PREVALENCE OF NOSOCOMIAL INFECTIONS AND ANTIBIOTICS SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATES IN SOME HOSPITALS IN ILORIN, NIGERIA

BY

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B.Sc (Microbiology), BUK; M.Sc (Microbiology), ILORIN.

Ph.D/Pharm.Sc/24906/2000-2001.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIRMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL MICROBIOLOGY.

DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

AUGUST, 2008

DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Prof. Y.K.E. Ibrahim and Prof. P.F. Olurinola in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

The work of other investigators referred to were appropriately acknowledged. I declare that no part of this dissertation has been submitted elsewhere for the purpose of a degree.

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CERTIFICATION

This thesis entitled: PREVALENCE OF NOSOCOMIAL INFECTIONS AND ANTIBIOTICS SUSCEPTIBILITY PROFILE OF BACTERIAL ISOLATES IN SOME HOSPITALS IN ILORIN, NIGERIA by RAZAQ FUNSHO ATATA meets the regulations governing the award of the degree of Doctor of Philosophy (Pharmaceutical Microbiology), Ahmadu Bello University, Zaria, Nigeria and approved for its contributions to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late father, Mallam Abdul-Kareem Ishola Atata, who though did not have western education, struggled hard against all odds and gave me the best of education available, but could not live long enough to reap the fruit of his labour. And

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DEFINITION OF TERMS

Mastectomy	-	Surgical removal of one or both breast.
Exploiratory laparatomy	-	Surgical procedure involving an incision through abdominal wall to gain access into abdominal cavity.
Appendicetomy	-	Surgical removal of appendix
Thyroidectomy	-	Surgical removal of all or part of thyroid gland.

Abstract

Prevalence of nosocomial infections and antibiotics susceptibility profiles of bacterial isolates in six private hospitals and University of Ilorin Teaching Hospital (UITH) in Ilorin town Kwara State, Nigeria were investigated. . A 1-year retrospective study of the private hospitals and 3-years retrospective analysis of the university of Ilorin teaching hospital were carried out. A 2-year prospective study surgical site nosocomial infection in the teaching hospital was also carried out.

Patients' folders and Microbiology laboratory records were used for the determination of prevalence of nosocomial infections in retrospective study. Bacteriological analysis of Floor and air of theater and surgical wards of the private hospitals and swabs of surgical wounds, floors and air of theater and surgical wards of the teaching hospital were done, using diagnostic media and biochemical tests.

Antibiotic susceptibility profiles of the bacterial isolates were determined using tube dilution and agar diffusion methods as described in official monographs. Multi Antibiotic Resistant (MAR) isolates were screened for β-lactamase production by iodometric method. Presence of plasmids in the MAR isolates was determined using the agar gel electrophoresis technique. Plasmid curing, to determine if antibiotic resistance was due to plasmids was also carried out by treating the isolates with acridine orange and re-determining of their MIC values. Susceptibility of the isolates to some commonly used hospital disinfectants were determined by the broth- dilution technique, for two years. In-situ efficacies of some hospital disinfectants indicated reduction level of microbial count of the floors and airs of theater and surgical wards.

Result of the retrospective study of the six private hospitals showed that nosocomial infections are common in the hospitals, with a prevalence rate of 15.23%. Analysis of incidence of nosocomial infection rates showed that Surgical site infection ranked the highest with prevalence rate of 26.1%, followed by infection of the blood stream, urinary tract and respiratory tract in descending order. In the university teaching hospital the average rate of nosocomial infection in the wards for the 3 years was 1.27%. With highest rate of prevalence of 67.42% also occurring in surgical site followed distantly by UTI, Blood stream and Respiratory tract infections in descending order.

Comparative analysis of results of retrospective study on leading causative agents of nosocomial infection in both private and the teaching hospitals showed that *S. aureus, Ps.*

aeruginosa, S. epidermidis and *E. coli* are the leading causative pathogens nosocomial infections in decreased order of frequencies. Isolates from both private and Teaching hospital environments equally incriminated *S. aureus, S. epidermidis* and *Ps. aeruginosa* as the leading causative agents.

The results of susceptibility tests showed that all the isolated bacterial species from both the private hospital and university teaching hospital environments were multiple antibiotics resistant (MAR). The MAR indices ranged from 0.4-0.8. Bacteriological study of surgical site infections at UITH showed nosocomial infection prevalence rate of 30-45.7% with appendicectomy having the highest figure of 46.7%. Other commonly infected surgical sites were laparatomy, mastectomy, thyroidectomy, and herniorrhaphy.

A total of ten (10) different bacterial species were isolated from the surgical sites, with *S. aureu, S. epidermidis, Ps. aeruginosa, E. coli, Kl. pneumonia,* and *Pr. mirabilis* constituting 89% of the isolates. Most of the isolates from the surgical sites were multi-antibiotic resistant, with MAR indices ranging from 0.6-0.8. Only the quinolones and aminoglycosides showed good inhibitory activities against most of the isolates.

Sixty- four percent (90 out of 149) of the bacteria isolated from the surgical sites were β-lactamase producers with Staphylococci constituted 90% of the β-lactamase producers.

Results of plasmid curing analysis showed that 61.2% of the multiple antibiotic resistant isolates, which also produced β-lactamase, harboured plasmids. After plasmid curing 61.2% of these isolates became sensitive to some of the antibiotics which they were initially resistant to. The result of electrophoresis analysis of the resistant isolates showed different plasmid numbers and sizes, with some isolates having the same number of plasmid bands and sizes confirming that resistance observed with the isolates were plasmid- mediated and the likely origin of the these isolates. There was decrease in efficacies of the disinfectants from 2004 to 2005 which resulted in high bacterial counts in most of the wards in the hospital.

Generally, surgical site infection was the leading nosocomial infection in the investigated hospitals in Ilorin. *S. aureus, S. epidermidis, Ps. aeruginosa, E. coli and Kl. pneumoniae.* Which were the causative pathogens They were resistant to commonly prescribed antibiotics. Antibiotic resistance in the isolates was largely due to production of β-lactamases and transfer of resistance plasmid among the isolates. Ineffective disinfection of the clinical environment was mostly responsible for the high number of the bacteria contaminants in the hospital wards.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

The World Health Organization offers several definitions of nosocomial infection or hospital-acquired infection. Two of such definitions are:

- An infection acquired in hospital by a patient who was admitted for a reason other than that infection, or
- An infection occurring in a patient in a hospital or other health care facility in whom the infections were not present or incubating at the time of admission, which include infections acquired in the hospital but appearing after discharge, and also occupational infections among the staff of the facility (Benson, 1995; Bolyard, 1998; Ducel, 2002; Coffin and Zaotis, 2005).

Nosocomial infection is developed by a patient while in a healthcare facility due to the introduction of pathogens whether or not the disease is expressed during the duration of the hospital stay (Johnson, 2002; Morley, 2002). Infections occurring more than 48 hours after admission are usually considered nosocomial (Garner, 1988). Nosocomial infections are most frequently infections of the urinary tract, surgical wounds, and lower respiratory tract. Several definitions have been developed to identify nosocomial infections in specific infection sites. A number of these were derived from those published by the Centre for Disease Control and Prevention (CDC) in the United State of America (Garner, 1988; Horan, 1992) and during international conferences and are used for surveillance of nosocomial infections. They are based on clinical and biological criteria, and include approximately 50 potential infection sites.

Nosocomial infections may also be considered either as endemic or epidemic (Coffin and Zaotis, 2005). Endemic infections are defined as sporadic infections that constitute the background rate of the infection at the healthcare facility; the rate of such infections usually fluctuates from month to month but overall, it is not statistically significantly different from the background rate of these infections (Jarvis, 1991). Of all nosocomial infections, endemic infections account for the majority of infections, meaning that they are at the level of usual occurrence within the setting and are the focus of most infection control activities (National Nosocomial Infections Surveillance (NNIS), 1995). Epidemic infections are defined as the occurrence of infection at a rate statistically significantly higher than the background rate of such infections or when there is an unusual increase in infection above baseline for a specific infection or organism (Ducel *et al.*, 2002). Four sites of nosocomial infections recognized are; urinary tract, surgical site, blood stream and respiratory nosocomial infections.

1.2 History of Nosocomial Infections

The history of hospital infections will find rich source materials in the scattered references of mediaeval chroniclers to pestilences and 'visitations'. The early hospital reformers met with little success with regards to curbing incidences of nosocomial infections in the monastic infirmaries, the leprosaria and the larger pre-renaissance hospitals of Western Europe. In the 13th Century, Theodoric of Bologna deplored the current teaching that 'laudable pus' was a perquisite for sound wound healing. He carefully cleaned all wounds and then sutured them to avoid contamination from the air. By this means, he apparently prevented suppuration in most of his cases (Major, 1954). As a result of his pioneer work on aseptic surgery, Theodoric was persecuted by de Mondeville, who also taught that suppuration hindered wound healing, but did not belief in 'laudable pus'. The next major

commentary on nosocomial infection in surgical practice was at the great Hotel- Dieu in Paris in the sixteenth Century (Major, 1954).

The scientific study of hospital or nosocomial cross infection began during the first half of the 18th century, and from that time until the beginning of the Bacteriological Era, many of the most notable contributions originated in Scotland. In 1966, Selwyn drew attention to the virtually unknown work of Sir John Pringle, who in the 1740s made the first important observation on hospital acquired infection while he was both physician to the British Army overseas and Professor of Pneumatical and Ethical Philosophy at Edinburgh (Selwyn, 1966). In his effort to improve the health of the expeditionary forces, Pringle introduced great sanitary reforms into his military hospitals to remedy the evils of overcrowding and poor ventilation. His observation led to a statement that, "**among the chief causes of sickness and death in an army, the reader will little expect that I should rank, what is intended for its health and preservation, the Hospitals themselves, and that on account of the bad air, and other inconveniences attending them**" His statement suggest that hospital is the first suspect of the factors responsible for nosocomial infections. Pringle's constant aim was that of preventing infections, the common and fatal consequences of a large and crowded hospital. His insistence on adequate ventilation and spacing of patients, as expressed in the following passage, appears unremarkable, but his view were revolutionary at the time: '**As to the disposition of hospitals, with regard to preserving the purity of the air, the best rule is, to admit so few patients into each ward, that a person unacquainted with the danger of bad air, might imagine there was room to take in double or triple the number. It will also be found a good expedient, when the ceilings (sic) are low, to remove some part of them, and to open the garret story (sic) to the tiles. It is surprising in how few days the air will be corrupted in close and crowded wards; and what makes it hard to remedy the evil, is the difficulty of convincing either the nurses or the sick themselves, of the necessity of opening the doors or windows at any time for air. I have generally found those wards the most**

healthful, when by broken windows, and other wants of repair, the air could not be excluded'.

Pringle's observation that hospitals were among the chief causes of sickness and death in army stimulated his military successor, Rechard Brocklesby to bring about further improvement in hospital conditions (Brocklesby, 1764). Brocklesby's forthright statement on the danger of hospitals is still relevant to the present day: "**infirmaries, or hospitals, in all countries, are for the most part of unclean and infectious places, and though every precautions is taken to purify them, such as washing with vinegar, burning brimstone, gunpowder, "**

Today, nosocomial infections occur worldwide and affect both developed and resources poor countries. Despite progress in public health and hospital care, infection continues to develop in hospitalized patients, and may also affect hospital staff. At least 5 to 10% of patients entering hospitals in United State of America acquire an infection that was not present or incubating on admission (Haley, *et al*; 1985); it may be higher in the developing countries. Such hospital acquired infections did not only add significantly to the morbidity and mortality in patients, but also a huge economic burden expected from underling disease (Dixon, 1987; Freeman *et al.*, 1979; Green and Wenzel, 1979; Haley *et al*; 1980; Martin, *et al*., 1989; Townsend and Wenzel, 1981). The greatest contributors to these costs are the increased stays in hospital that patients with nosocomial infections require. The increased length of stay varies from 3 days for gynecological procedures to 19.8 days for orthopedic procedures. Other costs include additional drugs, the need for isolation, and the use of additional studies and also indirect costs due to loss of work-days. These infections may cause increased functional disability and emotional stress and may lead to conditions that reduce quality of life. As a result, it can be stated with confidence that these infections represent a leading cause of death, and an important health care cost (Dixon, 1987;

McGowan, 1981). Specifically of the 40 million patients admitted to acute-care institutions in USA, 2 million to 4 million develop a nosocomial infection. If 100,000 to 400,000 episode of nosocomial blood stream infections alone occurred, there would be 40,000 to 160,000 death overall and 25,000 to 100,000 deaths directly attributable to the infections. By the same reasoning, there would be expected at least 20,000 death directly attributable to nosocomial pneumonias each year (Leu *et al*., 1989). Not only do they affect the general health of patients, they are also a huge burden financially. For instance, if infections added only an additional 5 days to the expected stay at an average cost of $750/day, the economic burden would be $7.5 billion to $15 billion dollars annually.

The most frequent nosocomial infections are infections of surgical wounds, urinary tract and lower respiratory tracts. World Health Organization (WHO, 2002) study and others have also shown that the highest prevalence of nosocomial infections occurs in Intensive Care Units (ICU), and acute surgical and orthopedic wards.

1. 3 Factors Influencing the Development of Nosocomial Infections.

1.3.1 Microbial Agents

Most nosocomial infections are caused by bacteria and are frequently associated with infection of blood, skin, surgical, the respiratory and urinary tracts and various other sites (WHO, 2002).

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not by itself necessarily result in the development of clinical disease; other factors influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (Inocula) of infective material. Many different bacteria,

viruses, fungi, and parasites may cause nosocomial infections... Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patients own flora (endogenous infection). Some organisms may be acquired from inanimate objects or substances recently contaminated from another human source (environmental infection).

Before the introduction of basic hygienic practice and antibiotics into medical practice, most hospital infections were due to pathogens of external origin (food borne and airborne diseases, gas-gangrene, tetanus, etc) or were caused by microorganisms not present in the normal flora of the patient (e.g. diphtheria, tuberculosis). Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in the hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients e.g. *Staphylococcus aureus,* coagulase-negative Staphylococcus, Enterococci, and Enterobacteriaceae (Ducel, 1995).

1.3.2 Patient Susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, diagnostic and therapeutic interventions. The extremes of life – infancy and old age – are associated with a decreased resistance to infection. Patients with chronic disease such as malignant tumours, leukaemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) have an increased susceptibility to infections with opportunistic pathogens. The latter are organisms that are normally innocuous e.g. part of the normal bacteria flora in the human, but may become pathogenic when the body's immunological defence are compromised. Immunosuppressive drugs or

irradiation may lower resistance to infection. Injuries to skin or mucous membranes bypass natural defense mechanisms. Malnutrition is also a risk. Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, intubations/ventilation and suction and surgical procedures increase the risk of infection. Contaminated objects or substances may be introduced directly into tissues or normally sterile sites such as urinary tract and lower respiratory tract (Dunagan, *et al*., 1991; Beek-Sagnue, *et al.*, 1996).

1.3.3 Environmental Factors

Environmental factor may either assist or impede the transmission of microorganisms (Canadian Communicable Disease Report, 1999). The environment may be conducive to growth and survival of the organism. Health care settings are an environment where both infected persons and persons at increased risk of infection congregate (Mayon-White, 1988). Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for patients and staff. Patients who get infected in the hospital are a further source of infection (Haley, *et al*., 1985). Crowded conditions within the hospital or sharing of equipments may, frequent transfers of patients from one unit to another, and concentration of patients highly susceptible to infections in one area (e.g. newborn infants, burn patients, intensive care) may increase the chances of exposure to the microbial flora of other patients there by contribute to the development of nosocomial infections (Kirkland, 1999; Canadian communicable Disease report, 1999). Microbial flora may contaminate objects, devices, and materials which subsequently contact susceptible body sites of patients. Respiratory viruses and rotaviruses persist for prolonged periods on inanimate objects, and transmission of RSV from inanimate environment has been documented (Bean *et al.*, 1982; Brady *et al.*, 1990; Hall *et al*., 1980; Hendley *et a*l., 1973; Ward *et al*., 1991; Keswick *et al*., 1983; Hall. 1981). Bedside tables

and bedrails may be contaminated by Vancomycin resistance enterococci –VRE (Boyce *et al.*, 1995; Lior *et al.*, 1996) and Methicillin Resistant *Staphylococcus aureus*-MRSA (Boyce *et al.*, 1997). Call bell may become contaminated by VRE (lLior *et al.*, 1996). Environmental contamination may be important in transmission of relatively hardy bacteria such as enterococci (Boyce *et al.*, 1994) and spore-forming bacteria such as *Clostridium difficile* (Gerding *et al.*, 1995; Johnson and Gerding, 1998). The mobil the equipment that is shared among patients may be source of transmission. Transmissions of VRE and C. difficile have been linked to contaminated thermometers (Livornese *et al*., 1992; Porwancher *et al*., 1997; Brooks *et al*., 1998; Jernigan *et al.*, 1998). Contaminated blood pressure cuffs have been implicated in transmission of *C. defficile* (Mania *et al*., 1996) and Klebsiella (Myers, 1978). Recent studies have shown that stethoscope diaphragms and otoscope specula become contaminated with microorganisms including MRSA (Smith *et al*., 1996; Cohen *et al*. 1997; Dias *et al*., 1997; Wright *et al*. 1995; Jones *et al.*, 1995). Many patients care items including bedside table and bed rails, may not be cleaned routinely. Although there have been much concern about the most appropriate cleaning agents, the actual method of cleaning may be more important than the agent used (Bonilla *et al*., 1997; Jackson and Lynch, 1996; Rutalla *et al.*, 1997). The environment may play a larger role in transmission of certain pathogens than previously appreciated, reinforcing the importance of minimizing environmental contamination by patient excretions and secretions, avoiding unnecessary hand contact with environmental surfaces, ensuring adequate resources for procurement of necessary items (2006).

1.3.4 Bacterial Resistance.

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistant elements, antibiotics usage promote the emergence of multidrug resistant strains of bacteria. The widespread use of antimicrobials for therapy or prophylaxis (including topical) is the major determinant of resistance. Microorganisms of the human normal flora which are sensitive to the drug are suppressed, while resistant strains persist and may become endemic in the hospital. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of Pneumococci, Staphylococci, Enterococci, and *Mycobacterium tuberculosis* are currently resistant to most or all antimicrobial agents which were once effective. Multi-resistant *Klebsiella* and *Pseudomonas aeruginosa* are prevalent in many hospitals. This problem is particularly critical in developing countries where more expensive second line antibiotics may not be available or affordable (Resource, 1990).

1.4 Statement of Research Problems.

Nosocomial infections continue to be a burden to health care system throughout the world. It increases risks to patients and employees. These infections have serious health and financial cost with an estimated incidence of 2 million infections and 20, 000 deaths per year and added cost of $2 billion per year in the American health care system. Even in developed countries with high propensity to adherence on rules and guidelines on health policy and management in hospitals, nosocomial infection has continued to pose serious healthcare problems, with occurrence rate well over 2%.

1.5 Justification

In Nigeria, public and hospital staff awareness of nosocomial infections either is very low or none with many healthcare institutions having no policy on disease management and

guidelines on combating nosocomial infections. If the state of Nigerian hospitals and healthcare institutions is to be used as a yardstick to measure prevalence of nosocomial infection, then surely nosocomial infection rate in Nigeria could be relatively high. It would thus imply that a significant number of patients that go on admission in hospitals in Nigeria often have to spend much more money and time getting relief than would have been otherwise needed.

In spite of the seriousness of nosocomial infections and the problems it poses to successful management of health in health institutions as well as the attendant effects on patients, little or no previous attempts have been made to investigate the problems of nosocomial infections in Nigeria.

1.6 Objectives of the Study

Objective of the study will include the following:

- Survey of the incidence of nosocomial infections (both retrospectively and prospectively) in selected private hospitals in Ilorin metropolis.
- Determine the prevalence of nosocomial infections in the selected hospital.
- Isolate and characterize the causative bacterial agents.
- Study the antibiotic resistance profile of the bacteerial isolates.
- Determine the nature and possibly the origin of resistance among the bacterial isolates.
- Examine the disinfection practices in the selected hospitals
- Initiate practical workable measures in place to reduce incidence of nosocomia in the selected hospitals.

1.6.1 The objective would be approached through;

- Analysis of patients' health records in the hospital will be carried out, from hospital to hospital and Ward to Wards in each hospital.

  The following information will be collected from each patient record.

  a. initial diagnosis of the patient on admission

  b. initial responsible pathogen

  c. duration of treatment

  d. Secondary diagnosis if any

  i. responsible pathogens

  ii. duration of treatment of the new infection

Analysis of information obtained from the above data will be used to select nosocomial infection type in the environment and hence enable the choice of the particular ward of the hospital where such occurs, for more detailed study.

1.6.2 Modalities

- Patients admitted into the hospital ward will be recruited for the study. The result of microbiological sample from the laboratories will be used to determine initial diagnosis and the pathogen isolated.
- The patients will be monitored on daily bases to know when a new diagnosis different from that initially made is established.
- Samples will be collected from patient for bacteriological analysis. The type of sample will depend on the site of infection.

- Samples will also be taken from air and floor of the patients environment to establish the likely source of the pathogen.
- Sample collected will be cultured, growths will b separated in pure culture, fully characterized and identified by using standard microbiological protocools. Fully characterized isolates will be stored in agar slants, as appropriate in refrigerator at $4^{o}C$ for subsequent use.
- Antibiotics susceptibility testing of the isolate will be carried out as specified by official standard. To determine the resistance profile of the isolates.
- Resistant isolates will be study for resistant factors such as β-lactamase production and presence of plasmids.
- Likely source and relationship of the isolates from different sources will be established by plasmid profiling of the resistant isolates.
- Disinfection procedures of the hospital will be evaluated.

1.8 Expected out come of the study

- The study would be able to provide information on the prevalence level of each type of nosocomial infections in hospitals in Ilorin, and thus provide base data for hospitals in Kwara State for the first time. This can be used to plan for control measure and the hospital management and Government alike.
- The results of the study would rank the hospitals for the prevalence of nosocomial infections.

- The study would determine the factors responsible for the observed prevalence, which can be used to adopt effective control of nosocomial infection, thereby preventing further spreading of the infections.
- Determine pathogen causative agents, characterized and the relationship among these pathogens.
- Establish antibiotic susceptibility practice of the pathogens.
- Establish the appropriateness of disinfection policy of the hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nosocomial Pathogens.

Many different types of pathogens can cause nosocomial infections. The most common are bacteria, including commensal bacteria, which are part of the normal floral and pathogenic bacteria which come from exogenous source. Commensal bacteria normally have a protective role against pathogenic bacteria but can cause infection when the host is compromised. Pathogenic bacteria are more virulent and can cause infection regardless of host status. Viruses including Hepatitis B and C, Respiratory Syncytial Virus (HSV), rotaviruses, and enteroviruses may also be transmitted nosocomially.

The organisms causing nosocomial infections can best be understood in terms of their selection on the basis of current antibiotics use and clinical practices. In the pre-antibiotic era, Streptococci (both *Streptococcus pyogenes* and *Streptococcus pneumoniae*) were the major nosocomial pathogens. Coincidence with the introduction and use of penicillin and sulfonamides, *Staphylococcus aureus* emerged as the most common offending agent in the 1950s. Subsequently, associated with the use of first-generation cephalosporins and aminoglycosides, aerobic gram-negative rods accounted for 70% of nosocomial bloodstream pathogens in the 1970s. In the late 1970s and early 1980s, there was great usage of broad-spectrum cephalosporins and an associated exponential use of vascular catheters and other medical devices. Associated with these major events, there was a major shift in pathogens from gram-negative to gram-positive species: coagulase-negative staphylococci, *Staphylococcus aureus,* and the enterococci. Not only were there endemic problems with the new organisms, but also important epidemics of methicillin-resistant *Staphylococcus aureus* occurred internationally in the 1980s (Thompson and Wenzel,

1982; Wenzel, 1982). As a result of both endemic and epidemic infections with gram-positive organisms frequently resistant to methicillin, the usage of vancomycin increased linearly in the mid-1980s. In most large hospitals, it became the major antibiotic prescribed and the most expensive item in the hospital pharmacy. Since vancomycin has been the mainstay of therapy for infections caused by coagulase-negative Staphylococci, methicillin-resistant *S. aureus*, and often the Enterococci, it became a matter of great concern when reports of vancomycin resistance began to appear in the late 1980s (Froggat *et al*., 1989; Schwalbe *et al*,. 1987) with *Staphylococcus haemolyticus* as the major pathogens.

By 1990, it became obvious that *Candida* spp. represented important nosocomial pathogens, accounting for almost 10% of all nosocomial bloodstream infections in some institutions in United States (Horan *et al.*, 1988; Wey *et al*., 1988). This organism is associated with an alarming crude case fatality rate and high attributable mortality (Wey *et al.*, 1988) as well as excess hospital stays, in large part because of the need for prolonged therapy with amphotericin B (Ducel, 2002).

2.2. Types of Nosocomial Site Infections.

Nosocomial infection is often classified on the basis of the sites in which it occurs. Most common nosocomial infections are grouped into the following;

- Surgical Site nosocomial infection
- Urinary tract nosocomial infection
- Respiratory tract nosocomial infection
- Blood stream nosocomial infection

2.2.1 Surgical Site Nosocomial Infections

Up to the end of the nineteenth century, nosocomial infection was the greatest risk associated with any surgical procedure (Dellinger and Ehrenkranz, 1998). Surgical site nosocomial infection is the most common infections in surgical patients, accounting for about 24% of the total number of nosocomial infections. Although the practice of surgery had spread rapidly following the introduction of ether at Massachusetts General Hospital in 1846, even relatively minor procedures could be complicated by severe systemic infection and death, while for major procedures this was the expected outcome. Increasing knowledge regarding the relationship between bacteria and infection advanced at the end of the Century by such legendary figures as Pasteur, Lister, and Koch, led to a series of discoveries and the development of techniques that paved the way for modern surgery. Lister recognized the role of bacteria in surgical wound infections and in 1867 introduced the practice of spraying antiseptics into wounds to combat bacteria. Subsequently, in 1881, Koch verified the superiority of heat over antiseptics for killing bacteria and preventing access of bacteria to wounds (Wangensteen and Wangensteen, 1978).

The role of the surgeon's hands in introducing bacteria into wound was slow to be recognized despite the work of Semmelweis in 1847. In 1882, when Ernst Bergman arrived at the Ziegelstrasse Clinic in Berlin and was asked what was new in surgery, he replied, "Today we wash our hands before operation" (Dellinger and Ehrenkranz, 1998).

In the 20th Century, the standardization of aseptic practices in the operating room has greatly improved the safety of clean operative procedures, but operations involving anatomic structures with a dense endogenous flora that cannot be eliminated before operation, such as the colon and rectum, continue to carry a very high risk of infections (Dellinger and Ehrenkranz, 1998). National Research Council (NRC) documented the rate of surgical wound infection following 15,613 operations carried out for 27 months starting

from 1959 to 1962 in 16 operating rooms of five university hospitals (Howard *et al*., 1964). The study was designed to investigate whether the reduction of airborne bacteria in the operating room, accomplished with ultraviolet light irradiation, could achieve a reduction in surgical wound infections. The study found that ultraviolet light produced a significant reduction in airborne bacteria, but this reduction had no effect on wound infections except in the class of refined clean wounds, which are wound with the lowest probability for contamination by endogenous bacteria. The infection rate in all other classes of wounds: other clean, clean contaminated, contaminated, and dirty wounds were unaffected by ultraviolet irradiation.

This study (Howard *et al*, 1964) was one of the earliest and one of the most convincing studies to document the importance of endogenous bacteria as primary etiological agent of surgical wound infections. This report also introduced a system for classifying wounds according to the risk of endogenous contamination (and thus of postoperative wound infection), and provided a basis for comparing wound infection statistics. Although more sensitive and specific wound classification system employing additional risk factors for wound infection have been developed since then (Haley *et al*., 1985; Culver *et al*., 1991), they all continue to incorporate element of this original scheme. This report was also the largest and most carefully conducted study in its day to examine a host of other factors related to the patient and the environment that influence the risk of postoperative wound infections.

Although antibiotics were introduced near the end of the Second World War, their use did not appear to result in a lower infection rate despite early optimism. The effective use of antibiotics for prevention of postoperative infection was made possible by the pioneering studies of John Burke, who used an animal model to demonstrate the critical importance of the timing of prophylactic antibiotic administration (Burke, 1961). Results of his study

showed that appropriate antibiotics given before bacteria contamination, could cause a significant reduction in the risk of infection, while the same antibiotic, given after bacteria contamination, was much less effective. The information was translated into trials demonstrating clinically and statistically significant effect in human patients undergoing scheduled operative procedures, first by Bernard and Cole (1964) and then by Polk and Lopez-Mayor (1969) in the 1960s. Work on prophylactic antibiotics since that time has focused on defining those procedures and circumstances most likely to benefit from the use of prophylactic antibiotics and on examining the relative efficacy of different drugs and different routes and regimens of administration. In the 1970s, reports of surveillance by surgical groups of large numbers of incisions validated the relationship between wound class and differing risk of infection as well as the beneficial effect of reporting wound infection data to the operating surgeons (Cruse and Foord, 1980).

Surgical wound infections are caused by bacteria, and in the absence of bacteria they do not occur (Dellinger and Ehrenkranz, 1998). However, surgeons have known for years that many other factors also influence the risk of infections. Burk demonstrated in 1963 that all (50 of 50) clean surgical incisions contain bacteria at the end of an operation, but only a small number (4% in that report) become infected (Burke, 1963). An animal study of the relationship between bacteria inoculums and infection risk showed an increasing risk of infection with increasing numbers of bacteria. It was further predicted that reductions in the incidence of postoperative infection could be achieved by techniques that reduce the number of bacteria that gain access to surgical wounds and by focusing on methods to increase the efficacy of host defenses in resisting those bacteria that gain access to the wound.

2.2.2 Urinary Tract Site Nosocomial Infections

According to data from numerous hospitals and from multi-hospital collaborative studies, nosocomial urinary tract infections have been responsible for 35% to 45% of all hospital-acquired infections (Walter, 1998).

Endemic acquisition account for most nosocomial urinary tract infections, but numerous examples of epidemic transmission have been reported (Schaberg *et al.,* 1976 and Stamm *et al.,* 1981). Epidemic of nosocomial bacteriuria have resulted from inadequately disinfected cystoscopes, non-sterile irrigating solutions used on multiple patients, contaminated disinfectants in catheter insertion trays, and most commonly person to person transmission on crowded hospital wards where aseptic catheter care practice were not being used (Schaberg *et al.*, 1980; Stamm *et al*., 1991).

Frequent catheter irrigation appeared to be an important causal factor in several epidemics. Multiply drug resistant strains of *Serratia, Proteus, Klebsiella, and Pseudomonas* possessing unique antimicrobial susceptibility pattern have afforded opportunities to trace the epidemiology of nosocomial urinary tract infections in the epidemic setting. Most often, epidemic investigations have demonstrated transmission of organisms from one person catheterized patient to another by the hand of hospital personnel (Walter, 1998). In several epidemics, patients with unrecognized asymptomatic bacteriuria lasting for weeks or even months served as an important reservoir, with temporal and spatial clustering of subsequent cases in proximity to these sources of patients (Walter, 1998).

Most likely, a proportion of infections classified as endemic actually resulted from clusters or microepidemics of cross infection on hospital wards. Using tertiary marker systems (e.g. pyocin typing, serotyping, and phage typing) to trace cross infection attributable to common organism such as *E. coli,* Schaberg and coworkers (Schaberg *et al*., 1980)

demonstrated that approximately 15% of endemic nosocomial infections occur in cluster suggestive of cross infection, *Pseudomonas* and *Serratia* infections were most often clustered, and *E. coli* infections least often. Thus attention to the same factors that prevent or terminate epidemics of nosocomial bacteriuria presumably would prevent some endemic infections as well (Walter, 1998).

Nearly all nosocomial urinary tract infections occur in patients with indwelling urinary catheters (approximately 80%) or after other types of transient urologic instrumentation (nearly 20%). Specific host factors associated with an increased risk of infection during or after instrumentation include female gender, old age, and an increasing degree of underlying illness (Walter, 1998). The risk of development of nosocomial bacteriuria in women exceeds the risk in men by approximately two fold in each decade of life (Hooton *et al.,* 1981). In addition, 95% of deaths and 83% of bacteremic episodes occur in patients older than 50 years (Stamm *et al.*, 1977).

Apart from host factors, the risk of urinary infection relates directly to the type and duration of urological instrumentation. After a single in and out catheterization, between 1% and 20% of patients acquired bacteriuria (Stamm, 1975; Truck *et al.,* 1962), lower rate occur in healthy outpatients and higher rates in older hospitalized patients. Indwelling urethral catheters draining into an open collecting vessel result in bacteriuria in 100% of patients within 4 days (Kass, 1956). With the sterile closed collecting systems used in most hospitals today, bacteriuria occurs on the average in 10% to 20% of catheterized patients (Finkelberg and Kunin, 1969, Kunin, 1985; Thornton, 1970). In prospective studies carried out in general hospital populations between 1966 and 1995 (Finkelberg and Kunin 1969; Garilbaldi *et al.*, 1974; Johnson, 1990; Kunin and McCormack, 1966; Platt *et al.*, 1982; Thomson, 1984; Warren, 1978; Riley, 1995), there has been an apparent trend

toward a reduced prevalence of catheter associated bacteriuria. Many factors may account for this decrease, including increased antimicrobial use in catheterized patients, decreasing length of catheterization and hospitalization, and more effective infection control efforts. The per day risk for development of bacteriuria appears comparable throughout catheterization (3% to 6%), but cumulative risk increases with duration of catheterization (Garibaldi, 1980; Garibaldi, 1982). Thus approximately 50% of hospitalized patients catheterized longer than 7 to 10 days contract bacteriuria (Kunin, 1985; Garibaldi, 1980). Bacteriuria develops more frequently in patients not receiving systemic antimicrobial and in patients in whom catheter care violation have occurred (Johnson, 1990; Platt *et al*. 1986).

Aerobic gram-negative rods account for most catheter associated urinary tract infections. In data collected by the National Nosocomial Infection Surveillance (NNIS) system (Javis and Martone 1992), *Enterobacteriaceae, Pseudomonads*, and enterococci, accounted for most of the infections, in both hospital wide and intensive care unit setting. Increasingly, the aerobic gram-negative rods causing nosocomial bacteriuria have been characterized by multiple antimicrobial resistances, often mediated by transmissible plasmids (Levy, 1983). Candidal infections of catheterized urinary tract appear to have increased since the early 1980, and now predominate as the most common cause of nosocomial urinary tract infection in the intensive care unit setting (Walter, 1998).

The relative proportion of infections accounted for by an individual species, as well as their antimicrobial susceptibility patterns, vary widely from hospital to hospital and fluctuate with time in each hospital. In two studies *E.coli* and Proteus species accounted for a progressively smaller percentage of nosocomial urinary tract infections as the period of hospitalization and catheterization lengthened (Stamm, 1977; Krieger *et al.*, 1983).

Conversely *Serratia* and *Pseudomonas aeruginosa* accounted for a progressively greater proportion of infection as hospitalization increased. These observations suggest that a proportion of catheter-associated infections are infact not newly acquired but may represent recognition of previous covert bacteriuria after insertion of urinary catheter (Walter, 1998)

Nosocomial urinary tract infections result from direct introduction of urethral microorganisms at the time of catheterization and other instrumentation, however, most catheter-associated infections arise later in the period of catheterization. Microorganisms causing these infections enter the catheterized urinary tract either through the lumen of the catheter (intraluminal route) or along its external surface in the mucous sheath between the catheter and urethral mucosa (transurethral route.

Women appear to be greater at risk of transurethral than men. In one study, approximately two third of women in whom catheter- associated bacteriuria developed were shown to have previous urethral and anal colonization with their infecting strain of organisms, compared with only one third of men (Daifuku and Stamm, 1984). Thus, the pathogenesis of catheter-associated infections in many women resembles the pathogenesis of non-catheter-associated infections. Studies of community-acquired bacteriuria in women suggest that faecal organisms establish introital, vaginal, and urethral colonization before urinary tract infection with these strains occurs (March *et al.,* 1972; Stamey, 1986). In either sex, however, establishment of urethral colonization with a gram-negative rod apparently confers an increased risk of subsequent infection. Garibaldi and coworkers (Garibaldi, *et al.*, 1974) showed that the increased risk of subsequent nosocomial bacteriuria associated with a positive meatal culture was approximately fourfold in women and twofold in men. However, periurethral colonization is not invariably followed by

bacteriuria, and, when bacteriuria does occur, it is often > 72 hours after the establishment of priurethral colonization (Warren, 1987).

Bacteriuria may also result from microorganisms that enter the collecting system and ascend through the lumen of the urinary catheter into the bladder (intraluminal route). In studies assessing this mode of entry, approximately 15% to 20% of infected patients can be demonstrated to have their infecting organisms in the collecting bag before entry into the bladder (Garibadi, *et al.*, 1974, Schaeffer, 1986; Warren, 1987). Retrograde spread of organism from the collecting bag to the bladder occurs within 24 to 48 hours in nearly all such patients (Garibaldi, 1974). Once in the bladder, small number of microorganisms (e.g., 100 colony-forming unit –cfu/ per milliliter) increase to large numbers (> 100,000 cfu/ml) in < 24 hours (Stark and Maki, 1984). Thus contamination of collecting bag or introduction of organisms by disconnection of the distal catheter and proximal collection tube can allow entry of organisms that produce nosocomial bacteriuria. Rarely, nosocomial bacteriuria arises secondary to haematogenous seeding of the genitourinary tract or as complication of surgical procedures involving the urinary tract or adjacent structures.

Studies since the mid-1980s have demonstrated an important role for attachment and growth of bacteria on the inner surface of the catheter in the pathogenesis of catheter-associated urinary tract infection (Cox, 1987, Cox, 1989, Nickel *et al*., 1985). Two populations of bacteria exist in the catheterized urinary tract, those growing within the urine itself (planktonic growth) and those growing on the surface of the catheter (biofilm growth). The growth of a bacterial biofilm on the urinary catheter progresses according to a well-defined sequence of events: bacteria attach to the urinary catheter, initiate a biofilm form of growth in which sheets of organisms coated the catheter, and secrete an extracelluar matrix of bacterial glycocalyces in which they become embedded (Walter,

1998). In addition, host urinary protein such as Tamm-Horsfall protein and urinary salts are incorporated into this biofilm. Subsequently, this material leads to encrucitation of the inner catheter surface. Certain genera of bacteria, particularly Proteus and Pseudomonas, are frequently associated with a propensity for biofilm growth and catheter obstruction (Mobley and Warren, 1987).

Although catheter-associated bacteriuria has been clearly linked to subsequent bacteriuria, the mechanism through which such bacteriuria occurs has not been elucidated. Bacteremia could occur secondary to mucisal ulceration resulting from bladder wall damage during catheterization. Cystoscopic examination of catheterized patients suggests such ulcerations are common after 7 days of catheterization (Sullivan, 1973). Alternatively the well known association of bacteremia with either insertion or withdrawal of urinary catheter or other urological instruments suggests that bacteremia may result from the mucosa trauma associated with these events (Sullivan, 1973).

Only 20% to 30% of patients with catheter-associated bacteriuria experience symptoms attributable to infection, and thus most cases can be classified as asymptomatic bacteriuria (Mooney *et al*., 1980). When present, symptoms include dysuria, urgency, frequency and hematuria. Fever, flank pain, or other clinical manifestations of pyelonephritis develop in less than 1% of patients with catheter associated bacteriuria (Musher *et al*., 1976) and Stamm, 1983). Despite the absence of symptoms in most patients with catheter associated bacteriuria the presence of pyuria suggests host invasion and actual infection rather than simple bladder colonization.

Most physicians diagnose catheter associated bacteriuria by obtaining a urine specimen for culture by aspiration through a sampling port on the catheter itself. Because specimen so collected presumably represents actual bladder urine, contamination is infrequent. Catheter

aspiration corresponded with cultures obtained by suprapublic aspiration in at least 90% of cases (Berquist *et al*., 1980). Urine culture obtained from the catheter sampling port may not always reflect true bacteriuria in patients who have organisms growing on biofilm on the inner surface of the catheter. Rather, bladder urine may be sterile but organisms from the catheter biofilm may contaminate the aspirated urine culture. Such contamination would be more likely to occur in patients with catheter in place for a week or more.

Quantitative culture results may demonstrate organisms ranging in quantity from 10 to > 10^5 cfu/ml to define infection in catheterized patients. However, prospective studies to establish a specific quantitative level as more or less indicative of infection have been carried out. For clinical purposes, bacteriuria of 10^2 cfu/ml or more, especially when associated with pyuria, can probably be taken as evidence of bladder infection (Stark and Maki, 1984). Catheter tip culture should not be used to diagnose urinary infection (Gross *et al*., 1979).

Guidelines for prevention of catheter associated bacteriuria have been published by the Centre for Disease Control and Prevention (Wong and Hooton, 1982). Until more have been learned about the pathogenesis of nosocomial bacteriuria, preventive efforts must be focus largely on aseptic care of the urinary catheter as highlighted bellow:

- Avoid catheterization
- Decrease duration of catheterization
- Use intermittent catheterization
- Insert catheter aseptically
- Use a closed sterile drainage system

- Use a condom catheter in cooperative patients
- Maintain gravity drainage
- Apply topical metallic antimicrobials in women
- Give systemic antimicrobials
- Separate infected and uninfected patients

2.3 Antibiotics and Nosocomial Infections

The era of chemotherapy for infectious disease is now over 50 years old. Those years were marked by the continuous development and introduction of new and potent antimicrobial agents and by the relentless development and spread of significant antibiotic resistance among pathogenic bacteria (Theodore, 1998). Contemporary clinicians now have at their disposal, a broad array of potent antibiotics agents of proven efficacy against susceptible pathogens. However, growing problems with antimicrobial drug resistance are beginning to erode the antibiotic armamentarium.

Since the introduction of antibiotics, significant changes have also occurred in the nature of nosocomial infections. Available data strongly suggest that in the 1940s and 1950s, nosocomial infection was generally synonymous with gram- positive coccal organisms, most notably with β-haemolytic streptococci and staphylococci (Theodore, 1988). In the 1950s, staphylococci particularly those resistant to the antimicrobial drugs in common use, such as Penicillin G, tetracycline, erythromycin, chloramphenicol, and streptomycin, emerged to cause epidemic infection in hospitals. For reasons that are poorly understood, staphylococci subsided somewhat in importance as the major cause of nosocomial infection in the early 1960s to be replaced by enteric gram-negative bacilli, enterococci, and fungi (Finland, 1970). During the 1970s and 1980s, staphylococci again became more

prominent. Important nosocomial pathogens in the 1990s include coagulase- positive and negative staphylococci, enterococci, enteric gram-negative bacilli, and gram-negative non-fermenting organisms such as Pseudomonas, Stenotropho-monas (formerly *Xanthomonas* or *Pseudomonas)*, *Achromobacter*, *Acinetobater*, and *Candida* species (NNIS, 1996; Swartz, 1994; Jone, 1996). The changing character of nosocomial infections is likely to be related to the sequential introduction of new antimicrobial agents, implying that there is a cause and effect relationship. While no one can seriously doubt that antimicrobial drugs have had a profound effect in shaping the character of nosocomial infections, one should not fail to take into account the enormous changes in the technology of medicine and patterns of health care delivery that have also taken place since the introduction of antibiotics (Theodore, 1998).

Data on the overall patterns of antibiotic use in hospitals have frequently appeared in the literature in the last several decades. The general thrust of such data indicates that from 25% to 40% of hospitalized patients receive systemic antibiotics at any given time.. Furthermore, a sequentially obtained data suggest that there is a trend toward increasing, rather than decreasing, antibiotic use in hospitals. Continuous and sharp increases in the use of extended- spectrum cephalosporin, vancomycin, metronidazole, and amphotericin B. have been observed (Pallares *et al*, 1993). In their report, it was found that the percentage of patients who received at least one parenteral antibiotics increased from 23% - 44% and the average number of agents used in patients receiving at least one antibiotic rose from 1.8 – 2.1 during the period of study.

Such massive use of antibacterial drugs in hospitals, whether appropriate or inappropriate, has profound effects on both the hosts who receive these drugs and the bacteria exposed to them. There are enormous economic consequences as well. The global antibiotic market was valued at over 20 billion dollars in 1994 (Fernandes, 1996), and this could well double

in the years ahead. There is enormous pressure on the part of the pharmaceutical industry to increase antibiotic use, both to recoup the cost of new drug development, and to accomplish the very legitimate goal of making profit

2.4 Hospital Bacteria and Antibiotic Resistance

Every major class of bacterial pathogens has so far demonstrated an ability to develop resistance to one or more commonly used antimicrobial agents (Theodore, 1998).

Current problems of resistance of bacterial to antimicrobial drugs becomes more understandable if one recalls some of the history of the development of antibacterial drugs resistance in Paul Ehrlichi's Laboratory; trypanosomes became resistant to Prosaniline after repeated exposure (Mitsuhashi, 1971). Similarly, it was shown that pneumococci could develop resistance to hydrocupreine derivatives after repeated exposure (Finland, 1972). In the mid 1940s, shortly after the introduction of Penicillin G, it was recognized that certain strains of Staphylococci elaborated a potent β-lactamase, an enzymatic inactivator of penicillin, and that Penicillin G had no therapeutic activity in patients with infections caused by such staphylococci. This recognition came as a major disappointment but not a total surprise to the scientific and medical community. Since that time, it has become abundantly clear that the major nosocomial pathogens are either naturally resistant to clinically useful antimicrobial drugs or possess the ability to acquire resistance. The best known examples are the staphylococci and aerobic gram-negative bacilli, which together regularly account for most nosocomial infections.

Selective pressures favouring drug resistant bacteria conferred by antibiotic therapy may indeed be principally focused in the institutional setting, but they extend widely into the community as well. The increasing prevalence of ampicillin- resistant *Haemophilus Influenzea*, the increasing frequency of β-lactamase- producing gonococci, the wide spread

dissemination of methicillin- resistant staphylococci, and the emergence of penicillin-reistance pneumococci are more recent examples. Maxwell Finland wrote in 1978, “little by little, we are experiencing the erosion of the strongest bulwarks against serious bacterial infection”.

Finland’s concerns have taken on a new urgency in the 1990s. With the development of extended spectrum cephalosporins and fluoroquinolones in the 1980s, many individuals, including some in the pharmaceutical industry considered bacterial infections to be controlled and consequently, hence effort and investment in new antibiotic development dwindled. Furthermore, all easy or obvious target of antibiotic activity had already been identified, hence new antibiotics development promised to be very costly. Today, we face problems of drug resistance in community acquired infections, best exemplified by the rapid emergence of penicillin- resistant pneumococci; we also face increasingly resistant nosocomial pathogens, best exemplified by methicilin-resistant *Staphylococcus aureus* (MRSA) and staphylococcus epidermidis. Vancomycin resistant entercocci (VRE) and multidrug resistant gram- negative bacilli (MDRGNB). Certain nosocomial infections caused by VRE and MDRGNB are already resistant to all available antibiotics and therefore essentially untreatable (Theodore, 1998).

The occurrence of nosocomial infection due to multidrug- resistance organisms is governed by a number of factors, including antibiotic selection pressure, the nature of the resistance determinant, whether the plasmid is conjugative or non-conjugative or is a transposon, and possible linkage with other antibiotic resistance and genetic determinant governing adhesion and pathogenicity (Davies, 1983). Evidence is conflicting regarding the altered virulence whether enhanced or diminished of drug resistance bacteria, compared with that of drug susceptible organisms.

Although it is generally true that serious infections, such as bacteremic infection caused by drug resistant bacteria results in higher mortality than would be true of comparable serious infections caused by drug susceptible bacteria, it is usually not clear whether the increased mortality is a reflection of increased virulence, diminished effectiveness of antibiotic therapy, or both (Davis, 1983).

2.5. Antibiotics resistance in Specific bacteria.

2.5.1 Enterobacteriaceae

Antibiotic resistant enterobacteriaceae are common in hospitals, especially resistant *Klebsiella, Serratia and Enterobacter* (Robert *et al.*, 1998). Drug resistant *Proteus, Providentia, and Morganella* are also very common, particularly ingeriatric wards and in patients transferred from nursing home (Gaynes *et al.*, 1985, Weinstein *et al.*, 1980; Gaynes *et al.* 1983).

Predisposing factors for and the epidermiology of, resistant enterobacteriaceae have differed somewhat from genus to genus (Schaberg *et al.*, 1976). Some organisms such as *Klebsiella* may be more viable on human skin and thus may have a greater potential for person to person spread on the hands of hospital personnel (Schaberg *et al.*, 1976*). Serratia* commonly cause asymptomatic colonization of urinary tract and respiratory tract, and chronically colonized patients are often a source for large number of cross-infections (Schaberg *et al.*, 1976). *Enterobacter*, as well as *Serratia, Citrobacter, Pseudomonas*, and some *Proteus* and *Providencia* strains often become resistant to second and third generation cephalosporins during therapy (Sanders and Sanders, 1988, Olson *et al.*, 1983). This genetic predilection and the ubiquity of *Enterobacter* may explain in part why *Enterobacter* species are a relatively common cause of mediastintis after open heart

surgery despite cephalosporin prophylaxis (Olson *et al.,* 1983, Flyn *et al.*, 1987; Flyn *et al.,* 1988).

In some hospitals 15% to 25% or more of all antibiotic-resistant Enterobacteriaceae are cultured from patients at the time of admission. Patients with chronic respiratory or urinary tract infections, those who have frequent admission, and patients transferred from nursing home or other hospitals are particularly likely to bring resistant strain into the hospital (Weinstein *et al*., 1980; Gaynes *et al.,* 1977). In many cases such patients have been the index cases in outbreaks (Casewell *et al.,* 1977; Edwards *et al.,* 1974; Gerding *et al*., 1979). It has been easy to contain resistant enterobacteriaceae in the past, however the more recent problems with emergence of chromosomal mutation that encode resistance to newer cephalosporins and with plasmids that encode novel β-lactamases (Jarlier *et al.*, 1988; Labia *et al*., 1988) suggest the need to heighten surveillance for such strains, tighten precaution for colonized patients, and possibly consider selective decontamination (Weinstein, 1989).

2.5.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa has intrinsic resistance to most available antibiotics, leaving aminoglycosides, anti-pseudomonal penicillins, newer cephalosporins, imipenem, and fluoroquinolones as treatment options for systemic infection. *Pseudomonas* is ubiquitous in the hospital, frequently colonizing patients before admission and contaminating ater and various foods (Olson *et al.,* 1985; Shooter, 1971; Griffth *et al.,* 1989; Olson *et al.,* 1984). Several studies have shown that aminoglycosides- resistant Pseudomonas may emerge from sensitive populations of the same serotype during treatment (Olson *et al*. 1985; Weinstein *et al.,* 1980; Gaman *et al.,* 1976; Amirak *et al*., 1977; Olson *et al.,* 1984). In fact

, some hospital have found that despite isolating patients with antibiotic-resistant *P. aeruginosa*, the incidenc of colonization and infection with these strain has continued to increase (Olson *et al.*, 1985; Gaynes *et al.*, 1983; Olson *et al.*, 1984; Meyer *et al.*, 1976; Panlilio *et al.*, 1992), in paralleling the increasing use of aminoglycoside antibiotics. In epidemic situation, *Ps. aeruginosa* has been noted to spread from contaminated common sources to patients as well as fromperson to person (Duncan *et al*., 1981, Griffth *et al*., 1989; Marrie *et al.*, 1978; Farmer *et al.*, 1982). Such transmission may be more amenable to control measure. However control of endemic *Ps. aeruginosa* infections is problematic and may well require more innovative strategies that interfere with the ability of Pseudomonas to colonize or invade.

2.5.3 Staphylococcus aureus.

Despite aggressive control efforts, resistance of *S. aureus* to methicillin, aminoglycosides, or clindermycin has been increasing in frequency in United State since early 1980s (Haley *et al*., 1982; Craven *et al*., 1981; Palilio *et al.*, 1992; Semel *et al.*, 1980). Ciprofloxacin resistance has also increased steadily, especially among MRSA. In some hospitals, nearly 100% of MRSA isolates are concomitantly resistant to ciprofloxacin (Coronado, *et al*., 1987). The epidemiology of drug resistant isolates of *S. aureus* appears to be similar to that of susceptible strains. In addition, particular reservoirs for MRSA include intravenous drug addicts, burn unit patients, nursing home patients, and hospital personnel with dermatitis. In some areas, the growing prevalence of MRSA in hospitals can be traced in part to admission to hospital of colonized patients from nursing homes (Craven *et al*., 1981).

Once drug resistant staphylococci have become established in the hospital, eradication is difficult (Robert, *et al.*, 1998). Control has required accurate laboratory identification, prospective surveillance for colonized and infected patients (including periodic culture of

high-risk patients), identification of previously colonized patients at admission, appropriate isolation of all identified patients, search for environmental and personnel sources, and aggressive antibiotic therapy of infected patients -vancomycin with or without rifampicin (Haley *et al.*, 1982, Craven *et al.* 1981; Thompson *et al*., 1982).

2.5.4 Coagulase negative Staphylococci

Coagulase negative staphylococci, re formerly believed to be an avirulent part of our normal flora, have become a problem pathogen in patients with implanted prosthetic joints, heart valves, or neurosurgical shunts in patients with intravascular catheters, particularly long duration central lines and immunosuppressed patients, particularly those who have received broad-spectrum antibiotics to treat or prevent infection with gram-negative bacilli (Robert, *et al.* 1998). Methicillin-resistant coagulase negative staphylococci may be present in low number on the skin of patients and emerge in hospital as predominant flora and potential pathogens, especially after surgical antimicrobial prophylaxis (Kernodle *et al*., 1988), which is very similar to the epidermiology of *Enterobacter* infection (Flynn *et al.,* 1987; Flynn *et al.,* 1988). In a study gentamicin resistance was used as marker, it was found that resistant coagulase-negative staphylococci are relatively uncommon in healthy non hospitalized people but are rapidly acquired in the hospital, related to either previously antibiotic exposure or ubiquitous environmental contamination (Weinstein *et al.,* 1982). The resistance was plasmid mediated and could be transferred in vitro or on skin to *S. aureus* (Jaffe *et al.*, 1980). In another study, most nosocomial isolates of gentamicin-resistant *S. aureus* appeared to result from in vivo plasmid spread from resistant coagulase-negative staphylococci (Weinstein *et al*. 1982).

Because drug resistant coagulase-negative staphylococci often become part of the patients' normal flora and are so ubiquitous, control is difficult (Jaffe *et al*., 1980; Bentley *et al*., 1970). More recent observation of wound infection due to dissemination of coagulase

negative staphylococci strains by colonized surgeon suggest the need to consider this mode of transmission when wound infection rate exceed expected norms (Boyce *et al*., 1990).

2.5.5 Enterococci

Since the mid-1980s, enterococci have emerged as significant nosocomial pathogens (Robert *et al*., 1998). The NNIS report list enterococci as the second most common isolate, after *E. coli* (Schaberg *et al*., 1991). Enterococci are not highly virulent bacteria and tend to cause infections in patients who are elderly, debilitated, or immunocompromised (Graham *et al.,* 1980). The increased rate of isolation of enterococci from clinical specimen is probably due partly to increasing numbers of patients with one or more predisposing factors for enterococci infection (Robert *et al.,* 1998). The wide spread use of antibiotics with little or no activity agaist enterococci may provide selective pressure for enterococcal survival. Enterococci are intrinsically resistant to all cephalosporins, semisynthetic penicillinase-resistant penicillins, erythromycin, and clindamycin. Moreover since the mid of 1970s, enterococci have acquired high level resistance to penicillin, ampicillin, aminoglycosides, and most recently vancomycin. For a growing number of strains, no effective antimicrobial regimen is available. The increase in the number of nosocomial enterococcal isolates coupled with increasing enterococcal resistance has led one observer to term multiple drug-resistant *E. faecium* "the nosocomial pathogen of the 1990s" (McGowan, 1994).

Although enterococcal infection has traditionally been considered to originate from patients endogenous flora, more recent evidence demonstrate that nosocomial acquisition and spread of enterocoi can occur. In one study the reservoir for gentamicin-resistant

enterococci appeared to be the gastrointestinal tract of asymptomatic patients and nursing home transfers, and spread was from person to person on the hands of hospital personnel (Zervos *et al.*, 1987a; Zervos *et al.*, 1987b).

Vancomycin resistance is probably the most worrisome antibiotic resistance to emerge in enterococci (Murray, 1995), for reasons, first, the incidence of vancomycin resistance in Europe and United State has increased dramatically since it was first described in France in 1988 (Leclereq *et al.*, 1988). In 1989, 0.3% of enterococcal isolates reported to the NNIS System were resistant to vancomycin, by 1995 >10% of enterococcal strains isolated in both ICU and non ICUs were resistant to vancomycin (Gaynes, 1989). A second reason for concern is that most vancomycin resistant enterococci are resistant to moderate or high levels of ampicillin and high levels of aminoglycosides. Treatment of infections due to these multiply resistant pathogens is often limited to unproven combination of antibiotics or investigational compounds (Livornese *et al.*, 1992; Lynn *et al.*, 1994).

2.6 Commonly Prescribed antibiotics, their mode of action and spectrum.

2.6.1 Penicillins

The penicillins comprise a group of natural and semisynthetic antibiotics containing the chemical nucleus 6- aminopenicillanic acid, which consist of a β-lactam ring fused to a thiazolidine ring. The naturally occurring compounds are produced by a number of *Penicillium* spp. The penicillins differ from one another in substitution at the 6 position, where changes in the side chain may modify their pharmacokinetic and antibacterial properties (Joseph and Robert, 1991).

The major antibacterial action of penicillins is derived from their ability to inhibit a number of bacterial enzymes, namely, penicillin-binding proteins (PBPs) that are essential

for peptidoglycan synthesis (Waxman and Stromiger, 1983). This ability to inhibit bacterial cell wall transpeptidases usually confers on the penicillins bactericidal activity against gram-positive bacteria. The bactericidal activity of the penicillins is often related to their ability to trigger membrane-associated autolytic enzymes that destroy the cell wall. Other minor mechanisms of action include inhibition of bacterial endopeptidase and glycosidase, enzyme involved in bacterial cell growth (Waxman and Stromiger, 1983). There is also recent evidence suggesting that penicillins may inhibit RNA synthesis in some bacteria, causing death without cell lysis, but the significance of these observations remains to be proven (McDowell and Reed, 1989).

The penicillins have antibacterial activity against most gram-positive and many gram-negative and anaerobic organisms. Penicillin G is very effective against penicillins susceptible *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, virridans streptococci, Streptococcus bovis, Neisseria gonorrhoeae, Neisseria meningitides, Pasteurella multocida*, anaerobic.

Table: 2.0 Types of Penicillins

Natural	**Semisynthetic, extended**
Benzylpenicillin (penicillin G)	Spectrum
Phenoxymethyl penicillin (penicillin v)	Continued
Semisynthetic	Carboxypenicillins
Penicillinase resistant	Carbenicillin
Methicillin	Ticarcillin
Nafcillin	Ureidopenicillins
Isoxazoly penicillins	Azlocillin
Oxacillin	Azlocillin
Cloxacillin	Mezlocillin
Dicloxacillin	Piperacillin
Extended spectrum	Amidinopenicillin
Aminopenicillins	Amdinocillin
Ampicillin	Penicillin+β-lactamase inhibitors
Amoxicillin	Ampicillin-sulbactam (Unasyn)
Cyclacillin	Amoxicillin-clavulanate
Bacampicillin	(Augmentin)
Pivampicillin	Ticarcillin-clavulanate (Timentin)

Joseph, D.CY. and Robert, C.M.Jr (1991).

2.6.2 Cephalosporins

Cephalosporins are derivatives of the fermentation products of species of the fungus *Cephalosporium.* They contain a 7 aminocephalosporanic acid nucleus, which consist of a β-lactam ring fused to a dihydrothiazine ring. Various substitutions at the 3 and 7 positions alter their antibacterial activity and pharmacokinetic properties. Addition of a methoxy groups at position 7 of the β-lactam ring results in a new group of compound called cephamycins, which are highly resistant to a variety of β-lactamases.

Similar to the penicillins, cephalosporins act by bining to PBPs of susceptible organism, thereby interfering with synthesis of peptidoglycan of the bacterial cell wall. In addition, these β-lactam agent may produce bactericidal effects by triggering autolytic enzymes in the cell envelope (Waxman and Stromiger, 1983).

Cephalosporins are classified by a well accepted but some what arbitrary scheme of grouping by generations, based o general features of their antibacterial activity. The first generation drugs, exemplified by cephalothin and cefazolin, have good gram positive activity and relatively modest againt gram –negative bacteria.

Generations of cephalosporines

1ST generation	***2^{ND} generations***	***3^{RD} generations***	***4^{TH} generations***
Cefadroxil	Cefaclor	Cefixime	Cefcline
Cephaexin	Cefamandole	Cefoperzone	Cefepime
Cephaloridine	Cefonicid	Cefotaxime	Cefluprenam
Cephalothin	Ceforande	Ceftazidime	Cefocelis
Cephapirin	Cefotiam	Ceftizoxime	Cefozopran
Cephradine	Cefuroxime	Ceftriaxone	Cefpirome
	Cefotetan	Moxalactam	Cefquinome
	Cefoxitin		

Are active against penicillin-susceptible and resistant *S aureus* as well *as S. pnumoniae, S. pyogenes,* and other aerobic and anaerobic streptococci (Bergeron *et al.,* 1973; Chang and Weistein 1963; Haristein *et al.,* 1977; Renzilin *et al.,* 1975; Sabath *et al.,* 1973) Methicillin- resistant S. *aureus, S epidermidis,* and enterococci are resistant. Some Enterobacteriaceae are susceptible, including many strain of *E coli, Klebsiella spp, and proteus mirablis. Pseudomonas,* including *P aeruginosa*, many *Proteus* spp. and *Serratia* and *Enterobacter* spp. are resistant. These agents are active against penicillin –susceptible anaerobes except *B. fragilis.* They have only modest activity against *H. influenzae*.

The second generation cephalosporins are stable to certain β-lactamases found in gram-negative bacteria and as a result have increased activity against gram negative organism. The agents are more active than first generation drugs against *E coli, Klebsiella* spp. And *Proteus* spp. Their activity also extends to cover some entrobacter and serratia strains, and they have some activity against *Haemophilus* spp, *Neisseria* spp., and many anaerobes (Eykyn *et al*. 1976, Morel *et al.,* 1983) Cefuroxime as well as its axetil ester (O'Callaghan *et al*., 1976), cefamandole and cefonicid (Actor, 1984) are active against ampicillin-resistant *Haemophilus* spp. However, cefamandole exhibits a significant inoculum effect and is not suitable for treating life threatening infections due to *H. influenzae.* Ceforanide and cefotiam have a spectrum of antibacterial activity similar to that of cefamandole. However, ceforanide is less active than cefamandole against gram positive cocci (Barriere and Mills, 1982), whereas cefotiam is more active against staphylococci (Watt and Brown, 1982) Cefoxitin and cefotetan are unique in that they have marked activity against anaerobes, including B fragilis (Wexler and Finegold, 1988). Cefotetan has the advantage of more prolonged serum half-life. Cefoxitin is also active against penicillin-resitant strain

of *N. gonorrhoeae* (Sanchez *et al.,* 1983) none of the second generation agents is active against *Pseudomonas* spp.

Third generation cephalosporins are generally less active than the first generation agents against gram-positive cocci, but they are much more potent against the enterobacteriaceae and *P. aeruginosa*. Their potent broad spectra of gram-negative activity are due to their stability to β-lamtamases and their ability to penetrate through the outer cell envelope of gram-negative bacilli (Kesado *et al.,* 1982, Neu, 1982, Thornsberry, 1985). There are two subgroup among these agents those with potential activity against *P aeruginosa* (ceftazidime and cefoperazone) and those without such activity (ceftizoxime, cefotaxime ceftriazone, and maxalactam).

Cefotaxime inhibits more than 90% of strain of enterobacteriaceae, including those resistant to aminoglycosides. The MICs for 90% of strain tested (MIC_{90S}) of *E. coli*, *Proteus* spp., and *Klebsiella* spp. are <0.5µg/ml. its activity against strain of *Serratia marcescens, Enterobacter cloacae*, and *Acinetobacter* spp is variable and it is inactive against *Ps. aeruginosa* (Richmond, 1980; Schrinner *et a*l., 1980). It has moderate activity against anaerobes but is inferior to cefoxitin and cefotetan against most of these isolates.

Ceftizoxime, ceftriaxone, and moxalactam have spectra of activity similar to that of cefotaxime (Cleeland and Squires, 1984; Fu and Neu, 1980), with a few exceptions. Ceftriaxone is the most active agent against penicillinase positive or negative strain of *N. gonorrhea* (Jones *et al*., 1983; Neu *et al.* 1981). It is effective as single dose therapy for infections caused by these organisms. Because of its long serum half-life (the longest of the currently available cephalosporins), cetriaxone is used frequently in outpatient antibiotic therapy of serious infections. Moxalactam, an oxaβ-lactam, is less active against gram-positive cocci, including staphylococci and tstreptococci. It has slightly more

activity against *Pseudomonas* spp. than cefotaxime does. It is the most active third generation agent against anaerobes, including *B. fragilis*, against which it is comparable to cefoxitin (Jorgensen *et al.,* 1980; Rolfe and Finegold, 1981).

Cefoperazone is less active than cefotaxime against many enterobacteriaceae and gram positive cocci However; it has activity against *P. aeruginosa*, with an MIC50 of ≤ 16 μg/ml (Chattopadhyay *et al*., 1981; Levermore *et al.,* 1981; Neu and Lubthavikul, 1982). It is more active than the ureidopenicillins against these strains. This agent has activity similar to that of cefotaxime against the enterobacteriaceae but is not as active against gram positive cocci. It has little activity against gram-negative anaerobes.

Cefixime, the first oral third generation cephalosporin, has activity against gram-negative aerobes similar to that of ceftizoxime (Barry and Jones 1987; Kamimura *et al*., 1984; Neu *et al.,* 1984). It is more stable than other oral cephalosporins (cephalexin, cephradine) bacterial β-lactamases. Compared with these agents, cefixime is equally active against streptococci but considerably less active against *S. aureus.* None of the currently available cephalosporins is active against enterococci.

2.6.3 Monobactam

2.6.3.1 **Aztreonam**

Aztreonam is the only monobactam antibiotics currently in clinical use the monobactams are β-lactams with various side chains affixed to a monocyclic nucleus

Aztreonam binds primarily to PBP3 of gram –negative aerobes, including *P. aeruginosa*, thereby disrupting bacterial cell wall synthesis. It is not hydrolyzed by most commonly

ocuring plasmid- and chromosomally mediatedβ -lactamases, and it does not induce the production of these enzymes (Bush *et al.*, 1982).

The antibacterial activity of aztreonam is limited to aerobic gram-negative bacilli, inhibiting most enterobacteriaceae, neisseria spp and *Haemophilus* spp with MIC90s of ≤0.5μ/ml (Barry *et al.*, 1985; Jacobus *et al.*, 1982; Sykes *et al*., 1982). It has significant activity against *P. aeruginosa*, *Enterobacter* spp and *Serratia marcescens*, with most strain being inhibited at ≤ 16 μg/ml. However, many *Acinetobacter* spp., *Pseudomonas cepacia*, and *Xanthomonas maltophilia* are resistant. It shows in vitro synergism when combined with aminoglycosides against 30 to 60% of aztreonam-susceptible organism, including *P. aeruginosa* and aminoglcoside resistant gram-negative bacilli. Bacterial tolerance and inoculum effect are generally not seen with this agent. Aztreonomam is not active against gram positive bacteria and anaerobes.

2.6.4.2 **Carbapenems**

Imipenem (N-formimidoyl thienamycin) is the first carbapenem antimicrobial agent developed for clinical use. It is a semisynthetic derivative of thienamycin which is produced by *Streptomyces* spp. Thienamycins differ from other β-lactams in having a trans configuration of the hydroxyethyl side chain at position 6 and lacking a sulfur or oxygen atom in the bicylic nucleus, the unique stereochemistry of the hydroxyethyl side chain provides stability against β-lactamases (Brza, 1985).

Imipenem binds to PBP1 and PBP2 of gram-negative and gram-positive bacteria, causing cell elongation and lysis (Kropp *et al.*, 1985). It is stable toward most plasmid or chromosomally mediated β-lactamases except those produced by x maltophilia and some strain of *B. fragilis* (Kropp *et al.*, 1985).

Imipenem has the widest spectrum of antibacterial activity of the currently available antibiotics. It has excellent in vitro activity against aerobic gram-positive species staphylococci (penicillin susceptible and resistant isolates) viridans streptococci, group A,B,C and G streptococci, *Bacillus* spp and *L. monocytogenes* methicillin –resitant staphylococci are usually resistant most enterococci are inhibited at <3µg/ml, but tolerance is common. Some strain of *Enterococcus faecium* are resistant.

More than 90% of enterobacteriaeae are susceptible to imipenem, including those resistant to other β-lactams and aminoglycosides. Most *Acinetobacter* spp, *Enterobacter* spp, *Citrobacter* spp, and *Serratia* spp are inhibited by ≤µg/ml. the activity against *P. aeruginosa* is similar to that of ceftazidime, with most strain inhibited at 1 to 6 µ/ml (Prince and Neu, 1981) while imipenem inhibits *P. cepacia* and *Pseudomonas stutzeri*, it is inactive against *X. maltophlia*. Emergence of resistant Pseudomonas spp has been observed during therapy with imipenem (Winston *et al.*, 1984). The drug may show in vitro antagonism when combined with third-generation cephalosporins or extended spectru penicillins as a result of its ability to induce β-lactamase production (Neu, 1985)

Imipenem is the most potent β-lactam against anaerobes, with activity comparable to those of clindamycin and metronidazole. The MIC_{90} for *B. fragilis, Bacteroides melaninogenicus,* other Bacteroides spp, fusobacterium spp and anaerobic gram positive cocci are ≤1µg/ml (Kesado *et al*., 1982) most *Clostridium* spp except some strains of Clostridium difficile are susceptible. Imipenem is also active against *Acrinomyces* spp., *Nocardioa asteriodes*, and *Mycobacterium* spp (Cynamon and Palmer, 1982). Other members of this group are panipenem, meropenem etc.

2.6.5 β- Lactamase inhibitor

(a) Clavulanic acid

Clavulanic acid is a naturally occurring weak antimicrobial agent found initially in cultures of *Streptomyces clavuligerus* (Neu and Fu, 1978; Reading and Cole, 1977). It inhibits β-lactamases from Staphylococci and any gram negative bacteria. This agent acts primarily as a "suicide inhibitor" by forming an irreversible acyl enzyme complex with β-lactamase, leading to loss of activity of the enzyme.

Clavulanic acid has been shown to act synergistically with various penicillins and cephalosporins against β-lactamase- producing staphylococci, Klebsiellae, *H influenzae, Moroxella catarrhalis, N gonorrhoeae, E coli Proteus spp.,* and *Bacteroides* spp (Bansal *et al.,* 1985; Fuchs *et al.,* 1984; Hunter *et al.*, 1980; Peter *et al.,* 1980; Sutherland *et al.*, 1985). Recently discovered plasmid-mediated TEM β lactamases in ceftazidime-resitant strain of *Klebsiella pneumoniae* and *E. coli* are inactivated by this agent (Wiedeman *et al.*, 1989) However, the inducible β-lactamases of *Enterobacter, Citrobacter, Acinetobacter, Serratia, and Pseudomonas* spp are not inhibited by clavulanic acid. The combination of clavulanic acid with ampicillin, amoxicillin, or ticarcillin is active in vitro against *Mycobacterium tuberculosis,* which is known to produce β-lactamases (Cyamon and Palmer, 1983; Sorg and Cyamon, 1987; Woodley and Kilburu, 1982).

(b). **Sulbactam**

Sulbactam is a semisynthetic 6 desaminopenicillin sulfone with weak with weak antibacterial activity (Englis *et al.*, 1978). It functions as effective inhibitors of certain plasmid and chromosomally mediated β-lactamases. Its inhibits the β-latamases of *S. aureus* many Enterobacteriaeceae, *H, influenzae, Moraxella catarrhalis. Neisseria* spp. *Legionella* spp. *Bacteroides* spp., *and Mycobacterium* spp (Greenwood and Eley, 1982)

sulbactam alone is active against *N. gonorrhoeae*, *N meningitides*,some*Acinetobacter* spp. and *P. ceptacia* (Jacob and Sutton, 1989; Retsema *et al*., 1986) it acts synergistically with penicillins and cephalosporins against organism that are otherwise resistant to the β-lactam drugs because of the production of β-lactamases. A combination of sulbactam (8μg/ml) and ampicillin (16μg.ml) inhibits most strains of staphylococci, *Klebsiella* spp., *E. coli*, *H. influenzae*, *Maraxella catarrhalis*, *Neisseria* spp., and *Bacteroides* spp. That are ampicillin resistant (Retsema *et al.,* 1980; Wise *et al.*, 1980) sulbactam is inactive against the β-lactamases of Enterobacter, citrobacter, and providencia spp., indole positive *Proteus* spp and *X. maltophilia*.

2.6.6 Aminoglycosides

Since the first amonoglycoside (amonoglycosidic aminocyclitol) streptomycin, was introduced in 1944, this class of antibiotic has played a vital role in the treatment of serious gram-negative infection. Among the unique features of the amonogluycosides are the bactericidal activity against aerobic gram-negative bacilli (including *Pseudomonias* spp) activity against *M. tuberculosis*, and relatively low incidence of bacterial resistance. The currently available aminoglycosides are derived from *Micromonospora* spp (gentamicin, sisomicin and netilmicin) or from streptomyces spp (streptomycin neomycin, kanamycin and tobramycin). The different in origin of these compound accounts for the differences of their suffixes “micin” versus “mycin” streptomycin, neomycin, kanamycin, tobramycin and gentamycin are naturally occurring aminoglycoisides, whereas amikacin and netilmicin are semi-synthetic derivatives of kanamycin and sisomicin, respectively. Isepamicinn which is presently undergoing clinical trial in the United States and elsewhere is a semisynthetic derivative of gentamicin B. Structurally each of these aminoglycosides

contains two or more amino sugar linked by glcosidic bonds to an aminocyclitol ring nucleus.

Aminoglycosides are bactericidal agents that inhibit bacterial protein synthesis by binding irreversible to the bacterial 30s ribosomal subunit. The aminoglycoside bound bacterial ribosomes then become unavailable for translation of mRNA during protein synthesis, thereby leading to cell death (Byan, 1984). They also cause misreading of the genetic code, with the resultant production of nonsense proteins. To reach the intracellular ribosomal binding targets, an aerobic energy dependent process is necessary to enable successful penetration of the aminoglycosides into the bacteria. The bacterial uptake of these agents is facilitated in the presence of inhibitors of bacterial cell wall synthesis such as β-lactams and vancomycin. This interaction aminoglycosides and β-lactam antibiotic (Davis, 1982; Giamarellou, 1986).

Spectinomycin acts similarly to the aminoglycosides by binding to the 30s ribosomal subunits and inhibiting protein synthesis. However, it does not cause misreading of the mRNA and is not bactericidal.

Aminoglycoside antibiotics are active primarily against aerobic gram-negative bacilli *S. aureus*. As a group they are particularly potent against the Enterobacteriaceae, *Ps. aeruginasa, Acinetobacter* spp., and *Providencia* spp. certain difference in antimicrobial spectra among the various aminoglycosides do exist. Kanamycin is limited in its spectrum because of the common resistance of *Ps. aeruginosa* and frequent occurrence of plasmid-mediated inactivating enzymes among other gram negative bacilli (Davis, 1983) it is now occasionally used as a “second line” drug in combination with other antibiotic in the therapy of tuberculosis similarly widestpread resistance among Enterbacterieaceae has limited the usefulness of streptomycin. As a single agent streptomycin is used in the therapy of infection due to *Francisella tularensis* (tularemia) and *Yersina pestis* (plague)

it is often used in conjunction with tetracycline for the treatment of brucellosis. It has the greatest in vitro activity of the aminoglycosides against *M. tuberculosis*. It may also be used in combination with penicillin or vancomycin for the treatment of infective endocarditis due to viridans streptococci or enterococci provided that the organism does not possess high level ribosomal or enzymatic resistance to steptomycin (Davis, 1983).

Although gentamicin and tobramycin have very similar antibacterial activity profiles, genetamicin is more potential in vitro against *Serratia* spp., whereas tobramycin is more potential against *P. aeruginosa* (Appel and Neu, 1978). However, these minor differences have not been correlated with greater efficacy of one agent over the other. For the most part, gentamicin and tobramycin are susceptible to inactivation by the same modifying enzymes produced by resistant bacteria with the exception that in contrast to gentamicin, tobramycin can be inactivated by 6-actyltransferase and 4 adenyltransferase and has variables susceptibility to 3-acetyltransferase and has viable susceptibility to 3-acetyltransferase. Netilmicin and amikacin are resistant to many of these aminoglycoside-modifying enzymes and therefore, are active against most Enterobacteriaceae that are resistant to gentamicin and tobramycin. Netilmicin is intrinsically less active than gentatmicin or tobramycin against *P. aeruginosa*, and most gentamicin-resistant *Setrratia*, *Proteus*, *Providencia* and *Pseudomonas* isolates are also usually resistant to netilmicin (Fu and Neu, 1976) Amikacin is used often as the aminoglycoside of choice when gentamicin and tobramycin resistance is prevalent. Aminoglycosides are only moderately active against *Haemphilius* and *Neisseria* spp.

Although active against staphylococci, aminoglycosies are not recommended as single agents for treatment of staphylococcal infections. Gentamicin is often combined with a penicillin or vancomycin for synergy in the treatment of serious infections due to

staphylococci, enterococi or viridans streptococci. The aminoglycosides are not active against anaerobes.

Spectinomycin is used primarily for uncomplicated anogenital infection due to *N. gonorhoeae (*Appel and Neu, 1978), including β- lactamase- producing strain. It is useful in patient with penicillin allergy. However, spectinomycin is ineffective for pharyngeal gonococcal infections syphilis, or chlamydail infections.

2.6.7 **Quinolones**

Quinolones belong to a novel group of potent antibiotics biochemically related to nalidixic acid, which was developed initially as a urinary antiseptic. Nalidixic acid and its early analogs, oxolinic acid and cinoxacin, have limited clinical application as a result of widespread emergence of bacterial resistance. Newer quinolones have been synthesized by modifying the original two-ring quinolone nucleus with different side chain substitutions (Wolfson and Hooper, 1985). These new agents also know as fluoroquinolones, contain a fluorine atom attached to the nucleus. A number of other fluoroquinolones including ofloxacin, enoxacin, lomefloxacin, temafloxacin, fleroxacin and others are undergoing clinical investigation in the United State and will likely be released for clinical use in the near future.

Quinolones posses excellent activity in vivo against enterbacteriace, *P. aeruginosa, Citrobacter, Serratia, and Acinetobacter spp.,* β-lactamase-postivie and –negative *H. influenzae* and gram-negative cocci such as *N.gonorrhoeae, N. Meningitidis*, and *Branhamella catarrhalis* (Phillips and King, 1988; Wolfson and Hooper, 1985). They are also active against *S. aureus*, including methicillin resistant strains and other staphylococcal species but are less potent against streptococci and enterococ. These agents

generally are in active against anaerobic bacteria, with little or no clinically useful activity against *B. fragilis* (Golstein and Citron, 1985) and *C. difficile* (Chow *et al.*, 1985; Delmee and Avesani, 1986).

Enteropathogeic gram-negative bacilli such as *Salmonellae, Shigellae, Yersinia enterocolitica, Vibrio* spp. Aeromonas and *Pseudomonas* spp. *Campylobacter jejuni* and Enterotoxigenic *E coli* are all susceptible to the quinolones. Clinical studies have shown these drugs to be effective in the prophylaxis as well as treatment of infectious diarrheas *Legionella* spp are susceptible to these agents with MICS of most fluoroquinolones of 0.12 to 10µml. Ciprofloxacin is the most active fluoroquinolone against

Ps. aeruginosa, with an MIC90 of < 0.5µg/ml (Phillips and King, 1988). However, *P. cepacia* and *X . maltophilia* are generally resistant to the quinolones.

The fluoroquinolones, especially ciprofloxacin, are active in virtro against *M. tuberculosis* Mycobacterium fortuitum-chelonae complex, *Mycobacterium kansasill*, and mycobacterium xenopi (Berln *et al.*, 1987; Davies *et al.*, 1987; Young *et al.*, 1987). Their activity against *Mycobacterium avium* intercellular complex is fair to poor. They also exhibit activity against *Chlamydia trachomatis* and *Mycoplasma hominis* but are less potent against *Ureaplasma urealyticum* (Sarma, 1989) ciprofloxacin and pelloxacin have been shown to inhibit *Rickettsia conorii* (Leigh, 1981) *Rickettsia rickettsii* (Raoult *et al*., 1987) and *Coxiella burnetii* (Yeaman *et al*., 1987) Quinolones have been found also to have in vitro activity against the malarial parasite *Plasmodium falciparum* alter achievable serum concentrations (Divos *et al*., 1988; Sarma, 1989) *Nocardia* spp are relatively resistant to the quinolones.

No significant inoculum effect has been observed among the bacteria susceptible to quinolones. Combinations of quinolones with β-lactam drug or aminoglycosides may be indifferent or additive in effects against gram-negative and gram positive bacteria and

mycobacteria. Synergism occurs particularly against *Ps. aeruginosa* in up 50% of strains tested (Neu, 1989; Wolfson and Hooper, 1989) However, bactericidal activities of quinolones can be antagonizxed by rifampin or chloramphenicol..

2.6.8 Tetracyclines

Tetracycliens are broad spectrum bacteriostatic antibiotic with the hydroonaphthacene nucleus which contain four fused rings. The congeners comprise three groups based on their duration of action

The tetracyclines act against susceptible microorganism by inhibiting protein synthesis. They enter bacteria by energy –dependent process and bind reversibly to the 30S ribosomal subunits of the bacteria this process blocks the access of aminoacyle t-RNA to the RNA-rbosomes complex, preventing bacterial poly peptide synthesis. Members of the tetracycline group include the following;

Short acting	**intermediate acting**	**long acting**
Tetracycline	Demeclocycline	Doxycycline
Chlortetracycline	Methacycline	Minocycline
Oxytetracycline		

All tetracyclines have similar antimicrobial spectra, with activity against many gram-positive and gram-negative with activity bacteria, Mycoplsmas, Chlamydiae, rickettsiae, and some protozoa.many gram-positive aerobic cocci including *S. asureus, S pyogenes* and *S pneumoniae*, are susceptible at concentrations achievable in the serum. However, emergence of tetracycline-resistant strain of *S pneumoniae* is frequent (Freeman *et al*., 1983). Although may *E.coli* are susceptible to tetracyclies, Pseudomonads and many

enterbacteriacea are resistant to these agent. Tetracyclines are used mainly for the treatment of acute, uncomplicated urinary tract infections due to *E.coli* and as effective prophylactic therapy for traveller's diarrhea caused by enterotoxigenic *E coli* (Freeman *et al.,* 1983; Merson *et al.*, 1980) with activity against Pseudomonas (Eickhoff *et al.,* 1970) *Brucella* spp (Farrell *et al.,* 1976) *Vibrio* spp (Morris and Black, 1985) and *Mycobacterium marimum,* they have been used successful in the treatment of infections due to these bacteria. Minocycline is active against *Nocardia* spp (Dewsnup and Wright, 1984). Tetracyclines are active against many anaerobic bacteria, including *B. fragilis* and *Actinomyces* spp (Chow *et al.,* 1975). They are commonly used with or without neomycin as oral bowel preparations preoperatively.

These drugs are useful in the treatment of urethritis and acute pelvic inflammatory diseases caused by *N. gonorrhoeae, Chlamydia trachomatis, U urealyticum,* and *Mycoplasma hominis*. Emergence of resistance to tetracyclies among *N. gonorrhoeae* is increasing (CDC, 1987) they are effective for the treatment of other chlamydial infections (psittacosis, lymphogranuloma venereum, and trachoma. other infections responsive to tetracyclines include grauloma inguinale, chancroid, relapsing fever tularemia and cholera Tetracyclines are the drug of choie for treating rickettisal infections (Rocky Mountain spotted fever, endemic and scrub typhus, and Q fever) Many pathogenic spirochetes, including treponema pallidum and borrelia burgdorferi, the agent of Lyme disease (Johnson *et al.,* 1984) are susceptible. Protozoans such as plasmodium spp and enteaemoeba histolytica are also inhibited by these drugs (Colwell *et al.,* 1972; Pang *et al.*, 1987)

2.6.9 **Macrolides**

Erythromycin is a macrolide antibiotic derived from *Streptomyces erythreus*. Its chemical structure consists of a macrocyclic lactone ring attached to two sugar moieties, desosamine

and cladinose. Other marcolides include trioleandomycin and oleandomycin, and those currently under investigation include dirithromycin, clarithromycin, roxithromycin, azithromycin and spiramycin (Hardy et al., 1988; Kirst and Sides, 1989).

Erythromycin is a bacteriostatic agent that inhibits bacterial RNA dependent protein synthesis. This agent may be bactericidal at high drug concentration and against a low inoculum of bacteria. It binds reversible to the 50S ribosomal subunits of susceptible microorganism, thereby blocking the translocation reaction of polypeptide chain elongation (Oleinick and Cororan, 1969).

Erytromycin is a relatively broad-spectrum antibiotic with activity against gram positive and some gram negative bacteria, mycoplasmas, chlamydiae, triponemes, and rickettsiae (Washington and Wilson, 1985). It shows good activity against pneumococci and groups-A streptococci, but emergence of resistance among these isolates (especially group -A streptococci) is a problem in certain areas of the world (Dixon and Lipinskl, 1972; Linares *et al.*, 1983). Erythromycin is also active against *S. aureus, Corynebacterium diphtheriae,L. monocytogees,* and *Actinomyces isrealii*. Its antibacterial activity against gram negative bacilli is infleucned by pH with increasing potency as the pH rises to 8.5. It is active against *N. gonorrhoeae, N meningitides, Bordetella pertussis, Campylabacter jejuni* (Vanhoff *et al.,* 1980) and *Helicobacter pylori* (McNulty *et al*., 1985). It has somewhat lower activity against *H. influenzae*. It is the drug of choice for the treatment of infection due to *Legionella pneumophila* and *Legionell micdade* (Dowling *et al.,* 1982; Edelstein and Meyer, 1980). Erythromycin has activity against some species of gram-negative anaerobes but not against B fragilis strains.

This drug may be used for the treatment of gonorrhea and syphilis in patients who can not tolerate penicillin G or tetracycline. It is effective as an alternatie to tetracycline for Chlamydia infections. It is 50 times more potent than tetracycline against *Mycoplasma*

pnuumoniae. Erytromycin is used occasionally in the therapy of infections with *Mycobacterium scrofulaceum*, *M kansaii* (Molavl and Weinstein, 1971) and *M. chelonae* (Wallace *et al*., 1982) and in combination with ampicillin against *Nocardia asteroid*.

Other macrolides, such as dirithromycin, clarithromycin, and azithromycin, exhibit pharmacokinetic advantage over erythromycin. With the possible exception of azithromycin which exhibits some enhanced activity against H influenzae and other gram-negative organism none of he other compound differs significantly from erythromycin in its spectrum of activity. Spiramycin has been reported to show efficacy in the treatment of toxoplasmosis and crypotosporidiasis, although its utility for the latter remains to be proven (Davey *et al.*, 1988). Other related compounds include; Azalides, Ketolides and Streptogramins.

2.6.10 Lincosamides

Lincosamide antibiotics (lincomycin and clindamycin) were initially isolated from *Streptomyces linocolnensis.* Each drug has a chemical structure consisting of an amino acid linked to an amino sugar. Clindamycin is a derivative of lincomycin with increased antibacterial activity and improved absorption after oral administration. Both drugs are available for parenteral and oral use, but lincomycin is now used very infrequently in the United states.

Lincosomides bind to the 50S ribosomal subunit of susceptible bacterial and suppress protein synthesis (Reusser, 1975). Their ribosomal binding sites are the same as or closely related to those for the macrolides and chloroamphenicol. Clindamycin can be concentration, bacterial species' and inoculum or bacteria dependent.

Lincosamides have a broad spectrum of activity against the aerobic gram-positive cocci and anaerobes clindamycin is more potent than lincomycin against methicillin susceptible staphylococcus spp S pneumoniae, and group A and viridans streptococci. The MIC_{50} are in the range of 0.01 to 0.1 μg/ml for those strain. However, lincosamide resistance has been reported among clinical isolates of these bacteria which are usually also resistant to erythromycin (Kohn and Evan, 1970; Linares *et al.,* 1983; Moellering, 1984). The prevalence of lincosamide resistance *S .aureus* may be 15 to 205 in some institutions. Enterococci are uniformly resistance to the linocosmides.. Clindamycin is one of the most active antibiotics available against anaerobes including *B fragilis* and *Clostridium perfringens* with MIC_{90} of ≤ 32μg/ml. However, clindamycin resistance (which appears to be increasing) is found in 5 to 10% of *B. fragilis* groups species, 10 to 25% of *Clostridial* species, 10% of Peptococci and most *Fusobacterium varium* strain (Retsema *et al.,* 1980; Sutter, 1977). All of the enterobaceriaceae are resistant to clindamycin. Clindamycin has been used to treat actinomycosis, babesiosis, malaria and toxoplasma encephalitis (Danneman *et al.,* 1988; Rolston and Hoy, 1987).

2.6.11 Glycopeptides and lipopeptides

Vancomycin a bactericidal antibiotic obtained from streptomycin orientates is the only glycopeptide currently available for clinical use. Initially introduced for it efficacy against penicillin resistant staphylococci it has become most useful against methicillin-resistant stapjhylococci and in patients allergic to penicillins or cephalosporins.It has a spectrum of activity similar to those of the glycopeptides.

Glycopeptides inhibit peptidoglycan synthesis in the bacterial cell wall by complexing with the D-alanyl-D alanine portion of the cell wall precursor (Nieto and Perkin, 1971) r. Daptomycin also acts by inhibiting bacterial peptidoglycan synthesis but at a synthesis

step earlier than that inhibited by the glycopeptides (Allen *et al*., 1987). The exact mechanism of action of daptomycin has yet to be determined.

Glycopepptides and daptomycin are active mainly against aerobic and anaerobic gram-positive organism including methicillin susceptible and resistant staphylococci streptococci enterococci *Corynebacteium* spp., *Bacillus* spp., *Lactobacillus* spp., *L moocytogenes, clostridium* spp., and *Actinomyces* spp. The MICS of vancomycin against *S. aureus, S. epidermidis,* streptococci, and enterococci are typically in the range of 0.25 to 2μg/ml (Moorhouse *et al.,* 1985; Takayama *et al.,* 1972; Watanakunakorn, 1984). MBCs at 20-fold of MICS for viridans streptococci and 50 times the MICS for enterococci. Teicoplsanin and daptomycin (Eliopoulos *et al.,* 1986; Fass and Helsel, 1986; Low *et al.,* 1989) are two to four times more active than vancomycin and teicoplanin, which are characteristically bacteriostatic against enterococci daptomycin exhibits modierate bactericidal activity towards this group of organism. Recently vancomycin-resistant isolates of *Enerococcus faecalis, Enterococus faecium, enterococcu gallinarum,* and coagulase-negative staphylococci have been reported. Cross resitance with teicoplanin is variables in these strains, but ore are susceptible to daptomycin. Other vacomycin-resistant gram positive organisms include *Leuconostoc, Lactobacillus and Pediococcus* spp.

Vancomycin is useful in the prevention and treatment of endocarditis due to gram-positive bacteia in patients who are allergic to penicillin. It is the drug of choice for treating corynebacterium group infrections and is useful for floavobacterium meningosepticum meningitis and antibiotic associated *Cl. difficile* colitis.

The glycopeptides and daptomycin are not active against gram-negative organism. Recently, vancomycin-resistant isolates of *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus gallinarum* and coagulase negative Staphylococci have been reported (Bingen, *et al*., 1989; Sahm et al., 1989; Shlaes *et al.,* 1989; Uttley *et al.,* 1988; Leclereq *et*

al., 1988; Williams *et al.,* 1989). Cross resistance with teicoplanin is variable in these strains but most and susceptible to daptomycin. Other vancomycin-resistant gram-positive organisms include leuconostoc lactobacillus and Pediococcus spp. (Buu *et al*., 1985; Colman and Efstratiou, 1987; Isenberg *et al.,* 1988; Rubin *et al.,* 1988; Ruoff *et al.,* 1988).

Vancomycin is useful in the prevention and treatment of endocarditis due to gram positive bacteia in patients who are allergic to penicillin. It is the drug of choice for treating *Corynebacterium* group JK infections and is useful for *Flavobacteium meningosepticum* meningitis and antibiotic associated *C. difficile* colitis.

The glcopeptides and daptomycin are not active against gram negative organism, mycobacteia, or fungi. They show no cross resistance with the other unrelated antibiotics. They can be synergistic with amino glycosides (and in some cases with rifampin) against Staphylococci, Streptococci, Enterococi and listeriae.

2.6.12 Sulfonamides and trimethoprim

Sulfonamides were the first effective systemic anti microbial agents used in the United States during the 1930s. They are derived from sulfanilamide, which is similar in chemical structure to para-aminobenzoic acid, a factor essential for bacterial folic acid synthesis. Various susbstitutions at the sulfonyl radical attached to the benzene ring nucleus enhance the antibacterial activity and also determine pharmacologic properties of the drug.

Trimethoprime (TMP) is a phyrimidine analog that inhibits the enzyme dihydrofolate reductase, interfering with folic acid metabolism and subsequent pyrindmidine synthesis and one-carbon fragment metabolism in the bacteria. Sine TMP and sulfonamides block the bacterial folic acid metabolic pathway at different sites, they potentate the antibacterial activity of one another and act synergistically against a wide variety of organisms. Such a

combination, TMP sulfamethoxazole (SMX), also called cotrimoxazole was introduced clinically in 1968 and has proven tobe very effective I the treatment of many infections.

Sulfonamides competitively inhibit bacterial modification of para-aminobenzoic acid into dihydrofolat, where as TMP inhibit bacterial dihydrofolate reductase. This sequential inhibition of folae metabolism ultimately prevents bacterial DNA synthesis. Since mammalian cells do not synthesize folic acid, human purine synthesis is not affected significantly by sulfonasmides or TMP. The antibacterial effect of these agents may be reduced in patients receiving high doses of folinic acid.

Sulfonamides are inhibitory to a variety of gram positive and gram-negative bacteria actinomycetes chalmydiae toxoplasmas, and plasmodia their in viro antimicrobial activity is irregular strongly influenced by inoculum size and composition of the test media. Susceptibility testing endopoints are often difficult to determine because of the presence of hazy growth within zones of inhibition in disk diffusion tests and because of the phenomenon of “trailing” in dilution test. Sulfadiazine and sulfisoxazole are effective for rheumatic fever prophylaxis but they are not useful in treating established group- A beta-hemolytic streptococcal pharyngistis. These drugs may be used for prophylaxis of close contact of patients with meningtitis due to sulfonamide susceptible N meningitides. Sulfisoxazole can be used to treat chlamydial urethrities, and sulfacetamide ophthalmic solution is effective for trachoma and inclusion conjuctivities.

Sulfadiazine is combined with pyrimethamine has been used successfully to treat toxoplasmosis and sulfadcoxine combined with pyrimethamine (fansidar) is effective in the prophylaxis and therapy of *Plasmodoim falciparum*, *malaria*. Sulfonamides are active against *Nocardia asteroids* and they show moderate activity against *M kansasill, M. fortuitum, M. marinum* and *M. scrofulaceum* . Other uses of sulfonamides include therpy of meliodosis dermatitis herpetiformis lymphogranuloma venerum and chancroid.

Among the gram negative bacilli *E. coli* strain are generally susceptible to the sulfonamides especially at levels achievable in the urine. Therefore these drugs are used primarily in the treatment of first episode acute urinary tract infection due to *E. coli.* However increasing bacterial resistance has limited their efficacy in recent years *Serratia marcescene P. aeruginosa* enterococci and anaerobes are usually resistant to the sulfonamides.

TMP is active in vitro against many gram positive cocci and ost gram negative bacilli p aeruginosa most anaerobes, *Mycoplasma pneumoniae* and Mycobactera are resistant. The MIC varies considerably with the test media used. As with the sulfonamdes, the invitro activity of TMP is lowered in the presence of thymidinc. Currently TMP alone is primarily used in the therapy of uncomplicated and recurrent urinary tract infections due to susceptible organism However, the prevalence of TMP resistance enterobacteriaceae is increasing.

Combinations of TMP with other agents such as rifampin polymyxins and aminoglycosides have demonstrated in vitro synergistic antibacterial activity against various gram-negative bacilli TMP combined with dapsone is effective in the treatment of *Pneumocystic carinti,* pneumonia immunocompromised patients (Joseph and Robert, 1991)

Many gram positive cocci including staphylococci and streptococci and most gram negative bacilli except *P. aeruginosa* are susceptible to TMP SMX. This drug combination has variables bactericidal effect on enterococci, depending on the media used. With excellent activity against *S. pneumoniae, Moraxella caterrhalis* and *H influenzae* including β-lactamase producing strain TMP SMX is useful for the therapy of actute otitis media sinusitis acute bronchitis and pneumonia. It has shown excellent result in the prophylaxis

and therapy of acute and chronic urinary tract infection. It is an effective alternative tyheraphy for uncomplicated urogenital gonorrhea, including cases caused by penicillinase producing *N.gonorrhoeae*. it can be used also for the treatment of nongonococcal urethritis due to *Chlamydia trachomatis* and chancroid. The drug combination is also useful in treating infections due to *Salmonellae, Shigellae*, enteropathogenic *E. coli* and *Y. enterocolitica*. It has been used successfully for prophylaxis and treatment of traveler diarrhea.

Other microorganisms susceptible to TMP-SMX include *Brucella* spp *P psuedomallei, P cepacia, X. maltophilia, M. kansasii , M. marinnum* and *M. scrofulaceum. M. tuberculosis* and *Mycobacterium chelonei* are generally resistant. It is valuable antibiotic for the treatment of *Nocardia asteroids* infections *P cepacia* and *X maltophilia* bactermia *L monocytogenes* meningitis isopora belli gastroenteritis and Whipple's disease. In immuno compromised host those with leukemiam organ transplant recipients and those with AIDS, TMP-SMX is effective for the prophylaxis and treatment of *Pneumocysis carinill* pneumonia.

2.6.13 Polypeptides

Polymyxins are a group of related cyclic basic polypeptides derived from *Bacillus polymyxa.* They have limited spectra of antimicrobial activity and significant toxicity. Ony polymyxins B and E (colistin) are available for therapeutic use in humans.

Acting like detergent or surfactants members of this group of antibiotics interact with the phospholipids of the bacterial cell membrane, thereby increasing cell permeability and disrupting osmotic integrity. This process results in leakage of intracellular constituents leading to cell death. The bactericidal action is reduced in the presence of calcium which interferes with the attachment of drugs to the cell membrane (Joseph and Robert, 1991).

Polymyxins are active only against gram-negative bacilli, especially *Pseudomonas* spp. The MIC90s for *Pseudomonas* spp, inclding *P. aeruginosa* are <8 μg/ml. *Proteus, Providecia Serratia* and *Neisseria* isolates are usually resistant. Emergence of resistance during therapy is rare and there is no cross resistance with other antibiotics. Polymyxins B and E have identical antimicrobial spectra and show complete cross resistance to one another.

Combination of polymyxin with TMP-SMX may be synergistic in the treatment of serious infection due to multiple resistant *Serratia spp., P. aeruginosa, P cepacia* and *X. maltophilia*. The polymyxins are usually reserved for serious life threatening pseudomonas or gram negative to other antibiotics Aerosolized polymyxins have been used successfully to treat *Ps. aeruginosa* respiratory infections in patients with cystic fibrosis or bronchiectasis.

Bacitracin was originally isolated from *Bacillus licheniformis* (formerly *B. subtilis*), it is a peptide antibiotic consisting of peptide-linked amino acids. Although it was introduced initially for the systemic treatment of sever staphylococcal infections, it is now mainly reserve staphylococcal infections, it is now mainly restricted to topical use because of its systemic toxicity.

Bacitracin inhibits dephosphorylation of a lipid pyrophosphate a step essential for bacterial cell wall synthesis. It also disrupts the bacterial cytoplastic membrane. The drug is active mainly against gram positive bacteria, particularly staphylococci and group- A beta-hemolytic streptococci. However, group C and G streptococci are less susceptible whereas group B streptococci are resistant *Neisseria* spp are also susceptible, but gram negative bacilli are resistant. Bacitracin is often combined with neomycin polymyxin B or both in topical preparation to provide broad spectrum antibacterial coverage. Orally administered

bacitracin has been shown to be effective in treating antibiotic-associated *C. difficile* colitis.

2.6.14 Chloramphenicol

Chloramphenicol is a unique antibiotic originally derived from *Streptomyces venzuelae*. Its chemical structure contains a nitrobenzene ring. It is a highly effective broad spectrum antimicrobial agent with specific indications for use in seriously ill patients. Thiamphenicol is an analog of chloramphenicol with a similar spectrum of antimicrobial activity. Only chloroamphenicol is available for clinical use.

The drug is a bacteriostatic agent that inhibits protein synthesis by binding reversibly to the peptidyltransferase component of the 50S ribosomal subunit and preventing the transpeptidation process of peptide chain elongation. At therapeutic concentrations achieveable in the serum, it can be bactericidal against common meningeal pathogens such as *S. pneumonie, N meningitides* and *H. influenzae*. Bacterial resistance occurs with plasmid-mediated production of chloramphenicol acetyltransferase enzyme which inactivates the drug.

Chloramphenicol is very active against many gram- positive and gram negative bacteria, chlmydiae, mycoplasmas and rickettsiae. $MIC_{90}S$ for most gram-posiive aerobic and anaerobic cocci are $\leq$ 12.5 µg/ml However; the drug is usually inactive against methicilin –resistance *S. aureus* and *S epidermidis* and against enterococci, *N. meningitides, H. influenzae* (ampicillin ressisant and susceptible strains) and most enterobacteriaceae is susceptible. Its activity against *Serratia* and *Enterobacter* isolates is variables and pseudomonas spp are usually resistant. Salmonellae, including *Salmonella typhi* are also susceptible but resistant isolates are being encountered with increasing frequency. Chloramphenicol has excellent activity against aerobic bacteria, including *B. fragilis.*

Almost all of these isolates are inhibited at concentrations of ≤10 μ/ml . it is also active against *Rickettisa* spp and *Coxiella burnetii.*

2.6.15 Metronidazole

Metronidazole is a nitroimidazole derivative that was first introduced in 1959 for the treatment of trichomonas vaginalis infections. It now has an impotant therapeutic role in the treatment of infections due to anaerobic bacteria and certain protozoan parasites. Metronidazole owes its bactericidal activity to the nitro group of its chemical structure. After gaining entry into the cells of susceptible organism, the nitrogen group is reduced by a nitroreductase enzyme in the cytoplasm generating certain short lived highly cytoxic intermediate compound or free radicals that disrupt host DNA

Metronidazole exhibits potent activity against almost all anaerobic bacteria including B fragilis group fusobacterium, and clostridium spp. (Bartlett, 1982; Eykyn *et al.*, 1976; Riddell *et al.*, 1960; Sutter, 1983) it is the only antimicrobial agent with consistent bactericidal activity against *B. fragilis* (Ralph and Kirby, 1975). However, the susceptibility of gram-positive anaerobic cocci is some what variable, with MIC90s is16 μg/ml for these organisms. Most strains of the genera actinomyces, Arachnia, and Propionibacterium are usually resistant. Frequencies of metronidazole-resistant Bacteroides isolate (MIC of > 16 μg/ml) in the range 2 to 5% have been reported from various institutions (Cuchural *et al.*, 1988; Dublanchet, *et al.*, 1986; Musial and Rosenblatt, 1989). Metronidazole has no activity against aerobic bacteria, including the enterobacteriaceae.

The drug is effective in the treatment of antibiotic associated colitis caused by *C. difficile* (Cherry *et al.*, 1982; Kleinfeld *et al.*, 1988), with efficacy equivalent to that of oral

vancomycin for this indication (Teasley *et al*., 1983) it is also useful in ombination with an aminoglycodise for treating polymicrobial soft tissue infections and mixed aerobic anaerobic intra abdominal and pelvic infections. Metronidazole is active against the *protozoa Trichomonas viginalis, Giardia lamblia* and *Entomoeba histolytica*. It is the drug of choice for the treatment of trichomoniasis giardiasis and intestinal and invasive amebiasis including amoebic liver abscess (Medical letter, 1988; Most, 1984; Swedberg *et al.,* 1985)

Tinidazole and ornidazole are other nitroimidazole derivatives with somewhat more potent antianaerobe activity than metronidazole. However, they are not available for clinical use.

2.6.16 **Nitrofurantoine**

Nitrofurantion belongs to a class of compounds consisting of a primary nitro group joined to a heterocyclic rings. Its role in human therapeutic is limited to the treatment of urinary tract infections (D'Arcy, 1985).

The precise mechanism of action of nitrofurantioin is unknown. The drug is believed to inhibit various bacterial enzymes and also damage DNA (McCalla, 1977).

Nitrofurantoin has a broad spectrum of antibacterial activity against gram-positive and gram-negative bacterial particularly the common urinary tract pathogens it is active against gram-positive cocci such as *S. aureus, S. epidermidis, S. saprophyticus* and *Enterococcus faecalis* with MICS in the range of 4 to 25 µg/ml (Hof, *et al.*, 1984) *S. pneumoniae, S pyogenes, Bacillus substilis,* and *Corynebacterium* spp are also susceptible, but they rarely cause urinary tract infections over 90% of *E.coli* and many coliform bacteria are sensitive to nitrofurantoin at MICs,32 µg/ml. However, only one third of enterobacter and klebsiella isolates are susceptible. *Pseudomonas* and most *Proteus* spp are resistant susceptible organism rarely became resistant to this drug during therapy.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Selection of Hospitals

Hospitals and healthcare institutions that had facilities for in-patients admissions in Ilorin, Offa, Omu-aran, Oke-ode and Jebba towns of Kwara State of Nigeria were visited in June 2001, to assess their suitability and readiness to serve as sample collection centres. Beside the General Hospital, Offa, and those located in Ilorin town, other hospitals and health institutions admit only few patients at a time (a number that is not significant enough for use in this study) or generally refer patients requiring hospital admission to Ilorin, particularly General Hospital, Ilorin and University of Ilorin Teaching Hospital because of lack of needed facilities. For this reasons, only hospitals located within Ilorin metropolis were used as sample collection centres. The following criteria were considered in selecting the prospective hospital for sampling:

- Location of the hospital; It was ensured that the selected hospitals were spread out so as to give a good representative of the hospitals in the metropolis.
- Bed size of the hospital: must not be less than thirty (30) in number.
- Separate male, female and surgical wards.
- Regular performance of surgical operations.

The following hospitals that met the above criteria were selected. And for convenience sake they are coded A, B, C, D, E, F.

A Olalomi Hospital and Maternity, Ilorin.

B Temitope Hospital, Ilorin.

C Ola Olu Hospital, Ilorin.

D Civil Service Clinic, Ilorin.

E Sobi Military Hospital, Ilorin

F Surulere Hospital and Maternity, Ilorin.

University of Ilorin Teaching Hospital (UITH).

Official requests were made to and approvals sought from the administrative heads of the hospitals. In the case of University of Ilorin Teaching Hospital, the research proposal was submitted and defended before the Ethical and Research Committee of the Hospital sequel to the giving of the approval. A consultant medical microbiologist was appointed by the Committee to serve as the hospital representative for the work to facilitate data and sample collection from patients as well as interactions with patients and the responsible consultant physicians. For convenience the University of Ilorin teaching Hospital have been abbreviated to UITH.

3.1.2 Description of Sample Collection Centres

A. Olalomi Hospital and Maternity, Ilorin.

The hospital is located within Ilorin metropolis. It is about 5km a way from University of Ilorin teaching hospital (UITH). It is a two storey building with capacity of 40 beds. The hospital has separate male, female, children and surgical wards. It is maned by qualified medical personnel.

B Temitope Hospital. Ilorin.

Temitope is located in the eastern part of Ilorin. It is a three storey building with bed size of 48. It has male, female, children and surgical ward. It performs surgical operations regularly.

C. Ola Olu Hospital, Ilorin.

This hospital is one of the oldest private hospitalsin the town. It has good personnel and well patronized by patients. It is located in the western part of Ilorin. It has male, female, children, and surgical wards. The bed size is about 45. It has medically qualified personnel.

D. Civil Service Clinic, Ilorin.

Civil service clinic is a government owned hospital, purposely for the civil servants in the state. It is located in the southern part of Ilorin; it has bed size of 45, with separate wards for male, female, children, surgical patients and ophthalmic ward.

E Sobi Military Hospital.

The hospital is for the military officers in 22-armourd brigade Sobi barrack Ilorin. It is located about 20km a way from UITH, in Ilorin northern side. It has about 30 bed size, with wards for male, female, surgical and children patients. All medical personnel in this hospital are medically qualified uniform officers.

F Surulere Hospital and Maternity

The hospital is located in the central part of Ilorin , about 3km a way from UITH. It has bed capacity of about 26, with male, female, surgical and children wards separately. The

hospital is owned by one of the UITH consultants. It is reputed for outstanding medical care. The of patients patronage is enormous.

University of Ilorin Teaching Hospital

University of Ilorin Teaching Hospital (UITH) is a referral Health institution for all other hospitals and health centers in Kwara State and villages and towns in adjoining States. The Hospital has a bed space for over 600 patients. It is located within the Centre of Ilorin metropolis. There are separate wards for in-patients, presenting of different health categories. It has a good number of medical personnel including consultants and specialists in various fields of medicine.

Both retrospective and prospective aspects of the study were carried out in this hospital.

3.1.3 Selection of Wards in the Hospitals

All available wards with patients were selected for evaluation of incidences of nosocomial infections. In the private hospitals, four wards were selected for study, namely:

- Male ward
- Female ward
- Surgical ward, and
- Children ward

In the University of Ilorin Teaching hospital, the data on nosocomial infections were collected from the following wards or units:

- Ward 1 (W1) adult male medical ward
- Ward 2 (W2) orthopaedic surgical (male) ward
- Ward 3 (W3) paediatric surgical ward

- Ward 4 (W4) medical male and female ward
- Ward 5 (W5) surgical male and female ward
- Ward 6 (W6) female medical ward
- Ward 7 (W7) neurosurgical ward
- Ward 8 (W8) female orthopaedic/ ENT/ophthalmology ward

The wards of the maternity wing of the Teaching hospital also surveyed are:

- Ward 1 (post-natal ward)
- Ward 2 (surgical post-natal ward)
- Ward 4 (Infection ward)
- NICU (Neonatal Intensive Care Unit)
- Amenity ward

3.1.4 Bacteriological Culture Media

- Nutrient agar (LAB M, LAB 8), Topley House, 52 Wash Lane, Bury, Lancashire, BL9 6AU, UK. www.idgplc.com.
- MacConkey Agar (LAB M-LAB 2), Topley House, 52 WashLane, Bury, Lancashire, BL9 6AU, UK. www.idgplc.com.
- Muller Hinton Agar (LAB M-LAB 39), Lab M Limited Topley House, 52 Wash Lane, Bury, Lancashire BL9 6AS, United Kingdom. www.labm.com.
- Chocolate Agar, prepared from nutrient agar
- Peptone Water (LAM M- LAB 104), Topley House, 52 Wash lane, Bury, Lancashire, BL9 6AU, UK. www.idgplc.com
- Triple Sugar Iron Agar (HI-MEDIA), REF M 0211. Himedia Laboratories Pvt. Ltd, 23, Vadhani ind.Est, LBS Marg, Mumbai-400 086, India.

- Simmons Citrate Agar (HIMEDIA) REF M 099. Himedia Laboratories Pvt. Ltd, 23, Vadhani ind Est, LBS Marg, Mubai-400 086, India.
- Laury Tryptose Broth (Oxoide-CM451, UK).

3.1.5 Equipment

- Electrophoresis Chamber (model BIA), Owl Separation System inc., Portsmouth, NH, USA.
- Autoclave, model EC2P2ER, Gallenkamp Co. Ltd England
- Incubator, ambient (EC2H3, Gallankamp. Co. Ltd England)
- Incubator, Carbon dioxide, (model- 490 ICE, Barnstead International, USA)
- Refrigerator, (Thermocol)
- Freezer (5^0C), (Thermocool).
- Freezer (-20^0C), model LEIIORG, Fissons Scientific England
- Mini-centrifuge (model sm800b, UNISCOPE, Surgifriend Medical, England).
- Centrifuge, (model 90034d , UNISCOPE)
- Binocular Microscope (Olympus)
- Hot-air Oven, model EC3P3ER GallenkampCo. Ltd England
- Pressure cooker
- Transformer (
- Micro-pipette, (M12, Fisher scientific Co. Spring field USA
- Water bath, model Gallenkamp Co. Ltd. England

Chemicals & Reagents

- Barium chloride ($BaCl_2$)
- Hydrochloric acid, concentrated
- Sulphuric acid, concentrated.
- Sodium Hydroxide (BDH)
- Phenol
- Acetone
- Di-Potassium hydrogen phosphate (K_2HPO_4), anhydrous. BDH Chemical Ltd. England
- Potassium chloride
- Ammonium oxalate
- Phosphate buffer
- Lysol Creosate
- Izal solution
- Acridine orange
- Bromophenol blue
- Ethidium bromide
- Buffer A (400mM Tris, 20mM, Na-EDTA, Acetic acid to pH 8.0)
- Buffer B (3M acetic acid, Acetic acid to pH 5.5)
- Buffer C (10mM Tris, 2mM Na_2EDTA, and Acetic acid to pH 8.0.
- Lysin solution (4% SDS, 100mM Tris).
- DNA Maker standard (DNA HIND ll, Supplied by Roach, Pharm. Nig Plc, Lagos).

3.1.6 Recruitment of Patients for Study at the University of Ilorin Teaching Hospital.

Patients that were newly admitted and slated for operations were recruited for the study. They were monitored from the day of admission to the time the operation was performed. The following parameters were taken from the patients and recorded.

a. The time spent in the hospital before operation.

b. Underlining diseases.

c. Age of the patient

d. Antibiotics usage before operation.

During the operation, the bacteria load of the air and floor of the theater were studied.

3.2 METHODS

3.2.1 Retrospective Study of Nosocomial Infections

Retrospective study was carried out by assessing the medical records of patients kept with the medical record department of the Teaching Hospital and the record units of the other hospitals. Approvals were given to the heads of the medical record units by the Chief Medical Director of the Hospitals and the Head of the Department of Medical Microbiology and Parasitology in case of the Teaching Hospital. Medical records of patients for the year 2002 were available for data collection in the six private hospitals because of improper record keeping. In the Teaching Hospital, however records for three years (2000, 2001 and 2002) were available for analysis.

The study was based on review of hospital records of patients admitted and discharged between the years 2000-2002 as contained in their hospitals folders and Microbiology laboratory records. For patients whose information about their specimen

microbiological test results not available in the folders, visits were made to the microbiology laboratory to retrieve the needed data. Attempts were also made to verify the microbiological test results in the folders by matching them with records kept in the hospital microbiology laboratory. The folders were arranged year by year and sorted out ward by ward.

The following information were obtained from the folders: *patients hospital numbers, names, age, sex, date of admission, diagnosis on admission, specimen sent to the microbiology laboratory and results, type of organism(s) isolated, susceptibility of the isolates to the antibiotics tested, second diagnosis and date and interval between the first and second diagnosis.*

To classify an infection to be nosocomial, the following criteria, as specified by WHO/CDC (2002) were used:

- Time interval between the first and second diagnosis is not less than 48 hours
- Positive culture results in the first and second diagnosis
- Isolated pathogen in second diagnosis is different from the first isolated pathogen.

3.2.2 Classification of Infections into Sites

Classification of infections into sites was based on the diagnosis in the folders and the type of specimen sent to the microbiology laboratory for investigations.

When record indicated surgical operation and wound swab was sent to microbiology laboratory for culture and the culture was positive, the infection acquired is considered as surgical site. Where the sample involved was urine, with significant bacteria level, the infection is considered urinary. Whereas, when the specimens cultured are sputum

and blood and the culture produced positive result, the patients were deemed to have developed respiratory and blood stream infections respectively.

3.2.3 Determination of Rates of Infections

Rate of infection was determined by dividing total number of patients infected by total number of patients discharged multiply by 100.

$$\text{Infection rate} = \frac{\text{No of patients infected}}{\text{Total no of patients discharged}} \times 100 \quad \ldots\ldots\ldots\ldots\text{Equation 1}$$

Hospital wards with high prevalence of nosocomial infections were selected for prospective study. Based on the analysis of retrospective study, Ward 3 and Ward 5 which had high rates were selected for the prospective study. The surgical site which had the highest prevalence rate of nosocomial infections was also selected for prospective study. .

3.2.4 Bacteriological Study of Theatre Air

The bacterial load of the air in the operation theater of University of Ilorin Teaching Hospital was studied using settling plate method. Plates containing Nutrient agar (NA), MacConkey agar, and Chocolate agar were exposed and placed in different areas of the theater at the start of each surgical operation and left exposed until the operation procedure was completed. Thereafter, they were covered and taken for incubation. The duration for each operation was recorded. The Nutrient and MacConkey agar plates were incubated at $37^{o}C$ in an ambient incubator while plates containing Chocolate agar were incubated in a carbon dioxide incubator containing 10% CO_2 for 24 hrs. Colonies that developed after 24hr of incubation in the plates were enumerated and recorded. Based on the number of colonies per plate, the number of exposed plates and the duration of the surgical operation, the number of colonies in the air per hour was

determined and recorded number of colonies forming unit per cubic meter of air (cfu/m^3). Colonies showing different morphological characteristics were isolated, purified into pure cultures, coded, sub-cultured onto nutrient agar slant and kept in refrigerator for subsequent characterization.

3.2.5 Bacteriological Study of Theatre Floor

While the operation was in progress, the bacteria load of the theater floor was studied using swabbing method. The floor of theater which has already been demarcated (one meter square) was sub divided into 16 sub-squares, and one of these squares was randomly chosen and swabbed using sterile cotton swab moistened with sterile normal saline. The swab was then put into test tube containing 9ml sterile normal saline and mixed properly as to discharge it contents. One milliliter (1ml) each of this mixture was then used to flood plates of Nutrient agar, MacConkey agar and Chocolate agar. Plates of nutrient agar and MacConkey agar were incubated at 37^{o}C for 24 hrs while the plates of Chocolate agar was incubated under 10% CO_2 for 24 hrs (Bond and Schulster, 2004 and Favero *et al*., 1984).

The colonies that developed after 24 hrs of incubation, were counted, streaked onto nutrient agar slants and stored at 7^{o}C in refrigerator for further processing.

3.2.6 Bacteriological Study of Operated Surgical Sites

Surgical operations in the theater were followed and the time taken to complete each surgical operation cycle (i.e. time between incision of the operation site and closure of sutured sites) was recorded. Immediately after completion of an operation and closure of the operated site, it was swabbed using sterile swab stick. The swab was then inoculated into 9ml sterile peptone water from which 1ml each was inoculated on Nutrient agar, MacConkey agar and Chocolate agar plates. The plates were taken to the

Microbiology Laboratory of the Hospital, incubated aerobically (for Nutrient and MacConkey agar plates) or under 10 % CO_2 (in case of Chocolate agar plates) at 37^{o}C for 24 hours. The colonies that that developed after incubation were sub-cultured onto nutrient agar slant and stored in refrigerator for further characterization.

3.2.7 Bacteriological Study of Patient's Environment in the Wards

The microbial loads of the air environment in the Wards to which the patients were transferred to after the surgical operations were also evaluated using the plate settling method as described in 2.2.4 above. The microbial levels of the floor of the Wards where the operated patients were kept were also determined using cotton swabs as described in 2.2.5 above. Resulting colonies were computed and sub-cultured onto nutrient agar slants, kept in refrigerator at 7^{o}C for further characterization.

3.2.8 Bacteriological Study of Environments in the Private Hospitals

Monitoring of patients for detection of bacterial agents responsible for nosocomial infection was not allowed in the private hospitals hence only sampling of the hospital environments such as the air and floor of theatres and wards was carried out as described in sections 2.2.4 and 2.2.5 above.

3.2.9 Detection of Surgical Site Nosocomial Infections (SSI)

The operated patients in the ward were visited on daily bases, and the sites of operations were inspected for signs of infection. Patient's folders were also checked on daily bases for information that might indicate signs of infection in the surgical sites. The classification of an operations site as positive for surgical site infection (SSI) is based on the definition of WHO/CDC (2002) which states that "SSI is an infection that occurred within 30 days after operative procedure". The patient is deemed to have developed SSI when at least one of the followings is noticed in the patient.

a. Purulent drainage from the superficial incision.

b. Organism is isolated from an aseptically obtained culture of fluid or tissue from superficial incision.

c. At least one of the following signs or symptoms of infection occurs: pain, tenderness, or heat and superficial incision deliberately opened by surgeon unless incision is culture negative.

Where there was occurrence of any of the above signs or symptoms on a patient, the consultant attention was drawn and decision on the status of the sign made. Such sites were then swabbed using sterile cotton swab stick, inoculated in 9 ml sterile peptone water broths, inoculated on Nutrient agar, MacConkey and Chocolate agar and appropriately incubated as described in section 2.2.4

3.2.10 Characterisation of Bacteria Isolates

All the isolated bacteria already sub-cultured onto Nutrient agar slants were characterized based on their cultural, morphological and biochemical reactions as described by Cowan and Steel, 1974 and Monica, 1991.

a. Cultural characterization

Cultural characterization of the isolates was based on their morphological and growth characteristics on media (Cowan and Steel, 1974). Odour and pigmentation were used for the preliminary identification of isolated bacterial species.

b. Gram Staining Reaction

Gram staining was performed on the isolates as described by (Monica, 1991). Following examination under a binocular microscope, isolates were classified as Gram positive or negative cocci, cocco-bacilli or rods.

c. motility Test

The hanging drop technique as described by (Cowan and Steel, 1974) was performed on all the isolates. Directional movement by the cells under the microscope was interpreted as evidence of motility.

d. Biochemical Characterization of isolates

Biochemical tests that were performed on the isolates included:

Catalase test: This test was used to differentiate those bacteria that produced the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci. This was performed using 3% hydrogen peroxide solution on agar slant cultures of the isolates as described in (Monica, 1991).

Citrate utilization: Simmons citrate agar was used for this test. Change in the colour of the agar medium from green to blue within 24hr or longer was indicative of ability of the isolate to utilize citrate as sole source of carbon.

Coagulase Test: This test was used to differentiate *Staphylococcus aureus* which produces the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* which are coagulase negative. The slide method was employed using human plasma (Monical,

1991). With the aid of microscope, evidence of recorded. Only coagulase positive isolates produced showed signs of clumping.

Sugar Fermentation: The types of sugars fermented by organisms are unique and characteristic of some species of bacteria. The ability of the isolates to ferment lactose, glucose, sucrose, adonitol, dulcitol, inositol, mannitol and salicin was evaluated in peptone waters containing inverted Durham tubes as described by (Monica, 1991).

Indole Test: Indole test was performed by adding Kovac's reagent on a 48 hrs broth culture of the isolates (Monica, 1991). Red colouration in the alcohol layer was taken as indicative of a positive reaction.

Voges-Proskauer Test: This test was carried out on 48 hrs glucose phosphate peptone culture of the isolate as described by (Monical, 1991). Production of pink-red colour following addition of creatine and 40% Sodium hydroxide is indicative of positive result.

Methyl Red Test (MR): This test was carried out on 48 hrs glucose phosphate peptone culture of the isolate as described by (Monical, 1991). Production of bright red colour was taken as evidence of the ability of the isolate to ferment glucose to mixture of acids.

Oxidase Test: This was performed by smearing colonies of the isolates on filter paper soaked with oxidase reagent (Monical, 1991). Development of blue-purple colour within few seconds is positive for the isolate.

3.2.11 Determination of the Antibiotics Susceptibility Profiles of the Isolates.

Combinations of agar ditch diffusion and disc diffusion methods were used for evaluating the susceptibilities of the isolates to various test antibiotics. Where disc of antibiotics in use are available, Disc diffusion method was used for those antibiotics

available in discs while agar ditch diffusion was used for the others that were not available as antibiotic discs. Susceptibilities of the isolates were tested against twenty (20) antibiotics, namely Ampicillin (10µg), Cloxacillin (5µg), Penicillin G (10µg), Cefuroxime (30µg), Cefotaxime (30µg), Ceftriaxone (30µ), Ceftizoxime (30µg), Ceftazidime (30µg), Gentamicin (10µg), Streptomycin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Perfloxacin (5µg), Spafloxacin (5µg), Azithromycn (10µg), Erythromycin (10µg), Chloramphenicol (30µg), Tetracycline (30µg), Oxytetracycline (30µg) and Doxycycline (30µg). The antibiotic discs were supplied by the department of Medical Microbiology and Parasitology, UITH, Ilorin.

a. Agar Ditch Diffusion Method

All isolated bacterial species stored in slant nutrient agar were standardized for the antibiotics susceptibility tests. Isolates were sub cultured into nutrient broth, and incubated overnight. The turbidity produced was adjusted by using sterile physiological saline to match 0.5 McFarland standards [ca 10^8 $cfuml^{-1}$) (NCCLS, 1990)]. This was further diluted to produce cell concentration of $10^6 cfuml^{-1}$.

Plates containing Mueller Hinton agar were dried and flooded with standardized inoculums of the isolates as described by Onaolapo (1997). Using sterile cork borer No.8 (8mm bore size), holes were created and the bottom partly covered with molten agar to prevent the antibiotic solution from draining away. With automatic micropipettes, 40µl of the desired concentration of different antibiotics was added, allowed to diffuse for 1 hour and thereafter incubated at 37^oC for 18-24hr. Controls were equally set up.

The diameters of the resulting inhibition zones were measured using metric ruler, and recorded in milliliter. Based on the diameters of zones of inhibition and the

recommendations of Kirby-Bauer (1966) and WHO (1983), the isolates were classified as sensitive or resistant.

b. Agar Disc Diffusion Test.

Antibiotics with commercially prepared antibiotics disc were tested by agar disc diffusion method. The Mueller Hinton agar was seeded as described above, and the discs containing antibiotics under test placed firmly on the surface of the agar using sterile forceps. The plates were allowed to stand for an hour to enable the antibiotics to diffuse into the agar. The plates were then incubated at 37^{o}C, after which the plates were observed for development of inhibition zones. The diameters of zones of inhibition were measured and compared as described above.

3.2.12 Determination of Multiple Antibiotics Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index was determined for each of the selected bacterial isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman, 1983; Paul *et al*., 1997).

3.2.13 Determination of Minimum Inhibitory Concentration (MIC) of the Isolates

The MICs of the antibiotics used against the isolates were determined using the macrobroth serial dilution technique (Lennette *et al*., 1990; Bary, 1976). Stock solutions of the antibiotic were prepared. For each antibiotic, twelve (12) tubes containing 1.0ml sterile peptone water were serially arranged. A 1.0 ml of antibiotic from the stock was introduced into tube 1 and 2. Serial dilutions were carried out from tube 2 to 10 such that each new dilution contains 50% less of the antibiotic contains in the preceding dilution. Standardized overnight culture of the isolates (inoculum size of ca 10^{6} cfuml^{-1}) were then inoculated into the tubes and incubated at 37^{o}C for 24 hrs.

Positive and negative controls were set up as recommended (Woods and Washington, 1995). After incubation, the tubes were observed for growth. Based on the pattern of growth in the tubes, the MIC of the antibiotic for the isolates was recorded.

3.2.14 Test for the β- lactamase production.

The presence of β-lactamase enzyme in isolates was tested by using iodometric method (Miller and Smith, 1979). Nutrient agar containing 2% soluble starch was prepared. Overnight agar surface flooded with freshly prepared 10,000 unit/ml of Penicillin (0.06mg/ml in 0.1M phosphate buffer, pH 7.0) and left for 15-60 minutes at room temperature. Thereafter, iodine solution was added. Isolates whose colonies turned blue-black with colourless halos were considered as a β-lactamase producing isolates and recorded.

3.2.15 Test for the Presence of Plasmids in the isolates

This acridine orange method described by Crosa *et al* (1994) was employed in this determination. The MICs value of acridine orange to twenty selected highly resistant isolates (including resistance to ampicillin and streptomycin) was determined as described in 2.2.13. Sub-MIC of acridine orange (750μg/ml) was used to cure the isolates of plasmid they might contain. Sterile Nutrient broths were inoculated with standardized overnight inoculum of the test isolates and solutions of acridine such that the final concentration of acridine in the broth became 750μgml^{-1}. These were then incubated at 37^{o}C for 18 hrs. Thereafter, growths from the tubes were sub-cultured and the MICs of the twenty isolates were re-determined as described in section 2.2.13. The MICs of the acridine-treated isolates were then compared with the values before plasmid curing with acridine.

3.2.16 Detection of Plasmids and Determination of their Sizes in the resistant Isolates

This test essentially involves application of biotechnology tools. It is in several stages. It was carried out in the laboratory of the Nigeria Medical Research Institute (NIMR) , Oshodi, Lagos.

a. Culture purification

Purity of bacterial cultures to be used for the test was ascertained by sub-culturing the isolates on Nutrient and MacConkey agars.

b. Plasmids separation

A twenty four hours (24hr) old culture of isolates was inoculated in 5ml of lauryl tryptose broth (Oxoid) and incubated at 37^{o}C for 18 hrs. The culture was spun at 4000 rpm for 10 minutes using laboratory centrifuge (model SM800B). The supernatant was discarded leaving the pellet. The pellet was further vortexed with little quantity of supernatant in a Stuart vortex mixer at 2,500 rpm for few second.

The mixture was emptied in Eppendorf tubes which have already been labeled with the name of each isolates. The Eppendorf tubes with its contents were further centrifuged at 11,000 rpm for 5 minutes and the supernatant discarded. With a little quantity of the supernatant, the suspended pellet was again vortexed. Using automatic pipette, excess supernatant was removed and the pellet immersed in pack of ice block. Thereafter, 150µl of 3M sodium acetate of pH 5.2 was added, and allowed to stand in ice pack for 5 minutes. The mixture was vortexed and further spun for 2 minutes in a micro-centrifuge to pellet out cell debris and chromosomal DNA.

The supernatant was removed to fresh Eppendorf tubes, and then 0.9ml of 100% ethanol which has been pre cooled to -20^{o}C, was added and vortexed for 2-5 minutes, spun again at 11,000rfm for 2 minutes to pellet out plasmid DNA and RNA. The

supernatant was discarded and the pellet was rinsed twice with 1.0 ml of 70% ethanol. The pellet was dried under vacuum for 3 minutes, re-suspended in 20µl of (TE) buffer for the electrophoresis stage. When not immediately used, the buffered pellet was kept in Freezer at -7^{o}C.

c. Elecrophoresis

A 50ml of 0.8% w/v of agarose gel was prepared and 2 drops (7µl) of ethidium bromide was added. The mixture was poured in an electrophoresis chamber with comb in place and allowed to set. After the gel had set, the comb was removed thereby creating wells on the starting point of the gel.

Twenty microliter (20µl) of the sample was mixed with 2µl of bromophenol blue as the tracking buffer. The mixture was loaded into each well for each sample. A 20µl of HIND II DNA molecular weight marker was also mixed with 2µl of bromophenol blue, and loaded in first well to serve as standard for calculating the molecular weight of isolated plasmids.

After loading the electrophoresis chamber, it was connected to a transformer and plugged to source of electricity. The electrophoresis gel was run at a voltage of 65-70 Volts for 1½ hr using (TBE) as running buffer.

After 1½hr of running the electrophoresis, the chamber was disconnected and taken to dark room for photographing and measurement of distance of migration of the DNA plasmids extracted from the isolates and the standard marker.

The gel was emptied on the stage glass and with illumination by ultraviolet light (Plate 1.0), different migrations levels of each plasmid were shown. These were photographed using a Polaroid camera attached to the photographing machine (plate 1). After

photographing, distance of migration of each isolate plasmid and that of the marker was measured using metric ruler and recorded.

d. Determination of Molecular Weight of Plasmids of the Isolates

Using the distance of migration of the bands (plasmid unit) in each isolate and matching the value of the marker in the standard with it, the plasmid sizes of the isolates were determined.

3.2.17 Determination of Sources of the Isolated Nosocomial Pathogens

Based on the morphological and biochemical characteristics of the isolate, the isolates were identified to species level. With this identification to species level, antibiotic resistance patterns and plasmid profiles, of isolates from the floors and air of theater and wards were matched with those isolated from the surgical sites. Similarities in at least two of the three characteristics were taken as being indicative of the organism coming from the same source.



Plate 1.0: Photographing and measurement of distance of migration of plasmids after electrophoresis

3.2.18 Determination of Susceptibilities of Isolates to Some Disinfectants

The disinfectants commonly used in University of Ilorin Teaching Hospital are Izal and Lysol (cresol). Effect of different concentrations of Izal and Lysol (cresol) were measured on isolated bacteria by preparing different concentrations of the two disinfectants. The following concentrations of Izal were prepared, 1:300, 1:400, 1:500, 1:600, 1:700, 1:800, 1:900, and 1:1000. For the Lysol the concentrations were prepared in percentage, 0.25%, 1.0%, 1.5%, 2.00, 2.5%, 3.0%, 3.5%, 4.0%, 4.5% and 5.0%.

The test procedures described by Lamikanra (1989) were followed. One milliliter of organism under test which had been standardized to 10^6cfu/ml was mixed thoroughly with 20ml of molten Mueller-Hintin agar in a sterile petridish and allowed to set. A sterile cork borer (8mm in diameter) was then pushed into the agar and agar plugs were removed with a sterile loop. The ditches formed were then filled with different concentrations of Izal solution, using a sterile dropping pipette and plates used for all isolate were incubated at 37oC for 24 to 48hours. The plates were observed for presence and absence of zones of inhibition. The inhibition zone diameters were measured and recorded.

The same procedure was followed for the test of Lysol.

3.2.19 Determination of rate of killing by disinfectants on most antibiotics resistant isolates.

Rate of kill study was carried out on *Staphylococcus aureus* and *Pseudomonas aeruginosa* the two being the most antibiotics resistant isolates found in this work by using Kelsey and Maurer (1974) method. A 0.5ml of different concentrations of 106 cfu/ml of test isolates was mixed with 4.5ml of different concentrations of izal solution. A 0.1ml from the mixture was taken with sterile 1ml syringe and needle and plated on dried sterile nutrient agar at time interval of 0, 5, 10, 15, 20, 25 and 30 minutes. The plates were incubated at 37^oC for 24hours, surviving bacteria were counted and logarithms of the survivors was taken and plotted against time.

3.2.20 Determination of effectiveness of disinfections carried out by both the Hospital cleaners and the researcher.

Effectiveness of disinfections carried out by the hospital cleaners and the researcher was carried out. This was done by swabbing a squared meter of the hospital floor before disinfection and 1hr after disinfection, by using sterile moistened cotton swab stick. The swab was then inoculated in 9ml peptone water broth, which was then serially diluted up to 10^{-5}. Each dilution was plated on nutrient agar and incubated at 35^{o}C for 24hrs. The numbers of colonies developed was counted and recorded.

CHAPTER FOUR

RESULTS.

4.1 Retrospective Studies on Nosocomial Infections in Private Hospitals in Ilorin.

The results of prevalence of nosocomial infections in six selected private hospital in Ilorin showed that, hospital E, had the highest prevalence rate (18.0%) and the lowest prevalence was recorded in hospital B(Table 4.1).

Table 4.1 Prevalence of nosocomial infections in six private hospitals in Ilorin.

Hospitals	No. of patients discharged	No. of patients infected	Infections rate (%)
A	80	13	16.3
B	86	8	9.3
C	74	12	16.2
D	127	20	15.7
E	111	20	18.0
F	106	16	15.0
Total	584	89	15.23

Key: A=Olalomi hospital, B=Temitope hospital, C=Olaolu hospital, D=Civil service Clinic, E=Sobi military hospital, F=Surulere hospital and maternity.

Analysis of the available medical records in the six private hospitals studied indicated that nosocomial infection was prevalent in the hospitals (Table 4.2) with a prevalent rate of 13.5%. Surgical site was the most commonly implicated nosocomial infection site, with an occurrence rate (19.6%) twice that in respiratory tract site (9.0%).

Table 4.2: Prevalence of Nosocomial Infections by Sites in Private Hospitals in Ilorin

Site of infections	No. of patients discharged	No. of patients infected	Infection rate (%)
Urinary tract	230	24	10.4
Surgical	153	40	26.1
Respiratory tract	77	7	9.0
Blood stream	124	18	14.0
Total	584	79	15.23

Table 4.3 showed incidences of nosocomial infections in the various wards of the six hospitals. As indicated in this table, surgical ward had the highest nosocomial infection rate with 16.0%. The infection rates in the three other wards were similar.

Table 4.3: Nosocomial Infections Rates in the Various Wards of the Six Private Hospitals in Year 2002

Wards	No of patients discharged	No infected (rate)*
Male	222	26 (12.0)
Female	194	28 (14.0)
Surgical	142	18 (16.0)
Children	52	7 (13.0)
Total	584	79 (14.0)

*Values in brackets are the infection rates

Table 4.4:Incidences of Nosocomial Infection in Surgical Ward of the Private Hospitals in Ilorin in Year 2002

Hospitals	**No discharged**	**No. infected (rate)**
A	19	3 (16.0)
B	21	2 (10.0)
C	18	3 (17.0)
D	31	5 (16.0)
E	27	5 (19.0)
F	26	4 (15.0)
TOTAL	142	22 (15.5)

Table 4.4 showed the nosocomial infection rates in the surgical wards of the six private hospitals studied. Hospital E had the highest prevalence rate of 19.0%. The prevalence rate in four other hospitals were similar. The nosocomial infection rate in Hospital B was comparably low.

Table 4.5: Monthly Incidences of Nosocomial Infections in the Surgical Ward of Hospital E in Year 2002

Months	No discharged	Infection rate
January	-	-
February	4	25
March	-	-
April	3	-
May	1	-
June	4	50
July	-	-
August	3	-
September	3	-
October	3	-
November	5	40
December	1	-
Total	27	19

Data presented in Table 4.5 showed that incidence of nosocomial infections was limited to only few months of the year in 2002, mainly February, June and November, and these are the few months that more surgical operations were carried out.

4.2 Retrospective Studies of Nosocomial Infections in the University of Ilorin Teaching Hospital

Results of analysis of medicals records of patients for three years (2000-2002) in University of Ilorin Teaching Hospital are shown Table 4.6 to 3.110.

As shown in Table 4.6, nosocomial infection rates changed from one month to another and from one year to another. In 2000, relatively high rates of nosocomial infections were recorded in January, August and November, with rates almost twice the figures for the months of February, May and June. In Year 2001, however, the highest nosocomial infection rate of 10.3% was recorded in July. This month also recorded the lowest discharge number, a value that was less than one-tenth of the figures for the other months. With the exception of March and August, the monthly infection rates were generally higher compared with those of the previous year (2000). The monthly nosocomial infection rates in the Year 2002 showed a discernible pattern. Relative high figures of nosocomial infection rates from January to May followed by comparably lower rates from the month of June to November (August figure was the exception). The highest infection rate of 3.4% was obtained in the month of May. The yearly average infection rate showed that year 2002 recorded the highest nosocomial infection rate among the three years.

The nosocomial infection rate in the different wards and units of the hospital in the three years of survey is presented in Table 4.7. As shown in this table, the values of the infection rates were virtually consistent for some of the wards or units of the hospital. Surgical

wards or wards housed patients that underwent surgical operations (Surgical (male and female), Paediatric surgical and Orthopaedic surgical wards) recorded highest infection rates compared with other wards. This was followed by Male (adult) Medical ward and Intensive care unit. There was consistently low incidence of nosocomial infection in Amenity, Obstetrics and Gynaecology, Neurosurgey and surprisingly, Accident and Emergency wards.

Table 4.6: Monthly Infections Rates in the University of Ilorin Teaching Hospitals in Year 2000-2002

	Monthly Incidences of Nosocomial Infections in Year					
Month	**2000**		**2001**		**2002**	
	No Dis-charged	Infection rate (%)	No Dis-charged	Infection rate (%)	No Dis-charged	Infection rate (%)
January	375	**1.6**	433	**1.6**	228	**1.8**
February	346	**0.8**	414	**1.7**	332	**1.8**
March	492	**1.2**	497	**1.2**	459	**2.6**
April	510	**1.2**	440	**1.6**	264	**2.3**
May	499	**0.8**	396	**1.5**	410	**3.4**
June	427	**0.7**	240	**1.3**	264	**0.8**
July	352	**1.1**	19	**10.5**	131	**0.7**
August	321	**1.6**	414	**1.2**	238	**1.7**
September	491	**1.0**	481	**1.7**	889	**0.9**
October	557	**1.3**	560	**1.4**	1076	**0.2**
November	529	**1.5**	633	**2.8**	1042	**0.3**
December	456	**0.9**	354	**1.4**	680	**1.9**
Total / Mean	5315	**1.12**	4881	**1.76**	6013	**1.24**

Table 4.7: Nosocomial Infection Rates in the various Wards of University of Ilorin Teaching Hospital in 2000-2002

Wards	Incidences of Nosocomial Infections in the Wards in Year					
	2000		2001		2002	
	No Dis-charged	Infection rate (%)	No Dis-charged	Infection rate (%)	No Dis-charged	Infection rate (%)
Medical Male (adult)	382	**1.8**	275	**2.2**	228	**1.8**
Medical (Female)	137	**0.7**	253	**1.2**	264	**0.8**
Medical Male/Female	244	**0.8**	269	**0.7**	264	**2.3**
Orthopedic (Female)	208	**0.5**	207	**1.4**	238	**1.7**
Surgical Orthopedic	312	**1.3**	237	**1.3**	332	**1.8**
Surgical Male/Female	304	**3.6**	306	**7.8**	410	**3.4**
Pediatric Surgical	405	**2.2**	540	**3.5**	459	**2.6**
Neuro-Surgery	185	**0.0**	117	**0.0**	131	**0.7**
Accident & Emergency	941	**0.3**	786	**0.6**	889	**0.9**
Obstetrics/ Gynaecology	798	**0.0**	662	**0.5**	1076	**0.5**
Amenity	790	**0.3**	663	**0.5**	680	**0.3**
NICU	609	**1.6**	564	**1.2**	680	**1.9**
Total / Mean	5315	**1.12**	4881	**1.76**	6013	**1.24**

The distribution of nosocomial infections according to infection sites in University of Ilorin Teaching Hospital in year 2000, 2001 and 2002 is given in Table 4.8. Sixty (60) out

of 5315, 86 of 4881 and 75 of the 6,013 patients admitted and discharged in 2000, 2001 and 2002 respectively were infected. Thirty-eight of the infections in 2000, 66 in 2001 and 45 in 2002 were surgical wound infections. These values made the incidence of nosocomial infection in wounds (surgical sites) highest in each of the three years, and accounting for 67.7% of total nosocomial infections recorded in the hospital in the three years of survey. This was distantly followed by infection of the urinary tract. Incidence of nosocomial infection was lowest in respiratory tract in each of the three years, constituting only 4% of the total cases of nosocomial infections in the period.

Table 4.8: Incidences of Nosocomial Infections at Different Sites in University of Ilorin Teaching Hospital in Years 2000-2002

Infection Sites	Nosocomial Infection Rates at Different Infection Sites in Year		
	2000 (n=60)	**2001** (n=86)	**2002** (n=75)
Wounds	63.3	77.0	60.0
Urinary Tract	20.0	16.3	26.7
Blood Stream	12.0	5.0	8.0
Respiratory Tract	5.0	2.3	5.0

n = total number of nosocomial infection cases

Table 4.9 shows the distribution of the causative agents of nosocomial infections in the hospital in the three years of survey. Pathogens that were isolated as causative agents of the nosocomial infections in the University of Ilorin Teaching Hospital in 2000 to 2002 are shown Table 3.8. A total of 60, 86 and 75 bacterial species were recorded at UITH in 2000, 2001 and 2002 respectively. Pathogens that were frequently isolated during the period were *S. aureus, S. epidermidis, Ps. aeruginosa, K. pneumonia, Proteus spp., Strept. Pyogenes, E. coli* and other coliforms.

S. aureus was the most prevalent bacterial pathogen causing nosocomial infection at University of Ilorin teaching Hospital in the three years, accounting for about one-quarter of all the pathogens. This was followed by *Ps. aeruginosa* and *E. coli*. *Klebsiella pneumonia* which ranked third among the frequently isolated pathogen in 2000 was isolated with least frequencies in 2001 and 2002.

.

Table 4.9: Frequency of Isolation of Nosocomial Pathogens in UITH Ilorin in Years 2000-2002

Pathogens	Percentage Occurrence of Nosocomial Pathogens in Year		
	2000 (n=60)	2001 (n=86)	2002 (n=75)
Staph. Aureus	28.2	23.3	24.0
Staph. Epidermidis	11.7	9.0	12.0
Kleb. Pneumoniae	13.3	10.5	6.7
E. coli	10.0	11.6	12.0
Ps. aeruginosa	16.7	12.0	18.0
Proteus sp.	10.0	7.0	11.0
Coli forms	5.0	11.6	5.3
Streptococcus sp.	5.0	10.5	9.3

Susceptibility profiles of the nosocomial infection causative pathogens from UITH in the three years under review are shown in Table 4.10. In general, percentages of isolates exhibiting resistance to the antibiotics were lowest in 2000 and dramatically increased in 2001, which only slightly decreased in 2002. In 2000, about one third of the isolated pathogens were resistant with the exception of *E. coli* and *Streptococcus species* which had higher percentage of resistant strains. In 2001, almost two-third of all the isolates was

found not susceptible to the antibiotics. *Klebsiella pneumoniae* was the most resistant nosocomial pathogen (78.0%), while the least resistant pathogens were the Coliforms (50.0%). In the year 2002, almost 50% of the pathogens were resistant with *S. aureus* being the most resistant nosocomial pathogen recorded.

Table 4.10: Antibiotic Resistant Pattern of the Isolated Nosocomial Pathogens at University of Ilorin Teaching Hospital (2000-2002)

Isolated Pathogens	Antibiotic Resistant Patterns of the NosocomialInfection Pathogens					
	2000		**2001**		**2002**	
	No Tested	% Resistant	No Tested	% Resistant	No Tested	% Resistant
S. aureus	17	35.0	20	65.0	18	61.0
S. epidermidis	7	43.0	7	57.0	9	56.0
K. pneumoniae	8	25.0	9	78.0	5	60.0
E. coli	6	50.0	10	60.0	7	43.0
Ps. aeruginosa	10	30.0	12	67.0	14	57.0
Proteus sp.	6	33.0	10	60.0	9	44.0
Coli forms	3	33.0	10	50.0	4	50.0
Streptococcus sp.	3	67.0	9	67.0	7	43.0

The Multiple antibiotic resistance index (MAR) of the nosocomial pathogens isolated in University of Ilorin Teaching Hospital is presented in Table 4.11. All the isolated pathogens were multiple antibiotics resistant. Most multiple antibiotics resistant isolates in 2000 were *Klebsiella pneumoniae, Ps. aeruginosa* and *Pr. mirabilis*. The same set of organisms except *Pr. mirabilis* exhibited the highest MAR indices in 2001 and 2002. *S. aureus* and *S. epidermidis* had similar MAR indices throughout the three years of review. Generally, higher MAR indices were recorded in 2001. The nosocomial isolates in 2002 were resistant to lower number of antibiotics.

Table 4.11: Multiple Antibiotics Resistance Index of Nosocomial Pathogens Isolated in University of Ilorin Teaching Hospital in 2000-2002

Isolates	MAR Indices of the Nosocomial Pathogens in Year		
	2000	2001	2002
S. aureus	0.6	0.6	0.6
S. epidermidis	0.6	0.6	0.6
Kleb. pneumoniae	0.7	0.8	0.6
E. coli	0.5	0.6	-
Ps. aeruginosa	0.7	0.7	0.6
Pr. mirabilis	0.7	0.6	0.4
Strep. pyogenes	0.4	0.7	0.5
Other Coliforms	0.6	0.5	0.5

4.3 Bacteriological Studies of Hospital Environments

The air and floor bacteria quality of the sampled theatres and wards of the hospitals (six private hospitals and University of Ilorin Teaching Hospital) are presented in Tables 4.12 – 4.17

Tables 4.12 and 4.15 present the bacterial level of the environmental air and floors of operating rooms, surgical, male and female wards of the six private hospitals in Ilorin. As shown in these two tables, the bacteria levels of the floors were higher than those of the corresponding airs, usually about ten times higher. The bacterial counts of the operating rooms and the surgical wards were considerably less than those of the Male and Female wards: about 1% less.

Table 4.12: Microbial Levels of Air and Floors of the Operating room and Surgical of the Private Hospitals in Ilorin

	Bacterial Levels			
Hospitals	Surgical Wards		Operating Room	
	Air cfu/m^3	Floor cfu/m^2	Air cfu/m^3	Floor cfu/m^2
A	1.0×10^2	6.9×10^2	5.3×10^2	4.1×10^2
B	6.8×10^1	4.3×10^2	2.4×10^1	1.8×10^2
C	7.6×10^1	6.0×10^2	3.5×10^1	2.4×10^2
D	9.3×10^1	5.8×10^2	4.1×10^1	3.3×10^2
E	5.9×10^2	4.0×10^3	3.4×10^1	2.6×10^2
F	3.0×10^1	4.7×10^2	3.0×10^1	2.9×10^2

Table 4.13: Microbial Levels of Air and Floors of the Male and Female Wards of the Private Hospitals in Ilorin

	Bacterial Levels			
Hospitals	Male Ward		Female Ward	
	Air cfu/m^3	Floor cfu/m^2	Air cfu/m^3	Floor cfu/m^2
A	3.0×10^3	6.1×10^3	4.2×10^4	3.62×10^5
B	2.4×10^3	4.2×10^2	3.1×10^3	6.10×10^4
C	8.4×10^3	6.9×10^4	8.1×10^3	4.19×10^4
D	5.8×10^3	6.8×10^3	3.2×10^4	5.26×10^5
E	2.9×10^3	7.3×10^3	7.1×10^4	6.18×10^5
F	4.6×10^3	5.0×10^3	6.6×10^2	4.20×10^5

The data presented in Table 4.14 shows that out of seven species of bacteria that were isolated from the theatres, *S. aureus* was detected in all the theatres of the six private hospitals. Other organisms that were frequently isolated in the theatres were *Kl. pneumoniae* and *Pr. mirabilis*. More species of organisms were isolated in the theatre of Hospital A.

Table 4.15 shows the distribution of bacterial species isolated from the surgical wards of the six private hospitals in Ilorin. *S. aureus, S. epidermidis, Ps. aeruginosa and E. coli* were present in the surgical wards of the six private hospitals. *Pr. mirabilis, Ser.*

marcescens and *Str. pyogenes* were isolated from the surgical wards of five of the six hospitals. All the eight bacteria species were isolated in the surgical wards of Hospital A while seven of the eight species were detected in the surgical wards of two hospitals (C and D).

Bacteria isolated from the Male ward of the six private hospitals (.Table 4.16) showed that *S. aureus, Pr. mirabilis* and *Ser. marscecens* were present in this ward in all the hospitals. *S epidermidis, Kl. pneumoniae, E. coli, Pr. vulgaris* and *Str. pyogenes* were also frequently isolated, being detected in five of the six hospitals. *Str. viridians* and Legionella were less frequently isolated. At least eleven out of the fourteen bacteria species isolated in the male wards were detected in five of the six hospitals.

Table 4.14: Bacteria Isolated in the Theatres of the Private Hospitals

Isolates	Hospitals					
	A	B	C	D	E	F
S. aureus	+	+	+	+	+	+
S. epidermidis	+	-	-	-	-	-
Kl. pneumoniae	+	+	+	-	+	-
Ps. aeruginosa	+	-	-	-	-	+
E. coli	-	-	-	+	-	+
Ent. Cloacae	-	-	-	-	+	-
Pr. mirabilis	+	+	+	+	-	+

+ indicates organism isolated

- indicates organism not isolated

Table 4.15: Bacteria Isolated in the Surgical Wards of the Private Hospitals in Ilorin

Isolates	Hospitals					
	A	B	C	D	E	F
S. aureus	+	+	+	+	+	+
S. epidermidis	+	+	+	+	+	+
Kl. pneumoniae	+	+	+	+	+	+
Ps. aeruginosa	+	+	+	+	+	+
E. coli	+	+	+	+	+	+
Ent. Cloacae	+	-	+	+	-	-
Pr. mirabilis	+	+	+	+	-	+
Pr. vulgaris	+	-	-	+	+	-
Ser. Marcescens	+	+	+	-	+	+
Str. Pyogenes	+	-	+	+	+	+

Table 4.16: Bacteria Species Isolates from Male Wards of the Private Hospitals in Ilorin

Isolates	Detection of Organism in Hospital					
	A	B	C	D	E	F
S. aureus	+	+	+	+	+	+
S. epidermidis	+	+	+	+	+	-
Kl. pneumoniae	+	+	-	+	+	+
Ps. aeruginosa	-	+	-	+	+	+
E. coli	-	+	+	+	+	+
Ent. cloacae	+	+	-	+	+	-
Pr. mirabilis	+	+	+	+	+	+
Pr. vulgaris	+	+	+	-	+	+
Ser. marscences	+	+	+	+	+	+
Str. pyogenes	+	+	-	+	+	+
Str. viridan	-	-	+	-	+	+
Legionella sp.	+	-	+	+	-	-

A substantial higher number of different bacteria species (fourteen of them), belonging mostly to Staphylococcus, Streptococcus and Enterobacteriaceae families, were isolated in the Female wards of the private hospitals (Table 4.17). Only S. aureus was detected in the female wards of all the six hospitals. *S. eipdermidis*, Ent. *cloacae, Kl. pneumonia, Kl. aerogene, Pr. mirabilis,* and *Lactobacillus sp* were isolated in the female wards of

five of the six hospitals. More species of bacteria (eleven in number) were isolated in Hospital F than in any other hospital.

. **Table 4.17: Bacterial Isolated from Female Wards of the Private Hospitals in Ilorin**

Bacterial isolates	HOSPITALS					
	A	B	C	D	E	F
S. aureus	+	+	+	+	+	+
S. epidermidis	+	+	+	+	-	+
Kl. pneumoniae	+	+	+	+	+	-
Kl. aerogene	+	+	-	+	+	+
Ps. aeruginosa	+	-	+	-	+	+
E. coli	+	+	+	-	+	-
Ent. cloacae	-	+	+	+	+	+
Pr. mirabilis	+	-	+	+	+	+
Pr. vulgaris	-	-	+	+	-	+
Ser. marscenes	-	-	-	-	+	-
St. pyogenes	+	-	+	-	+	+
St. varidans	-	+	-	+	-	+
Legionella sp.	-	-	-	-	-	+
Lactobacillus sp.	+	+	+	+	-	+

Table4.18: Antibiotics Resistance Pattern of Isolates from the Private Hospitals in Ilorin

Bacterial isolates	Antibiotics to Which Resistant	MAR Index
S. aureus	K-G-C-E-V-CX-A-NIT-CEF-CET	0.9
S. epidermidis	C-V-CX-A-NIT-CEF	0.5
Kl. pneumoniae	K-G-C-E-V-CV-A	0.6
Kl. aerogene	C-E-V-CX-A-NAL	0.5
Ps. aeruginosa	K-G-C-V-A-NIT-CEF	0.6
E. coli	C-E-V-CX-A	0.4
Ent. Cloacae	K-C-A-NIT	0.3
Pr. mirabilis	C-A-NIT	0.2
Pr. vulgaris	C-E-CX-A-NIT-NAL-CEF	0.6
Ser. Marcescens	K-G-C-E-CX-NIT	0.5
Strept. Pyogenes	A-NIT	0.2
Strept. Varidans	K-G-E-V-NIT-NAL	0.5
Legionella spp.	G-E-V-CX-A	0.4
Lactobacillus spp.	CX-A	0.2

Key

A = Ampicillin C = Chloramphenicol CEF = cefuroxime

CET = Ceftriaxone V = vancomycin K= kanamycin

CX =Cloxacillin C= Chloramphenicol

E = Erythromycin G = Gentamycin NIT = Nitrofurantoin

NAL = Nalidixic aci

Antibiotics resistance pattern of isolates from the six selected private hospitals in ilorin indicated that all isolates were multiple antibiotics resistant, with MAR index ranging from 0.2-0.9. Staphylococcus aureus was the most resistant isolate (Table 4.18).

Table 4.19: Monthly Bacterial Levels of Environmental Air and Floors of the Theater and Surgical Ward of the University of Ilorin Teaching Hospital in 2004

Month	**Microbial Count**			
	Theater		Ward	
	Floor (cfu/m^2)	Air ($cfu/m^{3)}$	Floor (cfu/m^2)	Air (cfu/m^3)
January	1.2×10^3	3	1.3×10^3	4
February	3.2×10^2	5	8.0×10^2	1
March	1.3×10^3	4	8.0×10^2	6
April	1.7×10^3	4	6.4×10^2	4
May	1.2×10^2	1	4.8×10^2	3
June	3.2×10^2	2	5.4×10^2	3
July	1.2×10^3	2	6.4×10^2	1
August	8.0×10^2	3	3.2×10^2	4
September	1.8×10^3	4	3.2×10^2	1
October	4.8×10^2	1	4.8×10^2	2
November	7.5×10^2	2	9.6×10^2	2
December	2.0×10^3	2	3.4×10^3	3

Table 4.20: Monthly Bacterial Levels of Environmental Air and Floors of the Theater and Surgical Ward of the University of Ilorin Teaching Hospital in 2005

	Microbial Count			
Month	Theater		Ward	
	Floor (cfu/m^2)	Air ($cfu/m^{3)}$	Floor (cfu/m^2)	Air (cfu/m^3)
January	$3.30 \times 1\ 0^2$	8	2.18×10^2	7
February	2.40×10^2	5	2.90×10^3	9
March	3.70×10^2	6	4.16×10^3	12
April	4.17×10^3	3	2.99×10^3	5
May	3.99×10^4	9	4.10×10^2	14
June	2.71×10^3	6	2.82×10^3	21
July	3.34×10^4	5	8.15×10^2	16
August	4.55×10^3	11	7.30×10^3	10
September	5.23×10^3	7	3.20×10^3	29
October	3.13×10^2	10	5.21×10^2	15
November	2.45×10^3	8	4.61×10^3	18
December	1.30×10^3	6	4.16×10^3	9

Monthly average bacterial counts in the theaters and wards of the university of Ilorin teaching hospital is shown in Table 4.19. The average bacterial counts from the floors in the theaters in the months of April, May, July, August and October were higher than the remaining months (Table 4.19). Bacterial counts from the air of the theater ranged from 3.0cfu/m3 (April) to 11.0 cfu/m3 in August. Similarly, bacterial counts from the floors of the ward ranged from 2.1 x 10^2 cfu/m2 to 4.61x10^3 cfu/m2. Bacterial count from the wards ranged from 5 cfu/m3 to 29cfu/m3 (Table 4.20).

Table 4.21:Frequency Distribution of Bacteria species Isolated from the Surgical Ward and Theater of University of Ilorin Teaching Hospital in 2005

Bacterial species	No. isolated	Percentage Frequency (n = 708)
S. epidermidis	113	16
S. aureus	127	18
Str. pyogenes	43	6
Streptococcus spp.	7	1
B. subtilis	21	3
B. cereus	35	5
B. megaterium	35	5
Bacillus (unidentified)	7	1
Pr. mirabilis	43	6
Citrobacter freundii	14	2
Ps. aeruginosa	78	11
Pseudomonas sp	21	3
Kl. pneumoniae	50	7
Klebsiella sp	14	2
E. coli	50	7
Ser. marcescens	35	5
Serratia spp	14	2

Of 708 species of bacteria isolated from the theaters and wards of the university of Ilorin teaching hospital in the year 2005, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* ranked the highest with percentage frequencies of 18%, 16% and 11% respectively (Table 4.21).

Table 4.22: Comparison of bacterial species isolated (Feb-March, 2004) from air, floor and patients Wound in the theater and ward.

	Theatre			**Ward**		
Isolate	Air	Floor	Wound	Air	Floor	Wound
S. epidermidis	+	+	-	+	+	+
Klebsiella sp.	-	-	-	-	+	-
Bacillus subtilis	+	-	+	+	-	-
Enterobacter sp.	-	-	-	+	+	-
Serratia marcescens	-	+	-	+	+	-
Pseudomonas sp.	-	-	-	-	+	+
E. Coli	-	-	-	+	-	-
Ps.aeruginosa	-	+	+	+	+	-
Serratia sp.	-	-	-	-	+	-
Bacillus cereus	-	-	-	+	-	-
Bacillus megaterium	-	-	-	+	-	-
Citrobacter freundii	-	+	-	+	-	-
Proteus mirabilis	+	+	-	-	+	+
Bacillus sp.	-	-	-	+	-	-
Streptococcus spp.	-	-	-	+	-	-
Staph. aureus	+	-	-	+	+	+

As shown in Table 4.22, while the patients was still in the theater, *Ps. aeruginosa* was isolated from both the floor and the patients' surgical site, while *B. subtilis* was isolated from the air and the patients surgical wounds. But in the ward, *S. epidermidis* and *S. aureus* were isolated from the air, floor and surgical wound of the patients.

Table 4.23 Distribution of bacterial species during operations in the theater and monitoring of patients in the surgical wards (Feb- May, 2005).

	Theatre			Surgical Ward		
Isolates						
	Air	Floor	Wound	Air	Floor	Wound
S. epidermidis	+	-	-	+	-	-
Klebsiella sp.	-	+	-	-	-	-
Ps. aeruginosa	-	-	-	-	-	+
Ser.marcescens	-	+	-	-	-	-
Streptococcus spp.	-	-	-	-	-	-
B. megaterium	-	+	-	+	-	-
E. coli	-	-	-	-	+	-
Cit.freundii	-	-	-	-	-	-
Serratia sp.	-	+	-	-	-	-
Proteus mirabilis	+	+	-	-	+	-
Bacillus cereus	-	-	-	+	-	-
Staph. Aureus	+	+	-	+	-	+
Bacillus spp.	-	+	-	-	-	-
Enterobacter spp.	-	+	-	-	-	-
Pseudomonas spp.	+	-	-	-	-	+
Bacillus subtilis	-	+	-	-	-	-

No organism was isolated in the wound after operation in the theater, but *Ps.aeruginosa* was isolated from wound in the surgical ward, though *Ps. aeruginosa* was not present in the floor and air of the ward (Table 4.23).

Table 4.24 Distribution of bacterial species during operations in the theater and monitoring of patients in the surgical wards (July-Dec, 2005).

Isolate	Theatre			Ward		
	Air	Floor	Wound	Air	Floor	Wound
S. epidermidis	+	+	+	+	+	+
Klebsiella sp.	+	+	+	+	+	+
Bacillus subtilis	-	+	-	-	-	-
Enterobacter sp.	-	+	+	-	-	-
Serratia marcescens	-	+	-	-	+	-
Pseudomonas sp.	+	-	-	-	-	-
E. Coli	-	+	-	+	-	-
Ps.aeruginosa	+	+	-	+	+	+
Serratia sp.	+	-	-	-	-	-
Bacillus cereus	+	-	-	+	+	-
Bacillus megaterium	+	-	-	+	-	-
Citrobacter freundii	-	-	-	-	+	-
Proteus mirabilis	+	+	-	+	+	-
Bacillus sp.	-	+	+	-	+	-
Streptococcus spp.	+	-	-	-	-	+
Staph. Aureus	+	+	+	+	+	+

S.epidermidis, *Klebsiella spp*, and *S.aureus* were isolated in the air, floor and wound of patients in both the thater and surgical ward. In surgical ward Ps. aeruginosa was isolated

in the air, floor and the patients wounds. Streptococcus was also isolated from the wound, though it was not isolated from the air and floor of the ward (Table 4.24).

Table 4.25: Prevalence of Surgical Site Nosocomial infections in University of Ilorin Teaching Hospital in 2004 (Feb -April) and 2005 (Feb-Dec)

Type of Operation	No of Operations	No infected	Infection Rate
Appendicectomy	64	30	46.9
Exploiratory Laparatomy	105	48	45.7
Herniorrhaphy	27	9	33.3
Simple Mastetomy	18	7	38.9
Thyroidectomy	38	14	36.8
Others (Unclassified)	47	16	30.0
Total	299	124	41.5

Out of 64 patients operated for appendicectomy, 30(46.9%) were infected. For exploiratory laparatomy, 48(45.7%) out of 105 patients operated developed infections. Infections due to herniorrhaphy, simple mastectomy, Thyroidectomy and other unclassified operations were 33.3%, 38.9%, 36.8% and 30.0% respectively (Table 3.25).

Table 4.26. Average bacterial counts from surgical wound surfaces before and after closure in the theater and surgical wards.

Type of operations	Bacterial counts CFU/ml		
	Before closure in theater	After closure in theater	Surgical wards
Appendectomy	$1.6x10^1$	$2.8x10^1$	$2.3x10^5$
Exploratory Laparatomy	-	$1.3x10^1$	$1.8x10^3$
Herniorrhaphy	3.0	$1.8x10^1$	$1.2x10^3$
Simple mastetomy	-	-	$1.3x10^3$
Thyroidectomy	-	$1.9x10^1$	$2.8x10^3$
Others (not classify)	6.0	$1.4x10^1$	$3.4x10^5$

Average bacterial counts from various surgical sites are presented in Table 4.26 above. Higher counts were observed in appendecectomy in the surgical ward ($2.3x10^5$ cfu/ml) than all other classified surgical operations. Bacterial counts before and after closure of surgical wound ranged between 3.0 to $2.8x10^1$ CFU/ml.

Table 4.27: Prevalent Pathogens in the Surgical Site at the University of Ilorin Teaching Hospital in 2004 (Jan-March) and 2005 (Feb.-Dec)

Isolated Pathogens	Prevalence	
	Number	Percentage
S. aureus	36	26
St. epidermidis	26	18
Enterobacter spp.	6	4
E. coli	20	14
K. pneumoniae	10	7
Pr.mirabilis	10	7
Ps.aeruginosa	24	17
Bacillus spp.	3	2
Strept. Pyogens	4	3
Proteus vulgaris	3	2
Total	162	100

S.aureus, S.epidermidis, Ps. aeruginosa and *E. coli* were the most prevalent surgical site pathogens isolated. Prevalent rate were respectively, 26%, 18%, 17% and 14% (Table 4.27).

Table 4.28: Monthly Frequency of Nosocomial Infection in Surgical Sites of the University of Ilorin Teaching Hospital in 2004 (Jan-April) and 2005 (Jan – Dec)

Month	Total patient operated	Incidences of Nosocomial Infection	
		Number	Rate (%)
Year 2004			
January	35	9	25.7
February	11	2	18.2
March	16	4	25.0
Year 2005			
January	-	-	-
February	17	4	24
March	27	8	30
April	37	8	22
May	16	3	19
June	27	5	19
July	28	6	21
August	18	4	22
September	27	7	26
October	17	6	35
November	15	3	20.0
December	8	1	12.5

The highest infection rate was recorded in March 2005(30%), while the lowest rate was found in December (12.5%).

Table 4.29: Age Distribution of Patients with Nosocomial Infections in Surgical sites of the UITH in 2004 (Jan-April) and 2005 (Jan-Dec)

Age Group of Patients	No of Patients operated	No Infected	% Infection
0 - 10	2	0	0
11 - 20	37	18	49
21 – 30	66	40	61
31 - 40	62	27	52
41 - 50	50	23	46
51 – 60	31	18	58
61 – 70	16	11	69
71 and above	23	12	52

Impact of the Age distribution on prevalence of surgical site nosocomial infection showed that. Age range of 61-70 years had the highest prevalence (69.0%), followed by 21-30 years (61%).

Table 4.30: Multiple Antibiotics Resistance Profiles and Indecies of Bacterial species Isolated from Theater, Surgical Ward and Wounds of Patients of University of Ilorin, Teaching Hospital.

Isolates	**MAR Profile**	**MAR**
S. epidermidis	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-MA	0.7
S. aureus	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-CEF-CEFT-AM	0.8
Strept. Pyogenes	A-P-CX-C-AZ-E-PEF-OFX-T-DOX-CEF-AM	0.6
Streptococcus spp.	A-P-CX-C-AZ-E-OXT-T-D0X-CEF-AM	0.6
Klebsiella spp.	A-P-CX-C-S-E-OXT-T-DOX-CEF-AM	0.6
E. coli	A-P-CX-C-S-AZ-E-SPA-CIP-OXT-T-CEF-AM	0.7
Enterobacter spp.	A-P-CX-C-AZ-E-CIP-OXT-DOX-CEF-AM	0.7
Pr. mirabilis	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	0.8
Cit. freundii	A-P-CX-C-AZ-E-SPA-OXT-T-DOX-CEF-AM	0.6
Ser. marcescens	A-P-CX-C-AZ-E-OFX-CIP-OXT-T-DOX-CEF-AM	0.7
Serratia spp.	A-P-CX-C-S-AZ-E-OFX-SPA-OXT-T-DOX-CEF-AM	0.7
Ps. aeruginosa	A-P-CX-C-S-AZ-E-PEF-SPA-CIP-OXT-T-CEF-CRO-EFT-AM	0.8
Pseudomonas spp.	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	0.8
B. cereus	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-DOX-CEF-AM	0.8
B. megaterium	A-P-CX-C-S-AZ-PEF-CIP-OXT-T-DOX-CEF-AM	0.7
B. subtilis	A-P-CX-C-AZ-E-SPA-CIP-OXT-T-CEF-AM	0.6

Antibiotic resistance profile of isolates presented in Table 4.30, showed that all isolates are multiple antibiotics resistant. The antibiotic resistance indices ranged from 0.6 to 0.8, with S. aureus, *Pr. mirabilis, Ps. aeruginosa, Pseudomonas spp. and B. cereus* having the highest indices (0.8 each).

Table 4.31: MICs of Penicillins for Bacteria Species Isolated from

University of Ilorin teaching hospital

Isolates	Minimum inhibitory concentrations μgl^{-1}			
	Penicillin G	Ampicillin	Amoxycillin	Cloxacillin
S. epidermidis	1350-1500	1400-1500	140-180	200-240
Kl.pneumoniae	850-1000	700-800	80-120	280-350
Klebsiella sp.	800-900	610-800	100-140	260-300
Bacillus subtilis	700-1000	750-800	120-180	240-300
Enterobacter sp.	680-780	560-710	100-180	140-180
Serratia marcescens	580-850	780-800	120-180	180-250
Pseudomonas sp.	1300-1500	1000-1200	220-240	260-300
E. Coli	700-800	400-500	140-180	240-300
Ps.aeruginosa	1400-1600	1250-2500	200-280	240-350
Serratia sp.	540-600	625-1250	110-160	160-200
Bacillus cereus	1000-1200	625-1250	120-180	210-290
Bacillus megaterium	1000-1100	710-900	120-180	240-300
Citrobacter freundii	780-980	610-700	100-180	210-300
Proteus mirabilis	1000-1100	940-1200	80-120	250-450
Strept.pyogen	980-1020	900-1100	120-180	140-200
Streptococcus spp.	950-1020	850-1000	110-150	120-200
Staph. aureus	1350-1450	1000-1200	160-200	260-300

MIC results for penicillins showed that penicillin G had the highest MIC, among the penicillins compared. The result showed that majority of isolates required high amount of

Penicillins (β – lactam antibiotic) in order to be inhibited, for example, MIC range for penicillin G required to inhibit growth of the isolates was between 540-1500 µg/ml, for ampicillin the MIC range was 400 – 1500 µg/ml, for amoxicillin (80 - 280µg/ml), cloxacillin (120 - 450µg/ml). *S. aureus, S. epidermidis and Ps. aeruginosa* showed the highest resistance among all isolates (Table 4.31).

Table 4.32 MIC of Cephalosporines and Macrolides for Bacterial species isolated From University of Ilorin teaching hospital.

Isolates	Minimum inhibitory concentration μgl^{-1}					
	cefuroxime	Cefotaxime	Ceftriaxone	Ceftazidime	Erythromycin	azithromycin
S. epidermidis	140-160	150-200	100-60	10-50	50-70	40-60
Kl.pneumoniae	100-130	100-250	10-50	15-50	30-40	30-60
Klebsiella sp.	100-130	100-200	5-40	10-40	40-60	40-60
Bacillus subtilis	100-140	50-150	5-30	5-20	60-90	60-80
Enterobacter sp.	90-140	100-250	5-50	5-20	70-90	50-70
Serratia marcescens	100-150	100-200	10-50	10-50	30-40	50-80
Pseudomonas sp.	100-180	100-200	10-40	5-30	60-90	50-70
E. Coli	100-160	150-250	10-70	10-20	80-100	40-80
Ps.aeruginosa	120-220	100-300	10-60	15-40	100-160	50-80
Serratia sp.	90-120	100-150	5-50	5-20	40-80	40-60
Bacillus cereus	100-140	50-150	10-60	5-30	60-80	30-40
Bacillus megaterium	100-160	100-150	5-30	5-20	20-40	80-120
Citrobacter freundii	100-120	50-150	5-50	10-30	50-70	30-60
Proteus mirabilis	110-160	50-200	5-60	5-20	100-140	40-60
Strept.pyogen	90-130	50-150	5-40	5-20	60-80	60-80
Streptococcus spp.	70-110	50-100	5-40	5-20	30-40	40-60
Staph. aureus	140-200	100-250	10-80	10-60	120-160	60-80

In Table 4.32, Cefuroxime and cefotaxime showed high MIC among the cephalosporines, MIC ranged from 70-200µg/ml (cefuroxime), and 50-250µg/ml (cefotaxime). Cefriaxone and ceftazidime were fairly active with MIC rang of 5-60µg/ml (Ceftriaxone) and 10-60µg/ml (ceftazidime). Azithromycin showed MIC rang of 30-120, while MIC range for Erithromycin was 30-80µg/ml. generally S.aureus showed higher MIC than other isolates in all drugs on this table (Table 4.32).

Table 4.33: MICs of Aminoglycosides and Tetracyclines for Bacteria

Species isolated from University of Ilorin Teaching hospital

Isolates	Minimum Inhibitory Concentration µgml^{-1}				
	Streptommycin	Gentamycin	Tetracycline	Doxycycline	Oxyteracycline
S. epidermidis	60-70	5-10	120-160	30-90	10-40
Kl.pneumoniae	50-90	10-20	110-160	60-70	20-40
Klebsiella sp.	70-90	5-10	60-80	70-90	10-30
Bacillus subtilis	50-70	10-30	40-60	40-60	20-40
Enterobacter sp.	10-20	5-10	10-20	100-110	15-40
Serratia marcescens	10-20	5-10	40-60	60-70	20-40
Pseudomonas sp.	60-80	10-30	40-60	70-130	15-70
E. Coli	30-50	5-10	120-140	30-40	10-50
Ps.aeruginosa	80-100	5-10	110-140	70-120	30-70
Serratia sp.	20-30	5-10	30-40	80-90	20-40
Bacillus cereus	10-20	10-20	100-130	100-120	10-40
Bacillus megaterium	50-70	5-20	100-120	90-100	20-40
Citrobacter freundii	30-50	5-10	80-120	40-50	20-40
Proteus mirabilis	60-90	5-10	100-140	70-80	15-40
Bacillus cereus	10-30	10-20	100-130	100-120	10-40
Strept.pyogen	10-20	5-10	80-140	60-70	15-40
Streptococcus spp.	10-30	5-10	70-130	40-50	10-40
Staph. aureus	20-60	10-20	90-140	60-70	30-40

Table 4.34: MICs of Quinolones and Chloramphenicol for Bacteria Species Isolated from Universiy of Ilorin Teaching Hospital.

Organisms	Minimum Inhibitory Concentration ($ugml^{-1}$)				
	Cprofloxacine	Ofloxacin	Perfloxacin	Sparfloxacin	Chloramphenicol
S. epidermidis	10-20	5-10	5-10	10-15	90-10-30
Kl.pneumoniae	10-15	2.5-5	5-10	5-20	60-70
Klebsiella sp.	5-10	2.5-5	5-15	5-10	50-70
Bacillus subtilis	10-30	2.5-5	5-10	10-15	50-70
Enterobacter sp.	5-10	2.5-5	0.5-5	5-10	60-70
Serratia marcescens	10-20	2.5-10	0.5-5	5-10	30-50
Pseudomonas sp.	10-40	5-10	5-10	15-25	80-100
E. Coli	10-15	2.5-15	5-10	10-15	80-120
Ps.aeruginosa	10-50	10-25	15-20	15-25	100-140
Serratia sp.	5-10	5-10	0.5-10	5-10	50-70
Bacillus cereus	20-40	5-10	5-10	5-10	90-110
Bacillus megaterium	5-15	2.5-5	0.5-15	30-50	100-120
Citrobacter freundii	5-20	2.5-5	0.5-10	5-10	40-60
Proteus mirabilis	10-25	5-10	5-15	5-10	30-40
Strept.pyogen	10-20	2.5-5	5-15	5-15	90-110
Streptococcus spp.	5-15	2.5-5	5-10	5-10	50-90
Staph. aureus	15-25	10-20	10-20	5-20	80-110

Table 4.33 showed MIC of Aminoglycoside and tetracyclines. Gentamycin showed low MIC to all isolates with MIC range of 5-30µg/ml. while streptomycin on the other hand

has MIC range of 10 – 90 µg/ml. for Tetracyclines, the MIC was 10 - 140µg/ml, for Doxycline, it was 30 – 120 µg/ml and Oxytetracycline had 10 – 70 µg/ml. Table 4.33).

In the quinolones group, Ciprofloxacin, ofloxacin, Perfloxacin and sparfloxacin showed MIC range of between 0.5 – 40 µg/ml. The MIC of Chloramphenicol was 30 – 140 µg/ml. generally, the result showed that quinolones group are best among groups of antibiotics tested, it is the only group with MIC range of 0.5 - 4.0 µg/ml (Table 4.34). Followed by aminoglycosides and macrolides in decreasing order of effectiveness.

Table 4.35: β-lactamase Production of some Antibiotic Resistant bacteria Species Isolated from the University of Ilorin Teaching Hospital

Isolates	**No. Tested**	**β-lactamase Producers**	
		n	**%**
S. epidermidis	20	18	90.0
S. aureus	25	21	84.0
Strept. pyogenes	6	5	83.3
Strept. spp	2	0	0.0
E. coli	8	6	75.0
Kl. pneumoniae	10	8	80.0
Kl. spp.	5	3	60.0
Pr. mirabilis	12	7	58.3
Cit. freundii	4	3	75.0
Ent. spp	4	3	75.0
Serratia marcescens	9	7	77.8
Serratia spp.	3	2	66.7
Ps. aeruginosa	16	6	38.0
Ps. spp	3	1	33.3
B. cereus	5	2	40.0
B. megaterium	5	1	20.0
B. subtilis	5	1	20.0
Total	147	94	64.0

Table 4.35, showed that Out of 147 antibiotics resistant isolates tested for β-lactamase production, 94(64.0%) possessed β-lactamase enzyme. Staphylococcus epidermidis, produced the highest amount of the enzyme (90%), followed by *S.aureus* (84.0%), *S. marscenses* (77.8%), *Cit. freundii* and *Enterobacter spp.* (75% each). The result also showed that P.aeruginosa, B. cereus, B. megaterium and B. subtilis showed low level of β-lactamase production (table 4.35).

Table 4.36: Occurrence of Plasmids in some Antibiotic resistant bacteria Species Isolated from University of Ilorin Teaching Hospital

Isolates	No tested	Plasmid Carrying Strains	
		n	%
S. epidermidis	20	17	85.0
S. aureus	25	20	80.0
Strept. Pyogenes	6	2	33.3
Streptococcus spp.	2	1	50.0
Klebsiella spp.	5	3	60.0
E. coli	8	5	62.5
Kl. pneumoniae	10	10	100.0
Enterobacter spp.	4	3	75.0
Pr. mirabilis	12	7	58.3
Cit. freundii	4	2	50.0
Ser. marcescens	9	3	33.3
Serratia spp.	3	1	33.3
Ps. aeruginosa	16	10	62.5
Ps. spp	3	1	33.3
B. cereus	5	1	20.0
B. megaterium	5	2	40.0
B. subtilis	5	2	40.0
Total	147	90	61.2

Table 4.36 showed occurrence of plasmids in antibiotic resistant isolates. Out of 147 isolates tested for presence of plasmid, 90(61.2%) were positive. *Klebsiella pneumoni*ae was strongly positive for plasmid harboring; it showed 100% positive for plasmids. For *Staphylococcus epidermidis*, S. aureus, *Enterobacter spp*., *E. coli and Ps. aeruginosa* it was 85%, 80%, 75%, 62.5%, and 62,5% respectively. Low levels of plasmid production were observed in *Stept. Pyogenes, Serratia marcenscens, B. cereus, B. megaterium and B. subtilis* (Table 4.36).

Table 4.37: Resistant pattern of some Antibiotic Resistant Bacteria Species Isolated from UITH Before and After Plasmid Curing

Isolates	Resistance Profile before curing	Resistance profile after curing
S. epidermidis	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-MA	C-E-OFX-SPA-OXT-DOX
S. aureus	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-CEF-CEFT-AM	C-OFX-SPA-OXT-DOX
Str. pyogenes	A-P-CX-C-AZ-E-PEF-OFX-T-DOX-CEF-AM	OFX-DOX-T
Streptococcus spp.	A-P-CX-C-AZ-E-OXT-T-D0X-CEF-AM	AZ-DOX
Klebsiella spp.	A-P-CX-C-S-E-OXT-T-DOX-CEF-AM	E-OXT-T
E. coli	A-P-CX-C-S-AZ-E-SPA-CIP-OXT-T-CEF-AM	AZ-SPA-OXT
Enterobacter spp.	A-P-CX-C-AZ-E-CIP-OXT-DOX-CEF-AM	AZ-OXT-DOX
Pr. mirabilis	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	AZ-E-OFX-SPA-OXT-DOX
Cit. freundii	A-P-CX-C-AZ-E-SPA-OXT-T-DOX-CEF-AM	AZ-OXT-DOX
Ser. marcescens	A-P-CX-C-AZ-E-OFX-CIP-OXT-T-DOX-CEF-AM	AZ-OFX-CIP-OXT-DOX
Serratia spp.	A-P-CX-C-S-AZ-E-OFX-SPA-OXT-T-DOX-CEF-AM	OFX-SPA-OXT-DOX
Ps. aeruginosa	A-P-CX-C-S-AZ-E-PEF-SPA-CIP-OXT-T-CEF-CRO-CEFT-AM	C-AZ-SPA-OXT
Pseudomonas spp.	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	AZ-E-OFX-SPA-OXT-DOX
B. cereus	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-DOX-CEF-AM	C-AZ-PEF-OXT-DOX
B. megaterium	A-P-CX-C-S-AZ-PEF-CIP-OXT-T-DOX-CEF-AM	C-AZ-PEF-OXT
B. subtilis	A-P-CX-C-AZ-E-SPA-CIP-OXT-T-CEF-AM	AZ-SPA-CIP-OXT

Resistance profile of isolates before plasmid curing and after is shown on Table 4.37. Before the curing *S.epidermidis* was resistant to 15 different antibiotics, this was reduced to 6 after curing the isolate of plasmids. Similar pattern were observed for the rest isolates.

Table 4.38: Maximum Dilutions of Izal that Inhibited Growth of the isolates from the Floor of the Surgical Ward and Theater of University of Ilorin Teaching Hospital in 2004 and 2005

Organism	Minimum Effective Dilution of Izal	
	2004	2005
S .epidermidis	1:800	1:500
Kl. pneumoniae	1:800	1:500
Klebsiella spp.	1:800	1:600
B .subtilis	not tested	1:400
Enterobacter spp.	not tested	1:800
Ser. marcescens	not tested	1:900
Ps. aeruginosa	1:500	1:400
Pseudomonas spp.	1:500	1:600
E. coli	1:600	1:700
Serratia spp.	Not tested	1:800
B. cereus	1:700	1:600
Cit. freundii	Not tested	1:700
Pr. mirabilis	1:1000	1:400
S. aureus	1:900	1:500
B. megaterium	Not tested	1:400
Str. pyogenes	1:600	1:600
Strept spp.	1:1000	1:600

Comparison of dilution ability of the disinfectant is shown on Table 4.38. Decrease in ability of the disinfectant was observed from 2004 to 2005 data. But, *Strept. Pyogenes* still required the same concentration of the disinfectant.

Table 4.39 Minimum Effective Concentration of Lysol on isolates from University of Ilorin Teaching Hospital in 2004 and 2005

Organism	Minimum Effective Concentration of Lysol (% v/v)	
	2004	2005
S.epidermidis	0.50	1.50
Kl.pneumoniae	0.50	1.00
Klebsiella spp.	0.25	0.50
B.subtilis	*	0.50
Enterobacter spp.	*	0.50
Ser. Marcescens	*	3.00
Ps.aeruginosa	0.50	3.00
Pseudomonas spp.	0.50	2.00
E.coli	0.25	2.00
Serratia spp.	*	1.50
B.cereus	0.50	2.50
Cit.freundii	*	3.00
Pr.mirabilis	0.25	2.00
S. aureus	0.25	2.50
B.megaterium	*	2.50
Strept. Pyogen	0.25	1.50
Strept spp.	0.25	1.50

* The isolates were not detected.

Percentage Minimum Effective Concentration (MEC) of Lysol disinfectant was compared (Table 4.39). Increase in MEC was observed from 2004 to 2005. *Pseudomonas aeruginosa*

with MEC of 0.50% in 2004 has increased to 3.59% in 2005. Results for other isolates were similar to the observation.

Table 4.40: Average bacterial counts of the Wards and Theater floors before And after disinfection with Izal by the hospital cleaners.

Wards	Bacterial counts cfu/ml	
	Before	After
W1	$3.14x10^3$	$3.36x10^3$
W2	$3.45x10^2$	$3.19x10^2$
W3	$4.23x10^3$	$4.91x10^2$
W4	$3.84x10^2$	$3.10x10^2$
W5	$2.14x10^2$	$2.93x10^2$
W6	$3.92x10^2$	2.27x102
W7	$4.82x10^2$	$3.98x10^2$
W8	$3.78x10^2$	$2.19x10^2$
Theater	$2.67x10^2$	$1.34x10^2$

Table 4.40 showed effectiveness of disinfections made by the hospital cleaners. T- test statistical analysis showed no significant difference before andafter disinfection ($p<0.05$). In W1 and W5, bacterial counts before disinfection were $3.14x10^3$ and $2.14x10^2$; these were increased to $3.36x10^3$ and $2.93x10^2$ after disinfections respectively. In other wards,

there were no significant observable changes in bacterial counts before and after disinfections.

Table 4.41: Average bacterial counts of the Wards and Theater floors before And after disinfection with Izal by the researcher.

Wards	Bacterial counts cfu/ml	
	Before	After
W1	$3.98x10^3$	$2.136x10^2$
W2	$2.995x10^2$	$1.86x10^1$
W3	$3.68x10^3$	$3.90x10^2$
W4	$4.10x10^2$	$3.98x10^1$
W5	$3.18x10^2$	$2.83x10^1$
W6	$3.00x10^2$	$1.48x10^2$
W7	$3.23x10^2$	$3.11x10^1$
W8	$2.88x10^2$	$2.91x10^1$
Theater	$2.10x10^2$	$1.5x10^1$

There were significant observable differences ($p>0.05$) in bacterial counts before and after disinfections (Table 4.41). Bacterial loads were drastically reduced after disnfection.

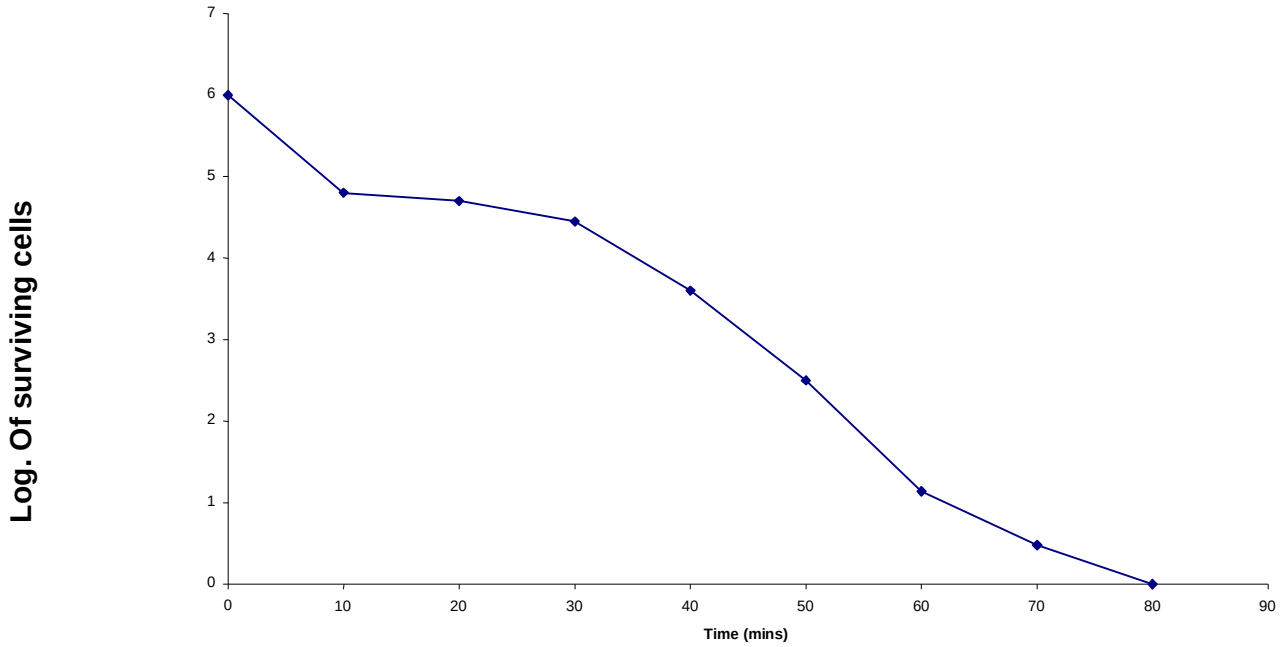


Fig 4.1 Effect of Izal on *S.aureus* (One of the antibiotics and disinfectants most resistant isolates)

Effect of exposing *S. aureus* to Minimum Effective concentration of Izal is shown in figure 4.1. The number of exposed cells decreased from 10^6 to zero within 90 minutes of exposure. Fig. 4.10showed the killing rate of *S. aureus* as one of the most resistant isolates to both antibiotics used and disinfectants. The curve showed that the total killing of the organism can only be affected eighty (80) minutes after exposure to the minimum concentration of the disinfectant.

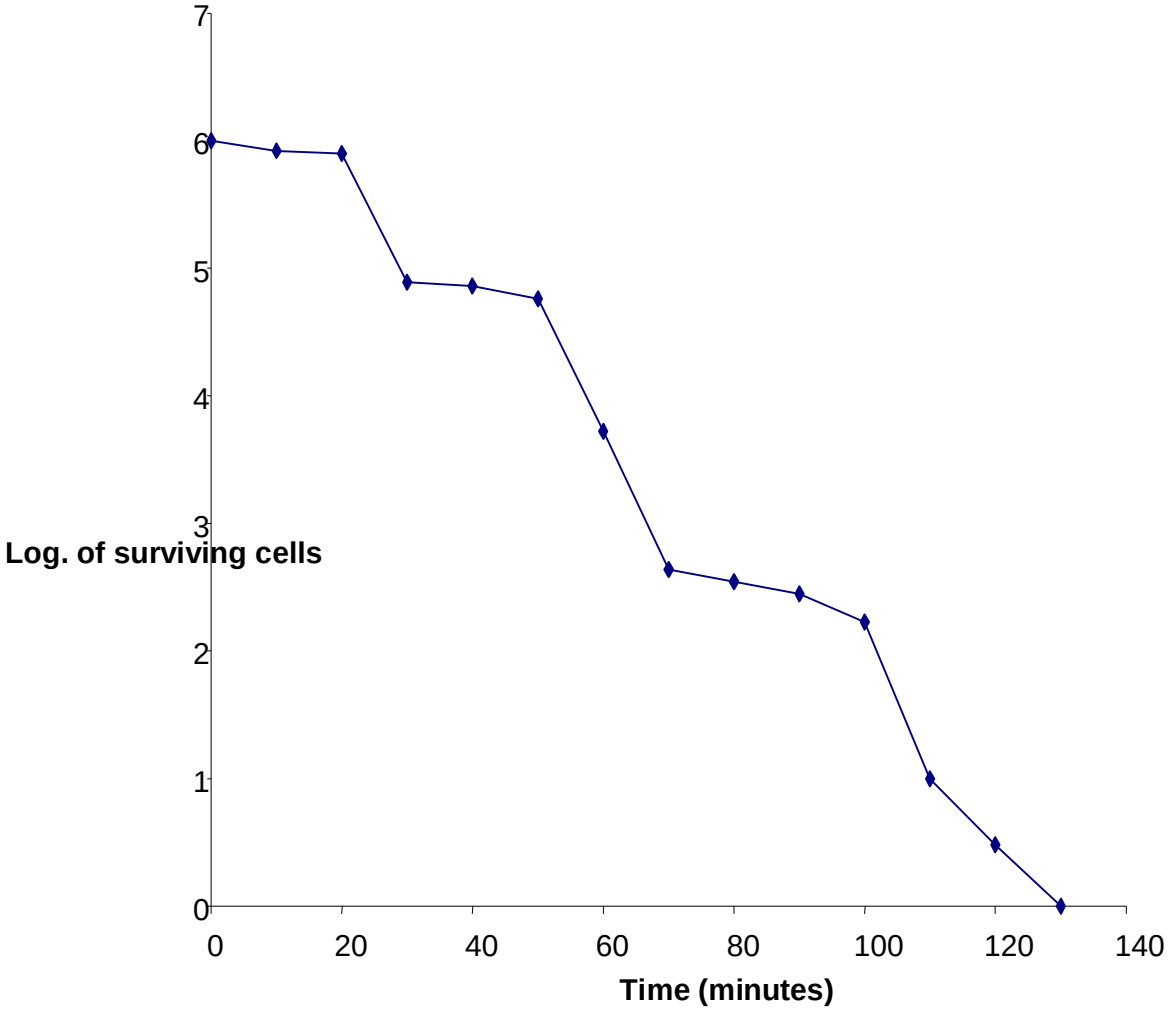


Fig 4.2 Effect of Izal on *Ps.aeruginosa*

Effect of Izal on *Ps.aeruginosa* is illustrated on fig 4.2. from this illustration, it can be observed that the number of surviving cells decreased from 10^6 cfu/ml to zero within 120-140 minutes of exposure to MEC of the disinfectant.

Table 4.42: **Relationship between β-lactamase producing and Plasmids carrying isolates from air, floor and wounds of patients in Theater and surgical wards.**

Code	Isolates	β -Lactamase	Plasmids Bands number
TA1	*Staphylococcus epidermidis*	+	2
TF1	*Klebsiella pneumoniae*	+	1
TF2	*Proteus mirabilis*	-	2
Twd1	*Staphylococcus epidermidis*	+	3
Wwd1	*Pseudomonas aeruginosa*	+	2
Wwd2	*Staphylococcus aureus*	+	2
Twd2	*Staphylococcus epidermidis*	+	1
TF3	*Proteus Mirabilis*	+	2
Twd3	*Staphylococcus aureus*	+	1
TA2	*Streptococcus pyogens*	+	3
TA3	*Staphylococcus epidermidis*	+	2
WF1	*Staphylococcus epidermidis*	+	1
WF2	*Staphylococcus epidermidis*	+	2
WF3	*Bacillus cereus*	+	Nil
TA4	*Proteus mirabilis*	+	1
Wwd3	*Staphylococcus epidermidis*	+	3
WF6	*Proteus mirabilis*	+	1
Wwd4	*Staph.epidemidis*	+	2
Wwd5	*Staphylococcus aureus*	+	2
WA1	*Staphylococcus aureus*	+	2

All isolates which produced β-lactamase enzymes also possessed plasmids, except, the isolate WF3 (*B. cereus*).

Table 4.43: Comparisons of plasmids number and sizes of isolates from air, floor and wounds of Patients in Theater and Wards of the UITH.Ilorin

Isolates	Sites sampled	No. of Plasmids	Plasmids sizes (Kbp)
S.epidermidis	Theater Air	2	23.130, 27.306
S.epidermidis	Theater Wound	3	16.962, 84.744, 20.462
S.epidermidis	Theater air	2	23.130, 54.9267
S.epidermidis	Theater wound	1	23.130
S.epidermidis	Ward floor	1	23.130
S.epidermidis	Ward floor	2	23.130, 54.9267
S.epidermidis	Ward wound	3	23.130, 39.2333, 42.785
S.epidermidis	Ward wound	2	23.130, 54.9267
S. aureus	Ward air	2	23.130, 59.47714
S. aureus	Theater air	1	23.130
S. aureus	Theater floor	1	23.130.
S. aureus	Ward wound	2	23.130, 59.47714
Strept. pyogen	Theater air	3	27.756.
Pr. mirabilis	Theater floor	2	23.130, 25.894
Pr. mirabilis	Theater wound	2	23.130, 25.894
Pr. mirabilis	Theater air	1	23.130
Pr. mirabilis	Ward air	1	23.130
Ps. aeruginosa	Ward wound	2	15.420, 22.5984
Kl. pneumoniae	Theater air	1	23.130
Bacillus cereus	Ward floor	nil	nil

Out of 18 multiple antibiotic resistant isolates subjected to agar gel electrophoresis analysis, 17 had plasmids, with number of band ranging from 1 to 3. Some of the isolates have the same number of plasmid bands and sizes (Table 4.43). All isolates except two had one plasmid common to them in size.

10 9 8 7 6 5 4 3 2 Marker standard

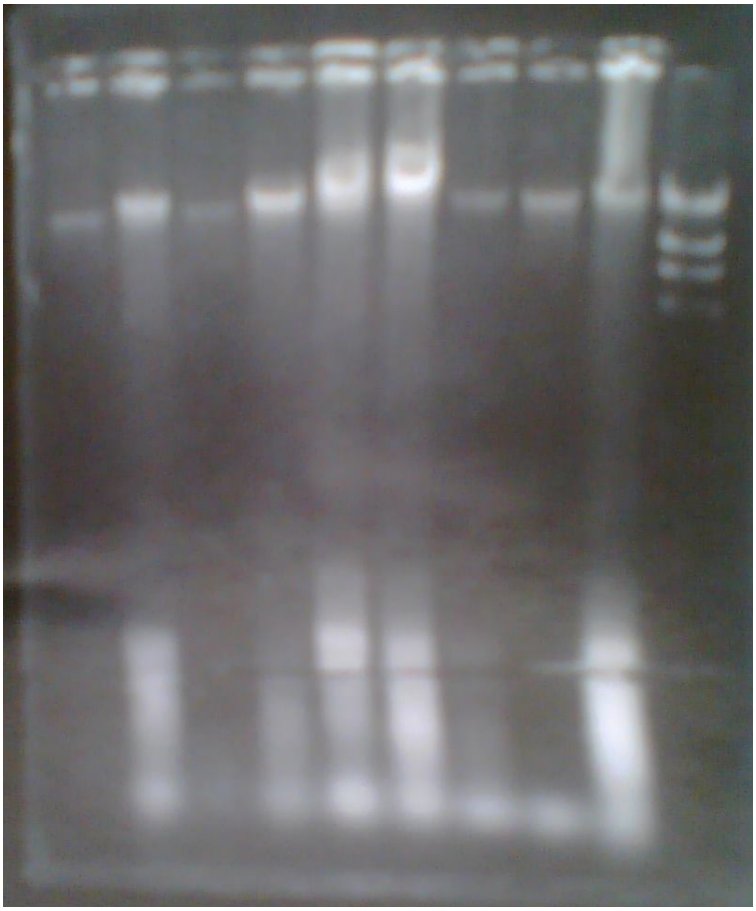


Plate 4.1 Diffrent Plsmid bands exhibited by some of the antibiotics resistant islates.

1. Marker standard
2. *Staphylococcus epidermidis* .
3. *Proteus mirabilis*

4. *Proteus mirabilis*
5. *Staphylococcus epidermidiss*
6. *Staphylococcus epidermidis*
7. *Pseodomonas aeruginosa*
8. *Proteus mirabilis*
9. *Staphylococcus epidermidis*
10. *Klebsiella pneumoniae*

In plate 4.1, isolates on lane 2, 5 and 6 have the same plasmid sizes and number. Isolates on lane 3 and lane 8 also have the same number of plasmids and size.

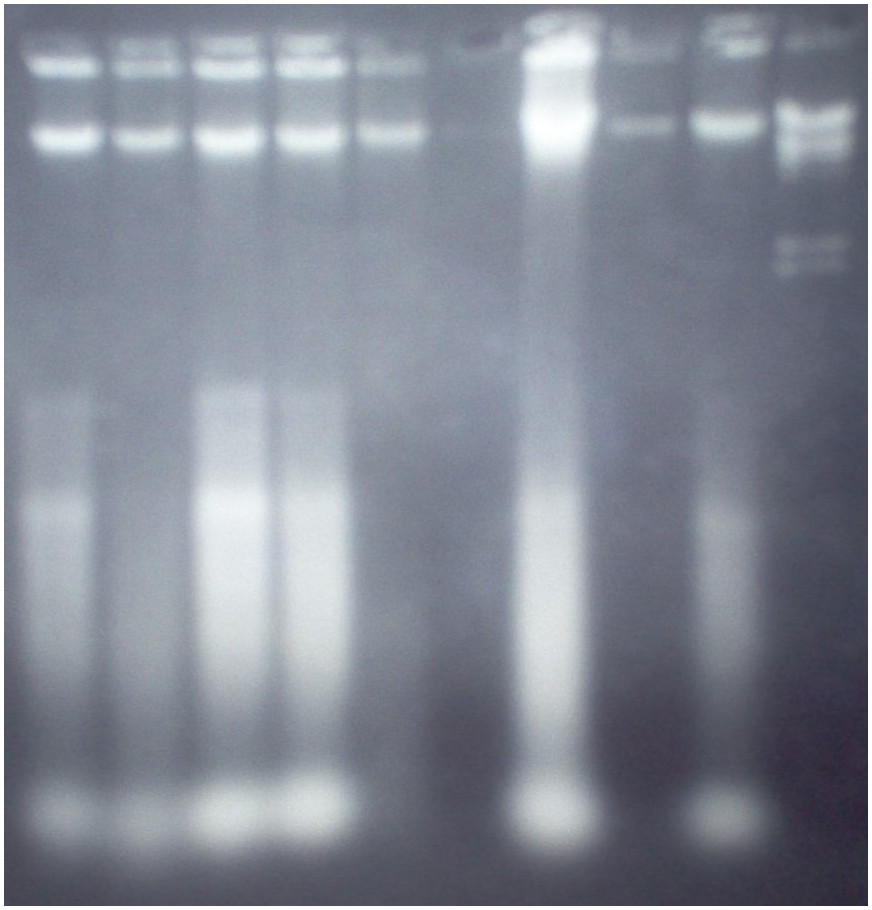


Plate 4.2. Different plasmid bands exhibited by some of the antibiotic resistant isolates.

11. *Staphylococcus aureus*
12. *Streptococcus pyogenes*
13. *Staphylococcuus aureus*
14. *Staphylococcus aureus*
15. *Staphylococcus aureus*
16. *Bacllus cereus*
17. *Proteus mirabilis*
18. *Staphylococcus epidermidis*
19. *Proteus mirabilis*
20. Marker standard.

In plate 4.2 isolats on lane 11, 13 and 14 have the same number of plasmids and the same plasmid sizes. Similar observation was made on isolate on lane 17 and 19.

CHAPTER FIVE

5.0 Discussion

5.1 BACKGROUND.

Nosocomial infections have become a major source of concern to health workers because it continues to hamper effective management of infections in hospitals all over the world. This study set out to address the prevalence of nosocomial infections in some health care institutions in Kwara state, will hopefully address some of these problems and an impetus to health-care providers in initiating appropriate health care policies on nosocomial infections.

Nosocomial infections have been reported to contribute to increase in duration of hospitalization of patients, increased in medical bills and spread of multi-antibiotic resistant pathogens in the hospital and the wide community (Francisco, *et al*., 2006).

The significant effect of nosocomial infections on the increased morbidity and mortality of infections has led to many advanced countries of the world to set up surveillance teams in health institutions and at national levels in efforts to address it resultant effects on health programmes of the populace (NNIS, 2002). Such efforts or measures are however non existent in Nigeria and many other developing countries of the world. This is probably due to some factors such as scanty scientific data on nosocomial infection in the society and lack of awareness of the impact of nosocomial infection on infection management. The results of this work therefore revealed as follow;

The retrospective study of prevalence of nosocomial infections in the six private hospitals in Ilorin metropolis studied gave a prevalence rate of 15.23%, which is higher than the World wide established rate of 5 to10 %(Haley *et al*,. 1985; WHO, 2002). Only one of the six private hospitals. had nosocomial rate within the average World wide rate (9.30%).

The reason for higher prevalence rate of nosocomial infections in private hospitals than World wide established rate may be adduced to the following;

- Level of hygiene in majority of the private hospitals is low.
- Unregulated entry into the wards of the hospitals by visitors. Visitors enter the wards severally at almost all times, contributing to microbial bioload of the wards and increasing the rate of nosocomial infections.

Classification of infections by sites in these hospitals showed that surgical site had the highest prevalence rate, compared with blood sream, urinary and resipiratoy nosocomial infection sites. This order is in contrast with the finding of Ogunsola *et al.*, (1998) who reported that Urinary tract infection site is the most prevalent infection site in University of Lagos Teaching hospital (LUTH). Prevalence rate of nosocomial infection in the wards which showed that surgical wards had the highest rate is a further testimony that surgical site was the most implicated in nosocomial infection.

Comparison of the infection rate among the private hospitals showed that, there is no significant difference in the infection rates except at Sobi Military Hospital where the rate was comparatively higher than the other private hospitals. This might be due to the fact that, Sobi Military Hospital, which caters for a whole army barrack, is usually over crowded with patients and visitors of in-patients, who were likely to increase the microbial burden of such wards. .

The results of retrospective study in University of Ilorin Teaching Hospital (UITH), Ilorin showed, prevalence of nosocomial infection over the years was generally low. This might be due to under-reporting of infections in this hospital. It was observed during collection of data that results of some culture positive test carried out in the Microbiology laboratory

were not reflected in the patients' folders in the wards and hence patients were not among those analysed for determination of rate of nosocomial infections in this hospital.

Monthly incidence of nosocomial infections showed that there was significant difference in rate of nosocomial infections between months and years (2000-2002) at $p>0.05$. The fact that there was no significant variation in the rate of nosocomial infection from month to months indicate that incidence of nosocomial infections in this hospital was most likely unaffected by seasonal changes like the observation in the private hospitals Analysis of prevalence of infections in wards of UITH also showed that surgical wards had relatively higher incidence of nosocomial infections in the 3-years examined. Neonatal Intensive Care Unit (NICU) which has been widely reported as epidemic jungle in teaching hospitals (Kappstein *et al*., 1999; Lizioli *et al*., 2003) had comparatively lower nosocomial infection rate in this hospital.

Classification of the incidence of nosocomial infections into sites also showed that, surgical wound infection is the leading infection site of nosocomial infections at that UITH. The order of of infection rate (from highest to lowest) in 2000 were

surgical > Urinary Tract Infectio (UTI) > Blood Stream Infection. In 2001 and 2002, the order were similar.

That surgical site is the leading nosocomial infection is corroborated by the work of Dumpis *et al*., (2003), as against the findings of result of Niculae *et al*., (2007) where surgical site infection was rated 4^{th} in the order of most common sites of nosocomial infections.

The observed higher prevalence rate of surgical site infections in this hospital could be due to the fact that records of patients that have undergone surgical operation were readily

available in several places, the theater, surgical ward and laboratory and therefore incidence of nosocomial infections are more likely to be noticed and reported.

The reason for the higher infection rate in surgical sites in the hospitals as reported in this work might not be unconnected to the unsatisfactory hygienic condition in the operating room in this hospital. lack of proper demarcation of surgical wards from other wards. Particularly in most of the private hospitals where surgical patients were often admittd in the same wards with medical patients.

Analysis of causative agents of nosocomial infection in the Teaching hospital indicated that *S. aureus* and *Ps. aeruginosa* were the leading nosocomical infection pathogensin the three years study period. This can be attributed to the fact that surgical site infection ranked the highest of all infection sites examined and most reported cases of surgical site infection are associated with *S. aureus, Ps aeruginosa* (Shittu, 2002;Dumpis, *et al.,* 2003; Onche and Adeayi, 2004; Oguntibegu and Nwobu, 2004).

The results of bacteriological sampling of the floors and airs wards and theater showed that Average bacterial count in the surgical ward and operting room of the six private hospitals in Ilorin metropolis were relatively high for environments where surgical operations are expected to be performed or where surgical patients are suppose to be managed. This is a further confirmation of the unsatisfactory hygienic conditions in most of the private hospitals.

Though the hospitals reported that there is daily disinfection in the wards, the effectiveness of such disinfection practices is questionable.

It was however observed that in some hospitals cleaners use only detergent and water. When disinfectants were used, the amount added to water was arbitrary.

It was also observed that the disinfection process itself could contribute to the contamination process. For example mopping sticks were not usually washed after use, and the same mopping sticks were reused repeatedly. Wet mops provide good and suitable media for bacterial growth and proliferation. The clinical significance of high microbial load in delaying wound healing had been described by Bendy *et al.,* (1964). He reported that healing in decubitus ulcers progressed only when the bacterial load was less than 10^6 cfu/ml organisms per quantities swab sample taken from open wounds are required to cause wound infections. Although, none of the bacteria from either floor or air was as high as 10^6cfu/ml the fact is that multiplication of these organisms to the required infectious level is not impossible.

All private hospitals sampled and analysed have *S. aureus, S. epidermidis, Ps. aeruginosa, E.coli, Kl. pneumoniae and Pr. mirabilis* in decreasing order as possible nosocomial pathogens. The predominat bacteria species are normal body microbial flora mostlikely originating from the hospital personnel, patients and visitors.

S. aureus, S. epidermidis, Ps. aeruginosa, and *E. coli* have been reported notorious causative agents of surgical wounds, Urinary tract and blood stream infections (Abdallah *et al.,* 1998; Liziol, *et al., 2003* and Thn *et al*., 2006).

Antibiotic resistant pattern of the isolated bacterial species showed that most of the isolates were multiple antibiotics resistant. *Staph. Aureus* was the most resistant of all the isolates. The implication of this is that most of the patients may not respond positively if they are infected with any of the isolated oganisms and treated with any of the antibiotics tested in this study. Several reasons have been advanced to explain why hospital isolates are multidrug resistant

- intrinsic nature of some isolates to be resistant to the drug either due to lack the target site for that drug, or inability of the drug to transit through the organism cell wall or membrane and reach its site of action (Neu, 1992).
- acquisition of plasmids, which carry resistance genes this is common among hospital isolates
- drug efflux,
- Target site modification or permeability (Speer, *et al.,* 1992).

Monthly bacterial loads of the theatre and surgical wards of UITH was observed to be lower than the value obtained in the six private hospitals. Very low bacterial count in both air and floor of both theatre and surgical wards in UITH is an indication that level of hygiene in this hospital was better than those of the private hospital.

Bacterial load levels in both the theatre and surgical wards were below the prescribed infectious dose of 10^6 cfu/ml of wound fluid swabs (Levine *et al.,* 1976; Raahave *et al.*, 1986; Breidenbach and Trager, 1995; Pruitt *et al*., 1998).

A total of seven hundred and eight (708) species of bacteria comprised of seventeen bacterial species were isolated in the hospital environment of UITH in 2004 - 2005. *S. aureus* and *S. epidermidis* remain the most predominant endemic bacteria species in UITH hospital environment. These predominace and presence of these two organisms which originated from human sources (patients, hospital personnels or visitors to the wards) should be a source of concern to hospital administrators and medical personnel. *S. aureus* especially methicillin resistant ones (MRSA) is currently a majour threat to the successful management of infections in hospitals all over the world.

Ps. aeruginosa another comparatively isolated nosocomial pathogen is a known causative agent of surgical wound and urinary tract infections, its presence in the hospital, environment should also be cause of concern. *Kl. pneumoniae, E. coli. Pr. mirabilis, Strept. Pyogenes, Ser. marcescens* isolated in the hospitals have also incriminated in surgical site infection. In this work, some of the isolated bacterial species, such as *B. cereus, Ps. aeruginosa, S. epidermidis, S. aureus,* and *Pr. mirabilis* which were found in the theater and surgical wards were also present in the patients wounds, implying that these environmental organisms are already colonizing the wound from the theatre. The organisms probably got into the patients wound through either the air or by direct inoculation during dressing, or other devices used on patients or the medical personnel.

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Between Feb-May 2005, *S. epidermidis S. aureu* that have been hitherto isolated from wounds of patient from the theater were no longer detected in the the patients wounds but between July–December, 2005, the distribution pattern of isolates in the theatre and surgical wards changed again. In the theatre *S. epidermidis, S. aureus* and *Klebsiella spp* were found in air, floor and the patient wound, *Enterobacter spp* and *Bacillus spp* were also isolated from the floor and the patients wound.

The reason for this new distribution pattern of isolates may not be unconnected with the fact that during this time large number of medical students undergoing training were in the theater on posting to observe all operation procedures. Many of them come with their overall, while some use theatre overall. In the surgical ward, *S. epidermidis, Klebsiella spp, Ps. aeruginosa* and *S. aureus* were found detected in air, floor and wound of the patients. These observed bacterial distribution have high tendency to cause serous wound infections in the patient, if the right dose of these organism are available.

Comparative analysis of nosocomial infection rate in surgical sites in Feb-April 2004 and Feb-Dec 2005 showed an incidence of 41.5% prevalence rate. The percentage increase in incidence of nosocomial infectio was highest in the case of appendicectomy. This was despite a decreased level of air and floor microbial burden in the theater and surgical ward of the hospital. This low microbial levels in the theater an surgical wards, would according to Bendy, *et al.,* 1964, and the report of some other researchers (Raahave *et al.,* 1976; Pruitt *et al.,* 1986; Breidenbach and Trager, 1995; Levine *et al.,* 1998), be too low to be responsible for contamination and infection of surgical site and wounds. However, Whyte *et al.,* (1991) and Garibaldi (1991) have reported that very low microbial levels in the environments could cause wound contamination and eventual infection, in certain surgical cases.

Whyte *et al.,* (1991) showed that during cholescystectomy, low number of species of bacterial culture from bile can be predictive of wound contamination and later wound infection. Garibaldi (1991) found that 30 or more colony forming units (cfu) of bacteria cultured from a wound are predictive of wound infection and is regardless of wound class, he further demonstrated in a prospective study of 190 colorectal surgery patient that a concentration of 5cfu/ml or higher number of bacteria in the peritoneal fluid is predictive of wound infection.

It means therefore that, the observed infections of surgical site and wound could have resulted from the relatively low microbial burden of the theater and surgical wards

The pathogens isolated from the surgical sites (*S. aureus, S. epidermidis, Ps. aeruginosa* and *E.coli)* have severally been implicated as major causative agents of nosocomial infection of surgical sites and wounds (CDC, 1999; Shittu, *et al*., 2002; Oguntibeju and Nwobu 2004). A number of these isolates (*S. aureus and S. epidermidid*) are indicative of contamination from personnel and therefore unsatisfactory hygienic practices.

Classification of incidence of nosocomial infection of surgical sites according to age of patients showed that patients of age at the two extreme ends (geriatric and paediatric) were more susceptible to nosocomial infection, which is in consonance with the reports of Rosineide and Paulo, (2003). This could be due to low immunity levels in these two categories of patients- under- developed organs and immune system in the very young and aging, debilitated organ system with resultant weakened immune system in the old.

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Antibiotic resistant profiles of bacteria isolated from the theatre, surgical ward and wounds of patients in UITH in the period under study showed that these were multiple antibiotic resistant to the majority of antibiotics tested. Multiply antibiotic resistance have been reported to occur through different mechanisms:

(i) Modification of drug target site or and reducing permeability of the cell to the drug. These mechanisms have been established with development of resistance to aminoglycoside of *Enterobactericeae, Pseudomonas spp. Staphylococci,* especially when gentamycin and Amikacin are used (Shaw *et al*., 1993).

(ii) Production of β-lactamse enzymes which destroy β- lactam ring of β-lactam

antibiotics, thereby rendering the drug useless. *Streptococci, Staphylococci, Pseudomonas spp* and *Enterococci* are recognized as among organisms normally developed resistance by these mechanisms.

(iii)Efflux pump to reduce antibiotics concentration in the cell is another mechanism used by Streptococci, Staphylococci and Pseudomonas spp. to develop resistance to tetracycline, especially doxycycline (Neu, 1989., Spear *et al.*, 1992).

Prevalence of these multidrug resistant isolates in the theatr, surgical wards and wounds is a cause of concern because of its attendant effect. It implied that;

- Most of the commonly used antibiotics will not be useful in the management of wound and surgical sites infected with such pathogens
- Infected wounds would take longer to heal
- Cost of treatment such as cost of drugs, test etc would increase
- Patients will stay longer in the hospitals
- Higher likehood of transmitting such pathogens to other patients.

It is, therefore, important that the hospital management adopt suitable policies to avoid epidemic out break of drug resistant bacteria infections in the hospital.

Resistance to multiple classes of antimicrobial agents such as chloramphenicol, tetracycline and quinolones in gram – negative and gram-positive bacteria have been described to be more often due to efflux of the drugs out of the bacterial cell (Nikaido, 1991 and Levy, 1992).

Sixty-four percent of the 149 MAR isolates were found to be β-lactamase producers, that most strains of the isolated bacterial species (*S. epidermidis, S. aureus, Strep. pyogen, E.*

coli, Kl. pneumoniae, Citrobacter freundii, Ser. Marcescens) were β-lactamase producers implying that substantial number of the MAR isolates developed resistance to the drugs as a result of β-lactamase production. Most isolates that produced β-lactamase enzymes were also found to habour plasmid.

Comparative analysis of data on β-lactamase production and presence of plasmid in some of the MAR isolates showed that all the isolates except one (*Pr.mirabilis*) produced β-lactamase also had plasmids. Also, one strain of the isolates that produced β-lactamase, had no plasmid. These findings confirmed that most of the β-lactamases produced by some of these isolates were plasmid encoded and theater most likely responsible for the resistance of the isolates to the antibiotics. Plasmid – mediated resistance to β-lactam agents is most often a result of β-lactamases (Fred and Denis, 1998). The result also provides explaination for the drug resistance of the isolates, since majority of the isolates harboured 1 – 3 plasmids.

This finding are in agreement with the conclution of some researchers that high percentage of many commonly encountered gram- positive and negative nosocomial pathogens have at least one and frequently multiple plasmids (Chang *et al.*, 1988, Costello *et al.*, 1980, Chow *et al.*, 1985, Cynamon and Palmer, 1983; Fred and Denis, 1998).

Comparison of plasmid sizes and numbers showed that some of the isolates have plasmid band of the same number and sizes which indicates tht they are likely of same origin, most probably the hospital environment. The plasmid profiling also showed that two isolates of S. aureus (one isolated from surgical ward and the other from a patients wound site in the ward) had identical plasmid band and sizes. This should established a linkage between

environmental contamination and nosocomial infection particularly of surgical sites. It is however in contrast to assertion by some researchers (Eickhoff, 1970; American Hospital Association Committee on infection within the Hospital, 1974; Rafferty and Pancoast, 1984) that there is no association between health care associated infection rate and microbial contamination of air or environmental surfaces..

Effectiveness of disinfection in UITH was evaluated and th results showed that disnfection did not follow standard procedure and the disinfectants were not standardized. These facts were observed when disinfection carried out by the researcher (myself) and one carried out by the hospital cleaners were compared. It was found that wrong procedure led to ineffective disinfections observed. Evaluation of different concentration of disinfectants over the year further demonstrated why disinfection remain ineffective. It was found that. Disinfectants were ineffective hence the need for potency evaluation periodically.

Wrong disinfection practices in the wards by the cleaners could have contributed to the continous prevalence of nosocomial infection in the hospital. Concentrations of disinfectant used in ward disinfection were not standardized; same arbitrary amount of disinfectant were used over the years whereas this study showed that the inhibitory concentrations of the disinfectant against the environmental contaminants increased over the period.

Mops used during cleaning and disinfection of ward floors were not constantly washed and dried before re-use. Such mops provide good menstrum for microbial proliferation, increasing the microbial bioburden of disinfectans during use and spreading such organisms all over the ward surfaces.

Adherence to good disinfection practices showed that when used properly , the commonly available disinfectants, particularly the phenolic would reduce environmental microbial levels of the wards, and substantially lower incidences of nosocomial infections.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION.

6.1 CONCLUSION.

Analysis of retrospective data on nosocomial infection obtained from six private hospital and the University of Ilorin Teaching Hospital showed that

(i) Nosocomical infection is prevalent in private hospitals in Illorin, with a rate much higher than the world estimate.

(ii) The nosocomical infection rate in the Teaching Hospitals is considerably lower than the rates in the private hospitals, as low as one-fifth to one-sixth.

(iii) The most frequent nosocomial infection site in these hospitals was the surgical site.

These findings therefore justify the need to carry out detailed studies on the prevalence of nosocomial infection in this town , with a view of finding the causes, attendant effect and remedial solutions.

Result of the prospective studies of nosocomial infection in the selected hospitals in Ilorin metropolis of Kwara State which involve monitoring of bacterial load of air, and floor in wards in the hospital, identification of the bacteria contaminants, their contamination potentials and evaluation of their antimicrobial susceptibilities profile among others, revealed the following:

- The hospital wards particularly the air and floors of theatre and surgical wards are loaded with relatively high number of bacteria contaminants.
- The bacterial environmental floras in the ward were highly varied with not less than seventeen bacteria species present in each ward.

- *Staphylococcus aureus* and *Staphylococcus epidermidis* which are often associated with personal hygiene were the major contaminant in these wards.
- A high number of the isolated bacteria in the wards were found as the major nosocomial infection causative organism in the wards and surgical sites in patients in surgical wards.
- Antimicrobial susceptibility tests showed that these nosocomical pathogens are not only resistant to the commonly used antibiotics, they are multi-drugs resistants.
- Most of the multi-antibiotic resistant nosocomical pathogens isolated were β-lactamase producers,
- These MAR isolates were also found to harbour plasmid that were responsible for the antibiotic resistance of the organisms.
- The beta lactamase enzymes in the resistant isolates wee plasmid encoded.
- Plasmid profiling showed that some of the nosocomical pathogens originated from the same source, most probably the hospital wards.
- These organisms were relatively susceptible to the inhibiting activities of commercially used hospital disinfectants such as izal and Cresol (Phenolic compounds).
- Institution of regular disinfection of the surgical ward using some of the disinfectant above, resulted in substantial reduction in the microbial load of air and floor in the wards.

6.2 RECOMMENDATION.

It was evident during the course of this study that if nosocomical infection in these hospitals is to be reduced, certain remedial steps and measures needs to be taken or adopted.

A number of issues that require attention are;

(a) Unrestricted mixing of visitors with patients in the wards. This is particularly the case in private hospitals, which is often compounded by keeping all patients in a large single room.

(b) Presence of large number of medical students during surgical operation in the theatre.

(c) Absence of antiseptic solutions or soap in wards and the resulting unhygienic practice by medical practitioners.

(d) Irregular floor disinfection of the wards.

(e) Absence of hospital disinfection policy.

To combat the problem of nosocomial infection and reduce the incidences, hospitals should put in place measures such as those recommended by WHO and ensure their compliance. WHO and CDC (2002) recommendation include the following;.

- Adoption of systemic programme for prevention of surgical wound infection. This include practice of optimum surgical techniques, a clean operating room environment with restricted staff and appropriate staff attire, use of sterile equipments, adequate pre-operative preparation of the patients and surgical wound surveillance.
- In operating room, air borne bacteria must be minimized and surface kept clean, through the following schedule;
- Every morning before any intervention between procedures and at the end of working day, cleaning there should be cleaning disinfection of all horizontal surfaces and all surgical items, e.g. bucket, table, etc.

- Complete cleaning of the theatre with standardized disinfectant is necessary after the days work.
- All items used within a sterile environment must be sterilized. Sterile drapes must be placed on the patients and on any equipment included in sterile room. These drapes must be handled as little as possible. Once a sterile drape is in position, it must not be moved.

For operating room staff, the following was recommended.

(b).Hand washing:

Surgical hand disinfection should be performed by all persons, participating in operative procedure.

(c) Operating room attire: -

Operating staff must wear sterile gloves. Double gloving is advisable for procedurse with high risk of puncture. Since glove puncture, is reported to occur frequently, 11.5 to 55%. All persons entering the surgical theatre must wear surgical attire restricted to be worn only within the surgical area. The design and composition of surgical attire should minimize bacterial shedding into the environment. All head, facial hair and neckline must be covered. All personnel entering the operating room must remove any jewellery, nail polish or artificial nails must not be worn. Full coverage of mouth and nose areas with a surgical mask is a must for every one entering the operating room. In addition,

- The number of persons entering the theatre during an operation must also be Minimized, while unnecessary movement or conversation should be avoided.

(d) Setting up infection control uni:t- Regular surveillance of infections in the hospital will go a long way in reducing rate of nosocomical infection. This can best be done by; Setting up Nosocomial Infection Control Team, comprising of the Chief Medical Director of the hospital, Pharmacist, Consultants, each from Medicine, Medical, Microbiology and Chemical Pathology, and Special Nurse trained in infection control.

The team will be responsible for surveillance and report of outbreaks of any particular type of nosocomical infection.

Although there is an infection control team, at UITH, their activities should not be limited to only routine inspection of hospital wards. It should be empowered to carry out real surveillance and report yearly occurrence of infection(s) in the hospital with recommendations for prevention.

Private Hospitals too should be made to follow operating procedures, inspection team should be put in place by the State Ministry of Health to carry out regular inspections of all hospitals, to monitor their compliance with laid down standards and regulations.

Studies of this nature are not without problems. Problems encountered in this study centered mostly on lack of cooperation from hospital authorities and patients.

Hospital managers were initially apprehensive because generally hospitals administrators are weary of studies that could expose inadequacies in their institutions and therefore tend to create unnecessary barriers. Surmounting such barriers can often be time consuming, resource depleting and inimical to the project success.

Most Patients are often uncoperative because of the traditional mindset which can only often be overcome by proper education/counseling and understanding of the social and psychological background of such patients by the researcher..

Need for further studies

Studies on nosocomial infections in this country are still at its infancy. Hospital administrators, medical personnel and the general public are not much worried about this problem, because generally, they are aware of its adverse effects and implications, particularly on patients. More studies need to be carried out to increase awareness of the public and particularly the hospital personnel on the inimical effects of nosocomial infection.

This study was unable to address the effect of nosocomial infection on duration of hospital stay in the investigated hospital and the cost implication of its treatment. Further studies are needed to address this.

A good knowledge of prevalence of nosocomial infection in this country, causative factors, antibiotic susceptibility profile and as well as cost implication will assist Health policy formulator to draw guidelines and regulation on measure to address them. Such information or data can only be available if similar studies are carried out in different parts of the country.

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APPENDIX

Minimum Inhibitory concentration of tetracycline to bacterial isolates from University of Ilorin teaching Hospital.

Isolates	**MIC(µg/ml)**
Staphylococcus epidermidis	120-160
Kl.pneumoniae	110-160
Klebsiella sp.	60-80
Bacillus subtilis	40-60
Enterobacter sp.	10-20
Serratia marscenses	40-60
Serratia spp.	30-40
Pseudomonas sp.	40-60
Escherichia coli	120-140
Pseudomonas aeruginosa	110-140
Bacillus cereus	100-130
Bacillus megaterium	100-120
Citrobacter freundii	80-120
Proteus mirabilis	100-140
Bacillus sp.	40-60
Strept. pyogenes	80-140
Streptococcus spp.	70-130
Staphylococcus aureus	90-140

Minimum Inhibitory Concentration of Cefruroxime to bacterial isolates from University of Ilorin Teaching Hospital.

Isolate	**MIC µg/ml**
S. epidermidis	140-160
Kl.pneumoniae	100-130
Klebsiella sp.	100-130
Bacillus subtilis	100-140
Enterobacter sp.	90-140
Serratia marcescens	100-150
Pseudomonas sp.	100-180
E. Coli	100-160
Ps.aeruginosa	120-220
Serratia sp.	90-120
Bacillus cereus	100-140
Bacillus megaterium	100-160
Citrobacter freundii	100-120
Proteus mirabilis	110-160
Strept.pyogen	90-130
Streptococcus spp.	70-110
Staph. aureus	140-200

Table 38.0 MIC values of Ampicillin to Bacterial isolates from University of Ilorin Teaching Hospital

Isolates	**MIC(µg/ml**
S. epidermidis	1400-1500
Kl. pneumoniae	700-800
Klebsiella sp.	610-800
B. subtilis	750-800
Enterobacter sp.	560-710
Ser. marscenses	780-800
Pseudomonas sp.	1000-1200
E. coli	400-500
Ps. aeruginosa	1250-2500
Serratia sp.	625-1250
B. cereus	625-1250
B. megaterium	710-900
Cit. freundii	610-700
Pr. mirabilis	940-1200
Str. pyogenes	900-1100
Streptococcus sp.	850-1000
S. aureus	1000-1200

Table 39.0 Minimum Inhibitory concentration of Penicillin to bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	**MIC µg/ml**
Staphylococcus epidermidis	1350-1500
Strept.pyogens	980-1,020
Klebsiella spp.	800-900
K.pneumoniae	850-1000
Bacillus subtilis	700-1000
Enterobacter spp	680-780
Staphylococcus aureus	1350-1450
Streptococcus spp.	950-1,020
Bacillus subtilis	850-1,000
Proteus mirabilis	1000-1,100
Citrobacter freundii	780-980
Bacillus megaterium	1000-1,100
Bacillus cereus	1,000-1200
Serratia spp.	540-600
Pseudomonas aeruginosa	1400-1,600
E. coli	700-800
Pseudomonas sp.	1300-1,500
Serratia marscenses	580-650

Table 40.0 Minimum Inhibitory concentration of Cloxacillin to bacterial isolates from University of Ilorin teaching Hospital.

Isolates	**MIC µg/ml**
S. epidermidis	200-240
K.pneumoniae	280-350
Klebsiella sp	260-300
B. subtilis	240-300
Enterobacter sp	140-180
Serratia marcescens	180-250
Ps.aeruginosa	240-350
Pseudomonas sp	260-300
E. coli	240-300
Serratia sp	160-200
B. cereus	210-290
B. megaterium	240-300
Cit. freundii	210-300
Pr. Mirabilis	250-450
Strept.pyogens	140-200
Streptococcus sp	120-200
S. aureus	260-300

Table 41.0 Minimum Inhibitory Concentration of amoxicillin to bacterial isolates from University of Ilorin Teaching hospital.

Isolates	**MICμg/ml**
S. epidermidis	140-180
Kl.pneumoniae	80-120
Klebsiella sp	100-140
Bacillus subtilis	120-180
Enterobacter sp	100-180
Serratia marcescens	120-180
Pseudomonas sp	220-240
Escherichia coli	140-180
Ps. aeruginosa	200-280
Serratia sp	110-160
Bacillus cereus	120-180
Bacillus megaterium	120-180
Citrobacter freundii	100-180
Proteus mirabilis	80-120
Strept.pyogens	120-180
Streptococcus sp	110-150
Staphylococcus aureus	160-200

Table 42.0 Minimum Inhibitory concentration of Chloramphenicol to bacterial isolates from university of Ilorin Teaching Hospital

Isolates	**MIC µg/ml**
Staphylococcus epidermidis	90-130
Klebsiella pneumoniae	60-70
Klebsiella spp.	50-70
Bacillus subtilis	50-70
Enterobacter spp.	60-70
Pseudomonas spp.	80-100
Serratia marscenses	30-50
Serratia spp.	50-70
Streptococcus pyogen	90-110
Escherichia coli	80-120
Bacillus cereus	90-110
Pseudomonas aeruginosa	100-140
Proteus mirabilis	30-40
Bacillus megaterium	100-120
Staphylococcus aureus	80-110
Citrobacter freundii	40-60

Table 43.0 Minimum inhibitory concentration of Streptomycin to bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	**MIC µg/ml**
S. epidermidis	60-70
Kl.pneumoniae	50-90
Klebsiella sp.	70-90
B. subtilis	50-70
Enterobacter sp.	10-20
Ser. marscenses	10-20
Pseudomonas sp.	60-80
E. coli	30-50
Ps. aeruginosa	80-100
Serratia sp.	20-30
B. cereus	10-20
B. megaterium	50-70
Cit. freundii	30-50
Pr. mirabilis	60-90
Bacillus cereus	10-30
Strept.pyogens	10-20
Streptococcus sp.	10-30
S. aureus	20-60

Table 44.0 Minimum Inhibitory Concentration of Gentamycin to bacterial isolates from University of Ilorin.

Isolates	**MIC µg/ml**
S. epidermidis	5-10
Kl.pneumoniae	10-20
Klebsiella sp.	5-10
B. subtilis	10-30
Enterobacter sp.	5-10
Ser. marscenses	5-10
Pseudomonas sp.	10-30
E. coli	5-10
Ps. aeruginosa	5-10
Serratia sp.	5-10
B. cereus	10-20
B. megaterium	5-20
Cit. freundii	5-10
Pr. mirabilis	5-10
Strept.pyogens	5-10
Streptococcus sp.	5-10
S. aureus	10-20

Table 45.0 Minimum inhibitory Concentration of Erythromycin to Bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	MIC µg/ml
Staphylococcus epidermidis	50-70
Klebsiella pneumoniae	30-40
Klebsiella spp.	40-60
Bacillus subtilis	60-90
Enterobacter spp.	70-90
Pseudomonas spp.	60-90
Serratia marscenses	30-40
Bacillus cereus	60-80
Serratia spp.	40-80
Streptococcus pyogen	30-40
Streptococcus spp.	20-30
Escherichia coli	80-100
Bacillus spp.	70-130
Pseudomonas aeruginosa	100-160
Proteus mirabilis	100-140
Bacillus megaterium	20-40
Staphylococcus aureus	120-160
Citrobacter freundii	50-70

Table 46.0 Minimum Inhibitory Concentration of Azithromycin to bacterial isolates from University of Ilorin teaching Hospital.

Isolates	**MIC µg/ml**
Staphylococcus epidermidis	40-60
Klebsiella pneumoniae	30-60
Klebsiella sp.	40-60
Bacillus subtilis	60-80
Enterobacter spp.	50-70
Pseudomonas spp.	50-70
Serratia marscenses	50-80
Bacillus cereus	30-40
Serratia spp.	40-60
Streptococcus pyogen	60-80
Streptococcus spp.	40-60
Escherichia coli	40-80
Bacillus spp.	60-80
Pseudomonas aeruginosa	50-80
Proteus mirabilis	40-60
Bacillus megaterium	80-120
Staphylococcus aureus	60-80
Citrobacter freundii	30-60

Table 47.0 Minimum Inhibitory concentration of sparfloxacin to bacterial isolates from University of Ilorin Teaching Hospital..

Isolates	MIC µg/ml
S. epidermidis	10-15
Klebsiella sp.	5-10
B. subtilis	10-15
Enterobacter sp.	5-10
Ser. marscenses	5-10
Pseudomonas sp.	15-25
E. coli	10-15
Ps. aeruginosa	15-25
Serratia sp.	5-10
B. cereus	5-10
B. megaterium	30-50
Cit. freundii	5-10
Pr. mirabilis	5-10
Bacillus sp.	10-15
Streptococcus sp.	5-10
S. aureus	5-20

Table 48.0 Minimum Inhibitory concentration of perfloxacin to bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	**MIC µg/ml**
S. epidermidis	5-10
Kl.pneumoniae	5-10
Klebsiella spp.	5-15
Bacillus subtilis	5-10
Enterobacter spp	0.5-5
Serratia marscenses	0.5-5
Pseudomonas spp	10-40
Escherichia coli	5-10
Ps. aeruginosa	15-20
Serratia spp	0.5-10
Bacillus cereus	5-10
Bacillus megaterium	0.5-15
Citrobacter freundii	0.5-10
Proteus mirabilis	5-15
Strept.pyogens	5-15
Streptococcus spp	5-10
S. aureus	10-20

Table 49.0 Minimum Inhibitory Concentration of Ofloxacin to bacterial isolates from University of Ilorin Teaching hospital.

Isolates	**MIC(µg/ml**
Staphylococcus epidermidis	5-10
Kl.pneumoniae	2.5-5
Klebsiella sp.	2.5-5
Bacillus subtilis	2.5-5
Enterobacter sp.	2.5-5
Serratia marscenses	2.5-10
Pseudomonas sp.	5-10
Escherichia coli	2.5-5
Pseudomonas aeruginosa	10-25
Serratia sp.	5-10
Bacillus cereus	5-10
Bacillus megaterium	2.5-5
Citrobacter freundii	2.5-5
Proteus mirabilis	5-10
Bacillus sp.	2.5-5
Strept.pyogens	2.5-5
Streptococcus sp.	2.5-5
Staphylococcus aureus	10-20

Table 50.0 Minimum inhibitory Concentration of Ciprofloxacin to bacterial isolates from University of Ilorin Teaching Hospital.

ISOLATES	**MIC µg/ml**
Citrobacter freundii	10-15
Klebsiella spp.	5-10
Kl.pneumoniae	10-15
Serratia spp.	5-10
Bacillus subtilis	10-30
Escherichia coli	10-15
Enterobacter spp.	5-10
Pseudomonas spp.	10-40
Bacillus megaterium	5-15
Proteus mirabilis	10-25
Pseudomonas aeruginosa	10-50
Serratia marcescens	10-20
Bacillus cereus	20-40
Streptococcus spp.	5-15
Strept.pyogens	10-20
Staphylococcus aureus	15-25
Staphylococcus epidermidis	10-20

Table 51.0 Minimum inhibitory concentration of Doxycycline to bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	MIC µg/ml
Staphylococcus epidermidis	30-80
Kl.pneumoniae	60-70
Klebsiella sp.	70-80
Ps. aeruginosa	70-120
Serratia marscenses	60-70
Strept.pyogen	60-70
Streptococcus sp.	40-50
Bacillus megaterium	90-100
Escherichia coli	30-40
Citrobacter freundii	40-50
Serratia sp.	80-90
Proteus mirabilis	70-80
Bacillus cereus	100-120
Staph. aureus	60-70
Enterobacter sp.	100-110
Pseudomonas sp.	70-130
Bacillus subtilis	40-60

Table 52.0 Minimum Inhibitory Concentration of oxytetracycline to bacterial isolates from University of Ilorin Teaching Hospital.

Isolates	**MIC(µg/ml**
Staph. epidermidis	10-40
Kl.pneumoniae	20-40
Klebsiella sp.	10-30
Bacillus subtilis	20-40
Enterobacter sp.	15-40
Serratia marscenses	20-40
Pseudomonas sp.	15-70
Escherichia coli	10-50
Pseudomonas aeruginosa	30-70
Serratia sp.	20-40
Bacillus cereus	10-40
Bacillus megaterium	20-40
Citrobacter freundii	20-40
Proteus mirabilis	15-40
Streptococcus sp.	10-40
Strept. pyogen	15-40
Staphylococcus aureus	30-40