ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

AEROBIC BACTERIA IN POST-SURGICAL WOUND INFECTION AND PATTERN OF THEIR ANTIBIOTIC SUSCEPTIBILITY IN HAWASSA TEACHING AND REFERRAL HOSPITAL, SOUTHERN ETHIOPIA

BY

LOPISO DESSALEGN TIRORE

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY

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ADVISOR: SOLOMON GEBRE-SELASSIE (MD, M.Sc)

CO ADVISORS: ATO TECHALEW SHIMELIS (B.Sc, M.Sc)

ATO ENDALE TADESSE (B.Sc, M.Sc)

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LIST OF ACRONYMS

- ATCC - American type culture collection
- $BaCl_2.2H_2O$ - Dihydrate barium chloride
- $BaSO_4$ - Barium sulphate
- CFU - Colony forming unit
- EDTA - Ethylenediaminetetra-Acetic Acid
- EHNRI - Ethiopian Health and Nutrition Research Institute
- HAI - Hospital Acquired Infection
- HIV - Human Immunodeficiency Virus
- H_2O_2 - Hydrogen peroxide
- H_2S - Hydrogen sulphide
- LDC - Lysine decarboxylase
- MSA - Mannitol salt agar
- MHA - Mueller–Hinton agar
- NINSS - Nosocomial Infection National Surveillance Service
- NNIS - National Nosocomial Infections Surveillance System
- PMN - Polymorphnuclear leukocytes
- SNNPR - Southern Nations, Nationalities and Peoples' Regional state
- SOPs - Standard Operating Procedures
- SSI - Surgical site infection
- SPSS - Statistical package for social science
- TSI - Triple sugar iron agar
- TMP-SXT (SXT) - Trimethoprim- sulphamethoxazole
- WHO - World Health Organization

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ABSTRACT

Background: Post-operative wound infections have been found to pose a major problem in the field of surgery for a long time. Advances in control of infections have not completely eradicated this problem because of development of drug resistance. This condition is serious in developing countries where irrational prescription of antimicrobial agents is common.

Objective: To determine the distribution of common aerobic bacteria in post-surgical wound infected patients and their antimicrobial susceptibility patterns.

Materials and Methods: This cross sectional study was carried out in a total of 194 patients with post surgical wound infections at Hawassa Teaching and Referral Hospital, from November 2010 to March 2011. Physicians collected data on socio-demography and clinical profiles using designed formats. Moreover, pus swabs were collected, processed and cultured using the standard bacteriological methods. Isolated organisms were tested for pattern of antimicrobial susceptibility using the standard disk diffusion method. The data were entered in to a computer and analyzed using SPSS Version-16 software.

Results: The prevalence of aerobic bacteria was 71.1%, and majority of the isolates (59.3 %) were Gram-negative organisms. The most frequently isolated aerobic bacteria was *S. aureus* (37.3%), followed by *E .coli* (25.4%) and *Klebsiella* species (13.6%). All bacterial isolates were resistant to at least one antibiotic, and 86.4 % were resistant to more than one antibiotic (multiple drug resistance).

Conclusion: Single and multiple drug resistance to the commonly used antibiotics in the study area was found to be very high leaving clinicians with a very few choices of drugs for the treatment of post surgical wound infected patients. Therefore, it is critical that use of antimicrobial agents with in hospitals, public healthcare providers as well as private ones should be reviewed and further studies to find out the overall resistance patterns and their possible causes and associated factors in the region at large need to be carried out.

Key words: Aerobic bacteria, post-surgical wound, antimicrobial, susceptibility.

1. INTRODUCTION

Post-operative wound infections have been found to pose a major problem in the field of surgery for a long time (Anguzu and Olila, 2007). Post-operative wound infection simply means wound infection after surgical operation. It may occur as a primary wound infections following surgical operation from sources in the ward or as a secondary wound infection due to some other complications (Masaadeh and Jaran, 2009). This clinical definition has advantages over culture based results because of several reasons. Firstly, a positive culture does not necessarily indicate infection. Secondly, many wounds are colonized by bacteria, whether infected or not. Thirdly, pathogens may not be necessarily isolated from infected wounds owing to their fastidious nature, or due to the effect of received antimicrobial therapy (Nwachukwu *et al.,* 2009). Most post-operative wound infections are hospital acquired (nosocomial infection) and varies from one hospital to the other (Jonathan *et al.,* 2008). Lack of standardized criteria for diagnosis presents a challenge to monitor the global epidemiology of surgical site infection (SSI). A survey by the World Health Organization (WHO) showed a global prevalence of nosocomial infections varying from 3-21%, with wound infections accounting for 5-34% of the total (Mayor-White *et al.,* 1988). SSIs are associated not only with increased morbidity but also with mortality. Seventy-seven percent (77%) of the deaths of surgical patients were related to surgical wound infection (Mangram *et al.,* 1999). Kirkland *et al* calculated a relative risk of death of 2.2 attributable to SSIs, compared to matched surgical patients without infection (Kirkland *et al.,* 1999).

The risk of developing a surgical wound infection is largely related to both bacterial and host factors (Nwachukwu *et al.,* 2009). The inoculum size, virulence and invasive capability of the organisms have been reported to influence the risk of infection. Moreover, the physiological state of the tissue in the wound and immunological integrity of the host seem to be of equal importance in determining occurrence of infection (Masaadeh and Jaran, 2009; Ranjan *et al.,* 2010).

Advances in control of infections have not completely eradicated the problem of post-operative wound infections because of development of drug resistance. The condition is serious particularly in developing countries owing to irrational prescription of antimicrobial agents, without the knowledge of specific drugs that are effective against the incumbent organisms (Andhoga *et al*., 2002). Emergence and rapid spread of resistant microbes presents

a challenge to the public, researchers, clinicians and drug companies looking for effective drugs. Measures to control this problem include development of new antimicrobial, better infection control program and more appropriate use of existing antimicrobial agents (WHO, 1996; Hart and Kariuki, 1998).

In Ethiopia, different studies reported that the prevalence of post surgical wound infection ranges from 14.8% - 60% (Taye, 2005; Endalafer *et al.*, 2011; Biadglegne *et al.*, 2009; Mulu *et al.*, 2006 and Tesfahunegn *et al.,* 2009). Bacteria including *S. aureus*, *Kelbsiella* species, *E. coli*, *Proteus* species, *Streptococcus* species, E*nterobacter* species, *Pseudomonas* species and *Coagulase negative Staphylococci* were the most common pathogens isolated from wound (Biadglegn *et al.*, 2009; Mulu *et al.,* 2006).

However, data on antimicrobial susceptibility pattern among bacterial isolates from post-surgical wound infections is limited in the study area. It would be crucial to describe epidemiology of bacterial pathogens and their drug susceptibility patterns in different localities to decide on appropriate treatment regimen. Therefore, this study aimed to determine the distribution of common aerobic bacteria in patients with post-surgical wound infections and their antimicrobial susceptibility patterns in Hawassa Teaching and Referral Hospital.

2. LITERATURE REVIEW

2.1. Definition and Etiologic agents of post-surgical wound infections

Surgical wound infections are those infections which are confined to the incisional wounds and involving structures adjacent to the wounds that were exposed during operation (Patherick and Dalton, 2006). They are divided into the following categories upon assessment at 30 days post operative surgery.

A. Superficial and incisional surgical wound Infection; these involve only the skin and subcutaneous tissue of the incision, with visible signs of inflammation.
B. Deep Incisional Infections; These are also defined at 30 days post operative surgery or at one year, if organ implant is involved. It involves infection present in the deep soft tissues of the incision.
C. Organ space Infections; these involve any part of the anatomy other than the incision itself (Howard and Lee, 1995).

A normal intact skin prevents microbial populations in general and potential pathogens in particular from colonizing and invading underlying tissue (Bowler *et al.*, 2001). But a loss of integrity by wounding provides a moist, warm, and nutritious environment for microbial colonization and proliferation which may be facilitated further by the presence of foreign materials and devitalized tissue in a traumatic wound (Bowler *et al.*, 2001). Wound contaminating organisms may originate from the environment, the surrounding skin and endogenous sources including primarily the gastrointestinal, oropharyngeal, and genitourinary mucosa (Lee *et al.,* 1997; Bowler *et al.*, 2001). Most surgical site infections (SSIs) have got the chance of being contaminated by the patient's own endogenous normal microbial flora, which are present on the skin, mucous membranes, or hollow viscera. The traditional microbial concentration quoted as being highly associated with SSIs is that of bacterial counts higher than 10,000 organisms per gram of tissue or in the case of burned sites, organisms per cm^2 of wound (Krizek and Robson, 1975). The usual pathogens on skin and mucosal surfaces are gram-positive cocci (notably staphylococci); however, gram-negative aerobic and anaerobic bacteria contaminate skin in the groin/perineal areas. The contaminating pathogens in gastrointestinal surgery are the multitude of intrinsic bowel flora, which include gram-negative bacilli (e.g., *Escherichia coli*) and gram-positive microbes, including enterococci and anaerobic organisms (NNIS, 1996). Gram-positive organisms, particularly staphylococci and streptococci, account for most exogenous flora involved in SSIs. Sources of such pathogens include surgical/hospital personnel and intraoperative circumstances, including surgical

instruments, articles brought into the operative field, and the operating room air. The most common group of bacteria responsible for SSIs are *Staphylococcus aureus* (Krizek and Robson, 1975). By far studies in Ethiopia and other parts of the world, *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Escherichia coli, Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus faecalis, Candida albicans* and *Candida tropicalis* have been implicated as etiological agents of wound infection.(Andhoga *et al.,* 2002; Jonathan *et al.,* 2008; Biadgelegne *et al.,* 2009 and Anguzu and Olila, 2007).

2.2. Epidemiology

Internationally, the frequency of SSI is difficult to monitor because criteria for diagnosis might not be standardized. A survey sponsored by the World Health Organization demonstrated a prevalence of nosocomial infections varying from 3-21%, with wound infections accounting for 5-34% of the total (Mayor-White *et al.,* 1988). A study which was conducted by Giacometti *et al.,* 2000 showed that out of 676 surgery patients with signs and symptoms indicative of wound infections, bacterial pathogens were isolated from 614 individuals with isolation rate of 90.8%. A single etiologic agent was identified in 271 patients, multiple agents were found in 343, and no agent was identified in 62. A high preponderance of aerobic bacteria was observed. Among the common pathogens were *Staphylococcus aureus* (191 patients, 28.2%), *Pseudomonas aeruginosa* (170 patients, 25.2%), *Escherichia coli* (53 patients, 7.8%), *Staphylococcus epidermidis* (48 patients, 7.1%), and *Enterococcus faecalis* (38 patients) (Giacometti *et al.,* 2000). Other studies also show that from a total of 45 patients with surgical wounds were enrolled for this study. Out of 45 patients studied 31(68.88%) were males and 14(31.11%) were females and the prevalence of the organisms present in the wound specimens was studied. Out of 45 surgical wound specimens examined, *Staphylococcus aureus* was isolated from 33 (42.30%), *Pseudomonas aeruginosa* from 25 (32.90%) *Proteus mirabilis* and *Escherichia coli* from 10 (12.80%) (Nwachukwu *et al.,* 2009). Study which was done by Mulu *et al.,* 2006 showed that Bacterial pathogens were isolated from 79 patients showing an isolation rate of 52%. *Staphylococcus aureus* was the predominant species 65% (51/79) followed by *Escherichia coli*, 8/79 (10%), *Klebsiella pneumoniae* 9% (7/79), *Proteus* species 4% (3/79) and *Streptococci* species 4% (3/7) (Mulu *et al.* 2006) and also Study which was done in Tikur Anbessa hospital also showed that a prospective study of surgical wound infection has been conducted on 1754 surgical patients operated from January 1, 1999 to Dec 31, 1999. Among the patients 1162 (66.2%) were males and 592 (33.8%) were females. Seven

hundred twenty eight (41.5%) wounds were classified as clean, 674 (38.4%) as clean-contaminated, 241 (13.7%) as contaminated and 111 (6.3%) as dirty and infected wounds. The overall wound infection rate was 14.8% (Taye, 2005). But Collated data on the incidence of wound infections probably underestimate true incidence because most wound infections occur when the patient is discharged, and these infections may be treated in the community without hospital notification (Mayon-White *et al.,* 1988).

2.3. Risk factors

Anyone who has got surgical operation can develop a wound or infection (Hunt and Hopt, 1997). The potential for the development of wound infection depends on a number of patient variables such as the state of hydration, nutrition and existing medical conditions as well as other extrinsic factors, for example factors related to pre-, intra-, and post-operative cares if the patient has undergone surgery (Heinzelmann *et al.,* 2002). Microbial factors that will influence the establishment of a wound infection include the bacterial inoculums, virulence, and the effect of the microenvironment. When these microbial factors are conducive, impaired host defences set the stage for enacting the chain of events that produce wound infection (Krizek and Robson, 1975). Oxygen tensions of between 5 and 20 mm Hg have been recorded in nonhealing wounds (Sheffield, 1988), and oxygen tension values of less than 30 mm Hg have been recorded in infected and traumatized tissue (Morykwas and Argenta, 1997); which correlates it with a reported pO_2 requirement of approximately 30 mm Hg for active cell division (Hunt and Hopt, 1997). "Factors that have a proven or probable influence on the frequency of wound infections are the use of antibiotic prophylaxis; the duration of surgery; the defence mechanisms of the host; the use of ultraclean air in the operating room; the patient's temperature in the operating room; the use of supplemental oxygen; the presence of hypovolemia, diabetes mellitus, or adiposity in the patient; the patient's nutritional status; the use of blood transfusion; and pain control (Gottrup, 2000; Gardland *et al.,* 2002 and Nwachukwu *et al.,* 2009)." Optimal antimicrobial prophylaxis in the appropriate dose, time and duration, which has been selected on the basis of the antimicrobial susceptibility pattern of the most common isolates in the hospital, would ensure a decreased rate of post-operative wound infections. Therefore the surveillance of nosocomial infections with an emphasis on antimicrobial audit will reduce the risk of postoperative wound infections (Amrita *et al.,* 2010).

2.4 Clinical features and complication

There are a number of indicators of wound infection; these include the classic signs related to the inflammatory. The classic signs of post-surgical wound infection include localised erythema, localised pain, localised heat, cellulites and oedema. Further signs include abscess, discharge which may be viscous in nature, discoloured and purulent, delayed healing not previously anticipated, discolouration of tissues both within and at the wound margins, friable and bleeding granulation tissue, abnormal smell and wound breakdown associated with wound pocketing/bridging at base of wound (Cutting and Harding, 1994). Post-operative complications may either be general or specific to the type of surgery undertaken, and should be managed with the patient's history in mind. Common post-operative complications include post-operative fever, atelectasis, wound infection, embolism and deep vein thrombosis. The highest incidence of post-operative complications is between 1 and 3 days after the surgery. However, specific complications occur in the following distinct temporal patterns: early post-operative, several days after the operation, throughout the post-operative period, and in the late post-operative period (Thompson *et al.,* 2003).

2.5. Laboratory diagnosis

The laboratory investigation of microbial diseases involves examining specimens to detect, isolate, and identify pathogens or their products using microscopy, culture techniques, biochemical methods, and immunological (antigen) and serological tests(antibodies) (Cheesbrough, 2006). Wound specimens should be cultured for both aerobic and anaerobic microorganisms in order to isolate which organism is responsible for the outcome of infection. A single microorganism in a Gram-stained smear from wound swab and swabs that provide more than 30 colonies on culture media both can possibly predict a microbial load of $>10^5$ CFU/g of tissue. Failure of isolating pathogenic organism from wound does not mean that infectious organism is absent rather it could be due to poor microbiological technique or sampling error, most commonly in those anaerobes (NNIS, 1996).

Collection and transport of wound swab

Surgical specimens may be obtained by aspiration of a localized abscess or other surgical procedures. The surgeon should be advised to obtain several small representative tissue samples and any purulent exudates. If possible, cotton swabs should be avoided. The exudates should be collected using a needle and syringe. If cotton swabs must be used, as much exudate should be collected as possible and dispensed into appropriate containers for dispatch to the

laboratory (NNIS, 1996). When collecting pus from abscesses, wounds, or other sites, special care should be taken to avoid contaminating the specimen with commensal organisms from the skin. As far as possible, a specimen from a wound should be collected before an antiseptic dressing is applied (Cheesbrough, 2006). The specimen should be transferred into the transport media and provided to the microbiology laboratory as soon as possible particularly for those anaerobic bacteria because they cannot survive in aerobic condition (NNIS, 1996).

Microscopic examination

In general, microscopy is used in microbiology for two basic purposes: the initial detection of microbes and the preliminary or definitive identification of microbes. The microscopic examination of clinical specimens is used to detect bacterial cells, fungal elements, parasites (eggs, larvae, or adult forms), and viral inclusions present in infected cells. Characteristic morphologic properties can be used for the preliminary identification of most bacteria (Cheesbrough, 2006). Gram-stain is performed to begin primary isolation of bacteria in order to differentiate whether the organism is gram positive or negative. It will be prepared with in less than 10 minutes. Visualization of bacteria on the smear by microscopic examination indicates that 10^6 or more bacteria per swab are present and reliably predict a microbial load of $>10^5$ CFU/g of tissue (Levine *et al.,* 1976).

Culture

If bacterial isolates are found on microscopic examination, appropriate culture media should be inoculated. Independently from the results of microscopy, all specimens of pus or exudates should preferably be inoculated onto these culture media: a blood agar plate for the isolation of *S. aureus* and *Streptococci* and MacConkey agar plate for the isolation of Gram-negative rods. The wound specimens can be inoculated on these medias and incubated at 35-37^{o}C overnight in appropriate gaseous atmosphere. Preliminary identification of bacteria is based on colony characteristics of the organisms i.e. haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms' biochemical tests are performed on colonies from primary cultures for final identification of the isolates (Cheesbrough, 2006).

Antimicrobial susceptibility tests

Antimicrobial agents include naturally occurring antibiotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents). Generally, however, the term 'antibiotic' is used to describe antimicrobial agents (usually antibacterial) that can be used to treat infection (Cheesbrough, 2006). Susceptibility tests should only be performed on well-isolated colonies of similar appearance that are considered significant according to the guidelines. A $BaSO_4$ turbidity standard is used (0.5 McFarland standard, approximately 10^5 organisms per ml). The turbidity of the standard is equivalent to the turbidity of sub cultured broth test microorganism (see Appendix V). Emulsify several colonies of similar appearance of the test organism in a small volume of sterile nutrient both, match the turbidity of the subculture against the turbidity standard, apply a loopful of the test organism subculture to the sensitivity testing plate using a sterile loop, spread the inoculums evenly across the plate using a sterile dry cotton wool swab, allow the inoculum to dry for a few minutes with the Petridis lid in place, place the anti microbial discs into the test organism in Petridis using a sterile forceps or dispenser, Incubate the plate aerobically at 37^oc over night and read the tests and interpret the result as Sensitive (S), Resistant (R) or Intermediate (I) comparing the chart of the sensitivity test (CLSI, 2005; NCCLS, 2002)

2.6. Treatment and prevention

The reporting by the microbiology laboratory of specific microorganisms isolated from a wound and the associated antibiograms may be interpreted by the practitioner as a diagnosis of wound infection that requires antimicrobial treatment (Bowler *et al.,* 2001). Although the primary purpose of antibiotics is to treat infection, prophylaxis associated with surgical practice accounts for up to half of all antibiotics prescribed (Periti *et al.,* 1998). The choice of prophylactic antibiotics should cover both facultative and anaerobic intestinal bacteria. The aim is to achieve high concentrations at the time of surgery and throughout the surgical procedure. Particularly in contaminated surgery, where excessive populations of gram-negative bacteria are likely to be present, careful selection of antibiotics is required since some are known to influence endotoxin liberation and hence septic shock (Periti *et al.,* 1998). Antibiotics that target the bacterial cell wall liberate larger amounts of endotoxin than do other classes of antibiotics, such as those that inhibit protein synthesis. Conversely, polymyxin B and the glycopeptide teicoplanin have the ability to neutralize endotoxin, and vancomycin has been

shown to down-regulate lipopolysaccharide-induced tumor necrosis factor alpha production from monocytes and is thus beneficial in the treatment of sepsis (Periti *et al.,* 1998).

Since *S. aureus* is considered to be the most problematic pathogen associated with infected traumatic wounds (Periti *et al.,* 1998; Eron, 1999), antibiotics like cephalosporins, macrolides, clindamycin, and semisynthetic penicillins such as flucloxacillin and oxacillin are often treatments of choice (Eron, 1999). If methicillin-resistant strains are involved, the glycopeptide antibiotics vancomycin and teicoplanin are alternatives (Periti *et al.,* 1998). In addition to antibiotic therapy, wound cleansing and surgical debridement may assist antibiotic treatment by reducing the microbial load, and hence the opportunity for infection, and enabling better penetration of antibiotics to where they are needed. Delayed wound closure may also be considered to allow time for antimicrobial therapy to reduce the microbial load, without which healing may not progress, and also to avoid the accumulation of a blood clot in tissue debris, which is ideal for microbial growth (Robson *et al.,* 1970; Robson *et al.,* 1968). As part of the surgical procedure, endogenous and exogenous microbial contamination must be minimized by ensuring good aseptic, skilled surgical techniques and minimizing the duration of surgery, while also optimizing the local wound conditions. This primarily involves removing any devitalized tissue to re-establish blood flow to the wound area, thereby maintaining adequate perfusion to enable the delivery of immune cells, oxygen, and nutrients and reducing the microbial load (Bowler *et al*., 2001).

2.7. Antimicrobial resistance

Antibiotics resistance among bacteria is a worldwide problem. The situation in developing countries like Ethiopia is particularly serious. Since the presence of drug resistant bacteria in the environment is threat to the public, up-to-date information on local pathogens and drug sensitivity pattern is very crucial to manage patients (Mulu *et al.,* 2006). Most of antimicrobial resistance is mainly due to the extensive use and misuse of antimicrobial drugs, result in emergence and survival of resistant strains. Bacteria become resistant to antimicrobial agents by a number of mechanisms, the commonest being: production of enzymes which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of an antimicrobial, modification of the target so that it no longer interacts with the antimicrobial and development of metabolic pathways by bacteria (Cheesbrough, 2006). Resistance in antimicrobial drugs in bacteria can result from two mutually non exclusive phenomenon's: mutations in housekeeping structural or regulatory genes and the horizontal acquisition of foreign genetic information (Courvalin and Trieu-Cuot, 2001). The rapid spread of

antimicrobial resistance genes on mobile genetic elements such as plasmids and transposons. Enterobacter isolates which are resistant to expanded spectrum cephalosporin is becoming a matter of concern for the probability of transmitting antimicrobial resistance from one microorganism to another worldwide (Gebre-Selassie, 2007).

2.8. Significance of the study

All surgical wounds are vulnerable to be contaminated by microbes. But in most cases, infection may not develop because of innate host defences that are quite efficient in eliminating microbial and other contaminants (Krizek and Robson, 1975). Surgical site infections (SSIs) are not an extinct entity; they account for 14-16% of the estimated 2 million nosocomial infections affecting hospitalized patients in the United States (Emori and Gaynes, 1993). The 2002 survey report by the Nosocomial Infection National Surveillance Service (NINSS), which covers the period between October 1997 and September 2001, indicates that the incidence of hospital acquired infection 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 (NINSS., 2002). In most studies in Ethiopia showed that the prevalence rate is in between 14.8- 60% (Taye, 2005; Mulu *et al.,* 2006 and Tesfahunegn *et al.,* 2009). Most infections are probably complex and polymicrobial; in which varying mixture were shown depending on laboratory methods used (Bowler *et al.,* 2001). Rates of nosocomial infections are markedly higher in many developing countries, especially for infections that are largely preventable (e.g. those following surgical procedures). Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antimicrobial drugs which have favoured the emergence and survival of resistant strains of micro-organisms (Mulu *et al.,* 2006). This might be a reflection of inappropriate use of the existing antimicrobials due to unavailability of guideline regarding selection of drugs. Though in Ethiopia there is published information about the bacteriology of wound infection in different parts of the country but there is no published information particularly about the bacteriology of post surgical wound infection in the area where this study was conducted. On the other hand antibiotics have been widely used to treat individuals who suffer with post operative wound infection. Given the high prevalence of this disease and the increasing incidence of microbial resistance to antibiotics (Mulu *et al.,* 2006) necessitate testing bacterial sensitivity to antibiotics. Since treatment is specific and based on the precise diagnosis testing antibiotic sensitivity is important for appropriate management.

Therefore the results of this study are important in terms of:

- Providing up-to-date information on frequently isolated aerobic bacterial species from patients with post surgical wound infection.
- Providing clinicians with the best antimicrobial agents to which the organisms are susceptible.
- Providing baseline information for further detailed and large epidemiological and drug resistance investigations in attempt to develop comprehensive treatment protocol.

3. OBJECTIVES OF THE STUDY

3.1. General Objective

- To determine the distribution of common aerobic bacteria in patients with post-surgical wound infection.

3.2. Specific Objectives

- To determine the magnitude and type of common aerobic bacteria in patients with post surgical wound infection.
- To describe the antibiotic susceptibility pattern of aerobic bacteria from post surgical wound infection.

4. MATERIALS AND METHODS

4.1. Study area and period

This study was conducted to identify common aerobic bacteria that cause post surgical wound infection and their pattern of antibiotic susceptibility at Hawassa Teaching and Referral Hospital, from November 2010 to March 2011. Hawassa is the capital city of the Southern Nations, Nationalities and Peoples' Regional state (SNNPR) in Ethiopia, and located 270 km South of Addis Ababa (Hawassa city administration socioeconomic profile, 2006). With 300 beds and good profile of qualified personnel, this hospital is the largest in the regional state and gives relatively better health services. Patients seeking medical care receive services at different outpatient and inpatient units (surgery, gynecology and obstetrics, internal medicine, pediatrics, ophthalmology, psychiatry, radiology, pathology). The laboratory in the hospital analyzes arrays of tests including parasitological, microbiological, immunological, hematological, and biochemical analysis.

4.2. Study design

A hospital based cross- sectional study was conducted.

4.3. Study population

The study population consisted of patients of all age groups and who had undergone surgery in Hawassa Teaching and Referral Hospital. Patients with surgical site infection that is, any patient with skin eruption or drainage at surgical site within 30 days post surgical procedure and who were admitted in surgical, gyn/obs and pediatrics wards were included in the study. A definite case of surgical site infection (SSI) is defined as any skin eruption or drainage that occurred at the surgical site and positive for pathogenic organisms by culture within 30 days of a surgical procedure.

4.4. Sample size and sampling technique

The sample size was computed using the formula:

$$n = \frac{Z^2 p (1-p)}{d^2}$$

Where n = sample size
z = statistic for level of confidence
p = estimated prevalence
d= precision

Considering 14.8 % estimated prevalence (Taye, 2005*),* 5% precision (d=0.05) and 95% level of confidence (z=1.96) the sample size was estimated to be 194. Sampling was done using non-probability technique, in which consecutive patients with post surgical wound infection attending Hawassa Teaching and Referral Hospital during the study period were included.

4.5. Data collection

4.5.1. Demographic and clinical profile

Physicians collected data on socio-demography and clinical profile related to sign and symptoms of post-surgical wound infections using designed data collection formats (see Appendix I).

4.5.2 Laboratory analysis

Sample collection

Pus swabs were aseptically collected from surgical sites prior to cleansing the wound with antiseptic solutions. Swabs were placed in sterile test tubes and transferred to the microbiology laboratory within 30 minutes after collection. Following reception in the laboratory, the specimens were registered and macroscopically examined for their physical appearances. All specimens were also processed for culturing and Gram-staining as specified (Cheesbrough, 2006).

Gram staining

Pus specimens were smeared on slides, air-dried, fixed with absolute methanol, and stained using Gram's staining technique (see Appendix VI). The Gram's staining technique helps to classify bacteria as Gram positive or Gram negative on the basis of bacterial cell ability to retain the primary stain, which is crystal violet. The stained slides were examined microscopically, and bacterial morphology, their arrangement as well as Gram reaction is determined. Gram staining was also performed to confirm type of bacteria grown on culture media.

Culture and biochemical tests

All pus specimens were inoculated on blood agar (BA), Mannitol salt agar (MSA) and MacConkey agar (Oxoid, Ltd., Basingstoke, and Hampshire, England). The plates were incubated in an aerobic atmosphere at 37°C for 24 - 48 hours. Growth of bacteria on media was confirmed based on characteristics including:

- Colony morphology (mucoid , raised, white , small, large)
- Pigment production (pyocyanine, pyoverdin)
- Haemolysis (beta-haemolysis, alpha-hemolysis, gamma-hemolysis)
- Motility (swarming motility)
- Biochemical utilization (fermenting lactose, mannitol, glucose, sucrose)
- Gram staining (gram positive or gram negative)

Colonies were counted using colony counter, and counts were categorized and interpreted as follows: counts <5 were considered as contamination; 5 -15 were colonization; 16-30 were critical colonization; and >30, infection (Cheesbrough, 2006).

Organisms that grew on both BA and MSA were suspected to be gram positive bacteria as MSA is selective media for Staphylococci. Catalase test was performed to differentiate (see Appendix IX) Staphylococci from Streptococci, in which catalase negative result exclude Streptococci species Further, coagulase test were performed (see Appendix X) to differentiate *S. aureus* from other species of genus Staphylococci, which are coagulase negative.

Bacteria that grew on BA and MacConkey agar were suspected to be gram negatives as MacConkey agar is selective to gram negative bacteria. Colonies on MacConkey agar were differentiated based on their characteristics to ferment lactose. Pink colour characterizes lactose fermenters whereas colourless colonies are non- lactose fermenter.

Gram negative bacteria were further tested for their motility and characterized using arrays of biochemical tests including indole, urea, Triple Sugar Iron agar (TSI), Simmon's Citrate Agar, and Lysine Decarboxylase (LDC). Colonies that produce pigment on blood agar and non-lactose fermenter on MacConkey agar were tested using oxidase (see Appendix XI) to confirm *P. aeruginosa,* which is oxidase positive. Interpretation for some of the biochemical tests used to differentiate gram negative bacteria is presented in Table 1.

Table 1: Interpretation of biochemical tests for some Enterobacteria and *P. aeruginosa.*

Biochemical reaction											Bacterial species
Lact	Ind	urea	Glu	Suc	Ox	Cit	Mot	H_2S	Gas	LDC	
+	+	-	+	+	-	-	+	-	+	+	*E. coli*
+	-	+	+	+	-	+	-	-	+	+	*Klebsiella* species
-	+/-	+	+	+/-	-	+/-	+	+	+/-	-	*Proteus* species
-	-	-	-	-	+	+	-	-	-	-	*P. aeruginosa*

Lact- Lactose, Ind- Indole, Glu- Glucose, Suc- Sucrose, Ox- Oxidase test, Cit- Citrate test, Mot- Motility test, H_2S- hydrogen sulphide, Urea- Urease and LDC- Lysine decarboxylase

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed for all aerobic bacterial isolates using agar disk diffusion method on Mueller-Hinton agar according to the criteria set by the Clinical and Laboratory Standards Institute formerly known as National Committee for Clinical Laboratory Standards (CLSI, 2005; NCCLS, 2002) to determine susceptibility patterns to commonly used antibiotics. In brief, 1-3 similar colonies of the isolates were inoculated on nutrient broth to prepare inoculums; broths were incubated at 37°C for 4hrs; thereafter turbidity of broths were standardized by suspending colonies in sterile phosphate buffered saline (pH, 7.2) until achieving turbidity 0.5 McFarland standards (see Appendix VII). A sterile cotton swab was dipped into the bacterial suspension and rotated several times against the inside wall of the tube to remove excess inoculum. Swabs were inoculated on Mueller–Hinton agar plate (Oxoid, UK) to obtain confluent growth. At the same time different antibiotic disks (Oxoid, UK), representing the most commonly used antibiotics in the study area, were placed on the media prior to incubation at 37^oC for 18-24 hrs (Cheesbrough, 2006).

The antibiotics used for gram positive isolates were: ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), penicillin G (10IU), erythromycin (15µg), amoxacillin-clavunilic acid (30µg), trimethoprim-sulphamethoxazole (25µg), ceftriaxone(30µg) and vancomycin (30µg). Similarly, antibiotics used for gram negative isolates were: ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), amoxacillin-clavunilic acid (30µg), trimethoprim-sulphamethoxazole(25µg) and ceftriaxone(30µg).

The diameter of growth inhibition zone was measured to the nearest whole millimetre using a sliding calliper. Based on measured zone size for each antibiotic, bacterial isolates were classified as susceptible, intermediate and resistant as described (CLSI, 2005; NCCLS, 2002).

4.6. Quality control

Laboratory analyses were carried out using standard operating procedures (SOPs) and under close supervision of experienced microbiologists. Prior to the actual work, reagents were checked for proper functioning. Reference strains of *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923) and *P. aeruginosa* (ATCC-27853), were used as a quality control throughout the study for antimicrobial susceptibility testing. All the strains were obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI).

4.7. Data analysis

Data was analyzed using statistical package for social science (SPSS, version 16) software. Percentages for proportion and odds ratio for categorical variable were used wherever appropriate. A p-value < 0.05 were considered as statistically significant.

4.8. Ethical consideration

The study was approved and ethically cleared by the Research and Ethical Review Committee of the Department of Microbiology, Immunology and Parasitology, School of Medicine; Addis Ababa University and College of Health Sciences, Hawassa University. Participants were informed about the purpose of the study and their consent was obtained from each study subjects or their parents (Appendix II and IV). Any information obtained from participants during the study was kept confidential. Doctors and other health professionals manage those patients with infection.

5. RESULTS

Patient profile

A total of 194 patients with post surgical wound infection from surgical, pediatrics and gynecology/obstetrics wards of Hawassa Teaching and Referral Hospital were assessed for the development of post operative wound infections between November 2010 and March 2011. Socio-demographic characteristics of study subjects are presented in Table 2. Out of 194 patients, 116(59.8%) were males and 78(40.2%) were females. The mean age of the study participants was 28 years with age range between 6/12 months to 100 years. The modal age group was 21-30 years, with frequency 56(28.9%). Majority of the study subjects 146(75.3%) were from the rural part of the country and the rest 48(24.7%) were urban residents. The predominant location of wound was abdomen, 107(55.2%), and wound on the back and thorax least frequently occurred, 6(3.1%) each (Table 2). Majority of the study subjects were admitted in surgical ward 87(44.8%).

Table 2: Socio-demographic characteristics of post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010-March 2011).

Characteristics	Frequency	Percent (%)
Sex		
Male	116	59.8
Female	78	40.2
Total	194	100.0
Age range(years)		
≤10	33	17.0
11-20	41	21.1
21-30	60	30.9
31-40	18	9.3
41-50	21	10.8
51-60	15	7.7
≥60	6	3.1
Total	194	100.0
Ward		
Surgery	87	44.8
Pediatrics	50	25.8
Gyn/Obs	57	29.4
Total	194	100.0
Residence		
Urban	48	24.7
Rural	146	75.3
Total	194	100.0
Site of wound		
Back	6	3.1
Thorax	6	3.1
Abdomen	107	55.2
Head and neck	9	4.6
Leg	66	34.0
Total	194	100.0

Culture results

Types of bacterial species

The type and percentage of aerobic bacteria isolated from the pus samples of post surgical wound infected patients is shown in Fig 1. The majority of cultured specimens 138(71.1%) showed bacterial growth within 48 hours of incubation. Out of 138 culture positive specimens, a total of 177 different aerobic bacteria were isolated. Majority of the isolates 105(59.3 %) were Gram-negative organisms, while 72 (40.7%) were Gram-positives. No growth of bacteria was observed in 56 (28.9%) samples. Of the 177 aerobic bacterial, *S. aureus* was most frequently isolated 66 (37.3%); followed by *E. coli,* 45 (25.4%), *Klebsiella* species, 24 (13.6%), *Proteus* species, 18 (10.2%), and *P. aeruginosa,* 18 (10.2%). *Coagulase negative Staphylococci (CoNS*) were the least isolated organism, with frequency of 6 (3.4%).

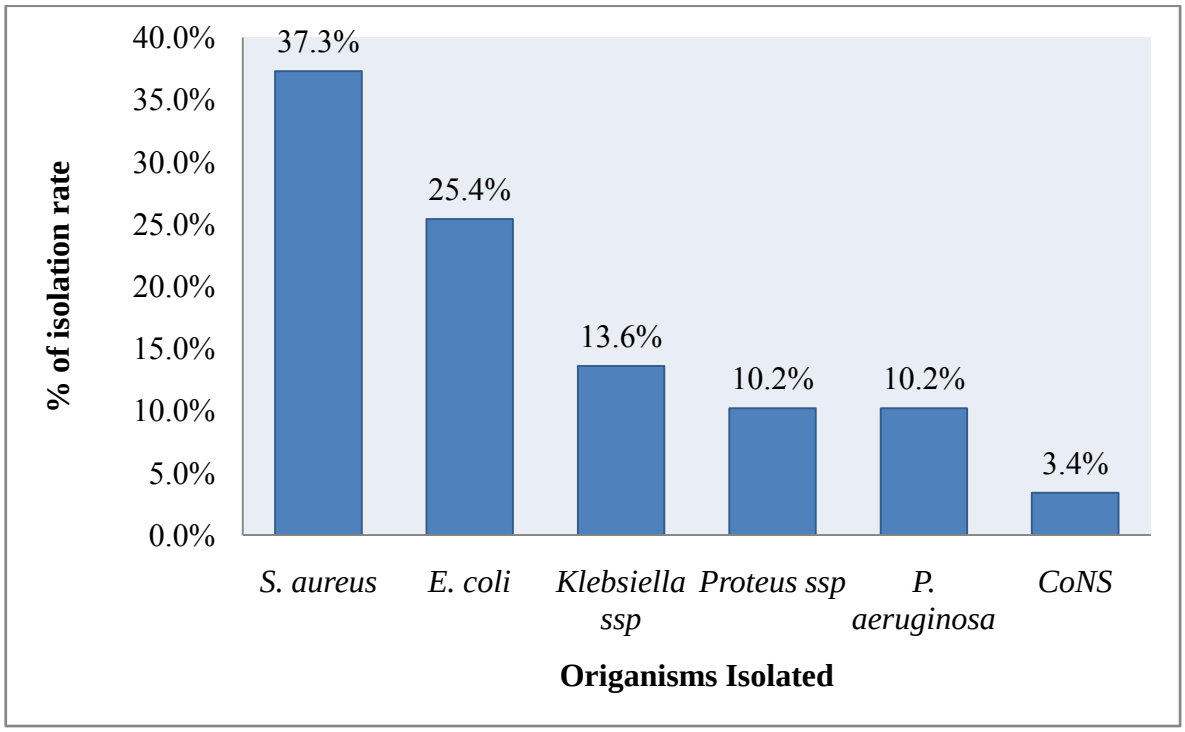

Figure 1: Characterization of organisms isolated from post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (Nov 2010-Mar 2011).

CoNS = Coagulase negative Staphylococci

Majority of patients 99 (51%) with post surgical wound infection were harbouring single organism. Dual infections were observed in 39(20.1%) of all 194 pus specimens and in 28.9% of samples there was no bacterial growth. *S. aureus* most frequently occurred in combination with *E. coli* and *Klebsiella* species in 9 specimens (23.1%) (Table 3).

Table 3: Multiple infections among post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010- March 2011).

Pattern of multiple infection	frequency	percentage (%)
S. aureus and *E. coli*	9	23.1%
S. aureus and *Klebsiella* species	9	23.1%
S. aureus and *Proteus* species	6	15.3%
S. aureus and *P. aeruginosa*	3	7.7%
E. coli and *Proteus* species	3	7.7%
E. coli and *P. aeruginosa*	3	7.7%
Klebsiella species and *CoNS*	3	7.7%
Proteus species and *CoNS*	3	7.7%
Total	39	100.0%

CoNS - coagulase negative staphylocicci

Prevalence and variables associated with post operative wound infection.

In the present study Significant aerobic bacteria was detected in 138(71.1%) of samples, and in 39 (20.1%) of the pus samples two types of bacteria each were isolated making the number of bacteria isolated to be 177 with the isolation rate of 91.2%. Of the total 194 wound swab specimens processed 56 (28.9%) had showed no significant bacterial growth. A significant aerobic bacteria was more common in males with an isolation rate of 93/116 (80.2%) than females 45/78 (57.7%) (Fig. 2) (P=0.001) and also there was statistical association among site of wound and development of post surgical wound infection (p=0.034). In this study there was no statistical significant association between age and development of post surgical wound infection (p= 0.787) similarly there was no statistical association between residence and development of post surgical wound infection (p=0.675). Statistical association was not also found among ward and developing post surgical wound infection (p=0.077).

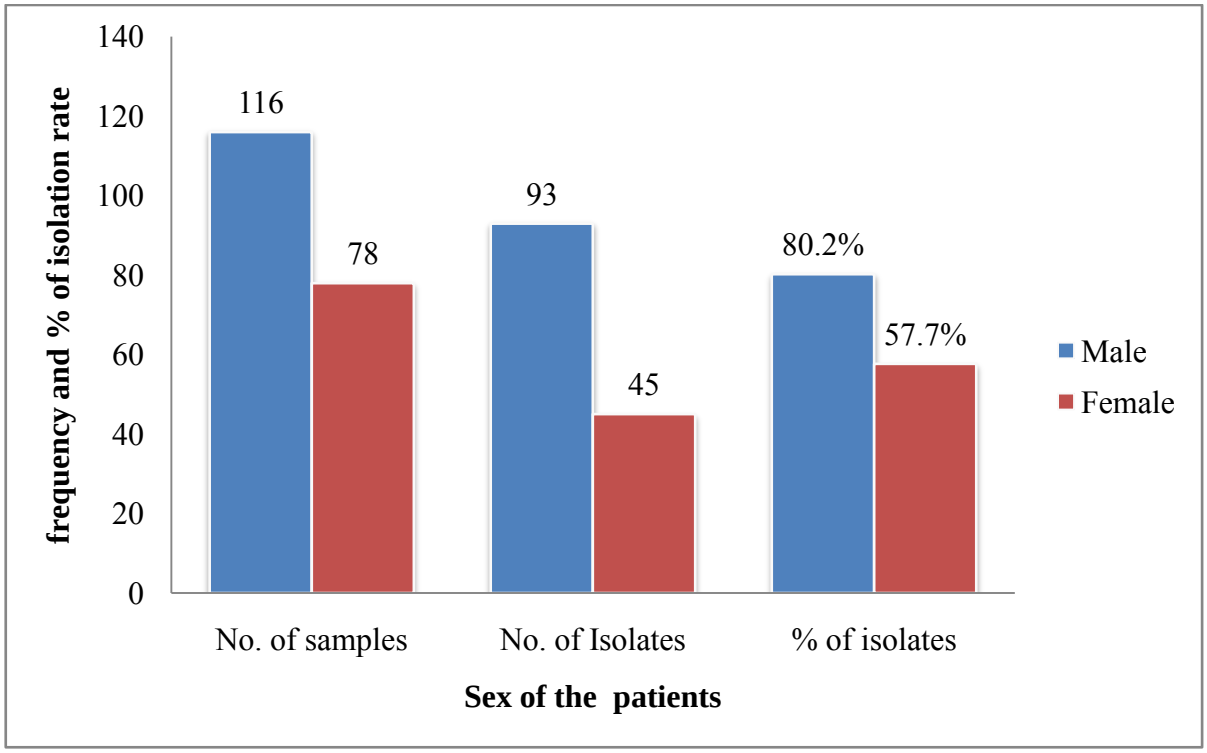

Figure 2: Occurrence of aerobic bacteria in post surgical wound infected patients in relation to sex attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (Nov 2010-Mar 2011).

Antimicrobial susceptibility

Gram negative bacteria

The result of antimicrobial susceptibility testing for Gram-negative bacteria isolated from wound swab culture of post surgical wound infected patients against chosen antimicrobial agents is presented in Table 4. Out of 105 Gram-negative isolates 45(42.9%) were sensitive to gentamicin, 72(68.6%) were sensitive to ciprofloxacin, 45(42.9%) were sensitive to amoxacillin-clavunilic acid (AMC) and only 3(2.86%) were sensitive to ampicillin. Out of 18 isolates of *P. aeruginosa* from post surgical wounds, 18(100%), 18(100%), 9(50.00%), 0(0%), and 12(66.7%) were resistant to ampicillin, ceftriaxone, gentamycin, ciprofloxacin, and trimethoprim-sulphamethoxazole, respectively (Table 4).

Gram positive bacteria

The result of antimicrobial susceptibility testing for Gram-positive bacteria (n=69) isolated from wound swab culture of post surgical wound infected patients against chosen antimicrobial agents is presented in Table 5. Out of 66 *S. aureus* isolated from the post surgical wounds, 26(39.4%) were resistant to ciprofloxacin, as 43(65.2%), 37(56.1%), 26(39.4%), 63(95.5%), 66(100%), 29(43.9%) and 20(30.3%) were resistant to vancomicin, trimethoprim-sulphamethoxazole, gentamycin, ampicillin, penicillin, erythromycin and amoxacillin-clavunilic acid, respectively (Table 5).

Among all bacterial isolates of both Gram-negative and Gram-positive a high level (>60%) of susceptibility was observed in and ciprofloxacin, while a high level of resistance (>95%) was observed against ampicillin (Table 4 and Table 5).

Table 4: Antimicrobial susceptibility pattern of gram-negative aerobic bacteria in post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010 - March 2011).

Bacteria isolated	Antimicrobial agents tested								
	Total N(%)	S/R	AMP Nº. (%)	CRO Nº. (%)	C Nº. (%)	CIP Nº. (%)	CN Nº. (%)	SXT Nº. (%)	AMC Nº. (%)
E. coli	45(42.9%)	S	-	27(60.0)	27(60.0)	27(60.0)	24(53.3)	18(40.0)	24(53.3)
		R	45(100.0)	18(40.0)	18(40.0)	18(40.0)	21(46.7)	27(60.0)	21(46.7)
Klebsiella spp.	24(22.9%)	S	3(12.5)	3(12.5)	3(12.5)	9(37.5)	-	3(12.5)	12(50.0)
		R	21(87.5)	21(87.5)	21(87.5)	15(62.5)	24(100.0)	21(87.5)	12(50.0)
Proteus spp.	18(17.1%)	S	-	12(66.7)	6(33.3)	18(100.0)	12(66.7)	15(83.3)	9(50.0)
		R	18(100.0)	6(33.3)	12(66.7)	-	6(33.3)	3(16.7)	9(50.0)
P. aeruginosa	18(17.1%)	S	-	-	-	18(100.0)	9(50.0)	6(33.3)	-
		R	18(100.0)	18(100.0)	18(100.0)	-	9(50.0)	12(66.7)	18(100.0)
Total	105(100%)	S	3(2.86)	42(40.0)	36(34.3)	72(68.6)	45(42.9)	42(40.0)	45(42.9)
		R	102(97.14)	63(60.0)	69(65.7)	33(31.4)	23(57.1)	63(60.0)	23(57.1)

S/R = Sensitive/Resistant; AMP = Ampicillin; CRO = Ceftriaxone; C = Chloramphenicol; CIP = Ciprofloxacin; CN = Gentamicin; SXT = Trimethoprim-sulphamethoxazole; AMC = Amoxacillin clavunilic acid.

Table 5: Antimicrobial susceptibility pattern of gram-positive aerobic bacteria in post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010-March 2011).

Bacteria isolated	Antimicrobial agents tested											
	Total Nº (%)	S/R	VAN No.(%)	SXT No.(%)	CN No.(%)	C No.(%)	AMP No.(%)	AMC No.(%)	CRO No.(%)	P No.(%)	E No.(%)	CIP No.(%)
S.aureus	66(91.7)	S	23(34.8)	29(43.9)	40(60.6)	23(34.8)	3(4.5)	46(69.7)	12(18.2)	-	37(56.1)	40(60.6)
		R	43(65.2)	37(56.1)	26(39.4)	43(65.2)	63(95.5)	20(30.3)	54(81.8)	66(100)	29(43.9)	26(39.4)
CoNS	6(8.3)	S	-	3(50.0)	3(50.0)	6(100)	-	3(50.0)	-	-	3(50.0)	3(50.0)
		R	6(100)	3(50.0)	3(50.0)	-	6(100)	3(50.0)	6(100)	6(100)	3(50.0))	3(50.0)
Total	72(100)	S	23(31.9)	32(48.5)	43(59.7)	29(40.3)	3(4.2)	49(69.1)	12(16.7)	-	40(51.5)	43(59.7)
		R	49(69.1)	40(51.5)	29(40.3)	43(59.7)	69(95.8)	23(31.9)	60(83.3)	72(100)	32(48.5)	29(40.3)

S/R = Sensitive/Resistant; VAN = Vancomycin, AMP = Ampicillin; CRO = Ceftriaxone;
C = Chloramphenicol; CIP = Ciprofloxacin; E = Erythromycin; CN = Gentamicin;
P = Penicillin; SXT = Trimethoprim-sulphamethoxazole AMC = Amoxacillin-clavunilic acid;
CoNS = Coagulase negative Staphylococci

Multiple drug resistance

Multiple drug resistance to two or more drugs was observed in 93/105 (88.6 %) of Gram-negative aerobic bacterial (Table 6) and in 60/72 (86.4 %) of Gram-positive aerobic bacteria (Table 7).

Generally multiple drug resistance accounted for 153/177 (86.4 %) for both groups (Gram-negative and Gram-positive), while none were found to be sensitive to all antibiotics tested and 11.9 % were resistant to all antibiotics.

Three bacteria 3/177 (1.7%) for *E. coli*, 15/177(8.5%) *Klebsiella* species and 3/177(1.7%) *S. aureus* were resistant to all antibiotics tested.

Table 6: Multiple drug resistance patterns of gram negative aerobic bacteria in post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010-March 2011).

	Antibiogram pattern%							
Organisms	**R_0**	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**
E.coli	-	9(20%)	9(20%)	-	6(13.3%)	6(13.3%)	12(26.7%)	3(6.7%)
Klebsiella ssp	-	-	-	-	3(12.5%)	-	6(25%)	15(62.5%)
Proteus ssp	-	-	6(33.3%)	6(33.3%)	-	6(33.3%)	-	-
P.aeruginosa	-	-	-	-	-	15(83.3%)	3(16.7%)	-
Total	0(0%)	9(8.6%)	15(14.3%)	6(5.7%)	9(8.6%)	27(25.7%)	21(20%)	18(17.1%)

R_0 - sensitive to all antibiotics, **R_1** - resistant to 1 antibiotic, **R_2** - resistant to 2 antibiotics, **R_3** - resistant to 3 antibiotics, **R_4** - resistant to 4 antibiotics, **R_5** - resistant to 5 antibiotics, **R_6** - resistant to 6 antibiotics and **R_7** - resistant to all antibiotics.

Table 7: Multiple drug resistance patterns of gram positive aerobic bacteria in post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010-March 2011).

	Antibiogrampattern %										
Organisms	**R_0**	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**	**R_8**	**R_9**	**R_{10}**
S.aureus	-	3(4.5%)	-	9(13.3%)	6(9.1%)	12(18.2%)	3(4.5%)	9(13.3%)	12(18.2%)	9(13.3%)	3(4.5%)
CoNS	-	-	-	-	3(50%)	-	-	-	-	3(50%)	-
Total	-	3(4.2%)	-	9(12.5%)	9(12.5%)	12(16.7%)	3(4.2%)	9(12.5%)	12(16.7%)	9(12.5%)	3(4.2%)

R_0 - sensitive to all antibiotics, **R_1** - resistant to 1 antibiotic, **R_2** - resistant to 2 antibiotics, **R_3** - resistant to 3 antibiotics, **R_4** - resistant to 4 antibiotics, **R_5** - resistant to 5 antibiotics, **R_6** - resistant to 6 antibiotics and **R_7** - resistant to 7 antibiotics, **R_8** - resistant to 8 antibiotics, **R_9** - resistant to 9 antibiotics, **R_{10}** - resistant to all antibiotics.

CoNS - *coagulase negative staphylococci.*

6. DISCUSSION

Post-operative wound infections have been found to pose a major problem in the field of surgery for a long time. Advances in control of infections have not completely eradicated this problem because of development of drug resistance (Anguzu and Olila, 2007). The surveillance of nosocomial infections with an emphasis on antimicrobial audit will reduce the risk of postoperative wound infections (Amrita *et al.,* 2010).

The data presented in this study could provide information to clinicians on major aerobic bacterial pathogens which are responsible for post surgical wound infection and selection of antimicrobial agents in treating these patients. In the present study samples taken from patients with surgical site infection, we found 71.1% culture positivity. Similar finding was found in previous study in Niger state showing 74.9% (Odedina *et al.,* 2007). However, it is much higher than other reports from Ethiopia with 14.8%, 44.1% and 53.0% (Taye, 2005; Tesfahunegn *et al.,* 2009 and Biadglign *et al.,* 2009) and it is lower than studies conducted elsewhere 98.5%- 100% (Jonathan *et al.,* 2008 and Adegoke *et al.,* 2010).

In this study a significant aerobic bacteria was more common in males with an isolation rate of 123/177 (69.5%) than females 54/177 (30.5%) (Fig. 2) (P=0.001). This finding is in line with report by Biadglign *et al.,* (2009) which showed that infection in males was 63.2% compared to females of 36.8% and it is similar with that reported by Rangan *et al.,* (2010) that the prevalence rate to be higher in males 58% patients compared to females 42% and it is also similar with that reported by Adegoke *et al.,* (2010). There was no difference in the frequency of SSI among different age groups (p= 0.787). This result is similar with a study conducted in Mekele (Tesfahunegn *et al.,* 2009).

The result of this research work shows that *S. aureus, E. coli, Klebsiella* ssp*., Proteus* ssp*., P. aeruginosa and CoNS* are associated with post surgical wound infections. Out of 177 isolates obtained from wound swab cultures 40.7% were gram positive organisms while 105(59.3%) were gram negative organisms. This result agrees with other studies that gram negative organisms are the predominant agents of surgical wound infection (Tesfahunegn *et al.,* 2009; Odedina *et al.,* 2007). In this study we also found that poly microbial pathogens were isolated from 20.1% of SSI patients which was higher than report from Ethiopia 11.7% (Tesfahunegn *et al.,* 2009) and lower than a report from Italy; where 55.8% (Giacometti *et al.,* 2000).

Out of 194 wound specimens examined by culture, *S. aureus* 66(37.3%), followed by *E. coli* 45 (25.4%), *Klebsiella spp*. 24(13.6%), *Proteus spp.* and *P. aeruginosa* both with 18(10.2%) and *Coagulase negative Staphylococci (CoNS*) was the least isolated organism with the frequency of 6(3.4%) (Fig.1). This result showed that *S. aureus* and the rest pathogens are the major microbial pathogens associated with post surgical wound infections in this region of study. This result is consistent with other studies which are done in Ethiopia and other countries (Biadglign *et al.,* 2009; Mulu *et al.,* 2006; Nwachukwu *et al.,* 2009; Anguzu and Olila, 2007; Jonathan *et al.,* 2008). The frequency of isolation of *E. coli*, *Klebsilla* species and *P. aeruginosa* was increased in this study while that of *Proteus* species was the same as compared to previous study (Biadglign *et al.,* 2009).

The results of our study showed that the predominant agents in wound swabs belongs to *S. aureus* which is in line with previous studies conducted in Ethiopia (Biadglign *et al.,* 2009), Uganda (Anguzu and Olila, 2007) and Nigeria (Nwachukwu *et al.,* 2009). The high prevalence of *S. aureus* infection may be because it is an endogenous source of infection. Nasal carriage of *S. aureus* is an important risk factor for infection of surgical site as the organism is a normal flora in the nostrils. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier *S. aureus*, which is a common bacterium on surfaces, easily find their way into surgical sites (Anguzu and Olila, 2007).

The proportion of gram negative bacteria was also high. This result is in agreement with other studies which were explained by the chronic nature of most infected wounds (Mulu *et al.,* 2006; Gottrup, 2000**).** The relative high number of Enterobacteria isolated in this study points to the fact that the presence of enteric organisms in the wounds at operation probably resulted to subsequent sepsis or may be indicative of faecal contamination and a reflection of poor personnel hygiene (Odedina *et al.,* 2007). Masaadeh and Jaran, 2009 reported similar findings therefore infer that enteric organisms are important determinants of healing in surgical wounds and also most of the patients have had abdominal surgeries that the opening of the gastrointestinal tract increases the likelihood of gram negative bacilli. These groups of organisms tend to be endemic in hospital environment by being easily transferred from object to object, they also tend to be resistant to common antiseptics and are difficult to eradicate in the long term and these group of organisms are increasingly playing a greater role in the many hospital acquired infections (Amrita *et al.,* 2010).

Fifty-six out of one hundred ninety-four swabs (28.9%) had no bacterial growth. This could be due to normal healing process where the bacteria have been overpowered by body's defence mechanism, antimicrobial activity in patients circulation since all of them had been on antibiotic therapy post operatively at time of collecting the samples which was confirmed by personal observation or adequate nursing care e.g. use of antiseptics for cleaning the wounds. It is also possible that some organisms could have been anaerobic bacteria or fungi that were missed as cultures were incubated aerobically or fungal culture media was not used. This condition could not therefore support growth of such organisms.

The in vitro antimicrobial sensitivity studies showed that organisms react differently to various antibiotics, as demonstrated by their sensitivity patterns (Table 4 and 5). In the present study a large number of the isolates were resistant to ampicillin, chloramphenicol, penicillin, SXT and as shown in Tables 4 and 5, which is consistent with reports in different studies conducted in Ethiopia (Biadglign *et al.,* 2009; Mulu *et al.,* 2006; and Gebre-Sealsssie, 2007). The remarkably higher prevalence of resistance to the commonly prescribed antibiotics such as ampicillin, chloramphenicol, SXT, and penicillin noticed in the present study may be due to the easily availability and indiscriminate use of the drugs without prescription (Ibeawuchi and Mabata, 2002). It was reported that, ciprofloxacin were effective for more than 90% of gram negative isolates in Gondar (Mulu *et al.*, 2006). However, in the present study ciprofloxacin was found to be effective for more than 68.6% of the isolates. This sharp fall in effectiveness may be due to overuse of it as empiric treatment option.

The resistance pattern of *S. aureus* for CIP, AMP and P is similar with Odedina *et al.,* (2007) which is 33.9%, 99.5% and 100% and similar with Anguzu and Olila, (2007) for CIP and AMP (31.3% and 97%). In this study the resistance pattern of AMP, CN, P, E and SXT is higher than Biadglign *et al* which was 50.7%, 24.3%, 56.4%, 30% and 26.4%, but similar for C 61.4% (Biadglign *et al.,* 2009). In this study also the resistant pattern of *S. aureus* is higher for AMP and CRO than Tesfahunegn *et al* which was 66.7% and 33.3%, but lower for C, E, CN and SXT (100%, 66.7%, 66.7% and 100%). In this study 65.2% of *S. aureus* was resistant to vancomicine which was in line with 66.7% (Tesfahunegn *et al.,* 2009).

In this study, *P. aeruginosa* was not appreciably susceptible to most antibacterial agents and it was 18(100%), 18(100%), 9(50.00%), were resistant to ampicillin, cloramphenicol, gentamycin which was in line with 18(100%), 18(100%), 9(50.00%) resistant to ampicillin, cloramphenicol and gentamycin (Tesfahunegn *et al.,* 2009). The only antibacterial agent effective was ciprofloxacin (100%) (Table 4). Surveillance of *P. aeruginosa* infections has revealed trends of increasing

multidrug resistance, because of its capability of affecting many mechanisms of antibacterial resistance including multidrug efflux pumps, β-lactamases, down regulation of outer membrane porins, enzymatic degradation and target structure alteration.

Multiple antibiotics resistance was seen in 86.4% of gram positive and 88.6% of the gram negative isolates. This is high when compared to previous studies (Biadglign *et al*, 2009; Mulu *et al.*, 2006). The high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics. Multiple antibiotics resistance to these commonly used antibiotics is found to be extremely high which makes the condition frustrating. Most of the isolates were resistant to these antibiotics. This finding is relatively higher as compared to other studies in Gondar (Mulu *et al*, 2006) and Bahir Dar (Biadglign *et al*, 2009). This may be explained by the fact that, irrational use of antibiotics for conditions that may not clinically indicate their use, over-the-counter sell of antibiotics , some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them hence antimicrobial resistance strains grow around.

7. CONCLUSION

A total of 138 culture positive pus specimens were detected from 194 patients suffering with post surgical wound infection resulting in the overall prevalence of 71.1%. However, a total of 177 different aerobic bacteria were isolated making the isolation rate of bacteria 91.2%.

S. aureus was the dominating bacterial isolates. The results of this study also showed that the etiologic agents of post surgical wound infection mainly belonged to Gram-negative enteric bacteria. More than one type of organisms was isolated in 39(20.1%) of pus specimens cultured. Single and multiple drug resistance to the commonly used antibiotics in the study area was found to be very high leaving clinicians with a very few choices of drugs for the treatment of post surgical wound infection. Therefore, it is critical that use of antimicrobial agents with in hospitals, public healthcare providers as well as private ones should be reviewed and further studies to find out the overall resistance patterns and their possible causes and associated factors in the region at large need to be carried out. In the present study, it is indicated that the majority of bacterial isolates were sensitive to ciprofloxacin, amoxacillin-clavulinic acid and gentamicin. Thus, these drugs appear to be effective against post surgical wound infection in the study area. These antibiotics should however be used with caution because of the emerging low level of resistance which may portent great danger for their future use. Limitation of the study was anaerobic bacteria and fungal pathogens were not investigated due to limited laboratory facilities.

8. RECOMMENDATIONS

The following recommendations are forwarded based on the findings of the present study:

- Further studies on a larger scale in the future is mandatory in order to monitor any changes in the sensitivity patterns and to explore the causes for increased drug resistance of pathogens causing wound infection among post operated patients in the study area and elsewhere.

- The use of antimicrobial agents with in hospitals and all other responsible health institutions should be reviewed and strict adherence to hospital disease control and antibiotics usage policy in the study area should be considered.

- Improvement of the laboratory services capable of doing culture and sensitivity in all wound isolates before subscribing any drugs in the study area and during follow up of post operated patients that may help to reduce the spreading of drug resistance.

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10. APPENDICES

Appendix I: Data collection format

Questionnaire for the investigation of bacterial isolates from post surgical wound infection and pattern of their antibiotic susceptibility in patients who have had surgery in Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia.

I. Patients Identification

1. Serial No_____
2. Patients name _____
3. Age _____
4. Sex (M/F) _____
5. Address _____
6. Ward_____
7. Location of wound_____

II. Clinical profile: signs and symptoms of post surgical wound infection

Localised erythema	YES/NO
Localised pain	YES/NO
Localised heat	YES/NO
Oedema	YES/NO
Abscess	YES/NO
Discharge which may be viscous in nature, discoloured and purulent	YES/NO
Discolouration of tissues both within and at the wound margins	YES/NO
Friable	YES/NO
Abnormal smell	YES/NO

III. Laboratory Data

1. Type of wound specimen: wound swab

2. Microscopic examination: gram positive --------- gram negative ----------

3. Culture: Name of organism/s isolated: bacteria (spp.) _____

4. Date and time of specimen collection _____

5. Antimicrobial susceptibility testing S (mm) I (mm) R (mm)

- Penicillin ------- -------- -------
- Ampicillin ----- ----- ------
- Amoxicillin-clavulanic acid ----- ------ ------
- Ceftriaxone ------ ------ ------
- Ciprofloxacin ------ ------ ------
- Chloramphenicol ------ ------- ------
- Erythromycin ------- -------- -------
- Gentamicin ------- ------- -------
- TMP-SMX ------- -------- -------
- Vancomycin ------- ------- -------

Comments:

_____

_____

Appendix II: Patient information sheet form (English version)

(To be translated in to the patient's language)

Purpose- We have planned to conduct a study with the objective of determining the distribution of bacterial pathogens in patients with post-surgical wound and their antimicrobial susceptibility patterns. Because the type of organisms and their pattern of antimicrobial susceptibility in surgical wound infection are different, the result of this study is believed to be important for appropriate management of post surgical wound infection.

Participation-We are asking you and others to participate voluntarily in this study, which would require your response to an interview, to be physically examined and to give wound swab sample for laboratory examination. All samples are collected using sterile containers and equipments.

Risks associated-There are no risks associated with the collection of wound swab, but if we us non sterile swab, there is a slight risk of introducing infection.

Benefits-If there is any positive finding in laboratory examination the result will be reported to your physician for appropriate treatment and management.

Confidentiality-Any information that is collected about you will be kept private and in a secured place.

Sharing the result-There will be a report which is written about the result of this study either through publication or any other means. The result will not bear any information relevant to your personality in anyway. Your permission is also needed to use the test results for writing a report.

Contact Address

If you have any question or doubt you can contact:

Lopiso Dessalegn
Department of Microbiology, Immunology and Parasitology
School of Medicine, Addis Ababa University
P.O.Box, 9086 Addis Ababa, Ethiopia
Tel: - 0912008633
0916665292
E-mail:- dlopiso@yahoo.com

Appendix III: Patient information form (Amharic version)

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ

ሀ. የጥናቱ ዓላማ ፡- ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ቀዶ ጥገና በተደረገ አካላት ላይ የሚያመጡትን ችግርና ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑት መድሃኒቶች ለመምረጥ ነው፡፡

ለ. ፈቃደኝነት፡- እርስዎንና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ለሚቀርብልዎትን መጠይቅ ምላሽ ከሰጡ በኋላ የቁስል ናሙና እንዲሰጡ ይጠየቃሉ::

ሐ. ሊያደርስ የሚችለው አደጋ፡- የለም ሆኖም ናሙናው በጥንቃቄ ካልተወሰደ መጠነኛ ጉዳት ሊደርስበት ይችላል፡፡

መ. የሚያገኙት ጥቅም ፡-በሽታ አምጪ ህዋሳት በላቦራቶሪ መኖራቸው ከተረጋገጠ በኋላ ተገቢውን መድሃኒት እንዲወስዱ ውጤቱ ለሀኪምዎ ተልኮ መድኒኑቱን በሀኪምዎ ትዕዛዝ ይሰጥዎታል፡፡

ሠ. ሚስጥራዊነት፡- የእርስዎ የግል መረጃ በሙሉ ሚስጥራዊነቱ የተጠበቀ ይሆናል፡፡

ረ. ውጤቱን ስለመጠቀም፡- ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይዳፋል፡፡ ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

አድራሻ

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድርሻ ይጠቀሙ ፡

የዋናው ተመራማሪ አድራሻ

ሎጲሶ ደሳለኝ

ህክምና ፋኩሊቲ አዲስ አበባ ዩኒቨርስቲ ማይክሮባይሎጂ፣ ኢሚዮኖሎጂና ፓራሳይቶሎጂ ት/ት ክፍል፡፡

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 0912 008633

0916 665292

ኢሜይል፡ - dlopiso@yahoo.com

Appendix IV: Consent form (English version)

(To be translated in to the patient's language**)**

I, the undersigned, confirm that, I give consent to participate in the study with a clear understanding of the objectives and conditions of the study.

I----------hereby give my consent for giving the requested information and wound swab specimen because the proposal has been explained to me in the language I understand.

Name of the patient---------- Patients signature----------

Date----------

Name of the researcher---------- researchers signature----------

Date----------

Appendix V: Consent form (Amharic version)

የፈቃደኝነት መጠየቂያ ቅፅ

እኔ /ተማሪ/አቶ/ወ.ሮ/ወ.ት _____ የተባልኩ ቀዶ ህክምና የተደረገልኝ ህመምተኛ በቁስል ላይ በሽታ አምጪ የሆኑትና ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስመመርመር በሚረዳው ምርምር ስምርምሩ የሚያስፈልጉ መጠይቆችን መረጃና የቁስል ናሙና ስመስጠት በሚገባኝ ቋንቋ የተብራራልኝ በመሆኑ በጥናቱ ስመሳተፍ በሙሉ ፍቃዴ የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የህመምተኛው ስም _____የህመምተኛው ፊርማ _____

ቀን _____

የተመራማሪው ስም_____የተመራማሪው ፊርማ _____

ቀን _____

Appendix VI: Gram staining technique

Method

1. After making a smear, leave the slide in a safe place for the smear to air-dry then fixed by heat, alcohol, or occasionally by other chemicals.
2. Cover the fixed smear with crystal violet stain for 30–60 seconds.
3. Rapidly wash off the stain with clean water. *Note*: When the tap water is not clean, use filtered water or clean boiled rainwater.
4. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.
5. Wash off the iodine with clean water.
6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.

   Caution: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.
7. Cover the smear with neutral red stain for 2 minutes.
8. Wash off the stain with clean water.
9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.
10. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

Appendix VII: Preparation of turbidity standard

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well.

   ***Caution*:** Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and never add the water to the acid.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water.
3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.
4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

Appendix VIII: Modified kurby-bauer susceptibility testing technique.

Method

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.
3. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately $60^0$to ensure even distribution.
4. With the Petri dish lid in place, allow 3–5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.
5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated.

   ***Note*:** The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35^0C for 16–18 h (temperatures over 35^0C invalidate results for oxacillin).
7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Appendix IX: Catalase test procedure

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required

Hydrogen peroxide, 3% H_2O_2 (10 volume solution)

Method

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

   Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.
3. Look for immediate bubbling.

Results

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Appendix X: Coagulase test procedure.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Required

EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.
2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar. Suspensions, and mix gently. Look for clumping

3. Add a loopful (not more) of plasma to one of the organisms within 10 seconds.

No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Results

Clumping within 10 secs . . . . . *S. aureus*

No clumping within 10 secs . . . . . .No bound coagulase

Appendix XI: Oxidase test procidure

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be. If the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Required

Oxidase reagent freshly

Method (fresh reagent)

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of *freshly* prepared oxidase reagent.
2. Using a piece of stick or glass rod (not anoxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.

Results

Blue-purple colour . . . . . Positive oxidase test (within 10 seconds)

No blue-purple colour . . . . . Negative oxidase test (within 10 seconds)

***Note*:** Ignore any blue-purple colour that develops after 10 seconds.

11. DECLARATION

The work provided in this thesis is the researcher's own original research work and has not been submitted elsewhere for any other degree or qualification.

M.Sc candidate: Lopiso Dessalegn Tirore (B.Sc).

Signature----------

Date of submission----------

Addis Ababa, Ethiopia

The work provided in this thesis is the researcher's own work, and we confirm that the research has been conducted under our supervision and completed as per the conditions of the technical and ethical requirements needed.

Advisor: Dr. Solomon Gebre-Selassie (MD, M.Sc).

Signature ----------

Date of submission ----------

Addis Ababa, Ethiopia