SURGICAL WOUND INFECTION IN TIKUR ANBESSA HOSPITAL WITH SPECIAL EMPHASIS ON *PSEUDOMONAS AERUGINOSA.*

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Table of contents **Page**

ACKNOWLEGEMENTS I
TABLE OF CONTENTS II
LIST OF TABLES V
ABBREVATIONS Vii
ABSTRACT Viii
CHAPTER 1: INTRODUCTION
1.1 .GENERAL INTRODUCTION 2
1.2. Microbiology of *Pseudomonas aeruginosa* *2*
1.3. Epidemiology of *Pseudomonas aeruginosa* *3*
1.4. Pathogenesis *of Pseudomonas aeruginosa* *4*
1.5. Clinical manifestations and diagnosis of *Pseudomonas aeruginosa* *7*
1.6. Treatment and prevention of *Pseudomonas aeruginosa* *12*
1.7. Mechanisms of *Pseudomonas aeruginosa* drug resistance 14
1.8. Surgical site infections 15
1.8.1. Surgical wound classification 17
1.8.2 .Microbiology of Surgical site infections 19
1.8.3. Pathogenesis of Surgical site wound infections 21
1.8.4. Epidemiology of surgical wound colonization and infection 21
1.8.5. Risk factor for surgical site colonization and infection 22
1.8.6. Antimicrobial prophylaxis 24
1.9.1. Relevance of the study 25
1.10. Objectives of the study 26
CHAPTER 2: MATERIALS AND METHODS
2.1. Study area 27
2.2. Study design 27
2.3. Data collection 28
2.4. Transport of specimen 28
2.5. Processing of specimens 29
2.6. Antimicrobial susceptibility testing 29
2.7. Standardized control reference strains 30

2.8. Stastical analysis _____ 30
2.9 Ethical clearance _____ 30
CHAPTER 3: RESULTS
3.1. Study population_____ 31
3.2. Rates of Post-operative wound infections_____ 32
3.3. Pathogens isolated from postoperative wound infections_____ 35
3.4. Antimicrobial susceptibility test_____ 40
CHAPTER 4: DISCUSSION _____ 44
Limitation of the study_____ 50
Conclusion and recommendation_____ 50
REFERENCES_____ 52
APPENDIX 1: Investigation form _____ 59
APPENDIX 2: Patient consent form_____ 62

LIST OF TABLES **PAGE**

Table 1.1. Factors associated with *Pseudomonas aeruginosa* infections 5

Table 1.2. Putative virulence factors of *Pseudomonas aeruginosa* *6*

Table 1.3. Antimicrobials with high activities against *Pseudomonas aeruginosa* *13*

Table 1.4. Surgical wound classification by class of wounds. *18*

Table 1.5. Distribution of pathogens isolated from surgical site infections,NNIS, 1986 to1996 20

Table 1.6. Patients and operation characteristics that may influence the risk of surgical site infection development 23

Table 3.1. Age and sex distribution of 173 operated patients at Tikur Anbessa hospital,AA, Ethiopia,2006 31

Table 3.2. Post-operative wound infection rate by age and sex: 32

Table 3.3. Post – operative wound infection rate by class of wounds 33

Table 3.4. Univariate analysis of potential predictors of wound infection 34

Table 3.5. Distribution of pathogens isolated from post-operative wound infections at Tikur Anbessa hospital, A.A, 35

Table 3.6. Frequency of isolates recovered from different wards at Tikur Anbessa Hospital, A.A. Ethiopia. 36

Table 3.7. Distribution of pathogens isolated from post-operative wound infections by wound category. 37

Table 3.8. *P. aeruginosa* infection rate by age group, Sex, Wound category and Antibiotic *prophylaxis* Antibiograms of P.aeruginosa isolates from surgical site wound infection 38

Table 3.9. Antimicrobial susceptibility tests on *Pseudomonas aeruginosa* isolates from surgical wound infection 40

Table 3.10. Antibiograms of *P.aeruginosa* isolates from surgical wound infection 41

Abbreviations

AMP: Antimicrobial Prophylaxis

ATCC: American Type Culture Collection

NNIS: National Nosocomial Infection Surveillance

MDR: Multi-Drug Resistant

SSI: Surgical Site Infection

CDC: Center for Disease Control

Abstract

Pseudomonas aeruginosa surgical site infection is a serious infection with significant patient mortality and health-care costs. The emergence of multidrug resistant strains of *Pseudomonas aeruginosa* has complicated treatment decisions and leads to treatment failures. In Ethiopia, few studies were conducted and even those studies were done generally on gram-negative bacteria not specifically on *Pseudomonas aeruginosa* from surgical sites despite the fact that *P.aeruginosa* is becoming serious cause of opportunistic infection in immunocompromised and hospitalized post operative patients. Nevertheless, the relationship between appropriate antimicrobial treatment and clinical outcome is not well established in Tikur Anbessa hospital. This is a prospective study of surgical wound infection on surgical patients operated from April to July 2006 in Tikur Anbessa hospital. A total of 173 patients were enrolled and clinical samples collected for analysis. Demographic data and laboratory analysis test on the occurrence of *Pseudomonas aeruginosa* wound infection rate and antimicrobial susceptibility patterns has been conducted in the first 30 days postoperatively. Among the patients, there were 97(56%) male patients and 76(44%) female patients. The mean age of the patients was 37.3(range: 1-80 years).

The infection rate was 10.3%, 13.6%, 22.7% and 36.8% for clean, clean-contaminated, contaminated and dirty wounds respectively. The rate of surgical site infection was 17.9% with *Pseudomonas aeruginosa* being 14.4% of all the isolates. On comparing infected patients (n=31) with non-infected patients (n=142), there was no significant difference in the frequency of infection by sex (*p= 0.11)*. *Pseudomonas aeruginosa* was the third dominant isolates following *Staphylococcus aureus* and *coagulase negative Staphylococci* respectively. The prevalence of drug resistance to Amikacin, Azeotronam, Ceftriaxone, Ceftazidime, Chloroamphenicol, Ciprofloxacine, Gentamicin, Imipene, Piperacillin, Tetracycline and Trimethoprim-Sulfamethoxazole was evaluated.

The minimum resistance rate was 25% for Amikacin and Imipenem and the highest resistance rate obtained was for Chloroamphenicol which was 100%. 87% of the isolates of *P.aeruginosa* were multidrug resistant. The outcome of this research emphasizes the use of first line drug therapy rather than random prescription of antibiotics with out susceptibility

testing. This will aggravate the ever increasing resistance not only to *Pseudomonas aeruginosa* but also to other microorganisms. Policies governing the use of antimicrobials in many institutions are lacking. Such policies must be implemented in order to limit the spread of resistance.

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CHAPTR I: INTRODUCTION

1.1. GENERAL INTRODUCTION

The methods for managing surgical site infection have evolved during the past 50 years. The evolution has been accompanied by causes in the etiology, epidemiology and approach to prevention of surgical site infections (Kiriz and Robson, 1975, Fry, 2003). The increasing trend of multi-drug resistant isolates of *Pseudomonas aeruginosa* has increased the mortality and health-care costs. Optimum application of surgical site infections prevention measures required that a variety of patients and operation characteristics be carefully considered (Nicholas 1997; Alicia *et al*, 1999). This causes for need of a more organized and effective infection control program that includes active infection surveillance as it has been done in developed countries (Chen *et al.,* 1995; Emine 2005; Spenur, 2002).The surgical site infection rate is much higher than the acceptable standard in Ethiopia on studies which were done in Gonder and Tikur Anbessa hospitals (Kotisso *et a*l., 1998; Taye, 2005).This under scores the need for establishing hospital infection control system.

Surgical infections are usually caused by bacteria, but fungal and viral infections can also occur, especially as postoperative infections in immuno-compromised hosts (Howard and Simmons, 1995). Staphylococcus species are the predominant gram positive Cocci of interest to cause postoperative infections (Linda *et al*., 2003). There are numerous gram-negative rods of surgical significance which are members of the family *Enterobacteriaceae.* These include the genera *Escherchia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Serratia* and *Providencia* (Mohamed, 2000).

The family *Pseudomonaceae* is composed of obligate aerobes that lack the ability to ferment sugars, unlike members of the *Enterobacteriaceae* (Mackie and Mcartney , 1989). *Pseudomonas aeruginosa* is the species in this family responsible for most postoperative infections (Howard and Simons1995; Kotisso *et al*., 1998). They cause infections similar to

those of gram-negative enteric bacteria in association to gastro-intestinal disease, pneumonia, urinary tract infections and burns (Harris *et al*., 1999). They are frequently found in immunologically compromised patients, especially if they have been hospitalized for some time. They cause necrotizing infection, especially pneumonia and vasculitis (Brook and Frazer, 1995). Ecthyma gangrenosam is the cutaneous manifestation of necrotizing vasculitis due to *Pseudomonas* bacteremia and is characterized by small, round, necrotic skin lesions (Nobel. 1993). Because of its resistance to antibiotic therapy, *Pseudomonas* infections are treated with a combination of two antibiotics (Daniel *et al*., 2004)

The frequent occurrence of *Pseudomonas aeruginosa* in surgical site infections is aggravated by risk factors of patients and operation conditions. Some of the risk factors include: age, nutritional status, obesity coexistent infection at remote body site, altered immune response due to HIV/AIDS and chronic cortico-steroid use (Fry, 2003). Beside this, operation conditions such as duration of surgical procedure, operation, antimicrobial prophylaxis and surgical technique compounded by increasing trend of anti-pseudomonal drug resistance have worsened the problems (Howard, 1991). . This study not only serve as baseline for further comparisons and monitoring, but lay the ground for wound surveillance and the increasing risk of resistance of *Pseudomonas aeruginosa* to different antibiotics.

1.2. Microbiology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a small, non-sporulating, aerobic gram-negative rod belonging to the family *Pseudomonadaceae*. It is motile by virtue of its single polar flagellum. More than half of all clinical isolates produce the blue-green pigment pyocyanin; this pigment is helpful in the identification of the organism and accounts for the species name *aeruginosa* (Harrison, 2005). Cultures of *P. aeruginosa* or wounds infected by this organism give off a distinctive grape-like odor due to one of their pigments, 2-aminoacetophenone (Boyed, 1995). *P.aeruginosa* grows well at 37-42^{0}C. Its growth at 42^{0}C helps differentiate it from other *Pseudomonas species*. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose (Jawits, 1998). *P.aereginosa* can produce arginine dihydrolase

and gelatinase and can use glucose but not trehalose as sole carbon and energy source (Chen *et al.,* 1995).

1.3. Epidemiology

P.aeruginosa is unable to cross the defense barrier of the healthy individual. However, this organism can cause life-threatening infection in immunocompromised hosts. (Boyed, 1995). Most *P.aeruginosa* infections are acquired in hospitals. According to the National Nosocomial infections surveillance (NNIS) system of USA, between 1992 and 1999, *P.aeruginosa* was the second most common cause of pneumonia, the fourth most cause of urinary tract infection, and the sixth most common blood stream isolate in intesive care units (ICUs). Many potential reservoirs of infection have been identified in hospital environment, including respiratory equipment, cleaning solutions, disinfectants, sinks, vegetables, flowers, endoscopes, and physiotherapy pools. Most reservoirs are associated with moisture (Harrison, 2005). Cross- transmission from patient to patient may occur via the hands of health care staff or through contaminated materials or reagents. Thus, a number of outbreaks of nosocomial infections due to *P. aeruginosa* have been reported; especially in intensive care units, burn wound units and cancer units (Veronique *et a*l., 2002). The incidence of *P. aeruginosa* in the post-operative wound infection is becoming more serious in developing countries because of relaxation in general hygienic measures, mass production of low quality antiseptic and medicinal solutions for treatment, difficulties in proper definition of the responsibility among the hospital staff (Oguntibeju and Nwobu, 2004). Generally, disruption of cutaneous or mucosal barriers by catheterization, intubations, immunosuppression due to AIDS, cancer, steroid therapy; disruption of normal bacterial flora by broad-spectrum antibiotic therapy and exposure to the hospital environment are factors predisposing to Pseudomonas infections (Harrison, 2005)

Epidemiological typing techniques that establish clonal relationships between individual isolates in hospital settings are warranted in order to recognize nosocomial transmission and hence to guide infection control practices (Ana *et al*., 2002). Typing can be used to test hypotheses about the reservoirs, sources and vehicles of transmission to verify the efficacy of control measures, and to study the epidemiology of out break strains, and in confirming their clonality. It can be performed by using a diversity of phenotypic and increasingly

today, genotypic methods. Among phenotypic methods currently used for hospital epidemiology, the antibiogram is still extremely useful as an outline technique to detect hospital infection with antibiotic resistant bacteria and determine the similarity of isolates in hospitals (Panzig *et al*., 1999)

1.4. Pathogenesis

Pseudomonas aeruginosa is well suited to colonization of the host once the primary defense barriers are no longer intact. The most important virulence determinants are pili, exotoxin-A, elastase, mucoid exopolysaccharide and phospholipase (Boyed, 1995).

Table 1.1**. Factors associated with *Pseudomonas aeruginosa* infections**

Disruption of cutaneous or mucus barrier	Immuno-suppression
Burn injury	Neutropenia
Dermatitis	Qualitative white blood cell defects
Penetrating trauma	Defective cell-mediated immunity
Surgery	Extremes of age
Endotracheal intubation	Diabetes mellitus
Indwelling catheterization	Steroid therapy
Urinary bladder catheterizations	Cystic fibrosis
Injection drug use	Cancer
Disruption of normal bacterial flora	AIDS
Broad-spectrum antibiotic therapy	
Exposure to the hospital environment	

Adapted from Fry, 2003.

TABLE 1.2: Putative Virulence Factors of *Pseudomonas aeruginosa*

Virulence Factors	Functions
Pili or fimbriae	Attachment to epithelial cells
Mucoid exopolysaccharide (Alginate)	attachment to epithelial cells,inhibition of mucociliary and opsonophagocytic clearance
Alkaline protease	Tissue break down,proteolysis of Immunoglobulin and complement.
Elastase	Destruction of elastic tissue, lamina blood vessels.
Phospholipase C	Break down of lipids and lecithin (tissue necrosis).
Lipopolysaccaride (endotoxin)	Fever,leukocytosis or leucopenia shock,hypotension.
Exotoxins	
Exotoxin A	Inhibition of protein synthesis.
Exotoxins S and T	Disrupt cellular actin cytoskeleton.
Exotoxin U and Y	Acute cytotoxicity

Adapted from Harrison, 2005.

P.aeruginosa produces infection of wounds and burns, giving rise to blue green pus: meningitis, when introduced by lumbar puncture: and urinary tract infection;when introduced by catheters and instruments or in irrigating solutions. Necrotizing pneumonia, mild otitis externa in swimmers, malignant otitis externa in diabetic patients, eye infection can be caused by *P.aeruginosa* (Jawits, 1998). *P. aeruginosa* is the predominant cause of chronic airway infection in cystic fibrosis and remains the primary cause of morbidity and mortality in this population (Samuel *et a*l., 2004).

1.5. Clinical manifestation and diagnosis.

Respiratory Tract Infections: Primary pneumonia, or non-bacteremic pneumonia, results from aspiration of upper respiratory tract secretions; often develops in patients with chronic lung disease, congestive heart failure, or AIDS; and is most common in an intensive care setting in association with mechanical ventilator use (Samuel *et a*l., 2004). Cavitary lesions are particularly common in AIDS patients with *P. aeruginosa* pneumonia. Pathologic lesions include alveolar necrosis, focal hemorrhages, and micro abscesses (Balch and Smith, 1994).

Chronic infection of the lower respiratory tract with *P. aeruginosa* is caused almost exclusively by mucoid strains, which produce alginate. Such infection is prevalent among older children and young adults with cystic fibrosis and also develops in some patients with AIDS. In patients with cystic fibrosis, mucoid strains invariably colonize and infect patients with increasing prevalence over time, contributing to the acute exacerbations and chronic progression that characterize pulmonary disease in these individuals (John *et al*., 2000). Airway obstruction appears to begin with bronchiolitis, which causes mucus plugging and predisposes to *P. aeruginosa* infection.

The infection produces more mucus plugging, chronic suppuration, bronchiectasis, atelectasis, and ultimately fibrosis. This process progresses to pulmonary insufficiency, hypoxemia, and alterations in cardiopulmonary dynamics resulting in pulmonary hypertension and cor pulmonale (Shepp, 1994).

Bacteremia: *P. aeruginosa* remains an important cause of life-threatening bloodstream infection in immunocompromised patients. Bacteremia is frequently iatrogenic and is

usually seen in hospitalized patients with various comorbid conditions. Bloodstream infection can be either primary (with no identifiable source) or secondary to a discrete focus of infection (Ohl and Pollack, 2004).

The clinical features of *P. aeruginosa* bacteremia are similar to those of other forms of bacteremia.Pathognomonic skin lesions termed ecthyma gangrenosum develop in a relatively small minority of patients with *P. aeruginosa* bacteremia. The lesions begin as small hemorrhagic vesicles surrounded by a rim of erythema and undergo central necrosis with subsequent ulceration (Hacker, 1994). They occur singly or in small numbers on the perineum, buttocks, and extremities; in the axillae; or elsewhere. Histologically, these lesions contain numerous bacteria invading blood vessels but few inflammatory cells. Bacteria are readily visible on Gram staining and may be cultured from aspirated material (Shepp, 1994).

Endocarditis: *P. aeruginosa* infects naive heart valves in injection drug users as well as prosthetic heart valves. The source of *P. aeruginosa* strains infecting drug users appears to be standing water contaminating drug paraphernalia (Balch and Smith, 1994).
Foreign materials mixed with heroin may cause injury to valve leaflets or mural endocardium, with resulting fibrosis and an increased risk for valve infection. Exposure of the tricuspid valve to both trauma and bacteria apparently accounts for the high incidence of tricuspid involvement in association with injection drug use (Pollack, 2000).

The diagnosis of *P. aeruginosa* endocarditis is based on positive blood culture in the absence of an extracardiac source; an indication of valvular dysfunction or vegetation on an echocardiogram; evidence of septic pulmonary lesions on a chest roentgenogram (in right-sided disease); and the actual demonstration of infected heart valves at the time of surgery (Howard and Simmons ,1995).

Ear Infections: *P. aeruginosa* is often found in the external auditory canal, particularly under moist conditions and in the presence of inflammation or maceration (as in "swimmer's ear"). Moreover, this organism is the predominant pathogen associated with external otitis, a

usually benign inflammatory process affecting the external auditory canal (Jawett, 1998). The ear is painful or merely itchy, there is a purulent discharge, and pain is elicited by pulling on the pinna. The external canal appears edematous and is filled with detritus that often prevents visualization of the tympanic membrane (Boyd, 1995).

Eye Infections: *P. aeruginosa* causes bacterial keratitis or corneal ulcer and endophthalmitis in the human eye. Keratitis due to *P. aeruginosa* may result from even minor corneal injury, which interrupts the integrity of the superficial epithelial surface and permits bacterial access to the underlying stroma (Harrison, 2005).
Corneal ulcer may complicate contact lens use, particularly when extended-wear soft contact lenses are involved. Contact lens solutions or the lenses themselves may be the source of the organism, which is probably inoculated into the eye at sites of minor lens-induced corneal damage (Pollack, 2000).

Patients who have sustained serious burns, have undergone ocular irradiation or tracheostomy, have been exposed to the intensive care environment, and/or are in a coma are also susceptible to *P. aeruginosa*-associated corneal ulcers. *P. aeruginosa* keratitis usually starts as a small central ulcer; spreads concentrically to involve a large portion of the cornea, sclera, and underlying stroma; and in some cases progresses to posterior corneal perforation (Harrison, 2005).

Bone and Joint Infections: Vertebral osteomyelitis due to *P. aeruginosa* is associated with complicated urinary tract infection, genitourinary instrumentation or surgery, and injection drug use (Pollack, 2000). Vertebral infections that are associated with a urinary tract source most often develop in the elderly and usually affect the lumbosacral spine. Presumably the route of infection in these patients is a shared venous plexus between the pelvis and spine. Injection drug use-related infections typically occur in younger patients and may affect the cervical or lumbosacral spine (Balch and Smith, 1994).

P. aeruginosa vertebral osteomyelitis is usually an indolent disease. Accordingly, symptoms may develop weeks or even months before diagnosis. Back or neck pain is generally

reported, while fever and systemic symptoms are relatively uncommon. Local tenderness and decreased range of motion of the affected spine are typical. Leukocytosis may be noted, the erythrocyte sedimentation rate is almost always markedly elevated, and blood cultures are sometimes positive (Morrison and Wenzel, 1984). *P. aeruginosa* is one of the most common causative agents in a variety of other, less specific syndromes involving nonhematogenous infections of bones and joints and collectively referred to as *chronic contiguous osteomyelitis* (Harrison, 2005). These infections may result, for example, from compound fractures, contamination associated with open reduction and fixation of closed fractures, sternotomy performed in conjunction with cardiac surgery, contiguous spread from infected ischemic ulcers related to peripheral vascular disease or diabetes mellitus, and cellulitis in general. The chronicity, indolence, and heterogeneity of these infections explain their varied clinical manifestations and the frequent need for complicated long-term management (Ohl and Pollack, 2004).

Urinary Tract Infections: *P. aeruginosa* is one of the most common causes of complicated and nosocomial infections of the urinary tract. These infections may result from urinary tract catheterization, instrumentation, surgery, or obstruction; they may arise from persistent foci (e.g., the prostate or stones) and may be chronic or recurrent (Jawits, 1998). The urinary tract may be a target for bloodborne infection in patients with *P. aeruginosa* bacteremia but more often is the source of bacteremia. Chronic *P. aeruginosa* infections of the urinary tract are relatively common among patients with indwelling urinary catheters, altered urinary tract anatomy secondary to diversionary procedures, and paraplegia (Boyd, 1995).

The clinical features of urinary tract infections due to *P. aeruginosa* are usually indistinguishable from those of other bacterial infections. However, *P. aeruginosa* infections exhibit a propensity for persistence, chronicity, resistance to antibiotic therapy, and recurrence. More unusual forms of urinary tract involvement peculiar to *P. aeruginosa* include (1) ulcerative lesions of the renal pelvis, ureters, and bladder that cause sloughing of vesical membranes in the urine; and (2) ecthyma like lesions of the renal cortex that are seen in association with *Pseudomonas* sepsis(Harrison, 2005).

Skin and Soft Tissue Infections: As indicated above, *P. aeruginosa* bacteremia may be associated with the disseminated skin lesions of ecthyma gangrenosum . Less common skin manifestations of *P. aeruginosa* sepsis include vesicular or pustular lesions, bullae, subcutaneous nodules, deep abscesses, and cellulites (Hacker, 1994). Metastatic lesions of the skin or mucous membranes complicate *Pseudomonas* sepsis and occasionally produce massive necrosis or gangrene of the extremities, perineum, face, or oropharynx (Nobel, 1993).

Primary *P. aeruginosa* pyoderma occurs when the skin breaks down secondary to trauma, burn injury, dermatitis, or ulcers related to peripheral vascular disease or pressure sores. Moist conditions and neutropenia may predispose to this condition (Morrison and Wenzel, 1984).

***P. aeruginosa* Infections in Patients with AIDS:** During the 1980s and 1990s, *P. aeruginosa* infections were increasingly associated with AIDS. The vast majority of these infections are currently seen in patients with advanced AIDS and previous opportunistic infections (Harrison, 2005). The specific immunologic factors that lead to *P. aeruginosa* infections in patients with AIDS are not well understood but are speculated to be a loss of mucosal integrity, defects in cellular and humoral immunity, and qualitative leukocyte abnormalities. The majority of *P. aeruginosa* infections in this population are community-acquired, in contrast to the nosocomial transmission documented for most *P. aeruginosa* infections in non-AIDS patients (Shepp , 1994).

1.6. Treatment and prevention.

In most severe or life threatening infections due to *P. aeruginosa,* two antipseudomonal antibiotics to which the infecting strain is sensitive should be administered together. The putative benefit of this combined therapy, as determined by in vitro studies, is to increase efficacy, achieve synergistic killing, and prevent the emergence of antibiotic resistance. (Harrison, 2005). Penicillin active against *P.aeruginosa* - ticarcillin, mezolocilin, or piperacillin is used in combination with aminoglycosides, usually gentamicin, tobromycin or

amikacin. Other drugs active against *P.aeruginosa* include aztreonam and imipenem (Jawitz, 1998). The fluoroquinolone, ciprofloxacin is effective for the treatment of serious nosocomial infections including those caused by *P. aeruginosa* (Ronald *et al*., 2004).

Table1.3: Antimicrobials with high activities against *Pseudomonas aeruginosa*

Penicillins	**Cephalosporins**
Piperacillin	Ceftazidime
Piperacillin/tozobactam	Cefoperazone
Mezlocillin	Cefepime
Ticarcillin	**Aminoglycosides**
Ticarcillin/clavulanate	Tobramycin
Carbapenems	Gentamycin
Imipenem	Amikacin
Meropenem	**Fluoroquinolones**
Monobactams	Ciprofloxacin
aztreonam	Levofloxacin

Adapted from Harrison, 2005

1.7. Mechanism of *Pseudomonas aeruginosa* drug resistance

Pseudomonas aeruginosa is an opportunistic pathogen that can cause serious and often life-threatening infections in immunocompromised and cystic fibrosis patients (Samuel *et a*l., 2004). Treatment of *P. aeruginosa* infections is a therapeutic challenge as a result of the organism's intrinsic resistance to a variety of antibiotics.
Although fluoroquinolones and carbapenems demonstrate potent antipseudomonal activity, clinical isolates of *P. aeruginosa* that are resistant to either class of agent are being isolated (Livermor, 2002).

The mechanisms associated with carbapenem resistance have included porin downregulation, carbapenem-hydrolyzing enzymes, and the overexpression of efflux pumps. Loss of the substrate-specific porin, OprD, causes resistance to imipenem and a decrease in susceptibility to meropenem (Trias and Nikaid 1990). Although the chromosomal cephalosporinase, AmpC, poorly hydrolyzes the carbapenems, the acquisition of class B enzymes, such as metallo-beta_-lactamases belonging to the IMP and VIM families (Nordman and Poirel, 2002) has been shown to confer high level resistance to these agents. In addition, overproduction of the MexABOprM efflux system in *nalB* mutants causes a decrease in susceptibility to meropenem but has no influence on imipenem susceptibility, because imipenem is not a substrate for this pump (Kohler *et al*., 1999). Fluoroquinolone resistance has been associated with target site mutations within DNA gyrase and topoisomerase IV, which are critical enzymes involved in regulating DNA topology during replication (Levine *et al*., 1998). Mutations within specific regions of each subunit (*gyr*A and *gyr*B for DNA gyrase; *par*C and *par*E for topoisomerase IV) known as the quinolone resistance–determining regions alter the affinity of fluoroquinolones for these enzymes. Several mutations within the quinolone resistance–determining regions (QRDRs) of both enzymes have been described in fluoroquinolone-resistant isolates of *P. aeruginosa (*Akasaka *et a*l., 2001). In addition, the over expression of multidrug efflux pumps has also been implicated in fluoroquinolone resistance.

Four efflux pumps belonging to the resistance nodulation cell division family, MexAB-OprM, MexCDOprJ, MexEF-OprN, and MexXY, have all been shown to extrude the fluoroquinolones resulting in resistant phenotypes (Poole, 2000). Typically, the over

expression of these efflux systems contributes to low-level fluoroquinolone resistance. Previous studies have only associated high level fluoroquinolone resistance with active efflux when combined with DNA gyrase or topoisomerase IV mutations (Nakajima *et al.*, 2002), Whereas resistance to either drug class remains a substantial problem, clinical isolates demonstrating simultaneous resistance to both fluoroquinolones and carbapenems have been encountered (Harris *et a*l., 1999) and this phenotype severely reduces therapeutic options. Dual resistance in *P. aeruginosa* isolates may be attributed to *nfxC*-type mutants that overexpress the MexEF-OprN efflux system and downregulate the production of the porin, OprD. This pathway to dual resistance raises a concern regarding the promotion of carbapenem resistance through the use of fluoroquinolones (Kohler *et a*l., 1997).

1.8. Surgical Site Infections (SSI)

Before the mid-19th century, surgical patients commonly developed postoperative irritative fever, followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was not until the late 1860s, after Joseph Lister introduced the principles of antisepsis, that postoperative infectious morbidity decreased substantially (Linda *et al*., 2003). The CDC's National Nosocomial Infections Surveillance (NNIS) system, established in 1970, monitors reported trends in nosocomial infections in U.S. acute-care hospitals. Based on NNIS system reports, SSIs are the third most frequently reported nosocomial infection, accounting for 14% to 16% of all nosocomial infections among hospitalized patients. (Emori and Gaynes ,1993). During 1986 to 1996, hospitals conducting SSI surveillance in the NNIS system reported SSIs following operations (Linda *et a*l., 2003). Among surgical patients, SSIs were the most common nosocomial infection, accounting for 38% of all such infections. Of these SSIs, two thirds were confined to the incision, and one third involved organs or spaces accessed during the operation.

When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection, and the majority (93%) was serious infections involving organs or spaces accessed during the operation (Horan *et al*., 1992).

Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial

prophylaxis. Despite these activities, SSIs remain a substantial cause of morbidity and mortality among hospitalized patients. This may be partially explained by the emergence of antimicrobial resistant pathogens like that of *P.aeruginosa* and the increased numbers of surgical patients who are elderly and/or have a wide variety of chronic, debilitating, or immunocompromising underlying diseases (Paulsen *et a*l.,1994).

The identification of SSI involves interpretation of clinical and laboratory findings, and it is crucial that a surveillance program use definitions that are consistent and standardized; otherwise, inaccurate or uninterruptible SSI rates will be computed and reported. Surgical site infection is defined as infection of the incision(up to 30 days post-operatively) affecting the skin ,subcutaneous tissue or muscle(Garner *et al* 1993).During this time the there may be pus draining from the incision, the patient may complain of pain ,the satures might rapture and/or the patient may have a fever of greater than 38^0c (Simmons ,1982).

The CDC's NNIS system has developed standardized surveillance criteria for defining SSIs. By these criteria, SSIs are classified as being either incisional or organ/space. Incision SSIs are further divided into those involving only skin and subcutaneous tissue (superficial incisional SSI) and those involving deeper soft tissues of the incision (deep incisional SSI). Organ/space SSIs involve any part of the anatomy (e.g., organ or space) other than incised body wall layers that was opened or manipulated during an operation. Failure to use objective criteria to define SSIs has been shown to substantially affect reported SSI rates (Garner et al., 1993). The CDC NNIS definitions of SSIs have been applied consistently by surveillance and surgical personnel in many settings and currently are a de facto national standard (Horan *et al*., 1992)

1.8. 1. Surgical Wound Classification

For years wounds have been classified in to four categories according to the theoretical number of bacteria that contaminate wounds: clean, clean-contaminated, contaminated and dirty. Wound infection rates in large series are approximately1.5 to 3.9 percent for clean wounds, 3 to 4 percent for clean-contaminated wounds and approximately 8.5 percent for contaminated wounds. Dirty wounds are generally left open, but wound infection rates for

dirty wounds of 28 to 40 percent have been reported (Linda *et al*., 2003 and Horan *et al*., 1992).

Table 1.4: Surgical Wound Classification by Class of Wounds

Class I/Clean: An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. Operative incisional wounds that follow no penetrating (blunt) should be included in this category if they meet the criteria.
Class II/Clean-Contaminated: An operative wound in which the respiratory, alimentary, genital or urinary tracts are entered under controlled conditions and with out unusual contamination. Specifically, operations involving the

	biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.
Class III/Contaminated:	Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g., open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.
Class IV/Dirty-Infected:	Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

Adapted from Garner, 1993 and Simmons, 1982.

1.8.2. Microbiology of surgical site infections.

According to data from the NNIS system, the distribution of pathogens isolated from SSIs has not changed markedly during the last decade (Table 1.5). *Staphylococcus aureus,* coagulase-negative staphylococci, *Enterococcus* spp., and *Escherichia coli* remain the most frequently isolated pathogens. An increasing proportion of SSIs are caused by antimicrobial-resistant pathogens, such as methicillin-resistant *S. aureus* or by *Candida albicans (*Schaberg *et al*., 1991*).* From 1991 to 1995, the incidence of fungal SSIs among patients at NNIS hospitals increased from 0.1 to 0.3 per 1,000 discharges. The increased proportion of SSIs caused by resistant pathogens and *Candida* spp. may reflect increasing numbers of

severely ill and immunocompromised surgical patients and the impact of widespread use of broad-spectrum antimicrobial agents (Jarvis, 1995).

TABLE 1.5. Distribution of Pathogens Isolated from Surgical Site Infection, National Nosocomial Infection Surveillance System, 1986 to 1996. * Adapted from Alicia et al., 1999

	Percentage of Isolates	
	1986-1989	1990-1996
	N=16,727	N=17,671
Pathogen	Percentage	Percentage
Staphylococcus aureus	17	20
Coagulase-negative staphylococci	12	14

Enterococcus spp.	13	12
Escherchia coli	10	8
Pseudomonas aeruginosa	*8*	8
Enterobacter spp.	8	7
Proteus mirabilis	*4*	3
Klebsiella pneumoniae	*3*	3
Other *Streptococcus* spp.	3	3
Candida albicans	*2*	*3*
Group D streptococci (Non-enterococci)	—	2
Other gram-positive aerobes	—	2
Bacteroides fragilis	—	2

*Pathogens representing less than 2% of isolates are excluded.

1.8.3. Pathogenesis of surgical site infection.

Microbial contamination of the surgical site is a necessary precursor of SSI. Quantitatively, it has been shown that if a surgical site is contaminated with $>10^5$ microorganisms per gram of tissue, the risk of SSI is markedly increased (Krizek and Robson, 1975). However, the dose of contaminating microorganisms required to produce infection may be much lower when foreign material is present at the site i.e., 100 staphylococci per gram of tissue introduced on silk sutures (Linda *et al*., 2003).Microorganisms may contain or produce toxins and other substances that increase their ability to invade a host, produce damage within the host, or survive on or in host tissue. For example, many gram-negative bacteria produce endotoxin, which stimulates cytokine production. In turn, cytokines can trigger the systemic inflammatory response syndrome that sometimes leads to multiple system organ

failure. One of the most common causes of multiple system organ failure in modern surgical care is intra-abdominal infection (Alicia *et al*., 1999 and Demling *et a*l., 1993). Some bacterial surface components, notably polysaccharide capsules, inhibit phagocytosis, a critical and early host defense response to microbial contamination. Certain strains of clostridia and streptococci produce potent exotoxins that disrupt cell membranes or alter cellular metabolism (Dellinge, 1997). A variety of microorganisms, including gram-positive bacteria such as coagulase-negative staphylococci, produce glycocalyx and an associated component called slime, which physically shields bacteria from phagocytes or inhibits the binding or penetration of antimicrobial agents. Although these and other virulence factors are well defined, their mechanistic relationship to SSI development has not been fully determined (Alicia *et al*., 1999).

1.8.4. Epidemiology of surgical wound colonization and infection

For most SSIs, the source of pathogens is the endogenous flora of the patient's skin, mucous membranes, or hollow viscera. When mucous membranes or skin is incised, the exposed tissues are at risk for contamination with endogenous flora (Altemeire *et al*., 1968). These organisms are usually aerobic gram-positive cocci (e.g., staphylococci), but may include fecal flora (e.g., anaerobic bacteria and gram-negative aerobes) when incisions are made near the perineum or groin. When a gastrointestinal organ is opened during an operation the source of pathogens, gram-negative bacilli (e.g., *E. coli),* gram-positive organisms (e.g., enterococci), and sometimes anaerobes (e.g., *Bacillus fragilis)* are the typical SSI isolates (Simons, 1982).

Seeding of the operative site from a distant focus of infection can be another source of SSI pathogens, particularly in patients who have prosthesis or other implant placed during the operation. Such devices provide a nidus for attachment of the organism. Exogenous sources of SSI pathogens include surgical personnel (especially members of the surgical team), the operating room environment (including air), and all tools, instruments, and materials brought to the sterile field during an operation (Alicia *et al*., 1999).

1.8.5. Risk factors for surgical site colonization and infection.

The term risk factor has a particular meaning in epidemiology and, in the context of SSI pathophysiology and prevention, strictly refers to a variable that has a significant, independent association with the development of SSI after a specific operation (Alicia et al, 1999). Risk factors are identified by multivariate analyses in epidemiologic studies. Unfortunately, the term risk factor often is used in the surgical literature in a broad sense to include patient or operation features which, although associated with SSI development in univariate analysis, are not necessarily independent predictors (Linda *et al*., 2003).

An SSI prevention measure can be defined as an action or set of actions intentionally taken to reduce the risk of an SSI. Many such techniques are directed at reducing opportunities for microbial contamination of the patient's tissues or sterile surgical instruments; others are adjunctive, such as using antimicrobial prophylaxis or avoiding unnecessary traumatic tissue dissection. Optimum application of SSI prevention measures requires that a variety of patient and operation characteristics be carefully considered (Fry, 2003).

TABLE. 1. 6. Patient and Operation Characteristics that may Influence the Risk of Surgical Site Infection Development.

Patient conditions	Operation conditions
Age	Duration of surgical procedure
Nutritional status, poor	Skin antisepsis
Diabetes, uncontrolled	Preoperative shaving
Smoking or use of other tobacco products	Duration of operation
Obesity	Antimicrobial prophylaxis
Coexistent infections at a remote body site	Operating room ventilation
Colonization with microorganisms	Surgical drains
Altered immune response (HIV/AIDS)	Surgical technique

Adapted from Alicia *et al.,* 1999.

1.8.6. Antimicrobial prophylaxis

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins (Nichols and Holmes 1995). AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intra-operative contamination to a level that cannot overwhelm host defenses. The benefit, however, must be weighed against the risks of toxic and allergic reactions, the emergence of resistant bacteria, drug interactions, super- infection and cost (Linda et al., 2003). AMP does not pertain to prevention of SSI caused by postoperative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice. Essentially all confirmed AMP indications pertain to elective operations in which skin incisions are closed in the operating room. (Page *et al*., 1993).

It is strongly recommended to choose an antibiotic effective against the pathogens most likely to be encountered an AMP agent must be safe, inexpensive, and bactericidal with an in vitro spectrum that covers the most probable intra operative contaminants for the operation. The infusion of the initial dose of antimicrobial agent must also be adjusted so that a bactericidal concentration of the drug is established in serum and tissues by the time the skin is incised and a few hours after the incision is closed in the operating room (Classen *et al*., 1992).

A simple way to organize AMP indications is based on using the surgical wound classification scheme which employs descriptive case features to *postoperatively* grade the degree of intraoperative microbial contamination. A surgeon makes the decision to use AMP by anticipating *preoperatively* the surgical wound class for a given operation.
AMP is indicated for all operations that entail entry into a hollow viscus under controlled conditions (Nichols et al., 1997). By definition, AMP is not indicated for an operation classified as contaminated or dirty. In such operations, patients are frequently receiving therapeutic antimicrobial agents perioperatively for established infections (Nichols and Holmes 1995).

Cephalosporins are the most thoroughly studied AMP agents. These drugs are effective against many gram-positive and gram-negative microorganisms. They also share the features of demonstrated safety, acceptable pharmacokinetics, and a reasonable cost per dose (Page et al., 1993). In particular, cefazolin is widely used and generally viewed as the AMP agent of first choice for clean operation. If a patient is unable to receive a cephalosporin because of penicillin allergy, an alternative for gram-positive bacterial coverage is either clindamycin or vancomycin. Cefazolin provides adequate coverage for many clean-contaminated operations (Nichols, 1995). But AMP for operations on the distal intestinal tract mandates use of an agent such as cefoxitin (or some other second-generation cephalosporin) that provides anaerobic coverage. If a patient cannot safely receive a cephalosporin because of allergy, a reasonable alternative for gram-negative cover age is aztreonam. However, an agent such as clindamycin or metronidazole should also be included to ensure anaerobic coverage. The aminoglycosides are seldom recommended as

first choices for AMP, either as single drugs or as components of combination regimens (Lee, 1995). An effective SSI surveillance program must be operational, with careful and timely culturing of SSI isolates to determine species and AMP agent susceptibilities). Simple protocols of AMP timing and oversight responsibility should be locally designed to be practical and effective (Simmon, 1982).

1.9.1 Relevance of the study

Several studies were undertaken in different countries on surgical site wound infection. This study will give special emphasis on the prevalence and drug susceptibility pattern of *Pseudomonas aeruginosa on* surgical site wound infection. It is found with different prevalence in different part of the globe. But in Ethiopia, few studies were conducted and even those studies were done generally on gram-negative bacteria not specifically on *Pseudomonas aeruginosa* from different anatomic sites (Asrat and Woldeamanuel 2001; Ferede et al., 2001). *P. aeruginosa* is becoming serious cause opportunistic infection in immunocompromised and hospitalized patients. Post-operative wound infections are the ideal sources of different microorganisms including *P. aeruginosa* (Linda *et al.,* 2003; Oguntibeju and Nwobu, 2004). Effective treatment and prevention of post operative wound infections is crucial. In Ethiopia, the rational use of drugs in not yet practiced. In this country there is also no trend of strict adherence to surgical site wound infection prevention guide lines. Because of limited facilities in most clinical settings; laboratory diagnosis and antimicrobial susceptibly testing are not practiced.

The aim of this study is to determine the rate of post operative wound infection with emphasis on *P.aeruginosa*, its susceptibility pattern to antipseudomonal drugs and to give concrete recommendations based on the out - come of this research. The finding of this study will also enable to determine the microbiology of surgical site wound infection, the rate of surgical site wound infection rate, drug resistant *P. aeruginosa* and identify potential risk factors for acquiring post operative wound infections.

1.10. Objectives of the study

1.10.1 General Objective

- To assess the microbiology of surgical wound infection at Tikur Anbessa Hospital.
- To determine the rate of occurrence of different post operative wound isolates at Tikur Anbessa Hospital.

1.10.2. Specific Objectives

- To determine the susceptibility pattern of *P.aeruginosa* to different antimicrobial drugs.
- To identify some potential risk factors for post operative wound infection.

CHAPTER TWO: MATERIALS AND METHODS.

2.1 Study area

This study was undertaken from April 2006 to July 2006 to study the surgical wound infection at Tikur Anbessa Specialized Hospital, Addis Ababa,with special emphasis on *P.aeruginosa* from hospitalized patient's clinical specimen who developed post-operative wound infection.

2.2 Study Design

A prospective study design was implemented starting from April 2006 to July 2006. All surgical patients operated during the study period and hospitalized up 30 days were considered eligible for the study. Those patients having oral or rectal operation only and patients who died or left before third post-operative days were excluded (Taye, 2005).Among the 451 patients operated during the study period, only the 173 form the basis of this study as result of the exclusion criteria. Since there was no previous study of this kind in Ethiopia to refer as base line, rate of *P.aeruginosa* infection from other countries was used to calculate sample size for the study (Giovanni et al., 1998).

Sample size was computed considering the 95% confidence level. Taking the proportion as 0.13 and tolerable error of 0.05, the sample size was considered to be 173. The formula used to calculate the sample size was as follow:

$$n_f = \frac{z\alpha^2 p(1-P)}{d^2},$$ the sample size to be taken will be 173 based on the above specification.

n_f = final sample size required

$Z\alpha^2$ = the standard normal deviation corresponding the specified size of total Population.

d = degree of accuracy desired, in this case 0.05 was taken.

P = Population to have a particular characteristics, in this Case 13%

$1-P$ = 87%

Accordingly, a total of 173 surgical patients were included during the study period. Out of the patients who developed post-operative wound infection, antimicrobial susceptibility testing was done for *Pseudomonas aeruginosa*.

2.3 Data and Specimen Collection

These eligible surgical patients were subjected to daily surveillance for the development of infection. This was done according to the clinical criteria for surgical site infection development (Simmons 1982, Garner et al., 1993). Based on these criteria, there may be pus draining from the incision, the patient may complain of pain, the sutures might rapture and/or the patient may have a fever of greater than 38 ^{o}C (Appendix-i). From these surgical patients who were suspected to develop infection, based on the criteria, wound swabs were taken for microbiological follow up. Wound swabs were taken from the lower extremities before wound dressing time to avoid skin flora contamination. The data wound swab was collected by the principal investigator and surgical wound nurses using data collection form and sterile cotton swab on sterile transport media. Patients were identified from the operating room log and daily operation schedule. Patient specific demographic characters and information on potential predicators of wound infection including the health condition

and wound class were recorded on the day of the operation from the patient card and the responsible surgeon, when necessary.

Patients were followed for the development of wound infection by the principal investigator. In addition each day the responsible ward nurse/resident was approached to find out patient with wound infection. All clinically suspected wounds were taken to the microbiology laboratory for identification on culture as well as on biochemical tests.

2.4 Transport of specimens

All post operative wound swabs were dipped in Amies transport media and taken to the bacteriology laboratory for culturing on MacConkey agar ,Blood agar, Sabourd's Dextrose Agar (Oxoid, England) and Pseudomonas agar base (Merck, Germany).

2.5. Processing of specimens-Culture and Identification

The wound swab specimens were inoculated on blood agar plate, MacConkey and Pseudomonas agar base and were incubated at 35 ^{o}C for 24-48 hours .In addition to this the swab specimens the wound swab specimens were inoculated on Sabouraud's Dextrose Agar and incubated at room temperature and at 37^{o}C for 7 days to observe the presence of fungus in the clinical specimen. Identification of bacterial isolates were done using colony morphology, Gram-stain and conventional biochemical tests. From the positive culture results of isolates, antimicrobial susceptibility testing was carried out for *Pseudomonas aeruginosa*. Clinically suspected surgical patient was considered infected if the presence of a pathogen was confirmed by culture results (Givovanni *et al*., 1998)

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using agar disc diffusion technique (Bauer *et al*., 196) for P.aeruginosa isolates only. Antibiotic testing was not done on other bacterial isolates in this study since the main focus of the study was on the susceptibility pattern of *Pseudomonas aeruginosa*.

When a pure culture was obtained a loop full of bacteria was taken from a colony and was transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension was adjusted to the optical density of McFarland 0.5 tube (0.14 – 0.15nm) measured at 500 nm absorbance using photometer in order to standardize the inoculum size. The standardized inoculums i.e. 10^4 CFU/ml of each isolate was swabbed on to Mueller-Hinton antibiotic sensitivity Medium. Discs containing appropriate concentrations were placed on the surface of the agar after drying the plates. The plates were incubated aerobically at 37^0C for 24 hours. The following antibiotic discs and concentrations were used. Ciprofloxacin 5µg, imipenem10µg, Trimethoprim-sulfamethoxazole 1.25/23.75µg ,Gentamicin10µg, Chloroamphenicol 30µg Ceftazidine 30µg, Piperacillin 100µg , Amikacin 30µg , Ceftriaxone 30µg , Tetracycline 30µg & Aztreonam 30µg (Oxoid, England) were used.

Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a metal caliper and classified as sensitive, intermediate, and resistant according to the standardized table supplied by the manufacturers and National Committee for Clinical Laboratory Standards (NCCLS, 2005).

Pseudomonas aeruginosa isolate was considered multidrug-resistant (MDR) when it was resistant to at least three of the agents: Amikacin, Azeotronam, Ceftriaxone, Ceftazidime, Chloroamphenicol, Ciprofloxacin, Gentamicin, Imipenem, Piperacillin, Tetracycline and Trimethoprim-Sulfamethoxazole (Elisabeth *et al*., 2004).

2.7 Standardized Control Reference Strains

All plates and disks were stored at 4^0C and the plates were incubated at 35^0C for 48 hours before use to assure sterility. *The* standard reference strains *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were tested weekly as control throughout the laboratory procedures on the biochemical tests.

2.8 Stastical analysis

The socio-demographic data, clinical data and microbiological data were obtained from the patient's chart, the patient and microbiology report which were collectively documented for each patient on a questionnaire (appendix-1). The information retrieved from these data was used to analyze the rate of surgical site infection, the rate of *Pseudomonas aeruginosa*

infection in surgical site and risk factors associated with surgical site infections. Results were analyzed using Stastical Package for Social Sciences (SPSS) version 14.0 and all p-values <0.05 were considered significant.

2.9. Ethical clearance

The MSc research project was approved by the Department of Microbiology, Immunology and Parasitology, and Faculty Research Publications Committee, and it was also endorsed by the Facultiy Academic Commission. Written informed consent was obtained from operated patients who developed infection prior to sampling by surgical ward nurses and the principal investigator (Appendix 2). Samples were taken during morning dressing and patients were not subjected for unnecessary sample collection unless it was warranted for the benefit of the patient.

CHAPTER 3: RESULTS

3.1. Study population

The data collected in this study consisted of 173 surgical patients admitted to Tikur Anbessa Hospital after being operated in operation room and hospitalized up to 30 days during the four months study period.

Table 3.1. Age and sex distribution of 173 operated patients at Tikur Anbessa hospital, A.A, Ethiopia, 2006.

Age in Years	Females (n)	Males (n)	Total
<5	11	13	24
6-14	17	17	34
15-29	12	17	29
30-44	13	12	25

45-60	18	18	36
>60	5	20	25
Total	76 (44%)	97 (56%)	173

The mean age of the patient was 37.3 years (range: 1- 80 years). There were 97 (56%) male patients and 76 (44%) female patients, with male to female ratio of 1.28:1.

3.2. Rates of Post-operative wound infections

Table 3.2. Post-operative wound infection rate by age and sex: .

Age in Years	Females (n) Infected %)	Males (n) Infected (%)	Total Infected (%)
<5	11	13	24
	4 (36.4)	1 (7.7)	5 (20.8)
6-14	17	17	34
	4 (23.5)	6(35.3)	10(29.4)
15-29	12	17	29
	5 (41.7)	3(17.6)	8 (27.6)
30-44	13	12	25
	1 (7.6)	3 (25)	4 (16)
45-60	18	18	36
	1 (5.6)	2 (11.1)	3 (8.3)
>60	5	20	25

	0 (0)	1 (5)	1 (4)
Total	76 (44)	97 (56)	173
Total infected	15 (19.7)	16 (16.4)	31(17.9)

Of the 173 patients studied who had undergone surgery, 31(17.9%) suffered from surgical site infections. There was significant difference in age distribution on comparing infected patients (n=31) with non-infected patients (n=142) (p= O.O2). However there was no significant difference in the frequency of infections by sex (p= 0.11).

The age groups were categorised into six: <5, 6-14, 15-29, 30-44, 45-60 and 60 and above. The result showed that the SSI was higher in the age group of 6-14 and 15-29 (57%) than in the age group of 30-60 years (35.9 %)

Table 3.3. Post – operative wound infection rate by class of wounds

Wound class	Frequency (%)	Infected (%)
Clean	29 (16.7 %)	3 (10.3%)
Clean- Contaminated	81 (46.8%)	11 (13.6%)
Contaminated	44 (25.4%)	10 (22.7%)
Dirty	19 (11%)	7 (36.8%)
Total	173	31 (17.9%)

29 (16.7%) of the wounds were classified as clean, 81 (46.8%) as clean-contaminated, 44 (25.4%) as contaminated and 19 (11%) as dirty. The infection rate in each wound category was 10.3 %(3), 13.6 %(11), 22.7 %(10) and 36.8 %(7) for clean, clean-contaminated, contaminated and dirty respectively.

Table 3.4 Univariate analysis of potential predictors of wound infection

Variables	Frequency	Infection rate	Relative R P Value
Mode of operation			
Elective	115	18.3%	R=1.1
Emergency	58	17.3%	P=0.009
Antibiotic prophylaxis			
Yes	80	20%	R=1.2
No	93	16%	P=1.49

Analysis of the association of potential risk factors for individuals with wound infection revealed that patients who were operated as emergency had 1.1 times risk with Chi^2 of 6.86 and p=0.009 at 95 % confidence interval. Patients who didn't take antibiotic prophylaxis are 1.2 times risk of developing surgical site infections but this does not attain stastical significance at 95 % confidence interval.

3.3. Pathogens isolated from post-operative wound infections

Table: 3.5. Distribution of pathogens isolated from post-operative wound infections at Tikur Anbessa hospital, A.A, Ethiopia, 2006.

Name of Isolates	Frequency n (%)
Staphylococcus aureus	14(25.5)
Coagulase negative Staphylococci	11(20)
Pseudomonas aeruginosa	8(14.4)
Eschercia coli	*7(12.7)*
Klebsiella species	*4(7.3)*
Enterobacter species	*2(3.6)*
Citrobacter species	*2(3.6)*
Enterococcus species	*2(3.6)*
Proteus species	*2(3.6)*
Streptococcus species	*1(1.8)*
*Pseudomonas species**	2(3.6)
Fungal isolates	0(0)
Othes	0(0)
Total Isolates	55(100)

*Pseudomonas species except *Pseudomonas aeruginosa*.

The proportion of gram positive and gram-negative organisms identified was almost equal. The gram-positive accounted for 51% of the isolates while gram negative accounted for 49%.The most predominant organism identified was *Staphyloccocus aureus* as it constituted 25.5% of all the isolates followed by Coagulase negative *Staphylococci* which are 20%. *Pseudomonas aeruginosa* accounted for 14.4% of all the isolates. *Pseudomonas aeruginosa* was the third dominant isolate.

Table.3.6. Frequency of isolates recovered from different wards at Tikur Anbessa Hospital, A.A. Ethiopia.

Microorganisms Identified	*Name of the Ward*					
	Orthopedics ward	Surgical emergency	Labor ward	Obstetrics &Gynecology	Pediatrics	Total
S. aureus	*4*	3	1	3	3	14
Coaguase-ve Staphylococci	2	4	0	2	3	11
P. aeruginosa	4	1	0	3	0	8
Escherchia coli	3	2	0	0	2	7
Klebsiella sp	*1*	*2*	*0*	*0*	*1*	*4*
Enterobacter sp	1	0	0	0	1	2
Citrobacter sp	1	0	0	1	0	2
Proteus sp	0	2	0	0	0	2
Pseudomonas sp	0	2	0	0	0	2
Enterococcus sp	*0*	*1*	*0*	*1*	*0*	*2*
Streptococcus sp	0	1	0	0	0	1
Total	*16*	*18*	*1*	*10*	*10*	*55*

From the above table, maximum proportions of the isolates were recovered from surgical emergency followed by orthopedics ward. The highest number of *S.aureus* isolates were from orthopedics ward. *Coaguase negative Staphylococci* isolates were recovered with high rate from surgical emergency ward whereas the highest number of *P.aeruginosa* isolates were recovered from the orthopedics ward.

Table 3.7. Distribution of pathogens isolated from post-operative wound infections by wound category.

Wound class	Pathogens isolated
Clean	*S. aereus, Cons*, Pseudomonas species*
Clean- Contaminated	*Citrobacter species,Cons*,E.coli,Proteus species,P.aeruginosa*
	S. aereus,Enterococcus species
Contaminated	*Klebsiela species, S. aereus, P.aeruginosa, E.coli, Cons*,*
Dirty	*P.aeruginosa, S. aereus, Cons*, E.coli , Proteus species*
	Streptococcus species, Klebsiela species

Cons*=Coaglase positive Staphylococcus

From table 3.7, it can be demonstrated that, the predominant isolates in this study, *are found in wound classes of* Clean- Contaminated, Contaminated and Dirty. *S. aereus* was recovered from all the wound classes whereas; *P.aeruginosa* is isolated from Clean- Contaminated, Contaminated and Dirty. Different isolates of the surgical site infection can be recovered irrespective of the wound classes. The pathogens isolated from infections differ, primarily depending on the type of surgical procedure. In clean surgical procedures, in which the gastrointestinal, gynecologic, and respiratory tracts have not been entered, *Staphylococcus aureus* from the exogenous environment or the patient's skin flora is the usual cause of infection. In other categories of surgical procedures, including clean-contaminated, contaminated, and dirty, the polymicrobial aerobic flora closely resembling the normal

endogenous microflora of the surgically resected organ are the most frequently isolated pathogens (Brook and Frazier 1990).

***Table 3.8 P. aeruginosa* infection rate by age group, Sex, Wound category and Antibiotic prophylaxis.**

Variables	Frequency (n)	***P. aeruginosa*** infection rate (%)
Wound category		
Clean	0	0
Clean - contaminated	1	12.5
Contaminated	3	37.5
Dirty infected	4	50
Sex of patients		
Male	5	62.5
Female	3	37.5
Antibiotic prophylaxis		
Yes	6	75
No	2	2
Age group		
<5	0	0
6 -14	5	62.5
15 - 29	2	25
30 - 44	1	12.5

Mode of operation		
Emergency	4	50
Scheduled	4	50

Considering wound category, the number of *P. aeruginosa i*solates recovered in dirty and contaminated is greater than these recovered from clean and clean-contaminated. The rate of *P.aeruginosa* infection is relatively higher in males than in females. The infection rate by *P. aeruginosa* is more in those patients who took antibiotic prophylaxis. In this study, relatively, the infection rate by *P. aeruginosa* is high in the age group of 6-14. Another observation from table 3.8 mode of operation whether it is emergency or scheduled has no effect on the rate of *P. aeruginoa* surgical site infection.

3.4. Antimicrobial susceptibility test

From this study, 6(75%) of the *P.aeruginosa* isolates were sensitive to amikacin and imipenem.5 (62.5%) isolates were sensitive to azeotronam, ceftazidime, gentamicin and piperacilin.

Table 3.9. Antimicrobial susceptibility tests on *P. aeruginosa* isolates from surgical wound infection

Antimicrobial agents	Resistant isolates Number (%)	Susceptible isolates Number (%)
Amikacin	2 (25)	6 (75)
Azeotronam	3 (37.5)	5 (62.5)
Ceftriaxone	4 (50)	4 (50)
Ceftazidime	3 (37.5)	5 (62.5)
Chloroamphenicol	8 (100)	0 (0)
Ciprofloxacine	6 (75)	2 (25)
Gentamicine	3 (37.5)	5 (62.5)
Imipenem	2 (25)	6 (75)
Piperacilin	3 (37.5)	5 (62.5)
Tetracycline	7 (87.5)	1 (12.5)
Trimethoprim-sulfamethoxazole	7 (87.5)	1 (12.5)

In the other hand only 1(12.5%) isolates were sensitive to tetracycline and trimethoprim-sulfamithoxazole. There was no *P. aeruginosa* isolate sensitive to chloroamphenicol as

shown in table 3.9. It can be observed, that most of the isolates resisted to four or more antibiotics tested. 87.5% of the isolates of *Pseudomonas aeruginosa* were found resistant to two or more antimicrobials and defined as multidrug resistant (MDR) isolates.

Table 3.10 Antibiograms of *P.aeruginosa* isolates (n=8) from surgical wound infection.

No of resisted Antibiotics	Types of antibiotics resisted	no. (%)
R-2	CIP, PIP	5(62.5%)
	CIP, CLP	6(75%)
	CIP, TTC	6(75%)
	CIP,SXT	6(75%)
	CLP, TTC	7(87.5%)
	CLP,SXT	7(87.5%)
	CLP,CRO	4 (50%)
	TTC,SXT	7(87.5%)
R-3	CIP,CLP,TTC	6(75%)
	CIP,AZT,SXT	3(37.5%)
	CLP,AZT,SXT	3 (37.5%)
	CLP,SXT,CRO	3 (37.5%)
	CAZ,CLP,TTC	3(37.5%)
	CAZ,SXT,CRO	3(37.5%)
	PIP,CLP,TTC	3(37.5%)
	TTC,SXT,CRO	3(37.5%)
	TTC,AZT,SXT	3(37.5%)
R4	CIP,CLP,TTC,AZT	3(37.5%)
	CIP,TTC,AZT,SXT	3(37.5%)
	CIP,CLP,TTC,SXT	5 (62.5%)
	GM,CAZ,PIP,CLP	3 (37.5%)

	GM,PIP,CLP,TTC	3 (37.5%)
	CLP,TTC,AZT,SXT	3 (37.5%)
	CLP,AZT,SXT,CRO	3 (37.5%)
	CIP,CLP, TTC,SXT	6(75 %)
	PIP,CLP,TTC,SXT	3(37.5%)
R5	CIP,CAZ,CLP,TTC,AZT	2 (25%)
	CIP,CLP,TTC,AZT,SXT	3(37.5%
	GM,CAZ,PIP,CLP,TTC	2(25%)
	CAZ,CLP,TTC,AZT,SXT	2(25%)
	CLP,TTC,AZT,SXT,CRO	2(25%)
	GM,PIP,CLP,TTC,SXT	3(37.5%)
R6	CIP,CAZ,CLP,TTC,AZT,SXT	2(25%)
	CIP,CLP,TTC,AZT,SXT,CRO	2(25%)
	CIP,CLP,TTC,AN ,AZT,SXT	2(25%)
	CIP,GM,PIP,CLP,TTC,SXT,	2(25%)
	CAZ,CLP,TTC,AZT,SXT,CRO	2(25%)
R7	CIP,CAZ,CLP,TTC,AZT,SXT,CRO	2(25%)
	GM,CAZ,PIP,CLP,TTC,SXT,CRO	2(25%)
R8	CIP,GM,CAZ,PIP,CLP,TTC,AN,AZT	1(12.5%)
	CIP,CAZ,PIP,CLP,TTC,AN,AZT ,SXT	1(12.5%)
R9	CIP,GM,CAZ,PIP,CLP,TTC,AN,AZT,SXT	1(12.5%)
	CIP,CAZ,PIP,CLP,TTC,AN,AZT,SXT ,CRO	1(12.5%)
R10	CIP,GM,CAZ,PIP,CLP,TTC,AN,AZT,SXT,CRO	1(12.5%)

R=Resistance to CIP-Ciprofloxacin, Gm-Gentamicin,CAZ-Ceftazidim,PIP-Piperacillin,CLP-Chloramphenicol,TTC-Tetracycline,AN-Amikacin,IMP-Imipenem,AZT-Aztreonam,SXT-Trimethoprim sulfamethoxazole,CRO-Ceftriaxone.

Table 3.10 shows antibiogram of *P.aeruginosa*. Among phenotypic methods currently used for hospital epidemiology, the antibiogram is still extremely useful as a routine technique to detect hospital infection with antibiotic resistant *P.aeruginosa*. This table shows the resistance pattern of *P.aeruginosa* to two or more antibiotics. Identical antibiograms are observed in most of the isolates.

CHAPTER IV

DISCUSSION

The aims of this study were to determine the rate surgical wound infection in Tikur Anbessa hospital with special emphasis on *Pseudomonas aeruginosa* in postoperative wound infections and to see the antimicrobial susceptibility pattern of this organism. The study has also attempted to determine the incidence of surgical wound infection rate and identify some of the potentially alterable predictors using simple available information.These data not only serve as baseline for further comparisons and monitoring, but lay the ground for wound surveillance and the increasing risk of resistance of *Pseudomonas aeruginosa* to different antibiotics.

In this study, out of the 173 surgical site patients, 31(17.9%) developed surgical site infection. This is in agreement with the findings of Taye (2005) and in study done in Nigeria (Michalopoulos *et al*:, 2003) which were 14.8% and 17.5% respectively. In the study done in India by Lilani *et al* 2005, the surgical site infection rate was as high as 22.41%. In most part of the world depending on the set up of their hospitals and their degree of adherence to aseptic techniques, the surgical site infection rate has varied from a low of 2.5 % to a high of 41.9%. In another study done by Darryl (2006), in the Surgical Site Infection Following Bowel Surgery, the rate of surgical site infection for all wound categories was 8.7%. This finding is not similar with the finding of the present study. In study done in Brazil by Aldo et al 2005, they showed that the over all incidence of surgical site infection was 8.8% in 1994, it decreased to 3.3% in 2003. This difference in surgical site infection rate as they stated is due to the use of education strategies, based on guide lines for surgical site infection incidence. In another study done in Iranian teaching hospital by Seyd *et al* 2005, the surgical site infection rate was 17.4% which was exactly equal to the rates of this study. In this study there is relatively higher rate of surgical site infection (17.4%) compared with the (14%) quoted in the literature (Linda *et al*., 2003). This similar result in developing countries indicates that there are similarities in their hospitals. This indicates the

need for implementing effective methods of hospital infection control system in these countries.

In this study, the extreme age is not associate with high infection rate (4%) for patients above 60 years and (20%) for patients below 5 years. The findings of this study is different from findings of Taye (2005) and Seyd *et al.*, 2005. This is in contrary to the fact that increasing age is correlated with greater likelihood of certain chronic conditions, malnutrition and fall in the body immunological efficiency, causing more extensive surgical site infection (Horan et al., 1992). A study done by Biscione *et al.,* 2007, confirmed that the rate of surgical site infection increased with age. In the present study surgical site infection is not correlated with sex. Although there were differences between the percentages of males and females, these differences were not statistically significant. The finding of this study was similar with the findings of other several studies (Seyd *et a*l., 2005, Biscione *et a*l., 2007 , Lilanil *et al.,* 2005).

It is also clear from this study that wound infection was significantly associated with class of wounds, with the highest rate being 36.8% for infected or dirty wound. It was found to be 10.3%, 13.6% and 22.7% for clean, clean - contaminated and contaminated respectively. Even though the dirty wound infection is comparable to the acceptable standard (i.e. 28 - 40%) in developed countries (Alicia *et al*., 1999), the rate of infections for the clean wound is about 10 times more than the acceptable standard (1.5% - 3.5%). The same finding was observed in a study by Taye (2005) and Shojae et *al*., 2006. These figures are greater than comparable data from developed countries reported by NNIS (2.1%, 3.3%, 6.4% and 7.1% respectively (Garner, 1993). As it is clearly stated in many literatures, the infection rate increases with classes of wounds, the highest rate being in dirty or infected wounds. The findings of this study also demonstrated this. The over all clean wound infection rate of 10.3% in this study was not comparable with the expected standard for clean wound and clean wound infection rate of studies done in Iran by Shojae (2005) which was 4.9%, and Taye (2005) which was 8%. But it was similar with the infection rate of the study done by Seyd et al 2007. The infection rate of clean contaminated wound n this study (13.6%) is comparable with the studies done by Taye (2005) and Seyd *et al*.,2007 which was 14.8% and 13.6% respectively. The findings of this study was not comparable with the rate of

clean-contaminated wound infection of the study done by Aldo et al., 2005 of Brazil which was 5.2% and Lilani *et al.*, 2007 of India which was much higher than this study (22.4%). Considering the contaminated wound class, the rate of infection obtained by this study (22.7%) is similar with the study done by Taye, (2005), which were 22%. The contaminated wound infection rates obtained by Seyd *et al.,* 2007 (45.8%) was higher than this study and the result obtained by Aldo *et al.,* 2005 which was only 11.2% was much lower than the present study. In the case of dirty or infected wound, the infection rate significantly increased (36.8%) in this study. This finding is similar with studies done by Biscione *et al.,* 2007 which was 30% and is comparable with the study done by Taye in 2005, which was 44.1%. In a study done in Gonder by Kotisso and Abraham in 1998 the infection rate for dirty wound was 61.4% which was significantly higher than the present study.

In the present study, analysis of the association of potential risk factors for individuals with wound infection revealed that patients who were operated as emergency had 1.1 times risk with Chi^2 of 6.86 and p=0.009 at 95 % confidence interval. The finding was similar with the study done by Taye in 2005, where patients who were operated as emergency had 1.6 times risk of developing surgical site wound infection. Similar results were obtained by Seyd *et al.,* 2007 and Biscione *et a*l., 2007. But the study done in Gonder by Kotisso and Abraham in 1998 was in contrast with the the present study where they demonstrated that operation procedures of either emergency or elective has no effect on the out come of wound infection. The findings of the present study also proved the risk of surgical site infection to be less in elective surgeries than those referred to emergency as cases of acute abdomen, which could result from lack of readiness for operation on the patients' side. We should reduce risk factors by preparing the patient for the urgent operation as much as possible.

In this study, patients who didn't take antibiotic prophylaxis are 1.2 times risk of developing surgical site infections but this does not attain statistical significance at 95 % confidence interval. This was in contrast with the study done by Taye in 2005, where patients who received preoperative antibiotics have a statistically significant higher wound infection rate ($p<0.001$). Another study done by Darryl in 2006 demonstrated that antibiotic has no effect on the out come of wound infection. But in a study done by Gupta *et al.,* 2000, antibiotic prophylaxis has been used to good effect in the prevention of post-operative wound

infections in patients undergoing gastrointestinal operations. In this study three hundred and thirty-four patients were recruited. Of the 164 receiving antibiotic prophylaxis 29 (17.7%) had wound infections compared with 32 (18.8%) in the placebo group (P=0.79). The findings of this study supported the literature by showing that administration of prophylactic antibiotic before the operation would bring about best results and the lowest surgical site infection.

In this study *Staphylococcus aureus* was the most dominant insolate. The predominance of *S. aureus* although supported by several workers (Kotisso and Abraham, 1998, Michalopoulos *et al.*, 2003, Linda et al., 2003), it is in contrast to the observations of numerous others (Oyuntibeju and Nwobu, 2004, Givovanni *et al*.,1998) where *P.aerusinosa* was most prevalent. Results of previous studies which are also confirmed in this study have shown that *P.aeruginosa* and *S.aureus* are the two most common isolated species in post operative wound infection, although gram negative bacilli are generally thought to prevail in surgical site infections (Habte et al., 1988). This predominance of *P. aeruginosa* is attributed to its resistance to different antibiotics and disinfectants in the hospital environment (Loughlin *et al*., 2002). *Enterococcus spicie*s and *Streptococcus species* is in agreement with some of the previous studies (Alicia *et al*., 1999, Hanberger *et al*., 2000). Among the gram-negative bacilli (other than *P.aeruginosa*), *E. coli* and *Klebsiella species* are the next important pathogens in this study. This in agreement within the work of Emine (2005) but in contrast to the study of Hanberger *et al* 2001 and Filiz *et al* 1999 where *Acinetobacter* species, *Klebsilla species* and *Enterobacter species* were dominant than *P. aeruginosa*. There was no fungal pathogen isolated in this study. This is in contrast with studies done by Schaberg et al 1991 and Alicia *et al* 1999.This is partly due to small sample size and biopsies and histological examination of surgical site wound infection being the most suitable technique for the diagnosis of fungal infection.

Concerning antimicrobial susceptibility pattern of Pseudomonas aeruginosa, although subject to frequent modifications, its assessment is important for clinical and epidemiological purposes. This is because the resistance of *Pseudomonas aeruginosa* to antimicrobials poses a challenge to post –operative wound treatment since it decreases the

effectiveness of treatment and increases morbidity, mortality and cost of care (Linda *et al*., 2003).

The overall resistance rates to all antimicrobial agents in this research was significant, as has been reported by others (Friendland *et al*.,2004, Mohamed.,2000, Shojae, 2006). The antimicrobial resistance of *P. aeruginosa* has been reported to be increasing in several studies (Friendland *et al.*, 2004, Mohamed, 2000, Spenur, 2002). In Europe, significant decline in susceptibility rates to β lactams, amino glycosides and quinolones was recently observed in this pathogen and nosocomial outbreaks of multi drug resistant (MDR) *P. aeruginosa* have been described in various European hospitals (Hanberger 2001, Spenur,2002). In a study done in Iran by Shojae.H (2006), the rate of multi drug resistant P. aeruginosa was 73.9%. This finding of multi drug resistant P. aeruginosa was similar to the finding of this paper but in contrast to what was found in a study done in United Kingdom and USA (Spenur, 2002, Flamm *et al*., 2004).

Combination therapy appears to prevent the emergence of multi drug resistant *P. aeruginosa.* This is because the mechanism of resistance to β-lactams, Quinolones and aminoglycosides are different so the emergence of resistance to two or three drugs should theoretically be low (Nordman and Poirel, 2002).

In this research high resistance rates to all the antimicrobials was found. The highest resistance was obtained for chloramphenicol which was 8(100%) followed by tetracycline and trimethoprim sulfamethoxazole which were 7(87.5%). This finding was in agreement with the study done in Nigeria by Oyuntigeju and Nwobu (2004). This is because in Ethiopia and Nigeria like any other developing countries, antibiotics such as tetracycline and Chloramphenicol are commonly prescribed although they are not first line single drug therapy for *P. acruginosa* infections. Of all the antibiotics tested in this study amikacin and imipenem were less resisted. Results of previous studies also confirmed this. But a study done in Turkey by Emine (2005) was in contrast to this study, where high resistance rate was observed to amikacin. Similar study done in Iran by Shojae (2006) found a high resistance rate to imipenem. Even though Ciprofloxacin was found to be the effective antipseudomonal agents in various studies (Chen *et al*., 1995, Elisabeth *et al*., 2004,

Giovanni et al., 1998), there is a high resistance rate in this study and study done in Turkey by Emine (2005). Ceftriazone also has high resistance rate in the present study and is in agreement with other studies done in Iran by Shojae (2006) and In Turkey by Emine (2005).

Rate of *P. aeruginosa* isolates resistance to azeotronam, ceftazidime, gentamicin and piperacilin is moderate in this research i.e. 37.5%. A similar trend was observed in various studies to gentamicin (Shojae, 2006, Emine 2005, Chen *et al.*, 1995). Even though ceftazidime was effective drug with less resistance pattern in studies done in developed countries (Chen *et al*., 1995, Elisabeth *et a*l., 2004 and Giovanni *et al*., 1998), high resistance rates were reflected in studies done in Iran by Shojae (2006) and Turkey by Emine (2005). For the antipseudomonal Penicillin; Piperacillin, the resistance rate were 65.5% for the study done in Turkey by Emine (2005) and 55.9% for the study done in Paris by Elisabeth et al (2004) and 12% in study done in Italy by Giovani *et a*l (1998). These three results are not in agreement with findings of this study. The most ideal antipseudomonal antibiotics recommended to developing countries like Ethiopia are Amipicillin, Imipeneum ceftazidime, Gentimicine and Piperacillin (Shojae, 2006, Emine 2005, Mohamed, 2000) .

Most of these drugs are used for first line single drug therapy and are relatively found effective in other developed countries. The outcome of this study emphasizes the use of first line drug therapy rather than random prescription of antibiotics with out susceptibility testing. This will aggravate the ever increasing resistance not only to *Pseudomonas aeruginosa* but also to other microorganisms. Resistance to Ciprofloxacin,Ceftriaxone and Ceftazidime which were the drugs of choice some years before they may present serious therapeutic problems with in the near future as the problem may be aggravated by different factors. Policies governing the use of antimicrobials in many institutions are lacking. Such policies must be implemented in order to limit the spread of resistance and also reduce the emergence of resistance to newly commissioned drugs.

Limitations of the study

The sample size used in this study is smaller than many of the studies done on post operative wound infections, as the duration of the study was also shorter than many. This might have

an impact on some of the results reported. Yet, there are studies including the present study which have used smaller number of patients than this study and demonstrated similar findings with those studies with larger sample size (Kotisso and Abraham 1998; Seyd *et al.,* 2005 and Lilani *et a.,l* 2005).

Another limitation of this study is that none of the specimens were cultured anaerobically and hence no comment can be made as to the rate of anaerobic surgical site infection in this study. However, as anaerobes account for a very small rate of infection in surgical site wounds, for the very nature of open wounds like surgical site wounds not allowing good growth of anaerobes (Brook and Frazier 1990). As a result, the absence of anaerobic culturing in this study may not have a detrimental effect on the overall pattern of bacterial isolates in post operative wound infection.

Conclusion and recommendation

This study has shown that the surgical site wound infection rate in this teaching and referral (tertiary level) hospital is high and control measure should be re-evaluated. The SSI incidence varies with the definition of wound infection, the intensity of surveillance, and the prevalence of risk factors for SSI in the patient group. *Pseudomonas aeruginosa* is the third predominant organism as colonizing agent at the surgical site next to S*taphylococcus aureus and Coagulase negative Staphylococci* in this study. *Pseudomonas aeruginosa* isolates were also found to be highly resistant to commonly used antibiotics in the setup of Tikur Anbessa hospital surgical unit. They are also found to be resistant to the most effective antipseudomonal agents commissioned and used world wide .The high rate of multidrug resistant *Pseudomonas aeruginosa i*solates found from this surgical unit of the hospital addresses that a proper use of effective disinfectants and antiseptics as well as strict adherence to the CDC recommended surgical site infection preventing guidelines is the best remedy for this deep-rooted problem. It can also concluded that the occurrence and rate of resistance to antipseudomonal agents among post-operative wound isolates of *P.aeruginosa* is increasing at considerable rate. The findings of this research support major rule for antibiotic with specific antipseudomonal activity in emergency of multi drug resistance *Pseudomonas aeruginosa*. The use and duration of treatment with these antibiotics should be

restricted as part of efforts to control the emergence of multidrug resistance *Pseudomonas aeruginosa* in post-operative wound infections. It can also be recommended that hospital infection control system as well as wound surveillance program has to be established to reduce the surgical wound infection rate to acceptable standard.

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APPENDIX- I

Addis Ababa University Medical Faculity Deprtment of Microbiology, Immunology and Parasitology

Questionnaire for investigation of Surgical wound infection in Tikur Anbessa Hospital with Special Emphasis on *Pseudomonas aeruginosa***.**

I. Patient Identification

1. Patient full name_____ Age_____ Sex____
2. Address_____ _____ Card number_____
3. Name of the ward _____Block_____
4. Reason for admission for the in-patient_____
5. Date of operation _____
6. Date of infection developed _____

7. Site of infection _____

8. Type of surgical procedure 1.Emergency_____ 2.Scheduled_____

9. Is antibiotic prophylaxis given? Yes___ No_____

II. Clinical Profile

	YES	NO
1. Fever (Temperature>38)	☐	☐
2. Redness (inflammation)	☐	☐
3. Swelling at incision site	☐	☐
4. Wound discharge	☐	☐
Type of discharge: Pus/exudates/serous/bloody/necrotic		
5. Gangrenous lesion	☐	☐

6. Surgical site wound category

Clean_____ Clean-contaminated_____ Contaminated_____Dirty and infected__

III. Laboratory Data

1. Date of specimen collection_____
2. Type of specimen: Pus aspirates /wound swab/exudates
3. Pus/exudates consistency: Clear_____ Yellowish_____ Greenish _____
   Bloody_____ Others: Specify_____

4. Culture and biochemical test identification

_____

_____

_____

5. Antimicrobial susceptibility testing

	S (mm)	I (mm)	R(mm)
• Ciprofloxacin	-------	--------	-------
• Gentamicin	-----	-----	-------
• Ceftazidime	------	------	---------

- Piperacillin ------ ------ ---------

- Chloramphenicol ------ ------- ----------
- Tetracycline ------- -------- ----------
- Amikacin ------- ------- ----------
- Imipenem ------- -------- ----------
- Aztreonam ------ ------- ---------
- TMP-SMX ------- -------- --------
- Ceftiriazone _____ _____ ---------

IV. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Appendix-II

CONSENT FORM

Serial no_…… Card no…..…….

Ward……..…… Date of admission……..

Reason of admission…….……..

I had been informed that the objectives of this study are to assess the prevalence and pattern of antimicrobial resistance of *Pseudomonas aeruginosa in post operative wound infection* .The results of this study have importance to treat me and other patients and to use as a guide for the control of this bacterium. I had been also informed about the confidentiality of the questionnaire. The principal investigator requested me to participate in the study that would require my willingness to respond to an interview, physical examination and to provide wound samples. Therefore, with full understanding of the

importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____here by give my consent for providing the requested information and specimens as the doctors find best for me.

Signature:_____ Date_____

<u>የስምምነት መግለጫ</u>

(ትርጉም በአማርኛ)

ተራ ቁጥር_____ ከርድ ቁጥር_____

የመታከሚያ ክፍል_____

በሽተኛው የተኛበት ቀን_____

በሽተኛው የተኛበት ምክንያት_____

የዚህ ጥናት ዋና ዓላማ ሆስፒታል ውስጥ ሲከሰቱ በሚችሉ በሽታ አምፈጪ ታህዋስያንና ስፀረ ታህዋስያን መድሃኒት የመቋቋም ባህርያቸው ስማጥናት መሆኑ፤ የጥናቱ ውጠ.ቱ ደግሞ ስኔና ስሌሎች ታከሚዎች ህክምና ጥቅም እንዲሁም ሆስፒታል ወስድ በሽታዎች ስመቆጣጠር እንደ አመላከች ሆኖ እንደሚያገለግል ተፈድቻለሁ።።

በተጨማሪም በጥናቱ ውስጥ ገብቼ የቃል መረጃ በመስጠት ፣ የአከል ምርመራ በማድረግ፣ እንዲሁም የቀዶ ጥገና ህክምና በተደረገበት የአከሌ ክፍል ኢንፌክሽን ከፈጠረ ከዚሁ ክፍል የሚወጣ ፈሳሽ ናሙና እንድሰጥ በአጥኚው ባለሙያ ፈቃደኝነቴን ተጠይቄያለሁ፡፡

በመሆኑም የጥናቱ ዓላማና ጥቅም በሚገባ ስለተገነዘብኩ ፤ ከጥናቱ የማገኘው ጥቅምም ነፃ የላቦራቶሪ ምርመራ ብቻ መሆኑንም ጭምር አውቄ ከላይ የተጠቀሱ ስጥናቱ የሚያስፈልጉትን ሁሉ ስመስጠት በሙሉ ፈቃደኝነት መስማማቴን በፈርማዬ ረጋግጣሉሁ፡፡

ስም_____

ቀን_____

ፈርማ _____