CHAPTER I

INTRODUCTION AND OBJECTIVES

1.1 BACKGROUND

Infection is the detrimental colonization of a host organism by a foreign species. It may be defined as lodgement and multiplication of an infectious agent in the body. All infections do not invariably result in disease (Chakraborty, 2005). Many individuals develop variety of infections but quickly overcome them. However, some individuals develop chronic or persistent infections. Among various types of infections, wound infection has been regarded as the most common nosocomial infections, in spite of technological advances that have been made in wound management (Dionigi *et al*., 2001; Iroha *et al*., 2008).

Wound infection has been a major complication of surgery and trauma. It has been demonstrated for at least 4000-5000 years (Leaper, 2004). In 1992, the Surgical Wound Infection Task Force replaced the term "Surgical Wound Infection" with "Surgical Site Infection" to include infections of organ or spaces deep in the skin and soft tissues such as peritoneum and bone (Horan *et al*., 1992). Any purulent discharge, abscess or spreading cellulitis at the surgical site during the month after the operation is termed as Surgical Site Infection (SSI) (Ducel *et al*., 2002). It occurs after an invasion (surgical procedure) in the part of the body where the surgery took place. Other types of Healthcare-Associated Infections (HAI) that mainly affect surgical patients are postoperative respiratory and urinary tract infections, bacteraemias and antibiotic-related diarrhoeas (particularly *Clostridium difficile* enteritis). SSIs have been shown to compose up to 20% of all HAI. At least 5% of patients undergoing a surgical procedure develop a surgical site infection (NICE clinical guideline, 2001). However, most patients who have surgery do not develop an infection.

Surgery primarily attempts the healing of wounds without serious complications. Infection that is a consequence of surgery is an important indicator of the quality of care. The incidence of wound infection is largely influenced by the type of surgical procedure.

However, infection is still the most serious postoperative complication despite the development of increasingly more powerful antimicrobial drugs. The data on surgical infection have an important impact on surgical development, in terms of hospital design, surgical technique and infection control measures. The growing focus of surgical research is, therefore, the understanding of these factors in order to prevent surgical infections. It seems that infections are more challenging even in the developed surgical centers (Singh and Rijal, 1998).

Some of the common symptoms of SSI are:

• Redness and pain around the area of incision

• Drainage of cloudy fluid from the surgical wound

• Fever

In the event of infection, a wound fails to heal, patient suffers increased trauma, treatment cost rises, and general wound management practices become resource demanding. Since wound colonization is most frequently polymicrobial involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected (Bowler *et al*., 2001).

Despite the fundamental role of antiseptics and antibiotics in the development of modern surgery, implementation of these practices have reduced but not eliminated post-surgical infections. The wound infection depends on a complex interaction between host factors like immunity, nutritional status and age; wound related factors like magnitude of trauma, dead space, devitalization and presence of hematoma and microbial factors like toxins, invasions and resistance to antibiotics (Misra *et al*., 2000). The abundance and diversity of microorganisms in any wound will be influenced by factors such as wound type, depth, location and quality, the level of tissue perfusion, and the antimicrobial efficacy of the host immune response (Bowler *et al*., 2001).

The infecting microorganisms are variable, depending on the type and location of surgery, and antimicrobials received by the patient (Ducel *et al*., 2002). The majority of SSI is caused by the native flora of the patient's skin, mucous membranes, or hollow viscera.

When skin is incised, underlying tissue is exposed to overlying endogenous flora. It is also caused by the organisms present in the hospital environment that are introduced to the patient by medical procedures. Most commonly, aerobic Gram-positive cocci such as *Staphylococcus* species serve as the contaminant, with resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) representing an increasing proportion of such infections in recent years. Entry into hollow viscera exposes surrounding tissue to Gram-negative bacilli such as *E. coli*, Gram-positive organisms such as *Enterococcus*, and occasionally, anaerobes such as *Bacteroides fragilis*. Yeast species and viral pathogens also pose a risk (Reichman and Greenberg, 2009).

The main risk factor is the extent of contamination during the procedure (clean, clean-contaminated, contaminated, dirty), which is to a large part dependent on the length of the operation, and the patient's general condition. Invasive therapeutic procedures, for example, intravenous cannulation, catheterization or other invasive surgical procedures, provide the opportunity for opportunistic organisms of low pathogenecity to invade the tissues. Other factors include the quality of surgical technique, the presence of foreign bodies including drains, the virulence of the microorganisms, concomitant infection at other sites, the use of preoperative shaving, and the experience of the surgical team (Ducel *et al*., 2002). Recent studies continue to report constant infection rates for general surgical services, despite the immense developments in surgery (Anderson *et al*., 1996; Culver *et al*., 1991). This continued rate causes the greatest delay in hospital discharge for all types of surgeries and ultimately invites an extra financial burden (Olson and Lee, 1990).

SSI is a postoperative complication occurring within 30 days following a surgical procedure and is an important cause of mortality and morbidity for patients undergoing surgery. According to Brachman (1981), a nosocomial surgical wound infection lengthens the hospitalization by an average of 7.4 days and raises the cost of hospitalization by more than 800 dollars. The 2002 survey report by the Nosocomial Infection National Surveillance Service (NINSS), which covers the period between Oct 1997 and Sept 2001, indicates that the incidence of HAI related to surgical wounds is as high as 10% and costs

National Health Service in United Kingdom approximately 1 billion pounds (1.8 billion dollars) annually (NINSS, 2002). It has been estimated that each patient with a surgical site infection will require an additional 6.5 days in hospital, which results in the doubling of hospital costs associated with that patients (Plowman, 2000). Hence, the delay in recovery and subsequent increased length of hospital stay has economic consequences as well.

This study aims to find out the prevalence of surgical wound infections and antibiotic sensitivity pattern of the isolated organisms among the patients admitted to the Shree Birendra Hospital, Chhauni. This will facilitate the clinicians to select the appropriate antibiotics for prophylaxis and treatment. It will also help to develop the bacterial database to assess the changes in bacterial resistance pattern in future.

1.2 OBJECTIVES

1.2.1 General Objective

To determine the prevalence of SSI among the inpatients of Shree Birendra Hospital and to assess the antimicrobial susceptibility pattern of the isolates from the infected surgical wounds.

1.2.2 Specific objectives

The specific objectives are as follows:

- To isolate and identify the etiological agents of surgical wound infections.
- To assess the antimicrobial susceptibility pattern of the isolated bacterial pathogen.
- To determine the prevalence of multi-drug resistant organisms in the infected wounds.

CHAPTER II

LITERATURE REVIEW

2.1 WOUND INFECTION

It is the deposition and multiplication of bacteria in tissue with an associated host reaction (Ayton, 1985). Wound infection is the invasion of organisms through tissues following a breakdown of local and systemic host defenses (Russell *et al.,* 2000). Infection occurs when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system. The subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound (Bowler *et al.,* 2001).

The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of microorganisms involved. Most acute and chronic wound infections involve mixed populations (Bowler *et al.,* 2001).

Wound infection has been a problem in the field of surgery for a long time. An infected wound complicates the post operative course and results in prolonged stay in the hospital and delayed recovery (Anguzu and Olila, 2007).

2.2 SURGICAL SITE INFECTION

The surgical wound is said to be infected if there is any purulent discharges, abscess or spreading cellulitis at the surgical site within 30 days after the operation. Surgical site infection (SSI) accounts for 15% of all nosocomial infections and among surgical patients, represents the most common nosocomial infection (Watanabe *et al.,* 2008). Wound infection has always been a major complication of surgery. Most post operative wound infections are hospital acquired, varies from one hospital to another and they cause significant post-operative morbidity, mortality and prolonged hospital stay (Bratzler,

2006; Jonathan *et al.*, 2008). Advances in control of infections have not completely eradicated the problem because of development of resistance (Anguzu and Olila, 2007). The inappropriate use of broad spectrum antibiotics or the prolonged courses of prophylactic antibiotics disposes all the patients at even greater risk of infection because of the development of antibiotic resistant pathogens (Khorvash *et al.*, 2008).

SSI is the infections of the tissues, organs or spaces contacted by surgeon. Intraoperative contamination of normally sterile tissues by pathogenic microbes is the most frequent triggering point of incision infection and it is unusual for SSI to prevent later than four weeks except in cases of surgical implants (hip replacement etc) where it can take up to one year (Ali *et al.*, 2009).

SSI may be major SSI or minor SSI. The wound that either discharges significant quantities of pus spontaneously or needs a secondary procedure to drain it is a major SSI. In this case the patients may have systemic signs such as trachycardia, pyrexia and a raised white blood cell count which is systemic inflammatory response syndrome (SIRS). Minor SSI may discharge pus or infected serous fluid but should not be associated with excessive discomfort, systemic signs or delay in return home (Willaims *et al.*, 2008).

In general, wound can be considered infected if purulent materials drain from it, even without confirmation of positive cultures. The clinical definition has advantages over culture based results because a positive culture does not necessarily indicate infection. Also many wounds are colonized by bacteria, whether infected or not. Infected wounds may not yield pathogens by culture owing to the fastidious nature of some pathogens, or if the patients have received an antimicrobial therapy (Patherick *et al.*, 2006).

2.3 CLASSIFICATION OF SURGICAL WOUND

According to traditional classification system by NRC (National Research council), operation is categorized as clean, clean contaminated, contaminated and dirty (Berard and Gandon, 1964).

Clean (Class I): A non- traumatic wound with no break in surgical technique during the procedure, and in which respiratory, gastro-intestinal and genito-urinary tracts are not

transected. e.g. Surgery on meninges and brain, joints, eye, heart and peripheral vessels or transplant surgery.

Clean-contaminated (Class II): A wound in which gastrointestinal, respiratory or urinary tract (non- infected) are entered without significant spillage. A clean operation with a major break in sterile technique also comes in this category. e.g. Enterotomy, Enterectomy.

Contaminated (Class III): There is a major break in technique during the operation, with a traumatic wound or gross spillage from an infected body cavity. e.g. Enterectomy, Cholecystectomy.

Dirty (Class IV): The wound surfaces are directly contaminated by purulent material or continuing discharges from hollow viscera. A hollow organ is ruptured. e.g. Ruptured gastrointestinal tract, gallbladder or pyometra.

2.4 CLASSIFICATION OF SURGICAL SITE INFECTION

According to the standardized surveillance criteria developed by Centers for Disease Control and Prevention (CDC), SSIs are classified as incisional and organ /space. Incisional SSI may be further classified as superficial or deep-incisional SSI (Horan *et al*., 1992).

Superficial incisional SSI

Such infection involves only skin and subcutaneous tissue of incision. It occurs within 30 days after the operation and patient has at least one of the following:

- Purulent drainage with or without laboratory confirmation, from the superficial incision
- Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision
- At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative
- Diagnosis of superficial incisional SSI made by a surgeon or attending physician

Deep incisional SSI

Infection involves deep tissues, such as facials and muscle layers. This also includes infection involving both superficial and deep incision sites and organ/space SSI draining through incision. It occurs within 30 days of the operation or within 1 year if an implant is present and patient has at least one of the following:

- A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain or tenderness, unless incision is culture-negative
- An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination
- Diagnosis of deep incisional SSI made by a surgeon or attending physician

Organ/space SSI

Infection involves any part of the anatomy in organs and spaces other than the incision, which was opened or manipulated during operation. Infection occurs within 30 days after the operation if no implant is left in place or within one year if implant is in place and patient has at least one of the following:

- Purulent drainage from a drain that is placed through a stab wound into the organ/space
- Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space
- An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination
- Diagnosis of organ/space SSI made by a surgeon or attending physician

2.5 RISK FACTORS FOR SURGICAL SITE INFECTION

There are multiple reasons for post operative wound infections, which have been validated and documented as risk factors. A risk factor is any recognized contribution to an increase in post operative wound infections (Masaadeh and Jaran, 2009).

According to the simple index developed during the Study on the Efficacy of Nosocomial Infection Control (SENIC) Project, the risk factors that predispose to the wound infection are:

- Surgery longer than 2 hours.
- Contaminated or dirty infected surgery (Wound contamination)
- Abdominal Surgery
- Three or more diagnoses at the time of discharge (excluding those related to surgical wound infections and their complications)

(Haley *et al.,* 1985)

The SENIC risk index that takes these four factors into account was replaced by the American Society of Anesthesiologists (ASA) pre operative assessment score, which was validated in a large study involving 44 hospitals from 1987-1990 (Garbaldi *et al.,* 1991).

The ASA classification scores patients on a five point scale from healthy to moribund on the basis of the presence of co-morbidities at the time of surgery. An ASA score >2 is associated with increased risk of wound infection and this risk is additional to that of classification of operation and duration of surgery (National Clinical Guideline, 2008).

Table 1: ASA classification of physical status

ASA score	Physical status
1	Normal healthy person
2	Patient with mild systemic disease that is not incapacitating
3	Patient with severe systemic disease that limits activity but is not incapacitating
4	Patient with incapacitating systemic disease that is a constant threat to life
5	Moribund patient who is not expected to survive with or without an operation

2.5.1 Factors influencing the risk of developing the SSI

The risk of developing an SSI is largely determined by three factors: the amount, type of microbial contamination of the wound and host susceptibility (Nwachukwu *et al*., 2009). The ultimate outcome of the interaction between wound and microbes depends on a number of factors which are shown in brief in table 3.

A. Patient's factor

a. Diabetes

Although the contribution of diabetes to SSI remains controversial, significant relationships have been demonstrated between elevated HgA_{1c} (glycated haemoglobin) level and SSI rates, as well as postoperative serum glucose levels higher than 200 mg/dl in cardiac surgery populations (Reichman and Greenberg, 2009). Also, increased glucose levels (>200 mg/dl) in the immediate postoperative period (<48 hours) were associated with increased SSI risk. More studies are needed to assess the efficacy of perioperative blood glucose control as a prevention measure (Mangram *et al*., 1999). Bibi *et al*. (2011) in his study showed that diabetic patients are 3.6 times more susceptible to infection as compared to non-diabetics.

b. Nicotine use

Nicotine use delays primary wound healing and may increase the risk of SSI. Some studies have corroborated cigarette smoking as an important SSI risk factor. The limitation of these studies, however, is that terms like current cigarette smoking and active smokers are not always defined. To appropriately determine the contribution of tobacco use to SSI risk, standardized definitions of smoking history must be adopted and used in studies designed to control for confounding variables (Mangram *et al*., 1999).

c. Obesity

Obesity has been identified as a risk factor for wound infection and complications after a wide variety of surgical procedures. It increases risk substantially when the subcutaneous abdominal fat layer exceeds 3 cm (1.5 inches). The risk is increased by the need for a

larger incision, decreased circulation to the fat tissue or the technical difficulty of operating through a large fat layer (Fuji *et al.,* 2011).

d. Infection at another site

It may increase the risk of spreading infection through the bloodstream.

e. Immunocompromised patients

Those patients with HIV/AIDS, those with chronic corticosteroid use such as occur with asthma and heavy smokers are at significantly greater risk of SSIs. The conditions such as transplant, immunosuppression or irradiation, agammaglobulinemia, administration of adrenocorticosteroids, drugs (nicotine) are also equally important factors that are responsible for wound infection. Patients with non- specific immunosuppression such as granulocytopenia, defective chemotaxis are prone to infection (Singh and Rijal, 1998).

f. Steroid use

Patients who are receiving steroids or other immunosuppressive drugs preoperatively may be predisposed to developing SSI but the data supporting this relationship are contradictory (Gil-Egea *et al.,*1987; Slaughter *et al.,* 1993). In a study of long-term steroid use in patients with Crohn's disease, SSI developed significantly more often in patients receiving preoperative steroids (12.5%) than in patients without steroid use (6.7%) (Mangram *et al*., 1999). In contrast, other investigations have not found a relationship between steroid use and SSI risk.

g. Malnutrition

Malnutrition may or may not be a contributing factor. Unfortunately, most studies have not been conducted in developing countries where severe malnutrition is more common. However, it causes depression of the immune system and hence wound infection and inflammatory response to this may delay healing (Russell *et al.,* 2000). For some types of operations, severe protein-calorie malnutrition is crudely associated with postoperative nosocomial infections, impaired wound healing dynamics, or death (Brown *et al.,* 1996).

h. Age, race, socioeconomic status and chronic diseases:

The conditions such as diabetes and malignancies are difficult to assess because they are frequently associated with other factors that independently contribute to risk. For example, age over 70 may be accompanied by decreased defense mechanisms, poor nutrition and anemia (Infection prevention guideline).

i. Prolonged preoperative hospital stay

Prolonged preoperative hospital stay is frequently suggested as a patient characteristic associated with increased SSI risk. It exposes patients to hospital flora, including multidrug-resistant organisms. However, length of preoperative stay is likely a surrogate for severity of illness and co-morbid conditions requiring inpatient work-up and/or therapy before the operation (Mangram *et al*., 1999).

j. Preoperative nares colonization with *Staphylococcus aureus*

S. aureus is a frequent SSI isolate. This pathogen is carried in the nares of 20% to 30% of healthy humans. It has been known for years that the development of SSI involving *S. aureus* is definitely associated with preoperative nasal carriage of the organism in surgical patients. A recent multivariate analysis demonstrated that such carriage was the most powerful independent risk factor for SSI following cardiothoracic operations (Mangram *et al*., 1999).

k. Perioperative transfusion

It has been reported that perioperative transfusion of leukocyte-containing allogeneic blood components is an apparent risk factor for the development of postoperative bacterial infections, including SSI. In three of five randomized trials conducted in patients undergoing elective colon resection for cancer, the risk of SSI was at least doubled in patients receiving blood transfusions (Mangram *et al*., 1999).

B. Hospital related factors

a. Operating room environment

It contributes a great deal to the surgical development, especially the surgical ward and operating room, because wounds are at high risk of contamination both in the operating room and ward.

A clean operating room with restricted staff entry, appropriate staff attire and sterile equipment should be maintained during operation in order to prevent microbial contamination. In busy surgical units, the risk of cross infection must be minimized. Air borne bacteria must be minimized and surfaces kept clean. The temperature of the operating room should be maintained at a reasonable level as the infection risk is reduced when the body is kept warm (Cluett, 2008).

b. Operating room staff

Persons entering the operating room should be minimized. Hand washing by the personnel involved in operation is must. Many hospital personnel fail to follow basic means of infection control, such as hand washing between patient contacts. In ICUs, asepsis is often overlooked in the rush of crisis care (Weinstein, 1991).

c. Duration of surgery

The risk of wound infection has repeatedly been shown to be proportional to the duration of the surgical procedure. Cruse and Ford (1980) found that the rate of wound infection increased for longer procedures, roughly doubling with every hour of the procedure. Operations lasting for one hour or less had a wound infection rate of 1.3% whereas those lasting three hours or more had a rate close to 4.0%. It has been found that an operative time for more than 2 hours as the second greatest independent predictor of risk (wound contamination being the first) (Nandi *et al*., 1999).

d. Type of operation

The principal factor responsible for wound infections is the type of operation. In the case of a clean operation, there is the least risk of infection, whereas in a contaminated

operation the risk of infection is quite high (Singh and Rijal, 1998). The often quoted infection rates for the traditional classification of operative procedures are as follows: clean (<2%), clean-contaminated (5% to 15%), contaminated ((15% to 30%), and dirty (>30%).

The incidence of primary wound infection is correlated to the bacteriological cleanliness of the operation. After contaminated operations, infection rates are more variable, depending on the type and number of organisms released from the membrane (Masaadeh and Jaran, 2009).

Table 2: Risk Factors for the development of SSIs

Patient Factors
⁾ Diabetes
⁾ Malnutrition (undernutrition and obesity)
⁾ Extremes of age
⁾ Skin disease at operation site
⁾ Irradiation at operation site
⁾ Peripheral vascular disease (for lower limb surgeries)
⁾ Hypoxemia
⁾ Postoperative anemia
⁾ Steroid therapy
⁾ Chronic inflammatory conditions
⁾ Infection at remote sites
⁾ Staphylococcal carriers
Treatment Factors
⁾ Emergency procedures
⁾ Inadequate and inappropriate antibiotic prophylaxis
⁾ Prolonged preoperative hospitalization
⁾ Prolonged operative time
⁾ Hypothermia
⁾ Surgical drains
Environmental Factors
⁾ Inadequate skin antisepsis
⁾ Inadequate sterilization of instruments
⁾ Inadequate ventilation
⁾ Contaminated medications

Source: Barie, 2005

C. Wound related factors

Specific wound related factors may predispose to the development of an infection. These include:

- Poor application of the aseptic principles at the time of wound dressing changes
- Presence of devitalized tissue within the wound margin, necrotic tissue or slough, particularly if over 50%
- Nature of wound and prolonged presence of exudates not managed by a closed wound drainage system (Collier, 2004)

D. Microbial factors

Microbial factors that influence the establishment of a wound infection are the bacterial inoculums, virulence, and the effect of the microenvironment. When these microbial factors are conducive, impaired host defenses become an advantage in enacting the chain of events that produce wound infection. The development of an infection will be influenced largely by the virulence of the organism and immunological status of the patient. Virulence describes both the pathogenecity and invasiveness of the relevant microorganism. When microorganisms are present to a degree of 10^5 per gram of tissue, an infection is likely to be present. Quantitatively wounds harboring bacteria that exceed 10^5 CFU per gram are considered infected wound (Heggars, 2003).

The virulence and invasion capability of the organisms have been reported to influence the risk of infection, but the physiological state of the tissue in the wound and immunological integrity of the host seem to be of equal importance in determining whether infection occurs (Heinzelmann *et al*., 2002). The wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and examination (Oguntibeju and Nwobu, 2004).

E. Antimicrobial administration

The practice of using prophylactic antibiotic in different surgeries is now becoming mandatory because of increased chance of hospital-acquired infection and also for

prevention of postoperative infectious morbidity. But the regimen for prophylactic antibiotic is different in hospitals. Higher generation of cephalosporins and combination of two antibiotics are found to be prescribed in hospitals in surgeries. Ideally, an appropriate spectrum of prophylactic antimicrobial agents for surgery should be saturated in the body fluids and at the surgical site during the operation but they should be terminated as soon as possible to avoid the occurrence of resistant organisms (Palikhe and Pokharel, 2003).

With the advent of antibiotics in the 1940s, it became obvious that all those microbes would be tackled successfully. Depending on the microbial response, a wide range of antibiotics have subsequently evolved. In surgical practice, antibiotics are also used to treat established infections after culture and drug sensitivity testing of the microorganisms. Antisepsis, including prophylactic antibiotics, is now reaching its zenith. However, genetic adaptations by these microbes have enabled them to survive in a hostile antibiotic environment. The incidence of multi-resistant staphylococcal infection, even today, is not uncommon (Singh and Rijal, 1998). Intravenous antibiotics should be given during the induction of anaesthesia with repeated doses for longer procedures.

In many surgical operations, patients will have previously received antibiotic prophylaxis. This varies according to the type of operation. Prophylaxis will affect the flora and thus the cause of any subsequent infection. It is therefore important to determine the nature of any prior antibiotic therapy, as this will determine the agents likely to be successful in any subsequent infection (Elsevier, 2010).

Patients who had received perioperative antibiotics and who developed infections were frequently infected with organisms that were resistant to the perioperative drug regimen, compared with patients who had not received antibiotics (Garibaldi, 1991).

Inadequate anti-microbial treatment defined as ineffective treatment of infection is an important factor in the emergence of antibiotic resistant bacteria. Factors that contribute to inadequate anti-microbial treatment of hospitalized patients include the prior antibiotic use, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical devices (Bhatt and Lakhey, 2006). Antibiotics especially those with broad

spectrum activity alter the normal flora of body. They destroy sensitive bacterial strain and select for resistant strains which are often multi-resistant strains, thus predisposing the patient to infection (Gyawali, 2007). In Nepal, there is no proper guideline for antibiotic prophylaxis in surgery. The practice is generally based on the influence of senior doctors, with the reference of books, and journals. The inappropriate use of antimicrobial agents has resulted not only in unnecessary expense or overuses of antimicrobial agents but also in the development of drug-resistant bacteria (Palikhe and Pokharel, 2003).

Antimicrobial resistance has been a problem in the field of surgery, as advances in the control of infections have not completely eradicated the problem of drug resistance (Nwachukwu *et al*., 2009).

2.6 ROUTES AND SOURCES OF TRANSMISSION OF SSI

There are a number of ways by which the micro-organisms can gain access to a wound, such as:

Direct contact: Micro-organisms may transfer from equipments or the hands of patients, doctors, nurses and other staffs or from independent environmental sources.

Airborne dispersal: Microorganisms are deposited from the surrounding air. Infected droplets originate in the nasopharynx and mouth which are expelled during talking, breathing and sneezing.

Self contamination: Microorganisms may contaminate the wound through physical migration from the patient's skin and GI tract. Most surgical infections are acquired intra-operatively and are endogenous arising from the flora of the patient's skin, gastrointestinal tract or mucous membrane.

Endogenous source: Endogenous infections are caused by the patient's own flora which is non-pathogenic under the normal condition (Chakravorty, 1990). Although the organisms of the normal flora of the various body surfaces are not pathogenic in their normal habitat, they may be pathogenic if they escape or are implanted elsewhere. Sources of endogenous contamination include the gastrointestinal and genitourinary

tracts, site of active infection remote from the wound (e.g. a urinary tract infection), the skin and the anterior nares (Bhatt and Lakhey, 2006).

Exogenous source: Many infections are exogenous. Skin and anterior nares are important sources of staphylococci, spread of organisms from hospital staffs and visitors occurs by direct and indirect airborne routes (Bhatt and Lakhey, 2006). Exogenous infections are mainly acquired from the nose or skin flora of the operating team and transmitted by the hands of the surgeon or through the air directly or indirectly from instruments (Sanjay *et al*., 2010).

2.7 PREVENTIVE MEASURES FOR SSI

A systematic programme for prevention of surgical wound infections includes the practice of optimal surgical technique, a clean operating room environment with restricted staff entry and appropriate staff attire, sterile equipment, adequate preoperative preparation of the patient, appropriate use of preoperative antimicrobial prophylaxis, a variety of preventive measures aimed at neutralizing the threat of bacterial, viral, and fungal contamination posed by operative staff, and the patient's endogenous skin flora and a surgical wound surveillance programme (Reichman and Greenberg, 2009; WHO, 2002).

The Guideline for Prevention of Surgical Site Infection, 1999, has provided recommendations concerning reduction of surgical site infection risk on the basis of existing scientific data, theoretical rationale, and applicability.

Preoperative preparation of the patient

For elective operative procedures, any existing infections should be identified and treated before surgery. The preoperative stay should be minimized. Any malnourished patient should have nutrition improved before elective surgery. The serum blood glucose level should also be adequately controlled in all diabetic patients and hyperglycemia should be particularly avoided. Smoking or use of other tobacco products should be stopped at least 30 days before elective surgery if possible.

Women using combined (estrogen- and progestogen-containing) contraceptives (oral or injectable) should be switched to a non hormonal method at least 30 days before major elective surgery to minimize the risk of deep vein thrombophlebitis and nonfatal pulmonary embolism.

The patient should normally be bathed or showered on the evening before the intervention, using an antimicrobial soap. If hair removal is required, this should be done by clipping or with a depilatory rather than by shaving immediately before the operation.

The operative site must be washed with soap and water, and then an antimicrobial preoperative skin preparation applied from the centre to the periphery. The area prepared must be large enough to include the entire incision and adjacent skin sufficient for the surgeon to work without contacting unprepared skin.

The patient must be covered with sterile drapes; no part is uncovered except the operating field and areas needed for the administration and maintenance of anesthesia (Mangram *et al.,* 1999; WHO, 2002).

Antimicrobial prophylaxis

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins AMP agent should be used for all operations or classes of operations in which its use has been shown to reduce SSI rates based on evidence from clinical trials or for those operations after which incisional or organ/space SSI would represent a catastrophe. An AMP agent that is safe, inexpensive, and bactericidal with an in vitro spectrum should be used that covers the most probable intraoperative contaminants for the operation (Mangram *et al.*, 1999).

The cephalosporin, Cefazolin is the antibiotic of choice in most clean procedures and is cost-effective, safe and effective against a broad spectrum of bacteria (Anonymous, 2006). Prophylaxis should be administered within 1 hr of initiation of the surgery to maintain an effective antibiotic serum concentration throughout the entire course of surgery (Florman and Nichols, 2007).

Ideally, an appropriate spectrum of prophylactic antimicrobial agents for surgery should be saturated in the body fluids and at the surgical site during the operation but they should be terminated as soon as possible to avoid the occurrence of resistant organisms (Palikhe and Pokharel, 2003).

Surgical hand antisepsis

The NICE guidelines for the prevention and treatment of SSI recommend that before the first patient on a theatre list, preoperative hand antisepsis should be performed with an aqueous antiseptic solution. NICE decision to recommend an aqueous antiseptic scrub for use prior to the first operation on a list is partly based upon the fact that alcohol rubs are less effective against the spores of *Clostridium difficile* (Casey and Elliott, 2009).

The optimum antiseptic used for the scrub should have a broad spectrum of activity, be fast acting, and have a persistent effect. Antiseptic agents commercially available in the United States for this purpose contain alcohol, chlorhexidine, iodine/iodophors, parachloro-meta-xylenol, or triclosan. Alcohol is considered the gold standard for surgical hand preparation in several European countries. Povidone-iodine and chlorhexidine gluconate are the current agents of choice (Mangram *et al.,* 1999).

Operating room environment

Positive-pressure ventilation should be maintained in the operating room with respect to the corridors and adjacent areas. A minimum of 15 air changes per hour should be maintained, of which at least 3 should be fresh air. UV radiations should not be used in the operating room to prevent SSI. The doors of operating room should be closed except as needed for passage of equipment, personnel, and the patient. Orthopedic implant operations should be performed in operating rooms supplied with ultraclean air (Florman and Nichols, 2007; Mangram *et al.,* 1999).

Postoperative incision care

An incision that has been closed primarily should be protected with a sterile dressing for 24 to 48 hours postoperatively. Hands should be washed before and after dressing changes and any contact with the surgical site. Sterile technique should be used while

changing an incision dressing. The patient and family should be educated regarding proper incision care, symptoms of SSI and the need to report such symptoms**.** (Florman and Nichols, 2007; Mangram *et al.*, 1999).

2.8 INDICATORS OF WOUND INFECTION

The inflammatory response is a protective mechanism that aims to neutralize and destroy any toxic agents at the site of an injury and restore tissue homeostasis (Collier, 2003). There are a number of indicators of infection; these include the classic signs related to the inflammatory process and further more subtle changes as highlighted by Cutting and Harding (1994). The classic signs of wound infection are:

- Localized erythema
- Pain
- Heat
- Cellulites
- Oedema

Further criteria include:

- Abscess
- Discharge which may be viscous in nature , discolored and purulent
- Delayed healing not previously anticipated
- Discoloration of tissues both within and at the wound margins
- Friable, bleeding granulation tissue despite gentle handling of and the non adhesive nature of wound management materials used
- Unexpected pain and/or tenderness
- Abnormal smell

2.9 ELEMENTS OF SURGICAL WOUND INFECTION

According to Way and Doherty (2003) three elements are common to surgical infections.

An infectious agent

Although few pathogens cause most wound infections, many organisms are capable of doing so. The bacteria generally encountered may be classified as exogenous or endogenous. Detailed microbiological analysis of wounds demonstrate close correlation between the species found in the normal flora of the gut or oral cavity and microorganisms present in wounds in the close proximity to those sites. Till today, widespread opinion among wound care practioners is that aerobic or facultative pathogens such as *S. aureus, P. aeruginosa* and β-haemolytic streptococci are the primary cause of delay in wound healing and infections (Bowler *et al*., 2001).

The susceptible host

Surgical infections are more common in immunosuppressed patients, and Acquired Immunodeficiency Syndrome (AIDS), transplant immunosuppression, and agammaglobulinemia are associated with a high risk of infection. Patients with nonspecific immunosuppression such as granulocytopenia, defective chemotaxis and malnutrition are prone to infection.

Both endogenous and exogenous factors are believed to affect the susceptibility of any wound to infection. Endogenous factors are the unique attributes of the patient which may or may not be variable prior to surgery. Exogenous factors are not unique to any patient, and can often be influenced by the surgeon, for example, the length of the operation. The host's ability to resist an infection can also be reduced by tissue destruction including clumsy surgery, prolonged anesthesia, and ischemia. Similarly, diabetic patients are also vulnerable to surgical infection.

An environment

An infective agent needs an environment to complete the triangle of infection in a susceptible host. The barrier, which keeps the infective agents away from the normally sterile tissue, such as the intact skin or bowel mucosa, is the key step in infection. In

general, poorly vascularized tissues are more susceptible to infection. Some natural spaces (lumen) in the body, for example, the appendix, gallbladder and intestines, are prone to become obstructed and infected. Foreign bodies, dead tissues, and injury interfere with the normal defense mechanisms of the sliding surfaces of the peritoneal and pleural cavities, and thus promote infection (Singh and Rijal, 1998).

2.10 PATHOPHYSIOLOGY OF SSI

By the end of an operation, bacteria and other microorganisms contaminate all surgical wounds, but only a small number of patients actually develop a clinical infection (Fry, 2002). Bowler *et al*. (2001) stated that infection occurs when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Infection does not develop in most patients because their defense mechanisms effectively eliminate the contaminating organisms at the surgical site. Whether a potential infection occurs depends on several factors, with the most important being (Fry, 2002):

- number of bacteria entering the wound;
- type and virulence (ability to cause infection) of the bacteria;
- host defense mechanisms (E.g., effectiveness of inflammatory response and status of the immune system); and
- external factors, such as being in the hospital several days before surgery or the operation lasting more than 4 hours.

In order to cause infection, a pathogen must accomplish the following**:**

Entry of pathogen into the host

The most frequent portals of entry of pathogenic bacteria into the body are the sites where mucous membrane meets with the skin. Abnormal areas of skin and mucous membrane (e.g. cuts, burns and other injuries) are also the frequent sites of infection. Normal intact skin provides the primary defense against infections (Brooks *et al.*, 2001).

For most SSIs, the source of pathogens is the endogenous flora of the patient's skin, mucous membranes, or hollow viscera. When mucous membranes or skin is incised, the exposed tissues are at risk for contamination with endogenous flora. These organisms are usually aerobic Gram-positive cocci (e.g., staphylococci), but may include fecal flora (e.g., anaerobic bacteria and Gram negative aerobes) when incisions are made near the perineum or groin. When a gastrointestinal organ is opened during an operation, it is the source of pathogens. Gram negative bacilli (e.g., *E. coli), G*ram positive organisms (e.g., enterococci), and sometimes anaerobes (e.g., *Bacteroide fragilis)* are the typical SSI isolates (Mangram *et al.,* 1999).

Adherence

Unless a pathogen is directly introduced into the tissue, the first step in initiation of infection is usually adherence or attachment of pathogen to some surface of the host. Many bacteria possess surface macromolecules that bind to receptors on the surface of host tissues. Capsules, glycocalyx, slime layer etc may be important for adherence not only to host tissue, but also between other bacteria. Fimbriae and pili may also function in the attachment process (Madigan *et al.,* 2000).

Some microorganisms may contain or produce toxins and other substances that increase their ability to invade a host, produce damage within the host, or survive on or in host tissue (Mangram *et al.,* 1999).

Invasion

A few microorganisms are pathogenic because of the toxins they produce. These microorganisms do not need to gain access to host tissue. However, the most pathogens penetrate the epithelium to initiate pathogenecity, a process called invasion. At the point of entry, usually at small breaks or lesions in the skin or in mucosal surfaces, growth is often established in the sub mucosa (Madigan *et al.,* 2000).

Colonization and growth

If the pathogen gains the access to the tissues, it may multiply and the process is called colonization. The initial inoculum is rarely sufficient to cause damage so the pathogen

must grow within host tissue in order to produce infection. Cellular damage to the skin and soft tissues may be mediated by toxins (endotoxins and exotoxins), degradative enzymes and the induction of the host cellular response that destroy tissues usually by immune mediated mechanisms (Schaechter *et al.*, 1989).

Host defense

The normal healing process begins the moment tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extra cellular matrix. This triggers the platelets to release clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and Transforming Growth Factor Beta (TGF-B) (Kim *et al*., 1998).

Neutrophils help in removing foreign material, bacteria and non-functional host cells and damaged matrix components that may be present in wound site. Neutrophils are attracted by the chemical signals given off by bacteria and ingest them. They will continue this until they are filled with bacteria and constitute laudable pus in the wound. The mast cells also help in wound healing. They release granules filled with enzymes, histamine and other active amines that are responsible for the characteristic signs of inflammation around the wound site (Diegelmann and Melissa, 2004).

The inflammatory response is a protective mechanism that aims to neutralize and destroy toxic agents at the site of injury and restore tissue homeostatis (Collier, 2003). The characteristic inflammatory response results in redness, swelling, pain and heat, which are localized at the site of infection (Madigan *et al*., 2000).

2.11 ORGANISMS COMMONLY ENCOUNTERED IN SURGICAL WOUND INFECTION

Data from the NNIS System reveals that the most common incisional SSI pathogens are *S. aureus, Enterococcus* spp., CONS, members of Enterobacteriaceae, *Pseudomonas* spp. and anaerobes (Horan *et al*., 1988).

The usual pathogens on skin and mucosal surfaces are Gram-positive cocci (notably staphylococci); however, Gram-negative aerobic and anaerobic bacteria contaminate skin in the groin/perineal areas The contaminating pathogens in gastrointestinal surgery are mostly intrinsic bowel flora, which include Gram-negative bacilli (e.g., *E. coli*) and Gram-positive microbes, including enterococci and anaerobic organisms. Gram-positive organisms, particularly staphylococci and streptococci, account for most exogenous flora involved in SSI. Sources of such pathogens include surgical/hospital personnel and intra-operative circumstances including surgical instruments, articles brought into the operative field, and the operating room air. The emergence of resistant strains has considerably increased the burden of morbidity and mortality associated with wound infections. MRSA is proving to be the scourge of modern day surgery (Singhal, 2009).

With the exception of clean operative procedures, surgical wound infections are recognized as having a polymicrobial etiology, involving both aerobic and anaerobic microorganisms, and intra-abdominal infections normally reflect the microflora of the resected organ. Rotstein *et al*. (1985) emphasized the polymicrobial nature of almost all surgical infections and commented that the critical importance of aerobic- anaerobic mixtures in these infections had received relatively little attention. The most common causative organisms associated with post surgical wound infection are shown in table 3.

Table 3: Commonly isolated pathogens from SSI

Gram positive cocci	*S. pyogens, E. faecalis, S. aureus, S. epidermidis, S. pneumoniae,* CONS
Gram negative aerobic rods	*P. aeruginosa, Acinetobacter* spp.
Gram negative facultative rods	*Enterobacter* spp., *E. coli, Klebsiella* spp., *Proteus* spp., *Citobacter* spp.
Anaerobes	*Bacteroides fragilis, Clostridium* spp.
Fungi	Yeasts (*Candida albicans*)

Source: Collier, 2004

The modern molecular survey done by Wolcott *et al*. (2009) using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) identified two previously uncharacterized bacteroidales in all of the SSIs and showed that it was the predominant population in the majority of the chronic wounds. The high prevalence of anaerobic bacilli and the overwhelming predominance of two previously uncharacterized bacteroidales suggest that such bacteria may be a leading contributor to such infections (Wolcott *et al*., 2009).

2.12 SOME OF THE COMMON SURGICAL PROCEDURES

Surgery (from the Greek: *cheirourgikē*, via Latin: *chirurgiae*, meaning "hand work") is an ancient medical specialty that uses operative manual and instrumental techniques on a patient to investigate and/or treat a pathological condition such as disease or injury, or to help improve bodily function or appearance.

An act of performing surgery may be called a surgical procedure, operation, or simply surgery. At a hospital, modern surgery is often done in an operating theatre using surgical instruments, an operating table for the patient, and other equipment. The environment and procedures used in surgery are governed by the principles of aseptic technique: the strict separation of "sterile" (free of microorganisms) things from "unsterile" or "contaminated" things. All surgical instruments must be sterilized, and an instrument must be replaced or re-sterilized if it becomes contaminated (i.e. handled in an unsterile manner, or allowed to touch an unsterile surface (Williams *et al.*, 2008).

Surgery may be done in the patients for different procedures such as, excision, resection, ligation, grafts, transplantation, spinal fusion, debridements, repair of fistula, hernia or proplapse etc.

Some of the commonly performed surgical operations that were included in the study are as follows:

Gastrointestinal surgery

- Appendicectomy
- Cholecystectomy
- Laparotomy
- Hepatectomy
- Gastrectomy
- Intestinal anastomosis
- Hemicolectomy
- Graham's omentopexy
- Abdominal rectopexy
- Splenectomy

Urogenital surgery

- Prostatectomy
- Nephrectomy
- Nephrolithotomy / pyelolithotomy

Gynaecological surgery

- Dilatation and Curettage
- Cesearean section
- Hysterectomy

Head and Neck surgery

- Cataract surgery
- Tonsillectomy
- Thyroidectomy
- Myringoplasty

Orthopedic surgery

- Amputation
- O.R.I.F.
- Implant surgery
- Total Hip Repalcement

Others

- Herniorrhaphy
- Free skin graft
- Mastectomy
- Debridement of wound , burn or infection

The details of the above terminologies are given in appendix IV

2.13 USE OF ANTIBIOTICS AND MULTIDRUG RESISTANCE

Antibiotic resistance has been a problem since the introduction of penicillin G and the sulphonamides in the 1940s (Norrby, 1995). Data reported by the National Nosocomial Infections Surveillance (NNIS) System for 1993- 1997 compared with January-November 1998 show a continuing increase in antimicrobial resistant pathogens; the increase is particularly marked for Vancomycin-resistant enterococci (VRE) (55%), methicillin-resistant *S. aureus* (MRSA) (31%), third-generation cephalosporin-resistant *E. coli* (29%), imipenem-resistant *P. aeruginosa* (32%), and quinolone-resistant *P. aeruginosa* (89%) (Hsueh *et al.*, 2002).

MDROs are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents. These highly resistant organisms deserve special attention in healthcare facilities. In addition to MRSA and VRE, certain GNB, including those producing extended spectrum beta-lactamases (ESBLs) and others that are resistant to multiple classes of antimicrobial agents, are of particular concern. These limitations may influence antibiotic usage patterns in ways that suppress normal flora and create a favorable environment for development of colonization when exposed to potential MDR pathogens (i.e., selective advantage). Increased lengths of stay, costs, and mortality also have been associated with MDROs. GNB resistant to ESBLs, fluoroquinolones, carbapenems, and Aminoglycosides also have increased in prevalence (Siegel *et al.*, 2006).

The increasing prevalence of resistant pathogens is one of the main reasons that patients still suffer adverse outcomes from postoperative infections. Methicillin-resistant *S. aureus*

now accounts for 59% of all *S. aureus* isolates with no evidence of a plateau occurring in this trend (NNIS report, 2004). Factors contributing to increased resistance include an increase in antibiotic usage as well as inappropriate usage. Total antibiotic exposure has been implicated as a risk factor in the emergence of MRSA (Harbarth *et al.*, 2000).

Inappropriate surgical prophylaxis continues to be a major problem nationally. Inappropriate use of antimicrobial agents not only adds to the cost of medical care, but also exposes the patient to potential toxicity and risks that promote the development and spread of antimicrobial resistance in health care facilities (Martone and Nichols, 2001).

The prescription of first-generation cephalosporins is heavy in surgical intensive care units, which probably reflects prolonged potentially inappropriate use of these agents for prophylaxis postoperatively in patients who are transferred to the intensive care unit. Second-generation cephalosporin usage is decreasing dramatically as those drugs leave the marketplace and become harder to find (Harbarth *et al.*, 2000). Surgical intensive care units tend to use more vancomycin than medical intensive care units.

The duration of antibiotic prophylaxis is also an important consideration in the development of postoperative infections (Barie *et al.*, 2006). In a retrospective review of 442 patients in a trauma unit in Miami, FL, transplant and non-transplant patients who had prolonged prophylaxis (>4 days) were more likely to develop bloodstream infections and other vascular catheter-related infections ($P \leq 0.0001$) (Namias *et al*., 1999).

2.14 MICROBIOLOGICAL ANALYSIS OF SURGICAL WOUND

In clinical practice, the presentation of a devitalized acute or chronic wound or a clinically infected wound is likely to prompt a practitioner to sample the wound for microbiological analysis (Bowler *et al.*, 2001).

Proper collection, transport, and storage of these specimens are of greatest importance, and compromises should be avoided. Once a specimen has been obtained, packaged, and dispatched to the laboratory, it should be processed as soon as possible. After the preliminary examinations have been completed and cultures made, the rest of the

specimen should be properly labeled, stoppered and refrigerated, until it is certain that no additional laboratory tests are needed (Vandepitte *et al.,* 2004).

i. Collection of specimen

Superficial wounds are always colonized by commensal micro-flora hence before swabbing wound should be cleaned with 70% alcohol or non-bacteriostatic sterile saline. Pus or fluid aspirate in a syringe, deep swabbing or punch biopsy of the leading edge of the lesion is preferred to the wound swab (Miller, 1999). Although the value of acquiring superficial swab samples has been seriously questioned, the procedure is simple, inexpensive, non invasive and convenient for most of the wounds. If anaerobic bacteria are suspected to be involved in an infectious process, fine needle aspiration or tissue biopsy is preferred since swabs are not valid transport media for these pathogens (Dedsite, 2006).

ii. Specimen transport

Following the acquisition of wound fluid or tissue for microbiological analysis, prompt delivery of the specimen to the laboratory is considered to be of utmost importance, particularly if anaerobic bacteria are being investigated. Since swab samples are susceptible to desiccation and oxygen exposure, a prereduced, nonnutritive transport medium is essential to maintain the viability of both aerobic and anaerobic microorganisms on cotton swabs (Bowler *et al*., 2001).

iii. Macroscopic examination of pus

Specimen of pus, received in a syringe or in a sterile container should be evaluated carefully for color, consistency and odour. The color of the pus varies green yellow to brown red. A red color is generally due to admixture with blood or haemoglobin. Pus from postoperative or traumatic wounds (burns) may be stained blue green by the pyocyanin pigment produced by *P. aeruginosa*. The consistency of pus may vary from a turbid liquid to one that is very thick and sticky. The presence of granules must also be observed. A foul feculent odour is one of the characteristic features of an anaerobic or a

mixed aerobic-anaerobic infection, although it may be lacking in some instances (Vandepitte *et al*., 2004).

iv. Gram stain

Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure *S. aureus*. Similarly, this procedure may also facilitate identification of the etiological agent of wound infection following clean surgery, where there is a higher probability of one microorganism being involved (e.g., clusters of Gram-positive cocci). However, in most other wound types that are characterized by a complex aerobic-anaerobic microflora, the Gram stain has little value, although the combined presence of leukocytes and bacteria is likely to be a good indicator of infection (Bowler *et al.*, 2001).

v. Culture of wound specimen

Routine analysis of wound specimens normally involves the use of selective and non selective agar media to culture aerobic bacteria and yeasts and, if the specimen is purulent and/or malodorous, anaerobic bacteria also (Bowler *et al.,* 2001). Independently from the results of microscopy, all specimens of pus or exudates should preferably be inoculated onto a minimum of three culture media. A blood agar for the isolation of staphylococci and streptococci, A Mac Conkey agar plate for the isolation of Gram negative rods, and a tube of broth that can serve as enrichment medium for both aerobes and anaerobes, e.g. thioglycollate broth or cooked meat medium (Vandepitte *et al.,* 2004).

vi. Identification

With the exception of contaminants from the environment or from the skin, all organisms isolated from wounds, pus, or exudates should be considered significant and efforts made to identify them. Full identification is however not always necessary, particularly in the case of mixed flora (Vandepitte *et al.,* 2004).

vii. Antibiotic sensitivity tests

The aim of antimicrobial therapy is to choose a drug which is selectively active against the most likely pathogens and least likely to cause adverse effects or promote resistance.

Hence it is necessary to determine the antibiotic susceptibility of organisms isolated from infected patients (WHO, 2002).

Antibiotic sensitivity testing is an *in vitro* method for estimating the activity of drugs which will assist clinician in selecting an antimicrobial agent effective in inhibiting the growth of an infecting microorganism *in vivo*. Antimicrobial activity of a drug and sensitivity of an organism could be measured by either

i. Serial broth dilution technique or
ii. By disc susceptibility testing method

(Gyawali, 2007)

WHO recommended Modified Kirby-Bauer disc diffusion technique is used by most laboratories for routine test of antimicrobial susceptibility of the organisms.

2.15 GLOBAL SCENARIO OF SSI

Surgical Site Infections (SSIs) are the most common cause of nosocomial infection, resulting in considerable morbidity and mortality, because patients who develop SSIs have a longer hospital stay, are more likely to be readmitted, and are more likely to die. The delay in recovery and increased hospital stay also has economic consequences. It has been estimated that each patient with a surgical site infection requires an additional hospital stay of 6.5 days, and hospital costs are doubled (Fehr *et al.*, 2006; NINSS, 2002).

The incidence of surgical site infections varies from 0.5% to 15% depending on the type of operation and underlying patient status. These are significant problems which limit the potential benefits of surgical interventions. The impact on hospital costs and postoperative length of stay (between 3 and 20 additional days) is considerable (WHO, 2002). The most common bacterial pathogens responsible for SSI are *S. aureus, P. aeruginosa, E. coli, K. pneumoniae, Enterobacter* spp., *Enterococcus* spp., CONS and viridans streptococci (Hsueh *et al.*, 2002; Gelfand *et al.*, 2005).

A recent prevalence survey demonstrated that SSI was the third-largest cause of Health Care Associated Infection (HCAI) in England, Wales, Northern Ireland and the Republic of Ireland. Indeed, SSI accounts for 14.5% of the total cases (Smyth *et al*., 2000). The

survey also revealed that SSI occurred in 4.7% of surgical patients and of these, 44.9% were superficial incisional, 35.4% deep incisional and 18.3% organ/ space. SSI is also associated with a significant mortality and therefore unfortunately still remains an important cause of sepsis.

The 2002 survey report by the NINSS (Nosocomial Infection National Surveillance Service), which covered the period between October 1997 and September 2001, indicates that the incidence of HAI related to surgical wounds in the United Kingdom is as high as 10.1% and costs the National Health Service in the United Kingdom approximately 1 billion pounds (1.8 billion dollars) annually. Mangram *et al.* (1999) reported that SSI is associated not only with increased morbidity but also with mortality as 77% deaths were related to surgical wound infection.

CHAPTER III

MATERIALS AND METHODS

A list of equipments, reagents, media and antibiotics used for the study is presented in appendix I. The study was carried out in microbiology section, pathology department, Shree Birendra hospital. The objective of the study was to study the surgical site infection in patients undergoing different types of surgery, to identify common types of organisms involved in SSI and to assess their antimicrobial susceptibility pattern.

The study was conducted over a period of 7 months, from October 2010 to May 2011 and a total of 200 pus samples were collected for culture and sensitivity from the patients of different wards of the hospital. The surgical wounds included in the study were from the patients who had undergone different surgical procedure at different sites.

3.1 Specimen collection

The sample collected for the study were pus and wound swabs from the surgical wounds that were clinically suspected as infected on the basis of common symptoms like redness, swelling, developing fever, pain at the operative site, wet dressing and later appearance of frank pus from the wound site usually within 5-7 days (Ali *et al.*, 2009). The area around the surgical wound was cleaned with 70% ethyl alcohol and the base was swabbed and placed in a sterile container. Duplicate swabs were collected from each surgical wound, one for Gram stain and another for culture.

The sample was taken to the laboratory immediately for processing, to avoid dessication of sample and to prevent the growth of some species at room temperature that may obliterate the true pathogens.

3.2 Sample processing

The sample was processed as soon as it reached the laboratory following the standard laboratory procedures. Of two samples taken from each patient, one was used for direct microscopic observation i.e. Gram stain and another for culture of the responsible pathogen (Collee *et al.*, 1999).

3.2.1 Macroscopic examination

The pus samples were examined for its appearance, color, consistency and presence of granules.

3.2.2 Microscopic examination

An evenly spread smear of the specimen was prepared on a clean glass slide. The smear was allowed to air dry, heat fixed and stained by Gram stain method. The smear was then examined for the presence of bacteria among pus cells using 40x and 100x objectives.

3.2.3 Culture of the specimen

The aerobic culture of the specimen was performed under aseptic condition as soon as the specimen reached the laboratory. The sample was inoculated on Nutrient Agar (NA), Mac Conkey Agar (MA), Blood Agar (BA) and Robertson's Cooked Meat Broth (RCMB).

The inoculated NA, MA and BA plates were incubated at 37˚C for 24 – 48 hours aerobically in ordinary incubator. The inoculated RCMB was incubated at 37˚C for up to 72 hours for enrichment of the responsible organism (Collee *et al.*, 1999). The turbidity in RCMB was observed. If the growth in culture plates were negative after 24 hours incubation at 37˚C but turbidity seen in the RCMB, then the sample was re-inoculated from the RCMB and again incubated. When the turbidity was not seen in the cooked meat broth after 72 hours incubation, the sample was reported as "no growth". The composition and preparation of media are given in the appendix II.

3.2.4 Isolation and identification of bacteria

The isolated organisms were identified using standard microbiological techniques. In every case the colony morphology was studied, Gram stained smear was prepared and observed under microscope. The isolated organism was subcultured in basal medium and the obtained pure colonies were subjected to different biochemical tests such as Catalase test, Oxidase test, Coagulase test, Oxidative-fermentative (OF) test, Methyl Red test, Vogues-Proskauer (VP) test, Indole test, Motility test, Hydrogen sulphide (H_2S) production test, Triple sugar iron (TSI) reactions, Citrate utilization test and Urease test.

The composition of media and reagents used for different biochemical tests and their procedures are given in appendix II.

3.2.5 Antibiotic susceptibility testing for isolated organisms

Antibiotic sensitivity test were performed using fresh broth culture of the isolates on MHA using commercially prepared antibiotic sensitivity disc. It was performed by using modified Kirby-Bauer disc diffusion method following NCCLS recommendations**.** The zone of inhibition around the disc was measured and the susceptibility pattern of the isolates was recorded. The isolates that were resistant to three or more types of antibiotics were considered as multi-drug resistant organisms (MDROs).

3.2.6 Quality control for tests

Quality control is absolutely essential for good operating procedure (Vandepite *et al.,* 2004). To maintain quality control all tests were performed in an aseptic condition. The samples were collected using sterile swab aseptically in order to avoid contamination. The sterility of each batch of the medium prepared were confirmed by incubating one uninoculated media and other inoculated with known culture for quality control. The positive and negative controls were incubated along with test for comparing the results. The control strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 were used for the quality control during antibiotic susceptibility testing.

3.2.7 Statistical analysis

The data were analyzed using SPSS ver. 16 for determining the association of incidence of SSI in patients of different age groups and gender. The P-value <0.05 was assumed to be significant for the analyses. Also the correlation between the results of Gram staining of direct smears and culture pattern were assessed.

CHAPTER IV

RESULTS

In this study, pus samples were collected from different types of surgical wounds. The samples were collected from indoor patients of different wards as well as outdoor patients of Shree Birendra Hospital. A total of 200 pus samples were collected within seven months study period. The results obtained are shown below.

4.1 Clinical Pattern of result

4.1.1 Pattern of samples from different operative procedures

Out of 200 pus samples, maximum number of pus samples 102 (51%) were collected from gastrointestinal surgery followed by 49 (24.5%) from orthopedic surgery and 22 (11%) from urogenital surgery as shown in table 4.

Table 4: Types and distribution of samples from different operative procedures

Types of operative procedures	**No. of samples**	**Percent (%)**
Gastrointestinal surgery	95	47.5
Urogenital surgery	22	11.0
Head and neck surgery	8	4.0
Orthopedic surgery	49	24.5
Gynecological and obstetric surgery	2	1.0
Hernioraphy	7	3.5
Others	17	8.5
Total	**200**	**100.0**

Out of 95 patients with infection at gastrointestinal surgical site, 54 (52.9%) were male and 48 (47.1%) were female. Among 22 patients with infection at urogenital surgical site, 13 (59.1%) were male and 9 (40.9%) were female. There were 8 cases of SSI in head and

neck surgery, out of which 4 (50%) were male and 4 (50%) were female. Out of 49 cases of SSI in orthopedic surgery, 38 (77.6%) were male and 11 (22.4%) were female while, both the cases of SSI in gynecological and obstetric surgery were female. Similarly, out of 17 cases of SSI in other surgeries like lump excision, skin grafting, incision and drain etc, 13 (76.5%) were male and 4 (23.5%) were female. The results are shown in table 5.

Table 5: Gender wise distribution of samples from different operative procedures

Type of operative procedure	**Male**		**Female**	
	No.	**%**	**No.**	**%**
Gastrointestinal surgery	47	49.5	48	50.5
Urogenital surgery	13	59.1	9	40.9
Head and neck surgery	4	50	4	50.0
Orthopedic surgery	38	77.6	11	22.4
Gynecological and obstetric surgery	0	0	2	100.0
Hernioraphy	7	100.0	0	0
Others	13	76.5	4	23.5
Total	**122**		**78**	

4.1.2 Pattern of Age and Gender wise distribution of patients

Out of 200 pus samples, 122 (61%) were collected from male patients and 78 (39%) were collected from female patients. The maximum number of patients 92 (46.0%) belong to age group 16 to 40 years, out of which 54 (58.7%) were male and 38 (41.3%) were female, followed by the age group 40 to 60 years. The median age group was 39.5±16.18. A significant association was found between the age and gender of the patients with incidence of Surgical Site Infection. The results were as shown in table 6.

Table 6: Age and Gender wise distribution of patients

Age	Male		Female		Total	Statistics
	No.	%	No.	%		
<16	7	63.6	4	36.4	11	
16-40	54	58.7	38	41.3	92	
40-60	39	54.2	33	45.8	72	P< 0.05
>60	22	88.0	3	12.0	25	
Total	**122**	**61.0**	**78**	**39.0**	**200**	

Median age= 39.5±16.18

4.1.3 Pattern of distribution of patients in different wards

Among 200 pus samples, highest number of samples 59 (29.5%) were collected from Surgical I followed by 53 (26.5%) from POP ward while 25 (12.5%) were from SOPD. Out of 123 male patients, higher number of males 55 (44.7%) were from Surgical I followed by 30 (24.4%) from POP. Similarly, more number of females 23 (29.9%) were from POP ward followed by 16 (20.8%) from gynae ward and SOPD as shown in table 7.

Table 7: Ward wise distribution of patients

Ward	Sex				Total	%
	Male	%	Female	%		
Post operative ward	30	24.4	23	29.9	53	26.5
Surgical 1	55	44.7	4	5.2	59	29.5
Orthopedic ward	15	12.2	2	2.6	17	8.5
High care unit	11	8.9	7	9.1	18	9.0
Paediatric ward	2	1.6	3	3.9	5	2.5
Family ward	0	0	6	7.8	6	3.0
Gynae ward	1	0.8	16	20.8	17	8.5
SOPD	9	7.3	16	20.8	25	12.5
Total	**123**	**61.5**	**77**	**38.5**	**200**	**100**

4.2 MICROBIAL PATTERN

4.2.1 Gram stain reaction of direct smear of samples

Out of 200 pus specimens/wound swabs analyzed 12 (6%) had no pus cells and no bacteria, 64 (32%) had pus cells but no bacteria and 124 (62%) had pus cells with plenty of bacteria. The results are shown in table 8.

Table 8: Gram stain reaction of direct smears of samples

Gram stain result	Number	Percent (%)
No pus cells and no bacteria	12	6.0
Pus cells but no bacteria	64	32.0
Pus cells with bacteria	124	62.0
	200	**100.0**

4.2.2 Pattern of culture positive and culture negative pus samples

Out of 200 samples analyzed 156 (78%) were culture positive and 44 (22%) were culture negative. Among positive cultures, 100 (64.1%) were from male patients while 56 (35.9%) were from female patients. Among negative cultures, 22 (50%) were from male patients and 22 (50%) from female patients. The result is shown in figure 1 and table 9.

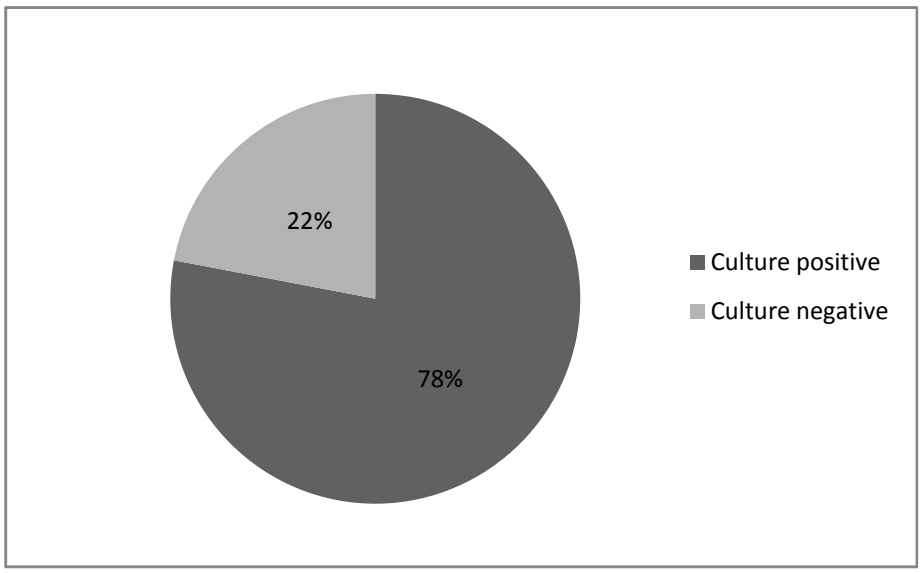

Figure 1: Pattern of culture positive and negative samples

Table 9: Gender wise distribution of culture positive and culture negative pus samples

Sex	Culture positive		Culture negative		Statistics
	No.	**Percent**	**No.**	**Percent**	
Male	100	64.1	22	50.0	P > 0.05
Female	56	35.9	22	50.0	
Total	**156**	**100**	**44**	**100**	

Out of 156 culture positive samples, highest number of samples (43.2%) were from the age group 16-40, followed by the age group 40-60 which was 38.6%. the results are shown in the table 10.

Table 10: Age wise distribution of culture positive and culture negative samples

Age groups	Culture positive		Culture negative		Total	
	No.	**Percent**	**No.**	**Percent**	**No.**	**Percent**
<16	9	5.8	2	4.5	11	5.5
16-40	73	46.8	19	43.2	92	46
40-60	55	35.3	17	38.6	72	36
>60	19	12.2	6	13.6	25	12.5
Total	**156**		**44**		**200**	

Out of 156 culture positive samples, 71 (45.5%) were from gastrointestinal surgery, followed by orthopedic surgery 42 (26.9%) and urogenital surgery 17 (109%). The results are shown in the table 11.

Table 11: Distribution of culture positive samples according to type of surgery

Type of operative procedure	Culture positive		Culture negative		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**
Gastrointestinal surgery	71	45.5	24	54.5	95	47.5
Urogenital surgery	17	10.9	5	11.4	22	11
Head and neck surgery	6	3.8	2	4.5	8	4
Orthopedic surgery	42	26.9	7	15.9	49	24.5
Gynecological and obstetric surgery	2	1.3	0	0	2	1
Hernioraphy	4	2.6	3	6.8	7	3.5
Others	14	9.0	3	6.8	17	8.5
Total	**156**		**44**		**200**	

4.2.3 Comparison of direct smear result with culture pattern

Gram stain of direct smear was performed in 200 wound specimens, among which 166 (83%) showed the similar results with that of culture result. The positive correlation was found between direct observation and culture pattern of the samples.

Table 12: Comparison of direct smear result with culture pattern

Direct observation	Culture pattern		Total
	Culture positive	**Culture negative**	
No pus cells and no bacteria	1 (8.3%)	11 (97.1%)	12
Pus cells but no bacteria	32 (50.0%)	32 (50.0%)	64
Pus cells with bacteria	123 (99.2%)	1 (0.8%)	124
	156	**44**	**200**

Correlation (r) = 0.691

4.2.4 Pattern of growth among culture positive samples.

Out of 156 culture positive samples, 130 (83.3%) showed single growth while 26 (16.7%) showed mixed growth. The single isolate was found in maximum number of samples as shown in figure 2.

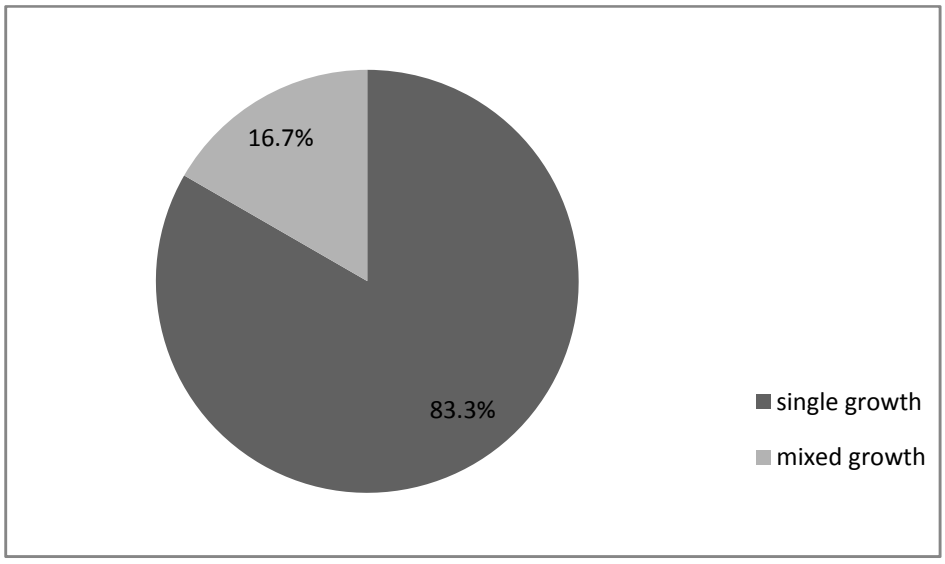

Figure 2: Pattern of single and mixed growth in total samples

4.2.5 Distribution of bacterial isolates in relation to age and sex

From 200 pus samples, 183 bacteria were isolated. Out of these 183 organisms, maximum number of organisms 86 (46.99%) were isolated from age group 16-40 years. This was followed by 65 (35.52%) from the age group 40-60 years and 23 (12.57%) from the age group above 60 years. The least number of organisms 9 (4.92%) were isolated from the age group below 16 years. Similarly, out of 183 organisms isolated, 120 (65.57%) were isolated from 122 male patients while 63 (34.43%) organisms were isolated from 78 female patients.

4.2.6 Pattern of Gram stain reaction in culture positive samples

From 156 culture positive samples, a total of 183 bacteria were isolated. Out of this 130 (71.04%) were from single growth and 53 (28.96%) were from mixed growth. A total of 99 (54.1%) isolates were Gram positive which consisted of 81.81% from single growth and 18.18% from mixed growth. Similarly, 84 (45.9%) isolates were Gram negative which consisted of 58.33% from single growth and 41.67% from mixed growth. The result is shown in table 13.

Table 13: Pattern of Gram stain reaction in culture positive samples

Type of growth	**Gram positive**		**Gram negative**		**Total**	
	No.	**Percent**	**No.**	**Percent**	**No.**	**Percent**
Single growth	81	81.81	49	58.33	130	71.04
Mixed growth	18	18.18	35	41.67	53	28.96
Total	**99**		**84**		**183**	

4.2.7 Pattern of common pathogens isolated from infected surgical wounds

From 156 culture positive pus samples, collected from patients undergoing different surgical procedures, 183 bacteria were isolated which was comprised of 99 Gram positive isolates and 84 Gram negative isolates. The most commomly isolated pathogen was *S. aureus* (26.78%), followed by CONS (23.5%), *E. coli* (21.31%) and *P. aeruginosa*

(9.29%). Other isolates were *K. pneumonaie* (7.65%), 2.73% *P. mirabilis* and haemolytic Streptococci each, 2.19% *M. morganii*, 1.1% Non haemolytic streptococci, *Enterobacter* spp. and *C. freundii* each and rest 0.6% *K. oxytoca.*

Table 14: Pattern of common pathogens isolated from infected surgical wounds

Organisms	No. of isolates	Percentage
S. aureus	49	26.78
CONS	43	23.5
β- haemolytic streptococci	5	2.73
Non haemolytic streptococci	2	1.1
E. coli	39	21.31
K. pneumonia	14	7.65
K. oxytoca	1	0.6
P. aeruginosa	17	9.29
M. morganii	4	2.19
Enterobacter spp.	2	1.1
P. mirabilis	5	2.73
C. fruendii	2	1.1
Total	**183**	**100**

4.2.8 Pattern of distribution of Gram positive bacteria

Out of 183 bacterial isolates, 99 were Gram positive bacteria. There were altogether 12 different types of organisms isolated from 156 culture positive samples. Among Gram positive bacteria *S. aureus* (49.5%) was the most common followed by CONS (47.67%), β-haemolytic streptococci (5.1%) and non-haemolytic streptococci (2%) as shown in table 15.

Table 15: Types and percentage of Gram positive bacteria

Gram positive isolates	No. in single growth	No. in mixed growth	Total	Percent
S. aureus	37	12	49	49.5
CONS	39	4	43	43.4
β-Haemolytic streptococci	3	2	5	5.1
Non-haemolytic streptococci	2	-	2	2.0
Total	**81**	**18**	**99**	**100**

4.2.9 Pattern of distribution of Gram negative bacteria

Out of 183 bacterial isolates, 84 were Gram negative bacteria. *E. coli* (46.43%) was the most common bacteria followed by *P. aeruginosa* (20.24%) and *K. pneumonia* (16.67%). Other, *P. mirabilis* (5.95%), *M. morganii* (4.76%), *Enterobacter* spp. (2.38%), *C. fruendii* (2.38%) and *K. oxytoca* (1.19%) were found lowest in number. The results are shown in table 16.

Table 16: Types and percentage of Gram negative bacteria

Gram negative isolates	No. in single growth	No. in mixed growth	Total	Percent
E .coli	22	17	39	46.43
K. pneumonia	9	5	14	16.67
P. aeruginosa	11	6	17	20.24
M. morganii	1	3	4	4.76
Enterobacter spp.	1	1	2	2.38
P. mirabilis	3	2	5	5.95
C. fruendii	1	1	2	2.38
K. oxytoca	1	-	1	1.19
Total	**49**	**35**	**84**	**100**

4.2.10 Distribution of bacterial isolates in different wards

Out of 183 bacterial isolates, 43 (23.5%) were isolated from POP ward, 53 (28.96%) from Surgical I, 15 (8.2%) from Orthopedic ward, 22 (12.02%) from HCU, 5 (2.73%) from Pediatric ward, 6 (3.28%) from NFW, 16 (8.47%) from GW and 23 (12.57%) from SOPD.

Most of the *S. aureus* (36.7%) were isolated from SI followed by 20.4% from POP and 12.2% from HCU. Other, 10.2%, 2.0%, 8.2% and 10.2% were from OP, PW, NFW, GW and SOPD respectively**.**

Out of 43 CONS, 23.3% were isolated from POP, 34.9% from SI, 16.3% from GW, 14.4% from SOPD and 7% from OP and 2.3% from HCU and PW each.

Likewise, among 39 *E. coli*, 23.08% were isolated from SI, 20.51% from POP and HCU each, 10.26% from GW and SOPD each and rest 7.69%, 5.13% and 2.56% were isolated from OP, PW and NFW respectively.

Out of 17 *P. aeruginosa*, 23.53% were isolated from POP, SI and OP each, 17.65% from NFW and 11.76% from SOPD. Out of 14 *K. pneumonia*, 35.71% were isolated from SI, 21.43% from POP, 14.29% from NFW and SOPD each and other 7.14% from OP and HCU each. The results are shown in the table 17.

Table 17: Types of bacterial growth found in different wards

Types of organism	P.O.P.		S.I.		O.P.		H.C.U.		P.W.		N.F.W.		G.W.		S.O.P.D.		Total
	No.	%	No.	%	No.	%	No.	%	No	%	No.	%	No.	%	No.	%	
S. aureus	10	20.4	18	36.7	5	10.2	6	12.2	1	2.0	-	-	4	8.2	5	10.2	49
CONS	10	23.3	15	34.9	3	7.0	1	2.3	1	2.3	-	-	7	16.3	6	14.4	43
β-Haemolytic streptococci	2	40	-	-	1	20	-	-	1	20	-	-	-	-	1	20	5
Non-haem streptococci	1	50	-	-	-	-	-	-	-	-	-	-	-	-	1	50	2
E. coli	8	20.51	9	23.08	3	7.69	8	20.51	2	5.13	1	2.56	4	10.26	4	10.26	39
K. pneumonia	3	21.43	5	35.71	1	7.14	1	7.14	-	-	2	14.29	-	-	2	14.29	14
K. oxytoca	-	-	-	-	-	-	1	100	-	-	-	-	-	-	-	-	1
P. aeruginosa	4	23.53	4	23.53	-	-	4	23.53			3	17.65			2	11.76	17
M. morganii	2	50	-	-	-	-	1	25	-	-	-	-	-	-	1	50	4
Enterobacter spp.	1	50	1	50	-	-	-	-	-	-	-	-	-	-	-	-	2
P. mirabilis	1	20	-	-	2	40	-	-	-	-	-	-	1	20	1	20	5
C. freundii	1	50	1	50	-	-	-	-	-	-	-	-	-	-	-	-	2
Total	**43**	**23.5**	**53**	**28.96**	**15**	**8.2**	**22**	**12.02**	**5**	**2.73**	**6**	**3.28**	**16**	**8.74**	**23**	**12.57**	**183**

4.2.11 Comparison of type of operation with microbial isolates

The majority of the organisms 85 (46.45%) were isolated from surgical sites after GI surgery followed by 25.7% from orthopedic surgery (Table 18). The commonest organism isolated after GI surgery was *E. coli* (64.1%, n=25) followed by *S. aureus* (44.9%, n=22). From orthopedic operations, the commonest organism isolated was *S. aureus* (31.9%, n=15).

Table 18: Distribution of microbial isolates with the type of surgery

Type of organisms	Type of operation							Total
	GI	**Urogenital**	**H/N**	**Ortho.**	**Gyn/obs.**	**Hernior.**	**Others**	
S. aureus	22	5	0	15	0	1	6	**49**
CONS	14	6	3	12	2	2	4	**43**
β-haemolytic Streptococci	2	0	1	1	0	0	1	**5**
Non-haemolytic streptococci	2	0		0	0	0	0	**2**
E. coli	25	4	0	6	1	0	3	**39**
K. pneumonia	4	1	1	7	0	0	1	**14**
Pseudomonas spp.	8	2	2	2	0	1	2	**17**
M. morganii	3	0	0	1	0	0	0	**4**
Enterobacter spp.	1	0	0	0	0	0	1	**2**
Proteus spp.	1	0	0	3	0	0	1	**5**
C. fruendii	2	0	0	0	0	0	0	**2**
K. oxytoca	1	0	0	0	0	0	0	**1**
Total	**85**	**18**	**7**	**47**	**3**	**4**	**19**	**183**

Note: GI- Gastrointestinal surgery
H/N- Head and Neck surgery
Ortho.- Orthopedic surgery
Gyn/obs.- Gynecological and obstetric surgery
Hernior.- Hernioraphy

4.3 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

4.3.1 Antibiotic susceptibility pattern of bacterial isolates as a whole

The common antibiotic discs used for all types of bacterial isolates were Amoxicillin, Cloxacillin, Gentamicin, Amikacin, Cefepime, Ciprofloxacin, Pefloxacin, and Piperacillin. Among these, Amikacin (77.60%) was the most effective drug. The second most effective drugs were

Gentamicin (61.20%) and Pefloxacin (60.66%). While the least effective drug was Cloxacillin (13.66%). The results are shown in table 19.

Table 19: Antibiotic susceptibility pattern of bacterial isolates as a whole

Total no. of isolates= 183

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin	66	36.07	26	14.21	91	49.73
Cloxacillin	25	13.66	13	7.10	145	79.24
Piperacillin	101	55.19	27	14.75	55	30.05
Ciprofloxacin	77	42.08	32	17.49	74	40.44
Pefloxacin	111	60.66	26	14.21	46	25.14
Cefepime	47	25.68	45	24.59	91	49.73
Amikacin	142	77.60	10	5.46	31	16.94
Gentamicin	112	61.20	25	11.48	46	25.14

4.3.2 Antibiotic susceptibility pattern of Gram positive bacterial isolates

Among Gram positive bacteria, the most effective antibiotic was Vancomycin (92.92%), followed by Amikacin (73.73%), Pefloxacin (67.68%), Methicillin (66.67%) and Gentamicin (63.63%). The least effective drugs were Erythromycin (9.9%), Cloxacillin (21.21%) and Cephalexin (25.25%).

Table 20: Antibiotic susceptibility pattern of Gram positive bacterial isolates

Total no. of isolates= 99

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin	51	51.51	17	17.17	31	31.31
Cloxacillin	21	21.21	12	12.12	66	66.67
Piperacillin	58	58.59	15	15.15	26	26.26

Methicillin	66	66.67	9	9.9	17	17.17
Cephalexin	25	25.25	22	22.22	52	52.52
Cefepime	25	25.25	21	21.21	53	53.53
Erythromycin	9	9.9	14	14.14	76	76.77
Ciprofloxacin	50	50.50	17	17.17	32	32.32
Ofloxacin	52	52.52	19	19.19	28	28.28
Pefloxacin	67	67.68	19	19.19	13	13.1
Amikacin	73	73.73	8	8.8	18	18.18
Gentamicin	63	63.63	12	12.12	24	24.24
Vancomycin	92	92.92	0	0	0	0

4.3.3 Antibiotic susceptibility pattern of Gram negative bacterial isolates

Among Gram negative bacterial isolates, the most effective drug was Imipenem (97.62%) followed by Amikacin (82.14%) and the least effective drugs were Amoxicillin (17.86%), Amoxiclave (14.29%) and Cloxacillin (4%). The results are shown in table 21.

Table 21: Antibiotic susceptibility pattern of Gram negative bacterial isolates

Total no. of isolates= 84

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin	15	17.86	9	10.71	60	71.43
Cloxacillin	4	4.76	1	1.19	79	94.05
Piperacillin	43	51.19	7	8.33	29	34.52
Ciprofloxacin	27	32.14	15	17.86	42	50
Pefloxacin	44	52.38	7	8.33	33	39.29
Amikacin	69	82.14	2	2.38	13	15.48
Gentamicin	49	58.33	13	15.48	22	26.19
Cefepime	22	26.19	24	28.57	38	45.24
Ceftriaxone	20	23.81	14	16.67	50	59.52
Cefotaxime	29	34.52	31	36.90	24	28.57
Ceftazidime	21	25.0	9	10.71	54	64.29
Amoxyclave	12	14.29	3	3.57	69	82.14
Imipenem	82	97.62	1	1.19	0	0

4.3.4 Antibiotic susceptibility pattern of *S. aureus*

The most effective drug was Vancomycin (100%) followed byAmikacin (71.43%), Methicillin (69.39%) and Pefloxacin (67.35%). The least effective drugs were Cefepime (26.53%), Cephalexin (24.29%) and Cloxacillin (8%). Out of 49 isolates of *S. aureus*, 9 (18.37%) were resistant to Methicillin and 5 (10.20%) were intermediate to it. The results are shown in table 22.

Table 22: Antibiotic susceptibility pattern of *S. aureus*

Total no. of isolates= 49

Antibiotic	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amoxicillin	27	55.10	6	12.25	16	32.65
Cloxacillin	8	16.33	8	16.33	33	67.35
Methicillin	34	69.39	5	10.20	9	18.37
Piperacillin	30	61.22	7	14.29	12	24.49
Ciprofloxacin	26	53.06	7	14.29	16	32.65
Ofloxacin	24	48.98	12	24.29	12	24.49
Pefloxacin	33	67.35	10	20.41	6	12.25
Cephalexin	12	24.29	11	22.45	26	53.06
Cefepime	13	26.53	14	28.57	22	44.90
Amikacin	35	71.43	3	6.12	11	22.45
Gentamicin	31	63.27	7	14.29	11	22.45
Erythromycin	5	10.20	9	18.37	34	69.39
Vancomycin	49	100	0	0	0	0

4.3.5 Antibiotic susceptibility pattern of *E. coli*

The most effective drugs against *E. coli* isolated from the pus samples was Imipenem (97.44%) followed by Amikacin (87.18%) and Gentamicin (58.97%). The least effective drugs were Cloxacillin (0%), Amoxyclave (7.69%), Ceftazidime (10.26%), Cefepime (12.82%), Amoxicillin (15.39%) and Ciprofloxacin (17.95%). The results are shown in table 23.

Table 23: Antibiotic susceptibility pattern of *E. coli*

Total no. of isolates = 39

Antibiotic	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amoxicillin	6	15.39	3	7.69	30	76.92
Cloxacillin	0	0	1	2.56	38	97.44

Piperacillin	17	43.59	8	20.51	14	35.9
Ciprofloxacin	7	17.95	6	15.39	26	66.67
Pefloxacin	16	41.03	2	5.13	21	53.85
Amikacin	34	87.18	2	5.13	3	7.69
Gentamicin	23	58.97	8	20.51	8	28.51
Cefepime	5	12.82	16	41.03	18	46.15
Ceftriaxone	6	15.39	6	15.39	27	69.23
Cefotaxime	8	20.51	14	35.9	17	43.59
Ceftazidime	4	10.26	5	12.82	30	76.92
Amoxyclave	3	7.69	2	5.13	34	87.18
Imipenem	38	97.44	1	2.56	0	0

4.3.6 Antibiotic susceptibility pattern of *P. aeruginosa*

The most effective drugs against *P. aeruginosa* isolated from the pus samples was Imipenem (100%) followed by Amikacin (82.36%), Polymixin B (76.47%), Pefloxacin (70.59%) and Piperacillin (70.59%). The least effective drugs were Cloxacillin (11.75%), Amoxicilllin (17.65%), Amoxyclave (23.53%) and Ceftriaxone (29.41%). The results are shown in table 24.

Table 24: Antibiotic susceptibility pattern of *P. aeruginosa*

Total no. of isolates = 17

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin	3	17.65	3	17.65	11	64.71
Cloxacillin	2	11.75	0	0	15	88.24
Piperacillin	12	70.59	2	11.75	3	17.65
Ciprofloxacin	9	52.94	4	23.53	4	23.53
Pefloxacin	12	70.59	1	5.88	4	23.53
Amikacin	14	82.36	0	0	3	17.65
Gentamicin	12	70.59	1	5.88	4	23.53
Polymixin B	13	76.47	0	0	4	23.53
Cefepime	9	52.94	1	5.88	7	41.18
Ceftriaxone	5	29.41	4	23.53	8	47.06
Cefotaxime	8	47.06	8	47.06	1	5.88
Ceftazidime	7	41.18	1	5.88	9	52.94
Amoxyclave	4	23.53	0	0	13	76.47
Imipenem	17	100	0	0	0	0

4.3.7 Antibiotic susceptibility pattern of CONS

The most effective drug against CONS was Vancomycin (100%) followed by Amikacin (79.07%) and Methicillin (72.09%). The least effective drugs were Erythromycin (6.98%), Cephalexin (18.61%) and Cloxacillin (20.93%). Out of 43 CONS, 8 (18.61%) were resistant to Methicillin. The results are shown in the table 25.

Table 25: Antibiotic susceptibility pattern of CONS

Total no. of isolates = 43

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin	19	44.19	9	20.93	15	34.88
Cloxacillin	9	20.93	2	4.65	32	74.42
Methicillin	31	72.09	4	9.30	8	18.61
Piperacillin	23	53.49	8	18.61	12	27.91
Ciprofloxacin	18	41.86	10	23.26	15	34.88
Ofloxacin	21	48.84	6	13.95	16	37.21
Pefloxacin	29	67.44	9	20.93	5	11.63
Cephalexin	8	18.61	10	23.26	25	58.14
Cefepime	11	25.58	6	13.95	26	60.47
Amikacin	34	79.07	4	9.30	5	11.63
Gentamicin	27	62.79	5	11.63	11	25.58
Erythromycin	3	6.98	5	11.63	35	81.40
Vancomycin	43	100	0	0	0	0

4.3.8 Pattern of multidrug resistance among different isolates

Out of 183 bacterial isolates 139 (75.96%) were MDR strains. Among 49 *S. aureus* isolated, 35(71.43%) were MDR strains. Similarly, 74.42% of CONS, 84.62% of *E. coli*, 78.57% of *K. pneumonia* and 70.59% of *P. aeruginosa* were MDR strains.

Table 26: Pattern of multidrug resistance among different isolates

Isolates	**No of isolates**	**No of MDR strains**	**Percent**
S. aureus	49	35	71.43
CONS	43	32	74.42
β- haemolytic streptococci	5	4	80
Non-haemolytic streptococci	2	0	0
E. coli	39	33	84.62
K. pneumoniae	14	11	78.57
K. oxytoca	1	1	100
P. aeruginosa	17	12	70.59
M. morganii	4	4	100
Enterobacter spp.	2	2	100
P. mirabilis	5	3	60
C. fruendii	2	2	100
Total	**183**	**139**	**75.96**

CHAPTER V

DISCUSSION

SSI is a major source of post-operative morbidity and mortality and is an important outcome indicator after surgery. SSIs are the most common HAI among surgical patients and the third most frequent HAI in the general hospital population (Poulako and Giamarello, 2007).

SSIs representing a global threat are associated with great complications (Hedrick *et al*., 2006). The most important ones for the patients who experience post operative complications are increased length of hospital stay, readmission rates, mortality rates, costs of care, (Bratzler, 2006) and most importantly the emergence of MDR bacteria (Poulakou and Giamarellou, 2007).

Different risk factors are associated with different bacterial colonization of surgical site and therefore different antibiotic resistant organisms. Thus different population groups should decide discretely upon their most usual present risk factors (as obesity, pre-hospitalization, ulcers and more) (Khorvash, 2008). In this study most of the patients had some underlying conditions such as diabetes, smoking habit, longer period of pre-hospitalization etc. which are the risk factors for SSI. The treatment of infected patient depends upon several factors including the severity of the infection, degree of antibiotic resistant pathogens, the sensitivity to alternative agents and the achievable concentration of antibiotic at the site of the infection. This study would help to some extent in administering appropriate drugs for the treatment of the infected patients.

In this study, particular emphasis was given to the post surgical wound infections and 200 patients with clinical symptoms of wound infection were enrolled in the study. All the patients in this study were on antibiotics, both during prophylaxis and after surgery. The etiological agents were isolated and identified on the basis of the colonial appearance and biochemical tests. The antibiotic susceptibility pattern of the isolates towards commonly used antibiotics was also studied. The results are presented in table 5 to 26.

Out of 200 pus samples, 95 (47.5%) were from gastrointestinal surgery, 49 (24.5%) were from orthopedic surgery, 22 (11%) were from urogenital surgery, 8 (4%) were from head and neck surgery, 7 (3.5%) from herniorraphy, 2 (1%) from gynecological and obstetric surgery and rest 17 (8.5%) from other surgeries. But Massadeh and Jaran (2009) collected highest number of samples from orthopedic surgery (19/115) followed by 16/115 from head and neck surgery, 8.6% from

gynecological and obstetric surgery, 6.96% from gastrointestinal surgery and 1.7% from hernioraphy. Likewise, Ranjan *et al*. (2010) in their study collected 23.67% from orthopedic surgery, 14.22% from GI surgery, 3.67% from head and neck surgery and 6.22% from hernioraphy. In both of these studies, SSIs were found to be more prevalent in orthopedic surgery which is in contrast to our study. SSI is found to be more prevalent in gastrointestinal surgery in this study.

Out of 200 patients studied, 122 (61%) were male patients and 78 (39%) were female patients. The SSI rate is found to be greater in male patients than in female patients. This is in accordance with the result found in other studies as well. In the study of Nwachukwu *et al.* (2009) 68.88% were males and 31.11% were females. Khosravi *et al.* (2009) in his study at Iran has found that 68.5% were males and 31.5% were females out of 155 patients. In the study of Luitel *et al.* (2009), among 245 patients, 42.4% were male and 57.6% female.

Similarly in the study conducted by Khan *et al.* (2008), out of 104 patients, 64.42% were males and 35.58% were females. Anguzu and Olila (2007) have also found the similar result with 59.6% male and 40.4% female patients out of 94 patients. But the result of Massadeh and Jaran (2009) is not consistent with the findings of other studies. In their study, male patient accounted for 52.2% and female patients accounted for 47.8% which are nearly equal.

In this study the ages of study groups ranged from 1-83 years. The highest number of samples 92 (46%) were collected from the age group 16-40 years followed by 72 (36%) from the age group 40-60 years. The median age group was 39.5±16.18 years. It is found that SSI is prevalent mostly in the working age group and the old age group. This result is similar to that of other studies as well. In the study of Anguzu and Olila (2007), the ages of study groups ranged from 1-77 and the modal age group was 11-20 years with frequency of 22.3%. Massadeh and Jaran, in their study collected highest number of samples (21.74%) from the age group 31-40 years and 61-70 years. In the study of Khosravi *et al.* (2009), the median age was 35 (± 15.8) years. Similarly in the study of Ranjan *et al.* (2010), the modal age group was 21-40 years with the frequency of 146.

Gram stain is the most important staining procedure in microbiology and is widely used as rapid technique for guiding antibiotic therapy in life threatening infections (Karkee, 2008). According to Bowler *et al.* (2001) the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure *S. aureus.*

In this study out of 200 pus/wound swabs, 12 (6.0%) had no any pus cells and bacteria but on culture one of them showed growth. 64 (32%) had pus cell but no any bacteria; however 32 of them showed growth. Likewise, 124 (62%) had pus cells with bacteria and all of them showed growth except one sample. Thus there was a positive correlation between Gram stain of direct smears and culture results.

In the study of Karkee (2008) out of 250 samples of which direct Gram stain was performed, 192 (76.8%) showed positive correlation with culture positive result. Similarly in the study carried out by Katuwal (1999) there was 60.83% correlation of direct smear Gram stain with culture results.

Out of 200 samples, 156 (78%) showed bacterial growth and 44 (22%) remained sterile even after 48 hours of incubation. High rate of bacterial growth (94.1%) was seen in the samples collected from male patients and 35.9% from the female patients. Highest rate of bacterial growth (46.8%) was found in the age group 16-40 years followed by 35.3% in the age group 11-30 years. Similarly, high rate of bacterial growth (45.5%) was seen in gastrointestinal surgery followed by orthopedic surgery (26.9%) and urogenital surgery (10.9%). Out of growth positive samples, 83.3% showed growth with pure bacterial isolate while 16.7% showed growth with mixed bacterial culture.

This study agrees with the study of Anguzu (2007) in which, 59.6% of total samples had bacterial growth within 48 hours of incubation and 72.7% of growth positive samples had pure growth while 27.3% had mixed growth.

This result can also be compared with the study carried out by Karkee (2008) where 88.63% of samples from surgical wounds showed single bacterial growth and 11.36% showed multiple bacterial isolate.

In the study of Khosravi *et al.* (2009), out of 165 patients, 93.9% were culture positive and 6.1% were culture negative. Among the positive cultures, less than 2% were mixed bacterial culture of two organisms and one culture was comprised of more than two organisms.

These results are similar to that of our study since less number of surgical wound has been found to be infected by mixed bacteria.

From 156 culture positive samples, a total of 183 organisms were isolated. The maximum number of bacteria 86 (46.99%) were isolated from the age group 16-40 years and 65 (35.52%) were from age group 40-60 years. Out of 183 isolates, 120 bacterial isolates were from male patients and 63

were from female patients. Likewise, 54.1% of the organisms were Gram positive and 45.9% were Gram negative. Pure Gram negative growth was seen in 31.4% samples, pure Gram positive growth in 51.9 % samples, mixed growth of Gram positive and Gram negative growth was seen in 11.5% samples and mixed growth of Gram negative bacilli was found in 5.1% of samples with growth positive. There were only four types of GPB isolated while eight different types of GNB isolated from the SSIs.

In the study of Sanjay *et al.* (2000), among 167 positive cultures, GNB was observed in 121 samples, mixed growth of GPB and GNB in 18 samples and GPB only were observed in 28 samples.

Similarly, among 44 growth positive samples from SSI, 49 bacteria were isolated. Out of these 65.30% were Gram positive and 34.70% were Gram negative. And a total of 5 types of GPB and 6 types of GNB were isolated (Karkee, 2008). In the study of Sonawane *et al.* (2010), 540 bacterial isolates were collected from 440 samples. Among 540 bacterial isolates, GNB were 63% and GPB were 36.48%.

These results show that GPB are mostly responsible for the surgical site infections, however, several types of GNB were isolated than the GPB.

But in the study of Knowhar *et al*. (2008) in an Indian hospital, GNB predominated with 58%, followed by 41.9% Gram positive isolates. This result is in contrast with that of our study.

S. aureus was found to be the most predominant bacteria 49 (26.78%) followed by CONS (23.5%), *E. coli* (21.31%), *P. aeruginosa* (9.29%) and *K. pneumonia* (7.65%) other isolates were *M. morganii, Enterobacter* spp., *P. mirabilis, C. fruendii, K. oxytoca,* β-haemolytic streptococci and non-haemolytic streptococci. This result is comparable to other studies from India and abroad that agrees *S. aureus* as the most common wound contaminant.

National Nosocomial Infection Surveillance (NNIS, CDC, 1996) found the prevalence of 20% *S. aureus*, 14% CONS, 12% Enterococci, 8% *E. coli*, 8% *P. aeruginosa*, 7% *Enterobacter* spp., 3% *P. mirabilis*, 3% *K. pneumonia*, 3% other streptococci and other 2% Gram positive rods.

Khorvash *et al.* (2008) in their study found that, out of 150 bacteria isolated from SSI, 43% were *S. aureus*, 18% were *E. coli*, 21% were *Klebsiella* spp., 13% were *Pseudomonas* spp., 10% were CONS, 5% were *Acinetobacter* spp., 5% were *Enterobacter* spp. and 13% *Citrobacter* spp.

In the study of Knowhar *et al.* (2008), the most common bacteria were *S. aureus* 37% and *P. aeruginosa* (37%), followed by *K. pneumonia* (8%), *Acinetobacter* spp. (3.2%), *Proteus* spp. (4.8%), *E. coli* (4.8%), *C. fruendii* (1.6%), *Edwardsiella tarda* (1.6%) and *E. faecalis* (1.6%).

In the study of Nwachukwu *et al*. (2009) also, *S. aureus* was isolated from 42.30% of samples, *P. aeruginosa* from 32.90%, *P. mirabilis* and *E. coli* from 12.80% of the samples. Anguzu and Olila (2007) in their study at Uganda also found that *S. aureus* was the most commonly isolated organism (45.1%) while the least isolated organism was *Enterobacter* species (2.8%).

Likewise in the study of Sonavane *et al*. (2010), the commonest pathogens were *S. aureus* (29.20%), *E. coli* (18.70%), *Pseudomonas* spp. (15.37%), *Acinetobacter* spp. (8.33%), *Enterococcus* spp. (7.22%) and other GNB were 7.04%. Hence, in most of the studies worldwide, *S. aureus* was the most commonly isolated organism however the relative rates varied from each other.

But, in the study of Ranjan *et al*. (2010), the most common isolated organism was *P. aeruginosa* (29.6%), followed by *E. coli* (20.3%), *Klebsiella* spp. (16.6%), *S. aureus* (14.3%), *Proteus* spp. (6.3%) and *C. fruendii* (0.6%).

The high prevalence of *S. aureus* in SSI may be because it is an endogenous source of infection. Nasal carriage of *S. aureus* is one of the important risk factor for SSI as it is a normal flora in the nostrils. It may also be due to contamination from the environment or surgical instruments which find their easy way into the surgical sites through the abrasions or the disruption of normal skin barrier.

In our study, majority of the isolates were from the surgical site after gastrointestinal surgery which was 46.45% and the most common isolate was *E. coli* (64.1%) This may be due to the contamination of surgical wounds with patient's endogenous flora. This is in contrary with the reports of Anguzu and Olila (2007) in which *S. aureus* (39.4%) was the most predominant organism. Most of the isolates of *S. aureus* were obtained from GI surgery (12.02%, n=22) and 8.2% (n=15) from orthopedic surgery. This can be due to the surface contamination of wounds by organisms on the skin and environment causing nosocomial infections.

In this study, out of 200 surgical wound specimens, 156 specimens were growth positive from which 183 bacteria were isolated. All of these isolates were tested for their antibiotic susceptibility pattern. Most of the patients with SSI that were enrolled in this study had pre- operative antimicrobial prophylaxis and also they were under antibiotic therapy. In-vitro antimicrobial

susceptibility profile of the aetiological agents of surgical site infection has revealed that there is a growing emergence of multi-drug resistant microbes.

Amikacin (77.60%) was found to be the most effective drug against both Gram positive and Gram negative orgainisms, followed by Gentamicin (61.20%) and Pefloxacin (60.66%). Cloxacillin was the least effective drug with 79.24% resistance, followed by Cefepime and Amoxicillin with 49.73% resistance.

In the study conducted by Knowhar *et al.* (2008) in an Indian Hospital, Ciprofloxacin was the most effective drug with 74.19% sensitive isolates followed by Amikacin with 61.29% sensitive isolates. While the least effective drug was Ampicillin with 25.81% sensitive isolates. This result concurs with that of Nwachukwu *et al*. (2009) but is in contrast to our findings. In our study Ciprofloxacin was sensitive against only 42.08% of the microbial isolates.

Against GPB, the most sensitive commonly used drug was Amikacin with 73.70% susceptible isolates followed by Pefloxacin (67.7%), Methicillin (66.7%) and Gentamicin (63.6%). The least effective drugs were Erythromicin (9.9%), Cloxacillin (21.21%), Cephalexin (25.3%) and Cefepime (25.3%). Knowhar *et al.* (2008) has found Ciprofloxacin to be effective against 50% of GPB which is similar to that of our result. But it is in contrast to that of Nwachukwu *et al.* (2009) in which 61.54% of GPB was sensitive to Ciprofloxacin. However, Amikacin stands to be second most sensitive drug and Erythromycin to be the least effective drug which is similar to our findings.

Perera and Hay (2005) observed that there was interesting geographical variation in the prevalence of Erythromycin resistance. There was 31.1% resistance overall with highest rate found in Asia (79.6%), France (57.6%), Hungary (55.6%) and Italy (42.9%).

Regarding GNB, 97.62% of the isolates were susceptible to Imipenem, 82.14% were sensitive to Amikacin, 58.33 % were sensitive to Gentamicin and 52.38% were sensitive to Pefloxacin. The least effective drugs were Cloxacillin (4.76%), Amoxyclave (14.29%), Amoxicillin (17.86%) and Ceftriaxone (23.81%).

Knowhar *et al*. (2008) and Nwachukwu *et al*. (2009) found Ciprofloxacin as the most effective drug against GNB followed by Amikacin. But in our study, Ciprofloxacin was sensitive against only 32.14% of the GNB. However, it can be compared with the study of Khorvash *et al*. (2008) that has reported the resistance of isolated organisms to be 41.7% in Amikacin, 78.6% in Ceftazidime, 85.7% in Ceftriaxone, 61.5% in Ciprofloxacin, 78.8% in Gentamicin and 6.4% in

Imipenem. Onche and Adedeji (2004) have found Gentamicin (68.75%) as the most sensitive drug against Gram negative isolates while most of them were resistant to cephalosporin and penicillin group of antibiotics as in our study.

Sanjay *et al*. (2010) in his study has reported Imipenem and Amikacin as the sensitive drug with 81.34% susceptible isolates and Ciprofloxacin as the less effective one with 32.09% susceptible isolates. This result is in accordance with the findings of our study.

The majority of the *S. aureus* (71.43%) were sensitive to Amikacin, 69.39% sensitive to Methicillin, 67.35% sensitive to Pefloxacin and 63.27% sensitive to Gentamicin. Similarly 69.39% of the isolates were resistant to Erythromycin and 67.35% resistant to Cloxacillin which were the least effective drugs. Out of 49 isolates of *S. aureus*, 69.39% were resistant to Methicillin and all the MRSA were sensitive to Vancomycin making it a choice of drug against MRSA. Our findings are similar to that of Giacometti *et al*. (2000) in which *S. aureus* was the most common isolates of SSI with Methicillin resistance in 54.4% isolates. Ali *et al*. (2009) has found all the *S. aureus* isolates to be sensitive to Vancomycin which is identical to our studies. In the study of Isibor *et al*. (2008) 63.2% isolates of *S. aureus* were sensitive to Gentamicin which concurs with our findings.

In the study of Adegoke *et al*. (2010), 46% of *S. aureus* isolated were resistant to Cloxacillin which is comparable to our study. Cloxacillin is a drug often used for initial and empirical treatment of Staphylococcal infections. This high level of resistance to Cloxacillin may pose problems in the treatment of SSI. The increase in resistance of the isolates against the commonly used antibiotics may be due to the widespread abuse of the drug which is usually available in combinations with Ampicillin for the treatment of infections in our society and can be obtained all over the country without a prescription.

Floroquinolones were also not a good choice against *S. aureus* as Ofloxacin resistance was 24.49% and Ciprofloxacin resistance was 32.65% however, it is lesser than that reported by Khorvash *et al*. (2008) in their study.

CONS were found to be highly sensitive to Vancomycin (100%) followed by Amikacin (79.07%) and Methicillin (72.09%). Erythromycin was the least effective antibiotic with (81.40%) resistivity followed by Cloxacillin (74.42%), Cefepime (60.47%) and Cephalexin (58.14%). Methicillin resistance was noted in 18.61% of the CONS isolated. In the study of Khorvash *et al*. (2008), all the 10 isolates of CONS were resistant to Methicillin however they were sensitive to Vancomycin.

Bhatt and Lakhey (2005) have reported that CONS were 100% sensitive to Ampicillin, Cephalexin and Gentamicin and equally sensitive to Ciprofloxacin and Ofloxacin (85%).

Regarding the antibiotic susceptibility pattern of Enterobacteriaceae family, 97.44% of the *E. coli* was susceptible to Imipenem followed by Amikacin (87.18%). Antibiotics of Penicillin and Cephalosporin groups were found to be less effective against *E. coli*. Majority of the *E. coli* isolates (97.44%) were resistant to Cloxacillin, 87.18% to Amoxiclave, 76.92% to Ceftazidime and Amoxicillin. Bhatt and Lakhey (2005) has reported 57.1% *E. coli* isolates sensitive to Gentamicin and Ciprofloxacin which can be compared to our study however only 17.95% of the isolates were sensitive towards ciprofloxacin in our study. In the study of Khorvash *et al*. (2008) also, Imipenem was the most effective drug with 77.8% sensitive *E. coli* isolates. Sanjay *et al.* (2010) has reported maximum resistance of *E. coli* isolates towards Cephotaxime, followed by Ciprofloxacin and minimum resistance was shown to Imipenem and Amikacin.

P. aeruginosa showed 100% sensitivity towards Imipenem followed by Amikacin (82.36%). Polymixin B, Pefloxacin and Piperacillin were also equally sensitive against it. But Cloxacillin (11.75%), Amoxicillin (17.65%), Amoxiclave (23.53%) and Ceftriaxone (29.41%) were found to be less effective against this organism. Cefepime was moderately effective with 52.94% sensitive and 41.18% resistant organisms. 52.94% were sensitive to Ciprofloxacin and 41.18% to Ceftazidime which can be compared with the study of Shampa *et al*. (2006) in which 58% *P. aeruginosa* were sensitive to Ciprofloxacin and 54% to Ceftazidime. Shampa *et al.* (2006) has also found 58% *P. aeruginosa* to be sensitive to Ciprofloxacin which nearly agreed to but differed from the finding of Anbumani *et al*. (2006) where only 12% of *P. aeruginosa* were sensitive to Ciprofloxacin. Hence, third generation Cephalosporins and Aminoglycosides have a potent anti-pseudomonas activity as reported by Ali *et al*. (2009). In the study of Khorvash *et al* (2008), Cefepime resistance was very high (87.5%), Ceftriaxone and Ceftazidime resistance were 88.9% and 57.1% respectively which is higher than that in our study. In the study of Masaadeh and Jaran (2009), Amikacin was the most effective drug with 78% sensitivity against *P. aeruginosa* followed by Gentamicin (72%) which is similar to our study however Cefepime resistance was higher than that in our study. According to the study of Banjara *et al*. (2003), *P. aeruginosa* was 100% sensitive to Imipenem and Polymixin B and 70% sensititve to Ceftazidime. This result also agrees with our study however the percentage of sensitive organisms is different.

Bacterial infections due to MDROs are being increased widely in many parts of the world. Multi-resistant organisms are highly responsible for SSI which is one of the important hospital acquired infection. This may be due to frequent use of antibiotics or due to its inadequate use. SSIs due to such MDROs may pose a serious threat to the vulnerable patients with treatment failure and high expense. In the current study, 75.96% of the bacterial isolates were found to be MDR strains. The MDR isolates *S. aureus* 49/35 (71.73%), CONS 43/32 (74.42%), β-haemolytic streptococci 4/5 (80%), *E. coli* 33/39 (84.62%) *K. pneumonia* 11/14 (78.57%), *K. oxytoca* 1/1(100%), *P. aeruginosa* 12/17 (70.59%), *M. morganii* 4/4 (100.0%), *Enterobacter* spp. 2/2 (100.0%), *P. mirabilis* 3/5 (60.0%) and *C. fruendii* 2/2 (100.0%). This result is comparable to the study of Banjara *et al.* (2003) in which the MDR isolates were *S. aureus* 18/49 (36.7%), *P. aeruginosa* 17/39 (43.6%), *E. coli* 26/47 (55.3%), *K. pneumoniae* 14/23 (60.9%), *C. fruendii* 4/9 (44.4%), *P. mirabilis* 3/5 (60.0%) and *K. oxytoca* 2/2 (100.0%). Adegoke *et al*. (2010) in their study has also found a maximum number of MDR isolates. These results have demonstrated the immediate need of management strategies for the patients with infections in order to minimize the therapeutic failure, high cost and the incidence of adverse drug reactions.

CHAPTER 6

SUMMARY AND RECOMMENDATION

6.1 SUMMARY

The results obtained can be summarized as follows:

- All together 200 wound specimens were studied. There were 51% (n=102) patients with GI surgery, 11% (n=22) with Urogenital surgery, 4% (n=8) with Head and Neck surgery, 24.5% (n=49) with Orthopedic surgery, 1% (n=2) with Gynaecological and Obstetric surgery and 8.5% (n=17) with other type of surgeries.
- Among total specimens, 61 % (n=122) were collected from male patients and 39% (n=78) from female patients.
- The age of the patients ranged from 1 to 83 years and highest frequency of patients with SSI was found in age group 16-40 years (46.0%, n=92) followed by the age group 40-60 years (36.0%, n=72). The difference in incidence of SSI among male and female patients of different age group was statistically significant.
- Most of the samples were collected from the Surgical I ward (29.5%, n=59) followed by 26.5% (n=53) from POP ward, 12.5% (n=25) from SOPD, 9% (n=18) from High Care Unit, 8.5% (n=17) from Orthopedic ward and Gynae ward, 3% (n=6) from Family ward and 2.5% (n=5) from Pediatric ward.
- Growth was seen in 78 % (n=156) pus specimens and 22 % (n=44) were found to be sterile. 64.1% (n=100) of males and 35.9 % (n=56) females were culture positive. There was no significant difference between the culture pattern of samples and gender of the patients. Among growth positive samples, 46% (n=92) were from age group 16-40 years followed by 36% (n=72) from the age group 40-60 years, 12.5% (n=25) from >60years and 5.5% (n=11) from <16 years.
- Of the total, 83.3% (n=130) showed monomicrobial growth while 16.7% (n=26) showed polymicrobial growth.
- A positive correlation was found between the Gram stain result of direct smears and culture pattern of the samples.
- A total of 183 bacteria were isolated from 156 culture positive samples. Among them 54.1% (n=99) were Gram positive and 45.9% (n=84) were Gram negative.

- Four different types of Gram positive bacteria were isolated among which *S. aureus* (49.5%, n=49) was the most frequently isolated organism followed by CONS (43.4%, n=43), β-haemolytic streptococci (5.1%, n=5) and non-haemolytic streptococci (2.0%, n=2). Similarly, eight different types of Gram negative bacteria were isolated. *E. coli* (46.43%, n=39) was the most common isolate followed by *P. aeruginosa* (20.24%, n=17), *K. pneumonia* (16.67%, n=14), *P. mirabilis* (5.95%, n=5), *M. morganii* (4.76%, n=4), *C. fruendii* (2.38%, n=2), *Enterobacter* spp. (2.38%, n=2) and *K. oxytoca* (1.19%, n=1).
- Most of the organisms were isolated from surgical site after GI surgery (46.45%, n=85), followed by Orthopedic surgery (25.7%, n=47), Urogenital surgery (9.84%, n=18), Head and neck surgery (3.83%, n=7), Hernioraphy (2.18%, n=4), Gynaecological and obstetric surgery (1.64%, n=3) and Others (10.38%, n=19)
- *S. aureus* was most commonly isolated from SI ward (36.7%, n=18) followed by 20.4% (n=10) from POP. Likewise, *E. coli* was also mostly isolated from SI ward (23.1%, n=9) followed by POP and HCU with 20.5% (n=8) isolates.
- Regarding antimicrobial susceptibility pattern, the most effective drug for Gram positive isolates was Amikacin (73.73%, n=73) and Pefloxacin (68.68%, n=67). Similarly, against Gram negative bacteria, Imipenem was most effective (97.62%, n=82) followed by Amikacin (82.14%, n=69).
- Most of the bacteria were resistant to Cloxacillin (79.24%, n=145) followed by Amoxicillin (49.73%, n=91), Cefepime (49.73%, n=91) and Ciprofloxacin (40.44%, n=74).
- Out of 49 isolates of *S. aureus*, 18.37% were MRSA and all of them were sensitive to Vancomycin.

6.2 RECOMMENDATIONS

- As this study is confined to Shree Birendra Hospital, Chhauni, it does not reveal the pattern of microbial isolates and their AST pattern of the whole country. Hence, this type of study should be conducted throughout the country in different hospitals.
- In this study only aerobic/facultative bacteria were isolated and their AST pattern was studied. Besides that, anaerobic bacteria and fungi should also be cultured as they are also responsible for SSI.

- SSI rate could be studied according to the types of surgical wound like clean, contaminated or dirty.
- *S. aureus* along with some MRSA and *E. coli* were found to be common bacteria causing SSI. Hence they should be considered as serious problem and precautions should be taken to minimize the wound contamination by using appropriate antibiotics with periodic monitoring of microbial study of hospital.
- According to the AST pattern of the organisms isolated in this study, most of the commonly used antibiotics have very low activity and the second line drugs have been found to be the effective drugs. This suggests the limited activity of those drugs for the prophylaxis or the empirical treatment of SSI. Hence reliable laboratory procedures should be used for monitoring changes in the resistance trends among clinically relevant bacteria and for managing the infected patients.

REFERENCES

Adegoke AA, Mvuyo T, Anthony O and Steve J (2010) Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. Sci. Res. Essays 5 (24): 3876-3881

Ali SA, Tahir AM, Memon AS and Shaikh NA (2009) Pattern of pathogens and their sensitivity isolated from superficilal surgical site infections in a tertiary care hospital. J. Ayub. Med. Coll. Abbottabad 21 (2): 80-82

Anbumani N, Kalyan J and Mallika M (2006) Epidemiology and microbiology of wound infections. Ind. J. Pract. Doct. 3 (5): 182-205

Anderson G, Boldistone C, Woods S and O'Brien P (1996) A cost effectiveness evaluation of three antimicrobial regimens for the prevention of infective complications after abdominal surgery. Arch. Surg. 131: 744-748

Anguzu JR and Olila D (2007) Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr. Health Sci. 7 (3): 148-153

Anonymous (2006) Antimicrobial prophylaxis for surgery. Treat. Guidel. Med. Lett. 4: 83-88

Antibiotic prophylaxis in surgery (2008) A National Clinical Guideline. Scottish Intercollegiate Guidelines Network, pp 6-8

Ayton M (1985) Wound care: wounds that won't heal. Nurs. Times 81 (46): 16-19

Banjara MR, Sharma AP, Joshi AB, Tuladhar NR, Ghimire P and Bhatt DR (2003) Surgical wound infections in Patients of Tribhuvan University Teaching Hospital. J. Nepal Health Res. Counc. 1 (2): 41-45

Barie PS and Eachempati SR (2005) Surgical site infections. Surg. Clin. N. Am. 85: 1115-1135

Barie PS, Nichols RL and Wilson SE (2006) Surgical site infections in the era of antimicrobial resistance. National foundation for infectious diseases 9: 1-10

Berard F and Gandon J (1964) Postoperative wound infections: The influence of ultraviolet irradiation of the operating room and of various other factors. Ann. Surg. 160 (1): 1-192

Bhatt CP and Lakhey M (2006) The distribution of pathogens causing wound infection and their antibiotic susceptibility pattern. J. Nepal Health Res. Counc. 5 (1): 22-26

Bibbings J (1984) Honey, Lizard dung and pigeon's blood. Nurs. Times 80 (48): 36-38

Bibi S, Channa GA, Siddiqui TR and Ahmed W (2011) Frequency and risk factors of surgical site infection in general surgery ward of a tertiary care hospital of Karachi, Pakistan. Int, J. Infect. Control 7 (3): 1-5

Bolenz, C, Gupta A, Hotze T, Ho R, Cadeddu J, Roehrborn C and Lotan Y (2010) Cost comparison of robotic, laparoscopic, and open radical prostatectomy for prostate cancer. European urology 57 (3): 453–458

Bowler P (1998) The anaerobic and aerobic microbiology of wounds: a review. Wounds 10 (6): 170 -178

Bowler PG, Duerdin BI and Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin. Microbiol. Rev. 14: 244-269

Brachman PS (1981) Nosocomial Infection Control: an overview. Rev. Infect. Dis. 4: 640-648

Bratzler DW (2006) The Surgical Infection Prevention and Surgical Care improvement projects: Promises and Pitfalls. Am. Surg. 72: 1010-1016

Brown IW, Moor GF, Hummel BW, Marshall WG and Collins JP (1996) Toward further reducing wound infections in cardiac operations. Ann. Thorac. Surg. 62 (6): 1783-1789

Brooks GF, Butel JS and Morse SA (2001) Jawetz, Melnick and Adelberg's medical microbiology, 22nd edn. Lange medical books, pp 133-144

Casey AL and Elliott TSJ (2003) Progress in the prevention of surgical site infection. Curr. Opin. Infect. Dis. 22: 370–375

Chakraborty P (2005) A Text Book of Microbiology, 2nd edn. New Central Book Agency (P) Ltd. 8/1 Chintamani Das Lane, Kolkata 700 009, India, pp 86-92

Chamberlain NR (2004) The microbiology of wounds. Ostomy/wound management 45 (8):23-40

Cluett J (2008) How to prevent Surgical infections. About.com Guide (http://orthopedics.about.com/od/boneinfections/ht/infection.htm)

Collee JG, Fraser AG, Marmion BP and Simmons A (1999) Mackie and McCartney Practical Medical Microbiology, 14th edn. Churchill Livingstone, pp 26-196

Collier M (2003) Understanding wound infection. Nurs. Times 99 (25): 63-64

Collier M (2004) Recognition and management of wound. World Wide Wounds 18 (4): 221-225

Cruse PJE and R Foord (1980) The epidemiology of wound infection: A 10 year prospective study of 62,939 wounds. Surg. Clin. North Am. 60 (1): 27–40

Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG, Banerjee SN, Edwards JR, Tolson JS, Henderson TS, Huges JM and the National Nosocomial Infection Surveillance System (1991) Surgical Wound infection rates by wound class, operative procedure, and patient risk index. Am. J. Med. 91 (3B): 152S-157S

Cutting K and Harding K (1994) Criteria for identifying wound infection. J. Wound Care. 3 (4): 198-201

Dahms RA, Johnson EM, Statz CL, Lee GT, Dunn DL and Beilman (1998) Third generation cephalosporins and vancomycin as risk factors for postoperative vancomycin-resistant enterococcus infection. Arch. Surg. 133: 1343-1346

Dedsite A (2006) Support of the laboratory for the diagnosis of skin infections: The lab point of view. SBIMC-BVIKM and society belgede/BelgischeVereniging voor dermatologie 1: 1-56

Diegelmann RF and Melissa CE (2004) Wound Healing: An overview of acute, fibrotic and delayed healing. Frontiers in Bioscience 9: 283-289

Dionigi R, Rovera F, Dionigi G, Imperatori A, Ferrori A, Dionigi P and Dominion I (2001) Risk factors in Surgery. J. Chemother. 13: 6-11

Ducel G, Fabry J and Nicolle L (2002) Prevention of Hospital acquired infections: A Practical guide, 2nd edn. WHO/CDS/CSR, pp 4-5

Dudely H, Carter DC and Russell RCG (1986) Atlas of general surgery, 2nd edn. Butterworths, pp 25-286

Elsevier BV (2010) Prevention of nosocomial infection, CDC. Am. J. Infect. Control 16 (3): 128-140

Falanga V, Grinnell F, Gilchrest B, Maddox YT and Motshell A (1994) Workshop on the pathogenesis of chronic wounds. J. Invest. Dermatol. 102 (1): 125-127

Fehr J, Hatz C, Soka I, Kibatala P, Urassa H, Battegay M, Jeffrey Z, Smith T, Mshinda H, Frei R and Widmer AF (2006) Antimicrobial prophylaxis to prevent surgical site infections in a rural sub-Saharan hospital. Clin. Microbiol. Infect. 12: 1224–1227

Florman S and Nichols RN (2007) Current Approaches for the Prevention of Surgical Site Infections. Am. J. Infect. Dis. 3 (1): 51-61

Fry DE (2002) The economic costs of surgical site infections. Surg Infec (Larchmt) 3 (1): S37-43

Fuji T, Tabe Y, Yajima R, Yamaguchi S, Tsutsuni S, Asan T and Kuwano H (2011) Effects of subcutaneous drain for the prevention of incisional SSI in high risk patients undergoing colorectal surgery. Int. J. Colorectal Dis. 26: 1151-1155

Garibaldi RA, Cushing D and Lerer T (1991) Risk Factors for post operative infection. Am. J. Med. 91 (3b): 158S-163S

Gelfand B, Popov T, Karabak V and Belocerkovsky B (2005) Epidemiology and etiology of nosocomial infections in a surgical intensive care unit. Critical Care 9: 16

Giacometti A, Cirioni O, Schimizzi AM, Prete MS, Barchiesi F, D'errico MM. Petrelli E and Scalise G (2000) Epidemiology and microbiology of surgical wound infections. J. Clin. Microbiol. 38 (2): 918-922

Gil-Egea MJ, Pi-Sunyer MT, Verdaguer A, Sanz F, Sitges-Serra A and Eleizegui LT (1987) Surgical wound infections: prospective study of 4,486 clean wounds. Infect. Control 8 (7): 277-80

Goel SC (2006) Current concept review: Infection following implant surgery. Indian J. Orthoped. 40: 133-137

Gyawali Y (2007) Study on bacteriological profile of infected wound from patients visiting to Lumbini Zonal Hospital, Butwal, Nepal. A dissertation presented to the Central Depatment of Microbiology, Tribhuvan University, Kathmandu, Nepal

Haley RW, Culver DH, Morgan WM, White JW, Emori TG and Hodon TM (1985) Identifying patients at high risk of surgical wound infection. A simple multivariate index of patient susceptibility and wound contamination. Am. J. Epidemiol. 121: 206-215

Harbarth S, Liassine N and Dharan S (2000) Risk factors for persistent carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 31: 1380-1385

Heggars JP (2003) Assessing and controlling wound infection. Clin. Plast. Surg. 30 (1): 25-35

Heinzelman MM, Scott M and Lam T (2002) Factors predisposing to bacterial invasion and infection. Am. J. Surg. 183: 179-190

Horan T, Culver D and Jarvis W (1988) Pathogens causing nosocomial infection surveillance system. Antimicrobic. Newsletter 5: 65-67

Horan TC, Gaynes RP, Martone WJ, Jarvis WR and Emori TG (1992) CDC definitions of nosocomial surgical site infections: A modification of CDC definition of surgical wound infection. Infect. Control. Hosp. Epidemiol. 13: 606-608

Hsueh PR, Chen ML, Sun CC, Chen WH, Pan HJ, Yang LS, Chang SC, Ho SW, Lee CY, Hsieh WC and Luh KT (2002) Antimicrobial drug resistance in pathogens causing nosocomial infections at a University Hospital in Taiwan. Emerg. Infect. Dis. 8: 63-68

Iroha IR, Amadi ES, Orji JU and Esimone CO (2008) Invitro evaluation of the activity of colloidal silver concentrate against *Pseudomonas aeruginosa* isolated from postoperatrive wound infection. Sci. Res. Essay 3 (5): 209-211

Isibor JO, Oseni A, Eyaufe A, Osagie R and Turay A (2008) Incidence of aerobic bacteria and *Candida albicans* in post-operative wound infections. Afr. J. Microbiol. Res. 2: 288-291

Karkee P (2008) Bacterial isolates and their antibiogram from wounds and abscesses of surgical outpatients visiting Bir Hospital. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Katuwal A (1999) Bacteriology of wound infection among inpatients at Bir Hospital. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Khan NS, Rehman SU, Ali Ma, Sultan B and Sultan S (2008) Infection in orthopedic implant surgery, its risk factors and outcome. J. Ayub. Med. Coll. Abbottabad 20 (1): 23-25

Khorvash F, Mostafavizadeh K, Mobasherizadeh S, Behjati M, Naeini AE, Rostami S, Memarzadeh M and Khorvash FA (2008) Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of Surgical Site Infection (SSI): A 1 year of surveillance. Pak. J. Biol. Sci. 11: 1940-1944

Khosravi AD, Ahmadi F, Salmanzadeh S, Dashtbozorg A and Montazeri EA (2009) Study of bacteria isolated from orthopedic Implant Infections and their antimicrobial susceptibility pattern. Res. J. Microbiol. 4 (4): 158-163

Kim WJ, Gittes GK and Longaker MT (1998) Signal transduction in wound pharmacology. Arch. Pharm. Res. 21: 487

Kingsley A (2001) A proactive approach to wound infection. Nurs. Stand. 15 (30): 50-58

Knowhar H, Shankar EM, Vignesh R, Sekar R, Velu V and Rao UA (2008) High isolation rate of *Staphylococcus aureus* from surgical site infections in an Indian Hospital. J. Antimicrob. Chemother. 85: 758-760

Leaper DJ (2004) Wound infection. Bailey and Love's Short Practice of surgery, 24th edn. Hodder Arnold, pp 118-132

Luitel BR, Kandel SP, Shrestha B, Sapkota R and Bhandari RS (2009) Prediction of surgical site infection and other adverse postoperative outcomes. J. Ins. Med. 31 (2): 3-6

Madigan MT, Martinko JM and Parker J (2000) Brock biology of microorganisms, 9th edn. Prentice Hall International, pp 773-798

Mangram AJ, Horan TC, Pearson ML, Silver LC and Jarvis WR (1999) Guideline for prevention of surgical site infection. Infect. Control Hosp. Epidemiol. 20: 250-280

Martone WJ and Nichols RE (2001) Recognition, prevention,surveillance and management of surgical site infections: Introduction to the problem and symposium overview. Clinical Infect. Dis. 33 (2): S67-S68

Masaadeh HA and Jaran AS (2009) Incident of *Pseudomonas aeruginosa* in postoperative wound infection. Am. J. Infect. Dis. 5 (1): 1-6

Miller JM (1999) A guide to specimen management in clinical microbiology, 2nd edn. pp 31-44

Misra RN, Chander Y, Debata NK and Ohri VC (2002) Antibiotic resistance pattern of isolates from wound and soft tissue infections. MJAFI 56: 205-208

Mousa H (1997) Aerobic, anaerobic and fungal burn wound infections. J. Hosp. Infect. 37: 317-323

Namias N, Harvill S and Ball S (1999) Cost and morbidity associated with antibiotic prophylaxis in the ICU. J. Am. Coll. Surg. 188: 225-230

Nandi PL, Rajan SS, Mak KC, Chan SC and So YP (1999) Surgical wound infection. HKMJ. 5 (1): 82-86

National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October (2004) Am. J. Infect. Control 32: 470-485

NICE Clinical Guideline 74 (2008) Surgical site infection: Prevention and treatment of surgical site infection. National Institute for Health and Clinical Excellence, Mid City Place, 71 High Holborn, London WC1V 6NA, pp 4-5

Nichols RL (2001) Preventing surgical site infections: A surgeon's perspective. Emerg. Infect. Dis. 7 (2): 220–224

NNIS System (1991) Nosocomial Infection rates for the inter hospital comparison: limitations and possible solutions. National Nosocomial Infection Surveillance (NNIS) report. Infect. Control Hospital Epidemiol. 12: 609-621

NNIS System (1996) Prevention of antimicrobial resistance in health care settings. Ntional Nosocomial Infections Surveillance (NNIS) report data summary from October 1986- April 1996. Am. J. Infect. Control 24 (5): 380-388

Norrby SR (1995) Emerging antibiotic resistance in Gram positive bacteria: return to the pre-antibiotic era? HKMJ 1: 129-135

Nosocomial Infection National Surviellance Service (NINSS) (2002) Surveillance of surgical site infection in English Hospitals 1997-2002: A national surveillance and quality improvement programme. Health Protection Agency, pp 3-12

Nwachukwu NC, Orija FA and Okike UM (2009) Antibiotic susceptibility pattern of bacterial isolates from surgical wounds in Abia State University Teaching Hospital (ABSUTH), Aba- Nigeria. Res. J. Medicine and Med. Sci., 4 (2): 575-579

Oguntibeju OO and Nwobu RA (2004) Occurrence of *Pseudomonas aeruginosa* in post operative wound infection. Pak. J. Med. Sc. 20: 187-191

Onche I and Adedeji O (2004) Microbiology of Post-operative wound infection in implant surgery, 23rd edn. Oxford Press, pp 87-98

Olson MM and Lee JI (1990) Continuous 10-year wound infection surveillance. Arch. Surg. 125: 794-803

Oluwatosin OM (2005) Surgical wound infection: A general overview. Annals of Ibadan Postgraduate Med. 3 (2): 26-31

Palikhe N and Pokharel A (2003) Prescribing regimens of prophylactic antibiotic used in different surgeries. Kathmandu University Medical journal 2 (3): 216-224

Patherick ES and JE Dalton (2006) Methods for identifying surgical wound infections after discharging from hospital. Clinical infectious diseases: 6: 170-178

Plowman R (2002) The socio-economic burden of hospital acquired infection. Euro. Surveill. 5 (4): 49-50

Perera G and Hay R (2005) A guide to antibiotic resistance in bacterial skin infections. J. Eu. Acad. Dermatol. Venerol. 19 (5): 531-545

Poulakou G and Giamarellou H (2007) Investigational treatments for postoperative surgical site infections. Expert Opin. Investig. Drugs 16: 137-155

Ranjan KP, Ranjan N, Bansal Sk and Arora DR (2010) Prevalence of *Pseudomonas aeruginosa* in post-operative wound infection in a referral hospital in Haryana, India. J. Lab. Phy. 2 (2): 74-77

Reichman DE and Greenberg JA (2009) Reducing surgical site infections: A review. Rev. Obstet. Gynecol. 2 (4): 212-221

Rotstein OD, Pruett TL and Simmons RL (1985) Mechanisms of microbial synergy in polymicrobial surgical infections. Rev. Infect. Dis. 7: 151-170

Rubin RH (2006) Surgical wound infection: epidemiology, pathogenesis, diagnosis and management. BMC Infect. Dis. 6: 171-172

Russell RC, William NS and Bulstrode CJ (2000) Bailey and Love's Short Practice of surgery, 23rd edn. Oxford Press, pp 87-98

Sanjay KR, Nagendra PMN and Vijay Kumar GS (2010) A study on isolation and detection of drug resistant Gram negative bacilli with special importance to post operative wound infection. J. Microbiol. Antimicrob. 2 (6): 68-75

Schaechter M, Medoff G and Schlessinger (1989) the establishment of infectious diseases: Skin and soft tissue mechanism of microbial disease. William and Wilkins Int. edition, Baltimore, USA 3 (16): 682-695

Shampa A, Bhattacharjee A, Garg A and Sen MR (2006) Antimicrobial Susceptibility of *Pseudomonas aeruginosa* isolated from wound infection. Ind. J. Dermatol. 51 (4): 286-288

Shittu AU, Kolawole DO and Oyedepo ER (2002) A study of wound infections in two health institutions in Ile-Ife, Nigeria. Afr. J. Biomed. Res. 5: 97-102

Siegel JD, Rhinehart E, Jackson M and Chiarello L (2006) Management of multidrug –resistant organisms in healthcare settings. Healthcare Infection Control Practices Advisory Committee (HICPAC), pp 7-9

Siguan SS, Ang BS, Pala IM and Baclig RM (1990) Aerobic surgical infection: surveillance on microbiological etiology and antimicrobial sensitivity pattern of commonly used antibiotics. Phil. J. Microbial. Infect. Dis. 19 (1): 27-33

Singh Y and Rijal B (1998) Surgical infection and development: An overview. J. Ins. Med.20 (1, 2) http://www.jiom.com.np / index.phb? journal & page=article & op= view & path[ ]=12 & path[ ]= 14)

Singhal H and Zammit C (2006) Wound infection. eMedicine 1: 1-11

Slaughter MS, Olson MM, Lee JT and Ward HB (1993) A fifteen-year wound surveillance study after coronary artery bypass. Ann. Thorac. Surg. 56 (5): 1063-68

Smyth ET, Emmerson AM (2000) Surgical site infection surveillance. J. Hosp. Infect. 45 (3): 173-184

Sonawane J, Narayan K, Swaminathan R and Dosoni K (2010) Bacterial profile of surgical site infections and their antibiogram in a Tertiary Care Hospital in Navi, Mumbai. Bombay Hosp. J. 52 (3): 358-61

Taye M (2005) Wound infection in Tikur Anbessa Hospital, Surgical Department. Ethiop. Med. J. 43: 167-173

Vandepitte J, Verhaegen J, Engback K, Rohner P, Piot P and Heuck CC (2004) Basic Laboratory Procedures in bacteriology, 2nd edn. World Health Organisation, Geneva, AITBS Publishers & Distributors (Regd.) Delhi, pp 86-120

Watanabe A, Kohnoe S and Shimabukuro R (2008) Risk factors associated with surgical site infection in upper and lower gastrointestinal surgery. Surg. Today 38:404–412

Way LW and Doherty GM (2003) Current surgical diagnosis and treatment, 11th edn. Mc Graw Hill, Medical publishing division, pp 86-141

Weinstein RA (1991) Epidemiology and control of nosocomial infections in adult intensive care units. Am. J. Med. 91: 179-184

WHO/CDS/CSR/EPH (2002) Prevention of hospital-acquired infections, a practical guide. 2nd edition

Whyte W, Hambraeus A, Laurell G and Hoborn J (1991) The relative importance of routes and sources of wound contamination during general surgery. J. Hosp. Infect. 18 (2): 93-107

Williams NS, Bulstrode CJK and O'Connell PR (2008) Bailey and Love's short practice of surgery, 25th edition. Hodder Arnold, pp 32-48

Wolcott RD, Gontcharova V, Sun Y, Ziaschakau A and Dowd SE (2009) Bacterial Diversity in SSI: not just aerobic cocci anymore. J. Wound Care.18 (8): 317-323

APPENDIX- I

A. CLINICAL PROFILE:

Sample No.: …… Ward:

Name: …… Bed No.:

Age/Sex: …….

Short Clinical History:

Antibiotics administered: …….

Type of surgery: …….……

Sample site: ……..

Date of collection: ……...

Time of collection: ……..

B. MICROBIOLOGICAL PROFILE

Day 1

Direct microscopic observation

Gram staining

Result

a. Gram positive cocci
b. Gram positive bacilli
c. Gram negative bacilli
d. Gram negative cocci
e. Pus cells/ WBC
f. Others

Culture of specimen

Culture on: a. Nutrient agar (NA)

b. Mac Conkey agar (MA)

c. Blood agar (BA)

Incubation temperature:

Incubation time:

Day 2

Reading of culture plates

Media used	**Shape**	**Size**	**Color**	**Texture**	**Opacity**	**Margin**	**Consistency**
NA							
MA							
BA							

Gram staining results: ……

Catalase test: …….....

Oxidase test: ……..

Coagulase test: a. Slide coagulase: ……..b. Tube coagulase: ……..…

Others: ……

Day 3

Biochemical tests

		Result
a.	Methyl red (MR)	
b.	Voges Proskauer (VP)	
c.	Triple Sugar Iron (TSI)	
d.	Sulphide Indole Motility (SIM)	
e.	Citrate Utilization	
f.	Urea hydrolysis	
g.	Oxidative fermentative (OF)	

Organism isolated: ……

Day 4

Antibiotic sensitivity profile

Antibiotics used	Zone of inhibition	Interpretation
Amoxicillin		
Ciprofloxacin		
Cloxacillin		
Gentamicin		
Amikacin		
Pefloxacin		
Cefepime		
Polymixin B		
Ceftriaxone		
Cefotaxime		
Ceftazidime		
Amoxyclave		
Imipenem		
Piperacillin		

Performed By: **Checked By:**

APPENDIX II

LIST OF MATERIALS

1. Equipments

Autoclave
Burner
Incubator
Hot air oven
Microscope
Refrigerator

2. Glass wares

Petri plates
Test tubes
Glass slides
Glass rods

3. Mirobiological media (Hi-Media)

Nutrient Agar
Nutrient Broth
Mac Conkey agar
Blood agar
Mannitol Salt Agar
Robertson's Cooked Meat Broth
Muller Hinton Agar
Simmon's Citrate Agar
TSI Agar
MR/VP broth
Urease Broth
SIM Media
Hugh and Leifson (OF) Media

4. Chemicals and Reagents

Catalase Reagent (3% Hydrogen Peroxide)
Oxidase Reagent (1 % Tetra methyl P-Phenylene diamine dihydrochloride)
Kovac's reagent (1% p-dimethyl aminobenzaldehyde)
Barrit's reagent (5% ℑ- napthol and 40% KOH in ratio 3:1)
Crystal Violet
Gram's Iodine
Acetone-alcohol
Safranin
Blood Plasma
Methyl red
Normal saline

5. Antibiotic discs (Hi- media)

Amoxicillin (10 mcg)
Cloxacillin (30 mcg)
Amikacin (30 mcg)
Cefepime (30 mcg)
Ceftriaxone (30 mcg)
Ceftazidime (30 mcg)
Ciprofloxacin (5 mcg)
Gentamicin (10 mcg)
Pefloxacin (5 mcg)
Polymixin B (100 units)
Cefotaxime (30 mcg)
Amoxiclave (20/30 mcg)

Imepenem (10 mcg)
Piperacillin (100 mcg)
Cephalexin (30 mcg)
Methicillin (5 mcg)
Vancomycin (30 mcg)
Erythromycin (15 mcg)

6. Miscellaneous

Inoculating loop
Cotton swabs
Distilled water
Lysol etc.
Straight wire
Dropper
Immersion Oil

APPENDIX III

A. COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF CULTURE MEDIA

1. Blood Agar (Hi-Media)

(Blood agar base infusion agar + 5% Blood)

Composition	Gram/lit
Beef Heart infusion	500
Tryptose	10
Sodium chloride	5
Agar	1.5
Final pH at 25˚C	7.3±0.2

Preparation

As directed by the manufacturing company, 40 gm of blood agar bse was dissolved in 1000 ml distilled water. The medium was then sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min. then the prepared medium was cooled to about 40-50˚C, to which 50 ml of sterile defibrinated blood was added aseptically. Then the medium was poured into petriplates. For chocolate agar, st,erile blood agar plates were heated at 80˚C for 10 min in an oven so that the color of the medium turned into chocolate color.

2. Mac Conkey Agar (Hi-Media)

Composition	Gram/lit
Peptone	20
Lactose	10
Sodium taurocholate	5
Sodium chloride	5
Agar	20
Neutral red	0.04
Final pH at 25˚C	7.4±0.2

Preparation

As directed by the manufacturing company, 55 gm of the media was dissolved in 1000 ml distilled water. It was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 min and then poured into sterile petriplates aseptically.

3. Robertson's cooked meat broth (Hi-Media)

Composition	Gram/lit
Beef heart	454
Proteose Peptone	20
Dextrose	2
Sodium chloride	5
Final pH at 25˚C 7.2±0.2	

Preparation

As directed by the manufacturing company, 1.25 gm of the medium was suspended in 10 ml distilled water and allowed to stand for 15 min until all the particles were thoroughly wetted. Then the medium was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

4. Nutrient Broth (Hi-media)

Composition	gram/lit
Peptone	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Final pH at 25˚C 7.4±0.2	

Preparation

As directed by the manufacturing company, 13 gm of the medium was dissolved in 1000 ml distilled water and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

5. Nutrient Agar (Hi-media)

Composition	gram/lit
Peptone	10
Beef extract	10
Yeast extract	1.5
Sodium chloride	5
Agar	12
Final pH at 25˚C 7.4±0.2	

Preparation

As directed by the manufacturing company, 28 gm of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

6. Mannitol Salt Agar (Hi-media)

Composition	gram/lit
Proteose peptone	10
Beef extract	1
Sodium chloride	75
D-Mannitol	10
Phenol red	0.025
Agar	15
Final pH at 25˚C	7.4±0.2

Preparation

As directed by the manufacturing company, 111 gm of the media was dissolved in 1000 ml distilled water. The medium was boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

7. Muller Hinton Agar (Hi-media)

Composition	gram/lit
Beef extract	300
Casein Acid Hydrolysate	17.5
Starch	1.5
Agar	17
Final pH at 25˚C	7.4±0.2

Preparation

As directed by the manufacturing company, 38 gram s of the medium was suspended in 1000 ml of distilled water and the medium was warmed to dissolve. It was then sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

B. COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF BIOCHEMICAL MEDIA

1. Sulphide Indole Motility (SIM) Medium (Hi-Media)

Composition	gram/lit
Beef extract	3
Peptone	30
Peptonized iron	0.2
Sodium thiosulphate	0.025
Agar	3
Final pH at 25˚C	7.3±0.2

Preparation

As directed by the manufacturing company, 36 grams of the medium was dissolved in 1000 ml distilled water and distributed into tubes. The medium was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

2. Simmon's Citrate Agar (Hi-Media)

Composition	gram/lit
Magnesium sulphate	0.2
Mono-ammonium phosphate	1
Dipotassium phosphate	1
Sodium citrate	2
Sodium chloride	5
Agar	15
Bromothymol blue	0.08
Final pH at 25˚C	6.8±0.2

Preparation

As directed by the manufacturing company, 24.2 grams of the medium was dissolved in 1000 ml distilled water. The medium was then dispensed in test tubes and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

3. MR-VP Medium (Hi-Media)

Composition	Gram/lit
Buffered Peptone	7
Dextrose	5

Dipotassium Phosphate	5
Final pH at 25˚C	6.9±0.2

Preparation

As directed by the manufacturing company, 17 grams of the medium was dissolved in 1000 ml distilled water. The medium was then dispensed in test tubes and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

4. Christensen Urea Agar

Composition	**gram/lit**
Peptone	1
Dextrose	1
Sodium chloride	5
Disodium phosphate	1.2
Monopotassium phosphate	0.8
Phenol red	0.012
Agar	15
Final pH at 25˚C	7.4±0.2

Preparation

As directed by the manufacturing company, 24 grams of the medium was dissolved in 950 ml distilled water and sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes. The media was cooled to about 45˚C and 50ml of sterile 40% urea solution was mixed aseptically. Then 5ml of the media was dispensed into test tubes and allowed to set in a slanted position.

5. Triple Sugar Iron Agar (TSI) (HI- Media)

Composition	**gram/lit**
Peptone	10
Tryptone	10
Yeast extract	3
Beef extract	3
Lactose	10
Sucrose	10
Dextrose	1
Ferrous sulphate	0.2
Sodium chloride	5

Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12
Final pH at 25˚C 7.4±0.2	

Preparation

As directed by the manufacturing company, 65 grams of the medium was dissolved in 1000 ml distilled water and distributed in test tubes. The media was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes. It was then allowed to set in a slanted position with a butt of about 1 inch.

6. Hugh – Leifson's (OF) Media

Composition	**gram/lit**
Peptone	2
Sodium chloride	5
Dipotassium phosphate	0.3
Agar	2
Bromothynol blue	0.08
Final pH at 25˚C 6.8±0.2	

Preparation

As directed by the manufacturing company, 19.35 grams of the medium was dissolved in 1000 ml distilled water and distributed in test tubes. The media was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

C. COMPOSITION AND PREPARATION OF STAINING REAGENTS

For gram stain

a. Crystal violet solution

Crystal violet	20 gm
Ammonium oxalate	9 gm
Ethanol	95 ml
Distilled water	1000 ml

b. Gram's iodine

Potassium iodide	20 gm
Iodine	10 gm
Distilled water	1000 ml

c. Acetone-alcohol decolorizer

Acetone	500 ml
Ethanol (absolute)	475 ml
Distilled water	25 ml

Preparation

To 25ml distilled water, 475ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500ml of acetone was added to the bottle and mixed well.

d. Safranin (counter stain)

Safranin	10 gm
Distilled water	1000 ml

D. COMPOSITION AND PREPARATION OF TEST REAGENTS

a. Catalase reagent (3% H_2O_2)

Hydrogen peroxide solution	3 ml
Distilled water	97 ml

Preparation

To 97ml of distilled water, 3ml of hydrogen peroxide was added and mixed well.

b. Oxidase reagent (Tetramethyl p-phenylene diamine dihydrochloride)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled water	100 ml

Preparation

1gm of TPD was dissolved in 100 ml of distilled water. The strips of Whatman's No.1 filter paper were soaked into this solution and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. Kovac's Indole Reagent

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2 gm
Hydrochloric acid	10 ml

d. Methyl red solution

Methyl red	0.05 gm
Ethyl alcohol (absolute)	28 ml
Distilled water	22 ml

e. Voges-Proskauer reagent (Barrit's reagent)

Solution A

ℑ- Napthol	5 gm
Ethyl alcohol (absolute)	100 ml

Solution B

Potassium hydroxide	40 gm
Distilled water	100 ml

APPENDIX IV

PROCEDURES FOR GRAM STAINING AND DIFFERENT BIOCHEMICAL TESTS

A. Gram-staining procedure

- A thin film of the material to be examined was prepared and air dried.
- The material on the slide was heat fixed and allowed to cool before staining.
- The slide was flooded with crystal violet stain and allowed to remain for 1 min.
- The slide was rinsed with tap water.
- It was again flooded with gram's iodine solution and allowed to remain on the surface for 1 min.
- The slide was then rinsed with tap water.
- The slide was flooded with alcohol- acetone decolorizer for 10 seconds and rinsed immediately with tap water.
- The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
- The slide was then blot dried and examined microscopically under oil immersion at 100X.

B. Catalase test

- Using a sterile wooden stick or a glass rod, a small amount of colony from pure culture was picked and placed on a clean glass slide.
- A drop of 3% H_2O_2 was added over the organism on slide.
- Observation of bubbling was done
- A positive reaction showed the production of gas bubbles almost immediately.

C. Oxidase test

- The colony of the test organism was smeared on the oxidase reagent strip and observed for the change in color.
- The appearance of blue-purple color within 10 seconds indicated the positive oxidase test.

D. Oxidation-fermentation test

- Two tubes of OF media were taken. Using a sterile straight wire, the test organism was inoculates in it.
- One of the inoculated tubes was covered with a layer of paraffin to exclude oxygen while the other was open to the air.
- Both tubes were then incubated at 37°C for up to 24 hours.
- Fermentative organism utilizes carbohydrate in both tubes changing the medium from green to yellow where as the oxidative organism utilizes carbohydrate of open tube only.

E. Sulphide Indole Motility (SIM) test

- The test organism was stabbed in the 5 ml SIM medium in test tube with a sterile straight wire and incubated at 37°C for 24 hours.
- For indole production test, a few drops of Kovac's reagent was added and observed for development of red color.
- For motility test, the medium was observed for the appearance of turbidity along the stabbed line or throughout the medium.
- For H_2S production test, the medium was observed for the occurrence of black precipitate which indicates the production of H_2S gas.

F. Citrate utilization test

- The organism was inoculated on the slope of the medium by streaking with sterile straight wire.
- It was then incubated at 37°C for 24 hours and then observed for the color change.

G. Methyl Red test

- 2.5 ml of sterile glucose-phosphate (MR-VP) broth was taken in a tube and the test organism was inoculated into it.
- After overnight incubation at 37 °C, few drops of Methyl red solution were added. Bright red color was observed in the positive test indicating acidity.

H. Voges-Proskauer (VP) test

- 2.5 ml of sterile glucose-phosphate (MR-VP) broth was taken in a tube and the test organism was inoculated into it.
- It was incubated at 37°C for 24 hours.
- After incubation, 0.6 ml of ℨ- Napthol and 0.2 ml of KOH was added and gently shaken and then allowed to stand for 15 minutes.

- It was observed for the development of pink color indicating a positive reaction whereas the negative test appeared colorless or yellow.

I. Urease test

- Christensen's urea broth was inoculated with the pure culture of the test organism and the tube was incubated at 37°C for 24 to 48 hours.
- The change in the color was noted. Pink color indicated the positive reaction.

J. Triple Sugar Iron Agar (TSI) Reaction

- The organism was inoculated by stabbing the butt with straight wire and streaking the surface of the slant.
- The tubes were then incubated at 37°C for 24 hours.
- After incubation, the tubes were observed for gas formation, carbohydrate utilization and H_2S production.

K. Coagulase test

1. Tube coagulase test for free coagulase

- The plasma was diluted 1 in 10 physiological saline (mixing 0.2 ml of plasma with 1.8 ml of saline).
- 3 tubes were taken and labeled as: "T" for test organism; "P" for positive control (*S. aureus*); "N" for negative control (sterile broth).
- 0.5 ml of diluted plasma was pipette into each test tube.
- 0.5 ml of an overnight broth culture or an agar culture suspension of test organism was added to tube "T", that of S. aureus to tube labeled "P" and sterile broth to tube labeled "N".
- After mixing gently, tubes were incubated at 35°C to 39°C. It was then observed for clotting after 3-6 hours by gently tilting the tubes.

2. Slide test for bound coagulase

- A drop of physiological saline was at two ends of a clean slide.
- A colony of test organism was emulsified in each of the drops to make thick suspensions.
- A drop of plasma was then added to one of the suspension and mixed gently.
- The appearance of agglutinating or clumping of the organism in the suspension with plasma is the indication of positive slide coagulase test.

APPENDIX V

A. ZONE SIZE INTERPRETATIVE CHART FOR ANTIBIOTICS

Antibiotic	**Symbol**	**Disc content**	**Diameter of zone of inhibition in mm**		
			Resistant (mm or less)	**Intermediate (mm)**	**Sensitive (mm or more)**
Amoxicillin Enterobacteriaceae Staphylococci Streptococci other than *S. pneumoniae*	AMX	30 mcg	13 28 18	14-16 - 19-25	17 29 26
Amoxycillin/ Clavulanic acid	AC	20/10 mcg (30 mcg)	13	14-17	18
Amikacin	AK				
Cefepime	CPM	30 mcg	14	15-17	18
Cefotaxime	CE	30 mcg	14	15-22	23
Ceftazidime	CA	30 mcg	13	15-17	18
Ceftriaxone	CI	30 mcg	13	14-20	21
Cephalexin	CP	30 mcg	14	15-17	18
Ciprofloxacin	CIP	5 mcg	15	16-20	21
Erythromycin Staphylococci Streptococci	**E**	15 mcg	13 15	14-22 16-20	23 21
Gentamicin	**GEN**	10 mcg	12	13-14	15
Imipenem	**I**	10 mcg	13	14-15	16

Methicillin	**MET**	5 mcg	9	10-13	14
Ofloxacin	**OF**	5 mcg	12	13-15	16
Piperacillin	**PC**	100 mcg	17	18-20	21
Polymixin B	**PB**	300 units	11	-	12
Vancomycin	**VA**	30 mcg	-	-	15
Pefloxacin	**PF**	5 mcg	-	-	16

B. PROCEDURE OF ANTIBIOTIC SUSCEPTIBILITY TEST (KIRBY- BAUER DISC DIFFUSION METHOD)

- MHA plate with depth of the medium 4 mm was taken.
- The inoculum was prepared by transferring 3-4 pure culture colonies into nutrient broth (5 ml). Moderate turbidity was obtained by incubating at 37°C for 4 hours. The suspension was standardized to match the turbidity of the 0.5 MacFarland turbidity standards (1.5X 10^8 cfu/ml) which is done by adding 0.5 ml of 1.175% $BaCl_2.2H_2O$ solution to 99.5 ml of 0.36 N H_2SO_4.
- A sterile cotton swab was dipped into the suspension and excess inoculum was removed by rotating and pressing it against the inner wall of the tube. Then uniform swabbing was done on the agar surface and allowed to dry for 10 minutes.
- With the help of flamed forcep, discs were carefully placed on the inoculated plate, atleast 15 mm away from the edge with about 30 mm distance between two discs so as to avoid the overlapping of ZOI. The plates were allowed to stand at room temperature for 30 minutes.
- The plates were incubated at 37°C for 24 hours.
- Then ZOI was measured and interpreted as sensitive, resistant or intermediate.

APPENDIX VI

A. DETAILS ON SOME COMMOM TYPES OF SURGERY INCLUDED IN THE STUDY

1. Gastrointestinal surgery

- **Appendicectomy:** An appendectomy is the surgical removal of the appendix, a small tube that branches off the large intestine, to treat acute appendicitis. Appendicitis is the acute inflammation of this tube due to infection.
- **Cholecystectomy:** A cholecystectomy is the surgery to remove the gallbladder (a pear-shaped sac near the right lobe of the liver that holds bile). A gallbladder may need to be removed if the organ is prone to troublesome gallstones, if it is infected, or becomes cancerous. Surgical options include the standard procedure, called laparoscopic cholecystectomy, and an older more invasive procedure, called open cholecystectomy.
- **Laparotomy:** This is a surgical procedure involving a large incision through the abdominal wall to gain access into the abdominal cavity for direct examination of its contents, for example, to locate a source of bleeding or trauma. It may or may not be followed by repair or removal of the primary problem. It is also known as coeliotomy.
- **Hepatectomy:** It is the surgical resection of the liver.
- **Gastrectomy:** It is surgery to remove part or all of the stomach. If only part of the stomach is removed, it is called partial gastrectomy and if the whole stomach is removed, it is called total gastrectomy. Gastrectomy is used to treat bleeding, inflammation, non-cancerous (benign) tumors and polyps.
- **Intestinal anastomosis:** It is the connection between the tubular structures such as loops of intestine. In this a segment of intestine is resected and the two remaining ends are sewn or stapled together (anastomosed).
- **Right and left hemicolectomy:** It refers to the resection of the ascending colon (right) and the descending colon (left). When part of the transverse colon is also resected , it may be referred to as an extended hemi-colectomy. A partial colectomy is the removal of part of the large intestine (colon) which may be performed to treat cancer of the colon or long-term ulcerative colitis.
- **Graham's omentopexy:** It is the surgical treatment done for the closure of duodenal perforations.
- **Abdominal rectopexy:** It aims to prevent the further rectal prolapsed. Rectal prolapse occurs when the normal supports of the rectum become weakened allowing the muscle of the rectum to

drop down through the anus to the outside. This operation involves an abdominal incision, through which the rectum is fixed back into the place.

- **Splenectomy:** It is the surgical removal of the spleen, which is an organ that is part of the lymphatic system. The spleen is a dark-purple, bean-shaped organ located in the upper left side of the abdomen, just behind the bottom of the rib cage.

2. Urogenital surgery

- **Herniorrhaphy**: It is the surgical repair of hernia, with suture of the abdominal wall. When the weakened area is very large, some type of strong synthetic material is sewn over the defect to reinforce the area; this type of repair is sometimes specifically called hernioplasty. Postoperative care is similar to that for any type of abdominal surgery. The patient is protected from respiratory infections, which may cause coughing and undue strain on the suture line. Ambulation is usually not restricted, and the physician instructs the patient in activities that can be resumed after discharge from the hospital.
- **Prostatectomy:** It is the surgical removal of all or part of the prostate gland, the sex gland in men that surrounds the neck of the bladder and urethra - the tube that carries urine away from the bladder. A prostatectomy may be performed for an enlarged prostate, benign prostatic hyperplasia (BPH), or if the prostate gland is cancerous. There are several forms of the operation such as open prostatectomy, transurethral resection of the prostate (TURP) and Laparoscopic radical prostatectomy (Bolenze *et al*., 2010).
- **Nephrectomy:** A nephrectomy is the surgical removal of a kidney, the organ that filters waste from the blood and produces urine. Depending on the reason for a nephrectomy, all or part of one kidney or both kidneys can be removed.

- Partial nephrectomy – It is the removal of the part of one kidney.
- Simple nephrectomy – It is the removal of all of one kidney.
- Radical nephrectomy – It is the removal of all of one kidney together with the neighboring adrenal gland (the adrenaline-producing gland that sits on top of the kidney) and neighboring lymph nodes.
- Bilateral nephrectomy – It is the removal of both kidneys.

- **Nephrolithotomy/ pyelolithotomy:** Laparoscopic nephrolithotomy and pyelolithotomy are similar procedures performed under general anaesthetic, using either a transperitoneal or retroperitoneal approach. In a nephrolithotomy, once the kidney has been mobilised, the stone is located by ultrasound or by evidence of a bulge, or depression secondary to scarring. The renal capsule and parenchyma are incised and the stone or stones are removed from the affected calices. The

nephrotomy site may or may not be closed with sutures. A double-J stent may be inserted through the kidney, running from the kidney to the bladder, and left in place for several weeks after surgery. In a pyelolithotomy, the stone is accessed through an incision in the renal pelvis (pyelotomy). Once the stone is removed, the pyelotomy is usually closed with sutures, with or without a stent.

3. **Gynaecological surgery**

- **Dilation and curettage (Also called D & C.):** A D & C is a minor operation in which the cervix is dilated (expanded) so that the cervical canal and uterine lining can be scraped with a curette (spoon-shaped instrument).
- **Cesarean section:** Cesarean section (also called a C-section) is the surgical delivery of a baby by an incision through the mother's abdomen and uterus. This procedure is performed when physicians determine it a safer alternative than a vaginal delivery for the mother, baby, or both.
- **Hysterectomy:** A hysterectomy is the surgical removal of a woman's uterus. This may be performed either through an abdominal incision or vaginally.

4. **Head and neck surgery**

- **Cataract surgery:** Cataracts cloud the normally clear lens of the eyes. Cataract surgery involves the removal of the cloudy contents with ultrasound waves. In some cases, the entire lens is removed.
- **Tonsillectomy:** It is the surgical removal of one or both tonsils. Tonsils are located at the back of the mouth and help fight infections.
- **Thyroidectomy**: It is the surgical removal all or part of the thyroid gland. It may be performed for patients with thyroid cancer, hyperthyroidism, and drug resctions to antithyroid agents; pregnant women who can not be managed with drugs; patients who do not want radiation therapy; and patients with large goiters who do not respond to antithyroid drugs. The two types of thyroidectomy include:

  - Total thyroidectomy: It is the complete removal of thyroid gland which is usually donr in the case of malignancy.
  - Subtotal thyroidectomy: It is the removal of up to five-sixths part of the gland when anti-thyroid drugs do not correct hyperthyroidism.

- **Myringoplasty:** A myringoplasty is an operation to patch a hole in the ear drum. It is usually done under general anaesthetic. Depending on the size and position of the hole in your eardrum, the

operation may be done through your ear canal, or sometimes through an incision (surgical cut) behind your ear.

5. **Orthopedic surgery**

- **Amputation:** It is the removal of a body extremity by trauma or surgery. The amputation of the limb below knee is termed as BK Amputation and that above the knee is termed as AK amputation.
- **O.R.I.F.**: O.R.I.F. is an abbreviation for Open Reduction Internal Fixation. Open reduction internal fixation is a method of surgically repairing a fractured bone. Generally, this involves either the use of plates and screws or an intramedullary (IM) rod to stabilize the bone (Cluette, 2008).
- **Implant surgery:** It is commonest orthopedic operation performed to alleviate the pain and improve mobility in people with damaged joints. Infections associated with prosthetic joints occur less frequently than aseptic failure but it represents the most significant complication (Goel, 2006).
- **Total hip replacement (THR)**: It is the replacement of painful parts of arthritic hip completely with metal and plastic surfaces.

6. **Others**

- **Free skin graft:** A skin graft involves detaching healthy skin from one part of the body to repair areas of lost or damaged skin in another part of the body. Skin grafts are often performed as a result of burns, injury, or surgical removal of diseased skin. They are most often performed when the area is too large to be repaired by stitching or natural healing.
- **Mastectomy:** A mastectomy is the removal of all or part of the breast. Mastectomies are usually performed to treat breast cancer. There are several types of mastectomies, including the following:
  - Partial (segmental) mastectomy, involves the removal of the breast cancer and a larger portion of the normal breast tissue around the breast cancer.
  - Radical mastectomy, involves removal of the entire breast (including the nipple, the areola, and the overlying skin), the lymph nodes under the arm, and the chest muscles.
- **Debridement of wound, burn, or infection:** Debridement involves the surgical removal of foreign material and/or dead, damaged, or infected tissue from a wound or burn. By removing the diseased or dead tissue, healthy tissue is exposed to allow for more effective healing.

Source: Williams *et al*. (2008) and Dudely *et al*. (1986)

APPENDIX VII

A. ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ALL THE ISOLATES

Isolates	AMX	CIP	CP	CX	G	M	OF	AK	V	PF	CPM	E	PB	CI	CTX	CE	AC	I	P
1a	S	R	R	R	S	S	I	S	S	S	S	I	S	-	-	-	-	-	S
2a	S	R	R	S	S	S	I	I	S	S	S	I	S	-	-	-	-	-	S
3c	R	R	-	R	S	-	-	S	-	S	I	-	I	R	R	R	R	S	S
5a	R	R	R	R	R	R	I	R	S	I	R	R	S	-	-	-	-	-	I
6b	R	R	R	R	R	R	S	I	S	S	I	R	S	-	-	-	-	-	R
7c	R	R	-	R	R	-	-	S	-	R	I	-	R	r	r	r	R	S	R
8a	R	R	R	R	R	R	I	R	S	I	I	R	I	-	-	-	-	-	I
8c	R	R	-	R	R	-	-	S	-	R	R	-	S	R	R	r	R	S	I
9a	I	S	R	R	S	I	S	S	S	S	I	S	S	-	-	-	-	-	S
10c	R	S	-	R	S	-	-	S	-	R	I	-	S	S	S	S	R	S	R
11a	S	S	R	I	S	I	S	R	S	S	S	R	S	-	-	-	-	-	I
11c	R	R	-	R	R	-	-	S	-	R	S	-	S	R	R	R	R	S	R
12c	R	S	-	R	S	-	-	S	-	S	S	-	R	R	R	R	R	S	I
13a	S	S	I	R	S	S	S	S	S	S	S	S	S	-	-	-	-	-	R
14d	R	R	-	R	R	-	-	R	-	R	R	-	R	R	R	R	R	S	R
15b	R	R	R	R	R	R	R	I	S	I	I	R	I	-	-	-	-	-	R
17a	R	R	R	R	R	R	R	R	S	I	I	R	R	-	-	-	-	-	R
17c	R	R	-	R	S	-	-	S	-	R	I	-	S	R	R	R	R	S	R
19e	R	S	-	R	S	-	-	S	-	S	S	-	S	I	I	R	R	S	S
19c	R	S	-	R	S	-	-	S	-	S	I	-	S	R	R	R	R	S	S
20b	R	R	R	R	I	R	I	S	S	I	I	R	S	-	-	-	-	-	R
20c	R	R	-	R	R	-	-	S	-	R	I	-	S	R	R	R	R	S	R
21a	R	R	S	R	R	S	S	S	S	S	R	R	I	-	-	-	-	-	R
23c	R	R	-	R	R	-	-	R	-	R	R	-	R	R	R	R	R	I	R
24e	R	I	-	R	S	-	-	S	-	S	S	-	R	R	I	R	R	S	R

25l	R	R	-	R	S	-	-	S	-	S	S	-	S	R	R	R	R	S	R
26b	I	R	R	R	I	S	R	S	S	I	R	S	I	-	-	-	-	-	I
28c	S	I	-	R	I	-	-	S	-	S	I	-	S	I	I	I	R	S	S
29a	S	I	S	I	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
30c	R	R	-	R	S	-	-	S	-	R	R	-	S	R	R	R	R	S	I
31e	I	I	-	R	S	-	-	S	-	S	S	-	S	R	S	R	R	S	S
32e	S	I	-	S	S	-	-	S	-	S	S	-	S	S	S	S	S	S	S
34a	R	R	R	R	S	R	R	S	S	I	R	I	S	-	-	-	-	-	I
35a	S	S	S	S	I	S	S	S	S	S	I	R	S	-	-	-	-	-	S
Isolates	**AMX**	**CIP**	**CP**	**CX**	**G**	**M**	**OF**	**AK**	**V**	**PF**	**CPM**	**E**	**PB**	**CI**	**CTX**	**CE**	**AC**	**I**	**P**
35c	S	R	-	R	R	-	-	S	-	S	I	-	S	S	S	R	I	S	S
36e	R	R	-	R	R	-	-	R	-	R	S	-	R	S	S	R	R	S	I
38c	R	R	-	R	S	-	-	I	-	R	I	-	S	R	R	R	R	S	S
38l	R	R	-	R	R	-	-	R	-	R	I	-	R	R	R	R	R	S	S
39c	R	R	-	R	S	-	-	S	-	R	I	-	R	R	R	R	R	S	S
39f	R	S	-	R	S	-	-	S	-	I	I	-	R	S	R	I	R	S	I
39e	R	S	-	R	S	-	-	S	-	S	I	-	S	R	R	R	R	S	I
41g	I	I	-	R	R	-	-	S	-	S	S	-	S	S	S	S	R	S	S
42c	I	R	-	R	R	-	-	S	-	R	I	-	S	R	R	R	R	S	R
43a	S	R	I	R	S	S	R	S	S	I	I	I	S	-	-	-	-	-	R
44d	R	I	-	R	R	-	-	S	-	S	I	-	S	R	I	R	R	S	S
44h	R	R	-	R	R	-	-	R	-	S	R	-	S	R	I	R	R	S	R
45i	S	S	S	S	S	-	I	S	-	S	R	-	S	-	-	-	-	-	S
46a	R	S	R	R	S	S	R	S	S	S	S	R	S	-	-	-	-	-	R
46c	R	R	-	R	S	-	-	R	-	R	R	-	S	R	R	R	R	S	R
47b	R	R	R	R	R	I	R	R	S	I	R	R	S	-	-	-	-	-	I
49a	R	S	R	R	R	R	I	R	S	S	R	R	I	-	-	-	-	-	S
50a	R	I	R	R	R	R	R	R	S	R	R	R	R	-	-	-	-	-	R

52c	R	S	-	R	R	-	-	R	-	S	R	-	R	I	R	R	R	S	R
53b	S	S	S	S	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
54a	R	I	R	R	R	S	I	I	S	I	R	R	S	-	-	-	-	-	R
55b	S	S	S	S	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
56c	S	I	-	R	I	-	-	I	-	S	I	-	S	I	S	I	R	S	I
59a	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-	-	-	-	S
61b	S	S	I	S	S	S	S	S	S	S	S	R	S	-	-	-	-	-	S
62a	I	I	R	R	S	S	R	S	S	R	R	R	S	-	-	-	-	-	S
62c	R	R	-	R	S	-	-	S	-	R	R	-	S	R	I	R	R	S	S
63a	S	S	I	I	S	S	I	S	S	S	I	R	S	-	-	-	-	-	S
66a	S	I	I	I	S	S	R	I	S	I	S	R	S	-	-	-	-	-	S
69b	S	S	I	I	S	S	S	S	S	S	R	R	S	-	-	-	-	-	R
70b	S	S	I	R	S	S	S	S	S	S	R	R	S	-	-	-	-	-	S
72a	S	S	S	I	S	S	S	S	S	S	I	S	S	-	-	-	-	-	S
73a	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-	-	-	-	S
74b	I	I	R	R	S	S	R	S	S	I	R	R	I	-	-	-	-	-	I
80a	R	R	R	R	R	R	I	R	-	I	R	R	S	-	-	-	-	-	R
81b	I	S	I	R	S	I	S	S	S	S	R	R	I	-	-	-	-	-	S
82c	R	R	-	R	S	-	-	S	-	S	I	-	S	R	I	R	R	S	R
Isolates	**AMX**	**CIP**	**CP**	**CX**	**G**	**M**	**OF**	**AK**	**V**	**PF**	**CPM**	**E**	**PB**	**CI**	**CTX**	**CE**	**AC**	**I**	**P**
82e	R	S	-	R	S	-	-	S	-	S	R	-	S	R	I	S	R	S	S
83b	R	R	R	R	R	R	R	R	S	R	R	R	S	-	-	-	-	-	R
84f	R	S	-	R	S	-	-	S	-	S	S	-	R	S	S	S	S	S	S
84e	S	S	-	R	S	-	-	S	-	S	S	-	R	R	S	S	R	S	S
85b	S	S	I	S	S	S	S	S	S	S	R	S	S	-	-	-	-	-	S
86d	S	I	-	R	S	-	-	S	-	S	I	-	S	R	S	S	S	S	S
87a	S	S	I	S	S	S	S	S	S	S	S	R	S	-	-	-	-	-	S
89c	S	S	-	I	S	-	-	S	-	S	S	-	S	S	S	S	S	S	S

91b	R	R	R	R	R	R	R	R	S	I	R	R	R	-	-	-	-	-	R
92e	I	R	-	R	R	-	-	R	-	R	R	-	R	R	I	I	R	S	R
94d	I	I	-	R	S	-	-	S	-	S	R	-	S	I	S	S	S	S	R
96b	I	S	R	R	I	I	R	R	S	I	R	R	R	-	-	-	-	-	I
97c	I	I	-	R	I	-	-	S	-	S	I	-	S	S	R	I	R	S	R
98e	R	S	-	R	S	-	-	S	-	S	R	-	S	I	I	S	S	S	S
99a	S	S	S	S	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
100c	I	I	-	R	S	-	-	S	-	S	I	-	S	I	S	I	I	S	I
101b	R	I	R	R	S	S	I	S	S	S	R	R	S	-	-	-	-	-	I
102d	R	S	-	R	S	-	-	S	-	S	S	-	S	I	S	S	S	S	R
104a	S	S	S	R	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
106d	S	S	-	R	S	-	-	S	-	S	S	-	S	S	S	I	I	S	S
108a	S	I	R	R	R	I	I	S	S	S	R	R	S	-	-	-	-	-	I
109a	R	R	R	R	I	I	R	S	S	I	R	R	I	-	-	-	-	-	I
110b	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-	-	-	-	S
111a	S	R	R	R	R	R	R	S	S	R	R	R	S	-	-	-	-	-	R
113e	R	R	-	R	I	-	-	S	-	R	R	-	S	R	I	R	R	S	S
114b	I	R	R	R	R	R	R	R	S	R	R	R	S	-	-	-	-	-	S
114c	R	R	-	R	S	-	-	S	-	S	R		S	R	I	R	R	S	I
115b	R	I	R	R	R	S	R	S	S	S	R	R	S	-	-	-	-	-	R
116h	S	S	-	R	S	-	-	S	-	S	S	-	S	S	S	S	R	S	S
117h	I	S	-	R	S	-	-	S	-	S	I	-	S	R	R	R	R	S	R
119b	S	S	I	S	S	S	R	S	S	S	R	R	S	-	-	-	-	-	R
120d	R	R	-	R	R	-	-	S	-	I	R	-	S	I	S	I	R	S	R
121h	S	I	-	S	S	-	-	S	-	S	S	-	S	I	S	S	R	S	S
122b	I	S	R	R	R	R	R	I	S	S	R	R	S	-	-	-	-	-	I
123b	S	I	R	R	S	S	I	S	S	S	S	R	S	-	-	-	-	-	R
124a	R	R	R	R	S	S	R	S	S	S	R	R	I	-	-	-	-	-	S

Isolates	AMX	CIP	CP	CX	G	M	OF	AK	V	PF	CPM	E	PB	CI	CTX	CE	AC	I	P
125b	I	R	R	R	R	R	R	I	S	S	R	R	S	-	-	-	-	-	R
126b	S	S	R	R	S	S	S	S	S	S	I	R	S	-	-	-	-	-	S
127a	I	R	I	R	S	S	S	S	S	S	I	R	S	-	-	-	-	-	R
128d	S	S	-	S	I	-	-	S	-	S	I	-	S	S	S	S	S	S	S
129c	R	R	-	R	I	-	-	S	-	S	I	-	S	R	I	R	R	S	S
130a	R	S	R	R	S	S	I	S	S	S	I	R	S	-	-	-	-	-	I
131e	R	S	-	R	S		-	S	-	S	S	-	S	S	S	S	R	S	S
132b	S	I	I	R	S	S	R	S	S	S	R	R	S	-	-	-	-	-	S
132g	R	R	-	R	R	-	-	R	-	I	R	-	R	R	I	R	R	S	S
134c	R	R	-	R	S	-	-	S	-	R	R	-	S	S	S	I	R	S	S
135a	S	S	I	I	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
136b	S	S	I	R	S	S	S	S	S	S	R	R	R	-	-	-	-	-	S
137b	R	S	I	I	S	S	S	S	S	S	S	R	S	-	-	-	-	-	S
138b	R	S	R	R	S	S	S	S	S	S	I	R	S	-	-	-	-	-	S
140c	R	R	-	R	S	-	-	S	-	R	R	-	S	R	I	R	R	S	S
141c	R	R	-	R	S	-	-	S	-	R	R	-	R	R	I	R	R	S	S
142d	R	S	-	R	S	-	-	S	-	S	I	-	S	R	I	R	R	S	S
143e	S	S	-	S	S	-	-	S	-	S	S	-	S	S	S	S	S	S	S
144a	S	S	R	R	S	S	S	S	S	S	I	R	I	-	-	-	-	-	S
145a	S	S	I	R	S	S	S	S	S	S	R	R	S	-	-	-	-	-	S
146a	S	S	I	R	I	S	S	S	S	S	R	R	S	-	-	-	-	-	S
148a	R	R	R	R	R	R	R	R	S	R	R	R	S	-	-	-	-	-	R
149i	I	S	S	R	S	-	S	S	-	S	R	R	S	-	-	-	-	-	S
150b	R	R	R	R	S	S	R	S	S	R	R	R	S	-	-	-	-	-	R
151b	S	S	I	S	S	S	S	S	-	S	S	I	S	-	-	-	-	-	S
153a	S	R	I	R	S	S	I	S	S	R	R	R	R	-	-	-	-	-	S
153f	R	R	-	R	I	-	-	S	-	R	R	-	S	R	I	R	R	S	R

155c	R	R	-	R	I	-	-	S	-	R	R	-	S	R	I	R	R	S	S
156a	S	S	I	R	S	S	S	S	S	S	I	R	R	-	-	-	-	-	S
157a	S	S	S	S	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
158a	S	S	S	S	I	S	S	R	S	S	R	R	S	-	-	-	-	-	S
158e	I	S	-	R	S	-	-	S	-	S	S	-	S	R	S	R	R	S	S
159a	S	S	S	I	S	S	S	S	S	S	I	R	S	-	-	-	-	-	S
161a	S	S	R	I	S	S	S	S	S	S	R	R	S	-	-	-	-	-	S
161d	R	S	-	R	S	-	-	S	-	S	R	-	S	R	S	S	S	S	I
162b	S	I	S	S	S	S	S	S	S	S	S	I	S	-	-	-	-	-	S
163b	S	I	I	R	S	S	S	S	S	R	S	R	R	-	-	-	-	-	S
164e	R	S	-	R	S	-	-	S	-	S	S	-	S	R	S	S	R	S	S
Isolates	**AMX**	**CIP**	**CP**	**CX**	**G**	**M**	**OF**	**AK**	**V**	**PF**	**CPM**	**E**	**PB**	**CI**	**CTX**	**CE**	**AC**	**I**	**P**
165i	S	S	S	S	S	-	S	S	-	S	I	R	S	-	-	-	-	-	S
165c	S	S	-	R	S	-	-	S	-	S	R	-	S	S	S	S	S	S	R
167a	I	S	R	R	S	S	I	S	S	S	R	I	S	-	-	-	-	-	S
168f	R	R	-	R	R	-	-	R	-	R	R	-	S	S	S	R	S	S	R
170e	R	R	-	R	R	-	-	R	-	R	R	-	R	I	S	R	S	S	R
171d	R	R	-	R	R	-	-	R	-	R	R	-	R	I	R	R	S	S	R
172b	S	S	S	R	S	S	S	S	S	S	S	R	I	-	-	-	-	-	S
173b	R	R	R	R	R	S	I	S	S	S	R	R	S	-	-	-	-	-	I
173e	R	I	-	R	R	-	-	S	-	I	R		S	I	I	R	R	S	S
175k	R	R	-	R	S	-	-	S	-	R	R	-	S	R	I	R	R	S	R
176a	I	S	R	R	S	S	S	S	S	I	I	R	S	-	-	-	-	-	S
176c	R	R	-	R	S	-	-	S	-	R	R	-	S	R	R	R	R	S	S
177a	I	S	R	R	I	S	S	S	S	S	R	R	S	-	-	-	-	-	S
179b	S	S	S	R	S	S	S	S	S	S	I	R	S	-	-	-	-	-	S
180a	R	S	I	R	S	S	S	S	S	S	R	R	S	-	-	-	-	-	S
181b	R	I	R	R	S	S	R	S	S	I	R	R	S	-	-	-	-	-	S

182c	R	R	I	R	S	-	S	S	-	R	R	-	S	I	I	R	R	S	I
183i	S	S	I	I	S	-	S	R	-	R	S	S	S	-	-	-	-	-	R
184j	S	S	S	I	R	-	S	I	-	S	R	R	S	-	-	-	-	-	S
185b	I	S	R	R	R	S	S	S	S	S	R	R	R	-	-	-	-	-	I
186c	S	S	-	R	S	-	-	S	-	S	S	-	S	S	S	S	S	S	S
187j	S	S	S	S	S	-	S	S	-	S	R	R	S	-	-	-	-	-	S
188i	I	R	R	S	R	-	S	R	-	R	R	R	R	-	-	-	-	-	R
188d	S	S	-	R	S	-	-	S	-	S	S	-	S	S	S	R	R	S	S
189b	R	R	R	R	I	I	S	S	S	R	R	R	S	-	-	-	-	-	R
190a	S	S	S	S	I	S	S	R	S	S	R	R	R	-	-	-	-	-	S
190c	R	R	-	R	I	-	-	S	-	R	R	-	S	R	R	R	R	S	S
192b	I	I	R	R	S	S	I	S	S	S	R	R	S	-	-	-	-	-	S
193a	R	R	R	R	I	I	R	R	S	R	R	R	S	-	-	-	-	-	S
193c	R	I	-	R	S	-	-	S	-	R	S	-	S	I	I	R	R	S	I
194b	R	R	R	R	S	S	R	S	S	I	R	R	S	-	-	-	-	-	S
196c	R	I	-	R	S	-	-	S	-	I	R	-	S	R	I	R	R	S	S
196d	R	S	-	R	I	-	-	S	-	I	R	-	S	R	I	R	R	S	S
197c	R	R	-	R	I	-	-	S	-	I	R	-	S	R	R	R	R	S	R
198c	R	R	-	R	I	-	-	S	-	R	R	-	S	I	I	R	R	S	R
199d	R	R	-	R	R	-	-	S	-	R	R	-	S	S	S	S	R	S	S
199h	R	R	-	R	I	-	-	R	-	R	R	-	R	R	S	I	R	S	R
200b	S	I	R	R	I	S	I	S	S	S	R	R	S	-	-	-	-	-	S

a. *S. aureus*
b. CONS
c. *E. coli*
d. *Klebsiella pneumonia*
e. *Pseudomonas aeruginosa*
f. *Morganella morganii*

g. *Enterobacter* spp.
h. *Proteus mirabilis*
i. B-haemolytic streptococcus
j. Non-haemolytic streptococcus
k. *Klebsiella oxytoca*
l. *Citrobacter fruendii*