CHAPTER-1

1. INTRODUCTION

Wounds break the continuity of the skin and allow organisms to gain access to the tissues and cause infection. This may be characterized by the classic signs of redness, pain, swelling, raised temperature and fever. From the microbiological perspective, the primary function of normal, intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissues from being 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 (Bowler *et al*, 2001).

The presence of microorganisms in a wound is not unusual but not all wounds support the same range and number of species (Richard *et al*, 2006). The outcome of wound infection depends on interaction of complex host and microbial factors (Shittu *et al*, 2003). Since wound colonization is most frequently polymicrobial 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 resource demanding. Some researchers reported that during an analysis of post surgical wound infections following head and neck surgery, there was an increase in the average hospitalization period from 14 days when wounds healed without complication to 24 days when the wounds became infected. In the similar analysis of 108 post surgical wounds, researchers concluded that 10.2 days per case were directly attributable to wound infection and that the associated hospital cost was $3,937 per infected patient.

Thus, concern among health care practitioners regarding the risk of wound infection is justifiable not only in terms of increased trauma to the patient but also in view of its burden on

financial resources and the increasing requirement for cost-effective management within the health care system (Bowler *et al*, 2001).

In general, a wound can be considered infected if purulent material drains from it, even without the confirmation of a positive culture (Howard *et al*, 1964). This clinical definition has advantages compared with those based on culture results, because 1) a positive culture does not necessarily indicate infection, since many wounds, infected or not, are colonized by bacteria, and 2) infected wounds may not yield pathogens by culture because some pathogens are fastidious, culture techniques are inadequate, or the patient has received antimicrobial therapy (Garner *et al*, 1988). Wound infection may occur as a result of penetrating trauma from plants, animals, guns, knives or other objects. Since plants are made of porous material, a thorn or other such objects can serve as a vehicle for the entry of microorganisms into the tissues (O'dell, 1998).

Wound infection is mainly caused by overcrowding, lack of general cleanliness, poor socioeconomic condition and lack of education too. Wound infection can occur in children, adults and old people. No age is spared, no sex is spared. Bacterial colonization and infection are important factors in compromised wound healing, particularly in chronic wounds.

Wound healing and infection is influenced by the relationship between the ability of bacteria to create a stable, prosperous community within a wound environment and the ability of the host to control the bacterial community. Since bacteria are rapidly able to form their own protective microenvironment (biofilm) following their attachment to a surface, the ability of the host to control these organisms is likely to decrease as the biofilm community matures. Within a stable, climax biofilm community, interactions between aerobic and anaerobic bacteria are likely to increase their net pathogenic effect, enhancing their potential to cause infection and delay healing (Bowler *et al*, 2001). Heavy microbial contamination has a negative effect on wound healing. Polymicrobial colonization and the presence of antibiotic-resistant bacteria may impede the healing of delayed closure surgical wounds, pressure ulcers, and diabetic foot ulcers (Motta *et al*, 2004). The host factors that are associated with an

increased risk of a surgical wound infection include very young or old age, marked obesity, the presence of a perioperative infection, and the use of steroids.

A breast abscess is a localized collection of pus within the breast that usually occurs as a complication of mastitis. As some authors suggest that up to 11% of women with mastitis will develop a breast abscess, this is a potentially significant health issue (Bedinghaus *et al*, 1997). Bacterial mastitis occurs in about 2.5 percent of nursing mothers, most commonly occurring between 2 and 5 weeks post-delivery (Montgomery, 2001).

Chronic wounds (i.e. 6 weeks duration) such as leg ulcers or pressure ulcers are inevitably colonized with a mixture of species, many of which are potential pathogens (Richard *et al*, 2006). Foot ulceration and infection are major causes of hospitalization in people with diabetes (Boulton & Bowker, 1985).

Post-surgical wound infections are a major cause of increased nosocomial morbidity and mortality and excess healthcare costs. A significant proportion of surgical wound infection (10–80% in various studies) does not develop until after patient's discharge from hospital (Society for Hospital epidemiology of America, 1992). Surgical wound infection is the second most common type of wound infection (Brachman *et al*, 1989).

The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin. Patient factors such as age, extent of injury, and depth of burn in combination with microbial factors determine the likelihood of invasive burn wound infection (Pruitt *et al*, 1998).

The number of accidental wound infection is increasing. The wounding agents may range from nail pricks from farm equipments to door slams on fingers and vehicle accidents. Infecting microorganisms may be derived either from exogenous (dust, dirt, soil and clothes) or the endogenous source (organisms that are present as commensals in the patient's body).

Wound infection is causing a great fear both in the developed and the developing countries. In rural areas of Nepal, the socioeconomic conditions sanitation, education and hygiene is very poor. People are not aware and knowledgeable about the prevention of injuries and disability resulting from it. People in Nepal are generally prone to agricultural wounds, traffic accidents, domestic accidents and these wounds later on become complicated due to poor management of wounds in the initial stage. Absence of facilities in district and peripheral hospitals, combined with traditional unscientific household practices and lack of safety system result in wound infection.

Hence the importance of wound infections, in both economic and human terms, should not be underestimated. Practitioners need to know how to recognize and manage the signs and consequences of clinically infected wounds. The present study was carried out at Bir Hospital with a target to find out the common type of wounds and the bacteria associated with them. Similarly the antimicrobial susceptibility testing result suggests that some antibiotics would have very limited usefulness for the prophylaxis or the empirical treatment of wound infection. The result might serve as a foundation for establishing empiric therapeutic approaches for the management of such infections in Bir Hospital.

CHAPTER 2

2. OBJECTIVES

2.1 General objective

To study the bacterial agents in different types of wounds and its antibiotic sensitivity pattern among the patients visiting the surgical outpatient department (SOPD) of Bir Hospital.

2.2 Specific objectives

- To study the different types of wounds commonly found among the patients visiting surgical outpatient department (SOPD) of Bir Hospital.

- To isolate and identify the bacteria encountered in wound specimens from different types of wounds.

- To compare the pattern of isolated bacteria from different types of wounds.

- To characterize the antibiotic susceptibilities of the isolated bacterial pathogen.

CHAPTER-3

LITERATURE REVIEW

3.1 Definition of wound

Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae (Pintu and Kamal, 2001). Shenoy (2001) considers wound as simple when only skin is involved but can be complex when it involves underlying nerves, vessels, tendons etc. According to Lumley *et al* (1997), a break in the skin is considered 'wound' irrespective of its etiology, e.g. surgery or accidental injury, or the amount of underlying tissue damage.

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result. The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced (Giacometti *et al*, 2000). Community-acquired wound infections are often preceded by injuries resulting from occupational exposure or recreational activities and are associated with a greater diversity of microorganisms due to the exposure of open wounds to inhabitants of the microbial biosphere (Janda *et al*, 2005). The latter category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices (Giacometti *et al*, 2000).

Kingsley (2003) state that four basic conditions exist in open wounds resulting from the level of bioburden present (bacterial contamination - normal but short-lived state, colonization - normal state, critical colonization - abnormal state, and infection - abnormal state).

3.2 Historical background

The management of wounds, their classification, surgical dressing, and subsequent healing is an ever evolving field based on better understanding of the pathophysiological principles behind them. From the time of Hippocrates and Galen who recognized that infection impaired

wound healing to the development of antiseptic and aseptic technique through the work of Semmelweis, Pasteur and Lister, research continues into the factor that affect the quality of healing, cosmetic outcomes and functional recovery of the damaged tissue (Kirk & Ribbans, 2004).

According to Collier (2002), wound infection is not a modern phenomenon. As early as 14-37 AD, there is documentary evidence that Cornelius Celsus (a Roman physician) described the four principal signs of inflammation and used 'antiseptic' solutions. Another Roman physician, Claudius Galen (130-200 AD) had such an influence on the management of wounds that he is still thought of by many today as the 'father of surgery'.

Table 1 : Historical background (1510-1994)

Ambrose Pare (1510-1590)	Encouraged wounds to suppurate
Semmelweis (1818-1865) Pasteur (1822-1895) & Lister (1827-1912)	Accepted germ theory and introduced antiseptics
Florence Nightingale (1894)	'Not in bacteriology but looking into drains (for smells) is the thing needed'. Held a firm belief in the benefit of hand-washing and strict hygiene
Mary Ayton (1985)	Defined terminology in current use for wound infection
Vincent Falanga (1994)	Identified the concept of 'critical colonization' with fresh insights into chronic wound healing and non-healing wounds

(Ellis, 1994)

3.3 Wound colonization

Chamberlain (2004) defines wound colonization as the presence of replicating microorganisms adherent to the wound in the absence of injury to the host. This is also very

common. Most of these organisms are normal skin flora, *S. epidermidis*, other coagulase negative Staphylococci, *Corynebacterium* spp., *Brevibacterium* spp. etc.

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3.4 Critical colonization

Multiplication of bacteria causing delay in wound healing is usually associated with an exacerbation of pain not previously reported but still with no overt host reaction.

3.5 Wound contamination

Wound contamination is described by Chamberlain (2004) as the presence of non replicating microorganisms in wound. All chronic wounds are contaminated. These contaminants come from indigenous micro flora and/or the environment. Most contaminating organisms are not able to multiply in wounds. Wound contamination and colonization can be differentiated as latter having greater microbial load than infected wound and heals faster.

3.6 Wound infection

Russel *et al* (2000) defines wound infection as the invasion of organisms through tissues following a breakdown of local and systemic host defenses. The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of organisms involved (Bowler *et al*, 2001).

O'dell (1998) stated that wound infections may occur as a result of penetrating trauma from plants, animals, guns, knives or other objects and can serve as a vehicle for the entry of *S. aureus* or other organisms into the tissues. Cellulitis commonly occurs after such breaks in the skin.

According to Chamberlain (2004),

Infection= (Dose x virulence)/ Host resistance where,

Infection: The presence of replicating microorganisms within the wound that causes host injury.

Dose: The number of organisms. It differs depending upon the type of microorganism involved. Some organisms would need to be in high concentrations (E.g. *Candida, Enterococcus*). Various combinations of bacterial species result in more host damage (synergy) (E.g. *S. agalactiae* and *S. aureus*).

Virulence: The virulence factors they produce. Some organisms produce few virulence factors however, synergy between different bacterial factors can cause host damage. Group B *Streptococcus* and *S. aureus* cause haemolysis by synergism of two toxins.

Resistance: Host resistance is the single most important determinant in wound infection. Local and systemic factors both play a role in increasing the chance of wound to become infected.

3.6.1 Signs of wound infection

Signs of wound infection are closely associated with the wound type. The more obvious infection signs, such as purulent discharge and spreading erythema, are generally recognized as diagnostic. However, these features are not always present in the early stages when diagnosis is important for treatment and the avoidance of complicating sequelae (Cutting & Richard, 2005). The signs of wound infection have been described by some authors as:

- Pus or cloudy fluid draining from the wound
- Pimple or yellow crust formed on the wound (impetigo)
- Scab has increased in size
- Increasing redness around the wound (cellulitis)
- Red streak is spreading from the wound toward the heart (lymphangitis)
- Wound has become extremely tender
- Pain or swelling increasing after 48 hours since the wound occurred
- Wound has developed blisters or black dead tissue (gangrene and myonecrosis)

- Lymph node draining that area of skin may become large and tender (lymphadenitis)
- Onset of widespread bright red sunburn-like rash
- Onset of fever
- Wound hasn't healed within 10 days after the injury

3.6.2 Factors that increase the chances of wound infection

A) General Factors:

- Malnutrition (obesity/weight loss): Malnutrition causes depression of the immune system and hence wound infection and the inflammatory response to this may delay healing (Russel *et al*, 2000). Kirk & Ribbans (2004) stated that deficient protein intake may inhibit collagen formation and so inhibit the regaining of tensile strength.

- Metabolic disease (diabetes, uraemia, jaundice): Diabetic patients are more prone to infection as they are immunocompromised (Shenoy, 2001). Similarly, Russel *et al* (2000) state that jaundice and uraemic patients have poor wound healing because fibroblastic repair is delayed.

- Immunosuppression (cancer, AIDS, steroids, chemotherapy and radiotherapy): Many studies show that glucocorticoids have an inhibitory effect on the healing process and on the production of fibrous tissue.

- Age: Neonates and elderly are more susceptible to infection than others, probably because of decreased antibody production, ineffective phagocytosis and loss of intracellular killing of neutrophils (Boyd & Hoerl, 1981).

B) Local Factors:

- Local infection: According to Shenoy (2001), organisms eat away the suture material, destroy granulation tissue and cause slough and purulent discharge. Collagen synthesis is reduced and collagenolysis is increased. Similarly, Chamberlain (2004) stated that

large wound area, increased wound depth, degree of chronicity, anatomic location (distal extremity, perineal region) and reduced perfusion of blood also affect wound infection.

- Presence of foreign bodies: The presence of foreign body increases the intensity and prolongs the duration of the inflammatory response to injury. It is worth remembering that fragments of dead tissue, such as bone, and other elements of the patients own tissue that have become misplaced, such as hair or keratin, act as foreign bodies. Haematoma also precipitates infection.(Kirk & Ribbans, 2004).

- Faulty/Poor surgical technique: Poor surgical technique may increase risk of wound infection.
    i. Preoperative: Skin infection, wound contamination with infecting organisms associated with chronic skin disease and nail bed, debilitated, elderly, diabetic patients and who are receiving steroids.
    ii. Operative: Bad / Infected OT, bad/infected instruments, infected staff, faulty surgery, operation on the heavily contaminated area as nose and large gut.
    iii. Post operative: from patients own skin, nose, perineum, hand, from ward environment, from staff, from other patients (Pintu and Kamal, 2001).

3.6.3 Fate of wound infection

- Spontaneous resolution.
- Wound sepsis
- Abscess formation
- Wound bursting /rupture
- Septicaemia and pyaemia
- Metastatic abscess formation
- Osteomyelitis and septic arthritis
- Delayed healing (Pintu and Kamal, 2001)

3.6.4 Tissues devitalization contamination and infection

Rintoul (2001) stated that whenever a wound is inflicted, some devitalization of tissue (cellular damage) is inevitable. When the wound is made with a sharp knife the damage is comparatively slight and interferes little with wound healing. The majority of accidental wounds, however, are inflicted by contact with some blunt or ragged object or by crushing and in these wounds, tissue devitalization is extensive. All wounds, which are not made under the aseptic conditions of an operating theatre, are potentially infected. Organisms lie on the surface of the wound for a period of time and multiply in that situation. During this period of 12 to 24 hours there will be no local evidence of infection. A certain number of wounds however will develop evidence of infection after this period if left untreated. Organisms will flourish locally, enter the lymphatics and the characteristic signs of local inflammation will appear along with the signs of a general systemic disturbance.

3.6.5 Elements of wound infection

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

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

(ii) The susceptible host: Patients with suppressed immune system are being seen with increasing frequency and their problems have become a major surgical problem (Way & Doherty, 2003). According to Robson *et al* (1997), wound infection results when bacteria endogenous to the patient or exogenous to the wound achieve dominance over the systemic and local factors of host resistance. According to Kingsley (2003),

[(Inoculum x Virulence) + Potentiating factors]/ Host resistance outcome = Infection/no infection

Host defense mechanisms include:

a) Specific immunity:-Includes cell-mediated and humoral immunity.

b) Non specific immunity: - Also known as innate immunity, it includes phagocytic system, complement, humoral factors and inflammatory response.

iii) The closed space: Most surgical infections start in a susceptible, usually poorly vascularized place in tissue such as wound or a natural space. The common denominators are poor perfusion, local hypoxia, hypercapnia, and acidosis. Foreign bodies, dead tissues, and injuries interfere with this mechanism and predispose to infection. Fibrin polymerizes around bacteria, trapping them, and encounters abscess formation but at the same time prevents dangerous spread of infection (Way & Doherty, 2003).

3.6.6 Pathophysiology of wound infection

Bowler *et al* (2001) stated that infection occurs when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system and subsequent invasion and dissemination of microorganism in viable tissue provokes a series of local and systemic host responses. In order to cause infection a pathogen must accomplish the following:

1) It must enter the host.

2) It must metabolize and multiply on or in the host tissue.

3) It must resist host defenses.

4) It must damage the host (Pelczar, 1993).

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

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

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

Colonization and growth: If the pathogen gain access to tissues, it may multiply, a process called colonization. Because the initial inoculum is rarely sufficient to cause damage pathogen must grow within host tissue in order to produce infection. Cellular damage to the skin and soft tissues may be mediated by toxins (exotoxins and endotoxins), degradative enzymes, and the induction of the host cellular response that destroy tissues usually by immune mediated mechanisms (Schaechter *et al*, 1989). E.g. Hyaluronidase (*S. pyogenes*), proteases (*S. aureus, P. aeruginosa*), toxins (*S. pyogenes, S. aureus*), endotoxin (Gram negative organisms). Similarly, synergy between bacterial factors cause host damage (Chamberlain, 1999).

Host defenses: Both specific and non specific mechanisms come into play. Inflammation is a general nonspecific reaction.

Inflammatory response

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). The characteristic inflammatory response results in redness, swelling, pain, and heat, which are localized at the site of infection (Madigan *et al*, 2000).

In addition to need to control wound microflora, unregulated inflammation caused by both microorganisms and underlying abnormal pathophysiological conditions is a major factor associated with poor healing in chronic wounds. Consequently, therapeutic strategies that target chronic inflammatory processes are critical to wound progression (Bowler, 2002).

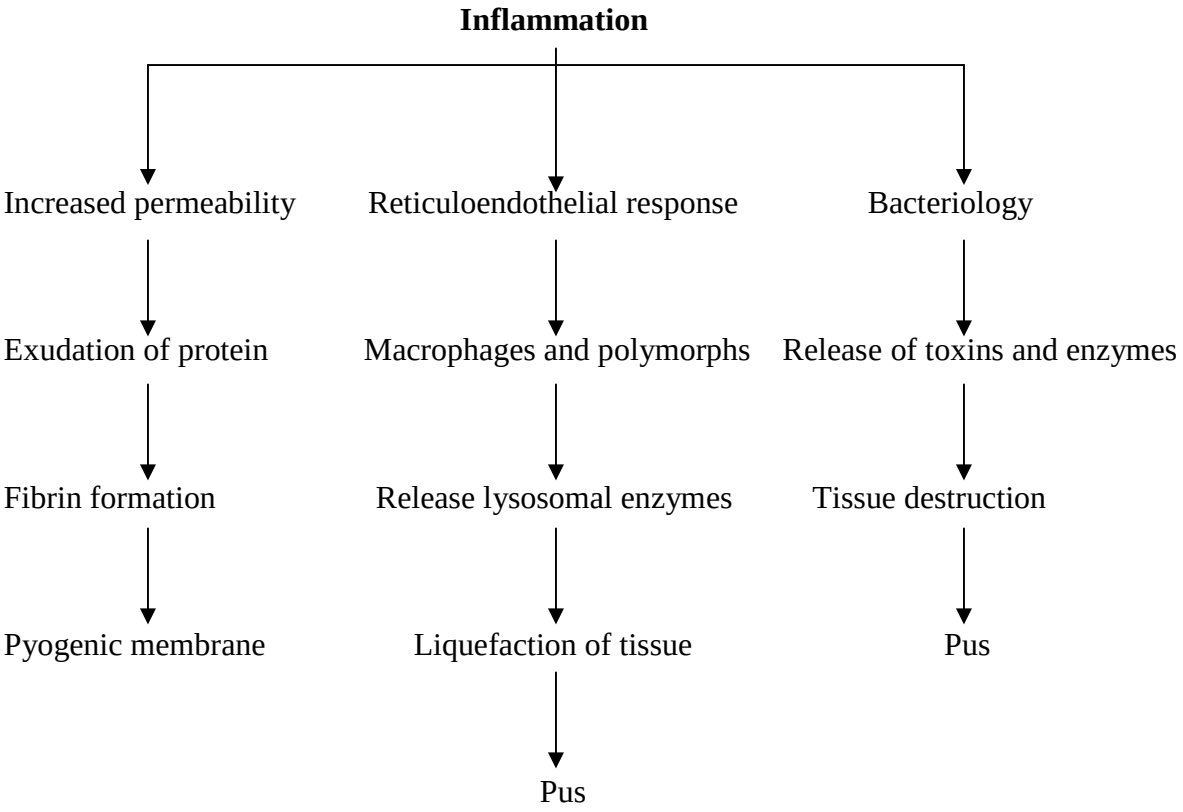

Figure 1: Pathological events during inflammation (Shenoy, 2001).

3.6.7 Origin of wound infection

Bowler *et al* (2001), in their literature stated that wound contaminant is likely to originate from three main sources:

i) The environment (exogenous microorganisms in the air or those introduced by traumatic injury),

ii) The surrounding (involving members of the normal skin microflora such as *S. epidermidis*, micrococci, skin diptheroids, and propionibacteria), and

iii) Endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae).

Endogenous sources appear to be responsible for most infections, especially if clean wound infections are excluded (Trilla and Mensa, 1993). Sources of endogenous contamination include the gastrointestinal and genitourinary tracts, sites of active infection remote from the wound (e.g. a urinary tract infection), the skin, and the anterior nares. The study was undertaken by Whyte *et al* (1991) to determine the relative importance of some sources, routes of transmission, and measures to prevent bacteria entering the wound during biliary tract surgery. They found when bacteria were growing in the bile they accounted for majority (greater than 99%) of the bacteria found in the wound. However, when the bile was sterile the skin bacteria at the incision site were found to make a substantial contribution to the wound flora.

Exogenous source for contamination of a surgical incision is responsible for a substantial proportion of infections in clean wounds. Exogenous contamination may come from any personnel or environmental source, although direct contact with the wound by the surgical team is probably the most common pathway for surgical wound infections. Epidemics due to *S. aureus* and group A *Streptococcus* suggest personnel carriers as a source. Epidemics due to Gram negative microorganisms may have been acquired from environmental sources, such as irrigating solutions and anesthesia or respiratory therapy equipment.

3.7 Wound types

There are a number of different classifications of wounds related to their position, their depth and the amount of tissue damage. Russell *et al* (2000) give the general classification of wounds as:

i**) Major wound:** It is defined as a wound which discharges pus and may need a secondary procedure to be sure of adequate drainage. There may be systemic signs of tachycardia, pyrexia and a raised white cell count (SIRS).

ii) **Minor wound:** It may discharge pus or infected serous fluid but should not be associated with excessive discomfort, systemic signs or delay in return home.

According to Russell *et al* (2000) the most useful classification of wounds from a practical point of view is that of Rank and Wakefield into Tidy and Untidy wounds.

i) Tidy wounds: Tidy wounds are inflicted by sharp instruments and contain no devitalized tissue. Examples are surgical incisions, cuts from glass and knife wounds. Tendons, arteries and nerves will commonly be injured in tidy wounds, but repair of these structures is usually possible. Fractures are uncommon in tidy wounds.

ii) Untidy wounds: Results from crushing, tearing, avulsion, vascular injury or burns, and contain devitalized tissue. Skin wounds will often be multiple and irregular. Tendons, arteries and nerves may be exposed, and might be injured in continuity, but will usually not be divided. Fractures are common and multifragmented.

Wounds can be broadly categorized as having either an acute or a chronic etiology

I) Acute wounds: Acute wounds are caused by external damage to intact skin. 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 (Bowler *et al*, 2001).

i) Closed wounds: Closed wounds result from blunt trauma and usual causes are falls, sporting injuries or assaults with a blunt weapon. A blunt injury may result in a bruise or contusion and there is danger of secondary infection (Russell *et al*, 2000).

a) Contusion: A contusion is the familiar bump or swelling which arises often quite quickly following a blow. The skin surface remains intact but small blood vessels may be injured or torn and bleeding may discolour the skin producing a bruise. Generally, a contusion requires no treatment (Kyle *et al*, 1992).

b) Haematoma: This result from rather more severe injury, particularly to the vessels, allowing the escape of larger volumes of blood which collect in the tissue or tissue planes. A subcutaneous haematoma may become infected, particularly if the overlying skin is damaged, and the resulting abscess will require incision (Kyle *et al*, 1992).

ii) Open wounds: Open wounds include following types of wounds:

a) Puncture wounds: A puncture wound is an open injury in which foreign material and organisms are likely to be carried deeply into the underlying tissue (Pintu and Kamal, 2001).

b) Bites: Infection is the major risk with bites due to mixed mouth organisms being deeply implanted into the tissues (Kyle *et al,* 1992). Due to complex nature of the oral microflora in humans and animals, the majority of bite wounds harbor potential pathogens, many of which are anaerobes as well as the common anaerobes in both human and animal bite wounds, such as *Bacteriodes, Prevotella, Porphyromonas,* and *Peptostreptococcus* spp., less common potential pathogens such as *Pasteurella multocida,* and *Eikenella corrodens* may also be involved (Bowler *et al*, 2001).

c) Abrasions and friction burns: An abrasion is a shearing injury of skin (due to horizontal force) in which the surface is rubbed off. Most are superficial and will heal but some may result in full thickness skin loss. A friction burn is similar but there will be an element of thermal damage as well as abrasion (Russell *et al*, 2000).

d) Laceration: A laceration or cut is the result of contact with a sharp object (the surgical equivalent is an incised wound). Once the cutting implement has gone deep to the dermis, there is less resistance in the subcutaneous tissues and the cut may therefore penetrate to a considerable depth (Russell *et al*, 2000). Frequently these wounds are grossly contaminated by clothing material or dirt forced into the tissues at the time of injury (Kyle *et al,* 1992).

e) Traction and avulsion: Avulsion injuries are open injuries where there has been a severe degree of tissue damage producing a degloving injury. Degloving is caused by shearing forces

that separate tissue planes, rupturing their vascular interconnection and causing tissue ischaemia. Skin necrosis may become slowly apparent in the following few days (Pintu and Kamal, 2001).

f) War wounds and gunshot injuries: Low-velocity injuries such as from a hand gun result in an entry and exit wound, the latter being the larger, and damage along the tract of the missiles. High-velocity injuries (from modern assault rifles) cause explosure pressure and decompression effect such that there is widespread tissue damage with injury to major limb vessels and nerves situated some distance from the tract of the missiles (Russel *et al*, 2000).

II) Chronic wounds: Chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromise the integrity of dermal and epidermal tissue (Davis *et al*, 1992). Pathophysiological abnormalities that may predispose to the formation of chronic wounds include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic disease such as diabetes mellitus (Bowler *et al*, 2001).

i) Ulcers: Chronic ulcers are wounds, which fail to heal and are particularly common in the lower third of lower limb and foots. Ulcers are common in diabetes and rheumatoid arthritis (Pintu & Kamal, 2001). Plantar ulcers associated with diabetes mellitus are susceptible to infection due to high incidence of mixed wound flora and the inability of the PMNs to deal with invading microorganisms effectively.

ii) Pressure sores/Decubitus ulcers: These are chronic wounds following tissue necrosis from pressure. They occur over bony prominences. Their pathogenesis is identical to compartment syndrome in that they arise where there is unrelieved pressure in the soft tissues overlying bone such that the external pressure exceeds capillary perfusion and ischaemic necrosis occurs (Russell *et al*, 2000).

Bowler *et al* (2001) commented that approximately 25% of decubitus ulcers have underlying osteomyelitis, and bacteria are also common. They demonstrated the microbial synergy in many decubitus ulcers who reported mixed aerobic and anaerobic microflora in 41% of 58 ulcers in children. *S. aureus*, *Peptostreptococcus* spp., *Bacteroides* spp., and *P. aeruginosa* were the predominant isolates.

Other acute infections: Other examples of acute infections include the following:

i) Cellulitis: Cellulitis is a spreading subcutaneous inflammation caused by haemolytic *Streptococcus*, *Staphylococcus*, *Clostridium* etc. Streptococcal infection produces diffuse inflammation because of production of hyaluronidase and streptokinase. Net result is that the inflammatory exudates spread in the fascial planes resulting in a gross swelling of the affected part. Sources of infection include major or minor injuries, graze or scratch, snake or scorpion bite. Common sites include the lower limb, face or scrotum. The affected part shows evidence of inflammation such as redness and itching followed by diffuse swelling. Skin is stretched and shiny. Pain, fever, toxaemia follow later. In untreated cases, suppuration, sloughing and gangrene can occur (Shenoy, 2001).

ii) Abscess: McLatchie and Leaper (2002) defined abscess as a localized collection of pus and contains live and dead PMNs, lymphocytes and macrophages, as well as bacteria and damaged tissue. Abscess usually liquefy (suppuration) following the acute inflammatory mediators. Shenoy (2001) classifies abscess into:

a) Pyogenic abscess: It is the commonest form of abscess, subcutaneous, deep, or it can occur within the viscera such as liver or kidney, etc. It is usually produced by staphylococcal infections. Signs of pyogenic abscess include calor (heat), rubor (redness), dolor (pain), tumor (swelling) and loss of function.

b) Pyaemic abscess: This is due to pus producing organisms in the circulation (pyaemia). It is multiple, deep seated with minimal tenderness with no local rise in temperature. Hence, it is also called as non-reactive abscess.

c) Cold abscess: Cold abscess means an abscess which has no signs of inflammation. Usually, it is due to tuberculosis e.g. following tubercular lymphadenitis or due to tuberculosis of spine. However, other chronic disease like leprosy, actinomycosis, madura foot etc. also produce abscess which are cold in nature.

iii) Boil/Furuncle: It is a hair follicle infection caused by *S. aureus* or secondary infection of a sebaceous cyst. Common locations of boils include face and back of the neck, axilla, and gluteal region. It starts with painful indurated swelling with surrounding oedema. After 1-2 days, softening occurs in the centre and pustules develop which bursts spontaneously discharging pus (Shenoy, 2001). Because the base of hair follicle may lie in subcutaneous tissue, the infection can spread as cellulitis or it can form a subcutaneous abscess (Rusell *et al*, 2000). Hot tub folliculitis is a special form of folliculitis caused by *Pseudomonas.* It occurs in people who bathe in poorly maintained hot tubs (O'dell, 1998).

iv) Carbuncle: A carbuncle is a deep-seated mass of fistulous tracts between infected hair follicles. This is an infective gangrene of the subcutaneous tissue caused by *S. aureus*. It usually occurs at the nape of neck, back and shoulder region. The initial lesion is similar to a boil in the form of hair follicle infection with perifolliculitis. Constitutional symptoms like fever with chills and rigors are severe (Shenoy, 2001).

v) Erysipelas: Erysipelas is an infection of the skin and subcutaneous tissues by a pathogenic *Streptococcus*. It spreads easily through the skin and produces a diffuse cellulitis. The erythrotoxins produced by the *Streptococcus* make the infected area red, hot, tender, and oedematous. Oedema of the reddened skin gives the involved area a raised border- a diagnostic clinical appearance (Browse, 1997).

vi) Impetigo: Chakraborthy (1995) defined impetigo as a superficial discrete crusted spot, especially in children, usually less than one inch in diameter. Lesions may appear singly or in clusters.

vii) Necrotizing fascitis: Necrotizing fascitis is the term used currently to describe the invasive soft tissue infection originally referred to as 'streptococcal gangrene'. This infection may be caused by bacteria other than group A streptococci (e.g. clostridia, staphylococci), by mixtures of different bacteria or by *S. pyogenes* alone. Infection is initiated after trauma that may be minor or even inapparent (Collier *et al*, 1998).

3.7.1 Surgical wound infection

Surgical wound infections cause suffering and increased costs. In her time, Florence Nightingale found the same problem and wrote in her book Notes on hospital,"It may seem strange principle to enunciate as the very first requirement of hospital is that it should do patient no harm" (Swenne, 2006).

Bowler *et al* (2001) stated that the risk of infection generally is based on the susceptibility of a surgical wound to microbial contamination. Clean surgery carries 1 to 5% risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27% risk of infection has been estimated. The Guideline for Prevention of Surgical Site Infection, 1999 issued by the Centers for Disease Control and Prevention classified surgical wound infections as being either incisional (involving skin, subcutaneous tissue, or deeper fascia and muscle tissue) or organ/space, involving any internal organs or anatomical spaces. With the exception of clean operative procedures, surgical wound infections are recognized as having a polymicrobial etiology, involving both aerobic and anaerobic microorganisms, and intra-abdominal infections normally reflect the microflora of the resected organ. CDC (1996) and Cruse & Foord (1980) has stated that there is <2% infective risk of wound infection in clean surgical wound, <10% infective risk in clean contaminated, about 20% in contaminated and about 40% in dirty infected surgical wound infection.

Global scenario of surgical wound infection

Singhal & Zammit (2006) has reported that internationally, the frequency of SSI is difficult to monitor because criteria for diagnosis might not be standardized. A survey sponsored by

WHO demonstrated a prevalence of nosocomial infections varying from 3-21% with wound infections accounting for 5-34% of the total.

The 2002 survey report by the Nosocomial Infection National Surveillance Service (NINSS), which covered the period between October 1997 and September 2001, indicates that the incidence of hospital acquired infection related to surgical wounds in the United Kingdom is as high as 10% and costs the National Health Service in the United Kingdom approximately 1 billion pounds (1.8 billion dollars) annually. Mangram *et al* (1999) reported that Surgical Site Infections (SSI) is associated not only with increased morbidity but also with mortality as 77% percent of the deaths were related to surgical wound infection.

Bowler *et al* (2001) state that despite the frequency and prevalence of endogenous anaerobes in surgical wound infections, the Centers for Disease Control and Prevention guideline for the prevention of surgical site infection has recognized *S. aureus*, CONS, *Enterococcus* spp., *E. coli, P. aeruginosa*, and *Enterobacter* spp. as the most frequently isolated pathogens.

Mitchell *et al* (1999) state that there are few published Australian data, but the Australian Nosocomial Infection Prevalence Survey published in 1984 showed that surgical wound infections accounted for 34% of nosocomial infections with an estimated excess annual cost of $60 million Australia wide.

In the study conducted by Reid *et al* (2002), at Christchurch hospital, New Zealand, a complete follow up of 1934 patients were done out of 1964 clean procedures performed, a total of 239 wound infections were recorded (12.6%). Wound infections occurred in 86(4.5%) patients prior to discharge and 153 (8.1%) after discharge. Infection rates varied with operation type: vascular (18.3%), breast (16.0%), abdominal (10.3%), hernia (8.0%), head and neck (7.1%).

A UK survey of 157 hospitals carried out in 1993/94 found that the prevalence of wound infection was 2.6% amongst 12,947 patients in eight surgical specialties, varying from 1.5% in neurosurgery to 6.2% in vascular surgery (Emmerson *et al,* 1996).

3.7.2 Burn wound infection

Burn is a tissue injury from thermal (heat or cold) application or from the absorption of physical energy or chemical contact. Each has its own distinctive features and management problems (Russell *et al,* 2000).

It is almost inevitable that a burned surface will become infected. The flora of the burn wound also influence the risk of infection and the invasive potential of the infection that does occur. The microbes in the wound immediately after burning is sparse and predominantly Gram positive. As time passes by, Gram negative organisms colonize the eschar, and by the end of first post burn week they have become the predominant inhabitants of burn wound (Pruitt *et al,* 1998).

Table 2: Classification of burns

Type of burn	**Tissue injury**
Scalds	Partial-thickness/ deep dermal skin loss
Fat burns	Usually full thickness skin loss
Flame burns	Patches of partial and full thickness
Electric burns	Full thickness with deep extensions
Cold injury	Ice formation, tissue freezing, vasospasm
Friction burns	Heat plus abrasion
Ionizing radiation	Early tissue necrosis, later tissue dysplastic changes
Chemical burn	Inflammation, tissue necrosis, systemic effects

(Russell *et al,* 2001)

Burn injury causes mechanical disruption to the skin, which allows environmental microbes to invade the deeper tissues. The usual skin barrier to microbes is replaced by a moist, protein-rich, avascular eschar that fosters microbial growth. The avascularity of the eschar prevents migration of immune cells and restricts the distribution of systemically administered antibiotics. Furthermore, toxic intermediaries released by the eschar can impede the immune response. The risk of burn wound infection is directly correlated to the extent of the burn and is related to impaired resistance resulting from disruption of the skin's mechanical integrity and generalized immune suppression (Schwarz & Dulchavsky, 2005).

Global scenario

Bowler *et al* (2001) reported that 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. Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts, many studies have reported there is prevalence of aerobes for e.g. *P. aeruginosa, S. aureus, E. coli, Klebsiella* spp., and *Enterococcus* spp.

Schwarz & Dulchavsky in 2005 reported that in the U.S. the risk of invasive burn wound infection is directly related to the extent of the burn. Burn wound infections account for 3-7% of all infections in patients with burns and occur most frequently in children, followed by elderly patients. Internationally, burn wound infections occur more frequently in countries with overcrowded burn units, fewer infection control barriers, and less access to immediate wound debridement or antimicrobial therapies.

3.7.3 Acute bacterial mastitis/Breast abscess

Although common in the lactating breast being called as lactational mastitis, abscesses are occasionally seen in the non-lactating phase (Kyle *et al,* 1992).

Predisposing factors

- Crack or fissure in the nipple
- Retracted nipple (hence cleaning of the breast is a problem)

- Oral cavity infection in child

The most common organism is *S. aureus*, which enters through the nipple, proliferates intradermally and produces clotting of the milk. Within the clot the organisms multiply which results in a cellulitic stage of the breast (mastitis) and in untreated cases, it may give rise to breast abscess. Initially only one lobule and duct get affected, later other lobules give rise to an intramammary abscess.

Clinical features

i) Severe pain in the breast due to spreading inflammatory exudates.

ii) Breast is swollen, tense, tender, and warm to touch. These are the signs of cellulitic stage.

iii) Once breast abscess develops, there is high grade fever with chills and rigors and a soft cystic fluctuant swelling can be felt in the breast. In the untreated cases, abscess may rupture through the skin resulting in necrosis of the skin of breast, ulceration and discharge.

iv) In deep seated abscess, in absence of immediate drainage, significant amount of breast tissue will be destroyed (Shenoy, 2001).

Global scenario

According to Montgomery (2001), bacterial mastitis occurs in about 2.5 percent of nursing mothers, most commonly occurring between 2 and 5 weeks post-delivery. It can be prevented by good breast hygiene and hand-washing, and by regular emptying of the breast. If a specific area of the breast does not drain well, manual expression of the milk from that duct may help avoid milk stasis. Accurate estimates of the proportion of women who develop a breast abscess following mastitis are difficult due to the varied definitions of mastitis used.

Amir *et al* (2004) estimated that 11% of women with mastitis develop a breast abscess however the current incidence in developed countries appear to be much lower (3%).

3.7.4 Ulcer

An ulcer is a discontinuity of the skin or mucous membrane, which occurs due to microscopic death of the tissues. The varying etiological factors, presence of complicated systemic

diseases, make the treatment of ulcers very difficult. Chronic ulcers in old people definitely cause considerable morbidity and diabetic ulcers of leg can cause life threatening complications such as diabetic ketoacidosis and septicemia (Shenoy, 2001).

Diabetic ulcer foot: Younes & Bakri (2006) stated that diabetic foot infection is suspected or documented infection of the tissues that comprise the foot of a diabetic person. Diabetic patients are more prone for development of ulcers in the foot because of resistance to infection. Uncontrolled diabetic patients are more susceptible for infection. Even though leukocytosis occurs in diabetic patients with infection, the phagocytic activity of the leukocytes is greatly reduced (Shenoy, 2001). As in most wound types, *S. aureus* is a prevalent isolate in diabetic foot ulcers, together with aerobes including *S. epidermidis*, *Streptococcus* spp., *P. aeruginosa, Enterococcus* spp., and coliform bacteria (Bowler *et al*, 2001).

3.7.5 Acute soft tissue infections

Infections of skin and soft tissues are common in community and hospital settings. The majority of patients with superficial infections can be treated as outpatients but patients with deep and spreading infections require a more aggressive approach involving surgical exploration and antimicrobial therapy. Acute soft tissue infections include cutaneous abscesses, traumatic wounds, and necrotizing infection. Microbiological investigations have shown that *S. aureus* is the single causative bacterium in approximately 25 to 30% of the cutaneous abscesses, and the same organism has also been recognized as being the most frequent isolate in superficial infections seen in hospital accident and emergency departments. However, other studies have demonstrated that approximately 30 to 50% of cutaneous abscesses, 50% of traumatic injuries of varied etiology, and 47% of necrotizing soft tissue infections have a polymicrobial aerobic-anaerobic microflora (Bowler *et al*, 2001).

3.8 Microbiology of wounds

The effect of specific types of microorganisms on wound healing and infection has widely been published and the majority of wounds are polymicrobial, involving aerobes and

anaerobes (Bowler *et al*, 2001). Breidenbach & Trager (1995) demonstrated that a critical level of bacteria of $\geq 10^4$ CFU/g of tissue must be reached to cause infection in complex extremity of wounds and that quantitative tissue cultures predict the likelihood of wound infection more effectively than swab cultures.

It is impossible to list all the microorganisms found in different types of wounds and the microbial flora of wound appear to change over time. In early acute wound, normal skin flora predominates. *S. aureus* and β-haemolytic *Streptococcus* soon follow. After about 4 weeks, facultative anaerobic Gram negative rods will colonize the wound, most common ones being *Proteus, E. coli, and Klebsiella*. As the wound deteriorates, deeper structures are affected. Anaerobes become more common. Oftentimes infections are polymicrobial. Long-term chronic wounds oftentimes contain more anaerobes than aerobes. Aerobic gram-negative rods also infect wounds late in the course of chronic wound degeneration. Organisms like *Pseudomonas* are not very invasive unless the patient is highly compromised. As the wounds go deeper and become more complex they can infect the underlying muscles and bones causing osteomyelitis. Coliforms and *S. aureus* are associated with osteomyelitis (Chamberlain, 2004). The potential pathogens commonly encountered in wound infection:

Gram positive cocci	*S. aureus, S. pyogenes, S. faecalis, S. epidermidis, S. pneumoniae,* CONS
Gram negative aerobic rods	*Pseudomonas* spp., *Acinetobacter* spp.
Gram negative facultative rods	*E. coli, Proteus* spp., *Klebsiella* spp., *Morganella* spp., *Enterobacter* spp., *Citrobacter* spp., and other enterobacteriaceae
Anaerobes	*Peptostreptococcus* spp., *Bacteroides fragilis* group, *Bacteroides melaninogenicus, Clostridium* spp.
Fungi	*Candida, Aspergillus*

(Forbes *et al*, 2002 & Collier, 2002)

3.9 Microbiological analysis of wounds

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

Proper collection, transport, and storage of these specimens are of greatest importance, and compromises should be avoided. Once a specimen has been obtained, packaged, and dispatched to the laboratory, it should be processed as soon as possible. After the preliminary examinations have been completed and the cultures made, the rest of the specimen should be properly labeled, stoppered, and refrigerated, until it is certain that no additional laboratory tests are needed (Vandepitte *et al*, 2004).

Collection of specimen: Superficial wounds are always colonized by commensal microflora hence before swabbing wound should be cleaned with 70% alcohol or non bacteriostatic sterile saline. Pus or fluid aspirate in a syringe, deep swabbing or punch biopsy of the leading edge of the lesion is preferred to the wound swab (Miller, 1999). Superficial swabs are the worst specimens for cultures as it yields many squamous epithelial cells and little PMNs on microscopic examination and the isolated organisms may only be the colonizing ones not involved in the infective process. But although the value of acquiring superficial swab samples has been seriously questioned, the procedure is simple, inexpensive, noninvasive and convenient for most of the wounds. If anaerobic bacteria are suspected to be involved in an infectious process, fine needle aspiration or tissue biopsy is preferred since swabs are not valid transport media for these pathogens (Dediste, 2006).

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

Macroscopic examination of pus: Specimens of pus, received in a syringe or in a sterile container should be evaluated carefully for color, consistency and odour. The colour of the pus varies from green-yellow to brown-red. A red colour is generally due to admixture with blood or haemoglobin. Pus from postoperative or traumatic wounds (burns) may be stained blue-green by the pyocyanin pigment produced by *P. aeruginosa*. The consistency of pus may vary from a turbid liquid to one that is very thick and sticky. The presence of granules must also be observed. A foul feculent odour is one of the most characteristic features of an anaerobic or a mixed aerobic-anaerobic infection, although it may be lacking in some instances (Vedepitte *et al*, 2004).

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

Culture of wound specimens: Routine analysis of wound specimens normally involves the use of selective and nonselective agar media to culture aerobic bacteria and yeasts and, if the specimen is purulent and/or malodorous, anaerobic bacteria also (Bowler *et al,* 2001). Independently from the results of microscopy, all specimens of pus or exudates should preferably be inoculated onto a minimum of three culture media.

- A Blood agar for the isolation of staphylococci and streptococci,
- A MacConkey agar plate for the isolation of Gram negative rods, and
- A tube of broth that can serve as enrichment medium for both aerobes and anaerobes, e.g. thioglycollate broth or cooked meat medium (Vandepitte *et al*, 2004).

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

Antibiotic sensitivity tests: 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. Routine susceptibility tests should not be performed on bacteria that have a known sensitivity pattern such as streptococci, *Pasteurella* and *Actinomyce*s, which are almost without exception susceptible to Benzylpenicillins. For Enterobacteriaceae, non-fermentative Gram negative rods, and staphylococci, the standardized disc-diffusion test (Kirby-Bauer) should be used (Vandepitte *et al*, 2004).

CHAPTER-4

4. MATERIALS AND METHODS

A list of equipments, reagents, media and antibiotics used for the study is presented in Appendix I. The study was carried out in microbiology section, pathology department, Bir Hospital. The objective of the study was to study the common types of wounds; to identify the common types of bacteria involved in causing infection of wound and their antibiotic sensitivity pattern among the patients visiting the outpatient department of surgery and dressing unit of Bir hospital.

In 10 months study from August 2006 to June 2007, a total of 400 samples were collected for culture and sensitivity from the patients with age range 6 to 89. The types of wound included surgical wounds, breast abscesses, Trauma, burns, ulcers, and other other pyogenic wounds.

4.1 Specimen collection

The sample collected was pus and wound swab on a sterile cotton swab or in a stoppered syringe without contamination with commensals or from external source. The specific anatomic site from which sample was collected was specified and whether the wound is deep or surface wound. Surface wound was cleansed with normal saline and the base was vigorously swabbed and placed in sterile container.

The sample was taken to the laboratory for processing as early as possible, to avoid desiccation of sample and to prevent the growth of some species at room temperature, which may obliterate the true pathogens. Two samples were taken from each patient, one for culture and another for direct Gram stain.

4.2 Sample processing

The sample was processed as soon as it reached the laboratory following standard laboratory procedures. Of two samples taken from each patient, one was used for Gram stain and other for culture (Collee *et al*, 1999).

4.2.1 Macroscopic examination

The colour, odour and whether it contained granules were noted. Specimens collected in a syringe were easy to evaluate but when obtained in swab were difficult to evaluate.

4.2.2 Microscopic examination

An even smear of the specimen was made on clean slide. The smear was heat fixed and stained by the Gram stain method. The smear was examined for bacteria among pus cells using 40x and 100x objectives.

4.2.3 Culture of specimen

Under aseptic technique, aerobic and anaerobic culture of the fresh wound exudates (pus) was done. Since a swab was generally used for the inoculation, it was applied to a small area of the plate the rest of the surface streaked out with loop. The sample was inoculated on Nutrient Agar (NA), MacConkey Agar (MA), two Blood Agar (BA) plates and Robertson's Cooked Meat Broth (RCMB).

For aerobic bacteria, BA, NA and MA plates were incubated at 37ºC for 24 to 48 hours in ordinary incubator. For anaerobic bacteria, a plate was incubated at 37ºC for 48 hours in anaerobic gas jar with anaerobic gas pack that reduced the oxygen level in the gas jar. The cooked meat broth medium was incubated at 37ºC for up to 72 hours at 37ºC for enrichment of exacting aerobes and anaerobes (Collee *et al*, 1999). The composition and preparation of media are given in appendix II.

4.2.4 Isolation and identification of bacteria

For aerobic organisms, the identification was done using standard bacteriological chart. The aerobic culture plates were examined after 24 hours incubation. In every case, colony morphology was studied, Gram-stained smear was prepared and observed under the microscope. Biochemical tests then followed for identification of bacteria. Different biochemcal tests performed were catalase test, oxidase test, coagulase test, oxidative-fermentative (OF) test, methyl-red (MR) test, voges-proskauer (VP) test, indole test, motolity test, hydrogen sulphide (H_2S) production test, triple sugar iron (TSI) reactions, citrate

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

For anaerobic bacteria, after 48 hours, the growth on the anaerobic blood agar was inspected and compared with the growth on aerobic plating media. Each colony type was examined with Gram stain. Bacteria with the same microscopic appearance that grew on aerobic and anaerobic agar were considered to be facultative anaerobes (Colonies that appear only on anaerobic culture are probable anaerobes and were subcultured).

The turbidity on cooked meat broth medium was observed. If the culture plate showed no growth but turbidity seen in the cooked meat broth, then loopful of the broth was again sub cultured into the blood agar plate. When the turbidity did not appear within 72 hours incubation in cooked meat broth and no organism seen on gram staining, the sample was reported as ”no growth”.

4.2.5 Antibiotic susceptibility testing for isolated organisms

The results of susceptibility testing was made available within 48 hours after receiving the specimen. Bacterial susceptibility to antimicrobial agent was done in vitro by modified Kirby-Bauer method using fresh broth culture of the isolates in Muller Hinton Agar Medium. The different antibiotics used and the test procedures are given in the appendix III.

4.2.6 Establishment of 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, which is equipped with a secure gas-proof lid, which can be easily removed and replaced. Commercially available disposable anaerobiosis-generating device, AnaeroGen sachet was used to absorb atmospheric oxygen in the jar with simultaneous generation of carbon dioxide. This novel method differs from those commonly used in that the reaction proceeds with no evolution of hydrogen, and therefore does not require a catalyst. Furthermore, no addition of water is needed to activate the reaction. AneroGen sachet reduces

the oxygen level in the jar to below 1% within 30 minutes. The resulting CO_2 level will be between 9% and 13%.

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Procedure:

- The inoculated Blood agar plates were placed in the appropriate anaerobic jar.
- AnaeroGen foil sachet was torn open at the tear nick indicated, and the AnaeroGen paper sachet was removed from within.
- Immediately, the AnaeroGen paper sachet was placed in the appropriate clip on the plate carrier within the jar.
- Then the jar lid was closed immediately. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute. Extended exposure will result in loss of reactivity and full anaerobic conditions may not be achieved in the jar.
- The gas jar was incubated at 37ºC for 48 hours.
- The plates were then removed and the presence of anaerobes was examined.

4.3 Quality control for tests

Quality control is absolutely essential for good operating procedure (Vandepite *et al*, 2004). An important criterion of quality for a microbiological test is how much it contributes to the prevention or cure of infectious diseases.

To maintain quality control, all tests were performed in an aseptic condition. Aseptic method was followed during sample collection using sterile swab and syringe in order to avoid contamination. The sample was also processed in aseptic condition. The sterility of each batch of test medium was confirmed by incubating one uninoculated tube and plate with the inoculated tests as quality control. During the test, one tube of each batch of medium was inoculated with known organism for positive reaction and another tube with stock culture known to give negative reaction. These positive and negative controls were incubated along with test and compared the results.

CHAPTER-5

RESULTS

A total of 400 pus samples (swabs and aspirates) were taken from many types of wounds, abscesses and boils from the Out Patient Department of surgery or dressing room of Bir Hospital. The results obtained are shown below.

5.1 Types and distribution of samples

The total number of 400 wound specimens was collected in sterile cotton wool swab and also aspirated in case of abscesses, boils and cellulitis. Different types of wound samples and their age and genderwise distribution are given in the tables 3, 4, 5 and figures 2, 3 and 4.

Table3: Types of samples

S. No.	Types of wound samples	No. of samples	Percentage (%)
1	Surgical wounds	61	15.25
2	Burn wounds	20	5
3	Breast abscess	65	16.25
4	Ulcers	25	6.25
5	Trauma	70	17.5
6	Other pyogenic wounds	159	39.75
	Total	**400**	**100**

There were 400 wound specimens collected and processed, out of which 61 (15.25%) were collected from patients with post discharge surgical wound infection. Similarly 20 (5%) specimens from persons with burn wounds were collected. Pus samples from patients with breast abscess were 65 (16.25%), ulcers were 25 (6.25%). 70 (17.5%) of the samples were collected from the patients with trauma and the maximum numbers of samples were of patients with other pyogenic wounds/abscesses, which were 159 (39.75%) in number.

Table 4: Genderwise distribution of patients in different wound specimens

S. No.	Types of specimens	Male		Female		Total patients
		No	%	No	%	
1	Surgical wound	42	68.85	19	31.1	61
2	Burn wound	14	70	6	30	20
3	Breast abscess	-	-	65	100	65
4	Ulcer	12	48	13	52	25
5	Accidental wound	51	72.86	19	26.40	70
6	Other pyogenic wound	108	67.9	51	32.07	159
	Total	**227**		**173**		**400**

The table 4 shows the higher number of male patients with wound infection than female patients. Out of 400 wound specimens, 227 (56.75%) samples were taken from male patients and 173 (43.25%) samples were taken from female patients. Out of 61 patients with surgical wounds, 42 (68.85%) were male and 19 (31.1%) were female. Among 20 patients with burn wounds 14 (70%) were male patients and 6 (30%) were female patients. All 65 (100%) cases with breast abscess were female. Out of 25 cases of ulcer, 12 (48%) were taken from male patients and 13 (52%) were taken from female patients. There were 70 cases of Trauma, out of which 51 (72.86%) were of male and 19 (26.40%) were of female patients. Similarly, out of 159 samples of patients with other pyogenic wounds 108 (67.9%) were male and 51 (32.075%) were female.

Table 5: Age and Genderwise distribution of patients

Age (years)	Male	Female	Total	Percentage (%)
0-10	9	4	13	3.25
11-20	53	45	98	24.5
21-30	63	58	121	30.25
31-40	37	28	65	16.25
41-50	27	13	40	10
51-60	16	9	25	6.25
61-70	14	11	25	6.25
71-80	5	4	9	2.25
81-90	3	1	4	1
Total	**227**	**173**	**400**	**100**

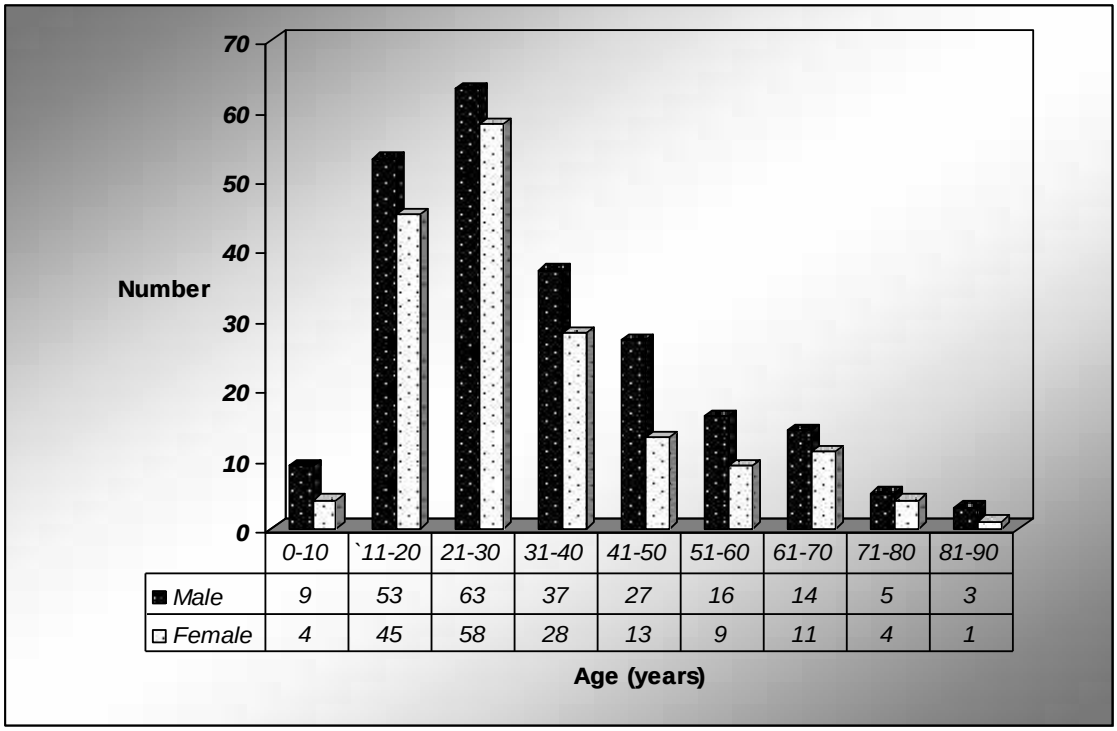

Figure 2: Age and Genderwise distribution of patients

Table 5 and figure 2 demonstrates that out of 400 wound specimens, 227 (56.75%) were collected from male patients and 173 (43.25%) were collected from female patients. The maximum number of patients belonged to the age group of 21 to 30 years 121 (30.25%) out of which 63 were male and 58 were female, followed by age group 11 to 20. The patients with age group 1 to 10 and above 80 were found to be less affected.

5.2 Pattern of growth in different types of wound specimens

Table 6: Growth pattern in different types of wound specimens

S. No.	Type of specimens	Growth		No growth		Total
		No	%	No	%	
1	Surgical wounds	44	72.13	17	27.86	61
2	Burn wounds	12	60	8	40	20
3	Breast abscess	4	69.2	20	30.77	65
4	Ulcers	18	72	7	28	25
5	Other trauma	39	55.71	31	44.28	70
6	Other pyogenic wounds	115	72.32	44	27.67	159
	Total	**273**		**127**		**400**

273 (68.25%) specimens out of 400 showed growth and 127 (31.75%) showed no growth. Among the 61 samples from postdischarge surgical wound infection 44 (72.13%) showed growth and 17 (27.868%) were sterile. Similarly out of 20 burn wounds 12 (60%) showed growth and the remaining 8 (40%) showed no growth. There were 65 cases of breast abscess where 45 (69.2%) were growth positive and 20 (30.778%) showed no growth. Among the 25 patients with ulcers, 18 (72%) showed growth and 7 (28%) showed no growth. There were 70 cases of other trauma, out of which, 39 (55.714%) were growth positive and 31 (44.28%) were growth negative. Among the 159 cases of other pyogenic wounds, 115 (72.327%) showed growth and 44 (27.67%) showed no growth.

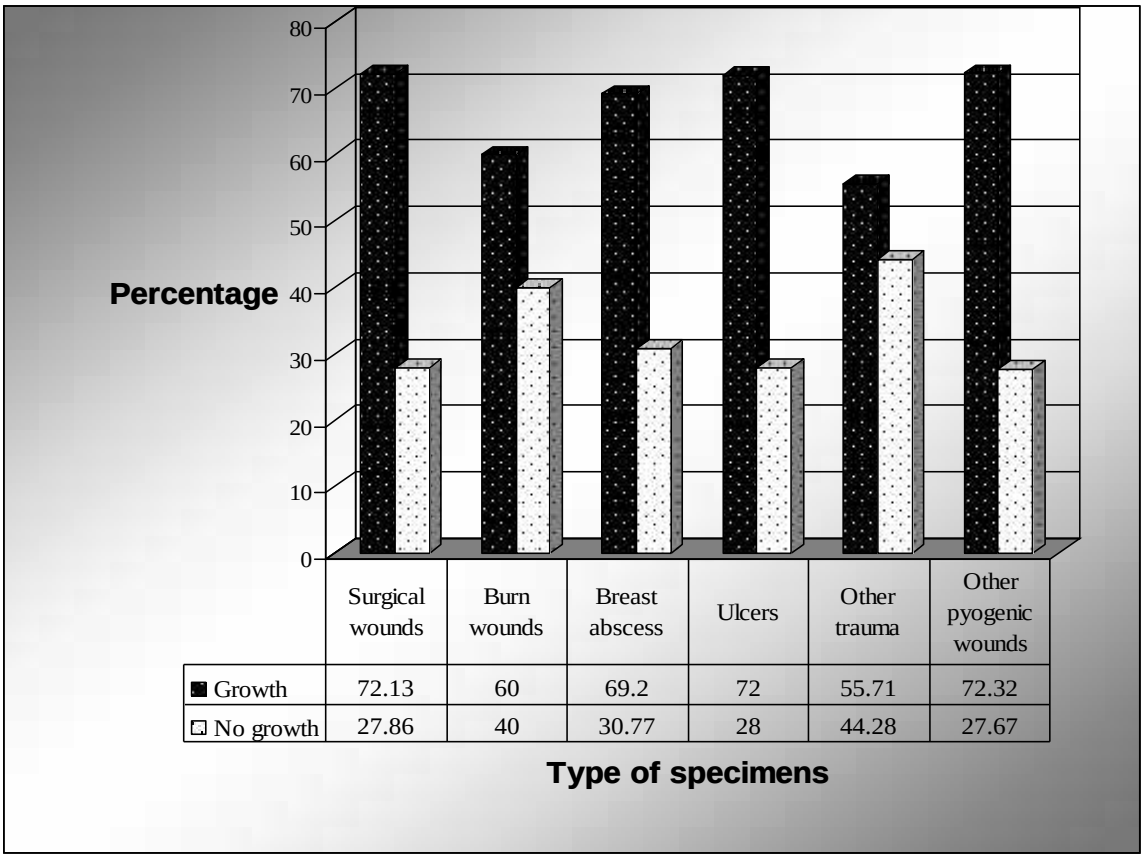

Figure 3: Growth pattern in different wound specimens

Table 7: Pattern of single and multiple microbial isolates in different wound specimens

S. No.	Types of samples	Single isolate		Multiple isolate		Total growth positive
		No.	%	No.	%	
1	Surgical wounds	39	88.63	5	11.36	44
2	Burn wounds	10	83.33	2	16.66	12
3	Breast abscess	41	91.11	4	8.89	45
4	Ulcers	15	83.33	3	16.66	18
5	Trauma	32	82.05	7	17.95	39
6	Other pyogenic wounds	102	88.69	13	11.30	115
	Total	**239**		**34**		**273**

Table 7 shows that out of 273 samples that showed growth, 239 (87.54%) showed growth of single bacterial isolate and 34 (12.40%) samples showed the growth of multiple bacterial isolate. Among samples of surgical wounds, 88.63% showed single isolate and 11.36% showed multiple bacterial isolate. 83.33% of specimens showing growth in burn wound showed the presence of single bacterial isolate and 16.67% showed multiple bacterial isolate. Among positive samples of breast abscess, 91.11% showed the growth of single bacterial isolates and 8.89% showed multiple isolate. 83.33% showed the presence of single bacterial isolate and 16.66% multiple bacterial isolate in ulcers. Among other trauma, 32(82.05%) showed the presence of single bacterial isolate and 7 (17.95%) showed multiple bacterial isolate. Similarly, among samples of other pyogenic wounds, 102 (88.69%) showed single bacterial isolate and the remaining 13 (11.30%) specimens showed the presence of multiple bacterial isolate.

5.3 Pattern of bacterial growth in different wound specimens.

Table 8: Pattern of Gram stain reaction in different types of samples

S. No.	Types of samples	Gram positive bacteria		Gram negative bacteria		Total bacteria
		No	%	No	%	
1	Surgical wounds	32	65.30	17	34.70	49
2	Burn wounds	5	35.71	9	64.28	14
3	Breast abscess	38	77.55	11	22.44	49
4	Ulcers	12	57.14	9	42.86	21
5	Other trauma	30	65.21	16	34.78	46
6	Other pyogenic wounds	87	67.71	41	32.28	128
	Total	**204**		**103**		**307**

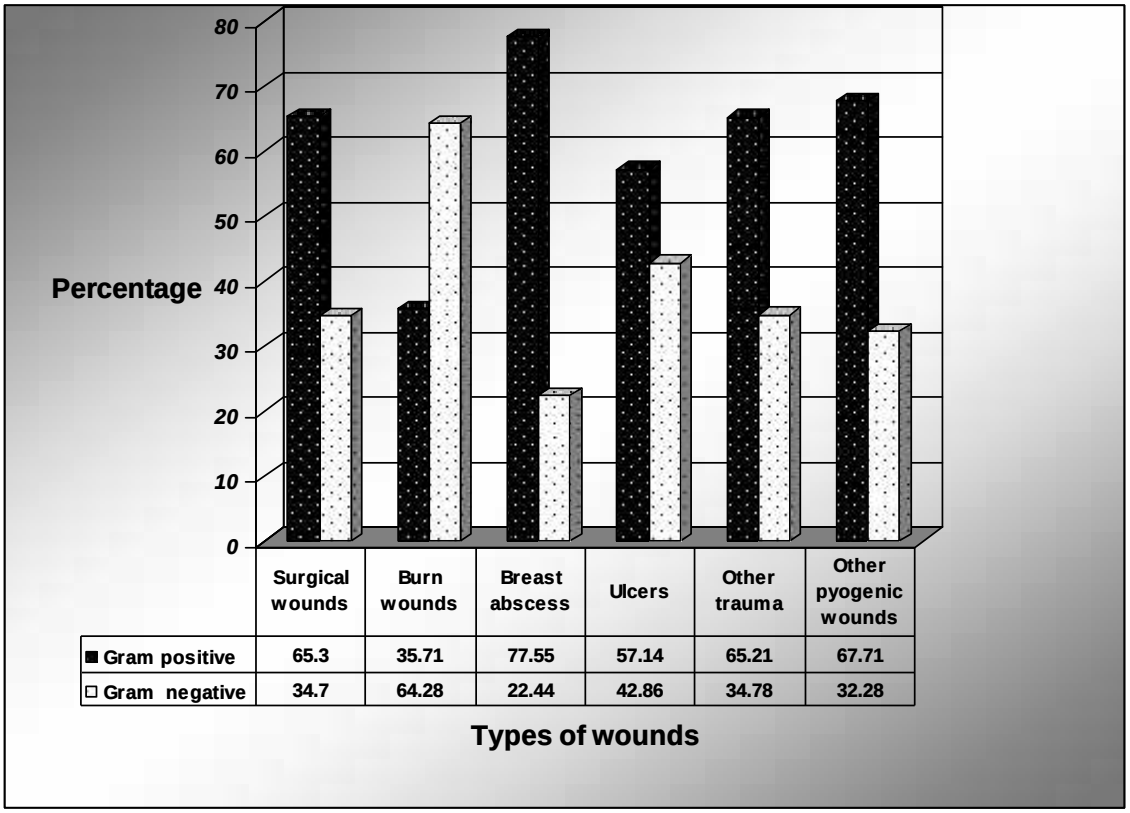

Figure 4: Pattern of Gram stain reaction in different wound specimens

There were 273 specimens that showed growth, which included single and multiple isolates, hence the total number of bacteria were 307. Out of 307 bacteria isolated 204 (66.45%) were Gram positive and 102 (33.55%) bacteria were Gram negative. Among 49 bacteria of surgical wounds 32 (65.30%) were Gram positive and 17 (34.7%) bacteria were Gram negative. Out of 14 bacteria isolated from burn wounds 5 (35.71%) were Gram positive and remaining 9 (64.28%) were Gram negative. Out of 49 bacteria isolated from breast abscess, 38 (77.55%) were Gram positive and 11 (22.44%) bacteria were Gram negative. In ulcers, 57.14% were Gram positive and 42.86% were Gram negative. Out of 46 bacteria isolated from Trauma 65.21% were Gram positive and 34.78% were Gram negative. Similarly, out of 115 growth positive samples of other pyogenic wound infection, 127 bacteria were isolated. Out of which 86 (67.71%) were Gram positive and 41 (32.28%) were Gram negative.

Table 9: Types of Gram positive bacterial isolates in wound specimens

Gram positive isolates	Number	Percentage (%)
S. aureus	143	70.10
CONS	35	17.16
S. pyogenes	5	2.45
Other β-haemolytic Streptococci	8	3.92
Non- heamolytic streptococci	6	2.94
Gram positive rod (unidentified)	7	3.43
Total	**204**	**100**

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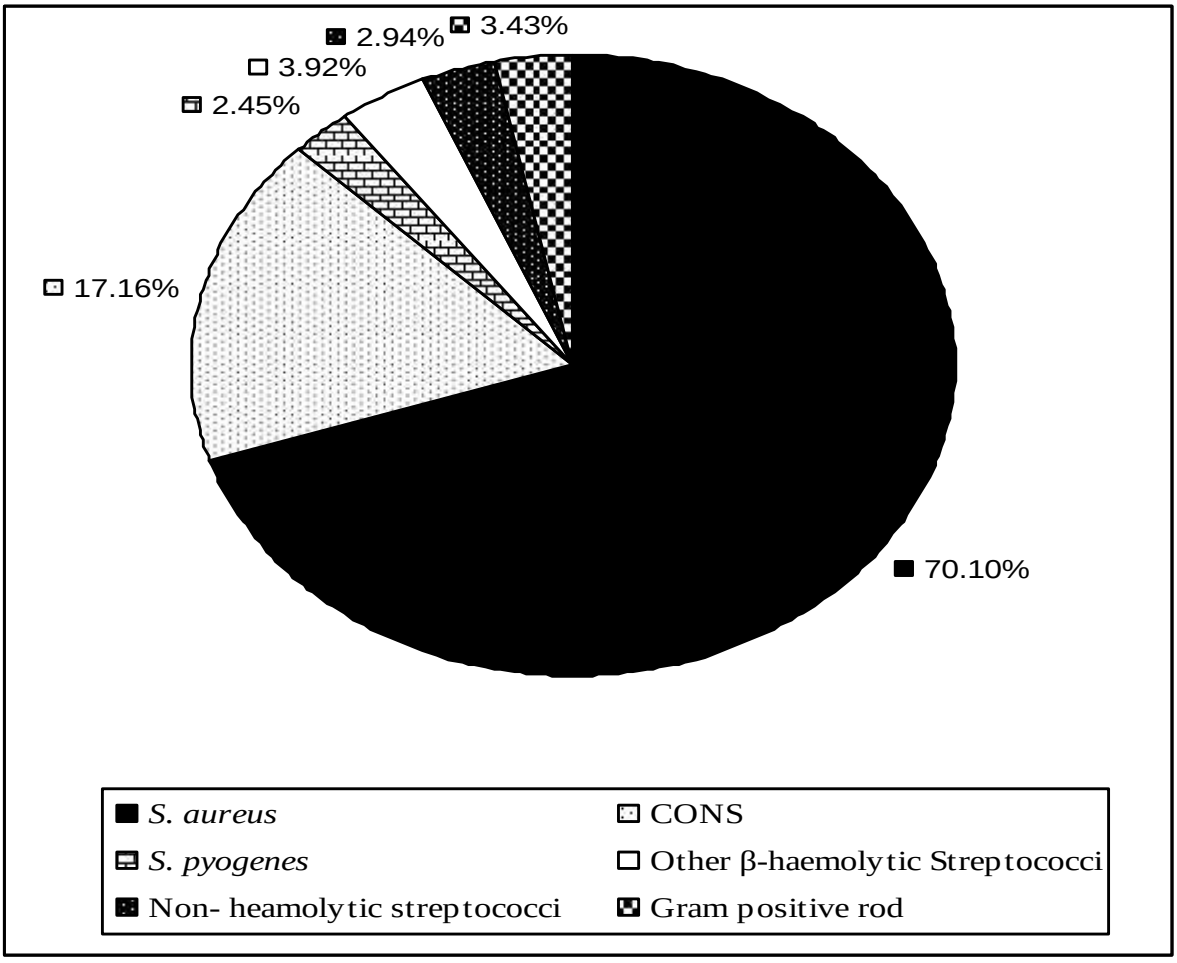

Figure 5: Gram positive isolates among different types of wounds

The above table and figure 5 shows that 204 Gram positive bacteria were isolated. *S. aureus* was present in highest number i.e. 143 (70%) followed by CONS i.e. 35 (17.16%) which was then followed by β haemolytic Streptococci other than *S. pyogenes* which was 8 (3.92%) in number. Non haemolytic streptococci was 6 (2.94%) in number, Gram positive aerobic rods (unidentified) were 7(3.4%) in number and finally *S. pyogenes* was 5 (2.45%) in number

Table 10: Types of Gram positive bacteria in surgical wounds

Gram positive bacteria	**No. in single isolate**	**No. in mixed growth**	**Total**	**%**
S. aureus	20	4	24	75
CONS	4	-	4	12.5
Other β-haem. streptococci	1	-	1	3.12
Non haem. streptococci	2	-	2	6.25
Gram positive rod (unidentified)	-	1	1	3.12
Total	**27**	**5**	**32**	**100**

Among surgical wounds, total Gram positive isolates were 32. *S. aureus was* the most frequently isolated bacteria i.e. 24 (75%) followed by CONS which was 4 (12.5%) in number.

Table 11: Types of Gram positive bacteria in burn wounds

Gram positive bacteria	**No. in single isolate**	**No. in mixed growth**	**Total**	**%**
S. aureus	3	1	4	80
CONS	-	1	1	20
Total	**3**	**2**	**5**	**100**

The above table shows that only Gram positive bacteria isolated from burn wounds was S. *aureus* and CONS.

Table 12: Types of Gram positive bacteria in breast abscess

Gram positive bacteria	No. in single isolate	No. in mixed growth	Total	%
S. aureus	30	3	33	86.84
CONS	2	-	2	5.26
S. pyogenes	2	-	2	5.26
Other β-haem streptococci	-	1	1	2.63
Total	**34**	**4**	**38**	**100**

The above table shows that among the Gram positive isolates, *S. aureus* was the most frequently isolated bacteria i.e 33(86.84%) in number followed by CONS together with *S. pyogenes* which were both 2(5.26%) in number.

Table 13: Types of Gram positive bacteria in ulcers

Gram positive bacteria	No. in single isolate	No. in mixed growth	Total	%
S. aureus	8	2	10	83.33
CONS	1	1	2	16.67
Total	**9**	**3**	**12**	**100**

Among the Gram positive bacterial isolates from patients with ulcers, only *S. aureus* (n=10) and CONS (n=2) were isolated.

Table 14: Types of Gram positive bacteria in other trauma

Gram positive bacteria	No. in single isolate	No. in mixed growth	Total	%
S. aureus	14	4	18	60
CONS	5	1	6	20
Other β haem. streptococci	3	-	3	10
Gram positive rod (unidentified)	3	-	3	10
Total	**25**	**5**	**30**	**100**

The above table shows that *S. aureus* was the most common Gram positive bacteria that was 18 (60%) followed by CONS which was 6 (20%) in number.

Table 15: Types of Gram positive bacteria in other pyogenic wounds

Gram positive bacteria	**No. in single isolate**	**No. in mixed growth**	**Total**	**%**
S. aureus	46	8	54	62.07
CONS	17	3	20	23
S. pyogenes	3	-	3	3.45
Other β haem. streptococci	2	1	3	3.45
Non haem. streptococci	3	1	4	4.6
Gram positive rod (unidentified)	2	1	3	3.45
Total	**73**	**14**	**87**	**100**

The above table demonstrates that *S. aureus* occurred in highest number i.e. 54 (62.07%) followed by CONS which was 20 (23%) among the Gram positive bacteria isolated.

Table 16: Types of Gram negative bacterial isolates in wound specimens

Gram negative isolate	**No. in single isolate**	**No in mixed growth**	**Total**	**%**
E. coli	22	16	38	36.89
P. aeruginosa	17	6	23	22.33
K. oxytoca	6	2	8	7.77
K. pneumoniae	3	-	3	2.91
E. aerogenes	7	3	10	9.71
P. mirabilis	1	5	6	5.82
P. vulgaris	4	3	7	6.8
Acinetobacter spp.	6	-	6	5.82
C. freundii	2	-	2	1.94
Total	**68**	**35**	**103**	**100**

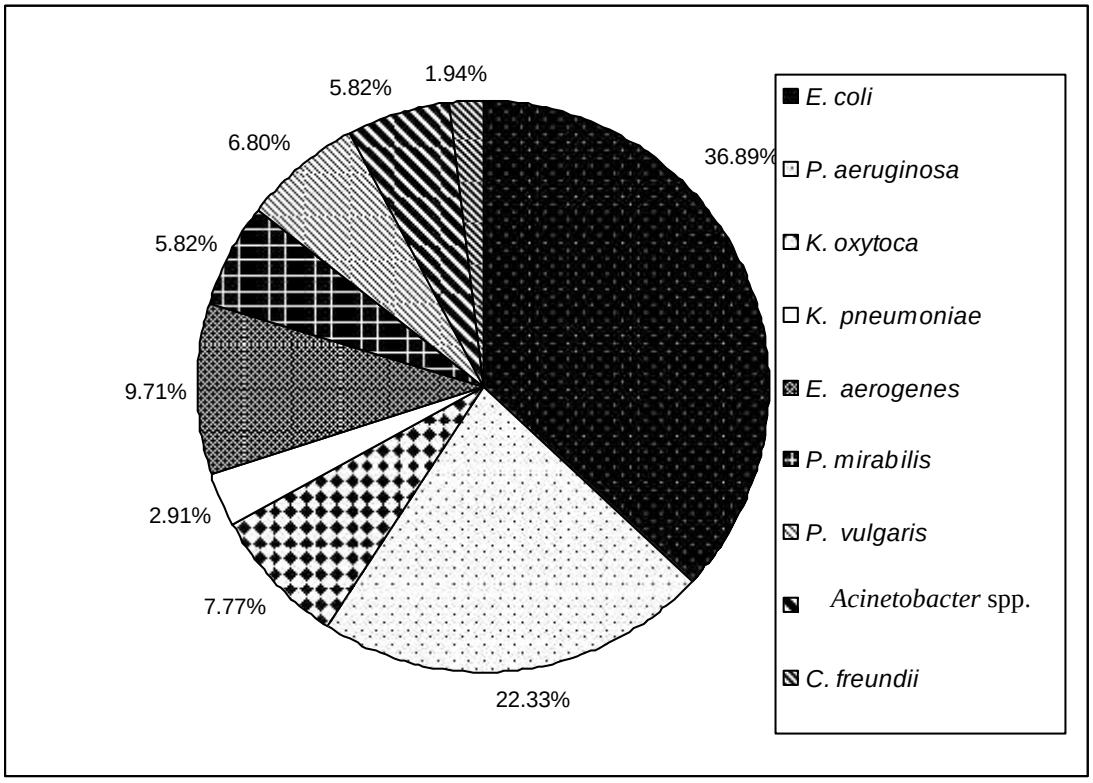

Figure 6: Percentage of Gram negative bacteria in different types of wounds

The table 16 and figure 6 demonstrates the presence of total 103 Gram negative bacteria. Out of which 68 were found as single isolate and 35 were isolated from mixed growth. *E. coli* accounted for 36.89% out of total bacteria. It was followed by *P. aeruginosa* 23 (22.33%), *E. aerogenes* 10 (9.71%), *K. oxytoca* 8 (7.77%), *P. vulgaris* 7 (6.8%), *P. mirabilis* 6 (5.82%), *Acinetobacter* spp. 6 (5.82%), *K. pneumoniae* 3 (2.91%) and *C. freundii* was lowest i.e. 2 (1.94%) in number**.**

Table 17: Types of Gram negative bacteria in surgical wounds

Gram negative bacteria	No. in single isolate	No. in mixed growth	Total	%
E. coli	7	3	10	58.82
P. aeruginosa	1	-	1	5.89
K. oxytoca	1	1	2	11.76
E. aerogenes	2	-	2	11.76
P. mirabilis	-	1	1	5.89
Acinetobacter spp.	1	-	1	5.89
Total	**12**	**5**	**17**	**100**

There were total 17 Gram negative bacteria isolated from the surgical wounds. 58.82% of *E. coli* was isolated followed by *E. aerogenes*, *K. oxytoca*, *P. aeruginosa*, *P. mirabilis*, and *Acinetobacter* spp.

Table 18: Type of Gram negative bacteria in burn wound

Gram negative bacteria	No. in single isolate	No. in mixed growth	Total	%
E. coli	1	1	2	77.78
P. aeruginosa	6	1	7	22.22
Total	**7**	**2**	**9**	**100**

The only Gram negative bacteria isolated from burn wounds were *P. aeruginosa* and *E. coli*.

Table 19: Types of Gram negative bacteria in breast abscess

Gram negative bacteria	No. in single isolate	No. in mixed growth	Total	%
E. coli	2	2	4	36.36
K. oxytoca	2	-	2	18.18
K. pneumoniae	1	-	1	9.09
E. aerogenes	2	1	3	27.27
P. mirabilis	-	1	1	9.09
Total	**7**	**4**	**11**	**100**

The above table shows the presence of 11 Gram negative bacteria out of which 7 were as single isolate and the remaining 4 occurred in mixed growth. *E. coli* was 4 (36.36%) in number followed by *E. aerogenes*, which was 3 (27.27%) in number.

Table 20: Types of Gram negative bacteria in ulcers

Gram negative bacteria	No. in single isolate	No. in mixed growth	Total	%
E. coli	2	1	3	33.33
P. aeruginosa	2	1	3	33.33
E. aerogenes	1	-	1	11.11
P. vulgaris	1	1	2	22.22
Total	**6**	**3**	**9**	**100**

The above table shows the presence of total 9 Gram negative bacteria out of which *E. coli* and *P. aeruginosa* were 3 in number and *P. vulgaris* was 2 in number

Table 21: Types of Gram negative bacteria in other trauma

Gram negative bacteria	No. in single isolate	No in mixed growth	Total	%
E. coli	3	4	7	43.75
P. aeruginosa	3	2	5	31.25
E. aerogenes	-	1	1	6.25
P. mirabilis	-	1	1	6.25
P. vulgaris	-	1	1	6.25
C. freundii	1	-	1	6.25
Total	**7**	**9**	**16**	**100**

The above table shows that *E. coli* was the commonest Gram negative bacteria isolated from the Trauma that was 7 (43.75%) in number followed by *P. aeruginosa* which was 5 (31.25%) in number.

Table 22: Types of Gram negative bacteria in other pyogenic wounds

Gram negative bacteria	No. in single isolate	No. in mixed growth	Total	%
E. coli	7	5	12	29.27
P. aeruginosa	5	2	7	17.07
K. oxytoca	3	1	4	9.76
K. pneumoniae	2	-	2	4.88
E. aerogenes	2	1	3	7.31
P. mirabilis	1	2	3	7.31
P. vulgaris	3	1	4	9.76
Acinetobacter spp.	5	-	5	12.19
C. freundii	1	-	1	2.44
Total	**29**	**12**	**41**	**100**

In other pyogenic wounds *E. coli* 12 (29.27%) was the most common followed by *P. aeruginosa* 7 (17.07%). *Acinetobacter* spp. 5 (12.19%) followed thenafter.

Table 23: Pattern of total bacterial isolates in different wound specimens

Type of organism	Surgical wound		Burn wound		Breast abscess		Ulcer		Other trauma		Other pyogenic wounds		Total	
	no	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**
S. aureus	24	48.9	4	28.6	33	67.34	10	47.61	18	39.13	54	42.19	143	46.58
CONS	4	8.16	1	7.14	2	4.08	2	9.52	6	13.04	20	15.62	35	11.40
S. pyogenes	-		-	-	2	4.08	-	-	-	-	3	2.34	5	1.63
Other β-haem. streptococci	1	2.04	-	-	1	2.04	-	-	3	6.52	3	2.34	8	2.60
Non haem. streptococci	2	4.08	-	-	-		-	-	-	-	4	3.12	6	1.95
Gram positive rod (unidentified)	1	2.04	-	-	-	-	-	-	3	6.52	3	2.34	7	2.28
E. coli	10	20.4	2	14.3	4	8.16	3	14.28	7	15.21	12	9.37	38	12.38
P. aeruginosa	1	2.04	7	50	-	-	3	14.28	5	10.87	7	5.47	23	7.49
K. oxytoca	2	4.08	-	-	2	4.08	-	-	-	-	4	3.12	8	2.61
K. pneumoniae	-	-	-	-	1	2.04	-	-	-	-	2	1.56	3	0.98
E. aerogenes	2	4.08	-	-	3	6.12	1	4.76	1	2.17	3	2.34	10	3.26
P. mirabilis	1	2.04	-	-	1	2.04	-	-	1	2.17	3	2.34	6	1.95
P. vulgaris	-		-	-	-	-	2	9.52	1	2.17	4	3.12	7	2.28
Acinetobacter spp.	1	2.04	-	-	-	-	-	-	-	-	5	3.90	6	1.95
C. freundii	-		-	-	-	-	-	-	1	2.17	1	0.78	2	0.65
TOTAL	**49**	**100**	**14**	**100**	**49**	**100**	**21**	**100**	**46**	**100**	**128**	**100**	**307**	**100**

The above table shows that there were 15 different types of bacteria isolated from different types of wounds. Among them *S. aureus* (46.58%) was the most common type of bacteria which was followed by *E. coli* (12.38%), CONS (11.40%) then followed. *P. aeruginosa* then followed that was 7.49%. The least common bacteria isolated was *C. freundii* (0.65%).

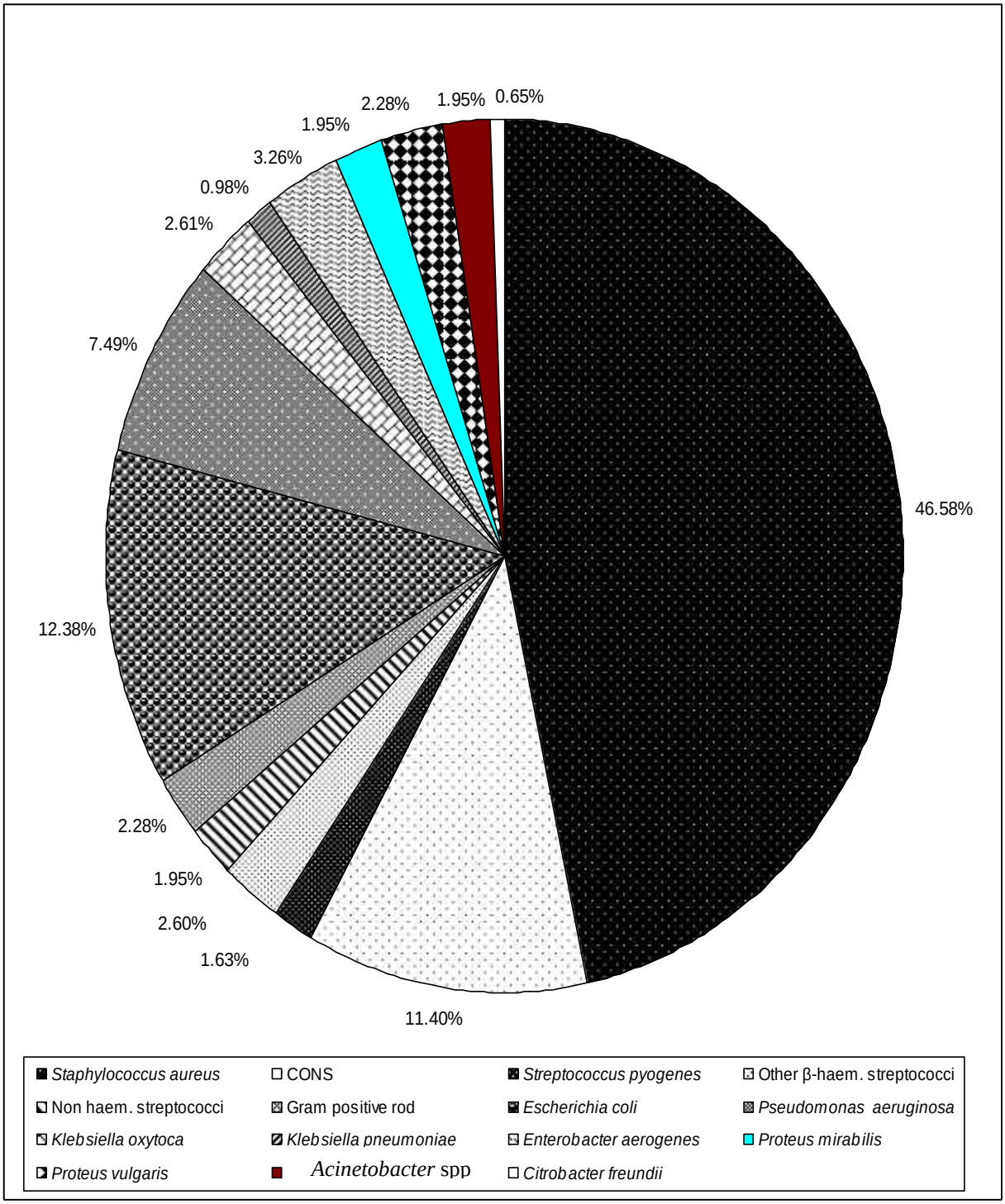

Figure 7: Total bacterial isolates in different wound specimens

5.4 Antibiotic susceptibility pattern of bacterial isolates

The common antibiotic discs used for all types of bacteria were Gentamicin, Ampicillin Ofloxacin, Ciprofloxacin, Amoxycillin, Cotrimoxazole, and Cloxacillin. Erythromycin was used for Gram positive bacteria only and Chloramphenicol for Gram negative bacteria only. Polymyxin B was used in case of *P. aeruginosa*.

Table 23: Antibiotic sensitivity pattern bacterial isolates as a whole

Antibiotic	Sensitive		Resistant		Total
	No.	%	No.	%	
Gentamicin	224	74.67	76	25.33	300
Ampicillin	117	39	183	61	300
Ofloxacin	247	82.33	53	17.67	300
Ciprofloxacin	211	70.33	89	29.67	300
Amoxycillin	143	47.67	157	52.33	300
Cotrimoxazole	132	44	168	56	300
Cloxacillin	201	67	99	33	300

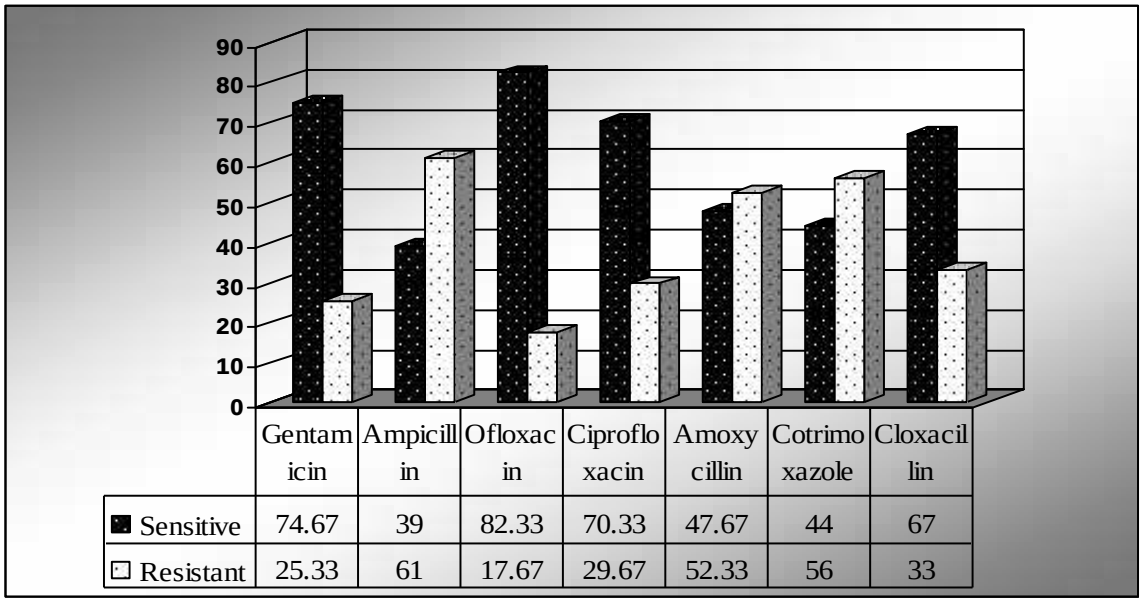

Figure 8: Antibiotic sensitivity pattern of bacterial isolates as a whole

Among the 300 bacterial isolates, the maximum bacteria were sensitive to Ofloxacin i.e. 82.33%. The second most effective drug was Gentamicin (74.67%) and Ciprofloxacin (70.33%). The least effective drug was Ampicillin (39%).

Table 25: Antibiotic sensitivity pattern of Gram positive bacterial isolates

Antibiotic	**Sensitive**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	
Gentamicin	149	75.63	48	24.36	197
Ampicillin	92	46.7	105	53.3	197
Ofloxacin	173	87.81	24	12.19	197
Ciprofloxacin	155	78.68	42	21.32	197
Amoxycillin	113	57.36	84	42.64	197
Cotrimoxazole	98	49.75	99	50.25	197
Erythromycin	139	70.56	58	29.44	197
Cloxacillin	146	74.11	51	25.89	197

For 197 Gram positive bacteria, the most effective drug was found to be Ofloxacin (87.81%) followed by Ciprofloxacin (78.68%), Gentamicin (75.63%) and Cloxacillin (74.11%). The least effective drug used for Gram positive bacteria were Ampicillin (46.7%) and Amoxycillin (57.36%).

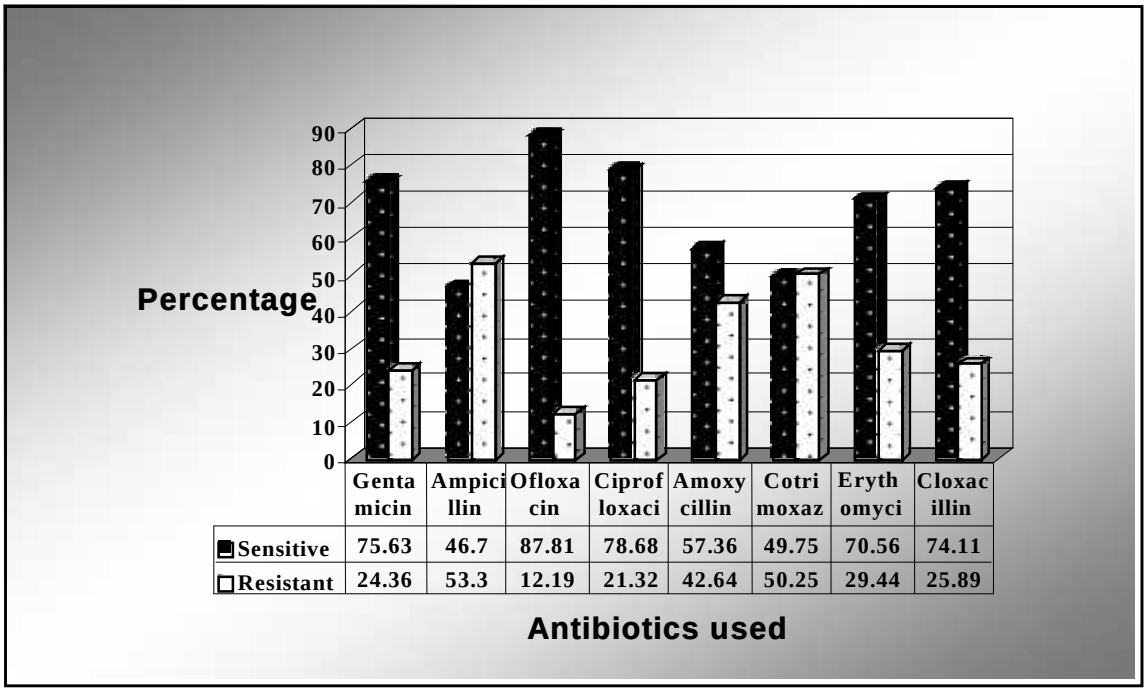

Figure 9: Antibiotic sensitivity pattern of Gram positive bacteria

Table 26: Antibiotic sensitivity pattern of Gram negative bacterial isolates

Antibiotic	**Sensitive**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	
Gentamicin	75	72.81	28	27.19	103
Ampicillin	25	24.27	78	75.73	103
Ofloxacin	74	71.84	29	28.15	103
Ciprofloxacin	56	54.36	47	45.63	103
Amoxycillin	30	26.13	73	70.87	103
Cotrimoxazole	34	33	69	67	103
Chloramphenicol	62	60.19	41	39.8	103
Cloxacillin	55	53.4	48	46.6	103
Polymixin B*(P. aeruginosa)*	16	69.6	7	30.43	23

Gentamicin was found to be the most effective drug for most of the Gram negative bacteria (72.81%). The second most effective drug was Ofloxacin (71.84%) and Chloramphenicol

(60.19%). The least effective drug was Ampicillin (24.27%) and Amoxycilin (29.13%). Polymixin B was used only for *P. aeruginosa*. Out of 23 *P. aeruginosa* 16 (69.6%) were sensitive to Polymyxin B.

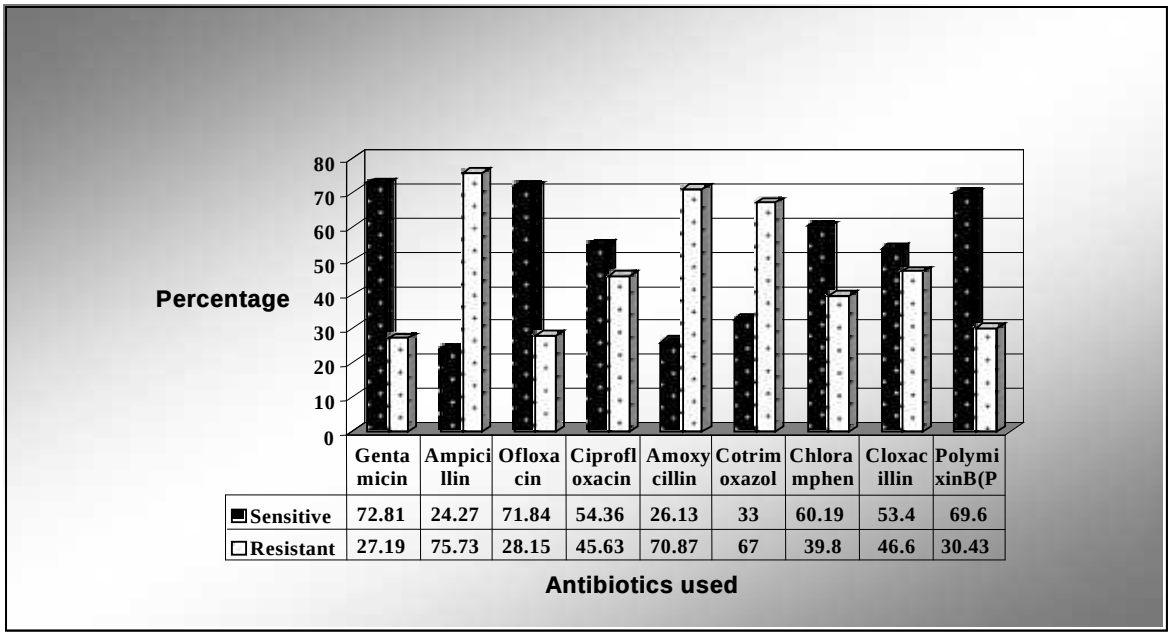

Figure 12: Antibiotic sensitivity pattern of Gram negative bacterial isolates

Table 26: Antibiotic sensitivity pattern of *S. aureus*

Antibiotic	Sensitive		Resistant		Total
	No.	%	No.	%	
Gentamicin	110	76.92	33	23.07	143
Ampicillin	66	46.15	77	53.85	143
Ofloxacin	128	89.51	15	10.49	143
Ciprofloxacin	116	81.11	27	18.89	143
Amoxycillin	76	53.15	67	46.85	143
Cotrimoxazole	70	48.95	73	51.04	143
Erythromycin	103	72.02	40	27.98	143
Cloxacillin	114	79.72	29	20.28	143

There were altogether 143 *S. aureus* .The most effective drug used was Ofloxacin (89.51%) followed by Ciprofloxacin (81.11%) and Cloxacillin (79.72%). The least effective drug used was Ampicillin (46.15%) and Cotrimoxazole (48.95%).

Table 28: Antibiotic sensitivity pattern of *E. coli, P. aeruginosa* and CONS (S= Sensitive; R= Resistant)

Antiibiotics	***E. coli***		***P. aeruginosa***		**CONS**	
	S (%)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Gentamicin	78.9	21.1	69.6	30.4	71.42	28.58
Ampicillin	26.31	73.69	8.69	91.31	34.28	65.72
Ofloxacin	61.3	38.7	60.87	39.13	74.3	25.7
Ciprofloxacin	44.7	55.3	65.2	34.8	62.85	37.15
Amoxycillin	34.2	65.8	21.73	78.27	54.28	45.72
Cotrimoxazole	34.2	65.8	26.08	73.92	57.14	42.86
Erythromycin	-	-	-	-	62.85	37.15
Chloramphenicol	69.05	30.95	39	61	-	-
Cloxacillin	57.9	42.1	43.47	56.53	60	40
PolymixinB	-	-	69.6	30.4	-	-

The above table shows the sensitivity pattern of common bacteria isolated which are *E. coli, P. aeruginosa,* and CONS. Gentamicin was the most effective antibiotic for *E. coli* (78.9%) and *P. aeruginosa* (69.6%) and second most effective for CONS (71.42%). The most effective drug for CONS was Ofloxacin (74.3%). The least effective drug for all three types of bacteria was Ampicillin which was effective to only 26.31% of *E. coli*, 8.69% of *P. aeruginosa* and 34.28% of CONS.

5.5 Validity of direct Gram stain in relation to culture

Table 29: Validity of direct Gram stain smear in relation to culture

Nature of result	**Wound specimen**	
	Number	**(%)**
1. Direct smear : Bacteria and pus cells observed Culture result: Growth	81	32.4
2. Direct smear : Only bacteria observed Culture result: Growth	52	20.8
3. Direct smear : Only bacteria observed Culture result: No growth	2	0.8
4. Direct smear : No bacteria and no pus cells observed Culture result: Growth	37	14.8
5. Direct smear : Only pus cells observed Culture result: Growth	19	7.6
6. Direct smear : No bacteria and no pus cells observed Culture result: No growth	59	23.6
Total	**250**	**100**

Gram stain of direct smear was performed in 250 wound specimens, where 192 (76.8%) showed similar results with culture result. Among these, 32.4% showed both bacteria and pus cells and 20.8% showed only bacteria. In both the cases culture positive result was obtained. Only bacteria were seen in Gram stain direct smear but culture result negative was seen in 0.8% cases. Among 14.8% cases, no bacteria and no pus cells was seen but showed culture positive result. Similarly, among 7.6% cases only pus cells were observed but showed growth. Among 23.6% cases no bacteria and no pus cells were observed and it also showed negative culture result.

CHAPTER -6

DISCUSSION

Wound infection extends the period of patient discomfort and inconvenience increasing health care cost of both individual and community and staff workload. Here wound cultures represent a general category for a group of extremely diverse anatomic samples that range from superficial specimens of cutaneous structures (folliculitis, cellulitis) to specimens revealing invasive infections involving deep fascial planes and muscle (myonecrosis).

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In this study, particular emphasis was placed on community acquired wound infections and the etiological agents associated with such conditions. 400 patients visiting surgical out patient department of Bir Hospital with wound infection were studied. The etiological agents were identified by biochemical tests and their susceptibility patterns to commonly used antibiotics were determined. The results were shown in Table 3 to 29.

6.1 Division of wound samples and growth pattern

Out of 400 wound samples collected and processed, 61 (15.25%) were collected from patients with post discharge surgical wound infection, 20 (5%) samples from patients with burn wound, 65 (16.25%) samples from patients with breast abscess. Specimens of ulcer were 25 (6.25%), samples from other trauma were 70 (17.5%) and maximum number of samples i.e. 159 (39.75%) were collected from patient with other pyogenic wounds.

After the culture in different media, out of 400 wound specimens, 273 (68.25%) specimens showed growth and 127 (31.75%) specimens showed no growth. Among the 61 samples from postdischarge surgical wound infection 44, (72.13%) showed growth and 17 (27.868%) were sterile. This is comparable to the study carried out by Gongal *et al* (1994) on postoperative wound infection in Bir Hospital which showed that 90% of wound swabs and 80% of drained pus showed microbial growth.

Similarly, 12 (60%) of burn wound specimens, 45 (69.2%) of cases of breast abscess, 18 (72%) of ulcer specimens, 39 (55.71%) of other trauma specimens and 115 (72.32%) of other pyogenic wound specimens showed growth and the remaining samples didn't show growth on culture. Olson & Lee (1990) obtained 97.5% growth in pus specimens of the infected incisions during continuous 10 year study.

In the study carried out by Bowler *et al* (2001) clean surgery carries a 1 to 5% risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27% risk has been estimated. Similarly, they estimated that up to 75% of deaths following burn injury are related to infection. Fehr e*t al* (2006) did a prospective cohort study from November 2003 to March 2004 in a rural Sub-Saharan district hospital to collect baseline data concerning SSI and antimicrobial prophylaxis. They reported SSI rate was 22% i.e. among 613 patients that underwent surgery 114 (21.6%) developed SSI. Saha *et al* (1995) found 31.37% prevalence rate of post operative infection.

In India Anbumani *et al* (2006) studied from July 2005 to Dec 2005 to find out the etiological agent of wound infection and collected 3333 samples of which 47% showed positive bacterial growth and 53% were culture negative.

6.2 Age and genderwise distribution of patients in different types of wounds

In this study, out of 400 patients, 227 (56.75%) were male and 173 (43.25%) were female. Out of these samples, 61 were collected from discharged patients after surgery among which 42 (68.86%) were male and 19 (31.14%) were female. Among burn patients 70% were male and 30% were female. All patients with breast abscess were female. Among patients with various types of ulcers, 48% were male and 52% were female. Similarly, 72.86% of patients with Trauma were male and 27.14% were female and in pyogenic wound infection 67.9% were male and 32.07% were female. This relatively higher percentage of male patients may be due to greater outdoor activity of male.

The age of patients ranged from 6 to 89 years old, out of which majority of patients were with age group 21-30 (30.25%) and only 1% of patients belonged to age group 81 to 90. Among the patients with breast abscess, 34 (52.3%) were collected from age group 21 to 30. This result is due to the prevalence of bacterial mastitis among lactating women. This agreed with the study of Ulttzsch *et al* (2002) in Sweden where the average age of 43 women with abscess was 30 years. Brooks *et al* (1990), in his study showed that 72.9% of patients with wound infection were male and age group ranged from 2 weeks to 76 years. Our finding differed from de Sa *et al* (1984) as they reported that the range of wound infection increased with increasing age, being maximum in the 51-70 years age group and infection rates in males and females being similar. In Nepal, Parajuli (1997) and Tuladhar (1999) also found that patients with age group 21-30 were more susceptible to wound infection.

Oslon & Lee (1990) reported that more than 99% of the affected patients by surgical wound infections were males and 90% were aged 50 years or older but in our study lesser number of patients belonged to this age group.

6.3 Pattern of single and multiple microbial isolates

There were 273 samples that showed growth. Out of which 239 (87.54%) showed growth of single bacterial isolate and 34 (12.40%) samples showed the growth of multiple bacterial isolate. This study agrees with the study of Gyawali (2007) at Lumbini Zonal Hospital that showed among total 172 growths, 147 (85.4%) pus samples showed single isolates and 25 (14.5%) pus samples showed multiple isolates. But in the study carried out by Dongol (1996), 17 cases (18.1%) out of 94 positive cases showed multiple isolates and Gongal *et al* (1994) found single isolates in 78.3% cases and mixed isolates in 21.62% cases.

Out of 44 samples showing growth of surgical wounds, 39 (88.63%) showed single isolate and 5 (11.36%) showed multiple bacterial isolate. This can be compared with the study carried out by Giacometti *et al* (2000) where 271/676 samples showed presence of single isolate, 343/676 showed multiple etiological agent and 62 samples were sterile. 12 samples of burn wounds showed growth, out of which 10 (83.33%) showed the growth of single bacterial isolate and 2

(16.67%) showed multiple bacterial isolate. Among the 45 samples of breast abscess, 41 (91.11%) showed the growth of single bacterial isolate and 4 (8.89%) samples showed multiple bacterial isolate.

Among the total growth of 18 ulcer specimens, 15 (83.33%) showed the presence of single bacterial isolate and 3 (16.66%) showed the growth of multiple bacterial isolate. This can be compared with the study carried out by Bowler & Davis (1999) for infected and non infected leg ulcers where 220 isolates were cultured from 44 infected leg ulcers and 110 isolates from 30 non infected leg ulcers. Among 39 specimens showing positive growth in other trauma, 32 (82.05%) showed the presence of single bacterial isolate and 7 (17.95%) showed multiple bacterial isolate. This differed from the literature of Bowler *et al* (2001) that states 50% of traumatic injuries of varied etiology have a polymicrobial microflora.

Among the total positive samples of other pyogenic wound infection, 102 (88.69%) showed single bacterial isolate and the remaining 13 (11.30%) specimens showed the presence of multiple bacterial isolate whereas Henderson *et al* (1996) found 78% of necrotizing soft tissue infection was polymicrobial. The study of Bowler *et al* (2001) demonstrated that approximately 47% of the necrotizing soft tissue infections have a polymicrobial aerobic-anaerobic microflora. In similar study in Greece, Paramythiotis *et al* (2007) found the presence of 15% single and 85% multiple isolates in necrotizing soft tissue infections.

In USA, Brook & Finegold (1981) performed a study in cutaneous abscesses in children and found that 4% of total 209 specimens were sterile, 24% yielded pure culture and the rest yielded mixed growth. Anbumani *et al* (2006) found the presence of 78.58% of single etiological agent and 21.41% of multiple bacterial isolate out of total 1569 growth positive samples.

6.4 Pattern of bacterial isolates from various wound specimens

Growth was shown by 274 samples, out of which 307 numbers of bacteria were isolated which included bacteria from single and multiple bacterial isolates. Out of 307 bacteria isolated 204

(66.45%) were Gram positive and 103 (33.55%) bacteria were Gram negative. But in the study carried out by Anbumani *et al* (2006), they found the equal presence of Gram positive cocci (49.6%) and Gram negative bacilli (49.5%) and found the negligible presence of Gram positive bacilli. However our study agreed with the study of Tuladhar (1999) and Parajuli (1997) at Kathmandu, which showed higher proportion of Gram positive isolates i.e. 67.47% and 59.25% respectively.

As a whole 15 different types of bacteria were isolated, out of which 6 were Gram positive and 9 were Gram negative bacteria. Most common bacteria (46.58%) were *S. aureus. E. coli* (12.38%) emerged as the next common organism causing wound infection in our study as in the other previously reported studies which is followed by, CONS (11.40%) and *P. aeruginosa* (7.49%). The least common bacteria isolated was *C. freundii* (0.65%). In Saudi Arabia, Abussaud (1996) isolated *S. aureus* (35%), *P. aeruginosa* (25%) and *Klebsiella* spp. (10%).

In the study conducted by Shah *et al* (1997) at TU Teaching Hospital, 11.43% of CONS was reported from pus sample, which was close to our finding. Shampa *et al* (2006), in their study to determine the prevalence of *P. aeruginosa* and its antimicrobial sensitivity pattern found 32% prevalence rate of *P. aeruginosa* of all pathogens isolated. This agreed with the study done in Africa by Oguntibeja *e*t *al* (2004) where 33% of the isolates were *P. aeruginosa* in post operative wound infections.

Giacometti *et al* (2000) studied microbiology of wound infection in Italy and found the prevalence of *S. aureus* (28.2%), *P. aeruginosa* (25.2%), *E. coli* (7.8%), *S. epidermidis* (7%), and *S. faecalis* (5.6%) which gave somewhat different result than our study. Our result can also be compared with the study of Anbumani *et al* (2006) which showed the common bacterial isolate was *S. aureus* (37%), *P. aeruginosa* (15%), *E. coli* (12%), *Enterococcus* (8%), *K. pneumoniae* (7%) and *Acinetobacter* (6%). In our study only 0.98% *K. pneumoniae* and 1.95% *Acinetobacter* was found.

According to data from the National Nosocomial Infections Surveillance System, *Acinetobacter* spp. was isolated in 1% of all nosocomial infections from 1990 to 1992 (Emori & Gaynes, 1993).

6.4.1 Pattern of bacterial isolates from surgical wound infection

Among 44 growth positive samples, 49 bacteria were isolated. Out of these 32 (65.30%) were Gram positive and 17 (34.70%) were Gram negative. Total 5 types of Gram positive bacteria and 6 types of Gram negative bacteria were found.

S. aureus was found to be the predominant bacteria (48.98%), followed by *E. coli* (20.40%) and CONS (8.16%). *K. oxytoca, E. aerogenes*, and non haemolytic Streptococci (4.08%) followed thenafter. Other isolates were β haemolytic Streptococci other than *S. pyogenes, P. aeruginosa, P. mirabilis, Acinetobacter* spp., and Gram positive rod.

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

Fehr *et al* (2006), in their study in SSIs in a rural Sub Saharan hospital found the presence of 36% *S. aureus*, 5% *E. coli* and 4% Enterococci. Other clinically relevant bacteria *Klebsiella* spp., *Proteus* spp., other Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* were found in 7% cases which was close to our finding.

In a retrospective study performed by Cantlon *et al* (2006) in USA to identify the microorganisms associated with SSIs in patients who underwent class I and II surgeries at a small urban to rural community hospital from January 2003 through December 2004, a total of 10,672 surgeries was performed, and 89 SSIs were identified. *S. aureus* was the most common pathogen (25.8%). Enterobacteriaceae were the second most frequently isolated organisms (12.4%), followed by streptococci species (11.2%), coagulase-negative staphylococci (10.1%),

enterococci species (7.9%), and *P. aeruginosa* (6.7%).

In Kualalumpur, Hanifah (1990) found that the most common bacterial isolate were *S. aureus* (36.1%), *P. aeruginosa* (15.4%) and *Klebsiella* spp. (10.1%) in the postoperative wound infection but in our finding *E. coli* and CONS followed *S. aureus*. In contrast, Bowler *et al* (2001) emphasized the polymicrobial nature of almost all surgical infections and commented that the critical importance of aerobic-anaerobic mixtures in these infections had received relatively little attention however no anaerobes were isolated.

6.4.2 Pattern of bacterial isolates from burn wounds

There were 12 samples that showed growth in burn wound samples, out of which, 14 bacteria were isolated. Gram negative bacteria were predominant (64.28%) than Gram positive bacteria (35.71%).

Among the bacteria, *P. aeruginosa* (50%) was found to be the most common bacteria followed by *S. aureus* (28.57%), *E. coli* (14.29%) and 7.14% of CONS. Our study agreed to some extent to the study carried out by Sharma *et al* (1996) in Ram Monohar Lohia Hospital, New Delhi where he found that *P. aeruginosa* was the most common (53.9%) followed by *Klebsiella* spp., *S. aureus* and *E. coli.*

At Jordan University Hospital, Karyoute *et al* (1989) showed that *P. aeruginosa* was the predominant pathogen which correlated with our study. Similarly in Delhi, Singh *et al* (2003) reported *P. aeruginosa* as the most common bacteria (31%) in burn wounds.

In the study carried out by Revathy *et al* (1998) in burn wound infection, there was prevalence of 36% *P. aeruginosa.* Our study varied from the study carried out by Shrestha (1997) as she found *S. aureus* and *K. pneumoniae* to be the most predominant organism in burn wound infection whereas in our study *P. aeruginosa* was the most common organism.

6.4.3 Pattern of bacterial isolates from breast abscess

Breast abscess was most common in the lactating women. Among 45 samples that showed growth, 49 bacteria were isolated. There were 38 (77.55%) Gram positive bacteria and 11 (22.44%) Gram negative bacteria. Total 4 types of Gram positive bacteria and 5 types of Gram negative bacteria were isolated.

The predominant bacterial pathogen isolated was *S. aureus* (67.34%). *E. coli* contributed 8.16%, *E. aerogenes* contributed 6.12%. Other isolates were CONS (4.08%), *S. pyogenes* (4.08%), *K. oxytoca* (4.08%), *K. pneumoniae* (2.04%), *P. mirabilis* (2.04%) and β-haemolytic Streptococci (2.04%) other than *S. pyogenes*.

In Sweden, Ulttzsch *et al* (2002) performed study in breast abscess among lactating women from 1996 to 1999 they found the prevalence of *S. aureus* (89%), 4% *S. pyogenes*, 4% *Peptococcus* and 2% both *S. aureus* and β haemolytic *Streptococcus* group A. Montgomery (2001) states that the most common bacteria isolated from bacterial mastitis are *S. aureus*, CONS, streptococci and *E. coli*.

6.4.4 Pattern of bacterial isolates from ulcer samples

From the 18 samples that showed growth, 21 bacteria were isolated. Out of which, 57.14% were Gram positive and 42.86% were Gram negative. 2 types of Gram positive and 4 types of Gram negative bacteria were found.

In ulcer samples also, the most common bacteria was *S. aureus* (47.61%), followed by *E. coli* and *P. aeruginosa* (14.28% each), CONS (9.52%), *P. vulgaris* (9.52%) and *E. aerogenes* (4.76%). In USA, Mertz and Ovington (1993) reported that *S. aureus* was the most frequently isolated organism from leg ulcer. *P. aeruginosa*, *Acinetobacter* and *Proteus* were other isolates.

Bowler & Davis (1999) in their study found the presence of 49% anaerobes in combination with aerobic and facultative anaerobic bacteria. They also found that isolation of *P.*

aeruginosa was generally low although *S. aureus* was a frequent isolate. However anaerobes were not isolated in our study but *S. aureus* was the most frequent isolate in our study as well.

In Japan, Konya *et al* (2005) detected in 17 patients with pressure ulcers, the presence of 47 microorganisms (11 species). The most common isolated species was *S. aureus* including MRSA, followed by β haemolytic streptococci. The opportunity for microbial synergy in many decubitus ulcers was demonstrated by Brook (1991), who reported mixed aerobic and anaerobic microflora in 41% of 58 ulcers in children.

6.4.5 Pattern of bacterial isolates from other trauma

In other trauma, 46 bacteria were isolated from 39 samples that showed growth. Among these 30 (65.21%) were Gram positive and 16 (34.78%) were Gram negative. 4 types of Gram positive bacteria and 6 types of Gram negative bacteria were isolated.

S. aureus (39.13%) was again the most common bacteria. *E. coli* (15.21%) followed thenafter which was again followed by CONS (13.04%) and *P. aeruginosa* (10.87%). *E. aerogenes, P. mirabilis, P. vulgaris* and *C. freundii* all contributed to 2.17% of the total isolates.

Dongol (1996) found the presence of *Clostridium septicum* and *Peptostretococcus* species from the wound resulted due to road traffic accident however no anaerobes were isolated in our study.

6.4.6 Pattern of bacterial isolates from other pyogenic wounds

There were total 115 samples that showed growth, out of which 128 numbers of bacteria were isolated. Among these 67.7% was Gram positive bacterial species and 32.28% were Gram negative bacterial species. Among 15 types of bacterial isolates, 6 types of Gram positive and 9 types of Gram negative bacteria were isolated. The most common bacteria was found to be *S. aureus* (42.19%) followed by CONS (15.62%) and *E. coli* (9.37%). Other isolated bacteria were *P. aeruginosa* (5.47%), non haemolytic streptococci (3.12%), *P. vulgaris* (3.12%), *K.*

oxytoca (3.12%), *S. pyogenes* (2.34%), other β haemolytic streptococci (2.34%), *E. aerogenes* (2.34%), *P. mirabilis* (2.34%), *K. pneumoniae* (1.56%) and *C. freundii* (0.78%).

This study was in good agreement with the study done by Mumtaz *et al* (2002) in aerobic pyogenic isolates from wounds and abscesses. *S. aureus* was the most common pathogen (49%) which was close to our finding, followed by *E. coli* (25.9%), *Klebsiella* (9.5%), *P. aeruginosa* (8.6%), *Proteus* spp. (4%) and *Acinetobacter* (2.7%).

In the study carried out by Brook & Finegold (1981) from 1977 to 1979 in cutaneous abscesses of children, they found the presence of 46% aerobic bacteria, anaerobes only in 26% cases and mixed aerobic and anaerobic in 28% but in our study anaerobes were not isolated. Similarly, among the aerobic bacteria, *S. aureus* was the most common bacteria (45.17%) which was close to our finding. Nonhaemolytic streptococci was the second most common bacteria (14.7%), 8.12% of β haemolytic streptococci, *Enterobacter* spp. (5.07%) and *E. coli* was found in only 4.06%.
Henderson *et al* (1996) reported 33% of *S. aureus* and 22% *S. pyogenes* in the necrotizing soft tissue infection.

6.5 Antibiotic susceptibility pattern of bacterial isolates

In this study, out of 400 wound specimens, 307 bacterial species were isolated including Gram positive rods. These Gram positive rods were considered as contaminants. Hence the antibiotic susceptibility testing was done for only 300 bacteria as aerobic Gram positive rods do not present uniform in vitro susceptibility patterns.

The antibiotics used were of Hi Media Laboratories Pvt. Ltd. From table 23 to 27, it was found that Ofloxacin was the most effective (82.33%) drug, followed by Gentamicin (74.67%) and Ciprofloxacin (70.33%). Cloxacillin inhibited 67%, Amoxycillin inhibited 47.67% and Cotrimoxazole 44% of the bacteria. The least effective drug was found to be Ampicillin (39%).

In our study Ciprofloxacin was effective to 70.33% of the isolates but it differed from the similar study carried out by Tuladhar (1999) in which 53.3% of isolates were susceptible to Ciprofloxacin. Fehr *et al* (2006) in their study for antimicrobial prophylaxis to prevent SSI detected Chloramphenicol resistance in 33% of *S. aureus*, 35 % of *E. coli* and 25% of enterococci.

Regarding Gram positive bacteria, Ofloxacin was found to be the most effective antibiotic (87.8%). It was then followed by Ciprofloxacin (78.68%), Gentamicin (75.63%), Cloxacillin (74.11%) and Erythromycin (70.56%). The least effective drug was Ampicillin (46.7%) and Cotrimoxazole (49.75%).

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

Among Gram negative bacteria, 72.81% were sensitive to Gentamicin, 71.84% were sensitive to Ofloxacin, 60.19% were sensitive to Chloramphenicol, 54.36% were sensitive to Ciprofloxacin and 53.4% were sensitive to Cloxacillin. The least effective drug was Ampicillin (24.27%), Amoxycillin (29.13%) and Cotrimoxazole (33%). Polymyxin B was used only for *P. aeruginosa* which was sensitive to 69.6% of *P. aeruginosa.*

Dongol (1996), in her study found that 96% of Gram positive cocci and 70% of Gram negative bacilli were sensitive to Ofloxacin, which was quite close to our finding.

S. aureus was found to be the most common pathogen isolated from wound specimens (46.58%) which were tested for antimicrobial sensitivity. Ofloxacin (89.51%) was found to the most effective drug against *S. aureus*, followed by Ciprofloxacin (81.1%), Cloxacillin (79.72%), Gentamicin (76.92%), and Erythromycin (72.02%). The least effective drug was Ampicillin (46.15%).

This study agrees with the study of Mumtaz *et a*l (2002) where *S. aureus* was found to be highly sensitive to Ofloxacin (82%), Ciprofloxacin was effective to 67%, Erythromycin to 62% and Amoxycillin to 33% of *S. aureus*. But in this study, the least effective drug was Cotrimoxazole (15%) but in our study 48.95% of *S. aureus* were sensitive to Cotrimoxazole.

In Nepal, Gongal *et al* (1994) reported that *S. aureus* was equally sensitive to Gentamicin and Cloxacillin (85.7%) and Erythromycin (59.5%). Fehr *et al* (2006) in their study for antimicrobial prophylaxis to prevent SSI found the ineffectiveness of Ampicillin in 95% of *S. aureus*. Abussaud (1996) found that Chloramphenicol was effective to 78% and Gentamicin was effective to 63% of *S. aureus*.
Our study also shows that Gentamicin was effective to 69.6% of *P. aeruginosa*, 78.9% of *E. coli* and 71.42% of CONS. Gongal *et al* (1994) found that 95% of *E. coli* were sensitive to Gentamicin. Katuwal *et al* (1999) found 62.7% of *E. coli* was sensitive to Gentamicin. Ofloxacin was effective to 74.3% CONS, 61.3% of *E. coli* and 60.87% of *P. aeruginosa*.

Although Ampicillin is ineffective to *P. aeruginosa*, in our study Ampicillin was effective 8.69% of *P. aeruginosa*. This may be due the presence of bacteria showing the similar characteristics like *Pseudomonas*. 34.2% of *E. coli* were sensitive to Ampicillin.. Also in the finding of Anbumani *et al* (2006), 50% of the Enterobacteriaceae were resistant to Ampicillin, Cloxacillin and Cephalexin. Our study can be compared with the study of Oguntibeju & Nwobu (2004) where *P. aeruginosa* isolated from post operative wound were found to be sensitive to Gentamicin but was resistant tot Ampicllin and Cotrimoxazole.

Shampa *et al* (2006) found 58% *P. aeruginosa* to be sensitive to Ciprofloxacin which nearly agreed to our finding in which 65.2% were sensitive but differed from the finding of Anbumani *et al* where only 12% of *P. aeruginosa* were sensitive to Ciprofloxacin. In the study of Mumtaz *et al*, she showed that Ofloxacin was effective to 78% of *E. coli*, 85% of *Klebsiella* and 57% of *Pseudomonas*. Similarly 52% *E. coli* and 42% of *P. aeruginosa* was sensitive to Ciprofloxacin, whereas in our study *P. aeruginosa* (65.2%) was more sensitive to

Ciprofloxacin than *E. coli* (44.7%). However, Katuwal (1999) reported that 79% *E. coli* was found to be susceptible to Ciprofloxacin.

Roberts & Chambers (2001) reported in their study to find out trends in quinolone susceptibility of Enterobacteriaceae from 1992 to 1998 that there was reduced susceptibility of *E. coli* to Ciprofloxacin (99 to95%). In the similar study carried out by Kresken *et al* (1994) there was a decrease in susceptibility of *S. aureus* and *P. aeruginosa* to Ciprofloxacin and Ofloxacin however *E. coli* did not change significantly.

6.6 Validity of Gram stain in relation to culture

Despite being used for over century, Gram's stain is still the most important stain in microbiology and is widely used as a rapid technique for guiding antibiotic therapy in life-threatening infections.

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

Both bacteria and pus cells with culture positive result was seen in 32.4% cases and in 20.8% cases only bacteria with culture positive result was seen. No growth but bacteria seen only in direct smear gram stain was observed in 0.8%. Culture positive but no bacteria and no pus cells were seen 14.8% cases. Among 7.6% cases only pus cells were observed but showed growth. Among 23.6% cases no bacteria and no pus cells were observed and it also showed negative culture result.

Bowler *et al* (2001) reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure *S. aureus*.

CHAPTER SEVEN

7. SUMMARY AND RECOMMENDATION

7.1 SUMMARY

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

- Altogether 400 wound specimens were studied. There were 15.25% (n=61) surgical, 5% (n=20) burn, 16.25% (n=65) breast abscess, 6.25% (n=25) ulcer, (n=70) 17.5% other trauma and 39.75% (n=159) other pyogenic wound samples.
- Among total specimens, 56.75% (n=227) were collected from male and 43.25% (n=173) from female patients. The age of the patients ranged from 6 to 89 years and the highest frequency of patients with wound infection was found in age group 21-30 (n=121). The difference in the incidence of wound infection in different age groups in both male and female was statistically significant.
- Growth was shown in 68.25% (n=273) of specimens and 31.75% (n=127) of the specimens were found to be sterile. 87.54% (n=239) showed single isolate and 12.40% (n=34) showed multiple isolate.
- Total 307 bacteria were isolated from 273 samples showing growth. Out of which, 66.45 % (n=204) were Gram positive and 33.55% (n=103) were Gram negative.
- Six different species of Gram positive bacteria were found among which *S. aureus* was the most common followed by CONS. Similarly, nine different species of Gram negative bacteria were found. *E. coli* was the most common followed by *P. aeruginosa*.
- Altogether 15 different species of bacteria were isolated. *S. aureus* 143 (46.58%) was the most frequently isolated bacteria followed by *E. coli* 38 (12.38%), CONS 35 (11.40%), and *P. aeruginosa* 23 (7.49%). Other bacterial isolates were *S. pyogenes*, other β haemolytic streptococci, non haemolytic streptococci, Gram positive rods, *K. oxytoca*, *K. pneumoniae, E. aerogenes, P. mirabilis*, *P. vulgaris*, *Acinetobacter* spp. *and C. freundii*.

- *S. aureus* was most commonly isolated from breast abscess specimens (n=33), followed by surgical wound specimens (n=24). Among burn wound specimens, *P. aeruginosa* (n=7) was the most frequently isolated organism.
- Regarding antibiotic sensitivity pattern, the most effective drug for Gram positive isolates was Ofloxacin (87.8%) followed by Ciprofloxacin (78.68%) and Gentamicin (75.63%). Similarly for Gram negative bacteria, Gentamicin (72.81%) was the most effective drug which was followed by Ofloxacin (71.84%).
- As a whole, Ofloxacin was effective to 82.33% of the total isolate, followed by Gentamicin (74.67%) and Ciprofloxacin (70.33%). Most of the bacteria were resistant to Ampicillin and Amoxycillin.
- 76.8% direct gram stain result showed similar results as culture results.

7.2 RECOMMENDATIONS

- Although no anaerobic bacteria were isolated in our study, routine anaerobic culture of the specimens should be performed as these may be involved in various wound infections.
- Before swabbing wound surface was cleansed with non bacteriostatic sterile saline. Similarly 70% alcohol could be used to reduce the colonizing commensal flora.
- *S. aureus* was the most common bacteria causing wound infection, hence they must be considered can serious problem.
- The susceptibility data collected in this study suggest that some antibiotics would have very limited usefulness for the prophylaxis or the empirical treatment of wound infections. Hence similar type of study should be undertaken regularly for establishing empiric therapeutic approaches for the management of such infections.
- In most cases direct Gram stain and culture results showed similar results hence Gram stain should always be performed to it is a rapid technique for guiding clinician in life-threatening infections.

CHAPTER-8

8. REFERENCES

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APPENDIX-I

A. CLINICAL PROFILE:

Sample No: Hospital
Name: a) Ward
Address: b) O.P.D.
Sex: Age: c) Cabin
Date: d) Bed No.

Short Clinical History:

Sample site:

Patient on Antibiogram:

Investigation requested for:

Type of wound:

Time of Collection:

Macroscopic examination:

B. MICROBIOLOGICAL PROFILE:

Day 1

Direct microscopic examination

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

Day 2

Culture of Specimens:

Media Used:	**Type of Colony**
Nutrient agar (NA)	
MacConkey agar (MA)	
Blood agar (BA)	

Chocolate agar (CA)
Cooked meat broth (CMB)

Day 3

Biochemical Test Employed **Result**

a. Catalase test
b. Oxidase test
c. Coagulase test
   i. Slide coagulase
   ii. Tube coagulase

d. Methyl Red (MR)
e. Voges Proskauer (VP)
f. Triple Sugar Iron (TSI)
g. Sulphide Indole Motility (SIM)
h. Citrate Utilization
i. Urea hydrolysis
j. Oxidative Fermentative (OF)
k. Bacitracin Sensitivity (0.04 unit)

ORGANISM ISOLATED:

Day 4

Antibiotic Sensitivity Profile

Antibiotics used **Zone of Inhibition** **Remarks**

a. Gentamicin
b. Ofloxacin
c. Ciprofloxacin
d. Cotrimoxazole
e. Ampicillin
f. Amoxycillin
g. Chloramphenicol
h. Erythromycin
i. Polymyxin B.

Checked by:

C. LIST OF MATERIALS

1. Equipments

Autoclave
Anaerobic gas jar
Burner
Incubator
Hot air oven
Anaerobic gas pack
Microscope
Refrigerator
Glass wares: Petri plates, tubes, slides, glass rod etc.

2. Microbiological media (Hi-Media)

Nutrient Agar
Nutrient Broth
Mac Conkey Agar
Blood Agar
Muller Hinton Agar
Robertson's Cooked Meat Broth
Simmon's Citrate Agar
TSI Agar
MRVP Broth
Urease Broth
SIM Media
Hugh and Leifson (OF) Media

3. Chemicals/Reagents

Catalase reagent (3% H_2O_2)
Oxidase regent (1% Tetramethyl p-phenylene diamine dihydrochloride)
Kovac's reagent
Barrit's reagent (40% KOH, 5% α-napthol in a ratio 1:3)
Crystal violet
Gram's iodine
Acetone-alcohol
Safranin
Blood plasma
Methyl red

4. Antibiotic discs (Hi-media)

Ampicillin (10 mcg)
Amoxycillin (10 mcg)
Bacitracin (10 units)
Chloramphenicol (5 mcg)
Ciprofloxacin (5 mcg)
Co-Trimoxazole (25 mcg)
Cloxacillin (5 mcg)
Erythromycin (15 mcg)
Gentamicin (10 mcg)
Metronidazole
Neomycin (30 mcg)
Ofloxacin (5 mcg)
Penicillin (10 units)

5. Miscellaneous

Inoculating loops, Straight wires, Cotton swabs, Distilled water, Immersion oil, Lysol, Oil, Dropper, etc.

APPENDIX-II

A. Composition and Preparation of Different Types of Culture Media

1. Blood Agar (Hi-Media)

(Blood agar base infusion agar) + 5% Blood

Composition	gram/lt
Beef heart infusion	500
Tryptose	10
Sodium chloride	5
Agar	1.5
Final pH at 25º C	7.3±0.2

Preparation

As directed by the manufacturing company 40 gm of blood agar base was dissolved in 1000 ml distilled water. The medium was then sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 min. Then the prepared medium was cooled to about 40-50 ºC, to which 50 ml of sterile defibrinated blood was added aseptically. Then the medium was poured into petriplates. For chocolate agar sterile blood agar plates was heated at 80º C for 10 minutes in an oven, the colour of the medium turns into chocolate colour.

2. MacConkey Agar (Hi-Media)

(With sodium taurocholate, without salt and crystal violet)

Composition	gram/lt
Peptone	20
Lactose	10
Sodium taurocholate	5
Agar	20
Neutral red	0.04
Final pH at 25 ºC	7.4±0.2

Preparation

As directed by the manufacturing company, 55 gm of the medium was dissolved in 1000 ml distilled water. It was sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 minutes.

3. **Cooked Meat Broth (Hi-Media)**

Composition	**gram/lt**
Beef heart	454
Proteose peptone	20
Dextrose	2
Sodium chloride	5
Final pH at 25 ºC	7.2±0.2

Preparation

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

4. **Nutrient Broth (Hi-Media)**

Composition	**gram/lt**
Peptone	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Final pH at 25ºC	7.4±0.2

Preparation

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

5. Peptone Water (Hi-Media)

Composition	**gram/lt**
Peptone	10
Sodium chloride	5
Final pH at 25°C	7.2±0.2

Preparation

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

6. Nutrient agar (Hi-Media)

Composition	gram/lt
Beef extract	10
Peptone	10
Sodium chloride	5
Agar	12
Final pH at 25º C	7.4±0.2

Preparation

As directed by the manufacturing company, 39 gm of the medium was dissolved in 1000 ml distilled water. It was sterilized by autoclaving at 15 lbs pressure (121º C) for 15 minutes.

7. Muller Hinton Agar (Hi-Media)

Composition	**gram/lt**
Beef extract	300
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17
Final pH at 25ºC	7.4±0.2

Preparation

As directed by the manufacturing company, 38 gm of the medium was dissolved in 1000 ml distilled water and was sterilized by autoclaving at 15 lbs pressure (121º C) for 15 minutes.

B. Composition and Preparation of Different Types of Biochemical Media and Procedures of tests

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

Composition	**gram/lt**
Beef extract	3
Peptone	30
Peptonized iron	0.2
Sodium Thiosulphate	0.025

Agar	3
Final pH at 25ºC	7.3±0.2

Preparation

As directed by the manufacturing company, 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 (121º C) for 15 minutes.

Procedure for indole test

- The test organism was inoculated in the 5 ml SIM medium with a sterile straight wire and incubated at 37°C for 48 hours.
- A few drops of Kovac's reagent was added, shaken gently and observed for development of red colour.

Procedure for motility test

- The test organism was stabbed in the SIM medium with a sterile straight wire and was incubated for 24 hours at 37°C.
- The medium was observed for positive growth by the appearance of spreading turbidity from the stab line or turbidity throughout the medium.

Procedure for Hydrogen sulphide production test

- The test organism inoculated by stabbing in the medium with a sterile straight wire and incubated at 37ºC for 24 hours.
- Observation was done for black iron-containing precipitate in the medium indicating the production of H_2S gas.

2. Citrate medium (Hi-Media)

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

Preparation

As directed by the manufacturing company 24.2 gm of the medium was dissolved in 1000 ml distilled water. The medium was then dispensed in test tubes and sterilized by autoclaving at 15 lbs pressure (121º C) for 15 minutes. The sterilized medium in the tubes were allowed to set in slopes.

Procedure for citrate utilization test

- Using a sterile straight wire, organism was inoculated by streaking on the slope of the medium.
- It was then incubated at 37ºC for 24 hours.
- The change in the colour was then observed It was then incubated at 37ºC for 24 hours.

3. MR-VP Medium (Hi-Media)

Composition	**gram/lt**
Buffered peptone	7
Dextrose	5
Dipotassium phosphate	5
Final pH at 25°C	6.9±0.2

Preparation

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

Procedure for Methyl Red (MR) test

- 2.5 ml of sterile Glucose-Phosphate broth (MR-VP broth) tube was taken and inoculated with the test organism.
- Overnight incubation at 37°C was done.
- Few drops of Methyl Red solution were added. Bright red colour was observed in the positive test indicating acidity.

Procedure for Voges-Proskauer (VP) test

- 2.5 ml of sterile MR-VP (Glucose-phosphate) broth was taken, to which was added the test organism.
- It was incubated for 24 to 48 hours at 37ºC.
- After incubation, 0.6 ml α-napthol and 0.2 ml. of KOH was added and gently shaken and allowed to stand for 15 minutes.

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

4. Urea Agar base Medium (Hi-Media)

Composition	**gram/lt**	
Peptone	1	
Dextrose	1	
Sodium chloride	5	
Disodium phosphate	1.2	
Monopotassium phosphate	0.8	
Phenol red	0.012	Agar
	15	
Final pH at 25°C	6.9±0.2	

Preparation

As directed by the manufacturing company, 24 grams of the medium was dissolved in 950 ml distilled water and sterilized by autoclaving at 10 lbs pressure (115°C) for 20 minutes. The medium was cooled to about 45°C to which 50 ml of sterile 40% urea solution was mixed aseptically and distributed into sterile test tubes. The medium was allowed to set in a slant position.

Procedure for urease test

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

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

Composition	**gram/lt**
Peptone	10
Tryptone	10
Yeast extract	3
Beef extract	3
Lactose	10
Saccharose	10
Dextrose	1
Ferrous sulphate	0.2
Sodium chloride	5
Sodium thiosulphate	0.3

Phenol red	0.024
Agar	12
Final pH at 25°C	7.4±0.2

Preparation

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

Procedure:

- The organism was inoculated by stabbing the butt with straight wire and streaking the surface of the slant.
- The tubes were then incubated at 37ºC for 24 to 48 hours.
- The tubes were observed for gas formation, carbohydrate utilization and H_2S production.

Interpretation

1) Production of gas ⟶ indicated by cracking of the media.
2) Production of H_2S ⟶ indicated by the formation of black iron containing precipitate in the butt.
3) Fermentative pattern ⟶ (a) Acid/Acid: - Lactose and sucrose fermented. (Both slant and butt-yellow)
   (b) Alk/Acid: - Non lactose fermenter but sucrose fermenter (Slant-red but butt-yellow)
   (c) Alk/Alk or Alk/NC: - Non glucose fermenter (both slant and butt-red)

6. Hugh-Leifson's (OF) Media (Hi-Media)

Composition	**gram/lt**
Peptone	2
Sodium chloride	5
Dipotassium phosphate	0.3
Agar	2
Bromothymol blue	0.08
Final pH	7.1

Preparation

As directed by the manufacturing company, 0.34 gm of the basal medium was dissolved in 100ml distilled water and was sterilized by autoclaving at 15 lbs pressure (121º C) for

15 minutes. Then 10 ml of sterile 10%dextrose solution was added to the medium and mixed thoroughly. Finally, the medium was dispensed in 5 ml amounts into sterile test tubes.

Procedure for Oxidative-Fermentative (OF) test

- Two tubes of OF medium were taken. Using a sterile straight wire, the test organism was inoculated in it.
- The inoculated medium in one of the tubes was covered with layer of paraffin oil to exclude oxygen while other was open to air.
- Both tubes were then incubated at 37°C for up to 14 days and the tubes were observed for carbohydrate utilization.
- Fermentative organism utilizes carbohydrate in both tubes changing the medium from green to yellow whereas the oxidative organisms utilize carbohydrate of open tube only.

C. Composition and Preparation of Staining Reagents

Gram stain reagents

1. Crystal Violet Stain

Composition	**gram/lt**
Crystal violet	20
Ammonium oxalate	9
Ethanol	95
Distilled water	

Preparation

20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. 9 gm of ammonium oxalate dissolved in 200 ml of distilled water was then added to the mixture. Finally, distilled water was added to make the volume 1000 ml.

2. Lugol's Iodine Solution

Composition	**gram/lt**
Potassium iodide	20
Iodine	10
Distilled water	

Preparation

To 250 ml of distilled water, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally, the volume was made 1 lt. by adding distilled water.

3. Acetone-alcohol decoloriser

Composition	**volume (ml)**
Acetone	500
Ethanol (absolute)	475
Distilled water	25

Preparation

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

4. Counterstain Solution

Composition	**gram/lt**
Safranin	10
Distilled water	

Preparation

10 gm of safranin was weighed and transferred to a clean bottle. Then 1lt. distilled water was added to the bottle and mixed well until safranin dissolves completely.

D Composition and Preparation of Test Reagents

1. Catalase Reagent (To make 100 ml)

Composition	**volume (ml)**
Hydrogen peroxide solution	3
Distilled water	97

Preparation

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

Procedure:

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

2. Oxidase Reagent (To make 10 ml)

Composition

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

Preparation

0.1gm TPD was dissolved in 10 ml distilled water. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these stripes were freeze dried and stored in a dark bottle tightly sealed with a cap.

Procedure for oxidase test

- A piece of filter paper soaked with few drops of oxidase reagent i.e. 1% solution of tetramethyl-p-phenylene diamine dihydrochloride was taken.
- Using a sterile stick or a glass rod, a colony of test organism was then smeared on the filter paper.
- It was observed for development of purple color within few seconds

3. Kovac's Indole Reagent (To make 40 ml)

Composition

4-dimethyl aminobenzaldehyde	2gm
Isoamyl alcohol	30 ml
Conc. Hydrochloric acid	10 ml

Preparation

To 30 ml isoamyl alcohol, 2 gm of 4-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. To it, 10 ml conc. HCl was added and mixed well.

4. Methyl Red Solution (To make 50 ml)

Composition

Methyl red	0.05 gm
Ethanol (absolute)	28 ml

Distilled water	22 ml

Preparation

To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml distilled water was added and mixed well.

5. Voges-Proakauer Reagent (Barrit's Reagent)

i. VP Reagent A (To make 100 ml)

Composition

α-napthol	5 gm
Ethanol absolute	100 ml

Preparation

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

ii. VP Reagent B (To make 100 ml)

Composition

Potassium hydroxide	40 gm
Distilled water	100 gm

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.

6. Procedure of coagulase test

i. Tube coagulase test for free coagulase

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

ii Slide test for bound coagulase

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

APPENDIX-III

A. Antimicrobial Susceptibility Test Discs

Antibiotic	**Symbol**	**Strength**	**Diameter of zone of inhibition in mm**		
			Resistant (mm or less)	**Intermediate (mm)**	**Sensitive (mm or more)**
Ampicillin Gram –ve enteric organism Staphylococci	A	10mcg	13 23	14-16 -	17 29
Amoxycillin Gram –ve enteric organism Staphylococci	Am	20/10 mcg	13 19	14-17 -	18 20
Bacitracin	B	10 units	8	9-12	13
Chloramphenicol	C	30 mcg	12	13-17	18
Ciprofloxacin	Cf	5 mcg	15	16-20	21
Co-Trimoxazole	Co	25 mcg	10	11-15	16
Cloxacillin	Cx	5 mcg	11	12-13	14
Erythromycin Staphylococci Streptococci	E	15 mcg	13 15	14-22 16-20	23 21
Gentamicin	G	10 mcg	12	13-14	15

Ofloxacin	Of	5mcg	12	13-15	16
Polymyxin B	Pb	300 units	8	9-11	12

Manufacturers: HiMedia Laboratories Pvt. Limited, Mumbai, India.

B. Procedure of Antibiotic susceptibility test

- MHA plate with medium depth of 4mm was taken.
- The inoculum was prepared by transferring 3-4 pure culture colonies into nutrient broth (5ml). Moderate turbidity was obtained by incubating at 37ºC for 4 hours. The suspension was standardized to match the turbidity of the 0.5 MacFarland turbidity standards (1.5 X 10 to the power 8 cfu/ml) which is done by adding 0.5ml of 1.175% $BaCl_2.2H_2O$ solution to 99.5 ml of 0.36N H_2SO_4.
- Inoculation was done by dipping a sterile cotton swab into the inoculum and it was rotated by pressing against the upper inside wall of the tube to remove excess inoculum. Then uniform swabbing was done on the agar surface (carpet culture). Then the plate was allowed to dry for 10 minutes.
- With the help of flamed forcep, discs were carefully placed on the inoculated plate, at least 15mm away from the edge , at equal distances and sufficiently separated (about 30 mm between two discs) to avoid overlapping of ZOI . The plates were allowed to stand at room temperature for 30 minutes (perfusion time).
- The plates were incubated at 37ºC for 24 hours.
- ZOI was measured.

APPENDIX IV

Statistical tools

1. x^2 test

Age (years)	Male	Female	Total	Percentage (%)
0-10	9	4	13	3.25
11-20	53	45	98	24.5
21-30	63	58	121	30.25
31-40	37	28	65	16.25
41-50	27	13	40	10
51-60	16	9	25	6.25
61-70	14	11	25	6.25
71-80	5	4	9	2.25
81-90	3	1	4	1
Total	**227**	**173**	**400**	**100**

a. For males:

We set up hypothesis as:

H_o: There is no significant difference in incidence of wound infections among males in different age groups.
H_1: There is significant difference in incidence of wound infections among males in different age groups.

Observed (O)	Expected (E)	O-E	$(O-E)^2$	$(O-E)^2/E$
9	25.22	-16.22	263.08	10.43
53	25.22	27.78	771.72	30.6
63	25.22	37.78	1427.3	56.59
37	25.22	11.78	138.76	5.50
27	25.22	1.78	3.16	0.125
16	25.22	-9.22	85.0	3.37
14	25.22	11.22	125.88	4.99

5 3 } =8	50.44	-42.44	1801.15	35.71

Cal. x^2= Σ (O-E)^2/E= 10.43+30.6+56.59+5.5+0.12+3.37+4.49+35.7=146.8
Level of significance (α) =0.05
Degree of freedom (d.f.) =9-1-1=7
Tab. $x^2_{0.05}$ at 7 d.f.= 14.07
Decision: Since cal. x^2> tab. x^2, H_o is rejected, the test is significant. Hence the incidence of wound infection among males in different age groups is significantly different.

b. For female:

We set the hypothesis as:

H_o: There is no significant difference in incidence of wound infections among females in different age groups.
H_1: There is significant difference in incidence of wound infections among females in different age groups.

Observed (O)	Expected (E)	O-E	(O-E)^2	(O-E)^2/E
4 45 } =49	38.44	10.78	116.2	3.02
58	19.22	38.78	1503.88	78.24
28	19.22	8.78	77.089	4.01
13	19.22	-6.22	38.68	2.97
9	19.22	-10.22	104.44	5.43
11	19.22	-8.22	67.56	3.51
4 1 } =5	38.22	-33.22	1103.56	57.41

Cal. x^2= Σ (O-E) ^{2}/E= 3.02+78.24+4.01+2.97+5.43+3.51+57.41=151.57
Level of significance (α) =0.05
Degree of freedom (d.f.) =9-1-2=6
Tab. $x^2_{0.05}$ at 6 d.f.= 12.59
Decision: Since cal. x^2> tab. x^2, H_o is rejected, the test is significant. Hence the incidence of wound infection among females in different age groups is significantly different.

4.4 PROTOCOL FOR LABORATORY EXAMINATION OF PUS SAMPLE