PHENOTYPIC AND MOLECULAR DETECTION OF MULTIDRUG RESISTANT BACTERIAL ISOLATES FROM SURGICAL WOUND INFECTIONS IN TWO NIGERIAN TEACHING HOSPITALS.

BY

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CERTIFICATION

I certify that this research work titled "Phenotypic and Molecular Detection of Multidrug Resistant Bacterial Isolates from Surgical Wound Infections in Two Nigerian Teaching Hospitals." was carried out by Ehiaghe Imuetiyan Joy with registration number 2012617002F under my supervision.

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APPROVAL PAGE

This dissertation titled "Phenotypic and Molecular Detection of Multidrug Resistant Bacterial Isolates from Surgical Wound Infections in Two Nigerian Teaching Hospitals." Carried out by Ehiaghe, Imuetiyan Joy has been approved for the award of Degree of Doctor of Philosophy in Microbiology, (Medical Microbiology unit), Nnamdi Azikiwe University, Awka.

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DEDICATION

I dedicate this dissertation to God Almighty for His grace, love, kindness and strength and above all for actualizing my dream, and to my loving husband for his financial support, and my blessed children.

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LIST OF ABREVATIONS

MDR-Multidrug resistant

SSI- Surgical Site Infections

DNA- Deoxyribonucleic acid

SENIC- Study on the efficacy of the nosocomial infection control

CFU- Colony forming unit

CDC- Centers for disease control and prevention

AMP- Antimicrobial prophylaxis

BMC- Bugando medical center

MRSA – Methicillin resistant *Staphylococcus aureus*

MSSA- Methicillin sensitive *Staphylococcus* aureus

ESBL - Extended spectrum beta lactamase

LPs- Lipopolysaccharide

TEM- Temoniera

SHV-Sulfhydryl variable

PER type- *Pseudomonas* extended resistance

VEB type- Vietnamese extended-spectrum beta–lactamase

ICUs- Intensive care units

PCR- Polymerase chain reaction

SCV- Small colony variants

PBPs- Penicillin binding proteins

EDTA- ethylene diamine tetra-acetic acid

MSCRAMMs- microbial surface components recognizing adhesive matrix molecules

UTI- urinary tract infections

SDS- sodium dodecyl sulfate

ABSTRACT

Postoperative surgical wound infection is a significant clinical challenge in hospitals in developing countries where proper healthcare delivery is hampered by limited resources. Currently there is paucity of documented information on the prevalence of multidrug resistant bacteria isolated from surgical wound infections at University of Benin Teaching Hospital (UBTH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH). Availability of such information would assist in the development or the review of in –use antibiotic policy of the institution. This study investigate the phenotypic and molecular characterization of multidrug resistant bacteria isolates implicated in surgical wound infections among hospitalized patients at UBTH and NAUTH in order to generate findings which could drive reformation of policies in management of patients with surgical wound infections. This study was conducted to investigate the phenotypic and molecular characterization of bacteria recovered from 362 swabs specimens of patients with clinically diagnosed postoperative surgical wound infections from UBTH (Edo state) and NAUTH (Anambra state) within a period of one year. A cross sectional study using the randomized sampling method and specimens were cultured on Blood and MacConkey agar media and incubated aerobically and anaerobically for 48 hours. Antibiotic susceptibility of isolates was determined by Kirby-Bauer disk diffusion technique on Mueller-Hinton agar. Plasmid DNA profiling were carried out on all multidrug resistant isolates on agarose gel. All plasmid positive isolates were cured of their plasmids (100%) and post plasmid antibiogram carried out increased susceptibility pattern of isolates to the previously used antibiotics. Chromosomally mediated multidrug resistant strains were targeted against four selected different antibiotics to detect the resistant genes (TEM, gyrA, ereA and Blaoxa) expression patterns, with the use of polymerase chain reaction. Bivariate analysis of variance (Anova) using GraphPad InStat version 3.05 were employed to determine the significant differences in isolation, distribution and multidrug resistance of bacterial isolates from surgical wound specimens. Differences were considered significant at $P < 0.05$ showed that one hundred and twenty two (33.7%) surgical wound bacteria isolates which included four different genera (*P. aeruginosa, E. coli, P. mirabilis and S. aureus*) were recovered from postoperative surgical wound swab specimens. There were no significant differences ($P>0.05$) in the isolation of pathogens with respect to locations. Most bacteria exhibited resistance to Augmentin, Cefuroxime and cefixime from both locations and least resistance to Imipenem among the antibiotic discs tested against Gram negative isolates. The most predominant isolates from both locations were *P. aeruginosa* (UBTH=60%; NAUTH= 36%) and *E. coli* (UBTH=34%; NAUTH=52%). They exhibited high preponderance of multidrug resistance in both locations {*P. aeruginosa*, UBTH (90%), NAUTH (92%); *E. coli*, UBTH (70%), NAUTH (95%)}. There were no significant difference in the prevalence of TEM resistant genes expressed by the isolates in both locations (P value = 0.6). However most isolates from UBTH expressed TEM gene (OR = 9.7). Meanwhile gyrA resistant genes expressed by the isolates were significantly different (OR = 16.10; P = 0.014). *S.aureus* isolated from NAUTH expressed both Blaoxa and ereA resistant genes. There was a positive evidence of multidrug resistant genes from both locations which may constitutes a significant cause of morbidity in the study areas. Though phenotypic characterization may be reliable but molecular method is conclusive. Findings in this study justify the need to strengthen infection control and drug dispensing policies, and together force greater collaboration between microbiologists and medical practioners to prevent the spread of multidrug resistant bacteria.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Surgical site infection (SSI) is defined as an infection occurring within 30 days after a surgical operation (or within 1 year if an implant is left in place after surgical procedure) and affecting either incision or deep tissues at the operation site. These infections may be superficial, deep incision or infections involving organ/body space. The clinical signs of wound infection are pain, tenderness, localized swelling, redness or heat. Infection may be defined as invasion and multiplication of microorganisms in body tissues, which maybe clinically in apparent or result in local cellular injury because of competitive metabolite intracellular replication, or antigen-antibody response. This series of events leads to progressive tissue destruction and eventual death of the host if left unchecked (ManGram *et al.*, 1999).

Any purulent discharge from a closed surgical incision, together with signs of inflammation of the surrounding tissues are considered as wound infection, irrespective of whether microorganisms had been cultured from it. In 1992, the surgical wound infection tasks force replaced the term ‘surgical wound infection’ with ‘surgical site infection to include infections’ of organs or spaces deep in the skin and soft tissues, such as peritoneum and bone. There are intermediate categories of wounds that may or may not be infected, namely wounds that have a small amount of clear discharge. These wounds may be considered as possibly or probably infected (Horan *et al.*, 1992). Postoperative surgical site infections remain a major source of illness and a less frequent cause of death in the surgical patient. The presence of a microorganism within the margin of a wound does not indicate that wound infection is inevitable (Taylor *et al.*, 1990).

Some bacteria produce proteins that kill or inhibit other bacteria while in some other cases, bacteria produce a variety of metabolites that inhibit the multiplication of other microorganisms. This is called protective colonization (Kingsley, 2001).

Postoperative surgical site is among the most common and the third most frequently reported nosocomial infection in the hospital population and surgical site infections are associated with increased morbidity ,mortality, prolonged hospitalization of patient and increased economic costs for patient care population (Weigelt *et al.*,2010). Surgical site infection (SSIs) is the most common nosocomial infection in surgical patients, accounting for 39.9% of all infections (Mohammed *et al.*, 2013). As most infections are the result of wound contamination by endogenous bacteria from the patient's skin, mucous membrane or hollow viscera, the concept of using a physical barrier to cover the out edges of the wound has been visited by surgeons many times over the past half century.

The development of an infection is influenced largely by the virulence of the organism and immunological status of the patient, when microorganisms are present to a degree of 10^5 per g ram of tissue, an infection is likely to be present. Quantitatively, wounds harboring bacteria that exceeds 10^5 colony forming unit per Gram are considered infected wounds (Heggars, 2003). However, Gram negative bacteria can contaminate skin wounds of the groin and perinea areas. The contaminating pathogens in gastro intestinal surgeries are the intrinsic bowel flora which includes Gram-negative bacilli and Gram-positive microbes, including enterococci and anaerobic organisms. Bacterial isolates colonizing surgical wounds vary in their carriage of genes encoding antibiotic resistance (Yah *et al.*, 2004).

However, the routine use of antimicrobial agents in both human and veterinary medicine has resulted in wide spread antibiotic resistance genes especially within the Gram negative bacteria

(Enabulele *et al.,* 2006). With the presence of antibiotic selective pressure, these resistant bacteria species tend to persist, enabling the organism to cause extra infections such as septicaemia (Prescott *et al.,* 2008).

Since the discovery of the first antibiotic (penicillin) by Alexander Fleming in 1929 bacterial resistance to antibiotics was barely a hindrance in antimicrobial chemotherapy as antibiotics worked effectively in the treatment of bacterial infections. This could be due to the improper use of drugs and the slow mutation rates of most bacteria strains to acquire resistance. However, over the years there has been great increase in resistance to antibiotics and this has brought about the need for the development of new antibiotics that will be more effective in chemotherapy. Most bacteria have developed resistance to antibiotics through various mechanisms which include production of enzymes that inactivate the antibiotics, efflux pumping machinery on the cell membrane, modification of drug structure, loss of porin proteins and acquisition of genes that harbor resistant plasmids (Lee *et al.,* 2000; Tenover, 2006).

1.2 The study Problem

Currently there is paucity of documented information on the prevalence of multidrug resistant bacterial isolated from surgical wound infections at University of Benin Teaching Hospital (UBTH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH). Availability of such information would assist in the development or the review of in –use antibiotic policy of the institutions. It is hoped that the study will give an insight and new information towards formulating a better policy toward improved patient care.

1.3 JUSTIFICATION OF THE STUDY

The developments of surgical wound infections are related to three factors which include; (1) the degree of bacterial contamination during the operation, (2) the duration of procedure, and (3) the underlying disease of the patients such as immune deficiency, diabetes, and malnutrition (Dunn and Beilman, 2005). Multidrug resistant bacteria isolates have posed serious challenges to the treatment of surgical wound infections worldwide (Mofikoya *et al.*, 2009).

In this study, the knowledge of types of bacterial pathogens and antimicrobial resistant pattern can optimize treatment and decrease disease morbidity and mortality rates of surgical wound infections. Also, bacterial resistance can result to complicated cases of surgical wound infection such as septicemia especially in immunocompromised patients leading to prolongation of therapy and even therapeutic failures. Getting to know the drug resistance strategies possessed by etiologic agents of surgical site infections will greatly improve chemotherapeutic approaches in the treatment of wound infections worldwide.

1.4 AIM OF THE RESEARCH

The aim of this study is to employ phenotypic and molecular technologies for characterizing multidrug resistant bacteria isolates incriminated in surgical wound infection from hospitalized patients in UBTH (Edo state) and NAUTH (Anambra state) with emphasis on identifying associated drug- resistant genes in the isolates.

1.5 OBJECTIVES OF THE RESEARCH

The objectives of this study are

1. To determine the prevalence of bacterial pathogens incriminated in surgical wound infections in UBTH and NAUTH.

2. To carry out detailed characterization of isolated pathogens in surgical wound infections in UBTH and NAUTH.

3. To determine the antibiogram patterns of bacterial isolates from surgical wound infections in UBTH and NAUTH.

4. To determine the plasmid DNA profiles of the multidrug resistant bacterial isolates.

5. To determine the effect of curing on the bacterial plasmid DNA profiles of the isolates.

6. To identify chromosomally mediated multidrug resistant bacteria isolates using polymerase chain reaction.

7. To detect some specific genes that confer chromosomally mediated multidrug resistance on the genes isolates using primers against selected antibiotics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical background of surgical wound infection

There is documentary evidence that the historical background of wound infection may be traced back to as far back as first century when a Roman physician, Cornelius census described the four principal signs of inflammation and used 'antiseptic' solution. Claudius Galen (130-200 AD), another Roman physician had such an infection on the management of wounds that is still thought of by many today as the 'father of surgery' Claudius. He and some of his followers instigated the 'laudable pus' theory, which incorrectly considered the development of pus in a wound as a positive part of the healing process (Bibbing and Honey 1984). This continued until the 16th century when Amboise pares encouraged wounds to suppurate. The 19th century witnessed the acceptance of the germ theory and introduction of antisepsis through Semmelweiss (1818-1865) and Lister (1827-1912) (Ayton, 1985). Terminologies such as wound contamination, wound colonization and wound infection which are currently in use have been defined (Ayton, 1985). Vincent Falanga in 1994 identified the concept of critical colonization with fresh insights into chronic wound healing and non healing wounds (Kingsley, 2001). These current terms are as follows

1. **Wound Contamination**: The presence of bacteria within a wound, without any host reaction.
2. **Wound Colonization**: The presence of bacteria in the wound which do multiply or initiate a host reaction.

3. **Critical Colonization**: Multiplication of bacteria causing a delay in wound healing usually associated with an exacerbation of pain not previously reported but still with no overt host reaction (Kingsley, 2001).
4. **Wound Infection**: The deposition and multiplication of bacteria in tissue with an associated host reaction.

2.2 **Associated Factors**

In practice, it appears as if health personnel use the term **critical colonization** to described wounds that are considered to be moving from colonization to local infection. However, the challenge is to ensure that most practitioners recognize this situation with confidence and for the bacterial bio- burden to be reduced as soon as possible (Collier, 2004). There are many factors that are thought to affect the susceptibility of any wound to infection, some of which strongly predispose to wound infection. These factors include pre-existing illness, length of operation, wound class and wound contamination. Other factors such as extreme of age, malignancy, metabolic diseases, malnutrition, immune suppression, cigarette smoking, emergency procedure and long duration of preoperative hospitalization are not considered as independent risk factors for wound infections (Sawyer and pruetts1994).

In the study on the efficacy of the Nosocomial infection control of 1970 (Haley *et al*., 1985),

498 patients undergoing operations were monitored for the presence and progress of wound infection and four independent risk factors were identified as

(1) Procedures lasting more than two hours.

(2) Wound contamination.

(3) Three or more diagnoses at the time of discharge (excluding those related to surgical wound infections and their complications), and

(4) Abdominal operations (Haley *et al*., 1985).

The risk of wound infection has repeatedly been shown to be proportional to the duration of the operative procedure. The rate of wound infection increased for longer procedure roughly doubling with every hour of the procedure (Cruse and Foord 1980). Operations lasting one hour or less had a wound infection rate of 1.3%, where as those lasting three hours or more has a rate close to 4.0% (Cruse and Foord 1980). An operative time of more than two hours is the second greatest independent predictor of risk, wound contamination being the first (Haley *et al*., 1985). On the basis of degree of microbial contamination, surgical site infections can be classified into four major groups: Clean site wounds, clean contaminated wounds, contaminated wounds, dirty or infected wounds (Anaya and Dellinger 2006).

Wound contamination, as shown by intra operative culture, is associated with later wound infection. During cholecystectomy, the number of and species of bacteria cultured from bile are predictive of wound contamination and later infection. Thirty (30) or more colony forming units (CFU) of bacteria cultured from a wound are found to be predictive of wound infection, regardless of wound class (Garibaldi, *et al.,* 1991). In addition, a prospective study of 190 colorectal surgery patients has shown that a concentration of 5 CFU per milliliter or higher of bacteria in the peritoneal fluids are predictive of wound infection. All surgical wounds are contaminated by both pathogens and body commensal ranging from bacteria and fungi to other parasites (Bowler 2001). The common Gram positive organisms are *Streptococcus pyogenes* and *Staphylococcus aureus*. The Gram negative aerobic rod is *Pseudomonas aeruginosa*. The facultative anaerobes include *Enterobacter* species, *Klebsiella* species, *Escherichia coli* and *Proteus* species. The fungi are *Candida* species and *Aspergillus* species (Mordi and Momoh, 2009), but the development of infection in the site depends on complex interplay of many factors (Olsen *et al.,* 2008). These Intrinsic factors include advanced age, malnutrition, metabolic

diseases, smoking, obesity, hypoxia, immune-suppression, and length of pre-operative stay. Extrinsic factors consist of application of skin antiseptics, pre-operative shaving, and antibiotic prophylaxis, and pre-operative skin preparation, inadequate sterilization of instruments, surgical drains, surgical hand's scrubs, and dressing techniques (Olsen *et al*., 2008).

Historically, the analysis of nosocomial pathogens has relied on a comparison of phenotypic characteristics such as biotypes, serotypes and antimicrobial susceptibility profiles. This approach has begun to change over the past 2 decades, with the improvement and functioning of new technologies based on DNA or molecular analysis (Snyder and Champness, 2007). Studies of microbial pathogenicity at the molecular level have made substantial contributions to the understanding of the epidemiology, clinical manifestations, diagnosis, treatment, and immune prophylaxis of infectious diseases. One of the most exciting and profound technical advances in the past years has been the development of nucleic acid amplification techniques and their application to the study of microbial pathogenesis and the diagnosis of infectious diseases (Snyder and Champness, 2007).

2.3 Classification of Surgical Site Infections

The Centers for Disease Control and Prevention (CDC) term for infections associated with surgical procedures was changed from surgical wound infection to surgical site infection in 1992 (Horan *et al.*, 1992). These infections are classified into incisional, organ or other organs and spaces manipulated during an operation; incisional infections are further divided into superficial (skin and subcutaneous tissue) and deep (deep soft tissue-muscle and fascia) (Horan *et al.*, 1992).

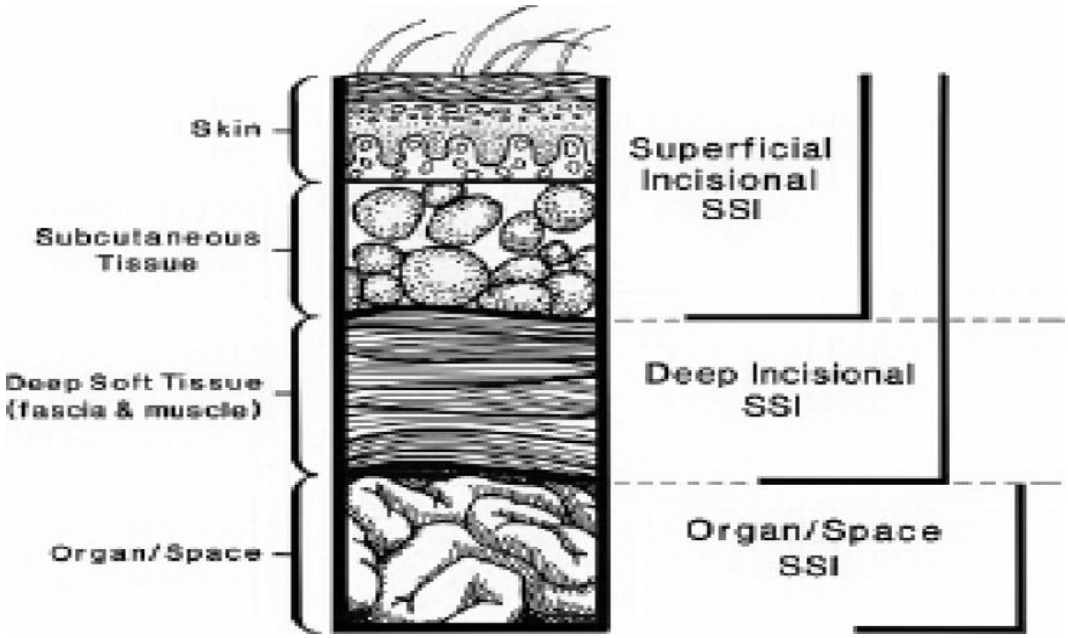


Fig: 1 Classification of surgical site infections (SSIs) according to the Centers for Disease Prevention and Control (CDC) (Mangram *et al.,* 1999*).*

2.4 Prevalence of Surgical Site Infections

Several literature sources report varying SSI prevalence and incidence rates in different parts of the world, ranging from 5.6% to 26%. A study at University Hospital in Brazil among general surgical patients reported high SSI rate of 16.9%, with high rate in clean contaminated (17.8%) as compared to contaminated surgery (12.5%), however majority of intervention involved the digestive tract (Santos *et al*., 1998). Few studies conducted in Africa have also reported varying magnitude of SSI depending on procedures and specialties in which it was performed. In one prospective multicenter study done at large hospitals in Lagos, Nigeria, low prevalence rate of surgical site infections 9.6% was reported among women who underwent caesarian section (Ezechi *et al*., 2009). The reason for this low prevalence was inferred to possibly be due to exclusion of cases with sub umbilical midline incision.

In a prospective study at a teaching hospital in Ethiopia among patients with abdominal surgical wounds, pathogenic organisms were isolated in 38.7% of patients, however on clinical grounds alone wound infection rate was 21% (Kotisso Aseffa 1991). This finding calls for the need to utilize laboratory techniques to confirm diagnosis of potentially infected wounds. Wound infection was significantly associated with class of wound, with highest rate being 64.1% for contaminated and dirty wound, and no difference in infection rate was observed between emergency and elective operations (Kotisso and Aseffa 1991).

A study at a district hospital in Kenya among women delivered by caesarian section reported high incidence rate (19%) of SSI, which was higher than that reported in Nigeria. The incidence was higher in single women (32%) when compared with married women (16%), but the difference was not statistically significant (Koigi-Kamau *et al*., 2005). Another study in Kampala, Uganda, called out among surgical patients reported a high SSI rate of 9.64% in pre-

intervention phase in which no antimicrobial prophylaxis (AMP) was administered, compared to 2.56% in intervention phase in which AMP was administered preoperatively (Tiberi *et al*.,2010). A similar study also done in Bugando Medical Centre (BMC), Mwanza observed a high rate of SSIs at 26%, of who 86.2% and 13.8% had superficial and deep SSIs respectively (Mawalla *et al.,* 2011). This rate was higher than that reported before, indicating increasing rate of surgical site infections among patients undergoing operations in this nation. In most SSIs, the responsible pathogens originate from the patient's endogenous flora. The most commonly isolated organisms were *S. aureus,* coagulase-negative staphylococci, *Enterococcus* spp. and *Escherichia coli*; however, the pathogens isolated depend on the procedure involved and increasing number of SSIs are attributable to antibiotic-resistant pathogens such as methicillin-resistant *S. aureus* (MRSA) (ManGram *et al*.,1999).

This may reflect the increasing number of severely ill or immunocompromised surgical patients, and the widespread use of broad-spectrum antibiotics. Pathogens may also originate from preoperative infections at sites remote from the operative site, particularly in patients undergoing insertion of prosthesis or other implant. In addition to the patient's endogenous flora, SSI pathogens may originate from exogenous sources such as members of the surgical team, the operating theatre environment, instruments and materials brought within the sterile field with attention to multiple patient-related and procedure-related risk factors. Several studies in a variety of clinical settings have shown that such approaches can produce significant reductions in SSI rates during follow-up periods of up to two years (Dellingner, 2005). In a prospective survey done in Central African Republic among orthopedic surgical patients it was found that methicilin-susceptible *S. aureus* was the most frequent species isolated followed by Enterobacteriaceae and *P. aeruginosa.* A strain of *E. cloacae* harbouring extended spectrum beta lactamase (ESBLs) was also isolated (Bercion *et al.,* 2007). Frequent isolation of *S. aureus*

(28.8%) and *Escherichia coli* (27.1%) have also been reported among patients with abdominal surgical wounds in Ethiopia (Kotisso and Aseffa 1991).

Surgical wounds sites with high bacterial contaminants constitute a serious problem in the hospital especially in surgical practice where clean operations can become contaminated and subsequently infected. The degree to which surface wounds are infected by surrounding bacteria contaminants have become clinically important (Taiwo *et al.,* 2002). In abdominal surgeries, the opening of the gastrointestinal tract increases the likelihood of coliforms and Gram negative bacilli as agents of wound infection. These groups of organisms tend to be endemic in hospital environment by being easily transferred from object to object and they also tend to be resistant to common antiseptics, often difficult to eradicate in the long term and Enterobacteriaceae are increasingly playing a greater role in the many hospital acquired infections (Mofikoya *et al.,* 2009). Surgical site infections are among the most common hospital acquired infections comprising 14-16% of inpatient infections (Ntsama, 2013). A survey sponsored by Who Health Organization demonstrated a prevalence of nosocomial infections varying from 3-21% with surgical site infections accounting for 5-34% (WHO, 2011).

2.5 Pathogenesis

Microbial contamination of the surgical site is a necessary precursor of SSI. The risk of SSI can be conceptualized according to the following relationship, exogenous and endogenous sources.

2.5.1 Endogenic Sources.

Microorganisms may contain or produce toxins and other substances that increase their ability to invade a host, produce damage within the host, or survive on or in host tissue. For example, many Gram-negative bacteria produce endotoxin, which stimulates cytokine production. In turn, cytokines can trigger the systemic inflammatory response syndrome that sometimes leads to

multiple system organ failure. Certain strains of Clostridia and Streptococci produce potent exotoxins that disrupt cell membranes or alter cellular metabolism (ManGram *et al.,* 1999). For most SSIs, the source of pathogens is the endogenous flora of the patient's skin, mucous membranes, or hollow viscera. When mucous membranes or skin is incised, the exposed tissues are at risk for contamination with endogenous flora. These organisms are usually aerobic Gram-positive cocci (e.g., *Staphylococci*), but may include fecal flora (e.g., anaerobic bacteria and Gram negative aerobes) when incisions are made near the perineum or groin. When a gastrointestinal organ is opened during an operation and is the source of pathogens, Gram negative bacilli (*E.coli*), Gram-positive organisms (e.g., *Enterococci*), and sometimes anaerobes (*Bacillus fragilis*) are the typical SSI isolates (ManGram, *et al*., 1999).

2.5.2 Exogenic Sources

These surgical personnel (especially members of the surgical team), the operating room environment (including air), and all tools, instruments, and materials brought to the sterile field during an operation. Exogenous flora is primarily aerobes, especially Gram-positive organisms (e.g. *Staphylococci* and *Streptococci*) (ManGram, *et al* 1999).

2.6 Risk Factors for Surgical Site Infections (SSIs)

There are four main factors which influence the infection rates in surgical wounds, they include Patient variables, Preoperative preparation, Operative procedure and Postoperative care (Flanagan 1997).

2.6.1 Patient Variables Diseases:

The contribution of diabetes to SSI risk is controversial, because the independent contribution of diabetes to SSI risk has not typically been assessed after controlling for potential confounding factors. Also, increased glucose levels in the immediate postoperative period were associated

with increased SSI risk. More studies are needed to assess the efficiency of perioperative blood glucose control as a preventive measure. Other diseases are like cancer of liver and kidney or lung conditions that may slow the healing process. Medical condition, such as low blood protein may also affect healing (Flanagan 1997).

Hyperglycemia and hypoglycemia:

Elevated blood sugar concentration impaired the function of phagocytic cells in experimental studies. Constant checking of blood sugar levels for patients with diabetes is important to maintain the blood sugar at a constant level. Intra-operative and postoperative blood sugar control remains a logistical problem. Furthermore, the ideal blood sugar level remains undefined. It generally is agreed that maintaining euglycemia (i.e., normal blood glucose concentration) for the patient is desirable. Future developments in real-time, on-line measurement of blood glucose will allow this problem to be resolved (Donald, 2007).

Weak Immune System:

The Immune system is the part of the body that fights infection. For some type of operation, severe protein-calorie malnutrition is crudely associated with postoperative nosocomial infection, impaired wound healing. The immune system may be weakened by radiation, poor nutrition, certain medications (anti-cancer medicines or steroids). Weight and age may also decrease the ability to respond to injury (Beaver, 2008).

2.6.2 Preoperative care

Prolonged Hospital Stay

Prolonged perioperative hospital stay is frequently suggested as a patient characteristic associated with increased SSI risk. However, the length perioperative stay is a likely surrogate for severity of illness conditions requiring inpatient work-up and therapy before the surgery.

Perioperative Transfusion

Mangram and co-workers (1999) states that, it has been reported that perioperative transfusion of leukocyte- containing allogeneic blood components is an apparent risk factor for the development of postoperative bacterial infection including SSI. However, there is currently no scientific basis for withholding necessary blood transfusion from surgical patients as a means of either incisional or organ/ space SSI risk reduction.

2.6.3 Operative procedure

When a surgery has to be done on an infected wound, the chances of SSI are increased. An emergency surgery on traumatic injuries and over 3 hour's surgery also increases the risks of SSI. It may also include surgeries also done on certain body organs, such as the stomach or intestines (bowels). The risk may be greater if an object pierced through the skin and into an organ. SSI is likely to occur after an open surgery than a laparoscopy surgery. Drains and blood transfusion may increase the chance of bacteria reaching the wound causing infection (Surgical Site Infection, 2008).

Foreign Objects

Patients involved in an accident, usually some foreign objects, such as glass or metal or dead tissues present in the wound may delay wound healing. It's also possible to have SSI if there is an infection on another part of the body or a skin disease (Donald, 2007).

2.6.4 Post Operative Care:

According to Mangram and co-workers (1999), the type of postoperative incision care is determined by whether the incision is closed primarily, left open to be closed later, or left open to heal by second intension. When the wound is closed primarily, it is covered by sterile dressing for at least 24-48 hours therefore reducing the chances of infection. When the surgical incision is left open for a few days before it is closed (delayed primary closure), it is likely for the site to be infected or patient condition may prevent primary closure, (e.g. edema at the site). When a surgical incision is left to heal by second intention, it is packed with sterile moist gauzes and covered with sterile dressing. It also recommended that when changing the dressings, it is appropriate to use sterile gloves to reduce the chances of infection. Nicotine use delays the primary wound healing also increasing the risk of SSI, at the same time patients receiving steroids or other immunosuppressive drugs perioperative may be predisposed to developing SSI. Even though nicotine is found to be a reason among other patients to be the cause of surgical site infection, it's not 100% proven to have relations with causes of SSI (Mangram *et al* 1999)

Asepsis:

There are several aseptic agents available for preoperative preparation of the skin at the incision site. Alcohol is considered to be the most available, inexpensive and the most rapid-acting skin antiseptic. Before the skin is prepared, it should be free of gross contaminations (i.e. soil or dirt). The skin is prepared by applying an antiseptic in concentric circles. The prepared area should be large enough to extend the incision or create new incisions or drain site if necessary.

Mobile phones:

Mobile phones have been the source of communication within the hospital. According to a recent research by (Ulger, *et al.,* 2009) hospital operating rooms and intensive care units (ICU) are the workplaces that need highest standard of hygiene, also the same requirements for the personnel working there and the equipment used by them. Risk of infection involved in using mobile phones has not yet been determined there are no cleaning guidelines available that meet hospital standards. However, mobile phones are used routinely all day long but not cleaned properly as health care workers may/do wash their hands as often as they should. They found out that health care workers hands and their mobile phones were contaminated with various types of microorganisms. Mobile phones used by healthcare workers may be the source of nosocomial infections in hospitals.

2.7 BACTERIA ISOLATES ASSOCIATED WITH SURGICAL WOUND INFECTIONS

The bacteria isolates often incriminated in surgical wound infections are *Pseudomonas aeruginosa, Staphylococcus, Klebsiella, Proteus* species, *and Escherichia coli* as well as anaerobes such as *Clostridium* and *Bacteroides*. It has been reported that *Pseudomonas aeruginosa, Staphylococcus* and *Klebsiella* are the most commonly isolated Pathogens in surgical wounds of patients attending the Ogun State Teaching Hospital, Nigeria. These bacteria pathogens have gained prominence in SSI due to their increased resistance to commonly used antibiotic drugs (Sule *et al.,* 2002). Multiple drug resistance (MDR) is defined as antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. Many different bacteria now exhibit multi-drug resistance, including staphylococci, enterococci, gonococci, streptococci, salmonella, as well as numerous other gram-negative bacteria and *Mycobacterium tuberculosis*. Antibiotic resistant bacteria are able to transfer copies of DNA that code for a

mechanism of resistance to other bacteria even distantly related to them, which then are also able to pass on the resistance genes and so generations of antibiotics resistant bacteria are produced (Hussain, 2015).

2.7.1 *Pseudomonas aeruginosa*

P. aeruginosa is a non-fermentative Gram negative bacteria widely distributed in nature and can survive on a wide variety of surfaces and in hospital environment, as the wards encourage bacterial growth (Arora *et al.,* 2011). It is oxidase positive, aerobic rod which can grow on many types of media (such as Blood Agar, Nutrient Agar, and MacConkey Agar) with a characteristic grape like odour. Some strains can haemolyse blood. Some strains of *P. aeruginosa* are known for their ability to produce a bluish green pigments, pyocyanin which diffuses into the agar media; others can produce pyoverdin a fluorescent pigment. *P.aeruginosa* grows well at 37-42^{0}C, its growth at 42^{0}C differentiate it from other members of the fluorescent group. It is able to switch to its dinitrification pathway under unfavourable conditions such as when placed under anaerobic condition in a nitrogen rich environment (Jawetz *et al.,* 2007). *P. aeruginosa* produces a variety of extracellular enzymes which contribute to its pathogenesis. Some of the enzymes produced by *P. aeruginosa* include alkaline protease, elastase, exotoxin A, exotoxin S and hemolysin. These enzymes are usually produced during the course of a clinical infection and contribute to the development of infections in animal models. The proteolytic enzymes help to breakdown physical barriers of the host and hemolysins lyses cells from varied so*urces.* The expression of these exoproducts does not occur until the late logarithmic phase of growth when the cell density is higher. These enzymes production occur through a phenomenon called quorum sensing that is involved in the activation of genes at high cell densities in response to chemical signals released by *P. aeruginosa*. Other virulence factors include Pilus expression and alginate production. The pili from *P. aeruginosa* are associated with adhesion and binding of GM_1

receptors on the surface of cystic fibrosis epithelial cells. The pilus genes responsible for these processes are all located in the bacterial chromosome and DNA sequence revealed the presence of three open reading frames designated as PilB, PilC and PilD that encode proteins of 62, 38, and 32 KDa, respectively. Alginate is a viscous exopolysaccharide consisting of D-manronic and L-gluronic acids. It is synthesized in response to environmental condition and prevents both opsonic and non opsonic phagocytosis, protecting the bacterium cell from the host immune response. Pyocyanine, a blue pigmented phenazine derivative produced by *P. aeruginosa* is known to play a role in virulence thereby enhancing pathogenesis of the bacteria and it is also known to have inhibitory and bactericidal effects. It is produced from the chorismic acid via the phenazine pathway where nine proteins encoded by a gene cluster phenazine – 1 – carboxylic acid are involved. The initial phenazine formed is converted to pyocyanin in two steps that are catalyzed by the enzymes PhzM and PhzS. Non production of pyocyanin may be due to lack of these phenazine genes (Jamileh *et al.,* 2012). PhzM is an adenosylmethionine dependent methyltransferase and PhzS is a flavin dependent hydroxylase. It has been shown that PhzM is active only in the presence of PhzS, suggesting that a protein – protein interaction is involved in pyocyanin formation. Such a complex would prevent the release of 5 methylphenazine – 1 – carboxylate, the putative intermediate and an apparently unstable compound (Greenhagen *et al.,* 2008). *Pseudomonas aeruginosa* is an important opportunistic pathogen, which is highly resistant to antibiotic therapy. Flouroquinolones, β-lactam, and amino glycosides are among the primary agents available for treatment of infections caused by this pathogen. Multidrug-resistant (MDR) phenotype is defined as resistant to one anti-microbial agent in three or more anti-pseudomonal anti-microbial classes (carbapenems, flouroquinolones, penicillins /cephalosporins and aminoglycosides) (Magiorakos *et al.,* 2011). *Pseudomonas aeruginosa* pathogenesis is regulated by the mex AB-OprM efflux system (Rahmatic *et al.,* 2002). The effects can however,

be counter balanced by the reduced fitness that may occur as a consequence of the extrusion of bacterial metabolites by a decreased intracellular concentration of auto inducing virulence factors. In *Pseudomonas aeruginosa,* four FQ-MDR efflux systems have been described to date. Two of these, MexEF-OprM and MxXY -OprM contribute significantly to intrinsic drug resistance of this organism; they are expressed constitively in wild type cells cultivated under usual laboratory condition (Zyurisk *et al*., 2000). These two efflux systems and two additional ones mexCD –OprJ and mexEF-OprM,mediate acquired MDR in *pseudomonas aeruginosa*, so these two system is quiescent in wild-type cells under the usual laboratory growth condition, that lead to MDR (Kohler *et al*.,1997). In case of *Pseudomonas aeruginosa,* resistance to all flouroquinolones tested show that, there is expression of the MexEF-OprM, MexCD-Oprl and MexEF-OprM deficient strains show hypersusceptibility to the flouroquinolones. It is unfortunate that resistance to FQs has increased in a number of Gram- negative organisms, most notably in *Pseudomonas aeruginosa*. Resistance is due usually to mutation in the genes for the bacterial targets of the FQs (DNA gyrase (gyrA) and topoisomerase lV (ParC) or to active efflux of the agents via antibiotic efflux pump (Kohler *et al*., 1997). *P. aeruginosa* develops resistance by various mechanisms like multidrug resistance efflux pumps, biofilm formation, production of β-lactamase and aminoglycoside modifying enzymes (Ahmed *et al*., 2013). The risk for acquiring MDR organisms is most likely to be related to the number of carriers in the same ward as well as to individual risk factors, such as patient characteristics and in-hospital events (invasive devices and antibiotic treatment (Carmeli *et al*., 2002). Although Köhler *et al*., (1997) demonstrated that loss of OprD is the first mechanism of resistance, and such mutants are resistant only to zwitterionic carbapenem antibiotics, plasmid mediated resistance to various antimicrobial drugs have been demonstrated by various workers (Shadid *et al*., 2003; Shadid and Malik, 2004; Olayinka *et al*., 2009; Yimaz *et al*., 2011). Extended spectrum beta lactamases

(ESβLs) have been described in *P. aeruginosa* only recently. β lactamases described in *P. aeruginosa* belong to various families as TEM and SHV types which are common among Enterobacteriaceae, PER type, VEB type which have been reported from various parts of the world (Amutha *et al*., 2009). A high rate of spread of resistant gene has been suspected as the cause of increased antibiotic resistance cases. Plasmid carry genes that could be spread by conjugation and transduction while the genome based resistant genes are also spread by replication. Intrinsic and acquired antibiotic resistance makes *P. aeruginosa* one of the most difficult organisms to treat. The high intrinsic antibiotic resistance of *P. aeruginosa* is due to several mechanisms: low outer membrane permeability, the production of an AmpC β-lactamase and the presence of numerous genes coding for different multidrug resistance efflux pumps (Livermore, 2006). Another study from the north east reported the occurrence of *Pseudomonas aeruginosa* in urine samples to be 4.6% (Jombo *et al*., 2008). According to the studies carried out by Wariso and lbe (2006), and Smith *et al*., (2012). Results obtain from south west *Pseudomonas aeruginosa* had 39.3% in wound swabs, 41.9% in ear swabs. Despite improvements in antibiotic therapy *Pseudomonas aeruginosa* is frequently resistant intrinsically to a number of antimicrobial agents including multiple classes of antimicrobial agents. Subsequently outbreaks due to multi resistant *P. aeruginosa* have been reported in various nosocomial settings, such as intensive care units (ICUs). *P. aeruginosa* adaptive ability causes difficulties for the sensitivity of microbial identification methods and it has become necessary to develop genotype-based characterization systems capable of accurately identifying these bacteria despite any phenotypic modifications. So molecular identification eliminates the problem of variable phenotype and allows for more accurate identification of bacteria (Drancourt *et al*., 2000). However, 16S rDNA genes are highly conserved among all organisms and they possess various unique species-specific regions that allow for bacterial identification. Polymerase chain

reaction (PCR) is a highly sensitive, specific and rapid method which vastly improved the detection of *P. aeruginosa* especially when using species-specific primer for 16SrDNA (Spilker *et al*., 2004). The resistance usually depends on expression of *bla* genes belonging to the interlaid *bla* TEM, *bla* SHV, and b*la* CTX -M genes family. The *bla* TEM, b*la* SHV, and *bla* CTX -M genes are responsible for production of TEM β-lactamase, SHV β-lactamase, and CTX-M β-lactamase, large families of enzymes with evolutionary affinity. Since the first TEM-1 β-lactamase was discovered, one hundred eighty-five new β-lactamase of the TEM family have been reported worldwide, whereas ninety-three variants are responsible for production of ESBLs. Among one hundred seventy-two enzyme types of the SHV family, forty-five have been reported as extended-spectrum β-lactamase (Al-Jassera, 2006). It is known that bla genes encoding antibiotic resistance may be placed on transferable elements such as plasmids or transposons. This localization of bla genes can facilitate a horizontal spreading of antibiotic resistance among bacterial strains (El-Salabi *et al*., 2012). In Turkey, Unan *et al.,* (2000) reported that 60% of his *P. aeruginosa* isolates were MDR. In Egypt, Gad *et al.,* (2007) observed high levels of MDR *P. aeruginosa* and that β-lactamase production is the main mechanism of resistance (36% were MDR and 95% were ESβLs producer) On the other hand, Zahra (2011) in Iran detected lower levels as 30% of their isolates were MDR and only 9.2% were ESβLs producer. Egypt is among the countries that reported high rates of antimicrobial resistance (El-Kholy *et al.,* 2003). MDR *P. aeruginosa* develops resistance by various mechanisms like multi-drug resistance efflux pumps, production of β-lactamase, aminoglycoside modifying enzymes, and decrease outer membrane permeability. Earlier studies reported that Imipenem was the most effective antibiotic against *P. aeruginosa* (Gales *et al*., 2002). However, recent studies demonstrated the evolution of Imipenem -resistant strains of *P. aeruginosa*. Carbapenem were considered to be the drug of choice against serious ESβLs associated infections; however resistance to Carbapenem,

especially in *P. aeruginosa,* results from reduced levels of drug accumulation, increased expression of pump efflux or production of β-lactamase. The study revealed moderate activity of ciprofloxacin 43.6%. There were variable data about ciprofloxacin action .Similar results about ciprofloxacin against *P. aeruginosa* were detected by Gad *et al.,* (2007) and Zahra (2011). Typing techniques such as PCR have been found to be useful for epidemiological study of *Pseudomonas aeruginosa* (Smith *et al.,* 2012).

2.7.2 The *Staphylococci*

The *Staphylococci* belong to the family *Micrococcaceae* and are broadly divided into two main categories of clinical importance: *Staphylococcus aureus,* which are coagulase positive; and a heterogeneous group of staphylococci that give a negative reaction with coagulase test. *S. aureus* is characteristically associated with acute pyogenic infections whereas CoNS cause infections in susceptible hosts with certain predisposing conditions. The most common species of CoNS that causes infection is *Staphylococcus epidermidis*. Besides the coagulase test, *S. epidermidis* differs from *S. aureus* in being negative for mannitol fermentation reaction and deoxyribonuclease test (Waldvogel, 2000). The cell wall of *S. aureus* comprises mainly of peptidoglycan layer whose function is to provide a rigid envelope for the cell content. The peptidoglycan chains are cross-linked by tetrapeptide chains bound to N-acetylmuramic acid and by a pentaglycine bridge specific for *S. aureus*. Peptidoglycan also has endotoxin properties, and has been reported to cause organ dysfunctions in experimental animals. Most *S. aureus* strains produce a slimy extracellular capsular polysaccharide. A total of eight capsular serotypes have been described, and serotypes 5 and 8 are predominant in isolates found in humans (O'Riordan and Lee, 2004). Capsules enhance microbial virulence by rendering the bacterium resistant to phagocytosis resulting in bacterial persistence in the bloodstream of infected hosts. Animal studies suggest that it also promotes bacterial colonization

and persistence on mucosal surfaces (O'Riordan and Lee, 2004). *S. aureus* produces a wide variety of exoproteins, most of them during the post exponential growth phase. These proteins degrade the host tissue to nutrients required for the growth of bacteria, and allow the bacteria to penetrate deeper into the host tissue. The majority of strains produce hemolysins, nucleases, proteases, lipases, hyaluronidase, and collagenase. Alpha-hemolysin (or alphatoxin) is dermonecrotic, neurotoxin, and lyses mammalian erythrocytes by forming a pore in the target membrane fatty acids. It is also able to stimulate apoptosis in lymphocytes (Dinges *et al*., 2000). Beta-hemolysin acts as sphingomyelinase, gamma-hemolysin has leucocytolytic activity, and it has been suggested that delta-hemolysin has surfactant or channel forming properties (Dinges *et al.*, 2000). Lipases act on the host immune response by inactivating the fatty acids that are intended to disrupt the bacteria. Proteases are involved in the inactivation of host defence peptides and also block antibodies. Staphylokinase is a plasminogen activator (Lahteenmaki *et al.*, 2001). Hyaluronidase digests hyaluronic acid present in the skin, bone, umbilical cord, vitreous body of the eye, and synovial fluid. *S. aureus* produces an extracellular protein known as coagulase. This protein protects the bacteria from the host defense by clotting fibrin around a focal infection. *S.aureus* is known to express a fatty acid modifying enzyme (FAME)which may be associated with abscesses where it could modify antibacterial lipids and prolong bacterial survival (Foster, 1996). Some *S. aureus* strains also produce additional exoproteins, which may have the evasion of host defense as their major function in vivo. *Staphylococcus aureus* is both a commensal organism and a pathogen. Approximately 30% of healthy individuals are colonized by *S. aureus* usually in the anterior nares, vagina and perinea area. Infection occurs when the organism is inoculated into the skin from a site of carriage when host defenses are breached whether by shaving, aspiration, insertion of an indwelling catheter or surgery. Colonization increases the risk of infection and allows *S. aureus* to be transmitted in

both hospital and community settings. The bacterial components and secreted products that affect the pathogenesis of *S. aureus* infections are numerous and include surface-associated adhesions, a capsular polysaccharide, exoenzymes, and serotoxins. This constellation of bacterial products allows *staphylococci* to adhere to eukaryotic membranes, resist opsonophagocytosis, lyses eukaryotic cells, and triggers the production of a cascade of host immunomodulating molecules (O'Riordan and Lee, 2004). In establishing an infection, *S. aureus* has numerous surface proteins called 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs) that mediate adherence to host tissues (Gordon and Lowy, 2008). The adherence of *S. aureus* to host tissue is an important step in pathogenesis as well as in colonization. Surface proteins such as protein A, clumping factors, fibronectin-binding proteins, and collagen-binding proteins can adhere to extracellular matrix components of the host (Foster and Hook, 1998). The main 16 function of protein A; is to bind the IgG Fc-domaine. Once *S. aureus* adheres to host tissues or prosthetic materials, it is able to grow and persist in various ways. *S. aureus* can form biofilms (slime) on host and prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials (Donlan and Costerton, 2002). In vitro studies have shown that *S. aureus* can invade and survive inside epithelial cells, including endothelial cells, which theoretically may also allows it to escape host defenses, particularly in endocarditic (Ogawa *et al*., 1985; Moreillon *et al.*, 2002). *S. aureus* is able to form small-colony variants (SCVs) and this may contribute to persistent and recurrent infection. In vitro, small-colony variants are able to "hide" in host cells without causing significant host-cell damage and are relatively protected from antibiotics and host defences. They can later revert to the more virulent wild-type phenotype which possibly results in recurrent infection (Gordon and Lowy, 2008). Small-colony variants are slowly growing organisms that exhibit a small, non-pigmented, non-hemolytic colony morphotype. They are usually dependent on various substrates (mainly thymidine, haemin and

menadione) supplementation for growth and are more resistant to antibiotics such as aminoglycosides and co-trimoxazole (Proctor *et al*., 2006). *S. aureus* has many other characteristics that help it evade the host immune system during an infection. Its main defense is production of an antiphagocytic microcapsule. Most human infections are due to capsular types 5 and 8, with a vast majority of MRSA isolates possessing capsular type 5 (Robbins *et al*., 2004). Together with intercellular polysaccharide adhesins, *S. aureus* capsular polysaccharides enhance biofilm constitution by augmenting adhesiveness. *S. aureus* has two main components in its cell wall, namely lipoteichoic acid and peptidoglycan. The hydrophobic domain of lipoteichoic acid plays a role in adherence, whereas peptidoglycan covalently links adhesive proteins (Lowy, 1998, Cheung *et al*., 2002). The zwitterionic capsule is also able to induce abscess formation (O'Riordan and Lee, 2004). The MSCRAMM protein A binds the Fc portion of 17 immunoglobulin and this may prevent opsonization. *S. aureus* is able to secrete chemotaxis inhibitory protein of *staphylococci* or the extracellular adherence protein, which interfere with neutrophil extravasation and chemotaxis to the site of infection. In addition, S. aureus produces leukocidins which cause leukocyte destruction by the formation of pores in the cell membrane (Gordon and Lowy, 2008). During infection, *S. aureus* produces numerous enzymes, such as proteases, lipases, and elastases, which enable it to invade and destroy host tissues and metastasize to other sites. The capacity to invade endovascular tissue also favors spread to other tissues. Apart from evasion of host immune defense, bacterial survival within the human host is dependent on successful acquisition of nutrients, particularly iron. During infection, 95% of iron is sequestered within host cells and serum iron is mostly bound to host proteins that are not easily accessed. *S. aureus* secretes high affinity iron-binding compounds (aureochelin and staphyloferrin) to make iron available for its use (Liu, 2009). Once in the blood, the organism

spreads widely to peripheral sites in distant organs causing septic shock. *S. aureus* causes septic shock by interacting with and activating the host immune system and coagulation pathways. Peptidoglycan, lipoteichoic acid, and α-toxin are known to play a part in the initiation of sepsis (Lowy, 1998). Invasion of the bloodstream results in a number of specific staphylococcal infections such as endocarditis, osteomyelitis, renal carbuncle, septic arthritis, or epidural abscess (Archer, 1998). Production of superantigens results in various toxinoses, such as food poisoning, scalded skin syndrome and toxic shock syndrome. Some strains are able to produce epidermolysins or exfoliative toxins which cause scalded skin syndrome or bullous impetigo (Gordon and Lowy, 2008). It is the most common cause of hospital-acquired infection, causes clinical disease in 2% of all patient admissions and is becoming increasingly resistant to antibiotics. In several industrialized nations, including parts of Europe, the USA and Japan, 40–60% of all hospital *S. aureus* are now resistant to methicillin (methicillin-resistant *Staphylococcus aureus*; MRSA) (Fluit *et al.,* 2001). *S. aureus* is associated with a variety of systemic infections such as septicemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and post-surgical toxic shock syndrome with substantial rates of morbidity and mortality (Shittu *et al.,* 2006). Hospitalized patients are particularly susceptible to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections (Lindsay and Holden, 2004). Phenotypic and genotypic methods have been of great advantage in classifying epidemiologically related strains. Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for MRSA phenotyping and for identifying specific clones (Amorim *et al.,* 2007). Surveillance data have shown that in hospital settings methicillin sensitive staphylococcus *aureus* (MSSA) tends to evolve into methicillin resistant staphylococcus *aureus* (MRSA). Globally the prevalence of MRSA is progressively increasing with significant regional variation

(Diekema *et al.*, 2001). Data in most African countries are scarce; in few surveys the prevalence of MRSA has been found to range from as low as 2% to as high as 41% (Kesah *et al.*,2003; Shittu and Lin 2006; Mawella, 2011 and Truong,2010).

In Uganda, previous studies at Mulago National Hospital showed that 28.7% of the SSI is due to *S. aureus*, and of these 31.5% are due to MRSA (Ojulong, 2009). The prevalence of MRSA in the study was low compared to a previous report of 20.23% in Southwestern Nigeria (Ghebremehin *et al.*, 2009). However an earlier multicenter study in Southwestern Nigeria reported 1.4% (Adesida *et al*., 2005). The difference in prevalence of MRSA in the three studies could be associated with the size of the study population (Ghebremehin *et al.*, 2009), the study population was significantly higher than that of Adesida *et al*.,(2005) and the present study. Despite the low MRSA rate reported in the study, it highlights the occurrence of multi-resistant MSSA in South Western Nigeria. Methicillin-resistant staphylococci are resistant to all other penicillins, carbapenems, cephems and beta-lactam/beta-lactamase inhibitor combinations (Clinical and Laboratory Standards Institute, 2006). It is therefore advisable that these antibiotics should not be used for treating of methicillin-resistant staphylococci infections. The antimicrobial susceptibility pattern showed that all the MRSA strains were resistant to at least ten antibiotics including Penicillins, penicillin/beta-lactamase inhibitor combinations, Oxacillin, Cephalosporins and 145 Carbapenem. Flouroquinolones were initially introduced for the treatment of Gram-negative bacterial infections but they have also been used to treat bacterial infections caused by Pneumococci and staphylococci (Lowy, 2003). The primary target of Quinolones is bacterial DNA gyrase, without which DNA replication is inhibited, quinolone resistance is as a result of spontaneous chromosomal mutations. These mutations are in the quinolone- resistance-determining region of the enzyme–DNA complex, reducing the affinity of quinolone for its targets (DNA gyrase and

topoisomerase IV). An additional mechanism of resistance in *S. aureus* is induction of the NorA multidrug resistance efflux pump. Increased expression of this pump in *S. aureus* can result in low-level Quinolones resistance (Waldvogel, 2000; Lowy, 2003). In vitro passage of both flouroquinolones-susceptible MSSA and MRSA in the presence of either ciprofloxacin or levofloxacin is associated with the frequent selection of clones resistant to these antibiotics (Limoncu *et al.,* 2003). Flouroquinolones use has also been associated with an increased risk of nosocomial acquisition of MRSA (but not of MSSA) (Weber *et al.*, 2011). The flouroquinolones with C8 substitutions, such as gatifloxacin and moxifloxacin, appear to be more potent against *S. aureus* than are older drugs of this class, and they may be less likely to select resistant mutants, an effect that may be strengthened by the addition of rifampin (Deresinski, 2005). The accurate detection of beta-lactam and mecA-mediated resistance in *S. aureus* is essential for the treatment of overt infections and the implementation of infection control practices. Resistance to penicillin in *S. aureus* is mediated by production of a Penicillinase, encoded by blaZ gene. About 95% of the *S. aureus* strains in this study harboured the blaZ gene. Reliable detection of Penicillinase production is important because penicillin is considered to be superior to Oxacillin against isolates that do not produce a Penicillinase. An erroneous report of penicillin susceptibility could result in potentially inadequate therapy of *S. aureus* infections (Kaase *et al.,* 2008). MRSA isolates are predominantly multi-drug resistant organism (i.e. resistant to three or more antimicrobial classes) compared to MSSA, with significant difference in Oxacillin among MRSA isolates respectively and MSSA. Confirmation of MRSA was based on PCR-detection of the *mecA* gene (usually the gold standard) through amplification and identification of a 162 bp amplicon upon agarose gel electrophoresis. The PCR program comprised of initial denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 53°C for 30 seconds, extension at 72°C for 45 seconds,

and a final extension at 72°C for 10 minutes. The amplicons were analyzed by electrophoresis on a 2% agarose gel (McClure *et al*., 2006).

2.7.3. *Escherichi.coli*

The *E. coli* genome is composed of a conserved core of genes that provides the backbone of genetic information required for essential cellular processes and a flexible gene pool that harbors genetic information which provides properties (e.g. virulence and fitness genes) that enables the bacterium to adapt to special environmental conditions (Dobrindt, 2005). The pathogenic ability of *E.coli* is largely afforded by the flexible gene pool through the gain and loss of genetic material. Virulence factors involve mechanisms that enable pathogenic bacteria to cause infections and the presence of several putative virulence genes has been positively linked with the pathogenicity of *E. coli* (Ulett, *et al.,* 2013). Antibiotics resistance of *E.coli* has become a major clinical concern worldwide. Recently, the use of second and third generation cephalosporins has led to the selection of Gram-negative organisms like *E.coli* resistance to *Beta*-lactamase (ESBL) stable cephalosporins. This resistance is attributed to the production of Extended spectrum Beta lactamases. Extended spectrum Beta-lactamases are enzymes that mediate resistance to extended spectrum (third generation) cephalosporins such as ceftazidime, Ceftriazone, cefotaxime and monobactam, i.e. aztreonam but do not affect cephamycins i.e.cefoxitin and of Carbapenems i.e. Meropenem or Imipenem (Rahamn *et al.,* 2004). Resistance pattern of antibiotics to microbial flora of surgical site infection revealed in the past study show that most of *the E. coli* isolates were resistance to at least three drugs and maximum isolates were resistance to 6 to 7 drugs where as some of them were found to be resistance to all drugs. Similarly Shamsuzzaman *et al.,* (2007), observed a gradual increase in resistance pattern of *E. coli* in different years against almost all the antibiotics except

Imipenem. The presence of ESBL producing *E. coli* stains in their study was 43.75%. In Bangladesh, a study by Rahman *et al.,* (2004) showed 43.21% E. coli ESBL producer and Alim *et al.,* (2009), found 41.39% ESBL producing organisms. So, the rates of ESBL producing *E. coli* in the three studies in Bangladesh are almost similar. Since antibiotic resistance is a major phenotypic trait particularly for the clinical isolates, it has a potential interest in exploring the characteristic of these ESBL producing isolates of *E. coli*. A study carried out by Pitout *et al.,* (1998), revealed that susceptibility test results of ESBL producers isolates were resistant. The susceptibility test results showed that all the ESBL producing isolates were resistant to 3rd generation Cephalosporin, This reflect the relationship between the ESBL and 3rd generation cephalosporins. Increased resistance might be due to extensive use of 3rd generation cephalosporins and other Beta-lactam drugs. This finding justifies that ESBLs producing *E.coli* are multidrug resistance. Infection caused by ESBL producing organisms have currently been treated with carbapenems such as Imipenem. Considering the strains of Gram negative bacteria, *E.coli* is mostly isolated from wound infection. Higher prevalence of *E. coli* in the past study might be due to its frequent presence in hospital environment from where study cases were selected and in most surgical site wounds *E. coli* is usually the predominant organism (Zafar, 1999).

2.7.4 *Klebsiella*

Klebsiella causes many types of infections; therefore the clinical symptoms only cannot be used during diagnosis. Culturing and identification are usually required for effective laboratory investigation and diagnosis. This organism has simple nutritional requirements and grows well on basic media commonly used for members of the Enterobacteriaceae making its isolation easy (Virella, 1997). Clinical specimens are plated for isolation on blood agar and a differential medium such as MacConkey or CLED (cysteine-lactose and electrolyte-deficient agar) (Alves *et*

al., 2006). *Klebsiella* species are strong lactose-fermenters (Virella, 1997). They therefore produce characteristic pigmented, large, mucoid colonies on lactose enriched media (Virella, 1997). *Klebsiella* species are Gram negative, facultative anaerobic, non-spore- forming rods, which are non motile. They are negative to oxidase test, but are able to reduce nitrates to nitrite. Klebsiellae cause a variety of infections in humans. These include pneumonia, urinary tract infections (UTI), rhinoscleromatis, and other soft tissue infections (Obiamiwe, 2006). All *Klebsiella* species ferment glucose, but fermentation of other carbohydrates varies. Lactose usually is fermented rapidly by most *Klebsiella* species. Some species of *Klebsiella* are positive to urease reaction, but they do so more slowly. Also with the exception of *K. pneumoniae, K. rhinoscleromatis* and *K. ozaenae* all the other known species are indole positive (Alves *et al*., 2006). *K ozaenae* is negative to malonate utilization test (Farmer, 1999). A battery of tests for biochemical properties is required to identify *Klebsiella* to the species level; these include indole test, methyl red test and malonate utilization test (Villera, 1997). They are usually opportunistic pathogens found in the environment and in mammalian mucosal surfaces, with the principal pathogenic reservoirs being the gastrointestinal tract of patients and the hands of hospital personnel (Podschun and Ullmann, 1998). *Klebsiella pneumoniae* and *K. oxytoca* are the commonest species responsible for human related *Klebsiella* infections, with *K. pneumoniae* accounting for 75 to 85% of *Klebsiella* infections reported worldwide (Farmer, 1999). *Klebsiella pneumonia* is very fatal, most cases occur in middle-aged and older men with underlying debilitating diseases such as diabetes and chronic lung diseases (Liam *et al*., 2001). The onset of *Klebsiella pneumonia* infection is usually sudden and symptoms associated with it are high fever, chills, malaise, body aches and productive cough with abundant, thick and blood-tinged sputum termed “currant jelly sputum” (Kobashi *et al*., 2001). Mortality rates can be as high as 50%, and in the case of alcoholics, mortality can be almost 100% (Branger *et al*., 2004). *Klebsiella* species

are ubiquitous in nature. They have two common habitats, one being the environment, where they are found in surface water, sewage, soil and on plants. They are also found on the mucosal surfaces of mammals such as horses, swine, and humans (Podschun and Ullmann, 1998). In humans, *Klebsialle* species are present in the nasopharynx and in the intestinal tract (Obiamiwe, 2006). The *Klebsiellae* possess a capsule, pilli and the ability to produce siderophores, which enhance its capacity to cause infection in human (Podschun *et al*., 2001). *Klebsiellae* also possess prominent capsules which are repeating subunits, consisting of four to six sugars. In addition to the sugar, it also contains uronic acids which confer on it a negatively charged component (Campos *et al.,* 2004). The capsular material forms thick bundles of fibrillose structures covering the bacterial surface in massive layer (Amako *et al*., 1988). This protects the bacterium from phagocytosis by polymorphonuclear anulocytes, (Brisse *et al*., 2004), and prevents killing of the bacteria by bactericidal serum factors, (Sahly *et al*, 2000). Pili, otherwise known as fimbriae are non flagellar, filamentous projections on the bacterial surface (Chung *et al.,* 2003). As a critical first step in the infectious process, microorganisms must come as close as possible to the host mucosal surfaces and maintain this proximity by attaching to the host cell (Schembri *et al*., 2005). Hospital acquired infections caused by *Klebsiella* are bronchitis, urinary tract infections, surgical wound infections, *pneumonia*, diarrhea and infection of the blood (Lynch *et al*., 1997). All of these infections can progress to shock and death if not treated early (Ryan and Ray, 2004). A study in Nigeria by Oguntibeju and Nwobu, (2004) on post-operative wound infection revealed that, out of 60 cases recorded, 10 were caused by *Klebsiella* species representing 16.7%, which ranked it third only to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In another study on catheter associated urinary infection in Nigeria, *Klebsiella* species were reported to be the commonest pathogen isolated giving it a prevalence rate of 36.6% (Taiwo and Aderoummu, 2006). Many hospital-acquired infections occur because of the invasive

treatments that are often needed in hospitalized patients. For example, intravenous catheters used for fluid administration, catheters placed in the bladder for urine drainage and breathing tubes for people on a breathing machine can all increase the susceptibility to infection .While these devices may be needed in certain patients, they allow bacteria to bypass the natural barriers into a person's body (Warren, 2001). According to Podschun and Ullmann (1998), hospital-acquired bacterial infections caused by Klebsiella species and their percentages worldwide are as follows: urinary tract infection 6-17%, pneumonia 7-14%, septicemia 4-15%, Neonatal septicemia 3-30% and wound infection 2-4%.Prevalence of Nosocomial Infections in Kosovo was studied by Raka *et al.,* (2006). They reported that out of 167 patients surveyed, 29 had nosocomial infections with the overall prevalence rate of 17.4%. There were fewer male patients (41.9%) than female patients. The mean age of the patients was 45 years (range, 0-80 years). The commonest nosocomial infection was bloodstream infection, which accounted for 62% of infections. The rest were surgical-site infection, nosocomial pneumonia, and nosocomial meningitis, each of which accounted for 10% of infections. The hospital wards with the highest prevalence observed were the intensive care units, both neonatal and adult accounting for 88.8% of infections, whereas the age group with the highest prevalence was newborns who had 77.7% of all registered infections. *Klebsiella pneumoniae* was the main pathogen isolated from blood cultures accounting for 36.8% of infections. Antimicrobial resistance is reported from all over the world, and resistance levels are increasing yearly, making the resistant strains new pathogens, capable of causing outbreaks. A publication on the outbreak of nosocomial infections due to *Klebsiella pneumoniae* by Alert *et al*., (2005) reported one hundred and fifty-four clinical isolates of *Klebsiella pneumoniae* resistant to broad- spectrum Cephalosporins, Aztreonam and Amikacin. These resistant strains were responsible for an outbreak of nosocomial infections in a university hospital in Paris. This outbreak affected 3931 patients in the intensive care unit, 8

patients in hematology units and 11 patients in surgical and medical units. These antibiotic resistant strains were responsible for 48% urinary tract infections, 21% wound and drainage fluids infection, 14% respiratory tract infections, 12% infection of blood and 5% of stool infections. According to a study carried out on the antimicrobial susceptibility patterns of *Klebsiella* species from the Lagos University Teaching Hospital by Abe-Aibinu (2000), isolates were obtained from urine, swabs of wounds, ear, throat and eye. Identification was carried out by conventional methods and antimicrobial susceptibility was investigated by a disk diffusion method. Seventy percent of the isolates were susceptible to, ceftazidime, ceftriaxone, aztreonam and nalidixic acid. However, ofloxacin and norfloxacin were observed to be more effective with 90- 93% sensitivity. Sixty-three percent of isolates were susceptible to Gentamycin. The Gentamycin- resistant *Klebsiella* species were mostly from urine samples. Isolates were in general, highly resistant (80%) to cotrimoxazole, tetracycline and amoxicillin-clavulanic acid. A study by Gangoue-Pieboji *et al*., (2006) on antimicrobial activity against Gram negative bacilli from Yaoundé Central Hospital, Cameroon reported that, high rates of resistance were found in most of the bacteria studied. Resistance to all isolates was mostly observed in amoxicillin (87%), 74% for piperacillin and 73% for trimethoprim/sulfamethoxazole. Susceptibilities to third generation cephalosporins (cefotaxime, ceftazidime) and monobactam (aztreonam) were 71% for *Klebsiella* species. Imipenem (98%) sensitivity was the most active antibiotic followed by the ofloxacin (88%). Susceptibility of *Klebsiella* species for Gentamycin was 67%. A study carried out in 11 hospitals in 7 regions in Ghana on the resistance to antimicrobial drugs by Newman *et al.,* 2006) reported that, 309 (57.6%) of 536 *Klebsiella* species isolated, showed 32 antimicrobial resistance. They concluded that the prevalence of drug resistance among the *Klebsiella* species was high. The report also revealed that, 82 % of isolates were resistant to tetracycline, 76 % to ampicillin, 75 % to chloramphenicol, and 73 % to cotrimoxazole. Lower prevalence of resistance

was found for Gentamycin, Cefuroxime and cefotaxime. Gentamycin recorded 28% resistant isolates with Cefuroxime recording 27%, (Newman *et al*., 2006). Infections caused by multiresistant strains of *Klebsiella* species are fatal (Kurupati *et al.*, 2007). In the 1970s, these strains were chiefly aminoglycoside-resistant *Klebsiella* strains. Since 1982, strains that produce extended spectrum beta lactamase, which render them resistant to extended-spectrum cephalosporins, have evolved (Anderson *et al.*, 2003).

2.8 ANTIBIOTICS USED IN TREATMENT OF WOUND INFECTION

2.8.1 PENICILLINS

Penicillin was discovered by Alexander Fleming in 1928. After observing that *Penicillium* colonies inhibited the growth of staphylococci on agar plates, Fleming made an extract from the mold and proved that it inhibited bacterial growth. Penicillin became available for general use in the 1940.

Mechanism of Action

Penicillin is bactericidal, killing bacterial cells by impairing cell wall synthesis. It impairs cell wall synthesis by preventing cross-binding of the peptidoglycan polymers necessary for cell wall formation and by binding the penicillin-binding proteins (PBPs) (carboxypeptidases, endopeptidases, and transpeptidases) that participate in cell wall synthesis. Although the exact mechanisms involved are not known, the end result is that the cell wall is structurally weakened and lyses, leading to cell death (Tomasz, 1979).Bacterial resistance to penicillins may take different forms. The most significant is the bacterial production of beta-lactamases, which can destroy the beta-lactam ring by means of hydrolysis, effectively preventing antimicrobial activity by the agents. In addition, some bacteria are able to prevent binding to the PBPs by various

means, including altered binding sites for the penicillins (Georgopapadakou, 1993). Various strategies have been employed to circumvent these microbial adaptations. Altering the structure of the penicillins to produce agents that are more resistant to the hydrolysis from the beta-lactamases has resulted in the development of the extended-spectrum penicillins. Another strategy has been to combine penicillins with other agents that either block bacterial beta-lactamases or have an alternate method for killing bacteria that are resistant to penicillin. Examples include amoxicillin plus clavulanic acid, ampicillin plus sulbactam, piperacillin plus tazobactam, and ticarcillin plus clavulanic acid. Clavulanic acid is produced by *Streptomyces clavuligerus*. Sulbactam and tazobactam are derived from the basic penicillin ring. These agents have little intrinsic antimicrobial activity, but they bind irreversibly to many beta-lactamases, preventing hydrolytic activity against the beta-lactam ring (Livermore, 1995).

Penicillinase-Resistant Penicillins

The Penicillinase-resistant penicillins were developed in response to the discovery of resistant staphylococcal bacteria that could deactivate available penicillins. These penicillins are resistant to hydrolysis by the lactamase produced by the staphylococci, and they include nafcillin and Oxacillin, which are parenteral formulations, and dicloxacillin, which is given orally. Methicillin and cloxacillin are no longer available in the U.S.While the Penicillinase-resistant penicillins are effective against many of the same Gram-positive organisms that the natural penicillins are effective against; they are not effective against Gram-negative or anaerobic organisms. They are, however, notable for their usefulness against penicillin-resistant *Staphylococcus* and *Streptococcus* species (Prescott *et al.,* 2008).

2.8.2 CEPHALOSPORIN

The first cephalosporin was discovered in 1948 by Giuseppe Brotzu, who observed that the fungus *Cephalosporium acremonium* produced a substance that inhibited the growth of *S. aureus* and other bacteria. The initial substance was identified and modified to create the cephalosporins that are now used. The cephamycins were created by adding a methoxy group on the beta-lactam ring of the original compound, based on the structure of cefoxitin, produced by *Streptomyces lactamdurans*. By altering the chemical groups substituted on the basic molecule, greater antimicrobial activity and longer half-lives have been obtained (kees *et al*., 1995).

Mechanism of Action

Like penicillins, the cephalosporins are beta-lactams in which the beta-lactam ring is joined to a dihydrothiazine ring. Their antimicrobial effect is based on the same mechanism of action that occurs in the penicillins. The cephalosporins inhibit bacterial cell wall synthesis by blocking the transpeptidases and other PBPs involved in the synthesis and cross-linking of peptidoglycan. Because each bacterial species has a unique chemical structure in its cell wall, the cephalosporins may have different mechanisms of action by which they inhibit cell wall synthesis (Fontana *et al*., 2000; wise, 1990).As with penicillins, resistance to the action of cephalosporins may result from mutations in the penicillin-binding proteins (preventing the cephalosporins from binding to them) and from the production of extended-spectrum beta-lactamases that deactivate the drug. An additional source of resistance in Gram-negative bacteria is alteration in the cell-membrane porins that normally allow passage of the cephalosporins of these mechanisms, the production of beta-lactamase is the most clinically significant. This form of resistance may occur through mutations or may be carried on plasmids (Gootz, 2004).The cephalosporins have been classified in different ways, based on chemical structure and pharmacologic activities. The most commonly

used classification system groups the agents into "generations" based on their similarities in antimicrobial coverage.

First-Generation Cephalosporins

The first-generation cephalosporins are most active against aerobic Gram-positive cocci. These agents include Cefazolin, cephalexin, and cefadroxil, and they are often used for skin infections caused by *S. aureus* and *Streptococcus*. They have activity against *E.coli* and some activity against *H. influenza* and *Klebsiella* species, but because of the limited Gram-negative coverage, they are not first-line agents for infections that are likely to be caused by Gram-negative bacteria.

Second-Generation Cephalosporins

The second-generation cephalosporins are more active against Gram-negative organisms, such as *Moraxella, Neisseria, Salmonella,* and *Shigella*. Cefoxitin and cefotetan, which are included in this group under this classification system although they are technically cephamycins, also have more coverage against anaerobic bacteria. The true cephalosporins that are also part of this class are cefprozil, Cefuroxime, cofactors, cefoxitin, and cefotetan. These drugs are used primarily for respiratory tract infections because they are better against some strains of beta-lactamase producing *H. influenza*.

Third-Generation Cephalosporins

The third-generation cephalosporins have the most activity against Gram-negative organisms, including *Neisseria* species, M. catarrhalis, and *Klebsiella,* while ceftazidime is active against *P. aeruginosa*. These agents have less coverage of the Gram-positive cocci, notably methicillin-sensitive *S. aureus*. In addition to the agent with antipseudomonas coverage, this class includes cefdinir, cefditoren, cefixime, cefotaxime, cefpodoxime, ceftibuten, and ceftriaxone. These drugs

are useful for more severe community-acquired respiratory tract infections, resistant infections, and nosocomial infections (because of the high incidence of resistant organisms (Neu, 1990).

Fourth-Generation Cephalosporins

Cefepime is classed as a fourth-generation cephalosporin because it has good activity against both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* and many *Enterobacteriaceae*. The Gram-negative and anaerobic coverage makes Cefepime useful for intra-abdominal infections, respiratory tract infections, and skin infections.

Fifth-Generation Cephalosporins

Ceftaroline fosamil is the only advanced generation cephalosporin to gain FDA approval to date. While the agent is not considered active against *P. aeruginosa*, *Enterococcus* spp., and extended-spectrum beta-lactamase producing Enterobacteriaceae or AmpC mutants, it has enhanced activity against many Gram-negative and Gram-positive bacteria. It is active against community-acquired pneumonia infections caused by *E. coli*, *H. Influenzae*, *Klebsiella*, *S. aureus* (methicillin-susceptible isolates only), and *S. pneumoniae* (including cases with concurrent bacteremia) and is effective and safe for treating skin infections caused by multidrug-resistant *S.aureus* .Clinical cure rates for moderate-to-severe community-acquired pneumonia were higher with Ceftaroline (83.3%) than with ceftriaxone (70%) in a phase III clinical trial, and the agent was well tolerated (Saravolatz. *et al.,* 2011; Low *et al.,* 2011).

2.8.3 CARBAPENEM

Meropenem, Imipenem/cilastatin, doripenem, and ertapenem are parenteral synthetic beta-lactams derived from thienamycin, an antibiotic produced by *Streptomyces cattleya*. They have a lactam ring, like the penicillins and cephalosporins, but have a methylene moiety in the ring.

Carbapenems are stable against the hydrolysis by the extended-spectrum β lactamases due to trans configuration of its hydroxyethyl side chain at position C (Schwabe *et al*., 2006).

Mechanism of Action

Like other beta-lactams, the carbapenem inhibit mucopeptide synthesis in the bacterial cell wall by binding to PBPs, leading to lysis and cell death. Bacterial resistance may occur due to a specific beta-lactamase that affects Carbapenems. Another significant source of resistance is a mutation that results in the absence of the outer membrane porin, thus not allowing transport of the drug into the cell. Cross-resistance may occur between the Carbapenems. Imipenem and ertapenem have a wide antimicrobial spectrum with excellent activity against anaerobic bacteria, including *Bacteroides* species. They also cover many Gram-positive cocci, such as *Enterococcus* and *Streptococcus*, as well as many Gram-negative bacteria. Meropenem has somewhat greater activity against Gram-negative bacteria, which are not affected by most beta-lactamases. Doripenem has good activity against *pseudomonas aeruginosa* (jone, 1985*).*

2.8.4 MONOBACTAMS

Monobactam has a single beta-lactam core, distinguishing them from the other beta-lactam drugs. Aztreonam is the only available example of this class of drugs. Aztreonam was originally extracted from *Chromobacterium violaceum*. It is now manufactured as a synthetic antibiotic (Ennis, 1995).

Mechanism of Action

As with other beta-lactams, aztreonam inhibits mucopeptide synthesis in the bacterial cell wall by binding to the penicillin-binding proteins of Gram-negative bacteria, leading to cell lysis and death. Aztreonam is resistant to most beta-lactamases. Treatment in combination with an aminoglycoside appears to be synergistic against *Pseudomonas*. Aztreonam does not have

significant activity against Gram-positive or anaerobic bacteria and is primarily used against Gram-negative aerobic bacteria, including *P. aeruginosa* and *Klebsiella*. It is indicated for use in pneumonia, soft-tissue infections, urinary tract infections, and intra-abdominal and pelvic infections that are caused by Gram-negative aerobic bacteria.

2.8.5 AMINOGLYCOSIDES

The aminoglycosides were developed during the 1940s. Actinomycetes were studied for possible antimicrobial by-products, and it was found that *Micromonospora* and *Streptomyces* produced useful agents. Streptomycin is derived from *Streptomyces griseus* and was the first of the aminoglycosides that was developed (Duma *et al.*, 1984).

Mechanism of Action

The basic structure of the aminoglycosides is an aminocyclitol ring. Different members of the family have different glycosidic linkages and side groups. The aminoglycosides have at least two effects on the bacterial cell that ultimately result in cell death. These agents bind negative charges in the outer phospholipid membrane, displacing the cations that link the phospholipids together. This leads to disruption in the wall and leakage of cell contents. In addition, they inhibit protein synthesis by binding to the 30S subunit of the ribosome, causing miscoding and termination (Moellering, 1984). Although resistance to aminoglycosides is less common than with many other antibiotics, it can develop as a result of three known mechanisms. The most common pattern of resistance is through modification of the aminoglycoside molecule itself by enzymes produced by some bacteria. After the aminoglycoside is altered, it cannot bind as well to the ribosome's. The genes that encode for these enzymes are carried on plasmids, allowing rapid transfer of resistance between bacteria. Of note, amikacin has an S-4 amino 2-hydroxybutyryl (AHB) side chain that protects it against deactivation by many bacterial enzymes

and is therefore less susceptible to this bacterial defense mechanism. The binding site for aminoglycosides on the rRNA of the ribosome may also be altered, reducing binding. In addition, mutations that cause reduced uptake of aminoglycosides have been documented (Kotra *et al.,* 2000). To combat resistances and overcome the relative natural resistance of enterococcus to aminoglycosides, other agents that target the cell wall are often used in conjunction with the aminoglycosides. Damage to the cell wall from the additional agents may be bactericidal in some cases and also makes the cell wall more permeable to the aminoglycosides (Gordon *et al.,* 1992). The bifunctional enzyme AAC (6′)/APH(2"), encoded by the aac(6′)-aph(2") gene, inactivates a broad range of clinically useful aminoglycosides such as gentamycin, tobramycin, netilmicin, and amikacin and is the most frequently encountered aminoglycoside resistance mechanism

among staphylococcal isolates (Martineau *et al.,* 2000).

2.8.6 MACROLIDES

The original macrolides, erythromycin, was discovered in 1952 by J.M. McGuire. It is produced by *Saccharopolyspora erythrae* (formerly known as *Streptomyces erythreus*). Semisynthetic derivatives (clarithromycin, azithromycin) have been produced from the original erythromycin, with modifications that improve acid stability, antibacterial spectrum, and tissue penetration.

Mechanism of Action

The macrolides are bacteriostatic, inhibiting protein synthesis by binding at the 50S ribosomal unit and by blocking transpeptidation and translocation. At high concentrations or with rapid bacterial growth, the effects may be bactericidal (Goldman *et al.,* 1990). Telithromycin is technically a ketolide, but it is structurally related to the macrolides. It also functions by binding the ribosomal subunit with subsequent inhibition of bacterial protein synthesis. By binding in two places, telithromycin remains active against bacteria that produce methylases, which alter

binding at the domain V site on the ribosomal subunit (File, *2005*). Many bacteria that are resistant to the penicillins are also resistant to erythromycin. Bacterial resistance may result from decreased permeability of the cell membrane; in addition, an increase in active efflux of the drug may occur by incorporating a transporter protein into the cell wall (Sun *et al.,* 2004*).* The gene for this mechanism is transferred on plasmids between bacteria. Mutations of the 50S ribosomal receptor site may also develop, preventing binding of the erythromycin. Lastly, bacterial enzymes have been described that may deactivate erythromycin. It is likely that this form of resistance is also transferred on plasmids (Matsuoka *et al.,* 2004).

2.8.7 QUINOLONES

The first Quinolones, nalidixic acid, was introduced in 1962. It was developed as a result of chloroquine synthesis. Later, derivatives with broader spectrum antimicrobial coverage were produced; leading to the current class of Quinolones drugs such ciprofloxacin, ofloxacin e.t.c. As with other classes of synthetic and semi synthetic antimicrobials, alterations of side chains affect antimicrobial activity and pharmacokinetics (Anderson, 2003).

Mechanism of Action

Quinolones cause bacterial cell death by inhibiting DNA synthesis. They inhibit DNA gyrase and DNA topoisomerase, enzymes that mediate DNA supercoiling, transcription, and repair .The exact mechanism by which this leads to cell death has not yet been determined (Drlica *et al.,* 1997). Bacterial resistance develops as a result of spontaneous mutations that change the binding sites for Quinolones on the DNA gyrase and the DNA topoisomerase. Mutations that decrease the ability of Quinolones to cross the cell membrane also occur. Some of these resistances may be transferred from other bacteria by means of plasmids (Willmott *et al.,* 1993).

2.9 ANTIBIOTIC PROPHYLAXIS

Burke in 1961 demonstrated the importance of the timely use of prophylactic antibiotics in Surgery. Antibiotics used are based on the clinical findings of both local and systemic involvement. The initial spectrum of coverage should include the organisms likely to be encountered from the type of operation recently performed. The coverage can be tailored when the pathogens are isolated and identified (Patel *et al.,* 2000). Topical antibiotics are safe on open wounds and promote wound healing possibly by providing moist wound environment (Mack *et al.,* 1967). The moist environment prevents desiccation and Escher formation and encourages granulation tissue growth leading to increased re epithelialization rates.

2.9.1 Choice of Antibiotics

The wound classification scheme devised by the National Research council serves as the basis for recommending antibiotic prophylaxis. The least toxic and most effective antibiotic regimen should be chosen. Excellent guidelines regarding the selection and use of prophylactic antibiotics to treat surgical wound have been published. The use of antibiotics as prophylaxis depends on the class of wounds. ln clean wounds where the risk of infection is less than 2%, there is generally no need for antibiotics prophylaxis except in procedures in which infection would be disastrous for example, prosthesis placements, central nervous system operations or cardiac procedure that use cardiopulmonary bypass. A first generation cephalosporin, such as Cefazolin, is commonly used, if the patient is allergic to penicillin, vancomycin is a good alternative (Nandi *et al*., 1999). In clean contaminated wounds, antibiotic prophylaxis used for treatment reduces the risk of infection from 30% to 10% (Sawyer *et al*., 1994). In contaminated, infected or dirty wounds preoperative antibiotics, which are usually continued in the past operative period are used for treatment (Ferraz *et al*., 1992).

2.9.2 Route and time of administration

A single bolus of intravenous antibiotic at the time of induction of anesthesia is considered adequate for most surgical procedure. This enables a high plasma and tissue concentration to be attained rapidly. The rate of infection increases if prophylactic antibiotics are given more than 2 hours preoperatively or postoperatively (Classen *et al*., 1992). Oral and intramuscular routes of administration produce a lower peak plasma level. For colorectal operations, both intravenous and oral routes of prophylaxis administration are necessary. In certain orthopedic procedures, local antibiotics are used for example; bone cement that is impregnated with Gentamycin is used in joints prosthesis implantation (Jorgensen *et al.*, 1991).

2.9.3 Dosage of antibiotics

The dose of prophylactic antibiotics should not be smaller than the standard therapeutic dose of the drug. A single prophylactic dose is effective and preferred to multiple doses (McDonald *et al*., 1998). The single dose approach has the advantage of low cost, less toxicity and less chance of developing antibiotic resistance. Limiting the use of antibiotic prophylaxis to the intra-operative period is one of the most significant changes in preventing infection and is dramatically different from the previously recommended 24 to 48 hour coverage. Prophylaxis is effective in most surgical procedures; although its use during cardiac operations remain debatable, prolonged antibiotic prophylaxis while lines, tubes and catheters are in situation is not necessary (Page *et al*., 1993). The longer the preoperative stay the greater the likelihood of infection from a more antibiotic-resistance organism. These point from the bases for the practice of prophylactic antibiotic administration to the surgical patient and suggest the advantage of the current practice of day case surgery (Oluwatosin, 2005). Inappropriate and indiscriminate use of prophylactic antibiotics may increase cost through unnecessary drug use, requisite laboratory monitoring and the emergence of resistant organism (Nandi *et al*., 1999). Resistant bacteria

posse's resistant genes that are more vulnerable in debilitated patients especially those with immune suppressed complications such as HIV/AIDS, cancers, diabetic wounds, burns etc (Frie *et al*., 1999).

2.10 The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a technique widely used in Molecular biology. It derives its name from one of its key components, DNA polymerase Used to amplify a piece of DNA by in-vitro enzymatic replication (David and Turlotte, 1998). As PCR progresses the DNA generated is used as a template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified. With PCR, it is possible to amplify a single or few copies of DNA across several orders of magnitude, generating millions for more copies of the DNA piece. Developed in 1984 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of application. These include DNA cloning for sequencing, DNA based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic finger prints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases . In 1993 Mullis was awarded the Nobel Prize in chemistry for his work on PCR (Saiki *et al.,* 1998*).*

2.11 BACTERIAL ANTIMICROBIAL RESISTANCE

Bacterial resistance to antibiotics can simply be defined as the ability of a bacteria to resist the effect of an antibiotic or groups of antibiotics to which it was formally susceptible to either by the production of enzymes that alter the structure of the antibiotics, by the modification of the antibiotics, by bypassing certain pathways or by acquisition of resistant plasmids from other bacteria strain. Bacterial resistance can either be plasmid or chromosome mediated. Plasmid mediated resistance due to plasmids to various antimicrobial drugs have been demonstrated by various workers (Shadid *et al.* (2003), Shadid and Malik (2004), Olayinka *et al*.(2009), Yimaz *et*

al., (2011). During the last five decades, the use and sometimes misuse of antimicrobials in both human and veterinary medicine has resulted in the emergence of strains of bacteria that no longer respond to antimicrobial therapy (McDermott *et al*., 2003).

Antibiotic resistance is the reduction in effectiveness of a drug such as an antimicrobial or an antineoplastic in curing disease or condition. When an organism is resistant to more than one drug, it is said to be multidrug-resistant (Fisher and Mobashery, 2010). Intrinsic resistance can be described as a natural phenomenon when it is displayed by all members of a species and is a function of the physiological or biochemical structure of that species (Harbottle *et al*., 2006). For example, *Enterococci* are intrinsically resistant to cephalosporins due to a decreased binding affinity to the penicillin-binding proteins. On the other hand, acquired resistance can result from the acquisition of a mutation in the regulatory or structural genes and/or the acquisition of a foreign resistance gene. Acquired resistance is not present in the entire species but within only a certain lineage of bacteria derived from a susceptible parent (Harbottle *et al*., 2006).

2.11.1 Plasmid-mediated resistance

This is when a bacteria or any other microorganism resists antibiotics due to the acquisition of resistant plasmids. It is the leading cause of resistance among many bacteria strains. Plasmid profile analysis examines the total bacterial plasmid content, or subjects plasmids to restriction endonucleases and separates the cleaved plasmid DNA by electrophoresis for analysis. This method is a powerful tool for following the spread of antibiotic resistance, because resistance is usually passed between bacteria on plasmids (Ogle *et al.,* 1987).

2.11.2 Chromosome-mediated resistance

This is when the resistant gene to antibiotics is already present in the organism. If an organism's resistant marker is retained after curing of plasmids, its resistance to antibiotics is said to be chromosomally borne. (Silver, 2003)

2.11.3 Horizontal transfer of antimicrobial resistance

Most bacterial genomes that have been sequenced contain DNA segments that have been acquired from other sources. It was found that this horizontally acquired DNA usually encodes functions that are of selective advantage to the organism such as antibiotic resistance, virulence and biodegradation pathways. The three common mechanisms for horizontal gene transfer are:

(1) Transformation, a process by which bacteria take up free DNA directly from their environment.

(2) Transduction, through which bacterial DNA is moved from one bacterium to another by a bacteriophage.

(3) Conjugation, a process by which a living bacterial cell transfers genetic material through cell-to-cell contact.

A number of different genetic elements have played major roles in the development of resistance in bacteria. Such genetic elements include plasmids, transposons, genomic islands, phage, integrons and gene cassettes (Normark and Normark. 2002).

2.12 MECHANISMS OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance of microorganisms may arise as an intrinsic resistance or as an acquired resistance via mutations or gene transfer. Knowing the mechanisms of resistance is important for implementation of strategies for control of spread of resistance such as antimicrobial restriction policies and infection control measures. Implementation of rigorous restriction of the use of

oxyimino β-lactam had led to the successful control of a large nosocomial outbreak due to ESBL-producing *K. pneumonia* (Pena *et al*., 1998). Some microorganisms and antimicrobials pairs and monotherapy with particular antimicrobial will cause the development of resistance; for example, treating active tuberculosis with isoniazid monotherapy resulted in higher rates of isoniazid resistance. Several mechanisms by which microorganisms confer resistance to antimicrobials have been described (Struelens, 2003).

2.12.1 Enzymatic inactivation or modification

Resistance may arise if the antimicrobial is inactivated before it reaches its target in the microorganisms. An example is the resistance to β-lactam antimicrobials via the production of inactivating enzymes- β-lactamases. These enzymes bind to β-lactam antimicrobials and cause hydrolysis of cyclic amide bonds of the β-lactam rings, β- Lactamases are present in Gram-positive (e.g. staphylococcal β-lactamases) and Gram-negative microorganisms (β-lactamases in *Enterobacteriaceae).* B - lactamases are increasing in prevalence among Gram-negative microorganisms in many countries (Harbarths *et al.*, 2005).Aerobic bacteria develop resistance to aminoglycosides by producing aminoglycoside-modifying enzymes such as acetyltranferases, phosphotranferases and nucleotidyl tranferases that modify the amino or hydroxyl groups of the aminoglycoside molecule.

2.12.2 Alteration of target site

Alteration in the target site so that it is no longer recognized by the antimicrobial agent, for example β-lactam resistance due to alterations of penicillin-binding proteins (PBPs) is another important mechanism of resistance in Gram-positive bacteria. MRSA produce PBP2a which has low affinity for methicillin and other P-lactams. This PBP2a is encoded by the *mecA* gene. Altered PBPs cause penicillin-resistance in Streptococcus *pneumoniae.*

2.12.3 Impaired permeability

Decreasing drug concentrations by reducing drug uptake (decreased Membrane permeability) is another mechanism by which antimicrobial resistance can occur. Some Gram-negative bacteria develop resistance to antimicrobial agents due to mutations which cause loss of specific porins. Porins facilitate drug entry in to the cell. Examples are those deficient in porins such as oprD2 porin leading to Imipenem resistance in *P. aeruginosa* and OmpF causing flouroquinolones resistance in *E.coli* (Lepper *et al.*, 2002).

2.12.4 Efflux pumps

Resistance may arise if the organism pumps back the antimicrobial out of the cell. Active efflux pumps which may be single or multiple have been described as another way of resistance mechanism. Some enteric Gram-negative microorganisms develop membrane transporter systems facilitating drug efflux (Struelens, 2003).

2.12.5 Alteration of metabolic pathway

Microorganisms may develop resistance by altering the metabolic pathway which bypasses the target. Resistant to sulfonamide may arise due to production of an altered enzyme that has reduced affinity for sulfonamide, and trimethoprim resistance may arise as a result of synthesis of dihydrofolate reductases with a decrease in the susceptibility to trimethoprim Two or more resistance mechanisms may be involved together to confer a resistance to a particular antimicrobial agent. For example in *P. aeruginosa* active efflux pumps, production of beta-lactamase enzymes and reduced membrane**.** Metabolic Pathway Permeability interplay together to cause resistant to (3-lactam antimicrobials and specifically Beta-lactamase activity and reduced membrane permeability due to loss of oprD2 porin which causes Imipenem resistance (Muto *et al*., 2003).

2.13 RISK FACTORS ASSOCIATED WITH ANTIMICROBIAL RESISTANCE

Resistant organisms pose a great challenge in the treatment of bacterial infections often leading to treatment failure, prolonged duration of illness and great risk of death. Bacteria have ability of undergoing mutation or acquiring a resistance gene when antimicrobial agents are inappropriately used. There has been an increase in the number of multidrug resistant organisms isolated from patients in hospitals worldwide. Infection with antibiotic resistant bacteria also increases the likelihood that the patients will receive inadequate therapy (Davies, 1994). Development of antimicrobial drug resistant pathogens occurs as a result of complex interactions, which favor the emergence, persistence and increased transmission of these resistant bacterial strains. Widespread and inappropriate use of antibiotics has been shown to increase the development of antimicrobial resistant pathogens (Davies 1994; Cohen 1992). Several literature sources have documented different risk factors associated with isolation of antimicrobial resistant pathogens from patients. In a case control study conducted in Denmark identifying possible risk factors for MRSA and methicillin susceptible *Staphylococcus aureus* (MSSA), prior to hospitalization for more than 7 days within the previous six months tended to be associated with MRSA (Bocher *et al.,* 2008). Another case control study in Australia, reported hospitalization within the preceding six months and residence in long care facility as being associated with higher risk of MRSA bacteremia (Ho and Robinson 2009). In a retrospective study at a university hospital in Malaysia, duration of hospitalization, previous antibiotic use, and bedside invasive procedures were significantly associated with MRSA than MSSA (Al-Talib *et al*., 2010). Prior exposure to antibiotics as a risk factor for emergence of drug resistant bacterial strains has also been reported in studies done in Asia countries (Cheol and Jae, 2013). In a study done in Thailand, patients with prior ESBL colonization and recent antibiotics exposures (<90 days), especially to third generation

cephalosporin and flouroquinolones were statistically significantly associated with risk of ESBL-producing compared with Non ESBL-producing *Escherichia coli* . Interestingly diabetes was not a risk factor with either type of infection. Use of ventilator, use of catheter and days of stay in hospital wards have also been significantly associated with acquisition of antibiotic resistant isolates. Multivariate analysis of data from a study in Madagascar showed that diabetes and use of an invasive procedure were independent risk factors for resistance to third-generation cephalosporins among ESBL-producing Enterobacteriaceae isolated from surgical wards and intensive care unit (Mansouri, 2011; Apisarnthanarak *et al.,* 2010).

2.14 BACTERIAL PLASMIDS

Plasmids are small, circular molecules of DNA in bacteria that are separate from the bacteria chromosome. They have their own origins of replication and replicate autonomously and are stably inherited. Some plasmids are episomes, plasmids that can exist either with or without being integrated into host chromosomes (Prescott *et al*., 2008). The plasmid DNA molecules enable genetic material to be exchanged within and between bacterial species through specialized sex pilli (Cheesbrough, 2000). Depending on the genes contained in the plasmid, one bacterium may confer on another, properties as antimicrobial resistance or toxin production. Different plasmids can be found in the same bacterium. Plasmids may be classified in terms of their mode of existence, spread and function (Cheesbrough, 2000). Conjugative plasmids are of particular note. They have genes for the construction of hair like structures called pilli and can transfer copies of themselves to other bacteria during conjugation perhaps the best studied conjugative plasmid is the F factor (fertility factor or F plasmid) of *E.coli*, which was the first conjugative factor to be described (Prescott *et al*., 2008). Other types of plasmid include; resistance plasmids, which confer antibiotic resistance on the cells that contain them, col plasmid, which contain genes for the synthesis of bacteriocins known as colicins. Other are Virulence plasmids, which

encode factors that make their host more pathogenic and the metabolic plasmids, which carry gene for enzymes that degrade substances such as aromatic compounds (toluene), pesticides (2, 4 dichlorophenoxyacetic acid), and sugars (lactose). In this study resistance plasmids are of particular note since they are responsible for the cause of antimicrobial resistance. The loss of a plasmid is called curing. It can occur spontaneously or be induced by treatments that inhibit plasmid replication. Some commonly used curing treatments are acridine mutagens, UV and ionizing radiation, thymine starvation, antibiotics and growth above optimal temperatures. Bacteria species however can be cured of their plasmid through *in vitro* treatment with sodium dodecyl sulfate (SDS) according to (Olukoya and Oni (1990). Plasmid may reside in cells either as a single copy or as multiple copies, large plasmid tends to have a lower copy number, whereas smaller plasmid may be present in more than 20 copies per cell and several studies have demonstrated that conjugative antibiotic resistant plasmids are widespread in nature (Olukoya and Oni (1990). Resistance in *P. aeruginosa* may be chromosomal born and in general, clinical strains may harbor multiple plasmids (Droge *et al*., 2000). To acquire these new properties, bacteria must undergo a genetic change, such genetic changes may occur by mutation or by the acquisition of new genetic materials. New genetic materials are acquired by transfer of resistance genes (plasmids) from one bacterium to another**.**

CHAPTER THREE

MATERIALS AND METHODS

3.1 Design of Study

3.1.1 Location

Two locations (University of Benin Teaching Hospital, Benin city, Edo State and Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State) were used in this study. Clinical samples of hospitalized post operative surgical patients were used in the study. Ethical approval was obtained from the ethics committees of both Hospitals (UBTH ADM/E22/A/VOL.VII/1013) and (NAUTH/CS/66/VOL.5/124) before the commencement of this study (Appendix C).

3.1.2 Duration

The study was conducted in the department of general surgery, obstetrics/gynecology and orthopedic wards of University of Benin Teaching Hospital (UBTH) Benin City, Edo State and Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi in Anambra state, Nigeria between February 2014 and February 2015.

3.1.3 Patients

The study subjects included a total of 164 male and 198 female hospitalized Patients having post operative surgical wounds from the department of general surgery, obstetrics/gynecology and orthopedic wards of University of Benin Teaching Hospital (UBTH) Benin City, Edo State and Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi, Anambra state, Nigeria ages ranging between 17 and 70 years.

3.1.4 Specimens

A total of 362 clinical wound swabs samples were collected from post operative hospitalized surgical patients. One hundred and eighty one of the samples were from UBTH in Edo state while one hundred and eighty one was from NAUTH in Anambra state.

3.2 Sample size Determination

Sample Size: The sample size was obtained using the formula of Naing *et al*., 2006, which is

$$N = Z^2 \times P\,(1-P)/d^2$$

Where: N = Minimum sample size

d = Desired level of significance (0.05)

Z = Confidence interval (1.96)

P = Prevalence rate of surgical wound infection (34%) (WHO, 2011).

Using this formula, the minimum number of sample size collected was: 345.

Sample collection

Random samplings of patients with post operative surgical wounds were used in this study. Each day samples were collected from postoperative patient by using even number or odd number such that patients with postoperative infections will have equal chances of been sample randomly. An informed consent form was signed before sample collection. Samples were collected in duplicate before surgical wound dressing and placed inside an Amies transport medium. All collections were done under strict aseptic conditions. Specimens were transported immediately to the Medical microbiology Department of Lahor Research Laboratories and Medical Centre, Benin City, Edo state for analysis within 3-4 hours of sample collection. Patient data collection Structured questionnaires were used to gather information from the patients.

3.3 Processing of Samples

3.3.1 Culture

The wound swab specimens were inoculated on Blood agar, MacConkey agar, Nutrient agar, and Mannitol salt agar plates and were incubated aerobically and anaerobically at 37^0C for 24 hours. Duplicate blood agar plates were incubated anaerobically at 37^0C for 24 hours. All the media used (Blood agar, MacConkey agar, Nutrient agar, and Mannitol salt agar) were prepared according to the manufacturers directives. (Appendix A).

3.3.2 Primary Gram Stain

Smear was made from the wound swab specimens after culturing and stained by Gram technique, and examined at X100 objectives for bacterial morphology (Appendix B).

3.3.3 Isolation

Macroscopic Isolates were identified based on their colonial appearances on MacConkey agar, and nutrient agar plates. *P.aeruginosa* produces pale coloured colonies on MacConkey agar while *E.coli* produces pink colonies on MacConkey agar. On nutrient agar, *P.aeruginosa* produces greenish colonies while *E.coli* appears colourless and *Staph aureus* produce golden yellow colour on mannitol salt agar plate (Cheesbrough, 2000). Morphological characteristics such as size, form, elevation, opacity, odour and edge were performed for identification of the organisms.

3.3.4 Secondary Gram Stain

The Gram staining reaction was used to identify pathogens in cultures by their Gram reactions. (Appendix B).

3.3.5 Identifications and pure culture preparation of isolates

A subculture of the colonies of each isolate was made. This was done by picking portions of the colonies with sterile wire loop and streaking on freshly prepared nutrient agar plates. The plates were incubated at 37ºC for 24 hours. All isolates were identified phenotypically and biochemically following standard procedures. The colonies on the MacConkey agar were classified into lactose and non-lactose fermenting colonies. The Non-lactose fermenting colonies and Lactose fermenting colonies were further subjected to the necessary conventional biochemical tests such as citrate utilization, urea production, indole production, Oxidase test, motility test, sugar fermentation (maltose, sucrose and mannitol) test.(Cowan and steel, 1974) (Appendix A).

3.3.6 Oxidase (Appendix B).

A strip of filter paper (Whatman No.1) was soaked with a freshly prepared solution of 1% Tetramenthyl 1 para-phenylen-diamine-dihydrochloride and with a clean slide edge, colonies of suspected organisms was streaked on it. Development of a blue purple colour within a few seconds indicated a positive test and where there was no colour change indicated negative test.

3.3.7 Indole Test (Appendix B).

Most strains of *Escherichia coli* and *Proteus* species are capable of breaking down tryptophan (Amino acid). The test organisms were inoculated in sterile bijou bottles containing 3 ml of sterile peptone water and incubated for 24 hours at 37^0c. Thereafter, 0.5ml of Kovac's reagent was added and it was shaken gently. A red colour ring was observed in the surface layer within one minute, which indicated a positive test while a yellow colour indicates a negative test.

3.4 Antibiotics Susceptibility Testing

Antimicrobial susceptibility testing was carried out on each isolates by the disc diffusion method using the Kirby- Bauer disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards (NCCL, 2003) guideline to evaluate the sensitivity of the test organisms to the various antibiotics. Test isolates were grown on Nutrient agar and incubated at 37^{o} C for 24 hours. Colonies were suspended into sterile normal saline and the inocula density was adjusted to 0.5 McFarland turbidity standards. A sterile cotton wool swab was inserted into each test tube containing the standardized inocula suspension, rotated with firm pressure on the inside wall of the test tube to remove excess fluid and then used to swab the surface of a freshly prepared dried Mueller- Hinton agar plate. The antimicrobial disc used included Ceftazidime (Caz 30µg), Gentamycin (GN 30µg), Ofloxacin (ofl 5µg), Ciprofloxacin (Cpr 5µg), Erythromycin (Ery 10ug), Imipenem (lmp 10ug), Oxacillin (oxa, 1ug,), Cefuroxime (Crx 30ug), Cefixime (CXM 5ug), and Augmentin (Aug, 30ug) (Oxide). The disc was placed on the surface of the inoculated Muller Hinton agar plate and incubated at 37^{0}C for 24 hours. After incubation, diameters of zones of inhibition were measured to the nearest millimeter using a transparent meter rule. The clinical isolates diameter zones were compared with reference control organism held at Lahor Research Laboratories (*E.coli* ATCC 25922, *P.aeruginosa* ATCC 27853 and *S.aureus* ATCC 6538) and interpreted as susceptible or resistant according to the CLSI (2009).

3.5 Molecular Assays

Plasmid analysis of the isolates that showed multidrug resistance was carried out to ascertain if multidrug resistance exhibited by the isolates were solely plasmid or chromosome mediated. The MDR classification was base on bacteria that were resistant to three or more different classes of antimicrobial agents (Magiorakos *et al.*, 2011).

3.5.1 Plasmid DNA isolation and Profiling.

Plasmid isolation was carried out on each of the multidrug resistant bacteria using a commercial plasmid isolation kit (ZR Plasmid Miniprep^{TM}- Classic Catalogue number. D4O15, D4016, and D4O54) according to the manufacturer's instructions. Zero point five millitre of the overnight broth culture (each isolates) was centrifuged. The supernatant was discarded. Two hundred micro litres of P1 Buffer was added to the pelleted cells followed by two hundred micro litres of P2 Buffer and mixed. It was incubated at room temperature for 2 minutes. Four hundred micro litres of P3 Buffer was then added and mixed. It was centrifuged at 16,000× g for 2 minutes. The Supernatant was loaded inside the Zymo-spin^{TM} IIN column and was centrifuged for 30seconds.The flow through was discarded. Two hundred micro litre of Endo- Wash Buffer was added to the column in a collection tube and centrifuged for 30 seconds. Four hundred micro litre of plasmid Wash Buffer was added and centrifuged for 1minutes. The spin Column was placed in a new micocentrifuge tube and 30ul of DNA Elution Buffer was added and centrifuged for 30 seconds (Sambrok *et al*., 2001; Ranjbar *et al*., 2007*).*

3.5.2 Preparation of 0.8% Agarose gel (Use for plasmid DNA detection)

Zero point eight percent agarose gel was prepared by dissolving 0.8g in 100ml Tris EDTA Buffer. The mixture was then heated in a microwave for 5 minutes to dissolve completely. It was then allowed to cool at 56^0C and 6 µl of ethidium bromide was added to it. The agarose gel was poured into the electrophoresis chambers with gel comb, and allowed to solidify (Bikandi *et al.*, 2004)

3.5.3. Electrophoresis

Ten microlitres of the molecular markers was loaded into the first well. Two microlitres of the loading dye mixed with 8µl of the plasmid DNA extract were loaded in the other wells. Electrophoresis was performed at 90v for 60 minutes. After electrophoresis the products were visualized by Wealtec Dolphin Doc UV transilluminator and photographed. Molecular weights were estimated using molecular weight standard of the maker.

3.5.4 Plasmid DNA curing

Procedure as described by Ehiaghe *et al.,* (2013) was used. Nine milliliter of freshly prepared nutrient broth was inoculated with 1ml overnight culture (each isolates) that was grown in Luria bertani broth containing antibiotics for 24 hours at 37^0C. The resultant mixture was grown for 4 hours to allow for minimal growth of the microorganisms. One milliliter of 10% Sodium Dodecyl Sulphate (SDS) curing agent was added to 9 ml nutrient broth culture, and was incubated at 37^oC for 24 hours. One milliliter of the cured culture was inoculated unto 9ml freshly prepared nutrient broth and incubated at 37^0C for 24 hours. The overnight broth culture was then used to carry out post susceptibility test on Muller Hinton agar plate with the necessary antibiotic disc placed and incubated for 24 hours at 37^0C.

3.6 Genomic DNA Extraction using Thermo Scientific Gene jet

A single colony of each isolates from Nutrient agar plate was inoculated into 3 ml of Luria broth and incubated at 37°C overnight with constant agitation at 120 rpm. One point five milliliters of the overnight culture was transferred into a micro centrifuge tube. The overnight culture was centrifuged at 13000rpm for 10 minutes and the supernatant was discarded. The pellet was resupended in 180µl digestion Solution. Two hundred micro litres of proteinase K solution was added and mixed thoroughly by vortexing to obtain a uniform suspension. The sample was incubated at 56°C in a thermomixers until the cells are completely lysed. Twenty microlitres of

RNaseA solution was added to the mixture and incubated for 10 minute at room temperature. Two hundred micro litres of Lysis Solution was added to the sample and mixed thoroughly by vortexing for about 15 seconds to obtain a homogeneous mixture. Four hundred micro litres of 50% ethanol was added to the mixture and mixed by vortexing. The resultant lysate was transferred to a Gene JET Genomic DNA Purification Column inserted in a collection tube and centrifuged at 6000 x g for 1 minute. The resultant flow-through solution was discarded. The Gene JET Genomic DNA Purification Column was placed inside a new 2ml collection tube. Five hundred micro litres of Wash Buffer I was added to the column and centrifuged at 8000 x g for 1 minute. The flow-through was discarded. Five hundred micro litre of Wash Buffer II was added to the Gene JET Genomic DNA Purification Column Centrifuged at 12000 x g for 3 minutes. Two hundred micro litres of the Elution Buffer was added to the center of the Gene JET Genomic DNA purification column membrane and incubated at 2 minutes. The column was centrifuged at 8000 x g for 1 minute to elute genomic DNA for PCR amplification.

3.7 Polymerase chain reaction detection (PCR)

The chromosomally mediated multidrug resistant isolates from both locations underwent molecular characterization using PCR techniques and four selected resistant genes primers were targeted against multidrug bacteria. Selected primers were used to identify the isolates and associated resistant genes were ascertained together with the sequences of their nucleotides. They were purchased from Inqaba Biotech industries, Harfield, South Africa.

Table 3.1: Summary of the primers used for the study.

Gene Symbol	Primers 5^{I}-3^{I} (forward, reverse)	n moles (mg)	Molecular weight	Melting temperature (TM) (^{0}C)
PA-GS-F	GACGGGTGAGTAATGCCTA	23.64	5892.3	60.16
	CACTGGTGTTCCTTCCTATA	20.02	6033.8	58.35
Pa16S-F	GGGGGATCTTCGGACCTCA	28.19	5844.3	64.48
	TCCTTAGAGTGCCCACCCG	33.6	5724.3	64.48
URL – 301	TGTTACGTCCTGTAGAAAGCCC	49.8	6709.8	62.67
	AAAACTGCCTGGCACAGCAATT	30	6711.6	60.81
16S-1	GTGCCAGCAGCCGCGGTAA	28.6	58.38	66.64
	AGACCCGGGAACGTATTCAC	49.09	6110.3	62.45
nuc-1	TCAGCAAATGCATCACAAACAG	26.29	6704.5	59.95
	CGTAAATGCACTTGCTTCAGG	30.9	6420.7	60.61
Blaoxa F	TATCTACAGCAGCGCCAGTG	39.53	6101.4	62.45
	CGCATCAAATGCCATAAGTG	36.13	6109.4	58.35
gyrA F	ATGACTGATATCACGCTGCCA	27.85	6389.6	60.61
	ATAACGCATCGCTGCCGGTGG	29.1	6446.5	66.47
TEMU1	ATGAGTATTCAACATTTCCG	38.98	6090.7	54.25
	CTGACAGTTACCAATGCT	32.02	5458.3	55.34
ereA-F	AACACCCTGAACCCAAGGGACG	32.19	6706.3	66.4
	CTTCACATCCGGATTCGCTCGA	27.1	6645.8	64.54

3.8 Preparation of the 100µM stock solution of the primers.

The lyophilized primers were spun down with the aid of the microfuge before opening it to ensure that the primer pellets are at the bottom of the tubes.

The primers were diluted as follows:

Genus oligonucleotide forward (PA-GSF): 236.36µl nuclease free water was added to the lyophilized primer and further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (PA-GS-R): 200.2ul nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Specie oligonucleotide forward (Pa 16S-F): 281.86µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Specie oligonucleotide reverse (Pa16S-R): 336.01µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Specie oligonucleotide forward (URL-30F): 497.96µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free (10µM)

Specie oligonucleotide reverse (URR-432 R): 299.9µl of nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Genus oligonucleotide forward (16S-1F): 285:97µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Genus oligonucleotide reverse (16S-1R): 285:97µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Specie oligonucleotide forward (nuc –1 F): 262:91µl nuclease free was added to lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Specie oligonucleotide reverse (nuc-2 R): 30ul nuclease free water was added to lyophilized primer. Further diluted 1:10 with nuclease free water (10µM.)

3.9 Resistant Genes Primers:

Oligonucleotide forward (Blaoxa F): 295.32µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (Blaoxa R): 361:28ul nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide forward (gyrA F): 278.47µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (gyrA R): 291.02µl nuclease free water was added to the lyophilized primer diluted 1:10 with nuclease water (10µM).

Oligonucleotide forward (TEM UI F): 389.8µl nuclease free water was added to the lyophilized. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (TEM LI R): 320.22µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide forward (ere A–F): 321:91ul nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (ere A- R): 270:98µl nuclease free water was added to the lyophilized primer. Further diluted 1:10 with nuclease free water (10µM).

3.10 Quick load One Taq One Step Polymerase Chain Reaction

Quick load One Taq one step PCR master (2X) with catalog number NEB MO486S was purchased from lnqaba Biotech Hartfield South Africa incorporated and used according to the manufacturer's instruction. See table 1 for details of the primers used. The system components were thaw and mixed by inverting ten times. The PCR was performed in 50µl reaction mixture containing 25µl Quick load One Taq one- step PCR master mix (2x), 1µL of each gene-specific forward primer (10µM), 1µL of each specific reverse primer10µM), 13ml of nuclease free water and 10µl of DNA template was added last. The PCR was started immediately as follows: Initial

denaturation at 94^0C for 1 minute, denaturation at 94^0 for 30secs, annealing at Tm-5 for 30secs, extension at 72^0C for 1 minute, go to the denaturation step for 39 cycles, final extension at 72^0 C for 15mins and final holding at 4^0C.

3.10.1 Preparation of agarose gel (1.5% Agarose gel use for genomic DNA detection)

One point five percent agarose gel was prepared by dissolving 1.5g in 100ml Tris EDTA Buffer. The mixture was then heated in a microwave for 5 minutes to dissolve completely. It was then allowed to cool at 56^0C and 6µl of ethidium bromide was added to it. The agarose gel was poured into the electrophoresis chambers with gel comb, and allowed to solidify.

3.10.2 Electrophoresis

Five microlitres of the amplified PCR products was analyzed on 1.5% agarose gel containing ethidium bromide in Tris EDTA buffer. Electrophoresis was performed at 90v for 60 minutes. After electrophoresis the PCR products were visualized by Wealth Dolphin Doc UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard of the maker.

3.11 Statistical Analyses:

Results obtained were analyzed with Bivariate analysis of variance (ANOVA) using GraphPad InStat version 3.05 statistical packages.

CHAPTER FOUR

RESULTS

4.1: **Prevalence of surgical wound infection in UBTH and NAUTH**

A total of three hundred and sixty two (362) post operative wound swabs specimens from hospitalized patients at UBTH and NAUTH were analyzed. One hundred and eighty one (181) patients from each location were investigated. Irrespective of location a total of 122 (33.7%) of patients studied had wound infection. A statistically higher prevalence (39.8%) of wound infection was recorded among patients in NAUTH. The prevalence of wound infection among study participants in UBTH was 27.6% (Table 4.1).

4.2 Distribution of etiologic agents of wound infection

Pseudomonas aeruginosa and Escherichia coli were the most isolated pathogens associated with wound infection in UBTH and NAUTH respectively (P=0.001). There was no significant difference in the prevalence of *E. coli* isolated from UBTH (34%) and NAUTH (52.8%). However, *P. aeruginosa* showed significantly higher prevalence of (60.0%) in UBTH as compared to NAUTH (36.1%) with P<0.0.05 (Table 4.2).

4.3 Antibiotic susceptibility profile of clinical isolates

The antibiotic susceptibility profile of clinical isolates from surgical wound infections in both locations showed that among UBTH samples, the isolates were more resistant to Cefixime (94%) while in NAUTH the isolates exhibited more resistant to Cefixime and Augmentin with 94% each (Table 4.3).

Table 4.1: Prevalence of surgical wound infection in UBTH and NAUTH

Locations	No. examined	No. infected (%)	OR	95%CI
UBTH	181	50(27.6)	0.569	0.365,0.890
NAUTH	181	72(39.8)	1.756	1.120,2.798
T0TAL	362	122 (33.7)		

Keys:

N =Number of sample examined

OR= Odd ratio

Cl = confidence interval

Table 4.2: Distribution of etiologic agents of wound infection

Locations	No. infected	*E. coli* N (%)	*P.aeruginosa* N (%)	*Proteus mirabilis* N (%)	*S.aureus* N (%)
UBTH	50	17(34.0)	30 (60.0)	0(0.0)	3(6.0)
NAUTH	72	38(52.8)	26(36.1)	2(2.8)	6(8.3)
p value		0.062	0.008	0.643	0.894

Keys:

N = Total number of bacteria infected (%)

P = P<0.05were considered significant

Class of Antibiotics	Type of antibiotics	UBTH (%) No. tested =50		NAUTH (%) No. tested = 72	
		R	S	R	S

Table 4.3: Susceptibility profile of isolates to tested antibiotics

Penicillin	Augmentin (30µg)	34(68)	16(32)	68 (94)	4 (6)
	Oxacillin (4µg)	0(0)	3(100)	1(17)	5(83)
Macrolides	Erythromycin (10µg)	0(0)	3(100)	1 (17)	5 (83)
Aminoglycoside	Gentamycin (30µg)	21 (42)	29(58)	38 (53)	34(47)
Cephalosporin	Ceftazidime (30µg)	24 (48)	26(52)	54 (75)	18(25)
	Cefuroxime (30µg)	34(68)	16(32)	57 (79)	15(21)
	Cefixime (5µg)	47(94)	3(6)	68 (94)	4 (6)
Carbapenem	Imipenem (30µg)	7(14)	43(86)	12 (17)	60(83)
Quinolones	Ofloxacin (5µg)	27(54)	23(46)	42(58)	30(42)
	Ciprofloxacin(5µg)	26 (52)	24(48)	50 (69)	22(31)

Keys:

No. = Number of bacteria tested

R= Number of bacteria resistant **(%)**

S= Number of bacteria sensitive **(%)**

4.4: Percentage Antibiogram of bacteria from the two locations

P. aeruginosa (90%) exhibited the highest resistance to antibiotics among isolates from UBTH while *E. coli* revealed highest resistance of 95% among the isolates from NAUTH (Table 4.4).

4.5 Prevalence of multi-drug resistant isolates by Kirby Bauer method

The prevalence of multidrug resistant isolates was higher in NAUTH (85%) when compared to UBTH 78% (Tables 4.5).

4.6 Plasmid profile of multidrug resistant surgical wound isolates

Bacterial isolates from surgical wound infections seen at UBTH were found to harbor more plasmids (72%) compared with those from NAUTH (66%). However, the difference in prevalence rate of plasmids among isolates from the two study institution was not statistically significant (P=0.329) (Table 4.6).

4.7 Plasmid curing profile of multidrug resistant isolates

Isolates harboring plasmid from both locations were cured of their plasmids (100.0%) respectively (Table 4.7)

4.8 **Antibiotics resistance profile of 28 chromosomally mediated MDR UBTH and 40 chromosomally mediated MDR NAUTH.** The antimicrobial resistance profile of Gram negative isolates from UBTH and NAUTH were not found to differ significantly against Augmentin, Gentamycin, Cefuroxime, Cefixime,Ofloxacin,Ciprofloxacin, and Imipenem (P >0.05). With respect to Ceftazidime however, isolates from NAUTH were found to have a significantly higher resistance profile as compared with those of UBTH (P = 0.044). This table also shows the antimicrobial resistance profile of Gram positive isolates from NAUTH to Oxacillin and Erythromycin (Table 4.8).

5.

Table 4.4: Percentage Antibiogram of bacteria from the two locations

	UBTH			NAUTH		
ISOLATES	N	R (%)	S (%)	N	R (%)	S (%)

P.aeruginosa	30	27 (90)	3(10)	26	24 (92)	2(8)
E.coli	17	12 (70.6)	5(29.4)	38	36(95)	2(6)
Proteus mirabilis	0	0 (0)	0 (0)	2	0 (0)	2(100)
Staph aureus	3	0 (0)	3 (100)	6	1(16.7)	5(83)
Total	50	39 (78)	11(22)	72	61(84.7)	11(18)

Keys:

N=Number of isolates

R= Number of isolates resistant **(%)**

S= Number of isolates sensitive (**%)**

Table 4.5: Prevalence of multi-drug resistant isolates by Kirby Bauer method

Locations	No. of isolates	No. with MDR (%)	OR	95%CI	P Value

UBTH	50	39(78.0)	0.982	0.492,2.106	1.000
NAUTH	72	61(85.0)	1.182	0.475,2.031	
Total	122	100(163.0)			

Keys:

No. =Number of isolates tested

P = P<0.05 were considered significant

N (%) = Percentage number of multidrug resistant isolates

Table 4.6: Plasmid profile of multidrug resistant surgical wound isolates

Locations	N	N.WT.P (%)	N.W.P. (%)	OR	95%CI	P value
UBTH	39	28(72)	11(28.2)	0.589	0.219,1.560	0.329
NAUTH	61	40(66)	21(34.4)	1.708	0.641,4.552	

Keys:

P = P<0.05 were considered significant

N=Number of isolates

N.W.P= No. with Plasmid

N.WT.P = No. without plasmid

Table 4.7: Plasmid curing profile of multidrug resistant bacteria isolates

Locations	No. with plasmid	No. cured (%)
Locations		
UBTH	11	11(100)
NAUTH	21	21(100)

Keys

No. =Number with plasmid

N=Number cured (%)

Table 4.8: Antibiotics resistance profile of 28 chromosomally mediated MDR UBTH and 40 chromosomally mediated MDR NAUTH bacteria isolates from surgical wound infection.

ANTIBIOTICS	N	No. Pos (%)	OR	95% CI	P. VALUE
AUGMENTIN					
UBTH	28	21(75.0)	1.38	0.378,3.425	1.000
NAUTH	40	29(72.0)			
GENTAMYCIN					
UBTH	28	13(46.4)	0.978	0.218,1.533	0.326
NAUTH	40	24(60.0)	1.731	0.6523,4.592	
CEFTAZIDIME					
UBTH	28	17(60.7)	0.272	0.086,0.864	0.044
NAUTH	40	34(85.0)	3.667	1.158,11.613	
CEFUROXIME					
UBTH	28	27(67.5)	1.444	0.240,8.49	1.000
NAUTH	40	36(90.0)	0.692	0.117,4.069	
CEFIXIME					
UBTH	28	27(96.4)	1.421	0.122,16.488	1.000
NAUTH	40	38(96.4)	0.7037	0.061,8.165	
OFLOXACIN					
UBTH	28	17(43.6)	0.832	0.307,2.280	0.801
NAUTH	40	26(42.6)	1.202	0.445,3.263	
CIPROFLOXACIN					
UBTH	28	19(67.9)	0.13	0.207,1.816	0.143
NAUTH	40	31(79.5)	1.632	0.551,4.875	
IMIPENEM					
UBTH	28	7(25.0)	0.500	0.137,1.795	0.367
NAUTH	40	10(10.0	2.00	0.557,7.179	
OXACILLIN					
UBTH	0	0 (0.0)	ND	ND	ND
NAUTH	1	1 (100.0)	0.(0.0)	0.(0.0)	ND
ERYTHROMYCIN					
UBTH	0	0 (0.0)	ND	ND	ND
NAUTH	1	1 (100.0)	0.(0.0)	0.(0.0)	ND

Key:

N= Number of isolates tested, **ND** = Not detected

No. pos = Number of isolates resistant to each antibiotics (%)

4.9 Phenotypic and Molecular characterization of chromosomal mediated multidrug resistant clinical isolates from UBTH and NAUTH

Molecular characterization revealed that multidrug resistant isolates (*P.aeruginosa, E.coli and S.aureus*) associated with surgical wound infection had same prevalence in NAUTH as compared with those from UBTH (100%)(Table 4.9).

4.10 Prevalence of resistance genes expressed

All 21 multidrug isolates from UBTH (100.0%) were found to express TEM gene as against 24(82.8%) out of 29 isolates from NAUTH. Indeed, Statistics however failed to show any significant difference in rate of expression of gene (P = 0.06). Similarly all Multi-drug resistant bacteria isolates in UBTH resistant to flouroquinolones were observed to express gyrA gene, as against 18(69.2%) out of 26 of the isolates resistant to flouroquinolones in NAUTH (P = 0.014) (Table4 .10).

4.11 Prevalence of expressed genes among *Escherichia coli* isolates

The prevalence of expressed genes among *E.coli* with respect to isolates all (100%) *Escherichia coli* isolates resistant to Augmentin in UBTH were found to express the TEM genes, in contrast to 9(69.0%) that was expressed respectively in NAUTH. With regards to expression of TEM gene, no statistically significant difference was observed between the two locations of MDR studied (P=0.249).A similar case was observed with the expression of gyrA gene in this study. Expression of gyrA gene by *E coli* was higher among MDR isolates in UBTH (100.0%) than value obtained in NAUTH (56.3%). The differences in expression of gyrA gene was found not significant (P=0.046) (Table 4.11).

Table4.9: Phenotypic and Molecular characterization of chromosomally mediated multidrug resistant clinical isolates from UBTH and NAUTH.

UBTH	PHENOTYPIC	MOLECULAR
P. aeruginosa N = 19	19(100)	19(100)
E. coli N=9	9 (100)	9 (100)
S.aureus N= 0	0 (0)	0 (0)
NAUTH *P. aeruginosa* N = 20	20 (100)	20 (100)
E. coli N= 19	19 (100)	19 (100)
S.aureus N= 1	1 (100)	1 (100)

Keys:

N = Number of isolates

P = Number identified phenotypically (%)

M = Number identified molecularly (%)

Table 4.10: Prevalence of resistance genes expressed

RESISTANCE GENES **LOCATIONS**	N	No_Expressed (%)	OR	95% CI	P value
TEM					
UBTH	21	21(100.0)	9.653	0.504,185.01	0.6
NAUTH	29	24(82.8)	0.104	0.005,1.986	
gyrA					
UBTH	17	17(100.0)	16.081	0.861,300.20	0.014
NAUTH	26	18(69.2)	0.0621	0.3425,1.567	

Keys

N=Number of isolates resistant to Augmentin (TEM) and Ofloxacin (gyrA).

CI=Confidence interval.

OR=Odd ratio.

P = P<0.05 were considered significant

No. = No. Positive expressed (%)

Table 4.11: Prevalence of expressed genes among *Escherichia coli* isolates

RESISTANCE GENES LOCATIONS	N	No. Pos expressed (%)	OR	95% CI	P value
TEM					
UBTH	7	7(100.0)	7.105	0.328,153.88	0.249
NAUTH	13	9(69.2)	0.142	0.006,3.048	
gyrA					
UBTH	6	6(100.0)	0.061	0.861,300.20	0.046
NAUTH	16	9(56.2)	16.467	0.793,341.61	

Keys

N=Number of *E.coli* isolates resistant to Augmentin (TEM) and Ofloxacin (gyrA).

CI=Confidence interval.

OR=Odd ratio.

P = P<0.05 were considered significant

No. = No. Positive expressed (%)

4.12: Prevalence of resistance genes among *P.aeruginosa* isolates

Among the 14, *P. aeruginosa* MDR isolates studied in UBTH, 14(100.0%) were observed to express TEM genes and in NAUTH, 15(93.8%) also expressed TEM genes respectively. Statistics however did not show any significant difference in expression rates of TEM (P=1.000) among isolates from both locations of study. The rate of Expression of gyrA genes among the population of MDR in UBTH and NAUTH was similar such that UBTH was (100.0%) while NAUTH was 90% (Table 4.12).

4.13: Prevalence of Blaoxa resistant genes among *Staphylococcus aureus* isolate

Among the multidrug resistant *S.aureus* isolated in NAUTH, the expression of Blaoxa gene was found to be (100%) in the bacterial isolates (Table 4.13).

4.14: Prevalence of ereA resistant genes among *Staphylococcus aureus* isolate

Among the multidrug resistant *S.aureus* isolated in NAUTH, the expression of ereA gene was found to be (100%) in the bacterial isolate (Table 4.14).

4.15 Plasmid DNA profiles of multidrug resistant bacteria isolates from UBTH.

Plates 4.1a, b, and c: Shows Plasmid DNA profiles of multiple drug resistant bacteria isolates from surgical wound patients in UBTH. These were analyzed with 0.8% agarose gel electrophoresis, stained with ethidium bromide. L is 100-1000bp molecular marker. Isolates 8, 23, 52, 55, 63, 84, 104, 119,154,113 and 124 all possessed plasmids genes with bands ranging from 700 to 1200bp.

4.16 Plasmid DNA profiles of multidrug resistant bacteria isolates from NAUTH.

Plates 4.2a, b, c, d and e: Shows Plasmid DNA profiles of multiple drug resistant bacteria isolates from surgical wound patients in NAUTH. These were analyzed on a 0.8% agarose gel electrophoresis, stained with ethidium bromide. L is 100-1000 base pair molecular marker Isolates 195, 199, 204, 205, 207, 344, 353, 211, 212, 219, 232, 254, 217, 221, 273, 242, 240, 215, 299, 322, and 320 were positive for plasmid genes with bands ranging from 450 to 1000 base pair.

Table 4.12: Prevalence of resistance genes among *P.aeruginosa* isolates

RESISTANCE GENES LOCATIONS	N	No Pos (%)	OR	95% CI	P. value
TEM					
UBTH	14	14(100.0)	2.806	0.106,74.624	1.000
NAUTH	16	15(93.8)	0.003	0.001,0.082	
gyrA	11	11(100)	3.632	0.132,99,927	
NAUTH	10	9(90.0)	0.275	0.01,7.577	0.476

Keys:

N = Number of *P.aeruginosa* isolates resistant to Augmentin (TEM) and ofloxacin (gyrA)

Cl = confidence interval

OR = Odd ratio

P = P<0.05 were considered significant

No. = No. Positive expressed (%)

Table 4.13: Prevalence of Blaoxa resistant genes among *Staphylococcus aureus* isolate

RESISTANCE GENE LOCATIONS	N	No Pos (%)	OR	95% CI	P value
Blaoxa					
UBTH	0	0(0.0)	ND	ND	ND
NAUTH	1	1(100.0)	ND	ND	

Keys:

N = Number of ***Staphylococcus aureus*** isolate resistant to oxacillin (Blaoxa)

Cl = confidence interval

OR = Odd ratio

ND =Not detected.

P = P<0.05 were considered significant

No. = No. Positive expressed (%)

Table 4.14: Prevalence of ereA resistant genes among *Staphylococcus aureus* isolate

Locations	N	No Pos (%)	OR	95% CI	P value
ere A					
UBTH	0	0(0.0)	ND	ND	ND
NAUTH	1	1(100.0)	ND	ND	

Keys:

N = Number of *Staphylococcus aureus* isolate resistant to Erythromycin **(ereA)**

Cl = confidence interval

OR = Odd ratio

ND =Not detected.

P = P<0.05 were considered significant

No. = No. Positive expressed (%)

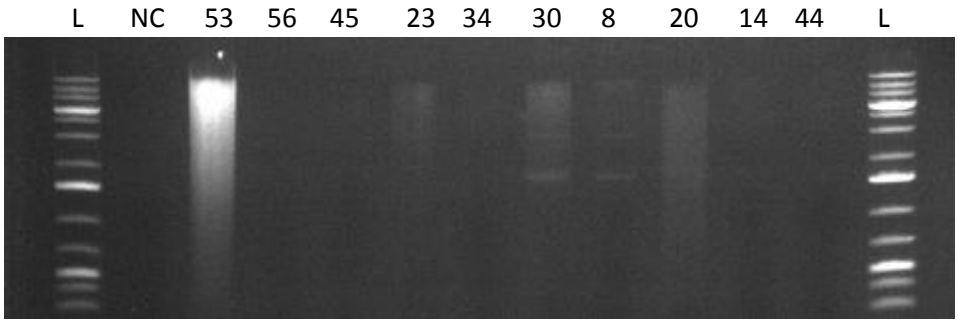


Plate 4:1a

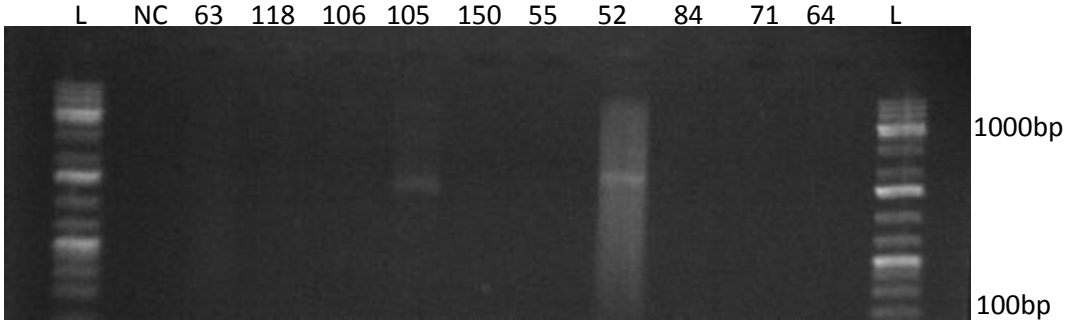


Plate 4:1b

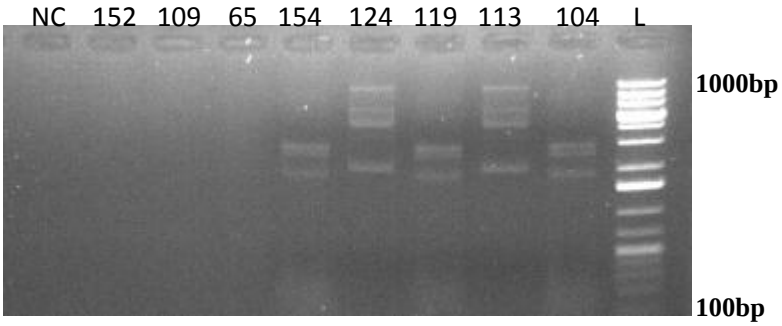
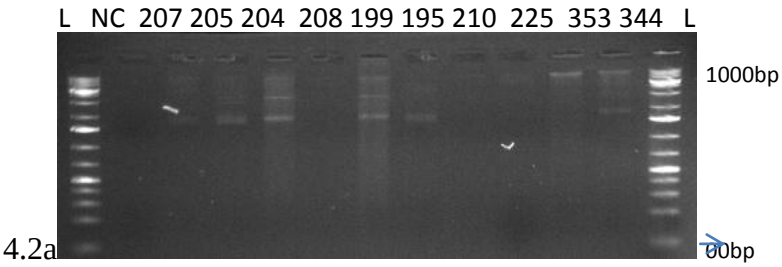


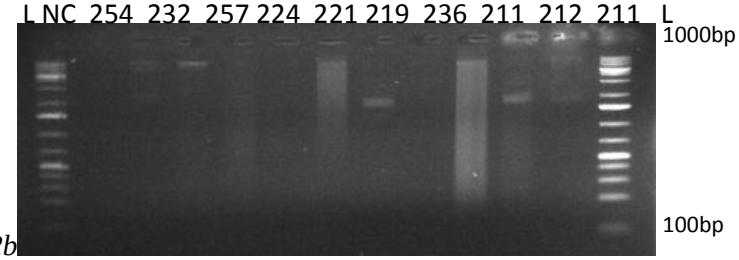
Plate 4:1c

Plates: 4.1a – 4.1c shows the plasmid DNA profiles from UBTH.

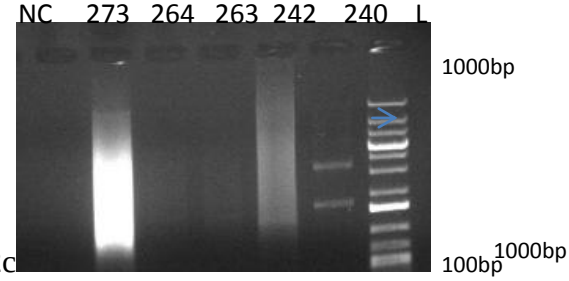
Plates 4.1a, b, and c: L was the DNA molecular marker (100bp-1000bp ladder) and NC as the no plasmid DNA template control. Samples were positive for plasmid genes at **plate a** 8 and 23 at 750bp, **plate b** 52 and 55 at 700bp, 63 and 84 at 1200bp, **plate c** 104, 119 ,154 had double bands at 900bp and 970bp with 113 and 124 have double bands at 950bp and 1000b.



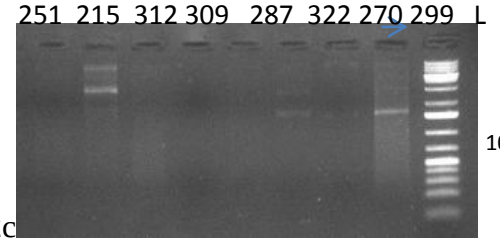
4.2a



4.2b



4.2c 1000bp



4.2c

4.2e

Plates: 4.2a – 4.2e shows the plasmid DNA profile from NA**UTH.**

(a) Lane 353 with band above 1000bp, 195, 199, 204, 205 and 207 are positive at 800bp, and 344 with double bands at 750bp and above 1000pb. (b)Samples 211,212, and 219 are positive for plasmid genes at 900bp, 232 and 254 bands above 1000bp and while sample 217 and 221 has a supercoiled plasmid ranging from 1000bp. (c) Lane 240 is positive for plasmid gene with double bands at 450bp and 670bp, samples 242 and 273 are supercoiled plasmid with bands ranging from 900bp to 200bp.(d) Lanes 299 and 322 are positive for plasmid genes with band at 850bp, sample 215 with double bands at 950bp and above 1000bp.(e) Sample 320 is positive for plasmid genes with band above 1000bp.

4.17 Molecular Identification of multidrug resistant bacteria isolates from UBTH and NAUTH using *Pseudomonas* genus and species oligonucleotide primers.

Plates 4.3 (a and b): Shows the Polymerase chain reaction results for bacterial isolates from UBTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. (L is 100-1000kb DNA molecular marker).Nineteen Multidrug resistant bacteria isolates were positive to *Pseudomonas genus* primer with bands at 580bp.

Plates 4.4 (a and b): Shows the Polymerase chain reaction results for clinical bacterial isolates from UBTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. (L is 100-1000kb DNA molecular marker). Nineteen Isolates were positive for *Pseudomonas aeruginosa primer* with bands at 1000 base pair.

Plates 4.5 (a, b and c): Shows the Polymerase chain reaction results for clinical bacterial isolates from NAUTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. (L is 100-1000kb DNA molecular marker).Twenty Multidrug resistant bacteria Isolates were positive to *Pseudomonas genus primer* with bands at 1000 base pair.

Plates: 4.6 (a and b) Shows the Polymerase chain reaction results for clinical bacterial isolates from NAUTH analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. (L is 100-1000kb DNA molecular marker).Twenty Isolates were positive for *Pseudomonas aeruginosa* with bands at 1000 base pair.

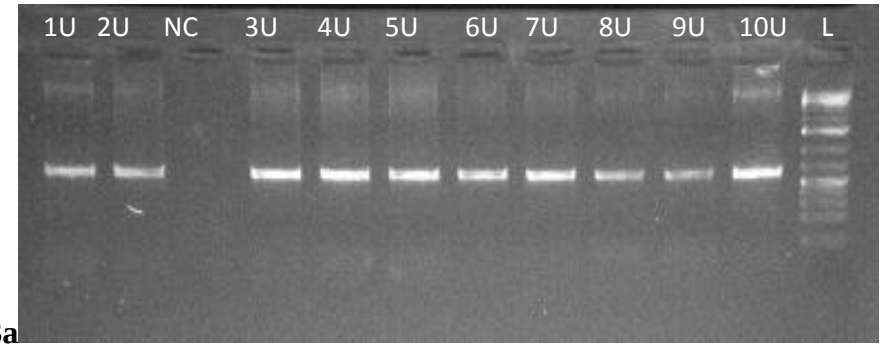


Plate4.3a

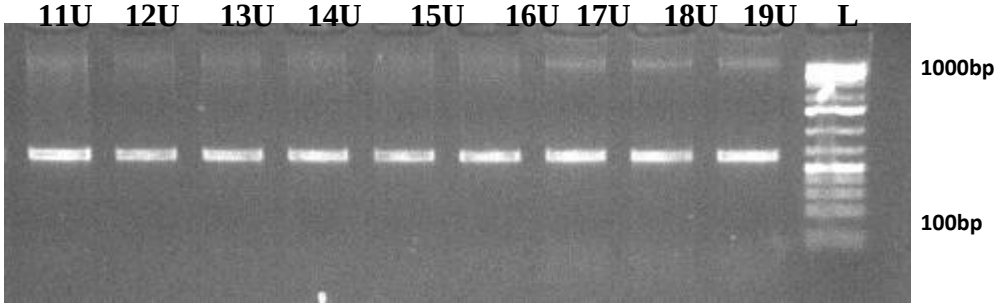


Plate4.3b

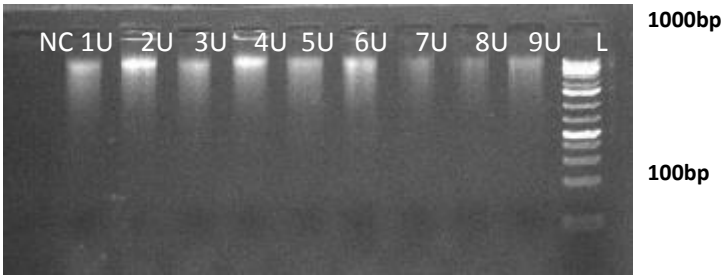


Plate4.4a

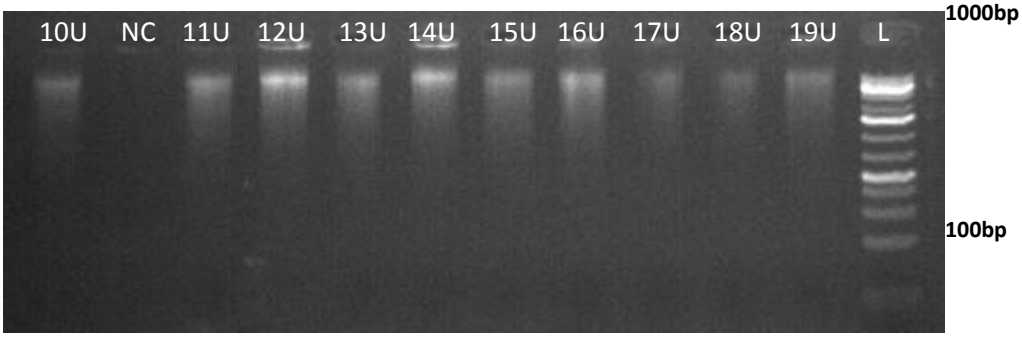


Plate4.4b

Plate 4.3a and b: L is 100bp-1kb DNA ladder (molecular marker). All samples 1U to 19U from UBTH are positive for ***Pseudomonas genus*** with bands at 580bp.

Plate 4.4a and b: L is 100bp-1kb DNA ladder (molecular marker). All samples 1U to 19U from UBTH are positive for ***Pseudomonas aeruginosa*** with bands at **1000bp.** NC is a no DNA template control.

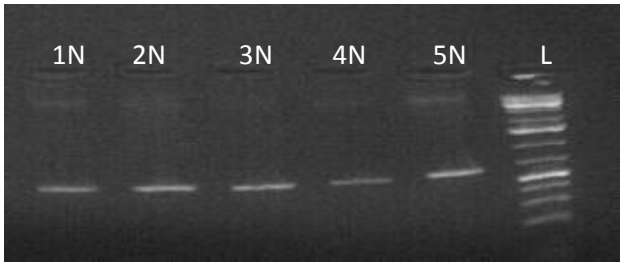


Plate4.5a

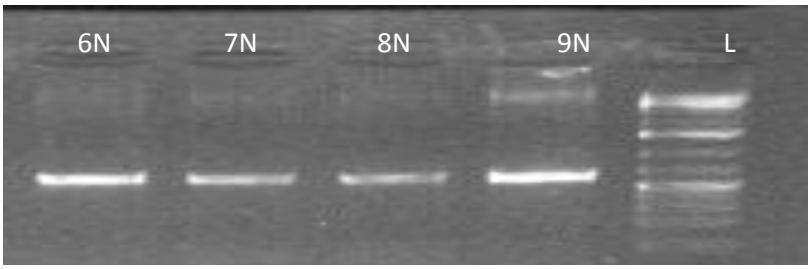


Plate4.5b

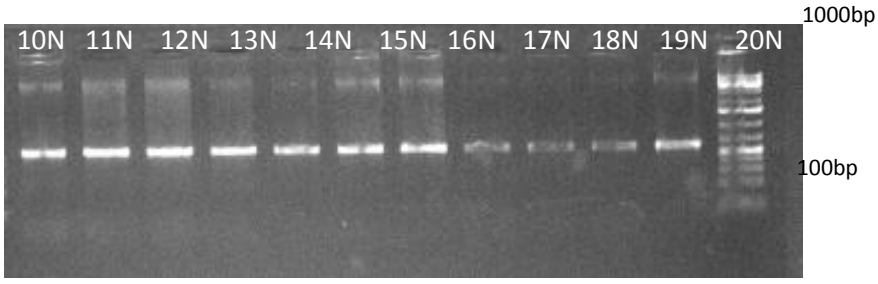


Plate 4.5c

Plate 4.5 a, b and c: Shows polymerase chain reaction results for clinical bacterial isolates from NAUTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Samples 1N to 20N are positive for ***Pseudomonas genus*** with bands at **580bp.**

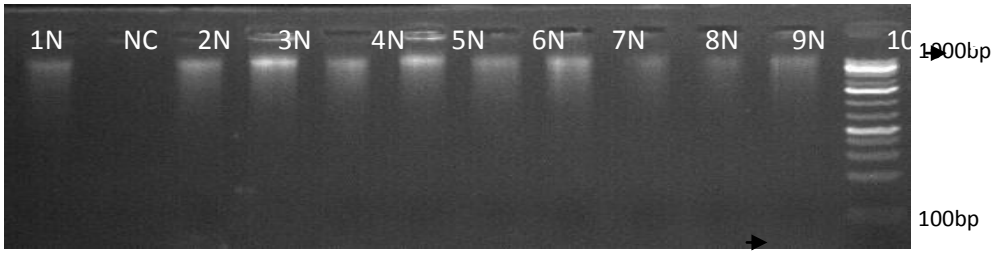


Plate 4.6a

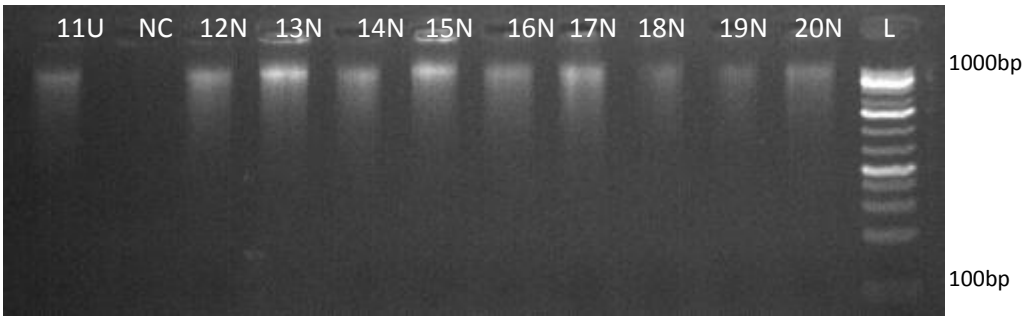


Plate 4.6b

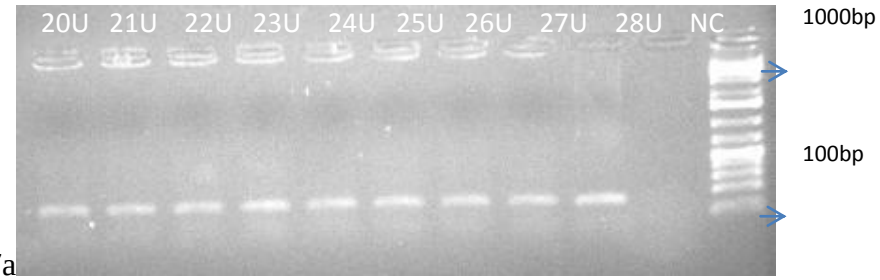
Plate4.6a and b: Shows Polymerase chain reaction results for *Pseudomonas* isolates from NAUTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Samples 1N to 20N are positive for ***Pseudomonas aeruginosa*** with bands at **1000bp**.

4.18 Molecular Identification of multidrug resistant bacteria Isolates from UBTH and NAUTH surgical wounds infections using *Escherichia coli* oligonucleotide primers.

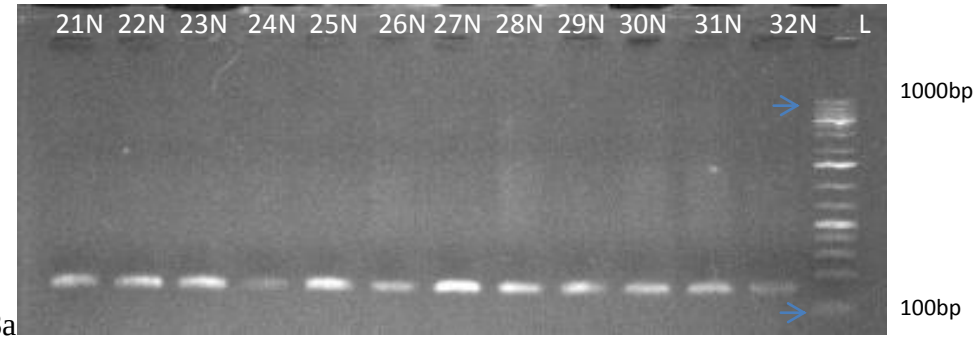
Plates 4.7a: Shows the Polymerase chain reaction results for clinical bacterial isolates from UBTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker. Nine Isolates were positive for ***Escherichia coli*** with bands at 160 base pair.

Plates 4.8a and b: Shows the Polymerase chain reaction results for clinical bacterial isolates from NAUTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. (L is 100-1000kb DNA molecular marker). 19 Isolates were positive for ***Escherichia* coli** with bands at 160 base pair.

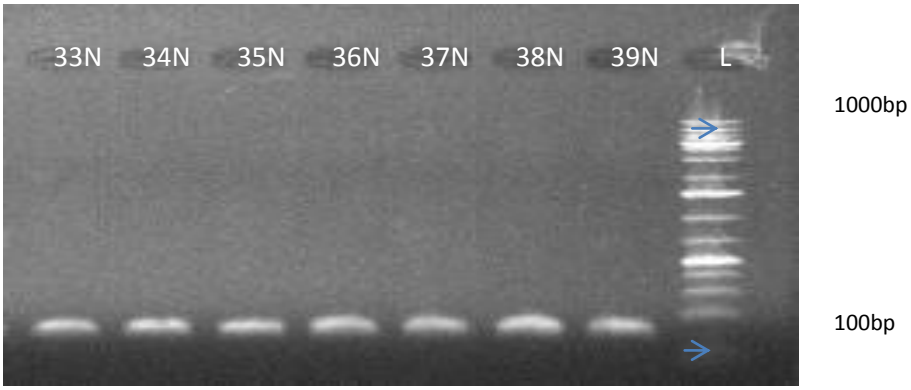
Plate4.9a and b: Polymerase chain reaction result for clinical *Staphylococcus* isolate from NAUTH analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Sample **(a)** 40N is positive for ***Staphylococcus genus*** with bands at 670bp and **(b)** ***Staphylococcus aureus*** with bands at 300bp.



4.7a



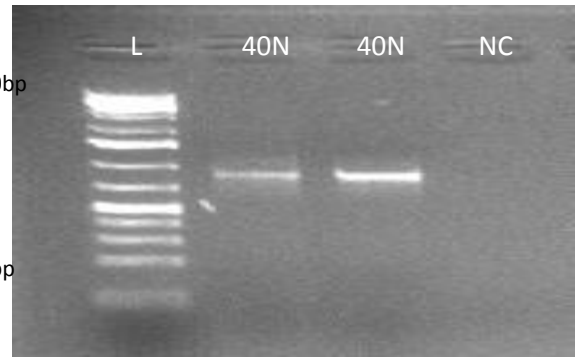
4.8a



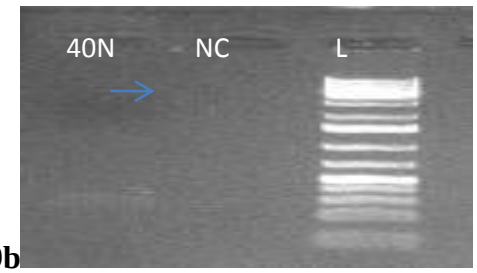
4.8b

Plate 4.7a Polymerase chain reaction results from UBTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. All Samples 20U to 28U are positive for *Escherichia coli* with bands at 160bp

Plate's 4.8a and b from NAUTH (21N to 39N *Escherichia coli*) are positive with bands at 160bp.



4.9a



4.9**b**

Plate4.9a and b: Polymerase chain reaction result for *Staphylococcus* isolate from NAUTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Sample **(a)** 40N is positive for ***Staphylococcus genus*** with bands at 670bp and **(b)** Positive for ***Staphylococcus aureus*** with band at 300bp.

4.19 Molecular detection of TEM resistant genes from chromosomally mediated multidrug resistant bacteria isolates from UBTH and NAUTH.

Plates 4.10a and b and plates4.11a and b: Shows the gene expression patterns for TEM genes detected at 650bp in *Pseudomonas aeruginosa* isolated from both locations analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker.

Plates 4.12a and 4.13a: Shows the genes expression patterns for TEM resistant genes detected at 650bp in *Escherichia coli* isolated from both locations analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker.

4.20 Molecular detection of gyrA resistant genes from chromosomally mediated multidrug resistant bacteria isolates from UBTH and NAUTH

Plate 4.14a and b and plate 4.15 a and b: Shows the gene expression patterns for gyrA genes detected in *Pseudomonas aeruginosa* from both locations analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker.

Plates 4.16a and b: Shows the genes expression patterns for gyrA genes detected in *Escherichia coli* isolated from both locations analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker.

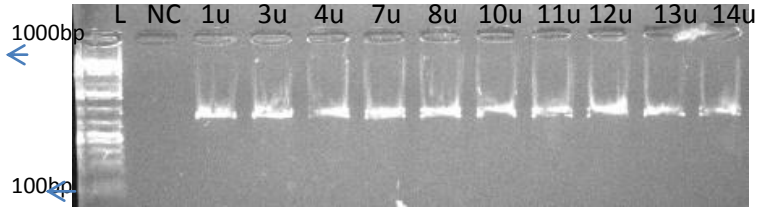


Plate 4.10a

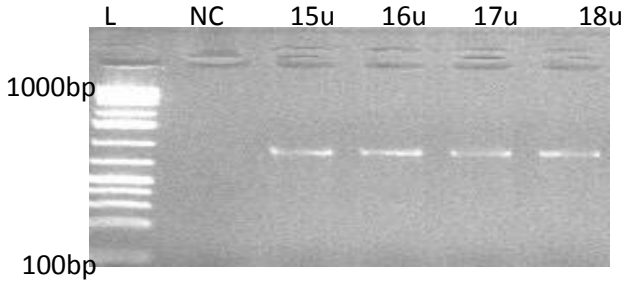
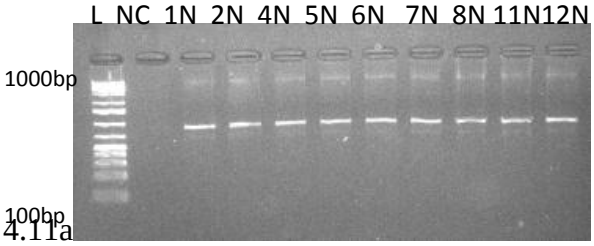


Plate 4.10b



4.11a

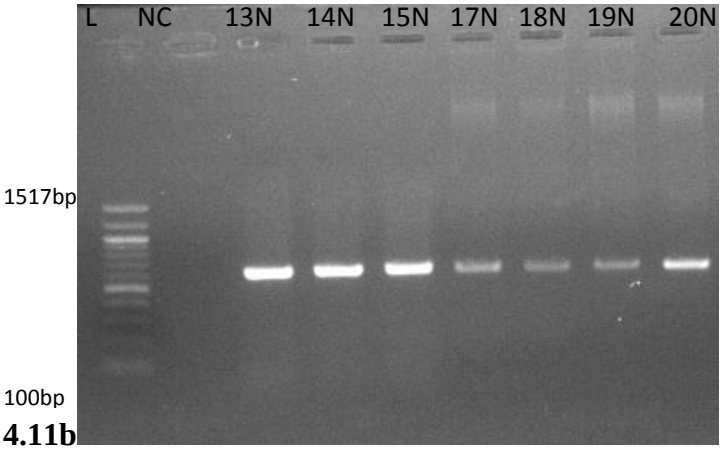


Plate 4.11b

Plates4.10a and b and plates4.11a and b: Shows TEM resistant genes detected in *Pseudomonas aeruginosa*. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Samples 1u to 18u from (UBTH) and 1N to 20N (NAUTH) are both positive for resistant genes with bands at 650bp.

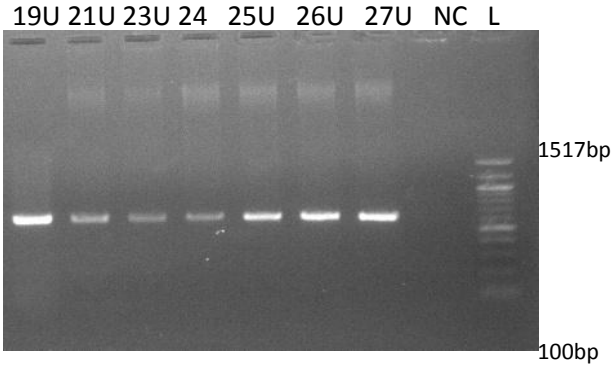


Plate 4.12a

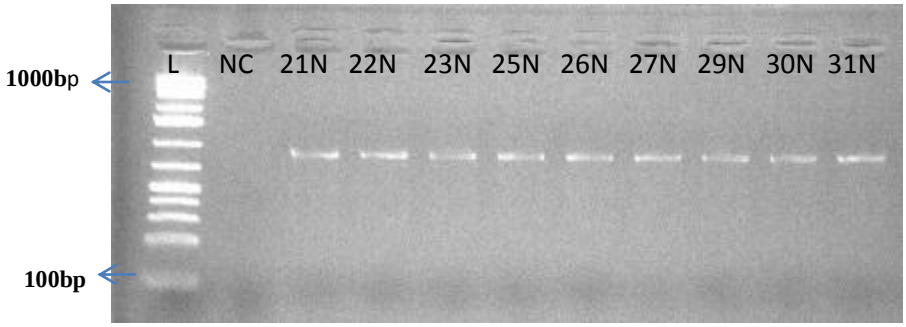


Plate 4.13a

Plates 4.12a and 4.13a: Shows Polymerase chain reaction results of TEM resistant genes detected in *Escherichia coli*. L is 100bp-1517bp DNA ladder (molecular marker). NC is a no DNA template control. Lanes 19u to 27u (UBTH) and 21N to 31N (NAUTH) are both positive for Augmentin (TEM) resistant genes with bands at 650bp.

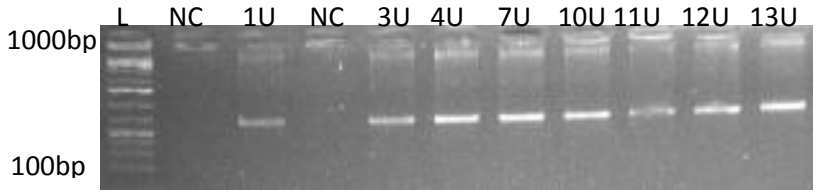


Plate4.14a

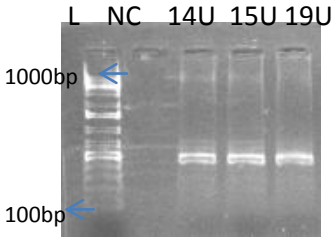


Plate4.14b

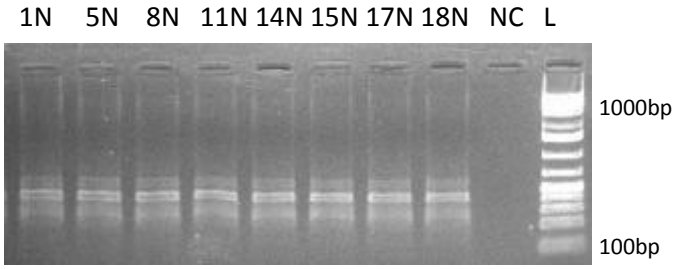


Plate4.15a

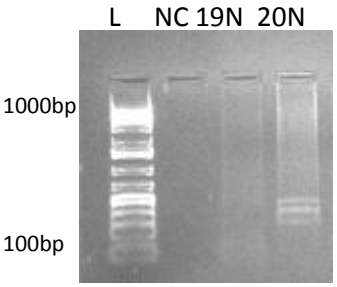


Plate 4.15b

Plate 4.14a and b and plate 4.15a and b: Shows polymerase chain reaction results of gyrA resistant genes detected *Pseudomonas aeruginosa*. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Samples 1U to 19U (UBTH) and 1N to 20N (NAUTH) are both positive for ofloxacin (gyrA) resistant gene with bands at 500bp. Negative to 19N.

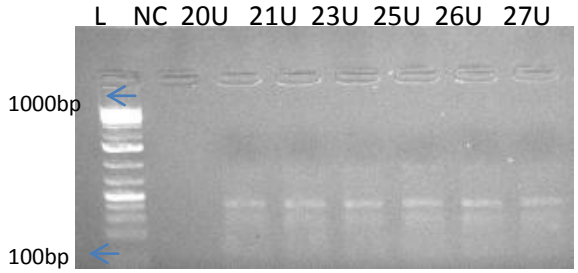


Plate4.16

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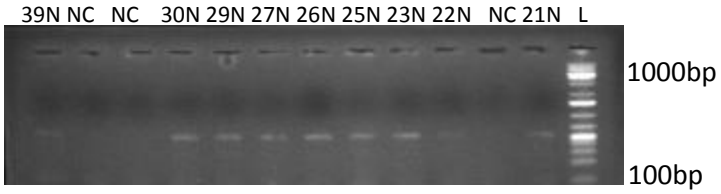

Plate4 .17

Plates 4.16 and plate17: Shows polymerase chain reaction results of gyrA resistant gene detected in *Escherichia coli* isolated. L is 100bp-1kb DNA ladder (molecular marker). NC is a know DNA template control. Samples 20U to 27U (UBTH) and 21N to 39N (NAUTH) are both positive for ofloxacin (gyrA) resistant gene with bands at 500bp.

4.21 Molecular detection of Blaoxa and ereA resistant genes from chromosomally mediated multidrug resistant bacteria isolates from NAUTH.

Plates 4.18: Shows the gene expression patterns for Blaoxa detected in *Staphylococcus aureus* isolated from NAUTH on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker. Positive to Blaoxa gene with band at 250bp.

Plates 4.19: Shows the gene expression patterns for ereA genes detected in *Staphylococcus aureus* isolated from NAUTH on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100-1000kb DNA molecular marker. NC is a no DNA template control. Positive to ere A gene with band at 90bp.

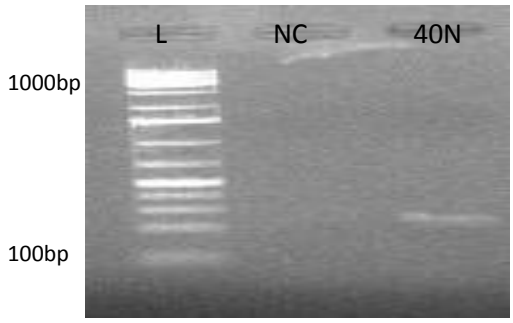

Plate 4.18

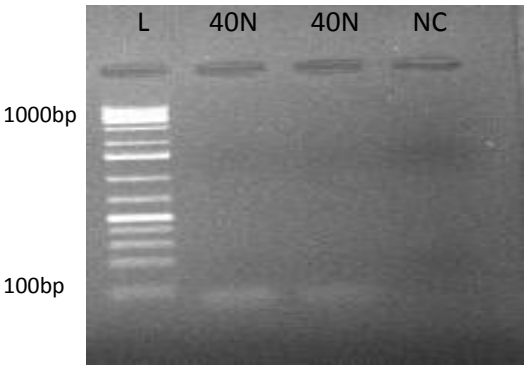

Plate 4.19

Plate 4.18: Polymerase results of Blaoxa resistant genes detected in *Staphylococcus aureus* from NAUTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Sample 40N is positive for Blaoxa resistant gene with band at 250bp.

Plate 4.19: Polymerase results of ere A resistant genes detected in *Staphylococcus aureus* from NAUTH. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Sample 40N is positive for ereA resistant gene with band at 95bp

CHAPTER FIVE

5. 1. DISCUSSION

Bacterial contamination of post operative wounds over the year has been a great threat in the hospitals and clinics. Contamination has been considered to be due to poor surgical operating procedure (ManGram *et al*., 1999). Surgical site infections depend on the host susceptibility, condition of the wound, and the amount and type of microbial contamination (Oni *et al.*, 2006). In most cases of surgical site infection the organism is usually patients' endogenous flora. In abdominal surgeries the opening of the gastrointestinal tract increases the likelihood of coliforms, Gram negative bacilli as agent of wound infection which was the finding in this study. These groups of organisms tend to be endemic in hospital environments by being easily transferred from object to object, they also tend to be resistant to commonly used antibiotics and antiseptics and are difficult to eradicate in the long term (Mofikoya *et al.,* 2009). In this study anaerobic organisms were not isolated from culture, despite measures taken to recover such organisms from surgical wounds infection. The probable reasons could be some patients received prophylactic metronidazole which kills anaerobes and the use of dry cotton wool swabs for specimen collection. This was in agreement with Sule *et al*., (2002) who documented that use of dry swabs for collection of specimens could hinder the isolation of anaerobes from wound infections.

A total of three hundred and sixty two (362) post operative wound swabs specimens from hospitalized patients at UBTH and NAUTH were analyzed. A total of 122 clinical bacterial isolates were isolated from both locations, giving overall prevalence of 33.7%. Although this is lower than an earlier report of 39.9% Mohammed *et al.,* (2013), it is still on the high side when compared to the result of Ezechi *et al.,* (2009) who reported a prevalence rate of 9.6%. However,

World Health Organization in 2011 gave a prevalence of 5 – 34% of SSI and this is in line with the result of this study. One hundred and eighty one (181) patients from each location were investigated. Irrespective of location a total of 122 (33.7%) of all patients studied had wound infection. A statistically higher prevalence (39.8%) of wound infection was recorded among patients in NAUTH while the prevalence of wound infection among study participants in UBTH was (27.6%). Though this is lower than that of Reiye, *et al*. (2014) who reported a prevalence rate of 75% in surgical wounds, it is higher than that of Sule *et al*., (2002) who reported a prevalence rate of (11%).

The distribution of etiologic agents of surgical wound infections showed that there was a variation in distribution of the isolates from the two locations with *E. coli* and *P. aeruginosa* ranking highest in distribution and this is in agreement with the works of Ezebialu *et al*., (2010) and Sulochana *et al*., (2014) who found *P. aeruginosa* and *E. coli* to be more prevalent in surgical site infections; even though other bacteria isolates such as *S. aureus* had predominated in other studies (Aisha, *et al.*, 2013; Christopher *et al.*, 2011 and Ojo *et al*., 2014). *E. coli* is reported extremely predominant in hospital acquired infections worldwide making it the first nosocomial pathogen (Teresa *et al*., 1986). In a study of health care associated infectious in Nigeria, Nejad *et al.*, (2011) *E. coli* (34.4%) was the predominant isolate from surgical wounds infections. However, in other studies Aisha, *et al.,* (2013); Dalhatu *et al.*, (2014) *P. aeruginosa* have been mostly recovered from post operative surgical wounds despite the site of infection and location of specimens due to its high survival characteristics in hospital environment. It is known to rank second among nosocomial pathogens isolated from hospitals, often contaminating hospital equipments such as wound dressing sinks and other surgical apparatus (Masaadeh *et al.,* 2009). Even antibiotic resistant strains can survive in supposedly sterile equipments used in the

hospitals making it a dangerous nosocomial pathogen widely distributed in the hospital environments where they are particularly difficult to eradicate.

All antibiotics used in this study displayed similar level of resistance to the isolates. This high resistance the isolates showed to the various antimicrobial agents used in this study may in part be due to various factors such as inappropriate usage of antibiotics and drug resistance mechanism possessed by the bacterial isolates. Cephalosporins and Penicillins have been found to be highly resisted by surgical wound pathogens. In the works of Eduardo *et al.,* (2008) and Yah *et al.,* (2010) Ceftazidime and Augmentin were mostly resisted by surgical wound etiologic bacteria. This is most likely due to the presence of Cephalosporinase and Penicillinase enzymes produced by these organisms *E. coli* and *P. aeruginosa* which prevent the action of the Beta-lactam ring structure of the antibiotics (Livermore, 1995 and Fontana *et al.,* 2000).

Here, *P. aeruginosa* (90%) ranked highest in resistance to antibiotics among UBTH isolates followed by *E. coli* (70.6%) and *S. aureus*, (0%) while in NAUTH, *E. coli* (95%) displayed highest resistance capacity followed by *P. aeruginosa* (92%) and *S. aureus* (16.7%). There was a high preponderance of resistance by the isolates from the two locations to the antibiotics (UBTH=78%; NAUTH= 84.7%) and this is largely due to the fact that bacterial isolates develop resistance by different mechanisms such as ability to modify the antibiotics target site, possession of efflux pumps, presence of inhibiting enzymes, acquisition of resistant plasmids and mutation of the drug receptor sites. The high prevalence of isolates resistant to antibiotic from NAUTH (84.7%) is probably due to poor hygienic practice among people living in this area which may have been the cause of high rate of multidrug bacterial surgical wound infections among patients in NAUTH. Akoachere *et al*., (2014) opined that bacterial infections of surgical wounds is attributed to overcrowding of hospital wards and lack of basic facilities for standard

hygiene condition which is common in sub-Saharan African countries including Nigeria. Prevalence of multidrug resistant isolates was found to be drastically high in both locations (UBTH= 78%, NAUTH= 85%; OR= 1.182; P value= 1.000). This high resistant pattern is an indication of the multidrug resistant strategies possessed by the clinical bacteria isolates. Many studies have been carried out on multidrug resistant bacteria and such resistance maybe due to the high selective pressure exerted on bacteria due to numerous reasons like poor adherence to hospital antibiotic policy and excessive and indiscriminate use of broad-spectrum antibiotics (Garba *et al.,* 2012; Akoachere *et al*., 2014). The odd ratio indicates that patients from both locations have equal chances of being infected with multidrug resistant strains.

This study reported the plasmid profile of multidrug resistant surgical wound isolates from the two locations and found that NAUTH (34.4%) isolates had a higher prevalence of plasmid mediated multidrug resistance than those from UBTH (28.2%). However, isolates from UBTH (72%) showed that resistance due to chromosomal mediation were higher compared to those from NAUTH (66%) indicating that statistic failed to showed significant difference among isolates with plasmid and isolates without plasmid from both locations (P value=0.329). This once again is highly attributed to poor hygienic practices by people living in these areas giving room to frequent acquisition of resistant plasmid and chromosome genes among bacterial isolates from surgical wound infections (Enabulele *et al.,* 2006). Bacteria resistance can be expressed through their ability to colonize new hospital environments where selective pressure prevails. Some opportunistic pathogens such as *E. coli* and *P. aeruginosa* are able to adapt to this new environment through the acquisition or development of mechanisms of resistance and persistence (Alejandro *et al.,* 2013). Multidrug resistance reported in this study is worrisome especially because the two most isolated surgical wound bacteria {MDR UBTH= *E.coli* (70.6%), *P. aeruginosa* (90%); MDR NAUTH= *E.coli* (95%), *P. aeruginosa* (92%) from both locations

possessed a very high percentage of Multidrug resistant genes. Isibor *et al*., (2013) reported a high percentage of multidrug resistance of *P. aeruginosa* isolate among aerobic bacterial isolates associated with diabetic wounds. While *E. coli* was the third highest multidrug resistant bacteria among Gram negative isolates as reported by (Girma *et al.,* 2013). All plasmid borne multidrug resistant bacteria isolates from surgical wounds infection in this study were cured of their plasmid upon treatment with Sodium Dodecyl Sulphate. This made them susceptible to the drugs to which they where once resistant as they have now lost their resistant markers. The importance of plasmid curing in the investigation of etiologic agent of diseases as a chemotherapeutic approach in hospitals cannot be overemphasized as it has improved the policy of antibiotics administration in chemotherapy Girma *et al.,* (2013). Thus, in cases of multidrug resistance in hospitals, the need for the knowledge of the resistant tendency of surgical wound bacteria isolates in chemotherapy is of paramount importance in order to discourage blind therapy. Post operative surgical procedures have been greatly implicated in Gram negative bacterial contamination of surgical wounds in which isolates rise to a substantial quantity ($> 10^5$cfu) at the surgical site making meticulous operative technique very paramount. Based on this also, used of prophylaxis administration of antibiotics before surgical procedure is also an implication of multidrug genes (Cruse *et al.,* 1980; Guiboux *et al.,* 1995).

In this study, the multi-drug resistant bacteria isolates were subjected to genetic identification using polymerase chain reaction method in order to give an in-depth genetic identification and the actual resistant pattern of MDR bacterial isolates from surgical wound infections. This helped in generating more accurate and conclusive reports on the molecular identity and the resistant patterns of MDR isolates to the antimicrobial drugs used.

The study revealed that *P.aeruginosa* had the highest incidence of all multidrug isolates, (39) in both locations, closely followed by *E.coli* (28) and lastly *S. aureus* (1). This implied that Gram negative opportunistic nosocomial pathogens were responsible for a wide range of infection associated with multidrug resistant patterns. Lambert, (2002) reported that *P.aeruginosa*, a Gram negative opportunistic nosocomial pathogen is responsible for a wide range of infections associated with antimicrobial resistance. It is also reported that the genome of *P.aeruginosa* is among the largest in the bacterial world allowing for great genetic capacity and high adaptability to environmental change via horizontal gene transfer (Slama, 2000). This showed that multidrug resistance pattern associated with Gram negative pathogens could result in increased morbidity and mortality. Georgios and Maria, (2013) opined that acquired resistant patterns of Gram negative pathogens was a consequence of mutational events associated with drug selective pressure. Also from this study, all sixty eight (68) phenotypically characterized MDR bacterial isolates from UBTH and NAUTH were confirmed molecularly to be (100%). This indicated high sensitivity and specificity of the molecular techniques used in screening and tracking of MDR bacterial isolates. Sibhghatulla *et al*, (2014) reported that molecular techniques undoubtedly had the potential to play an essential part in the laboratory setting for the screening, tracking and monitoring of spread of infective agents in the community and hospital setting.

All 21(100%) multi-drug resistant bacteria isolates from UBTH that were resistant to Augmentin were found to express TEM gene (at 650bp) as against 24 out (82.8%) of 29 the bacterial from NAUTH. Indeed, multi-drug resistant bacteria isolates in UBTH were more likely to express TEM gene when compared with those from NAUTH. Statistics, however failed to show any significant difference in the rate of gene expression ($P = 0.6$). Similarly, all 17(100%) multi-drug resistant bacteria isolates from UBTH that were resistant to Ofloxacin (flouroquinolones) were found to express gyrA genes at 500bp as against 18 (69.2%) of the bacteria isolates from

NAUTH. Interestingly, multi-drug resistant bacteria isolates in UBTH were more likely to express gyrA gene when compared with those from NAUTH (P = 0.014). This, however could be because UBTH isolates were more resistant to broad spectrum antibiotics due to constant exposure to the same. This relatively high level of resistance of the MDR to Augmentin and Ofloxacin clearly indicated misuse or abuse of these antibiotics in both locations. This often happens especially when antibiotic prescriptions in hospital are given without clear evidence of infection. When broad spectrum antibiotics are given in place of narrow spectrum antibiotics as substitute for culture and susceptibility testing the consequent risk is that of dangerous side effect (Prescott *et al*., 1999). Alekshun and Levy, (2007) reported that the continuous overuse and misuse of antibiotics including Augmentin and Ofloxacin in human medicine had contributed directly to the incidence of multi-drug resistant bacteria isolates. It has been reported that frequent exposure of bacteria strains to a multitude of β- lactam antibiotics including Augmentin has induced dynamic, continuous production and mutation of β-lactamase enzymes in bacterial isolates, expanding their activity even against newly developed β-lactam antibiotics (Paterson and Bonomo, 2005, Pitout and Lavpland, 2008).

All multi-drug *Staphylococcus aureus* isolates from NAUTH were found to express Blaoxa gene at 250bp and ereA gene at 95bp. This clearly indicated poor hygienic practices among health care providers including non-compliance to simple hand washing and overcrowding of the hospital wards which is common in most clinics and hospitals in Nigeria. Connie *et al..*, (2007) reported that the incidence of hospital-acquired methicilin resistant *Staphylococcus aureus* infection has been on the rise and it poses serious threat to health institutions. It is reported that health care providers-to-patient transfer is common with methicilin resistant *Staphylococci aureus* infection, especially when health care providers move from patient to patient without performing necessary hand-washing technique between patients (Tacconelli *et al.*, 2008).

Salgado *et al.,* (2003) opined that regular hand washing with soap and water and maintaining a clean environment will help in preventing hospital-acquired methicilin resistant *Staphylococcus aureus* infections. Also, as seen in this study, the prevalence of express genes for individual isolates were more prevalent in *P. aeruginosa* (≥90%) isolates from both locations. This may be due to the Multi drug resistant nature of *P. aeruginosa* especially as a nosocomial pathogen which is ubiquitous in hospital environments (Yimaz *et al.,* 2011).

5.2 CONCLUSION

This study employed the usage of polymerase chain reaction techniques in obtaining standard results from which positive evidence of multidrug resistant genes were established from both locations. However, this high incidence of multidrug resistant genes expressed in the bacterial isolates is very worrisome indicating that therapeutic failure can occur in both locations if appropriate measure is not taken. The overuse and misuse of antimicrobial chemotherapy may provide selective pressure for the spread of MDR strains of bacterial isolates. Therefore, efforts to promote the appropriate use of antimicrobials are paramount to avoid therapeutic failure in both locations. Thus, it is of essence to educate hospital staffs on the importance of good hygiene especially hand washing in order to curtail epidemiological spread of these hazardous bacteria in the environment. Though phenotypic bacteria characterization may be reliable but molecular method is conclusive

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5.3 Contribution to knowledge

1. The most predominant isolates from UBTH was *Pseudomonas aeruginosa* (60%)

2. The most predominant isolates from NAUTH was *Escherichia coli* (52%)

3. The susceptibility test result showed high level of multi-drug resistance in the two mostly isolated surgical wound bacteria pathogens.

4. The incidence of MDR bacterial isolate is higher in NAUTH when compared with UBTH.

5. The plasmid DNA profiling revealed that resistances to antibiotics were both plasmid and chromosome mediated.

6. Interestingly, the polymerase chain reaction results from this study revealed accurate and conclusive resistant gene expression patterns of MDR bacteria isolates to the respective antibiotics administered.

7. The study also revealed a life threatening resistant patterns of the MDR bacteria isolates, if measures are not taken to check the spread of these harmful pathogens.

8. The research showed clearly that *P. aeruginosa* and *E. coli* exhibited enormous MDR gene expression patterns.

9. Multi-drug resistant bacteria isolates in UBTH is more likely to express TEM and GyrA genes than those from NAUTH.

10. The study also showed that MDR strain of *S. aureus* isolated from NAUTH expressed the Blaoxa and ereA gene which is also an indicative of methicillin resistant *staph aureus.*

5.4 Recommendation

In other to curtail epidemic out-break of serious and harmful diseases, the following recommendations are made:

1. Molecular and phenotypic investigations of the suspected MDR bacteria isolates should be conducted before commencing treatment.

2. Treatment should be taken doggedly as prescribed by the physician.

3. Awareness campaigns, regarding the danger of MDR bacteria isolates to commonly used antibiotics should be made in our communities.

4. Good personal hygiene is highly recommended in order to curtail epidemiological spread of these hazardous bacteria.

5. A functional sanitary and waste management regulatory scheme should be established.

6. More research should be made towards developing new, cheap and available phenotypic based identification protocols for these microbial agents.

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APPENDIX A

COMPOSITION AND PREPARATION OF MEDIA USED

BLOOD AGAR BASE

Composition	Grams/litre
Hiveg infusion	10.00
Hiveg hydrosylate	10.00
Sodium chloride	5.00
Agar	15.00
Final pH (at 25^0C)	7.3±0.2

Directions

This was prepared by Suspending 40g in 1000ml of distilled water. The medium was heated to dissolve completely. It was then sterilized by autoclaving at 121^0C for 15 minutes. Cooled to 50^0C and aseptically 5% sterile difibrinated blood was added. The medium was well mixed and poured into sterile Petri dishes.

MACCONKEY AGAR

Composition	Grams/Litre
Peptone	20.00
Sodium chloride	5.00
Lactose	10.00
Bile chloride	5.00
Neutral red	0.05
Agar	13.50

Direction

This was prepared by weighing 52g of agar in the weighing balance in 1000ml of distilled water. This was dissolved with stirring. It was then sterilized by autoclaved at 121^0C for 15 minutes. The agar was allowed to cool to about 45^0c before poured into the sterile Petri dishes to solidify.

NUTRIENT AGAR

Composition	Grams/Litre
Peptone	5.00
Beef extract	1.00
Yeast extract	2.00
Sodium chloride	5.00
Agar	15.00
Final pH (at 25^0C)	7.4±0.2

Direction

This was prepared by weighing 28g of agar in the weighing balance in 1000ml of distilled water. This was dissolved with stirring. It was then sterilized by autoclaved at 121^0C for 15 minutes. The agar was allowed to cool to about 45^0c before poured into the sterile Petri dishes to solidify.

Mannitol Salt agar

Composition	Grams/Litre
Peptone	10.000
Meat extracts	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

Direction

This was prepared by weighing 110g of agar in the weighing balance in 1000ml of distilled water. This was dissolved with stirring. It was then sterilized by autoclaved at 121^0C for 15 minutes. The agar was allowed to cool to about 45^0c before poured into the sterile Petri dishes to solidify.

AMIES TRANSPORT MEDIUM

Composition	Grams/Liter
Charcoal pharmaceutical	10.0
Sodium chloride	3.0
Sodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Potassium chloride	0.2
Sodium thioglycollate	1.0
Calcium chloride	0.1
Magnesium chloride	0.1
Agar	4.0
Final pH (at 25°C)	7.2 + 0.2

Directions

This was prepared by suspending 20g in 1 liter of distilled water. It was then allowed to boil in order to dissolve the agar completely. This was distributed into small, screw cap bottles, while stirring to keep the charcoal evenly suspended. Screw down the caps firmly on the completely filled bottles. It was then sterilized by autoclaving at 121°C for 15 minutes. Bottles were inverted while cooling to distribute the charcoal uniformly and stored in a cool place.

Mueller-Hinton Agar

Composition	Grams/Liter
Beef extract	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25°C)	7.3 ± 0.1

Direction

This was prepared by weighing 38g of agar in the weighing balance in 1000ml of distilled water. This was dissolved with stirring. It was then sterilized by autoclaved at 121^0C for 15 minutes. The agar was allowed to cool to about 45^0c before poured into the sterile Petri dishes to solidify.

Preparation of McFarland Standard

A one percent volume by volume (1% v/v) solution of sulphuric acid was prepared by adding 1ml concentrated sulphuric acid to 99ml of water and mixed. 1% w/v solution of barium chloride was prepared by dissolving 0.5g of dehydrate barium chloride in 50ml of distilled water. 0.6ml of the barium chloride solution was subsequently added to 99.4ml sulphuric acid, and properly mixed. A small volume of the turbid solution was transferred into screw capped bijou bottles and store at room temperature (Cheesbrough, 2003).

10x Tris Borate EDTA Buffer

About 108g of Tris base, 55g boric acid were mixed in 20ml of 0.5M EDTA (pH 8) and dissolved in 500ml of distilled water. The volume was adjusted up to 1 liter. For use 1x concentration was prepared by taking 1 volume of 10x stock TBE buffer and 9 volume of distilled water.

Ethidium Bromide Stain

To 100ml of water 1g of ethidium bromide was dissolved and stirred on a magnetic stirrer for 6 hours for complete dissolution of the dye. The container was wrapped in aluminum foil or the solution was transferred to a dark bottle. This was then stored at room temperature.

APPENDIX B

BIOCHEMICAL IDENTIFICATION TEST

MOTILITY TEST

The Hanging drop method were used for the motility test. A ring of plasticine of about 2cm in diameter was made on a clean grease free glass slide. A loopful of test broth culture was transferred onto the center of a 22mm square cover slip. The slide was inverted quickly to prevent the displacement of the plastacine and this was examined microscopically at X 10 and X 40 objective.

GRAM' S REACTION

A loopful of the suspected organism was emulsified on a sterile grease free slide, fixed and air-dried. Smear were covered with crystal stain for 60 seconds and then washed off with clean tap water. Smear was covered with Lugol's iodine solution for 1 minute and was washed off with clean tap water, decolorized with acetone alcohol for 20 seconds and washed with clean tap water. Smear was covered with neutral red stain for 1 minute and then washed with clean tap water. It was allowed to air dry on a rack and then viewed microscopically using the oil-immersion objective (X100). The organisms that appeared red in color indicates Gram-negative organism and those with purple colour indicate Gram positive organisms.

OXIDASE TEST

A strip of Whatman No.1 filter paper was soaked with a freshly prepared solution of 1% Tetramenthyl para-phenylene-diamine-dihydrochloride and with a clean slide edge, colonies of suspected organisms were streaked on it. Development of a blue purple colour within a few seconds indicated a positive test while no colour change indicated a negative test.

INDOLE TEST

The test organism was inoculated in sterile bijou bottles containing 3ml of sterile peptone water and incubated for 24 hours at 37^0C.Half mill (0.5ml) of Kovac's reagent was added and it was shaken gently. A red ring colour was observed in the surface layer within 1 minute, which indicated a positive test while a yellow colour indicates a negative test.

CATALASE TEST

2ml of 3% hydrogen peroxide into a test tube and using a glass rod, colonies of each isolate were removed and immersed in the hydrogen peroxide solution. The occurrence of immediate bubbles (within 10 seconds) was recorded as a positive result.

COAGULASE TEST

A drop of normal saline was placed on microscopic slide and a colony of the test organism was emulsified. A loopful of plasma was added to the suspension and mixed gently. This was then observed for clumping within 10 seconds. Clumping within 10 seconds indicates a positive coagulase test.

CITRATE UTILIZATION TEST

The test is one of the several techniques used to assist in the identification of enterobacteria. This is based on the ability of an organism to use citrate as its only sources of carbon. A slope of the medium was prepared in bijou bottles. A sterile straight wire loop was used to pick the colonies and stab to the bottom of the bottle. The slopes were incubated for 24 hours at 37^0C. After incubation a bright blue colour indicated a positive citrate test.

UREASE TEST

The test organism was inoculated into bijou bottles containing 3ml sterile urea slopes. It was incubated at 37^0C for 24 hours. Pink colour in the medium indicated a positive urea test.

SUGAR UTILIZATION TEST

The following sugars namely; glucose, mannitol, lactose, and sucrose were tested separately to observe the ability of the isolates to utilized them. The isolates were inoculated into separate sugar broth (that is glucose broth, sucrose broth, mannitol broth, and lactose broth) and incubated at 37ºC for 24 hours, phenol red indicator was added to the tubes and Durham tubes immersed in them before inoculation and incubation. Sugar utilization was indicated by colour change from red to yellow with the production of acid. While in the Durham tube the presence of gas showed that the reaction was accompanied by gas.

APPENDIX

INFORMED CONSENT FORM

Dear Participant,

My name is Ehiaghe Imuetiyan Joy. I am a postgraduate student of the Department of Medical Laboratory Science (Medical Microbiology), Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus.

You are invited to participate in a research project entitled: "*Phenotypic and Molecular Detection of Multidrug Resistant Bacterial Isolates from Surgical wound Infections from Hospitalized Patients in Anambra and Edo States of Nigeria.*"

There are no identified risks from participating in this research. Participation in this research is completely voluntary and you may refuse to participate without any consequences.

The study will take approximately 1year to complete. The questionnaire and all its information will be treated with strict confidentiality. Please understand that your acceptance to participate in this study is absolutely priceless. You can contact me on Phone number **08060138954.** Thank you for your consideration.

Your signature below indicates that you have read the above information and agreed to participate in this study.

_____ _____

Signature Date

QUESTIONNAIRE (BIODATA)

(Please tick or fill appropriate)

SECTION A

1. Identification Number:
2. **Age**:
3. **Sex**: Male [ ] Female [ ]
4. **Status**: Married [ ] single [ ], Divorced [ ]
5. **Occupation**: Student [ ], Trader [ ], Farmer [ ]
   Teacher [ ], Civil servant [ ], others [ ]
6. **Tribe**: Bini [ ], Yoruba [ ], Igbo [ ] Hausa [ ] others [ ]
7. **Religion**: Islam [ ], Christianity [ ], Traditional [ ] others [ ]
8. **Duration of Stay in the Hospital:** 1 weeks [ ] 2 weeks [ ]
   3 weeks [ ] 4 weeks [ ] Over 4 weeks [ ]
9. Educational Status:_____
10. Personal Health Awareness:_____
11. Reason for Surgery:_____
12. Type of surgery:_____
13. Patient on drugs: Yes [ ] No [ ]
14. Type of Drugs:_____
15 Empirical improvement: _____
16 Route of administration of drug: I.V [ ] I.M [ ]

UNIVERSITY OF BENIN TEACHING HOSPITAL
P.M.B. 1111 BENIN CITY NIGERIA

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Telegram: UNITECHOS, BENIN
Telex: 41120 NG
Website: ubth.ng.org.

CHAIRMAN: RT. GEN. A.B. MAMMAN
Mni, OFR, FSS, Psc

CHIEF MEDICAL DIRECTOR: PROF.M.O. IBADIN *MBBS, FMC (Paed), FWACP*
MBBS (Benin), FMCP, (Paed) M.S, (IMMUNOLOGY & IMMUNOCHEM)
E-mail:mikobadin@yahoo.com;mikobadin@ubth.org

CHAIRMAN, MAC Dr. A.O. OGBEMUDIA
MBBS, FWACS (Ortho/Trauma)
E-mail: alfredoghogho@yahoo.com; alfredoghogho@ubth.org

DIRECTOR OF ADMINISTRATION: E.B. KOYENIKAN Esq.
LL.B (Hons), B.L. CHPM
E-mail: officeoftheda@ubth.org

ETHICS AND RESEARCH COMMITTEE CLEARANCE CERTIFICATE

PROTOCOL NUMBER: ADM/E 22/A/VOL. VII/1013

PROJECT TITLE: "PHENOTYPIC AND MOLECULAR DETECTION OF MULTIDRUG RESISTANT BACTERIAL ISOLATES FROM SURGICAL WOUND INFECTIONS FROM HOSPITALIZED PATIENTS IN ANAMBRA AND EDO STATES OF NIGERIA".

PRINCIPAL INVESTIGATOR(S) EHIAGHE I. JOY

DEPARTMENT/INSTITUTION: DEPARTMENT OF MEDICAL LABORATORY SCIENCE (MEDICAL MICROBIOLOGY) FAULTY OF HEALTH SCIENCES, NNAMDI AZIKIWE UNIVERSITY, NNEWI

DATE CONSIDERED MARCH 10TH, 2014
DECISION OF THE COMMITTEE: APPROVED
REMARK:
CHAIRMAN: PROF. M.N. OKOBIA SIGNATURE & DATE..... 10/3/20

SUPERVISOR(S): PROF. R.A.U NWOBU
DECLARATION BY INVESTIGATOR(S)
PROTOCOL NUMBER (please quote in all enquiries)
To be completed in four and three copies returned to the secretary, Ethics and Research committee, Clinical services and Training Division. University of Benin Teaching Hospital Benin City.
I/We fully understand the conditions under which I am/we are authorized to conduct the above mentioned research and I/We undertake to resubmit the protocol to the Ethics and Research Committee.

Signature..... Date. 20/03/2014

NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL

P.M.B. 5025, NNEWI, ANAMBRA STATE, NIGERIA

Professor Ivara E. Esu, OFR
B.Sc. (Ife), M.Sc. (Minnesota), PhD. (ABU)
Chairman
Board of Management

B. O. Chukwuma
B.Sc., MA, MHA, AHA,
Director of Administration/
Secretary to the Board

Professor Anthony O. Igwegbe
MBBS, FWACS, FICS, FISS
Chief Medical Director/
Chief Executive

Dr. E. A. E. Afladigwe
B.Sc (Hons) Nig. MBBS (NAU), FWACS, FICS
Ag. Chairman
Medical Advisory Committee

E-mail: nauthcmd@yahoo.co.uk
nauthnnewi@hotmail.com
Telegram: TEACHOS NNEWI

Our Ref: NAUTH/CS/66/VOL.5/124

Your Ref:

Date: 9th April, 2014

Ehiaghe I. Joy
Department of Medical Laboratory Science,
Nnamdi Azikiwe University Teaching Hospital,
Nnewi

ETHICS COMMITTEE APPROVAL

RE: PHENOTYPIC AND MOLECULAR DETECTION OF MULTIDRUG RESISTANT BACTERIAL ISOLATES FROM SURGICAL WOUND INFECTIONS FROM HOSPITALIZED PATIENTS IN SELECTED HOSPITALS IN ANAMBRA AND EDO STATES OF NIGERIA

We write to inform you that after due consideration of your revised research proposal, approval is hereby conveyed for you to commence the study.

The principal investigator is required to send a progress report to the Ethics Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Please note that this approval is subject to revocation if you fail to obtain proper authorization from your study site/unit.

.....
Prof. P.U Ele
Chairman, NAUTH Ethics Committee

.....
Udemezue N.O (Mrs)
Sec., NAUTH Ethics Committee

APPENDIX D

GENE EXPRESSION TABLES

UBTH		VARIABLE	AUGMENTIN	OFLOXACIN
Sample no.	S/NU	*P.aeruginosa*	TEM	gyrA
14	1u	*P.aeruginosa*	+	+
20	2u	*P.aeruginosa*	S	S
157	3u	*P.aeruginosa*	+	+
30	4u	*P.aeruginosa*	+	+
34	5u	*P.aeruginosa*	S	S
160	6u	*P.aeruginosa*	S	S
161	7u	*P.aeruginosa*	+	+
56	8u	*P.aeruginosa*	+	S
57	9u	*P.aeruginosa*	S	S
71	10u	*P.aeruginosa*	+	-
165	11u	*P.aeruginosa*	+	+
167	12u	*P.aeruginosa*	+	+
105	13u	*P.aeruginosa*	+	+
106	14u	*P.aeruginosa*	+	+
118	15u	*P.aeruginosa*	+	+
169	16u	*P.aeruginosa*	+	S
170	17u	*P.aeruginosa*	+	+
150	18u	*P.aeruginosa*	+	S
155	28u	*P.aeruginosa*	S	S
TOTAL	19	*P.aeruginosa*	R(14) P(14)	R(11) P(10)

UBTH		VARIABLE	AUGMETIN	OFLOXACIN
Sample no.	S/N U	*E. coli*	TEM	GyrA
4	19u	*E. coli*	+	S
45	20u	*E. coli*	S	+
163	21u	*E. coli*	+	+
64	22u	*E. coli*	S	S
109	23u	*E. coli*	+	+
152	24u	*E. coli*	+	S
181	25u	*E. coli*	+	+
65	26u	*E. coli*	+	+
168	27u	*E. coli*	+	+
TOTAL	9	*E. coli*	R(7)P(7)	R(6) P(6)

NAUTH		VARIABLE	AUGMENTIN	OFLOXACIN
Sample no.	S/N N	*P.aeruginosa*	TEM	GyrA
233	1N	*P.aeruginosa*	+	+
208	2N	*P.aeruginosa*	+	S
210	3N	*P.aeruginosa*	S	S
237	4N	*P.aeruginosa*	+	S
288	5N	*P.aeruginosa*	+	+
244	6N	*P.aeruginosa*	+	S
245	7N	*P.aeruginosa*	+	S
247	8N	*P.aeruginosa*	+	+
235	9N	*P.aeruginosa*	S	S
246	10N	*P.aeruginosa*	S	S
248	11N	*P.aeruginosa*	+	+
249	12N	*P.aeruginosa*	+	S
263	13N	*P.aeruginosa*	-	S
250	14N	*P.aeruginosa*	+	+
251	15N	*P.aeruginosa*	+	+
319	16N	*P.aeruginosa*	S	S
252	17N	*P.aeruginosa*	+	+
359	18N	*P.aeruginosa*	+	+
362	19N	*P.aeruginosa*	+	-
355	20N	*P.aeruginosa*	+	+
TOTAL		20	R(16) P(15)	R(10) P(9)S

NAUTH		VARIABLE	AUGUMETIN	OFLOXACIN	OXACILLIN	ERYTHROMYCIN
Sample no.	S/N N	*E. coli*	TEM	gyrA	Blaoxa	ere A
199	21N	*E. coli*	+	+		
205	22N	*E. coli*	+	+		
207	23N	*E. coli*	+	+		
224	24N	*E. coli*	S	S		
232	25N	*E. coli*	+	+		
236	26N	*E. coli*	+	+		
240	27N	*E. coli*	+	+		
257	28N	*E. coli*	S	S		
264	29N	*E. coli*	+	+		
270	30N	*E. coli*	+	+		
309	31N	*E. coli*	+	-		
312	32N	*E. coli*	S	S		
316	33N	*E. coli*	-	-		
333	34N	*E. coli*	-	-		
348	35N	*E. coli*	S	-		
326	36N	*E. coli*	S	-		
332	37N	*E. coli*	-	-		
353	38N	*E. coli*	-	-		
225	39N	*E. coli*	S	+		
TOTAL		19	R(13)P(9)	R(16) P(9)		
287	40N	*Staph aureus* 1	-	S	+	+
		20	1	0	1	1

Keys:

S/N = Serial Number

U = UBTH

N = NAUTH

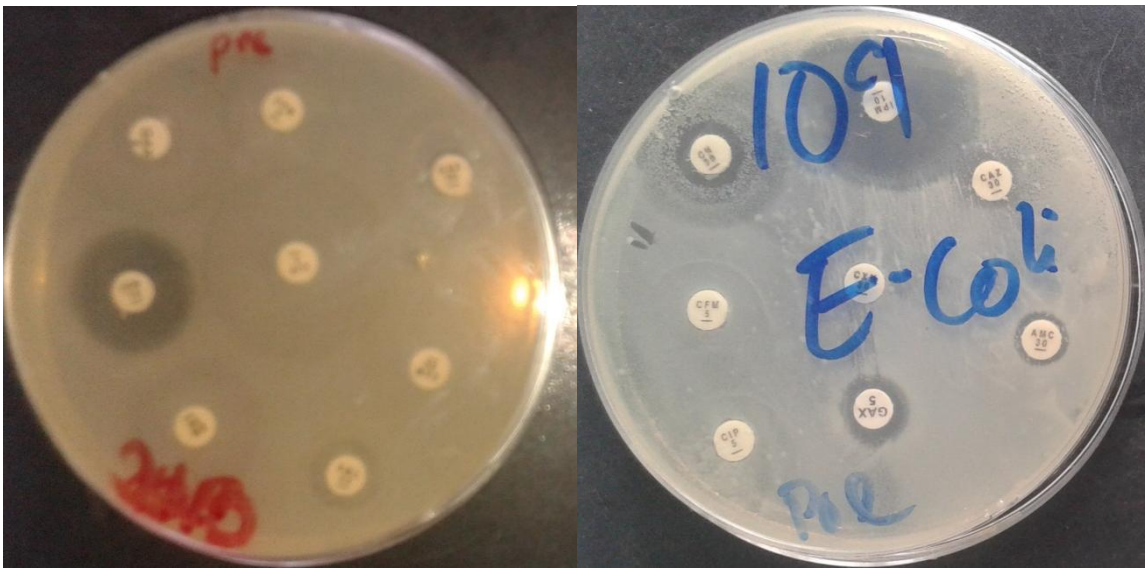
+ = Positive

- = Negative

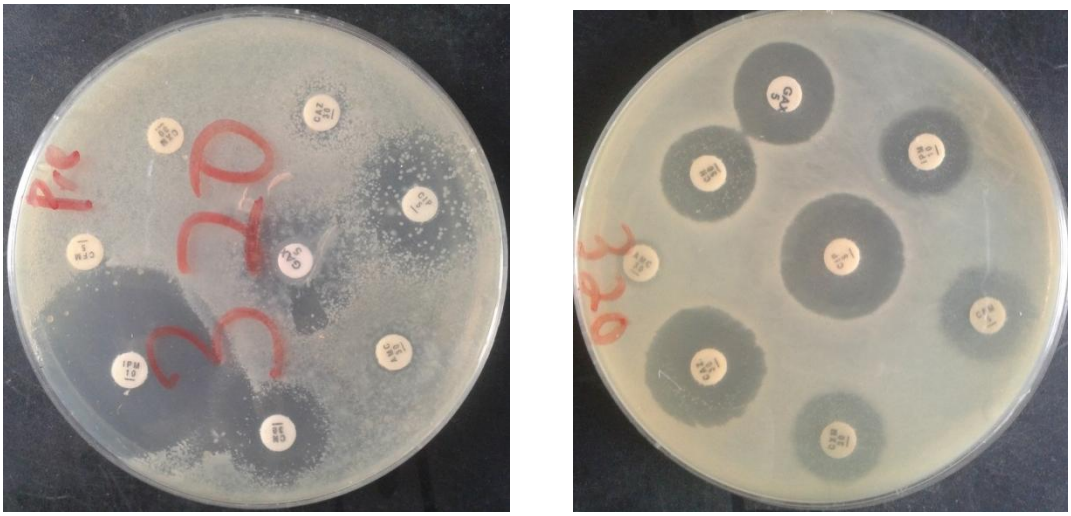
S = Sensitive

APPENDIX E

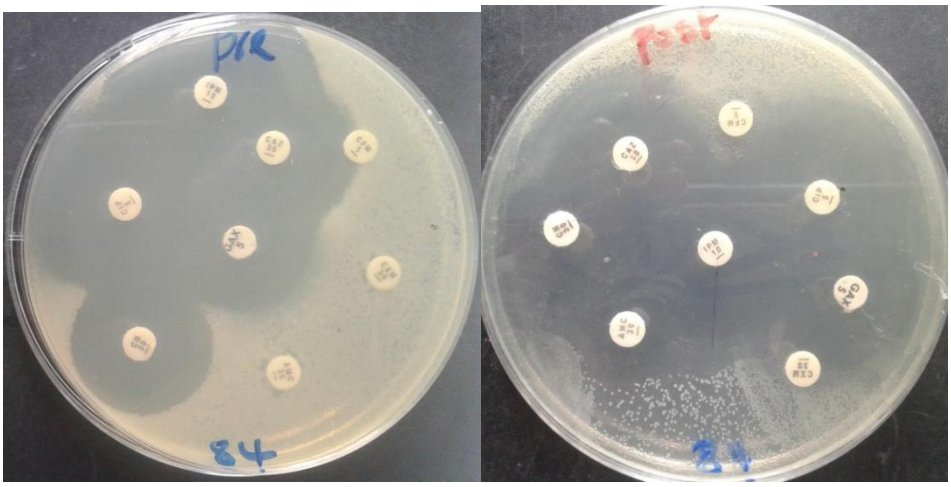
PHOTOGRAPHS OF ANTIBIOTICS SUSCEPTIBILITY TEST.



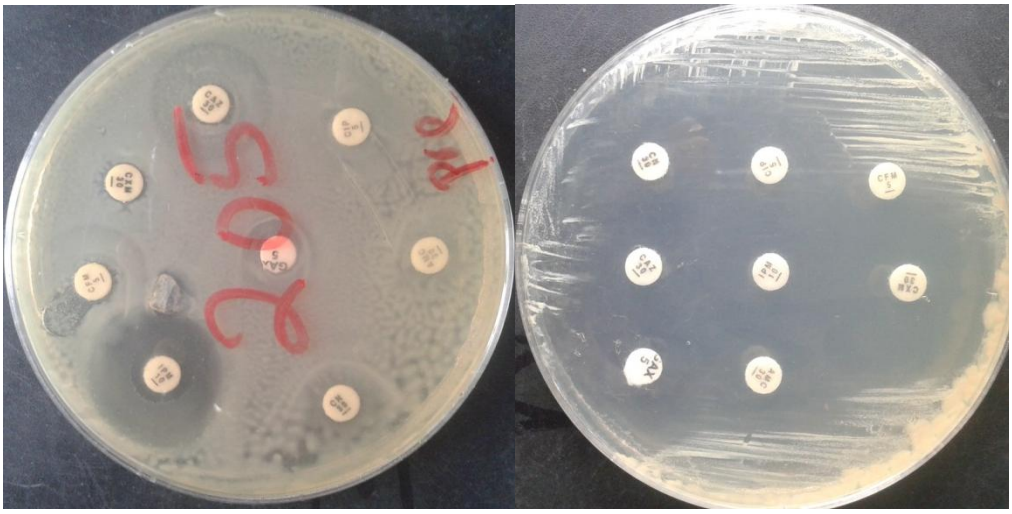
Chromosomally mediated cultured plate from both locations plate 248 from NAUTH and 109 from UBTH.



Precuring and Post curing Agar culture plate from NAUTH.



Precuring and Post curing Agar culture plate from UBTH



Precuring and Post curing Agar culture plate from UBTH

INTEPRETATIVE CHART FOR ZONE SIZES (MM)

Antibiotic (Antimicrobial Agent)	DISC CODE	Resistant	Intermediate	Susceptible
		< or = mm	Mm	= or > mm
Amoxicillin (other)	AMC	<13	14-17	>18
Amoxicillin (Staph)	AMC	19	-	20
Ampicillin (other)	AM	11	14-16	17
Ampicillin (Staph)	AM	28		29
Augmentin	AUG	19		20
Carbenicillin(Pseudomonas)	CB	13	14-16	17
Ceftazidime	CAZ	14	15-17	18
Ceftriaxone	CTR	14	15-19	20
Cefuroxime	CXM	14	15-17	18
Ciprofloxacin	CIP-5	15	16-20	21
Ofloxacin	OFX	12	13-15	16
Clindamycin	CC-2	14	15-20	21
Enoxacin (Flouroquinolones, 2nd generation)	ENX-10	14	15-17	18
Erythromycin	E	13	14-22	23
Gentamycin	GM	12	13-14	15
Kanamycin	K-30	13	14-17	18
Methicillin (Staph)	M(or DP)	9	10-13	14
Oxacillin (Staph)	OX	10	11-12	13
Penicillin G (Enterococcus)	P	14		15
Penicillin G (Staph)	P	28		29
Imipenem	IMP	13	14-15	1
Sulfamethoxazole-Trimethoprim	SXT	10	11-15	16
Tetracycline	Te-30	14	15-18	19
Tobramycin	NN-10	12	13-14	15
Vancomycin	Va-30	9	10-11	12

APPENDIX F

SAMPLE SIZE ESTIMATION

The following formula (Naing *et al*., 2006) was used to estimate expected sample size:

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

Where N = sample size,

Z = Z statistic for a level of confidence = 1.96

P = expected prevalence of surgical wound infection = 34%

d = precision = 0.05.

$$N = \frac{1.96^2 \times 0.34 (1-0.34)}{0.05^2}$$

$$N = \frac{3.8416 \times 02244}{0.05^2}$$

$$N = \frac{0.862055}{0.0025}$$

$$N = 345$$