes in the isolates, the Enterobacteria species isolated were subjected to antibiotic sensitivity test using multiple antibiotic discs. The resistivity of the bacterial isolate to the multiple antibiotic treatment lends support to Bolaji *et al*. (2011), that there is increase and widespread of antibiotic resistivity among nosocomial pathogens. According to Bereket *et al*. (2012), most of the prevalent

nosocomial bacteria are multi-drug resistant. From the results (Figure 4.6 to 4.9), it can be seen that the Enterobacteria species exhibited multidrug resistance. *E. coli*, *P. aeruginosa* and *K. pneumonia* showed high resistivity than *Shigella spp.* In line with this finding, Tolera *et al*. (2018), reported high level of resistivity for *E. coli*, *P. aeruginosa* and *Klebsiella spp.* towards multiple antibiotics. Also, Baka *et al.* (2014), reported *K. pneumoniae* susceptibility to majority of penicillin antibiotics. The increase in antibiotic resistivity can be attributed to the indiscriminatory use of antibiotics, poor infection control policies, overcrowding of hospitals and inefficient healthcare system (Nejad *et al*., 2011; Mbim *et al*., 2016).

Employing molecular assay, the present research investigated the prevalence of bla_{TEM}, *qnr*A, and aac(3)-1 MDR genes among the isolates. It was noticed that these genes investigated were present in all the Enterobacteria species ioslated. The bla_{TEM} was the most prevalent gene isolated from the bacteria, while the aac(3)-1 genes was the least prevalent (Figure 4.11). The dominant presence of the bla_{TEM} gene reflects the high resistance of the bacteria isolated to Cefalexin (CEP). *K. pneumoniae* was observed to contain most of the resistant gene, *E. coli* showed the second highest level of resistivity, whereas *Shigella spp*. showed the lowest level of resistivity (Figure 4.11). The presence of DNA bands for these resistant genes in bacteria isolates, suggests the presence of multidrug resistant gene band in the isolates. As it indicates that, the primers were relevant, and the genes were present in the chromosomal material. In contrast to the findings, Abd-Alfadil *et al*. (2018), reported the absence of bla_{TEM} genes in the chromosome of *E. coli*, *P. aeruginosa*, *Klebsiella spp.*, *Shigella spp.,* and *Salmonella paratyphi* isolated from banknotes despite their resistance to some beta-lactams.

The study indicated that the prevalence of antibiotic resistant Enterobacteria species is significantly different across the various hospital outfits they were isolated from ($p < 0.05$). The

difference in their prevalence rate based on the wards from where the species were isolated did not vary significantly ($p > 0.05$). The interaction between hospital wards and hospital equipment has no significant ($p > 0.05$) influences on the prevalence rate of antibiotics resistant Enterobacteria species isolated (Table 4.3).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Many microorganisms have been associated with hospital acquired infections; majority of these organisms have been reported to be bacteria, of which most species from the genus *Enterobacteriaceae* have been identified as the most prevalent. The increase in nosocomial infections, have been associated with the increase in antimicrobial resistance. This has been reported to be due to indiscriminate use of antibiotics, poor hospital hygiene and waste management system, hospital overcrowding, and insufficient professional hospital staffs. This study reported the presence of Enterobacteria species in hospital equipment (bed pan, bed cover, staff gloves) and environment (ward floors) from maternity ward, ICU, and surgical theatre. The Imo State University Teaching Hospital showed the highest prevalence of Enterobacteria species isolated. The biochemical and cultural assay of these isolates identified four Enterobacteria species: *P. aeruginosa, E. coli*, *K. pneumoniae,* and *Shigella spp.* the study also indicated that these species identified were resistant to multiple antibiotics. *K. pneumoniae* showed more resistivity compared to the other Enterobacteria species identified in the study. There was presence of resistance-genes: bla_{TEM}, *qnr*A, and aac(3)-1, in the chromosomes of the bacteria isolated. The bla_{TEM} gene was the most common resistant gene identified in the study. The prevalence rate of these isolates was not significantly different across the hospital equipment; but varied significantly across the various hospital wards. The interaction between hospital wards and hospital equipment insignificantly influences the prevalence rate of antibiotics resistant Enterobacteria species isolated. The increase in the prevalence of Enterobacteria species especially resistant strain in hospital environment can lead to prolonged hospital stays, long term disability in patients, and increased mortality rate.

5.2 Recommendations

Based on the results obtained from the study, the following recommendations are provided:

i. Antibiotics stewardship should be practised in healthcare settings. The practice of proper diagnosis and rational prescription of antibiotics should be imbibed in the health sector to reduce the selective pressure for resistant bacteria.

ii. Hospitals should adopt proper hygiene practices. Wastes should be adequately sealed (using biosafety waste bag) prior to disposal. Proper waste management system is recommended. Surface application of antibiotic can be applied when cleaning invasive devices and certain types of hospital equipment.

iii. The human resource for health should be educated on infection control, transmission-based precaution (transmission mechanisms of nosocomial pathogens), and proper hygiene in healthcare settings.

iv. There is need for further research encompassing other hospitals, wards, and medical equipment. The presence of other resistant genes should also be investigated.

5.3 Contribution to Knowledge

This research has provided knowledge on the specific Enterobacteria species involved in hospital acquired infection in the public healthcare centres in Imo State. It also has informed that the prevalence of these Enterobacteria species is significantly different across the various hospital wards. Although this prevalence is not dependent on the interaction between the hospital equipment and hospital wards.

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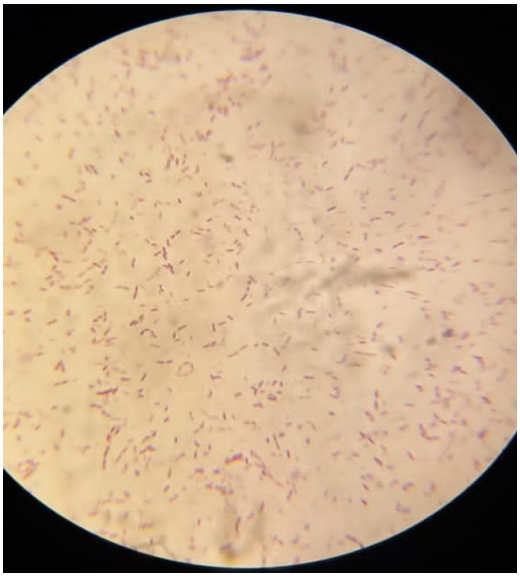
APPENDICES

APPENDIX I

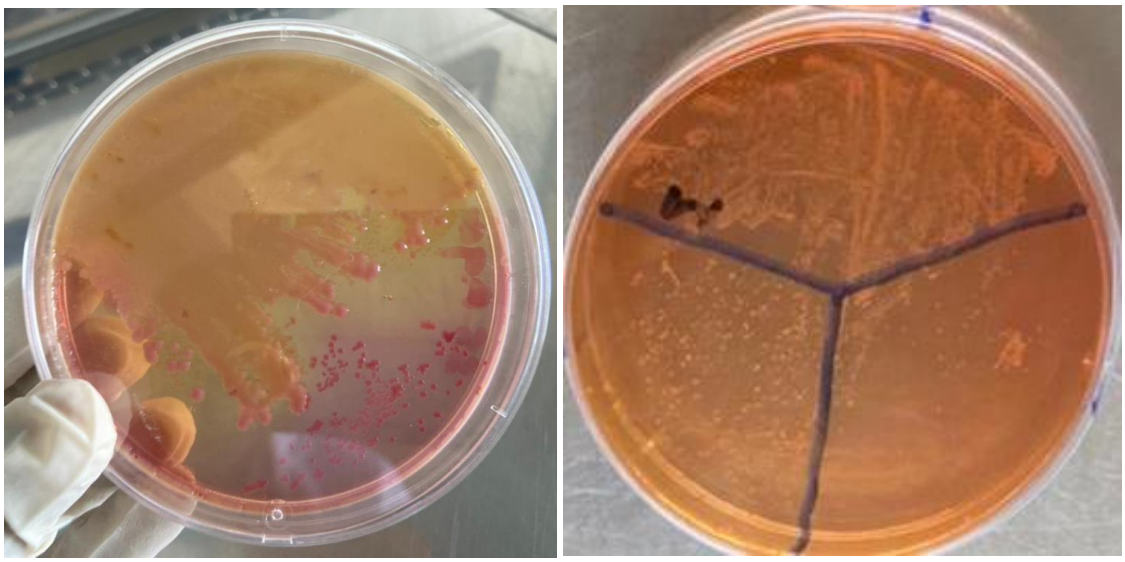
		Number of Enterobacteria species	
		Mean	Standard Error of Mean
Hospital	ISUTH	20.67	1.45
	FMC	18.00	1.15
	AMGH	18.67	1.20
	ISSH	19.67	.33

		Number of Enterobacteria species	
		Mean	Standard Error of Mean
Wards	MATERNITY	19.75	4.59
	ICU	17.75	5.63
	SURGICAL THEATRE	20.25	6.55

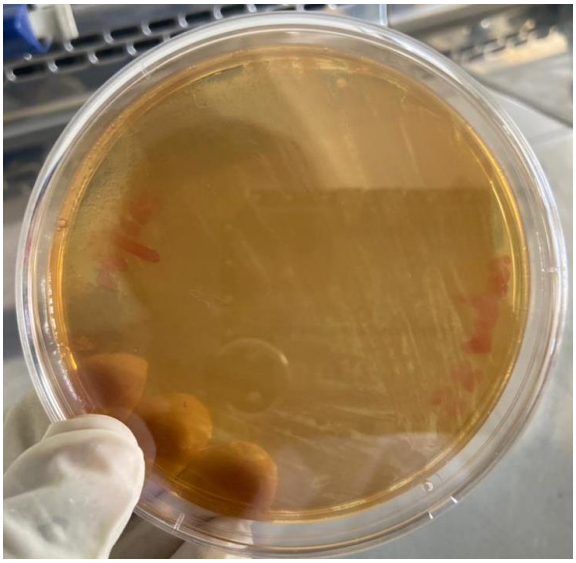
APPENDIX II



Microscopic imagine of isolate after Gram staining



Isolates on MacConkey agar showing reddish-pink colonies, brownish colonies and colourless colonies

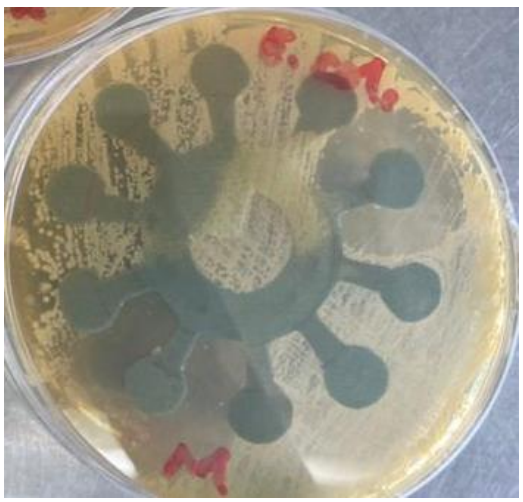


Isolate growth on Salmonella-Shigella agar

Biochemical assay: isolates reaction to indole production test

APPENDIX III

		Zone of Inhibition					
		Organisms					
		P. aeruginosa		E. coli		K. pneumoniae	
		Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
Antibiotics	Tarivid	20.33	2.33	18.00	1.00	.00	.
	Pefloxacin	15.67	1.33	18.50	1.50	.00	.
	Ciproflox	18.00	.	17.00	.00	.00	.
	Augmentin	6.67	6.67	.00	.	20.00	.
	Gentamicine	18.00	1.00	20.00	3.00	20.00	.
	Streptomycin	19.00	1.00	17.00	.00	20.00	.
	Ceporex	4.00	.	20.50	3.50	20.00	.
	Nalidixic acid	4.00	.	4.00	.	4.00	.
	Septrin	4.00	.	4.00	.	20.00	.
	Ampicillin	4.00	.	4.00	.	18.50	1.50



APPENDIX IV

SUMMARY	Maternity	ICU	Surgical Theatre	Total
Bed pan				
Count	4	4	4	12
Sum	25	27	31	83
Average	6.25	6.75	7.75	6.916667
Variance	6.916667	2.916667	2.916667	3.901515
Ward Floor				
Count	4	4	4	12
Sum	30	28	32	90
Average	7.5	7	8	7.5
Variance	1.666667	4.666667	0.666667	2.090909
Bed cover				
Count	4	4	4	12
Sum	12	8	11	31
Average	3	2	2.75	2.583333
Variance	3.333333	3.333333	2.25	2.628788
Gloves				
Count	4	4	4	12
Sum	12	8	7	27
Average	3	2	1.75	2.25
Variance	3.333333	3.333333	4.25	3.295455
Total				
Count	16	16	16	
Sum	79	71	81	
Average	4.9375	4.4375	5.0625	
Variance	7.2625	9.195833	10.59583	