CHAPTER 1

1. INTRODUCTION

The lodgment and multiplication of a microorganism in or on the tissue of a host constitute infection. Infection occurs when one or more of the contaminants evades the clearing effect of the host's defenses, replicates in large numbers and attacks and harms the host's tissues (Collee *et al*, 1996). However not all the infections lead to disease. Many pathogens can selectively attach to particular host surface and may remain localized, growing near the point of entry or may be transferred to other body site causing generalized infection or may damage the other body cells by elaborating toxins. The immune system of an infected person may be able to prevent the establishment, multiplication and spread of a pathogen and even resist the effect of toxins in tissue. The outcome of host-parasite relationship depends on pathogenicity of parasite and the resistance or susceptibility of the host to parasite. The risk of infection depend on several predisposing factors such as geography, external environment, age, sex, behavior, immunization, family history, coexisting illness, nutritional status etc. Among the various types of infections, wound infection is a common type.

Wound is a discontinuity or break of the surface of the body. A wound is simple wound when only the skin is involved. It can be complex when underlying tissue, nerves, vessels, tendons etc are involved (Shenoy, 2001). Any purulent discharge from a closed surgical incision together with the signs of inflammation of the surrounding tissue is considered as wound infection irrespective of whether microorganisms can be cultured or not (Mayhall, 1993). In 1992, the Surgical Wound Infection Task Force replaced the term 'surgical wound infection' with 'surgical site infection' to include the infections of the organs or the spaces deep in the tissues such as peritoneum and bone. Surgical site infection is classified into superficial site infection and organ or space infection (Horan *et al*, 1992).

From a microbiological perspective, the primary function of normal intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens. Exposure of subcutaneous tissue

following a loss of skin integrity (i.e. a wound) provides a moist, warm and nutritious environment that is conducive to microbial colonization and proliferation. However, 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. Whereas the micro flora associated with clean, surgical wounds would be expected to be minimal, the presence of foreign material and devitalized tissue in a traumatic wound is likely to facilitate microbial proliferation unless early prophylactic antibiotic treatment and surgical debridement is implemented (Robson, 1997).

Since wound colonization is most frequently polymicrobial (Bowler *et al*, 1999) involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected. In the event of infection, a wound fails to heal, the patient suffers increased trauma, treatment costs rise and general wound management practices become more resource demanding. The development of a wound infection depends on the complex interplay of many factors. If the integrity and protective function of the skin is breached through any means like abrasions (scrapes or scratches) or lacerations (rips in the tissue), large quantities of different cell types will enter the wound and initiate an inflammatory response. This may be characterized by the classic signs of redness, pain, swelling, raised temperature and fever (Calvin, 1998). This process ultimately aims to restore homeostasis.

Complications of wound infections include death of surrounding tissue, including muscle, connective tissue or bones, which may require surgical debridement (removal of dead tissue); spread of the infection to the bloodstream, involving other organs; septic shock, a critical illness involving the whole body, which may require intensive care and life support and lead to multiple organ failure or death.

The incision (cut) in the skin performed for an operation can become infected. Surgical wound infections can range from redness surrounding a small portion of the incision to deeper infections involving underlying muscles or to a severe infection spread through the bloodstream. Doctors take precautions to prevent surgical wound infections, including use of

sterile (free from germs) procedures and instruments and appropriate use of antibiotics. Risk factors for surgical wound infections include diabetes, emergency procedures, smoking, severe obesity, altered immune function, malnutrition, low body temperature and long operation times.

Infections of the surgical wound are one of the most common HAIs and are an important cause of morbidity and mortality. The delay in recovery and subsequent increased length of hospital stay also has economic consequences. 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 patient (Plowman, 2000). The 2002 survey report by the Nosocomial Infection National Surveillance Service (NINSS), which covers the period between October 1997 and September 2001, indicates that the incidence of hospital acquired infection (HAI) related to surgical wounds is as high as 10%. These infections complicate illness, cause anxiety, increase patient discomfort and can lead to death (NINSS, 2002).

Wound infection may occur after an accidental or intentional trauma of the skin or other tissue; the later is surgical or post operative sepsis (Collee *et al*, 1996). In general investigation, increased incidence of post operative infection are age over 60 years due to long pre operative stay in hospital, long duration of operation, pre existing infection at site of wound and organism in wound at the end of operation. The underlying disease such as diabetes, immunosuppressant or irradiation, malnutrition and administration of adrenocorticoids are also important (Speller and Humphreys, 1998).

An analysis of post surgical wound infections following head and neck surgery demonstrated an increase in the average hospitalization period from 14 days when wounds healed without complication to 24 days when the wounds became infected (Johnson, 1991). In a similar analysis of 108 post surgical wounds, Zoutman *et al* (1998) concluded that 10.2 days per case was directly attributable to wound infection and that the associated hospital cost was $3,937 per infected patient.

Wounds become contaminated by varying number and type of microbial pathogen; bacteria, fungi, virus, parasite, mycoplasma and rickettsia. Source of wound infection may be endogenous and exogenous. Endogenous infection or auto-infection are caused by organisms that have been leading a commensally existence elsewhere in patient's body. For example, an abdominal surgical wound may become infected with organism from large bowel after an operation involving incision of the colon. In exogenous infection the source of infecting organism is outside the body of patient who become infected; cross infection is a particular example of exogenous infection in which the causal organism is spread from one person to another (Collee *et al*, 1996).

Wound infection is a very common infection throughout the world. The socioeconomic condition of people, their knowledge on hygiene and sanitation etc reflect the status of health. Nepal, being a developing country, it is not surprising that people suffer from various kinds of illness due to illiteracy and poverty. Wound infection is very common in Nepal. Various kinds of wound infection that occur in Nepal include agricultural wounds, bite wound infection, accidental wounds, industrial wounds, burn wound, surgical wounds, etc. A large number of complications occur as a result of poor initial management of wounds and poor aseptic techniques used in hospitals. In this context, the present study was carried out for hospitalized patients suffering from wound infection in Bir Hospital. The goal of this study was to isolate the bacteria responsible for wound infection among the patients admitted at different wards of Bir Hospital. The knowledge of the causative agents of wound infection has therefore proved to be beneficial as guideline in early diagnosis and selection of empirical antimicrobial therapy and on the implementation of infection control measures in Bir Hospital.

CHAPTER 2

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

- To study the etiological agents of wound infection and their antibiotic susceptibility pattern from hospitalized patients of Bir Hospital.

2.2 SPECIFIC OBJECTIVE

- To isolate and identify the etiological agents responsible for wound infection in different wards of Bir Hospital.
- To determine the antibiotic susceptibility pattern of isolated bacteria.

CHAPTER -3

3. LITERATURE REVIEW

3.1 WOUND INFECTION

In medicine, a wound is a type of physical trauma wherein the skin is torn cut or punctured (an open wound) or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin.

Infection of a wound is difficult to define and no clear rules can be given to distinguish it from contamination and colonization (Collee *et al*, 1996). It is important to have a clear understanding of the terms used for wound infection. Since 1985, the most commonly used terms have included wound contamination, wound colonization, wound infection and more recently, critical colonization. These terms can be defined as:

- Wound contamination - The presence of bacteria within a wound without any host reaction is wound contamination (Ayton, 1985).
- Wound colonization - The presence of bacteria within the wound which do multiply or initiate a host reaction is wound colonization (Ayton, 1985).
- Critical colonization -The multiplication of bacteria causing a delay in wound healing, usually associated with an exacerbation of pain not previously reported but still with no overt host reaction is critical colonization (Falanga *et al*, 1994; Kingsley, 2001).
- Wound infection - The deposition and multiplication of bacteria in tissue with an associated host reaction is wound infection (Ayton, 1985).

The potential for infection depends on a number of patient variables such as the state of hydration, nutrition and existing medical conditions as well as extrinsic factors, for example related to pre-, intra- and post-operative care if the patient has undergone surgery. This often makes it difficult to predict which wounds will become infected. (Heinzelmann *et al*, 2002). Consequently the prevention of wound infection should be a primary management cbjective for all healthcare practitioners.

The presence of a micro-organism within the margins of a wound does not indicate that wound infection is inevitable (Bowler, 1998). Protective colonization may play a part whereby some bacteria produce highly specific proteins that kill or inhibit other, usually closely related, bacterial species or where certain bacteria produce a variety of metabolites and end products that inhibit the multiplication of other micro-organisms (Kingsley, 2001). Ultimately, the development of an infection will be influenced largely by the virulence of the organism and immunological status of the patient. Examples of potential wound pathogens include, *Streptococcus pyogenes, S. aureus,* MRSA, *P. aeruginosa* and enterococci.

3.2 RECOGNITION 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 infection include localized erythematic, localized pain, localized heat, cellulites and edema. Further criteria include:

- Abscess
- Discharge which may be viscous in nature, discoloured and purulent.
- Delayed healing not previously anticipated.
- Discolouration 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 either at the time of dressing change or reported by the patient as associated specifically with the wound even when the wound dressing is in place.
- Abnormal smell
- Wound breakdown associated with wound pocketing/bridging at base of wound, i.e. when a wound that was assessed as healing starts to develop strips of granulation tissue in the base as opposed to a uniform spread of granulation tissue across the whole of the wound bed.

3.3 MODE OF WOUND INFECTION

Pathogenesis, the ability of micro-organism to initiate infection includes, entry, colonization and growth of microorganism in the host, resulting in changes in host function that damage the host.

3.3.1 Entry

The human body is covered with many bacteria and other commensal organisms. These are commonly found in the skin of axillae and perineum and the mucosa of nose, mouth, pharynx and large bowel. Many of these commensals have potential to become harmful pathogen if they breach the body surface. The portal of entry may be abrasions or other trauma, such as surgery and instrumentation; the surgical and traumatic wounds are at particular risk. In general, size of inflammatory response is related to the number of bacteria and their ability to multiply. Several million organisms are required to produce an inflammatory response and many millions for abscess formation (Lumley, 1997).

3.3.2 Establishment

Once the bacteria enter the body of the host, they must adhere to the cells of host surface. Several factors like surface hydrophobicity and net surface charge, binding molecules on bacteria and host cell receptor etc play important role in adhesion process. Lipoteichoic acid on the surface of Gram positive cocci e.g. Streptococci; fimbriae or pillion on the surface of Gram negative rods and cocci etc mediate adherence of bacteria to host cell surface.

3.3.3 Spread and multiplication

Once microorganisms have penetrated the skin, they may spread locally and invade the lymphatic or bloodstream. The virulence of an organism is, therefore, related to its ability to cross resistant surface and to overcome non-specific tissue defense and specific immune response. To combat these body defenses, bacteria produce various enzymes and a number of toxins. Such toxins may be exotoxin when secreted by the organism and endotoxin, when released on the death of the organism. Harmful mechanisms include the enzymes such as hyaluronidase and streptokinase promoting tissue invasion, leukocidins inhibiting

phagocytosis, heamolysins destroying blood cells and neurotoxins such as those of polio, diphtheria and tetanus. Gram positive organism produces peptidoglycan and teichoic acid, giving rise to fever and general malaise. They do not usually have the lethal consequences of the endotoxins of gram negative organisms, which in large doses can induce the marked abnormalities of endotoxic shock. The bacteraemia and septicaemia may occur due to shedding of organisms in blood in large number (Lumbley, 1997).

3.3.4 Host defense mechanism

Inflammation is the body's response to injury and is an attempt to eliminate or minimize the harmful effect. The commonest injuring agent is a microorganism; the condition is then termed as infection. Other harmful modalities include trauma, physical and chemical agents and invading tumors. These mechanisms activate enzyme cascades; such as the production of compliment and fibrinolytic systems and activate circulating and fixed macrophages; giving rise to the local features of inflammation. The four cardinal signs of inflammation; redness, swelling, heat and pain; were described by Celsus in the first centuary A.D; Galen added 'loss of functon' a century later (Lumbley, 1997).

Inflammation produces systemic responses as well as local response in a patient. The term systemic inflammatory response (SIRS) include both sepsis and non-infective causes such as after major surgery, trauma, burns and pancreatitis. The tissue response is vasodilation, producing local redness, heat and increased capillary permeability, with extrusion of plasma and lymphocytes and increased extra cellular fluid (edema).

An inflammatory response may resolve by phagocytosis of bacteria and dead cells by neutrophil, polymorphs and macrophages. The damaged tissue is replaced by the proliferation of fibroblasts and capillary loops and covered by an ingrowth of epithelial tissue. Unresolved, subcutaneous dead tissue, organism and other foreign material give rise to an abscess; the dead material producing pus; the abscess cavity is surrounded by granulation tissue (Lumbley, 1997).

3.4 PREDISPOSING FACTORS TO WOUND INFECTION

Wound infection may occur preoperatively or after accidental trauma to the skin. Virtually, all wounds whether accidental or surgically induced, become contaminated with varying number and type of microbial pathogens. The ultimate outcome of the interaction between wound and microbes depend on a number of factors.

3.4.1 Patients factors

These include:

3.4.1.1 Age

Age is an important factor. Neonates and elderly are more susceptible to infection than others, because of their inefficient immunity further the skin of the older people is more easily damaged and older cells do not proliferate as fast and may not have an adequate response to stress in terms of gene up regulation of stress-related proteins (Mustoe, 2004).

3.4.1.2 Obesity

Obese individuals are more susceptible to infection rather than the lean patients (Boyd and Hoerl, 1981).

3.4.1.3 Malnutrition

Severe malnutrition is thought to be a factor in the acquit ion of pathogen and delay in wound healing. Wound healing is delayed probably because of vitamin C deficiency. Vitamin C is necessary for synthesis and maintenance of collagen. Zinc deficiency is also known to delay the healing (Shenoy, 2001).

3.4.1.4 Diabetes

Diabetes is one of the major causes of chronic wounds. Diabetes causes neuropathy, which inhibits the perception of pain (Snyder, 2005). Thus patients may not initially notice small wounds and may therefore fail to prevent infection or repeated injury. Further, diabetes causes

immune compromise and damage to small blood vessels, preventing adequate oxygenation of tissue, which can cause chronic wounds (Moreo, 2005).

3.4.1.5 Burns

Burns provide a suitable site for bacterial multiplication when this has taken place; the burn is richer and more persistent source of infection than surgical wound because a larger area of tissue is exposed for a longer time (Speller and Humphreys, 1998).

3.4.2 Hospital predisposing factors

Various factors in hospital contribute for the acquisition of infection. These include:

3.4.2.1 Antibiotic administration

Antibiotics especially those with broad spectrum activity alter the normal flora of the body. They destroy sensitive bacterial strain and select for resistant strains which are often multiresistant strains, thus predispose for infection.

3.4.2.2 Contaminated drugs and solution

Many hospital epidemics have been treated to contaminated drugs or fluids associated with therapeutic procedures. Example: *S. marcesens* and *P. aeruginosa* have been found in hand creams, ointments and other therapeutic solution.

3.4.2.3 Immunosuppressive drugs and therapy

Certain drugs may reduce the patient's resistance to infection by interfering with host defense mechanisms. Corticosteroids, immunosuppressive agents, cytotoxic drugs and prolonged antibody therapy are associated with an increased incidence of invasion by fungi and other organisms not commonly encountered in infection.

3.4.2.4 Surgical procedure

A number of specific factors have been identified in relation to infection rates in surgical wounds which includes presence of an existing chronic infection, time interval between skin

preparation and surgery, nature of the invasive procedure especially involving the bowel, extent of tissue loss and/or trauma to tissue during surgery, adequacy of wound drainage, inappropriate use of wound management materials.

3.5 ROUTES OF WOUND INFECTION

Wounds may acquire infection through exogenous and endogenous routes.

3.5.1 Endogenous route

Endogenous infection are those infection that are caused by the patient's own flora which are non pathogenic under normal condition (Chakravorty, 2003). Endogenous infection or auto-infection are caused by organisms that have been leading a commensally existence elsewhere in patient's body. It is usually difficult to decide whether the organism entered the wound at the time of operation or subsequently. Infection can originate from the patient's own skin when this is inadequately sterilized or when the edges of the incision are recontaminated during the operation (Speller and Humphreys, 1998).

3.5.2 Exogenous route

Exogenous infection is caused due to some microbial species acquired mainly or exclusively from the source outside the body of the person becoming infected. In exogenous infection, organisms spread from hospital staff or visitors by direct or indirect airborne routes. Exogenous infection in the operation theater may originate from the surgeon, by touching the wound or from the instruments (Speller and Humphreys, 1998). The sources of exogenous wound infection are as follows:

3.5.2.1 From the environment (soil)

Infection may occur whenever a laceration or other type of wound is contaminated with pathogenic microorganisms. Wounds can be contaminated with the object that inflicted the wound or from various other sources such as soil, clothing, faces or aerosols. For example the major agent of gas gangrene, *C. perfringens,* is normal inhabitant of intestinal tract of human and animals. Similarly, the spores of *C. tetani*, the causative agent of tetanus are most

common in animal faces and soil. Contamination from these sources frequently occurs in traumatic injuries e.g. automobile accidents, gunshot wounds, battle injuries and the like (Pelczar *et al*, 2001).

3.5.2.2 From the hospital environment

The wounds may become infected from environmental sources of hospital such as aerosols and infected dust in operating room in the hospital ward or from contact with articles such as bedpans and bedclothes (Pelczar *et al*, 2001). The bacteria may enter the wound in following ways:

I. From surgeon's hand

Microorganisms from the hands of staff may be resident (persistent over time and not removed by hand washing) or transient (recently acquired from another source). It is generally accepted that the hands of staff are an important vehicle and that hand-washing makes a significant contribution to the control of HAI (Larson, 1988; Reybrouck, 1983).

The relevant microorganisms can readily be demonstrated on the hands of staff and may easily be transferred to the skin of others by brief contact (Marples and Towers, 1979). The clothing of personnel can be shown to become contaminated with potential pathogens, such as *S. aureus* and less frequently Gram-negative bacilli, particularly after the hand washing of heavily colonized patients (Babb *et al*, 1983).

II. From equipment

Infection from surgical instruments is now extremely rare, but other items of equipment, even if they do not penetrate the tissue, may convey infection from one patient to another. Some of them escaped attention because the risks associated with them appeared to be low or had not been perceived. Others are pieces of equipments that are difficult to clean and disinfect adequately or are expensive and in short supply (Speller and Humphreys, 1998).

III. From airborne spread

Airborne spread includes transmission by droplets and dust particles. Infected droplets originate in the nasopharynx and mouth and are expelled during talking, breathing and sneezing, carrying microorganisms with them.

The bacteria found in the air of a properly ventilated operating theatre are rarely pathogens in usual sense; for example, they rarely include *S. aureus* or *S. pyogenes.* The effectiveness of this route depends on the source; on the number of microorganisms present and the degree of dispersal whether in droplets, in droplet nuclei or on the skin scales; on survival and retention of pathogenicity by microorganisms in air or environment (or their death, impairment or dilution there); on the size of infecting dose and on the local or general susceptibility of the persons exposed to infection (Speller and Humphreys, 1998).

3.6 TYPES OF WOUNDS

Wounds can be broadly categorized as either an acute or a chronic wound.

I. Acute wounds

Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries (Davis *et al*, 1992). Irrespective of the nature of the cutaneous injury, acute wounds are expected to heal within a predictable time frame, although the treatment required to facilitate healing will vary according to the type, site, and depth of a wound. The primary closure of a clean, surgical wound would be expected to require minimal intervention to enable healing to progress naturally and quickly. However, in a more severe traumatic injury such as a burn wound or gunshot wound, the presence of devitalized tissue and contamination with viable (e.g. bacterial) and nonviable foreign material is likely to require surgical debridement and antimicrobial therapy to enable healing to progress through a natural series of processes, including inflammation and granulation, to final reepithelialization and remodeling.

II. Chronic wounds

A chronic wound is a wound that does not heal in an orderly set of stages and in a predictable amount of time the way most wounds do; wounds that do not heal within three months are often considered chronic (Mustoe, 2005). Chronic wounds seem to be detained in one or more of the phases of wound healing. For example, chronic wounds often remain in the inflammatory stage for too long (Snyder, 2005; Taylor *et al*, 2005). Chronic wounds may never heal or may take years to do so. These wounds cause patients severe emotional and physical stress as well as creating a significant financial burden on patients and the whole healthcare system (Augustin and Maier, 2003). Chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue (Davis *et al*, 1992).

Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus. Advancing age, obesity, smoking, poor nutrition and immunosuppressant associated with disease (e.g. AIDS) or drugs (e.g. chemotherapy or radiation therapy) may also exacerbate chronic ulceration.

3.7 CLASSIFICATION OF WOUND

3.7.1 Classification according to etiology, time and severity

Wound infections have been subdivided according to the following clinically related subgroups (Peel, 1992).

I. Etiology

1. Primary wound infection: In a primary infection, the wound is the primary site of infection.
2. Secondary wound infection: It arises following a complication that is not directly related to the wound.

II. Time

1. Early wound infection: It presents within 30 days of a surgical procedure.
2. Intermediate wound infection: An infection is described as intermediate if it occurs between one and three months afterwards of a surgical procedure.
3. Late wound infection: It presents after more than three months after surgery.

III. Severity

1. Minor wound infection: Wound infection is described as minor if there is discharge without cellulites or deep tissue destruction.
2. Major wound infection: Wound infection is described as major if the discharge of pus is associated with tissue breakdown, partial or total dehiscence of the deep fascial layers of the wound or if systemic illness is present.

3.7.2 Open and closed wound

3.7.2.1 Open wounds

Open wounds can be classified into a number of different types, according to the object that caused the wound. The types of open wounds are:

I. Incisions or incised wounds – caused by a clean, sharp-edged object such as a knife, a razor or a glass splinter. Incisions which involve only the epidermis are legally classified as cuts, rather than wounds.

II. Lacerations - Irregular wounds caused by a blunt impact to soft tissue which lies over hard tissue (e.g. laceration of the skin covering the skull) or tearing of skin and other tissues such as caused by childbirth. Lacerations may show bridging, as connective tissue or blood vessels are flattened against the underlying hard surface.

III. Abrasions (grazes) - a superficial wound in which the topmost layers of the skin (the epidermis) are scraped off, often caused by a sliding fall onto a rough surface.

IV. Puncture wounds - caused by an object puncturing the skin, such as a nail or needle.

V. Penetration wounds - caused by an object such as a knife entering the body.

VI. Gunshot wounds - caused by a bullet or similar projectile driving into or through the body. There may be two wounds, one at the site of entry and one at the site of exit.

3.7.2.2 Closed wounds

Closed wounds have fewer categories, but are just as dangerous as open wounds. The types of closed wounds are:

I. Contusions (more commonly known as a bruise) - caused by blunt force trauma that damages tissue under the skin.

II. Hematoma (also called a blood tumor) - caused by damage to a blood vessel that in turn causes blood to collect under the skin.

III. Crushing injuries - caused by a great or extreme amount of force applied over a long period of time.

3.7.3 Superficial wound infections

Superficial wound infection mainly involves skin. Skin infections are common and may be caused by bacteria, fungi or viruses. Breaks in the skin integrity, particularly those that inoculate pathogens into the dermis, frequently cause or exacerbate skin infections.

3.7.3.1 Primary cutaneous infection

Primary cutaneous infection include bacterial infection, fungal and yeast infection which are as follows:

Bacterial skin infections

I. Impetigo

Impetigo is a common infection in children that may also occur in adults. It is generally caused by either *S. aureus* or streptococci. Impetigo may present in two forms: small vesicles with a honey-colored crust or purulent-appearing bullae.

II. Folliculitis

Patients with folliculitis present with yellowish pustules at the base of hairs, particularly on the scalp, back, legs and arms.

III. Furuncles and carbuncle

Furuncles are more aggressive forms of folliculitis. Patients present with a painful, often fluctuant swelling in a non- weight-bearing area, most commonly areas of friction, the nasal area or the external ear. A carbuncle is a collection of furuncles and typically occurs on the back of the neck in middle-aged and older men.

IV. Erysipelas

Erysipelas presents acutely as marked redness, pain and swelling in the affected area. The illness is generally believed to be caused by beta-hemolytic streptococci, but recently other organisms have also been implicated (Beutner, 1996; Franco, 1996).

V. Cellulites

It may be caused by a variety of organisms, including group A streptococci, *S. aureus*, *Haemophilus influenzae* and in coastal areas, halophilic *Vibrio* species. Cellulites are a relatively deep infection, generally resulting from a break in the skin. It involves subcutaneous spaces in addition to the dermis. The patient presents with pain, redness, warmth and systemic symptoms such as fever.

Fungal and yeast infections

Fungi and yeast are capable of causing many different forms of skin infections, broadly referred to as dermatomycoses. *Candida albicans* and *Trichophyton, Epidermophyton, Microsporum* and *Malassezia* species are the most common infecting organisms.

3.7.3.2 Secondary infection

I. Bite wound infections

The reported infection rate for human bite wounds ranges from 10 to 50% depending on the severity and location of the bite, and up to 20% of dog bites and 30 to 50% of cat bites become infected (Griegon *et al*, 1995). Goldstein (1998) reported that 74% of 39 human and animal bite wounds contained a polymicrobial aerobic-anaerobic micro flora with *S. aureus*, *Peptostreptococcus* spp. and *Bacteroides* spp. being the predominant isolates in both wound types.

II. Burn wound infections

Infection is a major complication in burn wounds and it is estimated that up to 75% of deaths following burn injury are related to infection (Revathi *et al*, 1998; Vindenes and Bjerknes, 1995). Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts, many studies have reported the prevalence of aerobes such as *P. aeruginosa, S. aureus, E. coli, Klebsiella* spp., *Enterococcus* spp., and *Candida* spp. (Vindenes and Bjerknes, 1995). In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to represent between 11 and 31% of the total number of microbial isolates from burn wounds (Brook and Randolph, 1981; Mousa, 1997; Ramakrishnan *et al*, 1986). Predominant anaerobic burn wound isolates were *Peptostreptococcus* spp., *Bacteroides* spp. and *Propionibacterium acnes* (Mousa, 1997).

III. Diabetic foot ulcer infections

Plantar ulcers associated with diabetes mellitus are susceptible to infection due to the high incidence of mixed wound micro flora (Diamantopoulos *et al*, 1998) and the inability of the PMNs to deal with invading microorganisms effectively (Armstrong, *et al*, 1995). As in most wound types, *S. aureus* is a prevalent isolate in diabetic foot ulcers, together with other aerobes including, *S. epidermidis*, *Streptococcus* spp., *P. aeruginosa*, *Enterococcus* spp. and coliform bacteria (Armstrong *et al*, 1995; Karchme and Gibbons, 1994; Pathare *et al*, 1998). With good microbiological techniques, anaerobes have been isolated from up to 95% of diabetic wounds (Gerding, 1995), the predominant isolates being *Peptostreptococcus*, *Bacteroides* and *Prevotella* spp. (Diamantopoulos *et al*, 1998; Gerding, 1995; Johnson *et al* 1995; Karchmer and Gibbons, 1994; Wheat *et al*, 1986).

IV. Leg and decubitus (pressure) ulcer infections

Bowler and Davies (1999) reported a significantly greater frequency of anaerobes (particularly *Peptostreptococcus* spp. and pigmenting and non pigmenting gram-negative bacilli) in clinically infected leg ulcers than in non infected leg ulcers. Decubitus ulcers develop as a consequence of continued skin pressure over bony prominences; they lead to skin erosion, local tissue ischemia, and necrosis, and those in the sacral region are particularly susceptible to fecal contamination. Approximately 25% of decubitus ulcers have underlying osteomyelitis (Brown and Smith, 1999) and bacteremia is also common **(**Lance, 1989).

3.7.4 Surgical wound infection

Surgical wound infections are the second most frequent type of nosocomial infection (Brachman *et al*, 1989). They can be classified according to whether they are superficial or deep. About 60% to 80% of surgical wound infections are incisional, while the rest occur in the tissues and structures below the fascial layer.

3.7.4.1 Classification of operative wounds

A system of classification for operative wounds that is based on the degree of microbial contamination was developed by the US National Research Council group in 1964 (Berard

and Gandon, 1964). The rate of infection varies depending on the type of surgery undertaken. Surgical wounds have been classified as clean, clean-contaminated, contaminated and dirty-infected (CDC, 1996; Cruse and Foord, 1980) (Table 1).

Classification on the basis of surgical procedure

Table 1: Surgical Wound Classification and Subsequent Risk of Infection (If no antibiotics used)

Classification	**Description**	**Infective Risk (%)**
Clean (Class I)	Uninfected operative wound No acute inflammation Closed primarily Respiratory, gastrointestinal, biliary and urinary tracts not entered No break in aseptic technique Closed drainage used if necessary	<2
Clean-contaminated (Class II)	Elective entry into respiratory, biliary, gastrointestinal, urinary tracts and with minimal spillage No evidence of infection or major break in aseptic technique Example: appendectomy	<10
Contaminated (Class III)	Nonpurulent inflammation present Gross spillage from gastrointestinal tract Penetrating traumatic wounds <4 hours Major break in aseptic technique	About 20
Dirty-infected (Class IV)	Purulent inflammation present Preoperative perforation of viscera Penetrating traumatic wounds >4 hours	About 40

(Source: CDC, 1996; Cruse and Foord, 1980)

3.7.4.2 Rates of infection

This classification scheme has been shown in numerous studies to predict the relative probability that a wound will become infected. Clean wounds have a 1% to 5% risk of infection; clean-contaminated wounds have an 8% to 11% risk of infection; contaminated wounds have a 15% to 17% risk of infection; and for dirty wounds, the risk of infection is over 27% (Cruse and Foord, 1980).

Infection rates in US National Nosocomial Infection Surveillance (NNIS) system hospitals were reported to be: clean 2.1%, clean-contaminated 3.3%, contaminated 6.4% and dirty 7.1% (Culver *et al*, 1991). There is, however, considerable variation in each class according to the type of surgery being performed (Ferraz *et al*, 1992).

3.7.5 **Nosocomial wound infection**

One of the main concerns in the care and treatment of hospitalized patients is that they should not be harmed in the course of diagnosis and treatment of their illness. Unfortunately, 5-10% of hospitalized patients do acquire an infection while in hospital. Nosocomial infections are costly and can usually be avoided or greatly reduced. Many hospital acquired infections are known to occur in surgical departments. The rate of post operative wound infection varies from hospital to hospital, and within a given hospital is likely to be highest in patients who have undergone abdominal, thoracic, or orthopedic surgery. Surgical wound infection may occur shortly after surgery or several days postoperatively. The site of infection may be limited to the suture line or may become extensive in operative site. *S. aureus* (usually MRSA) is the biggest offender, followed closely by *E. coli* and other enteric bacteria. Anaerobic bacteria from the patient's large bowel may gain access to the operative site, making a mixed infection a serious and fairly frequent occurrence in hospitals in which the postoperative wound care and infection prevention programmes are weak. *Bacteroides fragilis* and occasionally, *C. perfringens* may invade the bloodstream, resulting in a systemic and frequently fatal postoperative infection (Vandepitte *et al*, 2004).

3.8 COMPLICATIONS OF WOUND INFECTION

There are some complications of wound infection which include:

3.8.1 Septicaemia and pyaemia

The term septicaemia meant the presence of bacteria in blood with clinical signs and symptoms of infection. Their origin was from a focus of infection from which they entered the circulation. Pyaemia literally means presence of pus in bloodstream.

3.8.2 Tetanus

Tetanus is a serious disorder with very high mortality even with treatment. The disease is caused by *C. tetani*, an anaerobic spore-forming bacillus with terminal spore which has a drumstick like appearance. Having entered the wound, the organisms multiply and produce powerful exotoxins; tetanospasmin (neurotoxin) and tetanolysin (haemolysin), which produce the disease (Shenoy, 2001).

3.8.3 Gas gangrene

It is a highly fatal spreading infection caused by clostridial organisms which result in myonecrosis.The disease is caused by *C. welchii, C. septicum, C. histolyticum* etc. Gas gangrene develops in wound where there is heavy contamination with soil or foreign bodies or which is associated with laceration and devitalized muscle mass. Having entered the wound, organism multiply and produce toxin under favorable condition of low oxygen tension which cause further damage to the tissue and various pathological events occur like inflammation, edema, muscle necrosis and gangrene of the muscles (Shenoy, 2001).

3.8.4 Osteomyelitis

Osteomyelitis is an inflammatory process in bone and bone marrow. It is most often caused by pyogenic bacteria but may be caused by other microorganisms, including mycobacterium and fungi.

3.8.5 Toxemia

Toxemia is caused due to profound impact of toxin produced by the pathogen on one or more organ systems of the host frequently distant from the original site of infection. Examples include tetanus, gram negative sepsis with endotoxemia, pseudomonas exotoxemia etc.

3.8.6 Actinomycosis

Actinomycosis is a sub acute-to-chronic bacterial infection caused normally by *Actinomyces israelii*. When the bacterium is introduced into the soft tissues, it may form an abscess that develops into a hard red to reddish purple lump. When the abscess breaks through the skin, it forms pus-discharging lesions.

3.9 WOUND HEALING

Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. When an individual is wounded, a set of events takes place in a predictable fashion to repair the damage. These events overlap in time (Shibata *et al*, 2004; Stadelmann *et al*, 1998) and must be artificially categorized into separate steps: the inflammatory, proliferative and remodeling phases. Some authors consider healing to take place in four stages, by splitting different parts inflammation or proliferation into separate steps (Quinn, 1998).

3.9.1 Inflammatory phase

In the inflammatory phase, clotting takes place in order to obtain hemostasis, or stop blood loss, and various factors are released to attract cells that phagocytise debris, bacteria, and damaged tissue and release factors that initiate the proliferative phase of wound healing.

Because inflammation plays roles in fighting infection and inducing the proliferation phase, it is a necessary part of healing. However, inflammation can lead to tissue damage if it lasts too long (Midwood *et al*, 2004). Thus the reduction of inflammation is frequently a goal in therapeutic settings. Inflammation lasts as long as there is debris in the wound. Thus the

presence of dirt or other objects can extend the inflammatory phase for too long, leading to a chronic wound.

As inflammation dies down, fewer inflammatory factors are secreted, existing ones are broken down, and numbers of neutrophils and macrophages are reduced at the wound site (Scholar and Stadelmann, 2006). These changes indicate that the inflammatory phase is ending and the proliferative phase is underway (Scholar and Stadelmann, 2006).

3.9.2 Proliferative phase

About two or three days after the wound occurs, fibroblasts begin to enter the wound site, marking the onset of the proliferative phase even before the inflammatory phase has ended (Falanga, 2005). As in the other phases of wound healing, steps in the proliferative phase do not occur in a series but rather partially overlap in time. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction (Midwood *et al*, 2004). In angiogenesis, new blood vessels grow from endothelial cells (Chang *et al*, 2004). In fibroplasias and granulation tissue formation, fibroblasts grow and form a new, provisional extra cellular matrix (ECM) by excreting collagen and fibronectin. (Midwood *et al*, 2004). In epithelialization, epithelial cells crawl across the wound bed to cover it (Garg, 2000). In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis (Midwood *et al*, 2004).

3.9.3 Maturation and remodeling phase

In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. When the levels of collagen production and degradation equalize, the maturation phase of tissue repair is said to have begun (Greenhalgh, 1998).The maturation phase can last for a year or longer, depending on the size of the wound and whether it was initially closed or left open (Mercandetti and Cohen, 2005). During Maturation, type III collagen, which is prevalent during proliferation, is

gradually degraded and the stronger type I collagen is laid down in its place (Dealey, 1999). Originally disorganized collagen fibers are rearranged, cross-linked and aligned along tension lines (Lorenz and Longaker, 2003). As the phase progresses, the tensile strength of the wound increases, with the strength approaching 50% that of normal tissue by three months after injury and ultimately becomes as much as 80% as strong as normal tissue (Mercandetti and Cohen, 2005). Since activity at the wound site is reduced, the scar loses its erythematous appearance as blood vessels that are no longer needed are removed by apoptosis (Greenhalgh, 1998).

3.10 ORGANISMS COMMONLY ENCOUNTERED IN WOUND INFECTION

A wide variety of aerobic and anaerobic bacterial species may be present, either in single or in combination, in wounds and other soft tissue infections. In many cases there is a mixed infection with more than one bacterial species. Mixed infection with Gram positive cocci and coliform bacilli are not uncommon and polymicrobial infections with anaerobes such as bacteroides and fusiform or fuso-spirochaetal associations are also recognized (Collee *et al*, 1996).

Organisms generally encountered in wound infections and abscesses are as follows:

1. Gram positive cocci

Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pyogenes
Streptococcus pneumoniae
Streptococcus faecalis

2. Gram negative bacilli

Escherichia coli
Proteus spp.
Providencia spp.
Klebsiella spp.

Morganella spp.

Enterobacter spp.

Citrobacter spp.

Other Enterobacteriaceae

Pseudomonas spp.

Acinetobacter spp.

Haemophilus influenzae

3. Anaerobic bacteria

Gram positive cocci

Peptostreptococcus spp

Gram positive bacilli

Clostridium spp.

Non spore forming anaerobic Gram positive rods

Gram negative bacilli

Bacteroides fragilis group

Bacteroides melaninogenicus

4. Fungus

Candida spp.

3.11 LABORATORY DIAGNOSIS OF WOUND INFECTION

Laboratory diagnosis of wound infection includes the following (Vandepitte *et al*, 2004).

3.11.1 Collection and transportation of specimens

Surgical specimens may be obtained by aspiration of a localized abscess or other surgical procedures. The surgeon should be advised to obtain several small representative tissue samples and any purulent exudates. If possible, cotton swabs should be avoided. The exudates should be collected using a needle and syringe.

3.11.2 Macroscopic evaluation

Specimens of pus, received in a syringe or in a sterile container, should be evaluated carefully by an experienced technician for color, consistency and odour.

Colour: The colour of pus varies from green-yellow to brown red. A red colour is generally due to admixture with blood or hemoglobin. Pus from postoperative or traumatic wounds (burns) may be stained blue-green by the pyocyanin pigment produced by *P. aeruginosa*.

Consistency: The consistency of pus may vary from a turbid liquid to one that is very thick and sticky.

Odour: A foul feculent odour is one of the most characteristic features of an anaerobic or a mixed aerobic-anaerobic infection, although it may be lacking in some instances.

3.11.3 Microscopic examination

Gram-stained smear

A smear for Gram-staining and examination should be made for every specimen. Using a bacteriological loop, an even smear of the most purulent part of the specimen should be made on a clean slide. The cotton swab should then be gently rolled over the glass surface, without rubbing or excessive pressure. Allow the slide air dry, fix by heat, stain and examine the smear under the oil-immersion objective and note for the presence of:

- polymorphonuclear granulocytes (pus cells);
- Gram positive cocci arranged in clusters, suggestive of staphylococci;
- Gram positive cocci in chains, suggestive of streptococci or enterococci;
- Gram negative rods resembling coliform (*E. coli, Klebsiella,* etc), other Enterobacteriaceae (*Proteus, Serratia,* etc), non fermentative rods (*Pseudomonas* spp.*),* or obligate anaerobes (*Bacteroides* spp.);
- Large straight Gram positive rods with square ends suggestive of *C. perfringens*, the principal agent of gas gangrene, or *Bacillus anthracis*, the agent of anthrax;

- *Candida* or yeast cells, which are seen as ovoid Gram positive budding spores, often forming branched pseudomycelia.

3.11.4 Culture

All specimens of pus or exudates should preferably be inoculated onto a minimum of three culture media

- a Blood agar plate 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 aerobes and anaerobes e.g. Thioglycollate broth or Cooked Meat medium.

Routinely, all media should be incubated for two days and inspected daily for growth. If the growth appears in the broth, it should be Gram stained and sub cultured onto appropriate culture media. Additional culture media should be included if specially requested, or if indicated by results of microscopic examination, as in the following examples:

- If staphylococci have been seen, an additional mannitol salt agar is helpful in obtaining pure growth and in making preliminary distinction between *S. aureus* and other cocci.
- If streptococci have been observed, their identification may be hastened by placing a differential bacitracin disc on the initial streaking area.
- If yeast or fungi have been observed, the specimen should also be inoculated onto Sabouraud dextrose agar.
- Culture in a strictly anaerobic atmosphere is necessary when the Gram-stained smear shows mixed anaerobic flora and also when the specimen produces foul odour. Specimens from suspected anaerobic infection should be inoculated without delay onto the following media:
    - an anaerobic blood agar to be incubated in an anaerobic jar;
    - a tube of anaerobic broth (thioglycollate or cooked meat).

The aerobic cultures should be inoculated and processed as usual and examined after 24 and 48 hours for aerobic and facultative organisms. A small area of the anaerobic Blood agar plate should be inoculated and the inoculums streaked out with a loop. The plates should be incubated and the anaerobic jar opened after 48 hours for inspection. If growth is insufficient, the plates may be re-incubated for a further 24 to 48 hours. The broth culture should be heavily inoculated with a pipette so as to distribute the inoculums throughout the medium in the tube. After 48 hours, the growth on the anaerobic Blood agar should be inspected and compared with the growth on the aerobic plating media. Each colony type should be examined with Gram stain. Bacteria with the same microscopic appearance that grow on aerobic and anaerobic agar are considered to facultative anaerobes. Colonies that appear only on anaerobic agar are probable anaerobes and should be sub cultured on two Blood agar plate, and incubated anaerobically. If growth appears only in anaerobiosis, identification of a pure culture of the anaerobe should be attempted.

Establishing an anaerobic environment for incubating cultures

A variety of methods exist for creating an anaerobic environment. One that is simple and inexpensive is the use of an anaerobic jar made of thick glass or polycarbonate, with a capacity of 2.5-3.5 liters that is equipped with a secure gas proof lid which can be easily removed and replaced. After putting the inoculated Petri dishes into the jar, an anaerobic atmosphere is generated by introducing a commercially available disposable anaerobiosis-generating device and closing the lid. Disposable devices for generating anaerobiosis take the form of flat, sealed, foil envelopes that release hydrogen and carbon dioxide after addition of water. Thee devices, however, require a palladium catalyst fixed on the undersurface of the lid of the jar. The catalyst becomes inactivated during use and should be replaced at regular intervals. Disposable redox-indicator strips, which change from blue or red to colourless in an anaerobic atmosphere, are available from a number of manufacturers (Vandepitte *et al*, 2004).

3.11.5 Identification

The first step in identification; should be to examine well-separated colonies carefully, pick a single colony of each type, prepare Gram-stained smear, and then characterize the organisms

under the microscope. Bacteria and fungi isolated from pus and exudates belonging to any group or species are finally identified by performing various biochemical and serological tests (Vandepitte *et al*, 2004).

3.12. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotics are chemical substances produced by a microorganism that have the capacity, in dilute solutions, to selectively inhibit the growth of or to kill other micro-organisms (Cooper *et al*, 2003). Antimicrobial susceptibility tests measure the ability of an antimicrobial agent to inhibit bacterial growth in vitro. The results of susceptibility tests should be made available, however, within 48 hours after receiving the specimen. Antimicrobials may not always be needed for the management of patients with wounds, abscesses, or exudates. Proper surgical incision, drainage and debridement are generally more important than antimicrobial drugs.

For Enterobacteriaceae, non fermentative Gram negative rods and staphylococci, the standardized disc-diffusion test (Kirby-Bauer) should be used. The WHO meeting considered that the modified disc-diffusion technique of Kirby-Bauer, for which requirements had been established by WHO in 1976, could be recommended for clinical and surveillance purposes in view of technical simplicity and reproducibility (Vandepitte *et al*, 2004).

Problems are often encountered when testing the susceptibility of staphylococci, both *S. aureus* and *S. epidermidis*. Over 80% of isolates produce *B*-lactamase and are resistant to benzyl penicillin and ampicillin. Infection caused by benzyl penicillin resistant staphylococci is often treated with *B*-lactamase-resistant penicillin of methicillin-group (oxacillin, cloxacillin, etc). The oxacillin disc is currently recommended for testing the susceptibility to this group (Vandepitte *et al*, 2004).

Resistance to antibiotics has become a serious problem in recent years particularly with the rise of epidemic strains of MRSA. Methicillin-resistant *S. aureus* (MRSA) was first reported in the UK in the 1980s and remains a cause of concern for all healthcare practitioners. There are now many different strains of MRSA affecting a large number of individuals in many

different healthcare settings. The degree to which people are affected ranges in severity from simple wound colonization, which does not need to be treated aggressively, to systemic infection such as bronchopneumonia, which may prove to be fatal. Anecdotal evidence suggests that MRSA is no more pathogenic in a wound than the non-resistant version; however, it is accepted that if a wound is infected with MRSA it is difficult to manage with antibiotics (Collier, 2003). As a general rule, practitioners should follow the local protocol for the management of a wound colonized with MRSA, with ongoing treatment based on clinical signs.

CHAPTER-4

4. MATERIALS AND METHODS

4.1 MATERIALS

A list of materials used during the study is given in appendix I.

4.2 METHODS

The study was carried out in the laboratory of Microbiology, Bir Hospital in order to isolate the causative organisms of wound infection along with their antibiotic sensitivity pattern in hospitalized patients, from August 17, 2006 to May 14, 2007, thereby covering a total period of 9 months. A total of 305 pus samples were collected from the patients suffering from wound infection ranging in age from 5 to 92 years. The details of patients including age, sex, ward, and other clinical details were recorded from hospital chart of patients as given in appendix I

4.2.1 COLLECTION OF SAMPLE

For this study, pus samples were collected on a sterile cotton swab or aspirated in syringe and labeled with date, time, method of collection and the patient's name, age, sex, inpatient number, bed number and ward. For laboratory investigation, two pus swabs were collected; one for the direct smear stains microscopy and the other for culture.

4.2.2 PROCESSING OF SAMPLE

4.2.2.1 Macroscopic observation

The physical characteristics of the pus samples were noted.

4.2.2.2 Microscopic observation

The specimen was evenly spread on clean, sterilized glass slide to prepare smear. The smear was air dried, heat fixed and stained by Gram staining, observed under microscope and examined for the type and morphology of bacteria.

The composition and preparation of staining reagents are given in appendix II

4.2.2.3 Culture of sample

The samples were cultured for aerobic and anaerobic bacteria. The samples were inoculated on to Nutrient agar, Mac Conkey agar and Blood agar plates. The inoculated plates were incubated at 37^{o} C for 24 hours in ordinary incubator aerobically.

For anaerobic bacteria, the samples were inoculated in Robertson's cooked meat broth (RCMB) and on Blood agar plates. The antibiotics; Penicillin, Neomycin and Metronidazole were placed on the 'well' areas of the blood agar plates before incubation. The strict anaerobes but not the aerobes or facultative organisms are inhibited by Metronidazole whilst many anaerobes are resistant to Neomycin. The anaerobic plates and RCMB are kept in anaerobic gas jar with anaerobic gas pak and incubated at 37^{o} C for 48 hours in ordinary incubator (Collee *et al*, 1996).

The composition and preparation of culture media are given in appendix II.

4.2.3 ISOLATION AND IDENTIFICATION OF ORGANISMS

After overnight incubation, the culture plates incubated aerobically were examined for bacterial growth and identified using standard microbiological techniques which involve colony characteristics, staining reactions and biochemical properties. The colonies on Blood agar incubated anaerobically were compared with that of colonies on Blood agar incubated aerobically and also the sensitivity of antibiotics on Blood agar plates was noted.

RCMB were observed for turbidity. If turbidity appeared on the cooked meat broth medium, then a loopful of this medium was inoculated on each plate and incubated as before. If growth was observed after incubation, identification was carried out in the same manner as described before. If turbidity did not appear within 72 hours incubation in RCMB and no growth occurred on culture plates, the sample was discarded.

4.2.4 IDENTIFICATION WITH BIOCHEMICAL TESTS

To perform biochemical tests, the isolated organisms should be of pure culture. Organisms in primary culture were sub cultured on Nutrient agar and incubated at 37^{o} C for 24 hours to obtain pure culture. From the pure culture plate, catalase test, oxidize test and gram staining were performed. Isolated colony was then inoculated in peptone water and incubated at 37^{o} C for 4 hours to perform different biochemical tests. Next day, organisms were identified on the basis of results of biochemical tests using standard bacteriological chart (Cheesebrough, 2000). Different biochemical tests used for the identification of isolated organisms are as follows:

i. Catalase test
ii. Oxidase test
iii. Coagulase test
iv. Deoxyribonuclease (DNAse) test
v. Oxidative-fermentative (OF) test
vi. Motility test
vii. Hydrogen sulphide (H_2S) production test
viii. Indole test
ix. Methyl red (MR) test
x. Voges proskauer (VP) test
xi. Citrate utilization test
xii. Triple sugar iron (TSI) test
xiii. Urea hydrolysis test (Urease test)
xiv. Amino acid decarboxylase test

The composition and preparation of different biochemical media and reagents of these biochemical tests are given in appendix II. The detailed procedure of different biochemical tests are given in appendix III.

Purity plate

The purity plate was maintained to see whether the biochemical tests were processed in an aseptic condition or not. So after performing biochemical tests, the same inoculums were sub cultured in respective culture media plate and incubated. The maintenance of aseptic condition is indicated by the growth of the same organism in pure form in purity plate medium.

4.2.5 ANTIBIOTIC SENSITIVITY TEST BY DISC DIFFUSION METHOD

Bacterial susceptibility test to antimicrobial agents were done in vitro by modified Kirby-Bauer disc diffusion method using fresh broth culture of isolates in Muller Hinton agar medium. Commercially available antibiotic discs were used according to the nature of the organism and different zones of inhibition were measured and the results were interpreted as sensitive, intermediate or resistant by comparing with standard interpretative table given by different laboratories.

Use of different antibiotics along with zone of inhibition and test procedure are given in the appendix III.

4.2.6 Quality control for test

Quality control is considered as one of the important factor for the correct result interpretation (Cheesebrough, 2000). In our study, strict aseptic condition was maintained during collection and processing of the samples to maintain quality control. The sterility of each batch of test medium was confirmed by incubating one uninoculated tube and plate along with inoculated ones as quality control. The quality of media were assured by testing each batch of medium using control strains of bacteria known to give positive and negative reactions.

CHAPTER 5

5. RESULT

In this study, a total of three hundred five pus samples from hospitalized patients in different wards of Bir Hospital were studied for a period of nine months. After culture, isolated bacteria were identified using standard bacteriological techniques. The results obtained are shown below.

5.1 DISTRIBUTION OF SAMPLES AND PATTERN OF RESULTS

5.1.1 Pattern of distribution of pus samples from total patients

Out of 305 samples collected, 251 (82.29%) samples were pus swab and 54 (17.70%) were aspirated pus. The result is shown in figure 1.

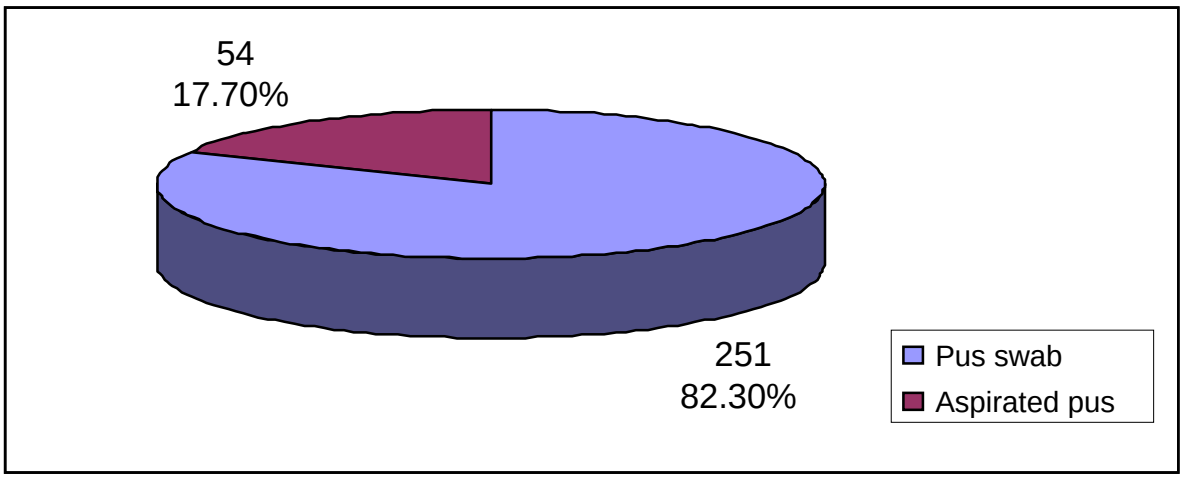

Figure1: Pattern of distribution of pus samples from total patients

5.1.2 Pattern of growth in different types of samples

Out of 305 samples studied, 197 (64.45%) samples showed growth while 108 (35.90%) samples showed no growth. 163 (64.94%) pus swab and 34 (62.96%) aspirated pus showed growth. The result is shown in table 1.

Table 1: Pattern of growth in different types of samples

S.N	Types of sample	Growth		No growth		Total	
		No.	%	No.	%	No.	%
1.	Pus swab	163	64.94	88	35.05	251	82.29
2.	Aspirated pus	34	62.96	20	37.03	54	17.70
Total		197	64.45	108	35.90	305	100

5.1.3 Pattern of single and multiple isolates from different pus samples

Out of 197 growth samples; 139 (70.55%) samples showed single isolate and 58 (29.44%) showed multiple isolates. The single isolate were found in 111 (68.09%) pus swabs and 28 (82.35%) aspirated pus while multiple isolates were found in 52 (31.9%) pus swab and 6 (17.64%) aspirated pus. The single isolate was higher than multiple isolates in both types of samples. The result is shown in table 2.

Table 2: Pattern of single and multiple isolates from different pus samples

S.N	Types of sample	Single isolate		Multiple isolates		Total
		No.	%	No.	%	
1	Pus swab	111	68.09	52	31.9	163
2	Aspirated pus	28	82.35	6	17.64	34
Total		139	70.55	58	29.44	197

5.1.4 Number and percentage of Gram positive and Gram negative bacteria from different samples

A total of 253 bacterial isolates were obtained of which 155 (61.2%) were Gram negative and 98 (38.8%) were Gram positive bacteria. 210 bacterial isolates were from pus swab of which 79 (37.6%) were Gram positive bacteria and 131 (62.4%) were Gram negative bacteria. In aspirated pus, out of 43 bacterial isolates, 19 (44.2%) were Gram positive and 24 (55.8%) were Gram negative bacteria. The result is shown in table 3.

Table 3: Number and percentage of Gram positive and Gram negative bacteria from different samples

S.N	Type of bacterial isolates	Gram positive bacteria		Gram negative bacteria		Total no. of bacterial isolates
		No.	%	No.	%	
1.	Pus swab	79	37.6	131	62.4	210
2.	Aspirated pus	19	44.2	24	55.8	43
Total		98	38.8	155	61.2	253

5.2 DISTRIBUTION OF PATIENTS IN HOSPITAL

5.2.1 Gender wise distribution of patients showing growth and no growth

Out of 305 cases, 185 (60.65%) were male patients and 120 (39.34%) were female patients. The growth was found to be higher in male patients (65.40%) than in female patients (63.33%). The result is shown in table 4.

Table 4: Gender wise distribution of patients showing growth and no growth

S.N	Gender	Total patients		Growth		No growth	
		No	%	No.	%	No.	%
1	Male	185	60.65	121	65.40	64	34.59
2	Female	120	39.34	76	63.33	44	36.66
		305		197		108	

5.2.2 Age and gender wise distribution of patients

In all age group, male patients (185) were affected more frequently than female patients (120). The highest number of patients belonged to age group 21-30 (76) followed by age group 31-40 (60) and 11-20 (58). The children (age gr. 0-10) and patients with age above 80 years were relatively found to be less affected. The result is shown in figure 2.

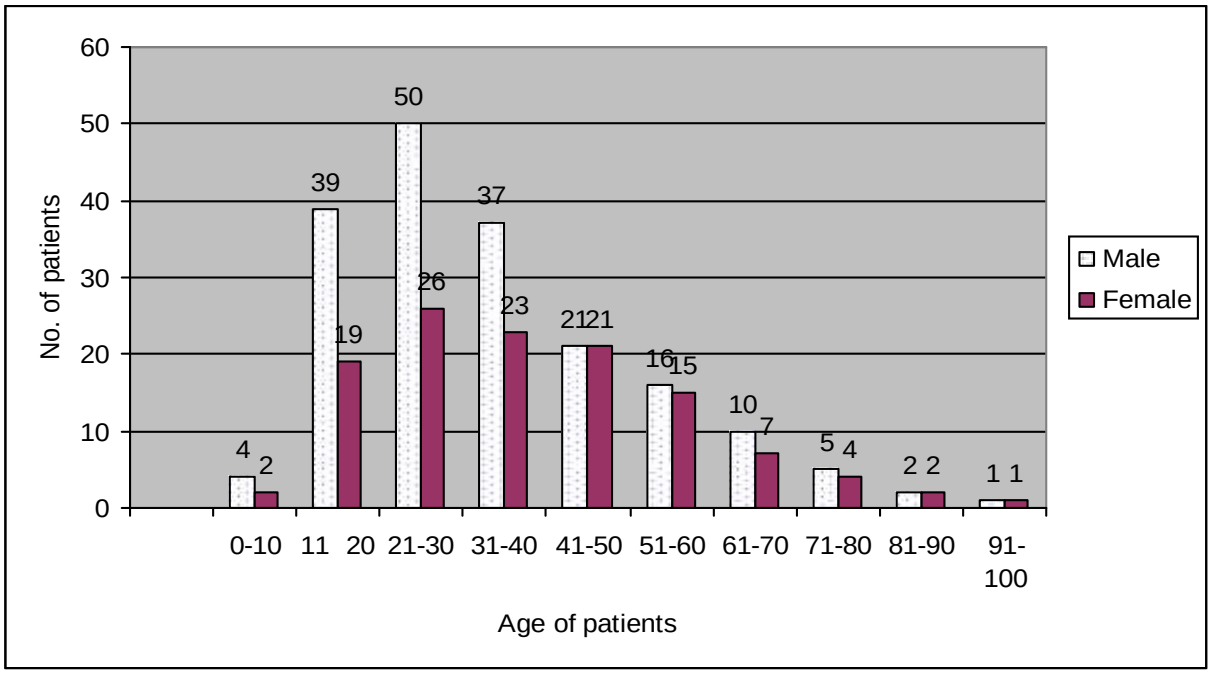

Figure 2: Age and gender wise distribution of patients

5.3 PATTERN OF MICROBIAL ISOLATES

5.3.1 Pattern of microbial growth in pus samples collected from different wards

Out of 305 samples, 197 samples showed growth. The growth was found to be highest in burn ward (90.90%), followed by post operative ward (80%) and lowest was in plastic surgery ward (33.33%). The result is shown in table 5.

Table 5: Pattern of microbial growth in samples collected from different wards

S.N	Wards	Total sample		Growth		No growth	
		No.	%	No.	%	No.	%
1	MSW	52	17.04	36	69.2	16	30.8
2	FSW	54	17.7	34	62.9	20	37.1
3	POW	20	6.55	16	80	4	20
4	Orthopedic	70	22.95	50	71.4	20	28.6
5	Burn	22	7.21	20	90.9	2	9.10
6	NSW	27	8.85	15	55.5	12	44.5
7	Plastic Surgery	3	0.98	1	33.3	2	66.7
8	Urology	7	2.29	4	57.1	3	42.9

9	**MMW**	**19**	**16.22**	**7**	**36.8**	**12**	**63.2**

S.N	**Ward**	**Single isolates**		**Multiple isolates**		**Total growth**	
10	**FMW**	**16**	**5.24**	**7**	**43.7**	**9**	**56.3**
11	**ENT**	**8**	**2.62**	**3**	**37.5**	**5**	**62.5**
12	**ICU**	**3**	**0.98**	**2**	**66.6**	**1**	**33.4**
13	**CTVS**	**4**	**1.31**	**2**	**50**	**2**	**50**
Total		**305**		**197**			**108**

5.3.2 Pattern of single and multiple isolates in pus samples collected from various wards

Infection due to single type of organisms was found to be more in all wards than due to multiple organisms. Infection due to multiple organisms was comparatively higher in NSW (53.3%). The result is shown in table 6.

Table 6: Pattern of single and multiple isolates in pus samples collected from various wards

		No.	%	No	%	No.
1	MSW	27	75	9	25	36
2	FSW	23	67.6	11	32.4	34
3	POW	10	62.5	6	37.5	26
4	Orthopedic	38	76	12	24	50
5	Burn	14	70	6	30	20
6	NSW	7	46.7	8	53.3	15
7	Plastic Surgery	1	100	-	-	1
8	Urology	3	75	1	25	4
9	MMW	6	85.7	1	14.3	7
10	FMW	5	71.4	2	28.6	7
11	ENT	2	66.7	1	33.3	3
12	ICU	1	50	1	50	2
13	CTVS	2	100	-	-	2
Total		139		58		197

5.3.3 Frequency of Gram positive and Gram negative isolates from samples collected from different wards

Out of 253 bacterial isolates the highest isolate was from orthopedic ward 62 (24.50%), of which 64.4% were Gram negative isolates and 34.4 % were Gram positive isolates, followed by MSW 46 (18.18%).The result is shown in table 7.

Table 7: Frequency of Gram positive and Gram negative isolates from samples collected from different wards

S.N	Ward	Total sample collected	No. of samples with growth	Total bacterial isolates		Gram positive isolates		Gram negative isolates	
				No.	%	No.	%	No.	%
1	MSW	52	36	46	8.18	17	36.9	29	63.1
2	FSW	54	34	44	17.39	14	31.8	30	68.2
3	POW	20	16	22	8.69	8	36.4	14	63.6
4	Orthopedic	70	50	62	24.50	22	35.4	40	64.6
5	Burn	22	20	26	10.27	14	53.8	12	46.2
6	NSW	27	15	22	8.69	11	50	11	50
7	Plastic Surgery	3	1	1	0.39	1	100	-	-
8	Urology	7	4	6	2.37	1	16.6	5	83.4

9	**MMW**	**19**	**7**	**7**	**2.7**	**3**	**42.8**	**4**	**57.2**
10	**FMW**	**16**	**7**	**8**	**3.16**	**2**	**25**	**6**	**75**
11	**ENT**	**8**	**3**	**4**	**1.58**	**2**	**50**	**2**	**50**
12	**ICU**	**3**	**2**	**2**	**0.79**	**1**	**50**	**1**	**50**
13	**CTVS**	**4**	**2**	**3**	**1.18**	**2**	**66.6**	**1**	**33.4**
Total		**305**	**197**	**253**		**98**		**155**	

5.3.4 Pattern of distribution of Gram positive isolates from total pus samples

Out of 253 bacterial isolates, 98 were Gram positive bacteria, of which *S. aureus* (83.67%) was most common followed by CONS (7.14%), Non haemolytic streptococci (4.08%), unidentified Gram positive rods (3.06%) and haemolytic streptococci (2.04%). The result is shown in figure 3.

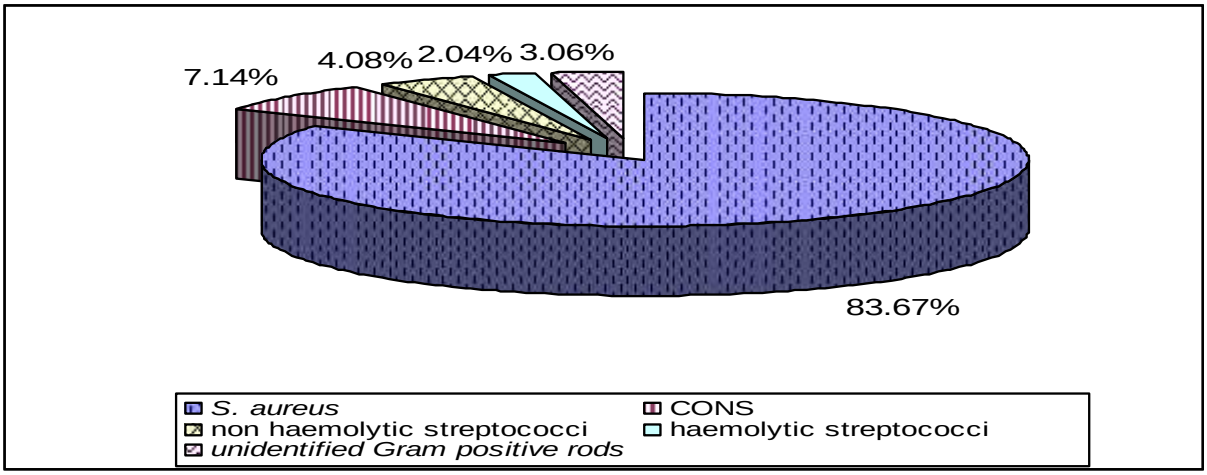

Figure 3: Pattern of distribution of Gram positive isolates from total pus samples

5.3.5 Pattern of distribution of Gram negative isolates from total pus samples

Out of 253 bacterial isolates, 155 were Gram negative bacteria of which *E. coli* (38.7%) was most common followed by *P. aeruginosa* (25.16%), *Acinetobacter* spp. (10.96%), *K.* oxytoca (7.74%), *P. mirabilis* (5.16%), *K. pneumoniae* (4.51%), *P. vulgaris* (2.58%), *Enterobacter*

spp. (2.58%), *C. freundii* (1.93%), and *Providencia* spp. (0.64%). The result is shown in figure 4.

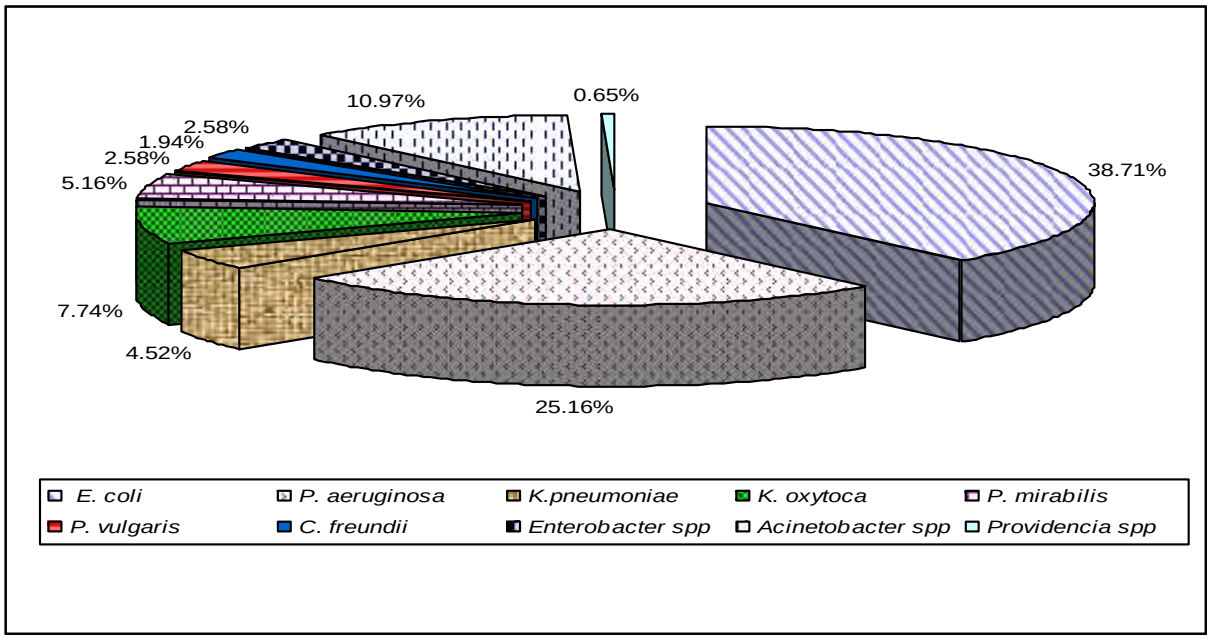

Figure 4: Pattern of distribution of Gram negative isolates from total pus samples

5.3.6 Pattern of microbial isolates in different pus samples

Out of 253 bacterial isolates, 210 bacteria were isolated from pus swab and 43 were from aspirated pus. Among Gram positive bacteria, *S. aureus* and among Gram negative, *E. coli* was the most common isolate in both the samples. The result is shown in table 8.

Table 8: Pattern of microbial isolates in different pus samples

S.N	Type of organism isolated	Total		Pus swab		Aspirated pus	
		No.	%	No.	%	No.	%
1	*S. aureus*	82	32.41	67	31.90	15	34.88
2	CONS	7	2.76	5	2.38	2	4.65
3	Non hemolytic streptococci	4	1.58	2	0.95	2	4.65

4	**haemolytic streptococci**	**2**	**0.79**	**2**	**0.95**	**-**	**-**
5	**unidentified Gram positive rods**	**3**	**1.18**	**3**	**1.42**	**-**	**-**
6	***E. coli***	**60**	**23.7**	**50**	**23.8**	**10**	**23.25**
7	***P. aeruginosa***	**39**	**15.41**	**35**	**16.66**	**4**	**9.3**
8	***K. pneumoniae***	**7**	**2.76**	**6**	**2.85**	**1**	**2.32**
9	***K. oxytoca***	**12**	**4.74**	**11**	**5.23**	**1**	**2.32**
10	***P. mirabilis***	**8**	**3.16**	**8**	**3.8**	**-**	
11	***P. vulgaris***	**4**	**1.58**	**3**	**1.42**	**1**	**2.32**
12	***C. freundii***	**3**	**1.18**	**2**	**0.95**	**1**	**2.32**
13	***Enterobacter* spp.**	**4**	**1.58**	**3**	**1.42**	**1**	**2.32**
14	***Acinetobacter* spp.**	**17**	**6.71**	**12**	**5.71**	**5**	**11.6**
15	***Providencia* spp.**	**1**	**0.39**	**1**	**0.47**	**-**	**-**
Total		**253**		**210**		**43**	

5.3.7 Pattern of microbial isolates in pus sample collected from different wards

In this study, pus samples were collected from different wards such as burn ward, surgical wards, post operative ward, orthopedic ward, medical wards, ENT, and other wards.

In burn ward, 26 organisms were isolated, out of which *S. aureus* (50%) was the most common, followed by *P. aeruginosa* (38.46%). In post operative ward (POW), 22 organisms were isolated, out of which *E. coli* (31.63%) was the most common, followed by *S. aureus* (22.72%), *P. aeruginosa* (13.63%) and *P. mirabilis* (9.09%). In male surgical ward (MSW), 46 organisms were isolated, out of which *E. coli* (30.43%) was the most common, followed by *S. aureus* (28.26%) and *Acinetobacter* spp. (8.69%). In female surgical ward (FSW), 44 organisms were isolated, out of which *S. aureus* (27.27%) was the most common, followed by *E. coli* (25%) and *P. aeruginosa* (22.72%). In neurosurgical ward (NSW), 26 organisms were isolated, out of which *S. aureus* (45.45%) was the most common, followed by *P. aeruginosa* (22.72%).In plastic surgery ward, only *S. aureus* was isolated. In urology, 6 organisms were isolated of which *E. coli* (33.33%) and *K. oxytoca* (33.33&) were common.

Similarly, 62 organisms were isolated from orthopedic ward of which *S. aureus* (32.25) and *E. coli* (30.64) were common. From male medical ward (MMW) and female medical ward (FMW), 7 and 8 organisms were isolated respectively, of which *S. aureu,* and *E. coli* were common. *S. aureus* and *K. pnemoniae* were common isolates from other wards like ENT, ICU and CTVS. The result is shown in tables 9(a) and 9(b).

Table 9(a): Pattern of microbial isolates in pus sample collected from burn ward, post operative ward and surgical wards

Types of organism	Burn		MSW		FSW		POW		Plastic Surgery		NSW		Urology	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. aureus	13	50	13	28.26	12	27.27	5	22.72	1	100	10	45.45	1	16.66
CONS	1	3.84	2	4.34	1	2.27	-	-	-	-	1	4.54	-	-
Non haemolytic streptococci	-	-	1	2.17	-	-	1	4.54	-	-	-	-	-	-
haemolytic streptococci	-	-	1	2.17	-	-	1	4.54	-	-	-	-	-	-
unidentified Gram positive rods	-	-	-	-	1	2.27	1	4.54	-	-	-	-	-	-
E. coli	-	-	14	30.43	11	25	7	31.81	-	-	2	9.09	2	33.33
P. aeruginosa	10	38.46	3	6.52	10	22.72	3	13.63	-	-	5	22.72	-	-
K. pneumoniae	-	-	-	-	-	-	1	4.54	-	-	2	9.09	-	-
K. oxytoca	1	3.84	2	4.34	-	-	-	-	-	-	1	4.54	2	33.33
P. mirabilis	-	-	3	6.52	3	6.81	2	9.09	-	-	-	-	-	-
P. vulgaris	-	-	1	2.17	1	2.27	1	4.54	-	-	-	-	1	16.66
C. freundii	-	-	-	-	-	-	-	-	-	-	1	4.54	-	-
Enterobacter spp.	-	-	2	4.34	-	-	-	-		-	-	-	-	-
Acinetobacter	1	3.84	4	8.69	4	9.09	-	-	-	-	-	-	-	-

spp.														
***Providencia* spp.**	**-**		**-**	**-**	**1**	**2.27**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total	**26**		**46**		**44**		**22**		**1**		**22**		**6**	

Table 9(b): Pattern of microbial isolates in pus sample collected from orthopedic ward, male and female medical wards, ENT, ICU and CTVS

Types of organism	**Orthopedic ward**		**Male medical**		**Female medical**		**ENT**		**ICU**		**CTVS**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
S. aureus	**20**	**32.25**	**2**	**28.57**	**2**	**25**	**1**	**25**	**1**	**50**	**1**	**33.3**
CONS	**2**	**3.22**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Non haemolytic streptococci	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
haemolytic streptococci	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**25**	**-**	**-**	**1**	**33.3**
unidentified Gram positive rods	**-**	**-**	**1**	**14.28**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
E. coli	**19**	**30.64**	**2**	**28.57**	**3**	**37.5**	**-**	**-**	**-**	**-**	**-**	**-**
P. aeruginosa	**7**	**11.29**	**-**	**-**	**1**	**12.5**	**-**	**-**	**-**	**-**	**-**	**-**

K. pneumoniae	**1**	**1.61**	**-**	**-**	**-**	**-**	**1**	**25**	**1**	**50**	**1**	**33.3**
K. oxytoca	**4**	**6.45**	**1**	**14.28**	**1**	**12.5**	**-**	**-**	**-**	**-**	**-**	**-**
P. mirabilis	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
P. vulgaris	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
C. freundii	**2**	**3.22**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
***Enterobacter* spp.**	**2**	**3.22**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
***Acinetobacter* spp.**	**5**	**8.06**	**1**	**14.28**	**1**	**12.5**	**1**	**25**	**-**	**-**	**-**	**-**
***Providencia* spp.**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total	**62**		**7**		**8**		**4**		**2**		**3**	

5.3.8 Correlation between direct smear Gram staining and culture

Out of direct smear Gram staining of 305 pus samples, 47 (15.40%) showed Gram positive cocci (GPC), 83 (27.21%) showed Gram negative bacilli (GNB) and 61 showed both Gram positive cocci (GPC) and Gram negative bacilli (GNB) while 114 showed no organism. On culture, GPC was seen in 51 (16.72%) cases, GNB on 85 (27.86%) cases and both on 58 (19.01%) cases while 108 showed no growth. Gram positive rods were isolated from 3 cases, which were assumed as contaminants. The calculation of correlation between direct smear Gram staining and culture are shown in appendix III. The correlation coefficient (r_{xy}) is 0.99. The result is shown in table 10.

Table 10: Correlation between direct smear Gram staining and culture

No. of pus samples	Type of bacteria	Results of direct smear Gram staining		Culture results	
		No.	%	No.	%
305	GPC	47	15.4	51	16.72
	GNB	83	27.21	85	27.86
	Both GPC and GNB	61	20	58	19.01
	Gram positive rods	-	-	3	0.98
	Organism not seen	114	37.37	108	35.4
Total		305		305	

5.4 ANTIBIOTIC SUSCEPTIBILITY PROFILE OF THE BACTERIAL ISOLATES

5.4.1 Antibiotic susceptibility profile of Gram positive isolates

Among 98 Gram positive isolates, the most sensitive antibiotic was Ofloxacin (70.40%) followed by Ciprofloxacin (65.32%), Cephalexin (63.26%), Cloxacillin (60.20%),

Chloramphenicol (55.10%), Erythromycin (53.06%) and Cotrimoxazole (44.89%). The least effective antibiotic was found to be Gentamicin (41.83%) and Ampicillin (39.79%). The result is shown in table 11.

Table 11: Antibiotic susceptibility profile of Gram positive isolates

Antibiotic used	Sensitive		Resistant		Total
	No.	%	No.	%	
Ofloxacin	**69**	**70.40**	**29**	**29.59**	**98**
Ciprofloxacin	**64**	**65.32**	**34**	**34.67**	**98**
Cephalexin	**62**	**63.26**	**36**	**36.73**	**98**
Cloxacillin	**59**	**60.20**	**39**	**39.79**	**98**
Chloramphenicol	**54**	**55.10**	**44**	**44.89**	**98**
Erythromycin	**52**	**53.06**	**46**	**46.93**	**98**
Cotrimoxazole	**44**	**44.83**	**54**	**55.10**	**98**
Gentamicin	**41**	**41.83**	**57**	**58.16**	**98**
Ampicillin	**39**	**39.79**	**59**	**60.20**	**98**

5.4.2 Antibiotic susceptibility profile of Gram negative isolates

Among 155 Gram negative isolates, the most sensitive antibiotic was Ciprofloxacin (69.03%) followed by Ofloxacin (63.22%), Chloramphenicol (53.54%), Gentamicin (40.64%), Amikacin (28.38%) and Cotrimoxazole (26.45%). The least effective antibiotic was found to be Ampicillin (12.90%) and Cephalexin (15.48%). The result is shown in table 12.

Table 12: Antibiotic susceptibility profile of Gram negative isolates

Antibiotic used	Sensitive		Resistant		Total
	No.	%	No.	%	
Ciprofloxacin	107	69.03	48	30.96	155
Ofloxacin	95	63.22	60	38.70	155
Chloramphenicol	83	53.54	72	46.45	155
Gentamicin	63	40.64	92	59.35	155
Amikacin	44	28.38	111	71.61	155
Cotrimoxazole	41	26.45	114	73.54	155
Cephalexin	24	15.48	131	84.51	155
Ampicillin	20	12.90	135	87.09	155

5.4.3 Antibiotic susceptibility profile of the bacterial isolates as a whole

Among 253 bacterial isolates, the most effective antibiotic was Ciprofloxacin (67.58), followed by Ofloxacin (64.82%), Chloramphenicol (54.15%), Gentamicin (40.31%), Cephalexin (33.99%) and Cotrimoxazole (33.59%). The least effective antibiotic was found to be Ampicillin (75.88%). The result is shown in figure 5.

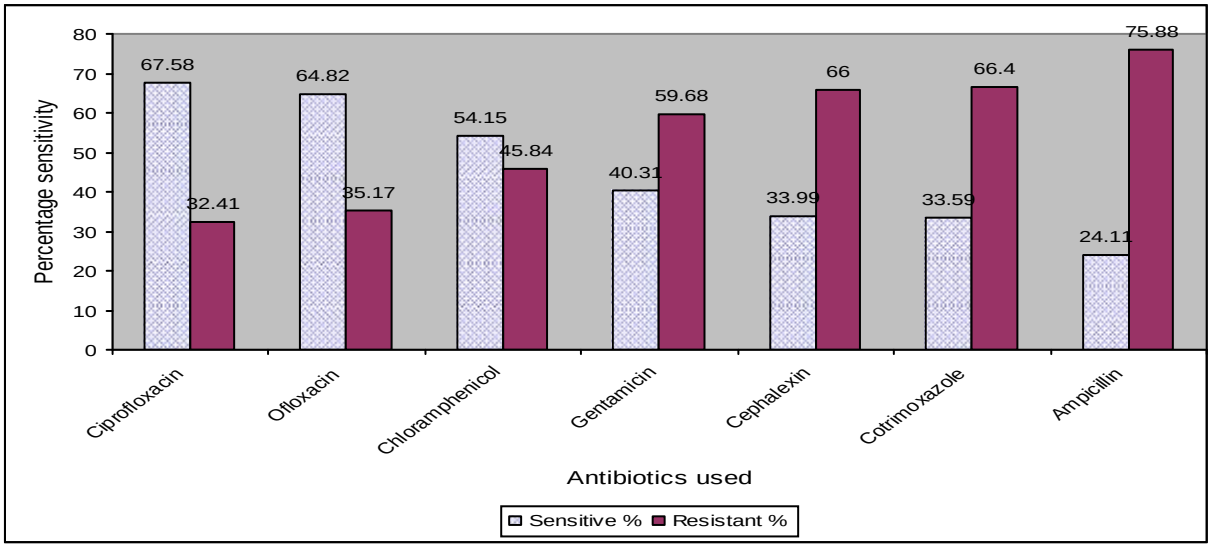

Figure 5: Antibiotic susceptibility profile of the bacterial isolates as a whole

5.4.4 Antibiotic susceptibility profile of *S. aureus* only

For *S. aureus*, the most sensitive antibiotic was Ofloxacin (69.51%) followed by Ciprofloxacin (63.41%), Cephalexin (60.97%), Cloxacillin (60.97%), Chloramphenicol (54.87%), Erythromycin (52.43%) and Cotrimoxazole (43.90%). The least effective antibiotic was found to be Gentamicin (37.8%) and Ampicillin (40.24%). The result is shown in figure 6.

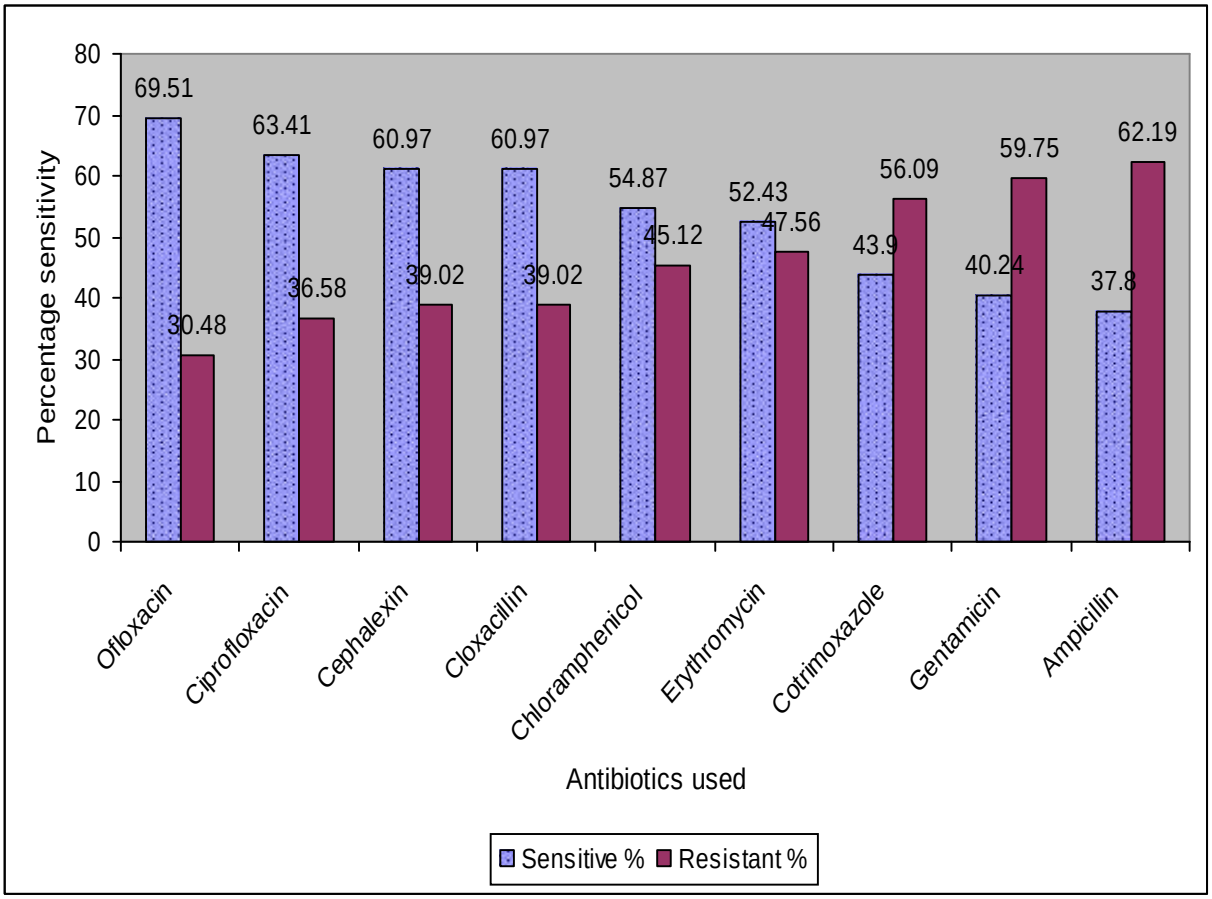

Figure 6: Antibiotic susceptibility profile of *S. aureus* only

CHAPTER 6

6. DISCUSSION

Wound infection is an ongoing problem for many patients. Infected wounds can cause great distress in terms of associated morbidity and mortality, increased length of hospital admission, delayed wound healing and increased discomfort and have long been known to increase healthcare cost significantly. Wound infection is one of the most common nosocomial infections among surgical patients.

This study was conducted with an aim to identify the etiological agents causing wound infection with their antibiotic susceptibility pattern at Bir Hospital among inpatients. In this study 305 pus samples from different types of wounds were collected and processed. The etiological agents were identified by culture and biochemical tests and their susceptibility pattern to commonly used antibiotics were determined. Pus swabs collected were high in number than aspirated pus. The male patients were high in number than females and majority of patients belonged to age group 21-30. The results obtained were tabulated in the previous chapter. In this chapter, the results are discussed and compared with the findings of other investigators.

6.1 Distribution of samples and pattern of results

6.1.1 Nature of pus samples and pattern of growth

Out of total 305 pus samples collected, 251 (82.29%) samples were pus swabs and 54 (17.70%) were aspirated pus. Out of 305 samples studied, 197 (64.45%) samples showed growth and 108 (35.90%) samples showed no growth. Among 251 pus swab, 163 (64.94%) and among 54 cases of aspirated pus, 34 (62.96%) showed the growth.

In a similar study carried out in Nepal by Gongal *et al* in 1994 on post operative wound infection at Bir Hospital, Nepal, it was found that 90% of wound swab specimens and 80% of drained pus showed microbial growth. However, Sa *et al* (1984) reported that overall rate of

wound infection was 18.92% (53 of 280 cases). Similarly, 20% rate of wound infection was reported by Agarwal (1972), 25% by Rao and Harsha (1975), 22.3% by Venkatraman *et a1* (1978), 16.9% by Shaw *et al* (1973) and 10.19% by Shrivastava *et al* (1969). Cruse and Foord (1973), however, in a prospective study of 23,649 surgical wounds reported an infection rate of only 4.8%.

6.1.2 Age and gender wise distribution of patients

Out of 305 cases, 185 (60.65%) were male patients and 120 (39.34%) were female patients. The growth was found to be higher in male patients (65.40%) than in female patients (63.33%). A similar study carried out by Parajuli (1997) at TUTH showed that pus samples were collected more from male patients 119 (55.3%) than female patients 96 (44.6%).

In our study pus samples were collected from patients ranging in age from 5 to 92 years old. In all age groups, male patients were affected more frequently than female patients. Therefore wound infection is higher in males than in female patients. The highest number of patients belonged to age group (21-30) years followed by age group (31-40) years and (11-20) years. Children (age gr. 0-10) and patients with age above 80 years were relatively found to be less affected. Our finding agrees with the study carried out by Parajuli (1997) and Tuladhar (1999) where it was found that patients with age groups (21-30) years were more susceptible to different types of wound infections. However, Sa *et al* (1984) reported that the rate of wound infection increased with increasing age, being maximum in the age group 51-70 years and the infection rate in males and females was almost similar, being 19.1% in the former and 17.64% in the latter.

6.1.3 Pattern of single and multiple isolates

Out of total pus samples, 197 samples showed growth; of which 135 (68.52%) cases showed single isolates and 62 (31.47%) cases showed multiple isolates. The single isolate was higher than multiple isolates in both pus swab and aspirated pus. The result agrees with the study carried out by Gongal *et al* (1994) where single isolates were seen in 78.3% cases and mixed isolates in 21.6% cases. Similarly in the study conducted by Giacometti *et al* (2000) including

676 surgery patients, bacterial pathogens were isolated from 614 individuals in which single etiologic agent was identified in 271 patients, multiple agents were found in 343 and no agent was identified in 62. However, Sa *et al* (1984) reported that mixed infections accounted for the maximum number of infected cases.

If only one type of organism is isolated from the specimen, it is considered as single isolate where as if more than one organism is isolated, it is considered as multiple isolates. Single isolates is assumed to be the only invading microbe whereas in mixed infection, if pyogenic organisms are found, they are usually considered to be the primary cause of infection and rest organisms are considered as the secondary invaders.

6.2 Pattern of microbial isolates

6.2.1 Frequency of Gram positive and Gram negative isolates

In this study, a total of 253 bacterial species were isolated of which 155 (61.2%) were Gram negative and 98 (38.8%) were Gram positive bacteria. Among Gram positive bacteria, *S. aureus* (83.67%) was most common followed by CONS (7.14%), non haemolytic streptococci (4.08%), unidentified Gram positive rods (3.06%) and haemolytic streptococci (2.04%). Among Gram negative bacteria, *E. coli* (38.7%) was most common followed by *P. aeruginosa* (25.16%), *Acinetobacter* spp. (10.96%), *K.* oxytoca (7.74%), *P. mirabilis* (5.16%), *K. pneumoniae* (4.51%), *P. vulgaris* (2.58%), *Enterobacter* spp. (2.58%), *C. freundii* (1.93%) and *Providencia* spp. (0.64%). Therefore in this study the frequency of Gram negative bacteria was higher than Gram positive bacteria.

However, a similar study carried out by Shrestha (1995) showed the higher prevalence of Gram positive bacteria (73.74%) than Gram negative bacteria (26%). The higher prevalence of Gram positive bacteria were also depicted in researches carried out by Tuladhar (1999) and Parajuli (1997) at TUTH. But while considering the indoor patients in their studies, the rate of Gram negative bacteria were found to be higher than Gram positive bacteria. These studies support the fact that Gram negative bacteria are more prevalent than Gram positive bacteria in

indoor patients and that Gram negative bacteria are isolated in higher proportion than Gram positive bacteria in case of hospital acquired infection (HAI). Banjare (2002) at TUTH showed that high rate of Gram negative bacteria are found in HAI. In the same way Gongal *et al*, 1994 reported that majority of the organisms causing wound infection in post operative patients in Bir Hospital were Gram negative bacteria (75%). Therefore, Gram negative bacteria are one of the most problematic nosocomial pathogens, associated with HAI.

Altogether, 15 different bacterial species were isolated from pus samples in our study with *S. aureus* (32.41%) being the most prominent followed by *E. coli* (23.7%) and *P. aeruginosa* (15.41%).

Abussaud (1996) found that in a total of 2,331 wounds examined, one hundred and ninety-three of them were infected (8%) and 283 strains were isolated from the infected wounds. Thirty five percent of these strains were identified as *S. aureus*, 31% as *E. coli*, 25% as *P. aeruginosa* and 10% as *Klebsiella* spp. The prevalence of *S. aureus* was found in various other studies. In researches carried out by Tuladhar (1999) and Parajuli (1997) at TUTH, *S. aureus* was most commonly isolated. *S. aureus* are ubiquitous and are the most common cause of localized suppurative lesions in human beings.

CONS was prominent after *S. aureus*, constituting 7.14% of total Gram positive isolates. In a study carried out by Shah *et al* (1997) at TUTH, Nepal, 11.43% of CONS was reported from pus samples.

In this study *Streptococcus* spp. constituted 6.12% of total Gram positive isolates. *Streptococcus* spp. are commonly found in the throat and in the skin and may cause infections that may range from mild to severe and even life threatening.

E. coli was the second principal isolate after *S. aureus*, constituting 38.7% of total Gram negative isolates. Similar result was found in the study conducted by Gongal *et al* (1994) in Nepal where *E. coli* (23%) was the most common isolate. *E. coli* is commonly associated with

abdominal wounds and abscess. It is more common in wounds following operations of urinary tract and close to perineum.

The third predominant organism in this study was *P. aeruginosa* constituting 25.16% of total Gram negative bacteria. This agrees with the study done by Tuladhar (1999) and Shrestha (1995) at TUTH. *P. aeruginosa* is one of the most troublesome agents causing nosocomial infection. It may cause suppurative otitis, localized or generalized infections in human. It is most common and most serious cause of infection in burns.

In this study, although the attempts were made to isolate anaerobes, no any anaerobes were isolated.

6.2.2 Pattern of microbial isolates in burn ward

22 samples were collected from burn ward, out of which 20 (90.90%) samples showed growth; 14 samples showed single isolates while 6 samples gave multiple isolates. In burn ward, 26 organisms were isolated, of which *S. aureus* (50%) was the most common, followed by *P. aeruginosa* (38.46%). The growth was found to be highest in burn ward (90.90%) than any other wards. Infection is a major complication in burn wounds and it is estimated that up to 75% of deaths following burn injury are related to infection.

Singh *et al* (2003) in their retrospective study of bacterial isolates from the wounds of patients admitted to burns unit, at Guru Tegh Bahadur Hospital, Delhi, over a period of 5 years reported *Pseudomonas* spp. (31%) to be the most common pathogen in the burn unit. Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts, many studies (Bariar *et al*, 1997; Lawrence, 1985; Mayhall, 1993; Revathi *et al*, 1998; Vindenes and Bjerknes, 1995) have reported the prevalence of aerobes such as *P. aeruginosa*, *S. aureus, E. coli*, *Klebsiella* spp. and *Enterococcus* spp.

6.2.3 Pattern of microbial isolates in surgical wards

From surgical wards, altogether 143 pus samples were collected; 52 from MSW, 54 from FSW, 27 from NSW, 3 from plastic surgery and 7 samples from urology ward. From male surgical ward (MSW), 46 organisms were isolated, of which *E. coli* (30.43%) was the most common, followed by *S. aureus* (28.26%) and *Acinetobacter* spp. (8.69%). In female surgical ward (FSW), 44 organisms were isolated, of which *S. aureus* (27.27%) was the most common, followed by *E .coli* (25%) and *P. aeruginosa* (22.72%). In neurosurgical ward (NSW), only 26 organisms were isolated, out of which *S. aureus* (45.45%) was the most common, followed by *P. aeruginosa* (22.72%). In plastic surgery ward, only *S. aureus* was isolated. In urology, 6 organisms were isolated of which *E. coli* (33.33%) and *K. oxytoca* (33.33%) were common followed by *S. aureus* (16.66%) and *P. vulgaris* (16.66%).

In the study conducted by Tuladhar (1999) at TUTH, Kathmandu, the predominant organisms in surgical wards were *E. coli* (31.7%) followed by *S. aureus* (29.2%) and *P. aeruginosa* (10%). In a prospective study of postoperative wound infection carried out over a 12-months period by Twum *et al* (1992) in which 1770 wounds studied, 167 (9.4%) became infected. Wound infection rates, according to clinical wound types, were clean 5.9%, clean-contaminated 10.7%, contaminated 24.3% and dirty 52.9%. The figures according to microbiological wound types were clean 4.7%, and potentially, lightly and heavily contaminated 15.3%, 22.1% and 30.2% respectively. In their study, the common causative organisms were *S. aureus* (23.7%), *E. coli* (16.9%), *S. epidermidis* (13.5%) and *P. aeruginosa* (13.0%). In a study of the aerobic and anaerobic microbiology of surgical-site infections (SSI) following spinal fusion at the Naval Hospital in Bethesda by Brook and Frazier (1999) from 1980 to 1992; aspirates of pus from 25 infection sites showed bacterial growth. Aerobic bacteria only were recovered from 9 (36%) specimens, anaerobic bacteria only were recovered from 4 (16%) and mixed aerobic and anaerobic bacteria were recovered from 12 (48%). The predominant aerobes were *E. coli* (n=8), *Proteus* spp. (n=7), *P. aeruginosa* (n=5), *Enterococcus* spp. (n=4), *K. pneumoniae* (n=3) and *S. aureus* (n=3). The predominant anaerobes recovered were *Bacteroides fragilis* (n=9) and *Peptostreptococcus* spp. (n = 6).

Similarly in the study of Giacometti *et al* (2000) which included 676 surgery patients with signs and symptoms indicative of wound infections, who presented over the course of 6 years, the common pathogens isolated from 614 individuals were *S. aureus* (191 patients, 28.2%), *P. aeruginosa* (170 patients, 25.2%) and *E. coli* (53 patients, 7.8%).

6.2.4 Pattern of microbial isolates in post operative ward (POW)

From post operative ward (POW), 20 samples were collected out of which 16 showed growth with 22 different organisms isolated, of which *E. coli* (31.63%) was the most common, followed by *S. aureus* (22.72%), *P. aeruginosa* (13.63%) and *P. mirabilis* (9.09%).

Similarly, in a study conducted by Murthy *et al* (1998) at Karturba Medical College, Manipal, *S. aureus* (32%) and *Pseudomonas* spp. (21%) were common organism recovered from post operative wound infection. However, Shaw *et al* (1973) reported that 72.3% post-operative wound infections were caused by Gram negative bacilli and 28% due to *S. aureus* alone. In a similar study done by Gongal *et al* (1994) in Nepal; *E. coli* was found to be the most common isolate (23%) causing wound infection in post operative patients. In a study carried out by Oguntibeju and Nwobu (2004), out of the 60 bacterial isolates found in post-operative wound infection, 20 (33.3%) were *P. aeruginosa* followed by *S. aureus* 13 (21.7%), *Klebsiella* spp. 10 (16.7%), *E. coli* 7 (11.7%), atypical coliform 4 (6.7%), *Proteus* species 4 (6.7%), *Streptococcus pyogenes* 1 (1.7%) and *Enterococcus faecalis* 1 (1.7%) in that order. Onche and Adedeji (1994) recruited 254 patients of which 19 had post-operative wound infection. The infection rate was 7.5%. Thirty-six bacterial isolates were recovered. *S. aureus* was the most common 16 cases (44%) followed by *Bacteroides fragilis* 4(11%), *E. coli* 4(11%), *Proteus* spp. 4 (11%). Others were *Pseudomonas* spp., *Klebsiella* spp. and *Peptostreptococcus*.

6.2.5 Pattern of microbial isolates in orthopedic wards

From orthopedic ward, 70 pus samples were collected, out of which 50 samples (71.4%) showed growth. The number of organisms isolated was 62, of which *S. aureus* (32.25) and *E. coli* (30.64) were most common followed by *P. aeruginosa* (11.29%) and *Acenetobacter* spp. (8.06%). However in the study done by Gongal *et al* (1994) in Nepal, *Klebsiella* spp. and

Proteus spp. were common in traumatic wounds. In study carried out by Parajuli (1997) at TUTH, the predominating organisms isolated from orthopedic ward were *P. aeruginosa* (9.83%), *S. aureus* (3.28%), *Proteus* spp. (3.28%) and *Klebsiella* spp. (1.63%).

A retrospective survey of 703 patients undergoing elective orthopaedic surgery in the year 1991 was carried out in Malaysia by Dhillon *et al*, who reported an overall post-operative infection rate of 6.8% and a deep infection rate of 3.3%. Haider *et al* (1996) carried out a prospective study to determine the prevalence and role of anaerobic bacteria in pyogenic osteomyelitis. In their study, anaerobic organisms were isolated from 39 cases, out of 134 cases (29%). The total number of aerobic and anaerobic isolates was 224, of which 50 were anaerobes (22%).

6.2.6 Pattern of microbial isolates in medical wards and other wards

Out of 19 and 16 pus samples collected from male medical ward (MMW) and female medical ward (FMW) respectively, 7 and 8 organisms were isolated respectively, of which *S. aureus*, and *E. coli* were common. Similarly, the study conducted by Tuladhar (1999) at TUTH, Kathmandu, observed that *S. aureus* (60%) was the most common isolate followed by *E. coli* (20%) and *Bacillus* spp. (10%) from medical wards. In this study *S. aureus* and *K. pneumoniae* were the common isolates from other wards like ENT, ICU and CTVS.

6.3 Correlation between direct smear Gram staining and culture

Out of direct smear Gram staining of 305 pus samples, 47 (15.40%) showed Gram positive cocci (GPC), 83 (27.21%) showed Gram negative bacilli (GNB) and 61 showed both Gram positive cocci (GPC) and Gram negative bacilli (GNB) while 114 showed no organism. On culture, GPC was seen in 51 (16.72%) cases, GNB on 85 (27.86%) cases and both on 58 (19.01%) cases while 108 showed no growth. Gram positive rods were isolated from 3 cases, which were assumed as contaminants. Hence, direct smear Gram staining and culture were correlated ($r_{xy} = 0.99$). Similarly, the study conducted by Tuladhar (1999) at TUTH, showed Gram stain result positively correlated with 95% of culture results.

In certain emergency cases where patients has to be treated immediately without waiting for time consuming culture result and also in the situation where facilities for culture are unavailable, the results of direct smear Gram staining prove to be very helpful. Initial Gram staining result can guide the clinician in selecting an empirical antimicrobial therapy.

6.4 Antibiotic susceptibility profile of the bacterial isolates

In this study, the antibiotic sensitivity test was performed for 253 bacterial isolates. The antibiotic discs used were Ciprofloxacin, Ofloxacin, Chloramphenicol, Gentamicin, Cephalexin, Erythromycin, Cotrimoxazol and Ampicillin for all the bacterial isolates. Cloxacillin was used only for Gram positive isolates and Amikacin was used only for Gram negative isolates.

Among the antibiotics used for the bacterial isolates as a whole, the most effective antibiotic was found to be Ciprofloxacin with a susceptibility of 67.58%, followed by Ofloxacin (64.82%), Chloramphenicol (54.15%), Gentamicin (40.31%), Cephalexin (33.99%) and Cotrimoxazole (33.59%). The least effective antibiotic was found to be Ampicillin (75.88%). However, Tuladhar (1999) reported that Gentamicin was found to be most effective (81.6%) drug followed by Ciprofloxacin (73.2%), Cephalexin (64.8%), Cotrimoxazole (58.7%) and Ampicillin (12.9%). However, Sa *et al* (1984) reported that Gentamicin was found to be the most effective antimicrobial drug tested, to which 87.57% of organisms were sensitive. Cloxacillin was very effective against staphylococci and streptococci, 93% being sensitive.

In our study, among Gram positive isolates, the most sensitive antibiotic was Ofloxacin (70.40%) followed by Ciprofloxacin (65.32%), Cephalexin (63.26%), Cloxacillin (60.20%), Chloramphenicol (55.10%), Erythromycin (53.06%) and Cotrimoxazole (44.89%). The least effective antibiotic was found to be Gentamicin (41.83%) and Ampicillin (39.79%). However, Tuladhar (1999) reported that Gentamicin was found to be most effective (89.53%) drug followed by Ciprofloxacin (83.72%) while only 16.27% of Gram positive cocci were sensitive to Ampicillin.

Among 155 Gram negative isolates, the most sensitive antibiotic in our study was Ciprofloxacin with sensitivity of 69.03% followed by Ofloxacin (63.22%), Chloramphenicol (53.54%), Gentamicin (40.64%), Amikacin (28.38%) and Cotrimoxazole (26.45%). The least effective antibiotic was found to be Ampicillin (12.90%) and Cephalexin (15.48%). In the study by Gongal *et al* (1994), less than 10% of Gram negative isolates were sensitive to Ampicillin which is more or less similar to the result of this study. Similarly, Parajuli (1997) reported that Gram negative organisms isolated from pus samples was 86% resistant to Ampicillin.

In a study carried out by Oguntibeju and Nwobu (2004), the sensitivity pattern of *P. aeruginosa* isolated from post-operative wound showed that it was sensitive to Gentamicin, but was resistant to Ampicillin and Cotrimozaxole.

For *S. aureus*, the most sensitive antibiotic in our study was Ofloxacin (69.51%) followed by Ciprofloxacin (63.41%), Cephalexin (60.97%), Cloxacillin (60.97%), Chloramphenicol (54.87%), Erythromycin (52.43%) and Cotrimoxazole (43.90%). The least effective antibiotic was found to be Gentamicin (37.8%) and Ampicillin (40.24%). Gongal *et al* (1994) reported that 61.9% of *S. aureus* was sensitive to Cloxacillin (85.7%), Gentamicin (85.7%), Cephalexin (61.9%), Erythromicin (59.5%) and Chloramphenicol (64%). Abussaud (1996), found that the most effective antibiotic against *S. aureus* was Chloramphenicol (78%) followed by Gentamicin (63%).

In study of Onche and Adedeji (1994), Cephalosporins were found to be most potent against *S. aureus* as 10 of 17 cultures (58.8%) responded favourably to Cephalexin. The traditionally potent Cloxacillin was completely resisted by it. This is because of the emergence of highly resistant strains of *S. aureus* in hospital setting. The Gram-negative aerobic rods like *E. coli*, *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp. were found to be sensitive to Gentamicin while essentially resistant to the Cephalosporins tested.

CHAPTER 7

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

The overall result of this study can be summarized as follows:

1) Out of total 305 samples collected, 251 (82.29%) samples were pus swabs and 54 (17.70%) were aspirated pus. 163 (64.94%) pus swab and 34 (62.96%) aspirated pus, showed growth while out of 305 samples studied, 197 (64.45%) samples showed growth.
2) Out of 197 samples with growth; 139 (70.55%) samples showed single isolate and 58 (29.44%) showed multiple isolates.
3) Out of 305 cases, 185 (60.65%) were male patients and 120 (39.34%) were female patients. The growth was found to be higher in male patients (65.40%) than in female patients (63.33%).
4) In all age group, male patients (n=185) were affected more frequently than female patients (n=120). The highest number of patients belonged to age group 21-30 (n=76) followed by age group 31-40 (n=60) and 11-20 (n=58).The children (age gr. 0-10) and patients with age above 80 years were relatively found to be less affected.
5) A total of 253 bacterial species were isolated of which the predominant bacteria were Gram negative bacteria (n=155) constituting 61.2% while Gram positive bacteria (n=98) represented 38.8% of total bacterial isolates.
6) All together 15 different species of bacteria were isolated. Among Gram positive bacteria, *S. aureus* (82/253) was found to be the most predominant isolate representing 32.4% of total bacterial isolates and among Gram negative bacteria *E. coli* (60/253) was most predominant isolate representing 23.7% of total bacterial isolates.
7) The growth was found to be highest in burn ward (90.90%), followed by post operative ward (80%) and lowest was in plastic surgery ward (33.33%).
8) Out of 253 bacterial isolates the maximum number of organisms were isolated from orthopedic ward (n=62) representing 24.50% of total bacterial isolates, followed by MSW 46 (8.18%), FSW 44 (17.39%), burn 26 (10.27%) etc.

9) From orthopedic ward, *S. aureus* and *E. coli* were most predominant representing 32.25% and 30.64% of total bacterial isolates respectively. *E. coli* representing 30.43% was common from MSW and *S. aureus* (27.27%) was common from FSW. In burn ward, *S. aureus* was most predominant representing 50% followed by *P. aeruginosa* (38.46%).
10) Direct smear Gram stain result positively correlated with culture results (r_{xy} = 0.99).
11) Among Gram positive isolates, Ofloxacin (70.40%) and among Gram negative isolates, Ciprofloxacin (69.03%) was the most sensitive antibiotic. Among 253 bacterial isolates, the most effective antibiotic was Ciprofloxacin (67.58) while the least effective antibiotic was found to be Ampicillin (75.88%).
12) For *S. aureus,* the most sensitive antibiotic was Ofloxacin (69.51%) while the least effective antibiotic was found to be Gentamicin (37.8%) and Ampicillin (40.24%).

7.2 RECOMMENDATIONS

1) In this study, both pus swab and aspirated pus samples from wound infections showed growth. So, both samples are benificial for analyzing wound infection.
2) This study shows that Gram negative bacteria are more common than Gram positive bacteria in indoor patients with wound infection. Among Gram positive bacteria, *S. aureus* and among Gram negative bacteria, *E. coli* was found to be the most predominant isolates respectively. So, both should be considered as serious problem. Anaerobes were not isolated which indicate that anaerobes might not be involved in wound infection.
3) The growth was found to be highest in burn ward (90.90%) than any other wards as infection is a major complication in burn wounds.
4) Direct smear Gram staining and culture results were correlated which indicate that direct smear Gram stain can be applied in certain emergency cases.
5) Among Gram positive isolates and among Gram negative isolates, the most sensitive antibiotics were Ofloxacin and Ciprofloxacin respectively and among 253 bacterial isolates as a whole, the most effective antibiotic was Ciprofloxacin while the least effective was found to be Ampicillin. So, proper antibiotics should be used.

CHAPTER 8

8. REFERENCES

Abussaud MJ (1996) Incidence of wound infection in three different departments and the antibiotic sensitivity pattern of the isolates in a Saudi Arabian hospital. Acta. Microbiol. Immunol. Hung. 43(4):301-305

Agarwal SL (1972) Study of post-operative infections. Ind. J. Surg. 34:314-320

Armstrong DG, Liswood PJ and Todd WF (1995) Prevalence of mixed infections in the diabetic pedal wound. In: A retrospective review of 112 infections. J. Am. Pediatr. Med. Assoc. 85:533-537

Augustin M and Maier K (2003) Psychosomatic aspects of chronic wounds. Dermatology and Psychosomatics 4:5-13

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

Babb JR, Davies JG and Ayliffe GAJ (1983) Contamination of protective clothing and nurses uniforms in isolation ward. J. Hosp. Infect. (4):149-157

Banjara MR (2002) Study of air, water and wound infection in different wards of TUTH. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Bariar LM, Vasenwala SM, Malik A, Ansari GH and Chowdhury TE (1997) A clinicopathological study of infections in burn patients and importance of biopsy. J. Ind. Med. Soc. 95:573-575

Bauer AV, Kirby WM, Sherris JC and Turck M (1996) Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clinic. Pathol. 45:493-496

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

Beutner KR (1996) Human papillomavirus infection of the vulva. Semin Dermatol. 15:2-7

Boulton AJ, Meneses P and Ennis WJ (1999) Diabetic foot ulcers a framework for prevention and care. J. Wounds 7:7-16

Bowler P, Duerden B and Armstrong D (1999) Wound microbiology and associated approaches to wound management. Clin. Microbiol. Rev. 14(2):244-269

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

Bowler PG (1999) The prevalence and significance of anaerobic bacteria in wounds. M. Phil. thesis, University of Wales, College of Medicine, Cardoff, United Kingdom

Bowler PG and Davies BJ (1999) The microbiology of infected and non infected ulcers. Int. J. Dermatol. 38:101-106

Boyd FR and Hoerl GB (1981) Basic Medical Microbiology. 2nd edition, Little Brown and Company, Boston pp 310-423

Brachman PS, Dan BB, Haley RW, Hooten TM, Garner JS and Allen JR (1989) Nosocomial surgical infections: Incidence and cost. Surg. Clinics. N. Am. 60:15-25

Brook I (1987) Microbiology of human and animal bite wounds in children. Pediatr. Infect. Dis. J. 6:29-32

Brook I and Frazier EH (1999) Aerobic and anaerobic microbiology of surgical-site infection following spinal fusion. J. Clin. Microbiol. 37(3):841–843

Brook I and Frazier EH (1990) Aerobic and anaerobic bacteriology of wounds and cutaneous abscesses. Arch. Surg. 125:1445-1451

Brook I and Frazier EH (1998) Aerobic and anaerobic microbiology of chronic venous ulcers. Int. J. Dermatol. 37:426-428

Brook I and Randolph JG (1981) Aerobic and anaerobic bacterial flora of burns in children. J. Trauma 21:313-318

Brown DL and Smith DJ (1999) Bacterial colonisation/infection and the surgical management of pressure ulcers. Ostomy Wound Management 45:119S-120S

Calvin M (1998) Cutaneous wound repair. J. Wounds 10(1):12-32

CDC (Centers for Disease Control and Prevention) (1996) Prevent antimicrobial resistance in health care settings

Chakraborty P (2003) A text book of microbiology. 2nd edition, New Central Book Agency Pvt. Ltd., Kolkota, India pp 690-692

Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K, Chi JT, Rijn M, Botstein D and Brown PO (2004) Gene expression signature of fibroblast serum response predicts human cancer progression: Similarities between tumors and wounds. Public Library of Science 2(2):32-48

Cheesbrough M (2000) District laboratory practice in tropical countries. Part 2, Cambridge University Press, London pp 26-196

Collee JG, Duguid JP, Fraser AG, Marmion BP and Simmons A (1996) Laboratory strategy in the diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP and Simmons A (eds.) Mackie and McCartney practical medical microbiology. 14th edition, Churchill Livingstone pp 53-94

Collee JG, Miles RS and Watt B (1996) Test for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP and Simmons A (eds.) Mackie and McCartney practical medical microbiology. 14th edition, Churchill Livingstone pp 131-149

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

Cooper R and Lawrence JC (1996) The isolation and identification of bacteria from wounds. J. Wound Care 5(7):335-340

Cooper R, Kingsley A and White R (2003) Wound infection and microbiology. Ostomy Wound Management 49(7A):1-7

Cruse PJ and Foord R (1980) The epidemiology of wound infection: A ten-year prospective study of 62,939 wounds. Surg. Clinics. N. Am. 60(1):27-40

Cruse PJ and Foord R (1973) A 5-year prospective study of 23,649 surgical wounds. Arch. Surg. 107:206-210

Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvis WR and Emori TG (1991) Surgical wound infection rates by wound class, operative procedure and patient risk index. National nosocomial infections surveillance system. 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

Dagan R and Bar DY (1992) Double-blind study comparing erythromycin and mupirocin for treatment of impetigo in children: Implications of a high prevalence of erythromycin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. 36:287-290

Davis MH, Dunkley P, Harden RM, Harding K, Laidlaw JM, Morris AM and Wood RAB (1992) Cause: Types of wound and ulcers. The Wound Programme Centre for Medical Education, Dundee, United Kingdom pp 109-132

Dealey C (1999) Care of wounds. 2nd edition, Blackwell Science, Oxford pp 256

Dhillon KS and Kok CS (1995) The incidence of post-operative wound infection in orthopaedic surgery. Med. J. Malaysia 50(3):237-240

Di Rosa E and Panichi G (1996) Anaerobic bacteria in postsurgical infections: Isolation rate and antimicrobial susceptibility. J. Chemother. 8:91-95

Diamantopoulos EJ, Haritos D, Yfandi G, Grigoriadou M, Margariti G, Paniara O and Raptis SA (1998) Management and outcome of severe diabetic foot infections. Exp. Clin. Endocrinol. Diabetes 106:346-352

Falanga V (2005) Wound healing. J. Mol. Biol. 345(5):1083-1097

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

Ferraz EM, Bacelar TS, Aguiar JL, Ferraz AA, Pagnossin P and Batista JE (1992) Wound infection rates in clean surgery: A potentially misleading risk classification. Infect. Cont. Hosp. Epidemiol. 13(8):457-462

Fleischer GR (1999) The management of bite wounds. N. Engl. J. Med. 340:138-140

Forbes BA, Sahm DF and Weissfeld AS (2002) Bailey and Scott's diagnostic microbiology. 11th edition, Mosby Inc., USA pp 972-982

Franco EL (1996) Epidemiology of anogenital warts and cancer. Obstet Gynecol. Clin. N. Am. 23:597-623

Garg HG (2000) Scarless wound healing. New York Marcel Dekker Inc. 6:213-226

Gerding DN (1995) Foot infections in diabetic patients: The role of anaerobes. Clin. Infect. Dis. 20:S283-S288

Giacometti A, Cirioni O, Schimizzi AM, Delprete MS, Barchiesi F, Errico MMD, Petrelli E and Scalise G (2000) Epidemiology and microbiology of surgical wound infections. J. Clinic. Microbiol. 38(2):918-922

Gilchrist B (2000) Taking a wound swab. Nurs. Times 96(4):2

Goldstein EJ (1998) New horizons in the bacteriology, antimicrobial susceptibility and therapy of animal bite wounds. J. Med. Microbiol. 47:95-97

Gongal ND, Shrestha MB, Gurubacharya LV and Shrestha J (1994) Pattern of post operative wound infection and their antibiotic sensitivity pattern in Bir Hospital. J. Nep. Med. Assoc. 32:231-236

Greenhalgh DG (1998) The role of apoptosis in wound healing. Int. J. Biochem. and Cell Biol. 30(9):1019-1030

Griegon RD, Rosen T, Orengo IF and Wolf JE (1995) Dog, cat and human bites: A review. J. Am. Acad. Dermatol. 33:1019-1029

Haider A, Mousa L, Sundus S, Bakr R and Thamer AH (1996) Anaerobic osteomyelitis. Eastern Mediter. Health J. 2(3):494-500

Hansson C, Hoborn J, Moller A and Swanbeck G (1995) The microbial flora in venous leg ulcers without clinical signs of infection. Acta. Dermatol. Venereol. (Stockh.) 75:24-30

Heinzelmann M, Scott M and Lam T (2002) Factors predisposing to bacterial invasion and infection. Am. J. Surg. 183(2):179-190

Horan TC, Gaynes RP, Martone WJ, Jarvis WR and Emori TG (1992) CDC definations of nosocomial surgical site infections: A modification of CDC definitions of surgical wound infections. Am. J. Infect. Cont. 20:271-274

Johnson S, Lebahn F, Peterson LR and Gerding DN (1995) Use of an anaerobic collection and transport swab device to recover anaerobic bacteria from infected foot ulcers in diabetics. Clin. Infect. Dis. 20:S289-S290

Johnson JT (1991) Role of anaerobic bacteria in postoperative wound infections following oncologic surgery of the head and neck. Ann. Otol. Rhinol. Laryngol. 15(4):46-48

Jones V and Milton T (2000) When and how to use iodine dressings? Nurs. Times 96(45):2-3

Karchme AW and Gibbons GW (1994) Foot infections in diabetics: Evaluation and management. Curr. Clin. Top. Infect. Dis. 14:1-22

Kingsley A (2001) A proactive approach to wound infection. Nurs. Stand. 15(30):50-54, 56, 58

Laing P (1994) Diabetic foot ulcers. Am. J. Surg. 167:31S-36S

Lance GW (1989) Other infections of skin, soft tissue and muscle. In: Finegold SM and Lance GW (eds.) Anaerobic infections in humans. Academic Press Inc. pp 1491-1492

Larson E (1988) A causal link between handwashing and mode of infection: Examination of the evidence. Infect. Cont. 9:28-36

Lawrence C (1985) The bacteriology of burns. J. Hosp. Acquired Infect. 6:3-17

Lorenz HP and Longaker MT (2003) Wounds: Biology, pathology and management. Stanford University Medical Center pp 26-52

Lumley John SP (1997) Hamilton Bailey's physical signs demonstrations of physical signs in clinical surgery. 18th edition, Butterworth Heinemann pp 61-71

Macmillan BG (1980) Infections following burn injury. Surg. Clin. N. Am. 60:185-196

Marples RR and Towers AG (1979) A laboratory method for the investigation of contact transfer of microorganisms. J. Hyg. 82:237-248

Mayhall CG (1993) Surgical infections including burns prevention and control of nosocomial infections. 2nd edition pp 614-664

Mercandetti M and Cohen AJ (2005) Wound healing: Healing and repair. J. Craniofac. Surg. 15(1):181-185

Midwood KS, Williams LV and Schwarzbauer JE (2004) Tissue repair and the dynamics of the extracellular matrix. Int. J. Biochem. and Cell Biol. 36(6):1031-1037

Moreo K (2005) Understanding and overcoming the challenges of effective case management for patients with chronic wounds. The Case Manager 16(2):62-67

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

Murthy R, Sengupta S, Maya N and Shivananda PG (1998) Incidence of post operative wound infection and their antibiogram in a Teaching and referral hospital. Ind. J. Med. Sci. 52(12):553-555

Mustoe T (2004) Understanding chronic wounds: A unifying hypothesis on their pathogenesis and implications for therapy. Am. J. Surg. 5(1):S65-S70

Mustoe T (2005) Dermal ulcer healing: Advances in understanding. Presented at meeting: Tissue repair and ulcer/wound healing: Molecular mechanisms, therapeutic targets and future directions, Paris, France pp 1-6

NINSS (2000) Surveillance of surgical site infection in English hospitals: A national surveillance and quality improvement programme. Public Health Laboratory Service

Oguntibeju O and Nwobu RAU (2004) Occurrence of *Pseudomonas aeruginosa* in post-operative wound infection. Pak. J. Med. Sci. 20(3):187-191

Onche A and Adedeji O (2004) Microbiology of post-operative wound infection in implant surgery. Nigerian J. Surg. Research 6(1):37-40

Parajuli K (1997) Study of causative organisms from pus sample and its antibiotic sensitivity pattern. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Pathare NA, Bal A, Talvalkar GV and Antani DU (1998) Diabetic foot infections: A study of micro-organisms associated with the different wagner grades. Ind. J. Pathol. Microbiol. 41:437-441

Peel ALG (1992) Definition of infection: Infection in surgical practice. Oxford University Press pp 82-87

Pelczar MJ, Chan ECS and Krieg NR (2001) Microbiology. 5th edition, Tata McGraw-Hill Publishing Company Ltd, New Delhi pp 779

Plowman R (2000) The socioeconomic burden of hospital acquired infection. Euro. Surveill. 5(4):49-50

Quinn JV (1998) Tissue adhesives in wound care. B.C. Decker Publishing, Hamilton, Canada pp 137-140

Ramakrishnan KM, Jayaraman V, Ramachandran K and Madhivanan T (1986) The management of anaerobic infection in extensive burns. J. Burns 12:270-272

Rao AS and Harsha M (1975) Postoperative wound infections J. Ind. Med. Assoc. 64:90-93

Revathi G, Puri J and Jain BK (1998) Bacteriology of burns. J. Burns 24:347-349

Robson MC (1997) Wound infection: A failure of wound healing caused by an imbalance of bacteria. Surg. Clin. N. Am. 77:637-650

Reybrouck G (1983) Role of the hands in the spread of nosocomial infection. J. Hosp. Infect. 4:103-110

Sa LA, Sathe MJ and Bapat RD (1984) Factors influencing wound infection (a prospective study of 280 cases). J. Postgrad. Med. 30:232-236

Scholar A and Stadelmann W (2006) Wound healing: Chronic wounds. Clinical Techniques in Equine Practice 3(2):110-122

Shah PK, Pokhrel BM and Sharma AP (1997) The incidence of coagulase negative staphylococci in Nepalese people. Nep. Med. Assoc. 123 (36):296-300

Shaw D, Doig CM and Douglas D (1973) Is airborne infection in the operating theatre an important cause of wound infection in general surgery? The Lancet 1:17-21

Shenoy RK (2001) Manipal manual of surgery. CBS publishers and distributors, New Delhi, India pp 1-28

Shibata A, Kato M and Masukawa T (2004) Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. Int. Immunopharmacol. 4(14):1873-1880

Shrestha ML (1995) Microbiology of wound infection. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Shrivastava SP, Atal PR and Singh RP (1969) Studies on hospital infection. Ind. J. Surg. 31: 612-621

Singh NP, Goyal R, Manchanda V, Das S, Kaur I and Talwar V (2003) Changing trends in bacteriology of burns in the burn unit Delhi India. J. Burns 29(2):129-132

Snyder RJ (2005) Treatment of non healing ulcers with allografts. Clinics. Dermatol. 23(4):388-395

Speller DCE and Hilary H (1998) Hospital acquired infection. In: Collier L, Balows A and Sussman M (eds.) Topley and Wilson's microbiology and microbial infections. 9th edition, Bacterial infections, Arnold Euston Road, London 3:187-230

Stadelmann WK, Digenis AG and Tobin GR (1998) Physiology and healing dynamics of chronic cutaneous wounds. Am. J. Surg. 176(2):26S-38S

Summanen PH, Talan DA, Strong C, McTeague M, Bennion R, Thompson JE, Vaisanen ML, Moran G, Winer M, and Finegold SM (1995) Bacteriology of skin and soft-tissue infections: Comparison of infections in intravenous drug users and individuals with no history of intravenous drug use. Clin. Infect. Dis. 20:S279-S282

Taylor JE, Laity PR, Hicks J, Wong SS, Norris K, Khunkamchoo P, Johnson AF and Cameron RE (2005) Extent of iron pick-up in deforoxamine-coupled polyurethane materials for therapy of chronic wounds. Biomaterials 26(30):6024-6033

Tuladhar P (1999) A prospective study on bacteriology of wound infection at TUTH. A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Twum DK, Grant C, al-Suleiman SA, Abdel-Khader S, al-Awami MS, al-Breiki H, Taha S, Ashoor AA and Wosornu L (1992) Microbiology of postoperative wound infection: A prospective study of 1770 wounds. J. Hosp. Infect. 21(1):29-37

Vandepitte J, Verhaegen, Engbaek K, Rohner P, Piot P and Heuck CC (2004) Basic laboratory procedures in clinical bacteriology. 2nd edition, WHO, Geneva, AITBS Publishers and Distributors (Regd.) Delhi pp 86-120

Velander PE, Theopold C, Gheerardyn R, Bleiziffer O, Yao F and Eriksson E (2004) Autologous cultured keratinocytes suspensions accelerate re-epithelialization in the diabetic pig. J. Am. College Surg. 199(3):58

Venkatraman MS, Bhaskaran KS and Sunderaraman S (1978) Personal factors in wound sepsis. Ind. J. Surg. 40:618, 623

Vindenes H and Bjerknes R (1995) Microbial colonisation of large wounds. J. Burns 21:575-579

Way LW and Doherty GM (2003) Current surgical diagnosis and treatment. 11th edition, Lange Medical Books/McGraw-Hill pp 6-21

Wheat LJ, Allen SD, Henry M, Kernek CB, Siders JA, Kuebler T, Fineberg N and Norton J (1986) Diabetic foot infections: Bacteriologic analysis. Arch. Intern. Med. 146:1935-1940

Zoutman D, McDonald S and Vethanayagan D (1998) Total and attributable costs of surgical-wound infections at a Canadian tertiary-care center. Infect. Cont. Hosp. Epidemiol. 19:254-259

APPENDICES

APPENDIX-I

(A) CLINICAL PROFILE

Sample no.:
Name:
Address:
Sex / Age:
Ward / Bed no.:
Short clinical history:
Patients on antibiogram:
Type of specimen:
Method of collection:
Time of collection:
Date:

(B) MICROBIOLOGICAL PROFILE

DAY I
Direct microscopic evaluation

S.N	Gram staining	Results
a.	Gram positive cocci	
b.	Gram positive bacilli	
c.	Gram negative bacilli	
d.	Gram negative cocci	
e.	Pus cells/ WBC	
f.	Epithelial cells	
g.	Others	

DAY II
Culture of specimen

Colonial Characteristics

S.N	Media used	Characteristics of isolated colonies

a.	**NA**	
b.	**MA**	
c.	**BA**	
d.	**RCMB**	

DAY III
Biochemical Tests

S.N	**Biochemical tests employed**	**Result**
a.	**Catalase test**	
b.	**Oxidase test**	
c.	**Coagulase test**	
d.	**DNAse test**	
e.	**OF test**	
f.	**SIM test**	
g.	**MR test**	
h.	**VP test**	
i.	**Citrate test**	
j.	**TSI test**	
k.	**Urease test**	

DAY IV
Antibiotic sensitivity profile

S.N	**Antibiotics used**	**Zone of inhibition (ZOI)**	**Remarks**
1.	**Ciprofloxacin**		
2.	**Ofloxacin**		
3.	**Chloramphenicol**		
4.	**Gentamicin**		
5.	**Cephalexin**		
6.	**Cotrimoxazole**		

7.	**Ampicillin**		
8.	**Cloxacillin**		
9.	**Amikacin**		

Checked by:

(C) LIST OF MATERIALS

1. Equipments

Autoclave
Burner
Microscope
Hot air oven
Incubator
Refrigerator
Anaerobic gas jar
Gas Pak (Oxoid, anaeroGen)
Glasswares: Petriplates, glass tubes, glass slides, glsss rod etc.

2. Microbiological media (Hi-Media)

Nutrient Agar
Nutrient broth
Mac Conkey Agar
Blood Agar
Muller Hinton Agar
Robortson's Cooked Meat Broth
Simmons Citrate agar
TSI Agar
MRVP Broth
Urease Broth

SIM Media
Hugh and Leifson Media

3. Chemicals/Reagents
Catalase reagent (3% H_2O_2)
Oxidase reagent (1% Tetramethyl p-phenylene diamine dihydrochloride)
Kovac's reagent
Barritt's reagent (40% KOH, 5% *a*-naphthol in a ratio of 1:3)
Crystal violet
Gram's iodine
Acetone-alcohol
Safranine
Blood plasma
Methyl red

4. Antibiotics Discs (Hi-Media)

Ciprofloxacin (5mcg)	Penicillin (10 units)
Ofloxacin (5mcg)	Neomycin (30mcg)
Chloramphenicol (30mcg)	Metronidazole
Gentamicin(10mcg)	Cephalexin(30mcg)
Cotrimoxazole (1.25mcg)	Ampicillin (10mcg)
Cloxacillin (5mcg)	Amikacin (30mcg)

5. Miscellaneous
Cotton swabs, Distilled water, Lysol, Inoculating loop, Inoculating wire, Immersion oil, dropper, etc.

APPENDIX-II

(A) Composition and preparation of different types of culture media (Hi-Media)
(Note: All compositions are given in grams per litre and at 25^{o}C temperature.)

1. Nutrient Agar (NA)

Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Agar	1.5
Final P^{H}	7.4=0.2

2.8 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^{o}C for 15 mins.

2. Nutrient broth (NA)

Peptone	5.0
Sdium chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5

Final P^H	7.4=0.2

1.3 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^{o}C for 15 mins.

3. Mac Conkey Agar (MA)

Pancreatic digest of gelatin	17.0
Peptone	3.0
Lactose	10.0
Sodium Chloride	5.0
Bile salt	1.5
Agar	13.5
Neutral red	0.03
Crystal Violet	0.001
Final P^H	6.9-7.3

5.5gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^{o}C for 15 mins.

4. Blood Agar (BA)

Composition of Blood Agar base	15.0
Proteose peptone	2.5
Liver digest	5.0
Yeast Extract	5.0
Sodium ChlorideAgar	15.0
Final P^H	7.4

Blood agar base medium was prepared and autoclaved at 121^{o}C for 10 mins. It is then cooled down to 48^{o}C and blood (7-10%) is added aseptically and mixed thoroughly. About 18-20 ml. of the media was then poured on Petri-plates. If bubbles appear in the poured plates, a flame is passed over the bubbled before the media sets.

5. Muller Hinton Agar

Beef infusion Broth	300.0
Casein Acid Hydrolysate	17.0
Starch	1.0
Agar	17.0
Final P^H	7.0=0.2

3.8 gms of media was suspended in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^{o}C for 15 mins. It was then poured while at 45-48^{o}C into sterile petriplates in 25 ml quantity.

6. Cooked Meat Broth

Beef heart	454
Proteose peptone	20
Dextrose	2
NaCl	5
Final P^H	7.2=0.2

1.25 gm of the medium was suspended in 10 ml distilled water and allowed to stand for 15 mins until all the particles were thoroughly wetted. Then the medium was sterilized by autoclaving at 15 lbs pressure at 121^{o} C for 15 mins.

7. Peptone water

Peptone	10
NaCl	5
Final P^{H}	7.2=0.2

15 gm was dissolved in 1000 ml distilled water and was sterilized by autoclaving at 15 lbs pressure at 121^{o} C for 15 mins.

(B) Composition and preparation of different types of biochemical media

1. MR-VP medium

Buffered peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final P^{H}	6.9

1.7 gms of media was dissolved in 100 ml distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121^{o}C for 15 mins.

2. Simmons Citrate Agar

Magnesium sulphate	0.2
Mono-ammonium phosphate	1.0
Dipotassium phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Bromothymol Blue	0.08
Agar	15.0
Final P^{H}	6.8=0.2

2.42 gms of media was dissolved in 100 ml distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121^{o}C for 15 mins. It was then allowed to cool in standing position to prepare slant.

3. Triple Sugar Iron (TSI) Agar

Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2

Sodium chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
Final P^H	7.4=0.2

6.5 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was distributed in the amount of 5 ml each into several test tubes and sterilized by autoclaving at 15 Ibs pressure at 121^{o}C for 15 mins. The media was allowed to set in slope form to from a slant with butt 1 inch long.

4. Urea Broth Base

Monopotassium Phosphate	9.1
Dipotassium Phosphate	9.5
Yeast Extract	0.1
Phenol red	0.01
Final P^H	6.8=0.2

1.85 gms of media was dissolved in 95 of distilled water and hence to dissolve the media. The media was sterilized by autoclaving at 15 Ibs pressure at at 121^{o}C for 15 mins. It was then cooled to 55^{o}C and 5ml of sterile 40% urea solution was aseptically added. The contents were then mixed well and distributed into sterile test tube.

5. Hugh and Leifson Media (O/F media)

Peptone	2.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	0.3
Bromothymol Blue	3ml
Agar	3
Distilled water	1 litre
Final P^H	7.1

The P^H is adjusted to 7.1 before adding the bromothymol blue and the medium is autoclaved in a flask at 121^o C for 15 minutes. The carbohydrate to be added is sterilized separately and added to give a final concentration of 1%. The medium is then tubed to a depth of about 4 cm.

6. Sulphide Indole Motility medium (SIM)

Beef extract	3
Peptone	30
Peptonized iron	0.2
Sodium thiosulphate	0.025
Agar	3
Final P^H	7.3=0.2

36 gm of the medium was dissolved in 1000 ml distilled water and distributed into tubes. The medium was sterilized by autoclaving at 15 lbs pressure at 121^o C for 15 mins.

(C) Composition and preparation of different reagents

I. Gram staining reagents

1. Crystal violet Gram stain

Crystal violet	20g
Ammonium oxalate	9g
Ethanol or methanol, absolute	95ml
Distilled water	1 litre

Preparation:

i. Crystal violet is weighed and transferred to a clean bottle and absolute ethanol is added and mixed until dye is completely dissolved.

ii. Ammonium oxalate is weighed and dissolved in about 200 ml of distilled water. Then it was added to the stain and total volume is made 1 litre by adding distilled water and mixed well.

2. Iodine Solution

Potassium iodide	1.5g
Iodine	1.0g
Distilled water	150ml

Preparation:

i. Potassium iodide is weighed and transferred to a clean bottle

ii. 30-40 ml of distilled water is added to Potassium iodide and mixed until it is fully dissolved.

iii. Iodine is weighed and added to potassium iodide solution and mixed well.

iv. Final volume is made 150ml by adding distilled water and mixed well.

3. Acetone-alcohol decoloriser

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

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

4. Counterstain solution

Safranine	10gm
Distilled water	1 lit

In a piece of clean paper, 10 gm of safranine was weighed and transferred to a clean bottle. Then 1 lit. distilled water was added to the bottle and mixed well until safranine dissolves completely.

II. Test reagents

1. Kovac's Reagent

Para-Dimethylaminobenzaldehyde	2g
Isoamyl alcohol	30ml

Hydrochloric acid	10ml

Preparation:

i. Para-Dimethylaminobenzaldehyde is weigh and dissolved in Isoamyl alcohol.
ii. Concentrated Hydrochloric acid is added and mixed well.

2. Methyl Red Solution

Methyl red	0.05g
Ethanol, absolute	28ml
Distilled water	22ml

Preparation:

1. Methyl red was weigh and dissolved in ethanol and water.

3. Voges-Proskauer reagent (Baritt's reagent)

(i) VP reagent A (To make 100 ml)

a-napthol	5gm
Ethanol, absolute	100ml

Preparation:

To 25 ml distilled water 5 gm *a*-napthol was dissolved and transferred to a clean brown bottle. Then the final volume was made 100ml by adding distilled water.

(ii) VP reagent B (To make 100 ml)

Potassium hydroxide	40gm
Distilled water	100ml

Preparation:

To 25 ml distilled water, 40 gm of KOH was dissolved and transferred to a clean brown bottle and final volume was made 100 ml by adding distilled water.

4. Catalase reagent (To make 100 ml)

Hydrogen peroxide solution	3ml
Distilled water	97ml

Preparation:

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

5. Oxidase reagent (To make 100 ml)

Tetramethyl P-Phenylenediamine dihydrochloride (TPD)	0.1gm
Distilled water	10ml

Preparation:

This reagent was made by dissolving 0.1 gm TPD in 10 ml distilled water. To that solution strips of Whatman's no. 1 filter paper were soaked and drained for 30 secs. Then these stripes were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

7. Hydrochloric acid (1M/L HCL) solution (To make 100 ml)

HCL (conc.)	8.6ml
Distilled water	100ml

Preparation:

8.6 ml of conc. HCL was added to 25 ml distilled water into a clean bottle. Then the final volume was made 100 ml by adding distilled water.

APPENDIX-III

(A) Procedure of different biochemical tests

i. Catalase test

A small amount of isolated colony from pure culture was transferred to the surface of clean dry glass slide.

1. A drop of 3% H_20_2 was placed onto the inoculum.
2. The evolution of oxygen bubbles was recorded immediately.
3. The slide was then discarded into a disinfectant.

ii. Oxidase test

1. A piece of filter paper was placed in a clean petridish and 2-3 drops of freshly prepared oxidase reagent was added.
2. Using a glass rod, a colony of test organism was smeared on the filter paper.
3. It was observed for the development of blue purple colour within a few seconds.

iii. Coagulase test

Slide test (to detect bound coagulase)

1. A drop of physiological saline was placed on end of a slide and colony of test organism was emulsified in each of the drops to make two thick suspensions.
2. A drop of plasma was added to one of the suspensions and mixed gently. It was looked for clumping of the organism within 10 seconds. But no plasma was added to second suspension. This is used to differentiate any granular appearance of the organism from the coagulase clumping.

Tube test (to detect free coagulase)

1. The plasma was diluted 1 in 10 physiological saline (mixing 0.2 ml of plasma with 1.8 ml of saline)
2. 3 tubes were taken and labeled as:

T = test organism (18-24 hour broth culture),

P = Positive control (*S. aureus* broth culture),

N = Negative control (sterile broth).

3. 0.5 ml of diluted plasma was pipetted into each tube.

4. About 5-5 drops each of test organism, S. aureus culture, and sterile broth was added to the tubes labeled 'T', 'P' and 'N' respectively.

5. After mixing gently, 3 tubes were incubated at 37^{o}C. It was examined for clotting after 1 hour. If no clotting occurs tubes were examined at 30 minutes intervals for up to 6 hours.

iv. Deoxyribonuclease (DNAse) test

1. A DNAse plate was divided into required number of strips by marking the underside of the plate.
2. Using a sterile loop or swab the test and control organisms were spot inoculated and labeled clearly.
3. The plates were incubated at 37^{o}C for 24 hours.
4. After incubation; the plates were flooded 1 mol/liter HCL solution and excess acid was tipped off.
5. It was observed for clearing around the colonies within 5 minutes of adding the acid.

v. Oxidative-fermentative (O/F) test

1. Using a sterile straight wire, the test organism was inoculated to the bottom of two tubes of sterile O/F media.
2. The inoculated medium in one of the tubes was covered with a 10mm deep layer of sterile paraffin oil.
3. The tubes were incubated at 37^{o}C for 24 hours and then examined for carbohydrate utilization.

vi. Motility test

1. The test organism was inoculated by stabbing in the medium with a sterile straight wire.
2. Then the medium was incubated at 37^{o}C for 24 hours.

3. After incubation, the appearance of spreading turbidity from the stab-line or the turbidity throughout the medium is the indication of the positive motility test.

vii. Hydrogen sulphide (H_2S) production test

1. The test organism was inoculated by stabbing in the medium with a sterile straight wire and the medium was incubated at 37^{o}C for 24 hours.
2. After incubation, a black coloured iron-containing precipitate in the medium indicates the production of H_2S gas.

viii. Indole test

1. Using a sterile straight wire, sterile SIM medium was inoculated with pure culture of test organism and incubated at 37^{o}C for 24 hours.
2. Then few drops of Kovac's reagent was added into the medium and observed for the development of pink red colour.

ix. Methyl red (MR) test

1. A colony of test organism was inoculated in 0.5 ml of sterile MR-VP broth.
2. After overnight incubation at 37^{o}C, a drop of methyl red solution is added.
3. A positive MR test is shown by the appearance of a bright red colour, indicating acidity, while a yellow or orange colour is indicative of negative test.

x. Voges-Proskauer (V-P) test

1. The test organism was inoculated in the broth medium and incubated at 37^{o}C for 24 hours.
2. After incubation, 1 ml of 40% potassium hydroxide and 3 ml of 0.5% solution of *a*-naphthol in absolute ethanol was added.
3. A positive reaction is indicated by the development of a pink colour in 2-5 minutes, becoming crimson in 30 minutes.

xii. Citrate utilization test

1. Using a sterile straight wire, the test organism was inoculated by streaking on the slope of the medium and incubated at 37^{o}C for 24 hours.
2. The appearance of growth on the streaked line and change in colour of the medium from green to blue was the indicator of the positive citrate test.

xiii. Triple Sugar Iron (TSI) test

1. Using a sterile straight wire, the test organism was first stabbed down the centre of the agar butt and then streaked on the slanted surface.

2. The TSI agar tubes were incubated at 37^{o}C for 24 hours.

3. The tubes were observed for gas formation, fermentation of sugars, and H_2S gas production.

Interpretation of results

From this test three different results can be drawn.

i. Production of gas: indicated by the cracking of the media.

ii. Production of H2S: indicated by the formation of black coloured iron containing precipitate in the butt.

iii. Fermentation of sugars: Fermentation pattern is shown in table 1:

Table 1: Interpretation of TSI agar test

Slant/butt	**Colour**	**Utilization**
Alkline/acid	**Red/Yellow**	**Glucose only fermented; peptone utilized**
Acid/acid	**Yellow/Yellow**	**Glucose fermented; lactose and/or sucrose fermented**
Alkaline/alkaline	**Red/Red**	**No fermentation of glucose, lactose or sucrose; peptone**

		utilized

(Source: Collee *et al*, 1996)

xiv. Urea hydrolysis test (Urease test)

1. Heavy inoculums of test organism was inoculated into Christensen's urea broth and incubated at 37^{o}C for 24 hours.
2. The change in the colour of the medium into pink indicates urease positive test.

xv. Amino acid decarboxylase test

1. The organism was inoculated lightly through the paraffin layer with a straight wire and incubated at 37^{o}C for upto 4 days.
2. The appearance of violet colour of the medium is the indication of the positive test.

(B) Antibiotic disc used and procedure of susceptibility test

(1) Antibiotic disc used

Antibiotics used	**symbol**	**Disc Content (mcg)**	**Diameter of Zone of inhibition (mm)**		
			Resistant	**Intermediate**	**Sensitive**
Ciprofloxacin	**Cf**	**5**	**15**	**16-20**	**21**
Ofloxacin	**Of**	**5**	**12**	**13-15**	**16**
Chloramphenicol	**C**	**30**	**12**	**13-17**	**18**
Gentamicin	**G**	**10**	**12**	**13-14**	**15**
Cephalexin	**Cp**	**30**	**14**	**15-17**	**18**
Cotrimoxazole	**Co**	**1.25**	**10**	**11-15**	**16**
Ampicillin	**A**	**10**	**13**	**14-16**	**17**
Cloxacillin	**Cl**	**5**	**11**	**12-13**	**14**
Amikacin	**Ak**	**30**	**14**	**15-16**	**17**

(2) Procedure of sensitivity test (Kirby-Bauer's Disc Diffusion Method)

i. Preparation of inoculum: For inoculum preparation, 3-4 pure culture colonies were transferred into Nutrient Broth abd incubated at 37^{o}C for 2-4 hours to obtain turbidity.

ii. Inoculation: A sterile cotton swab was dipped into the inoculum and rotated by pressing it against the upper inside wall of tube to remove excess inoculum. Then it was allowed to dry for 10 mins.

iii. Application of discs: With the help of flamed forcep, disc were carefully placed on the agar surface at least 15 mm away from the edge and pressed lightly to make contact with the surface of the medium and plates were allowed to stand at room temperature for 30 mins. (prediffusion time).

iv. Incubation: The plates were incubated at 37^{o}C for 24 hrs.

v. Interpretation of result: After incubatin, diameter of ZOI was measured.

(C) STATISTICAL TOOLS

(1) Association of positive growth and negative growth among gender

S.N	Gender	Total patients		Growth		No growth	
		No	%	No.	%	No.	%
1	Male	185	60.65	121	65.40	64	34.59
2	Female	120	39.34	76	63.33	44	36.66
		305		197		108	

Here, Ho: There is no significance association of positive growth and negative growth among gender

$H_{1:}$ There is significance association of positive growth and negative growth among gender

single and multiple isolates among pus samples.

Observed value (O)	**Expected value (E)**	**$(O-E)^2$**	**$x^2_{cal}=\frac{(O-E)^2}{E}$**
121	**119.49**	**4**	**0.03**
76	**77.50**	**1**	**0.01**
64	**65.50**	**1**	**0.01**
44	**42.49**	**4**	**0.09**
			$\sum x^2_{cal}=\frac{(O-E)^2}{E}= 0.14$

Calculated value of x^2 (x^2_{cal}) = 0.14
Here, the degree of freedom (d.f.) = (r-1) (c-1) = (2-1) (2-1) = 1
According to table value of x^2 (x^2_{tab}) at 5% level of significance (P> 0.05) for 1 d.f. = 3.84

Since the calculated value of x^2 is lesser than the corresponding tabulated value, the null hypothesis (Ho) is accepted.

(2) Correlation between direct smear Gram staining and culture

No. of Pus samples	**Type of bacteria**	**Results of direct smear Gram staining**		**Culture results**	
		No.	**%**	**No.**	**%**
305	**GPC**	**47**	**15.4**	**51**	**16.72**
	GNB	**83**	**27.21**	**85**	**27.86**
	Both GPC and GNB	**61**	**20**	**58**	**19.01**
	Gram positive rods	**-**	**-**	**3**	**0.98**

	Organism not seen	**114**	**37.37**	**108**	**35.4**
Total		**305**		**305**	

Let, result of direct smear Gram staining be X,
and Culture result be Y.

X	**Y**	**X^2**	**Y^2**	**XY**
47	**51**	**2209**	**2601**	**2397**
83	**85**	**6889**	**7225**	**7055**
61	**58**	**3721**	**3364**	**3538**
-	**3**	**-**	**9**	**3**
114	**108**	**12996**	**11664**	**12312**
		$\sum X^2 = 25815$	**$\sum Y^2 = 24863$**	**$\sum XY = 25305$**

Here,

Karl Pearson's correlation coefficient (r_{XY}) = $\sum XY / \sum X^2 \sqrt{\sum Y^2}$

= 0.99

≈ 1

Hence, Correlation between direct smear gram staining and culture is positively correlated.

SUMMARY OF LABORATORY EXAMINATION OF PUS SAMPLES