

**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES**



ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF COMMON BACTERIA ISOLATED FROM WOUND INFECTION IN PAEDIATRIC SURGICAL PATIENTS AT YEKATIT 12 HOSPITALS, ADDIS ABABA, ETHIOPIA.

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Addis Ababa, Ethiopia
June, 2017

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List of abbreviations

AST-----	Antimicrobial Susceptibility Test
BHS-----	Beta hemolytic <i>Streptococcus</i>
CDC-----	Center for Disease control
CLSI-----	Clinical and Laboratory Standards Institute
CoNS-----	Coagulase negative <i>Staphylococcus</i>
HAI-----	Hospital Acquired Infections
ICU-----	Intensive Care Unit
IQR-----	Interquartile range
MRSA-----	Methicillin Resistant <i>Staphylococcus aureus</i>
MSSA-----	Methicillin Susceptible <i>Staphylococcus aureus</i>
NLF-----	Non lactose fermenters
PBP- -----	Penicillin binding protein
QC-----	Quality control
SOPs -----	Standard Operating Procedures
SPP-----	Species
SPSS -----	Statistical Package for the Social Sciences
SSI- -----	Surgical Site Infection
WHO -----	World Health Organization

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Operational definition

Clean wound: - a superficial wound produced by uncontaminated sharp objects, electively, surgical procedure or by accident.

Contaminated wound: - presence of microorganism in a wound due to unsterile condition.

Dirty wound: - an external wound primarily untreated cut or tear of the skin, easily susceptible to infection as result of exposure to unsanitary condition.

Co-morbidity:- is the presence of one or more additional disease or disorders co-occurring with a [that is, concomitant or concurrent] primary disease or disorders.

Methicillin Resistant Staphylococcus aureus: - is a bacteria that is resistant to methicillin antibiotics.

Multidrug resistance :- Bacteria those are resistant to antimicrobials for a minimal of two or more of classes of antibiotics tested.

Resistant :-bacterial isolates that are not inhibited by the usually achievable concentrations of antimicrobial agent.

Susceptible :-bacterial isolates that are inhibited by the usually achievable concentrations of antimicrobial agent.

Abstract

Background: Wound infections contribute significantly to morbidity and mortality in surgical patients. Factors that increase the risk of wound infection include patient conditions such as; age, obesity, malnutrition, smoking, and the state of the wound which includes nonviable tissue in the wound, foreign bodies, long surgical procedures, and others. However microorganisms are the major causes with bacteria being the most prevalent. Severe and poorly managed infections can lead to gas gangrene and tetanus which may cause long-term disabilities. Bacterial wound infections are a common finding in open injuries..

Objective: The study aimed to identify bacteria that cause wound infections and to determine their antimicrobial patterns in the pediatric surgical wards at Yekatit 12 Hospital, Addis Ababa, Ethiopia.

Method: A cross sectional study was conducted from December 2016 to April. A total 150 clinical specimens were collected from study participant. All wound samples were cultured on Blood agar and MacConkey agar. All culture positive samples were characterized by gram stain and biochemical tests using the standard procedure. Antimicrobial susceptibility test was performed using Kirby-Bauer method. All demographic, laboratory and risk factors data obtained were entered and analyzed using SPSS version 20.

Result: The burden of wound infection among pediatric patients was 123 (82%). Of which 90 [65.7%] were gram positive and 47 [34.3%] were gram negative bacteria. *Staphylococcus aureus* 79[52.7%] was the most prevalent followed by *Pseudomonas aeruginosa* 26 [17.3%] and *Proteus* spp 14 [9.3%]. Patients who had mixed infections were 8.67% of the total participants. *Staphylococcus aureus* was highly sensitive to ceftriaxone but resistant to ceftazidime [91.1%]. Methicillin Resistant *Staphylococcus aureus* formed 50.6% of the *Staphylococcus aureus* isolates. Beta hemolytic *Streptococcus* was highly sensitive to amoxicillin clavulanate and resistant to cefuroxime. *Escherichia coli* was sensitive to ciprofloxacin but resistant to amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem and ceftazidime. *Klebsiella*spp was sensitive to all the antibiotics that were tested. *Proteus mirabilis* was sensitive to all the antibiotics except ceftazidime. The Non lactose fermenters were only sensitive to imipenem, ciprofloxacin and cefoxitim. *Pseudomonas aeruginosa* was highly sensitive to ciprofloxacin and imipenem but less sensitive to ceftazidime and resistant to ceftriaxone. Ceftriaxone, cefuroxime, flucloxacillin and amoxicillin clavulanate were widely used beside other antibiotics for either prophylaxis or treatment of the wound infections.

Conclusion: The prevalence of wound infection remains high despite wide use of antibiotics in the paediatric surgical wards. Resistance to new antibiotics like imipenem was observed.

Key terms: Bacterial isolates, antimicrobial susceptibility pattern, surgical site infection,

1. Introduction

1.1 Background

A wound results following disruption of the skin which can be intentional or accidental. Wound infections cause a burden of disease and morbidity for both the patient and the health services. To the patient it causes pain, discomfort, inconvenience, disability, financial drain, and even death due to complications such as septicemia. It causes financial strain on the health services due to the required high cost of hospitalization and management of the patients. [1]

A number of factors contribute to wound infection; however microorganisms are the major cause with bacteria being the most prevalent [2]. Early recognition of wound infection and appropriate management is important. Antibiotic therapy and surgical management are the cornerstone measures whereby antibiotics offer adjuvant treatment. Wound infection can be caused by single bacteria or multiple microorganisms. Surgical site infections are the second most common cause of nosocomial infections after urinary tract infections [3, 4]. Most surgical site infections occur in ambulatory patients after discharge from the hospital and therefore beyond the hospital infection control surveillance programs [3].

Prolonged preoperative hospital stay and exposure to diagnostic procedures has been associated with increased rate of SSI. In clean surgical procedures, *Staphylococcus aureus* is the most common pathogen while *Pseudomonas aeruginosa* is the most common gram negative bacilli. A number of studies indicate an increase in antibiotic resistant microorganisms in surgical patients. Resistant bacteria causes severe infections that are expensive to diagnose and difficult to treat. The mechanism by which resistance develops is complex and can result in multi-drug resistant bacterial strains due to simultaneous development of resistance to several antibiotics. Determination of local bacterial sensitivity patterns to antibiotics is important in providing a guide for antibiotic selection [2].

There are factors that increase the risk of wound infection which include patient characteristics such as; age, obesity, malnutrition, endocrine and metabolic disorders, smoking, hypoxia, anaemia, malignancies and immune suppressants [5]. Other factors are the state of the wound which includes nonviable tissue in the wound, foreign bodies, tissue ischaemia, and formation of haematomas, long surgical procedures, and contamination during operation, poor surgical techniques, hypothermia and prolonged pre-operative stay at the hospital. [2]

Wound infections can be prevented by restoring blood circulation as soon as possible, relieving pain, maintaining normal body temperature, avoiding tourniquets, performing surgical toilet and debridement of the wound as soon as possible, administration of antibiotic prophylaxis for deep wound and high risk infections [5]. High risk wounds include contaminated wounds, penetrating wounds, abdominal trauma, compound fractures, wounds with devitalized tissue; high risk anatomical sites such as hands and feet. Antibiotic prophylaxis should be started two hours before the surgical procedures. Establishment of the causative microorganism is important and treatment should be initiated based on the bacterial sensitivity patterns. Topical silver dressings have been used to treat infected wounds however; there is no evidence for their efficacy due to multiple microbial aetiologies [6]. To achieve optimum antimicrobial therapy, the biofilm load should be reduced to enhance drug concentration at the wound site [7].

Bacterial wound infections are a common finding in open injuries. Severe and poorly managed infections can lead to gas gangrene and tetanus which may cause long-term disabilities [5, 7]. Chronic infection can cause septicemia or bone infection which can lead to death. Sepsis associated encephalopathy increases morbidity and mortality especially in the ICU patients [8].

1.2 Statement of the problem

A wound results following disruption of the skin which can be intentional or accidental. Wound infections cause a burden of disease and morbidity for both the patient and the health services. To the patient it causes pain, discomfort, inconvenience, disability, financial drain, and even death due to complications such as septicemia. It causes financial strain on the health services due to the required high cost of hospitalization and management of the patients. [1]

Septic wounds are a common cause of morbidity. Despite improvement in the practice of Medicine and attempts to provide aseptic conditions in the surgical wards, the incidences of Wound infection are increasing. Management of wound infection remains a challenge in the surgical areas with the increasing resistance to antimicrobials [9]. Antimicrobial resistance can lead to complications which depending on severity can cause disability or death and increased cost of hospitalization and management. In children this impacts negatively on the quality of life at a tender age. The antibiotic sensitivity patterns have not been studied fully especially in the surgical patients at Yekatit 12 Hospital. It has been noted that inappropriate use of antibiotics can lead to development of resistance to antibiotics [10]. There are factors that increase the risk of wound infection which include patient characteristics such as; age, obesity, malnutrition, endocrine and metabolic disorders, smoking, hypoxia, anaemia, malignancies and immune suppressants [5]. Other factors are the state of the wound which includes nonviable tissue in the wound, foreign bodies, tissue ischaemia, and formation of haematomas, long surgical procedures, and contamination during operation, poor surgical techniques, hypothermia and prolonged pre-operative stay at the hospital. [2]

Bacterial wound infections are a common finding in open injuries. Severe and poorly managed infections can lead to gas gangrene and tetanus which may cause long-term disabilities [5, 7]. Chronic infection can cause septicemia or bone infection which can lead to death. Sepsis associated encephalopathy increases morbidity and mortality especially in the ICU patients [8].

1.3 Significance of the study

Knowledge of the distribution of antimicrobial susceptibility pattern of common bacteria that cause wound infection in the pediatric surgical patients provide information on the frequency of bacteria among pediatric surgical patients, in addition it add information for clinicians on the safe and effectiveness of empirical therapies for the development of rational prescription programs. Furthermore, it provide information for policy makers for controlling surgical site infections among pediatric patients, monitoring usage of antibiotics and their level of resistance among such patients as well as in the community.

2. Literature review

This literature analyses relevant studies that had been carried out in different parts of the world with reference to bacteria that cause wound sepsis, the sensitivity patterns and the antibiotics that are used in surgery.

2.1 Prevalence of wound infection

Worldwide, there is increasing prevalence of MRSA wound infections that affect the entire community; it is estimated at 60.1% while MSSA is at 30.2% [11, 12]. A study done at a teaching hospital in Sudan found the prevalence of aerobic hospital acquired wound infection post-surgery to be 25.23% [13]. Other studies have found that before the introduction of routine use of antibiotic prophylaxis clean wound infection rates were 1-2%, for clean contaminated wounds 6-9%, contaminated wounds rates were 13-20% while for dirty wounds rates were 40% [14, 15, and 16].

In Algeria, a study reported a decrease in HAI prevalence following introduction of antibiotic prophylaxis from 9.0% in 2001 to 4.0% in 2005 [17]. In Nigeria, a cumulative incidence of 23.6 per 100 operations was reported [18]. The incidences by wound classification ranged from 6.5% to 20.2% in clean wounds, 10.1% to 23.8% in clean-contaminated wounds, 13.3% to 51.9% in contaminated wounds and 44.1% to 83.3% in dirty wounds. In Tanzania, 19.4% of patients developed SSI post-surgery[19]. In Uganda the prevalence of SSI was found to be 10%, 9.4% of whom being in women who underwent caesarian section [20]. A study done in Kenya found the prevalence of wound infection among women who had undergone caesarian section to be 19% [22]. In Ethiopia, the prevalence of SSI 21% based on clinical criteria and 38.7% based on bacteriological criteria in patients who had undergone abdominal surgery [21].

2.2 Type of bacteria that cause wound infections

It has been found that wound infections are caused mostly by *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus epidermidis*[23]. Other studies have shown that the prevalence of Methicillin resistant *Staphylococcus aureus* which were initially hospital acquired is steadily increasing in the community [24]. The common bacteriological findings in chronic wounds are found on the skin as normal flora, in faeces, water and in the environment [7]. Evidence suggests that chronic wounds result due to a combination of structural damage and establishment of a chronic biofilm infection which stimulates host immune

response that cause further damage generating a vicious cycle. Aerobic bacteria are commonly encountered in surgical wound infections the prevalence was higher for: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus epidermidis* and *Enterococcus faecalis*. Most of the *Staphylococcus aureus* are MRSA [23]

.A study carried out in Algeria found the bacteria causing wound infection were: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* in decreasing frequency. Another study in Senegal reported *Enterobacter cloacae*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [25]. A report on HAI cumulative incidence in surgical patients showed the following distribution in decreasing frequency: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [26].

A study in Kenya gave a cumulative incidence of 19% SSI post Caesarian section delivery [22]. A study carried out in Tanzania and Ethiopia found *Staphylococcus aureus* and *Escherichia coli* to be the major cause of SSI with others being, *Klebsiella* spp, *Enterococcus* spp, *Pseudomonas* spp and *Enterobacteriaceae* [19, 21, 27]. In the Central Republic of Africa, a survey in the surgical orthopedic patients showed that the common organisms were *Staphylococcus aureus* and *Proteus mirabilis* [28]. In Nigeria the prevalence of the bacteria that cause SSI in paediatric patients was found to be higher for *Escherichia coli* followed by *Klebsiella* spp, *Pseudomonas* spp, *Staphylococcus* spp, and *proteus* spp [18]. A study done in Kenya at the Kenyatta National Hospital, orthopedic ward showed the prevalent bacteria in SSI are: *Staphylococcus aureus*, *Enterobacteriaceae*, *Streptococcus faecalis*, *Streptococcus pyogenes*, and *Pseudomonas* spp [29]. The pathology resulting from *Staphylococcus aureus* and *Pseudomonas aeruginosa* polymicrobial wound infections is of great importance due to their ubiquitous nature, increasing prevalence, growing resistance to antimicrobial agents, and ability to delay healing [46]. No study has been done in the pediatric surgical patients at the Yekatit 12 Hospital, to identify the bacteria that cause both SSIs and chronic wound infections.

2.3 Antimicrobial susceptibility of bacteria causing wound infection

Most *Pseudomonas aeruginosa* isolates are sensitive to piperacillin, ceftazidime, and imipenem, a gradual emergence of resistance to β -lactams. A few isolates are resistant to netilmicin, and there is decreased ciprofloxacin activity [23]. *Staphylococcus aureus* is the most prevalent in surgical wound infections. MRSA forms 54.4% of *Staphylococcus aureus* isolates [23]. Amoxicillin-clavulanate, cefazolin, and imipenem

have shown in vitro activity against more than 60% of the MRSA isolates, and are considered resistant to all β -lactams, cephalosporins, β -lactam- β -lactamase inhibitor combinations, and carbapenems. *Enterococci*, causes frequent surgical wound infections and almost all of the *Enterococcus faecalis* isolates are susceptible in vitro to glycol peptides and gentamicin. However, some are resistant to cefazolin and good in vitro sensitivity was shown by amoxicillin-clavulanate and imipenem. Gram-positive anaerobes are sensitive to most drugs while gram-negative anaerobes are resistant to ampicillin and cefazolin. *Streptococcus pyogenes* was found to be resistant to macrolides [30]. No resistance to macrolides was reported in Indonesia, Australia, Belgium, the Netherlands and United Kingdom. *Streptococcus pyogenes* showed resistance to all beta lactams except cefaclor. In chronic wound infections, once a biofilm has been established, causes the bacteria to resist antibiotics and other antimicrobials like silver sulphadiazine even the host defense [7].

The biofilm promotes higher mutation rates hence resistance to antibiotics. A study carried out at Kenyatta National Hospital showed Gentamicin was active against *Escherichia coli*, *Pseudomonas*, *Klebsiella* spp, *Enterococci*, *Alcaligenes* spp, *Citrobacter freundii*, *Serratia* spp and *Acinetobacter baumannii* [29]. Amoxicillin-clavulanic had significant activity against *Enterobacteriaceae*, *Streptococcus pyogenes*, *Streptococcus faecalis* except *Klebsiella* which showed activity of 44%. Piperacillin, ticarcillin and tazobactam showed good activity against *Pseudomonas* spp, and *Enterobacteriaceae*; however *Proteus* spp, *Escherichia coli* and *Klebsiella* spp showed resistance. Ceftazidime had 80% activity against most organisms. Cefuroxime had moderate activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter* spp, *Proteus* spp and *Klebsiella* spp, while *Streptococcus pyogenes* and *Citrobacter* spp showed high sensitivity. Ceftriaxone showed activity against *Enterobacteriaceae* but resistance was seen with *Pseudomonas* spp. Ciprofloxacin had good activity against *Enterobacteriaceae*, moderate activity Against *Staphylococcus aureus* and inactive-against *Streptococcus faecalis*

A study done in Uganda, the prevalence of *Staphylococcus aureus* was 59.4% in the inpatient with an average antibiotic susceptibility of ampicillin and higher, chloramphenicol but low for ciprofloxacin and erythromycin [47]. In a study carried out in Northeast Ethiopia, *Escherichia coli* showed high resistance to erythromycin and amoxicillin but high sensitivity to nitrofurantoin, norfloxacin, gentamicin, and ciprofloxacin [49]. In Khartoum, resistance was high for amoxicillin, cefuroxime, ceftriaxone, ciprofloxacin, amoxicillin clavulanate and ceftazidime [50]. In a study on in vitro selection of resistance in *Escherichia coli*, frequencies for mutations for levofloxacin and ciprofloxacin were less than 10-11 at peak concentrations [51].

In a study on natural antibiotic susceptibility, *Klebsiella* spp were found to be sensitive to penicillins, cephalosporins, quinolone, trimethoprim and cotrimoxazole [52]. An evaluation of antimicrobial susceptibility of *Klebsiella* spp found a sensitivity of 50-100% for ciprofloxacin [53]. The infections can therefore be treated with ceftazidime, cefepime, ampicillin/sulbactam, levofloxacin and meropenem [54]. A study on the trends in the susceptibility of *Proteus mirabilis* showed there is a steady increase to ciprofloxacin resistance [55]. *Proteus mirabilis* causes different infections and imipenem has shown the highest activity followed by amikacin and ceftazidime [56, 57]. In a study on antimicrobial sensitivity of *Pseudomonas aeruginosa*, high resistance was observed with piperacillin, ticarcillin, ceftazidime, imipenem, amikacin and cotrimoxazole 66.6%. Cefotaxime showed a susceptibility of 83.3% and an intermediate resistance was seen with ciprofloxacin [58]. In another study, isolated pathogens were resistant to amikacin, ciprofloxacin and ceftriaxone [59]. CoNS are of low virulence but are increasingly recognized as clinically significant [62]. The risk factors include foreign bodies such as indwelling devices and immunosuppressants. Resistance to semisynthetic penicillins has been observed in more than 80% of the cases. It is resistant to multiple antimicrobial agents used to treat *Staphylococcus aureus*. High resistance rate has been observed with penicillin G, erythromycin and oxacillin [63]. Clindamycin, cotrimoxazole and ciprofloxacin have shown medium resistance whereas rifampicin, ceftizoxime and gentamicin have shown low resistance. Ampicillin is the drug of choice for monotherapy treatment of susceptible *Enterococcus faecalis*, combination therapy with a cell wall active agent provides more synergy [64].

In a study, the isolated strains of *Enterococcus* were absolutely sensitive to vancomycin, teicoplanin and nitrofurantoin. Penicillins showed 96% sensitivity, ciprofloxacin 43% and tetracycline 28% [65].

2.4 Management of septic wounds with antibiotics

It has been noted that inappropriate use of antibiotics can lead to development of resistance to antibiotics [10]. Inappropriate use includes; no indication, incorrect choice, incorrect application of drugs and divergence from institutional guidelines. Antibiotic prophylaxis has been shown to significantly reduce rate of wound infection [31, 32]. A ground was laid for antibiotic prophylaxis as early as 1960s [33, 34]. However, a study done in the United Kingdom showed there was no benefit in using flucloxacillin prophylaxis in patients with open fracture [35]. A systematic review done in New Jersey, found that short course of first generation prophylaxis administered as soon as possible after the injury provided adequate prevention against wound infections [36]. A national advisory for prophylaxis recommends use of cefazolin, cefuroxime or vancomycin for knee, hip, cardiothoracic or vascular surgery prophylaxis while for the colon,

aminoglycosides, macrolides or metronidazole should be considered [37]. Even though antibiotic use in clean wounds is not clearly indicated, infection rates of 40% post-surgery have been reported [16]. Selection of antibiotics should be based on the infecting organism, tissue penetration ability, low toxicity and absence of allergies. In a study carried out in Ireland, the antibiotics that are mostly used are combinations of penicillins and beta lactamase inhibitors and macrolides [38].

The rates of using second generation cephalosporins use was 6% while third generation cephalosporins were 13%. In another study, cephalosporins use for antibiotic prophylaxis was at 67% [39]. A systematic review found that for MRSA eradication, linezolid performed better than vancomycin however amoxicillin clavulanic offered better prophylaxis against MRSA infections [40, 41]. *Klebsiella pneumonia* responds well to polymixin combinations and aminoglycosides [42]. A study carried out in Clayton, Australia; found that flucloxacillin continuous infusion offered good activity against wound infections with MSSA [43].

Antimicrobial treatment of non-healing polymicrobial and/or clinically infected wounds should be targeted to cover most of the potentially synergistic aerobic or facultative and anaerobic microorganisms and not simply target specific common pathogens e.g. *Staphylococcus aureus* and *Pseudomonas aeruginosa*[44]. The International working group on the diabetic foot recommends intravenous or oral use of empirical broad spectrum antibiotics in deep foot infections. The regimens that can be used include; ampicillin/ salbactam, ticacillin/clavulanate, amoxicillin/ clavulanate clindamycin and a quinolone, a second or third generation cephalosporins with a quinolone or metronidazole with a quinolone” [45]. A study carried out at Cardiff, Wales University found that antibiotic prescribing for wound infection was based on expert opinion and not scientific facts [9]. The antibiotics that were commonly used included; flucloxacillin, co-amoxi-clav, cefaclor, cefalexin, erythromycin, trimethoprim, metronidazole and ciprofloxacin. Flucloxacillin, coamoxiclav and metronidazole were mostly prescribed.

3. Objectives

3.1 General objective

To identify the bacteria that cause wound infection and to determine their sensitivity patterns in the pediatric surgical wards of Yekatit 12 Hospitals, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of bacteria from surgical site infection among pediatrics.
- To determine antimicrobial sensitivity patterns of the isolated bacteria.

4. Materials and methods

4.1. Study area

The study was conducted, at Yekatit 12 Hospitals Addis Ababa, Ethiopia. the hospital is provide various services such as OPD, surgery, family planning, ART, laboratory, pharmacy, ANC and delivery dental and neonatal care.

4.2 . Study Design and Period

A cross-sectional study was conducted from December 2016 to April 2017.

4.3 . Population

4.3.1. Source Population

All patients who developed surgical wound infection at yekatit 12 hospital Addis Ababa Ethiopia during the study period.

4.3.2. Study Population

All postoperative surgical pediatric patients, aged 13 years and below admitted in the surgical wards were considered. The parent or guardian was approach to give any other relevant information about the patient.

4.4. Eligibility

4.4.1. Inclusion Criteria

- a surgical patient who have a clean wound that was at least 72 hours old post-surgery and 24 hours old for contaminated and dirty wounds,
- whose parents or guardian consented to participate in the study were include
- Patients who was given ascent to participate in the study.

4.4.2. Exclusion Criteria

- Patients do not on antibiotics within the last 2 weeks
- All children above thirteen years old.

4.5. Sample Size

The sample size is calculated based on single population proportion sample size estimation as shown below by taking a value of P as 11% from a previous study [73].

$$n = \frac{Z^2 P [1 - P]}{d^2}$$

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion [P = 0.11], and d = precision [d = 0.05], Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

$$\begin{aligned} \text{Thus; } N &= \frac{1.96^2 \cdot 0.11 \cdot [0.89]}{0.05^2} \\ &= 150.4 \\ &= 150 + 15\% \text{ non-respondent} \end{aligned}$$

Where: N=sample size, Z= Standard normal deviate at 95% confidence interval. P= Proportion of target population with infected wounds, q= 1-p, d= degree of freedom, Z=1.96, p=0.11, q=1-0.11=0.89. d=0.05.

Therefore the sample size was: 150 + 15% non-respondent

4.6. Sampling Method and procedure

A total of 150 study participants were recruited using convenient sampling technique.

4.7. Study Variable

4.7.1. Dependent Variable

- Bacterial isolates from surgical site wound culture
- Antimicrobial susceptibility pattern.

4.7.2. Independent Variable

- Age, sex, , duration of wound healing, hospital stay, educational status

4.8. Data collection

Permission was sought from the consultant in charge of the ward before commencing on data collection. Two research assistants helped in the data collection; a clinical officer who help in getting wound swabs during wound dressing and a laboratory technician who carried out the microbiological work. Data was collected using a well-structured questionnaire [Appendix III]. The parent or guardian of the child was interviewed to get information on the biodata and the data on the prescribed antibiotics was taken from the patient records by the researcher. Isolation, identification and the antimicrobial sensitivity patterns were done in yekatit 12 hospital microbiology laboratory [Appendix IV][74]. The information on sensitivity patterns were filled in the questionnaire after the culture and sensitivity patterns is available. All data was kept under lock and key, with accessibility limited to the researcher only.

4.9. Specimen collection

The specimen was collected from each wound using sterile cotton swabs after interviewing the parent or guardian. It was collected before wound cleaning, label and deliver to the laboratory within one hour for bacteriological examination. A precaution was taken to avoid cross contamination at all stages.

4.9. Isolation and identification

At the laboratory, the culture media was prepared and poured in petri dishes up to a depth of 4mm then allowed to cool. The inoculums were applied to a small area then spread using a sterile loop of wire to provide for single colonies. Blood agar, Chocolate blood agar and MacConkey media were used. All inoculate plates were labeled and incubated at 37°C for 24 hours for the organisms to grow. Identification of the bacteria was done by using the recommended standard procedures [Appendix IV][74].

4.10. Antimicrobial sensitivity test

Kirby and Bauer Disc Diffusion sensitivity test was used to determine sensitivity patterns [Appendix 3]. Appropriate sensitivity discs were placed on the media and the drug activity was shown by zones of inhibition of growth around the discs. The diameter of the zones was compared to a standard and categorize as resistant or sensitive [Appendix IV][74].

The drugs that were tested include: amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem, ciprofloxacin, cefoxitin, cloxacillin and ceftazidime. To determine the prescribing patterns of the different antibiotics, an evaluation of the participants' medical records was done by where the information on the prescribed antibiotics was obtained.

4.11. Quality Control and data quality Assurance

Standard Operating Procedure[SOP]was strictly followed verifying that media meet expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination will be performed. QC will be performed to check the quality of medium. Each new lots was quality controlled before use by testing the *E. coli ATCC 25922* and/or *Staphylococcus aureusATCC25923* standard strains. A questioner was checked for its completeness, readability and clearness using pretest that was delivered for 5% of participants.

Socio-demographic characteristics of the patient was collect appropriately using questioner after getting consent form which was prepared carefully. Samples were collected in accordance with SOPs and. Culture results was recorded carefully before entry to statistical tool. Before analysis the data was double checked.

4.12. Statistical analysis and interpretation

The data was entered and double checked before analysis. Then the data was exported to SPSS version 20 for analysis. The descriptive statistics [mean, percentages or frequency] was calculated. The bi-variant logistic regression analysis was used to seen the relation between dependent variable and independent variables. Finally, the result was presented in words, charts, and tables.

4.13. Ethical considerations

The study was approved by “Department Research and Ethical Review Committee[DRERC]” of the Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants within the study period. Those patients who were give informed consent was selected and enrolled as the participants of the study. A patient result was communicated to the attending physicians. Information obtained at each course of the study was keep confidential.

4.14. Dissemination of the result

The finding of this study will be presented and submitted to Addis Ababa University, yekatit 12 hospitals. It will also be presented for scientific community elsewhere and manuscript will be submitted to peer reviewed national or international journal and it will be presented in relevant workshops, seminars and scientific conferences.

5. Result

5.1. Study subjects

There were 150 children admitted at Yekatit 12 pediatric surgical units who were recruited in the study. The mean age of the participants was 5.3 years. Approximately one-half [51.3%] of the participants were below 5 years of age [Table 1]. There were 78 [52%] males and the ratio of male-to-female patients was 1: 1.1. Sixty two percent of the participants were primary school children the rest were preschool children. Burns and accidents were the most common causes of wounds and most cases were found within the burns wards and orthopedics wards.

Table 1: Baseline characteristics of study subjects at Yekatit 12 Pediatric Surgical Units, 2017

Variable	Frequency [n]	Percent [%]
Age group		
0-4 years	77	51.3
5-9 years	49	32.7
10-13 years	24	16.0
Sex		
Male	78	52.0
Female	72	48.0
Attending school		
Yes	93	62.0
No	57	38.0
Ward admitted to		
Burns ward	84	56.0
Orthopedics wards	37	24.7
Surgical wards	23	15.3
Burns unit	6	4.0
Cause of wound		
Burns	88	59.9
Accident	34	23.1
Surgical	21	14.3
Bites	7	7
Total	150	100%

5.2. Duration of wound and hospitalization

The median [IQR] durations of hospitalization and wound healing among pediatric surgical patients were 23 days [Table 2]. Fifty three [35.5%] patients had been hospitalized between 8 and 29 days, and the duration of wound healing for 64 [42.7%] patients was between 8 and 29 days. Most patients 137 [91.3%] had no other illness beside the primary surgical diagnosis.

Table 2: Duration of wound healing and hospital stay among pediatrics patients at Yekatit 12, Hospital, 2017

Variable	Frequency [n]	Percent [%]
Hospital stay		
1-7 days	31	20.7
8-29 days	53	35.3
30-59 days	29	19.3
60-89 days	14	9.3
90 days and above	23	15.3
Wound duration		
1-7 days	25	16.7
8-29 days	64	42.7
30-59 days	23	15.3
60-89 days	14	9.3
90 days and above	24	16.0
Co morbidity		
Yes	13	8.7
No	137	91.3
Total	150	100%

5.3. Bacteriology of wound infection

The prevalence of bacteria causing wound infection is summarized in Table 3. Out of 150 participants, 110 [73.3%] of them had wound infection caused by bacterial agents. *Staphylococcus aureus* was the most common bacteria which causes wound infection accounting, [52.7%], followed by *Pseudomonas aeruginosa*, *Proteus mirabilis*, and coagulase negative *Staphylococcus*, *Beta hemolytic Streptococcus*, *Klebsiella* spp, *Non lactose fermenters*, and *Enterococcus faecalis*. Thirteen [8.7%] patients had mixed infections; 12 [8%] had two bacteria causing wound infection, 5 [3%] had *Staphylococcus aureus* and *Pseudomonas aeruginosa*, 3 [2%], had *Staphylococcus aureus* and *Proteus mirabilis*, 2 [1.3%], had

Staphylococcus aureus and *Klebsiella* spp, 1 [0.7%], had *Staphylococcus aureus* and Beta hemolytic streptococcus, 1 [0.7%], had *Proteus mirabilis* and *Pseudomonas aeruginosa*. One patient had three bacteria, namely; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*.

Table 3: Bacteriology of wound infection among pediatric patients at Yekatit 12, Hospital, 2017

Lists of isolates	Frequency [n]	Percentage [%]
<i>Staphylococcus aureus</i>	79	[52.7%]
<i>Pseudomonas aeruginosa</i>	26	[17.3%]
<i>Proteus mirabilis</i>	14	[9.3%]
BHS	5	[3.3%]
CoNS	5	[3.3%]
<i>Klebsiella</i> spp	3	[2.2%]
<i>Escherichia coli</i>	2	[1.3%]
NLF	2	[1.3%]
<i>Enterococcus faecalis</i>	1	[0.7%]
No growth	27	[18%]
Total	164	100%

5.4. Antimicrobial susceptibility patterns

5.4.1. Antimicrobial susceptibility of gram positive bacteria

The antimicrobial sensitivity patterns for *Staphylococcus aureus* summarized in table 4. Out of the 79 [52.9%] isolates, highest sensitivity was seen with ceftriaxone, 41 [51.9%], ceftazidime 39 [49.4%], cloxacillin, 37 [46.8%], ciprofloxacin, 37 [46.8%], amoxicillin clavulanate, 34 [43%], imipenem, 30 [38%], cefuroxime, 29 [36.7%]. Ceftazidime, showed the highest resistance. Forty [50.6%] of the cultures showed resistance against ceftazidime and therefore they contained MRSA.

The sensitivity pattern of *Beta Hemolytic Streptococcus* is summarized in table 4. Out of 5 [3.3%], isolates, highest sensitivity was seen with amoxicillin clavulanate, 5 [100.0%], ceftriaxone, imipenem and cloxacillin, cefuroxime, 3 [60.0%], showed the highest resistance.

The antimicrobial susceptibility patterns of coagulase negative *staphylococcus* are summarized in table 4. Out of 5[3.6%] isolates, highest sensitivity was seen with amoxicillin clavulanate, cefuroxime and imipenem, 60%. Ceftriaxone showed resistance of 60% with cloxacillin [80%], showing the highest resistance.

The antimicrobial susceptibility patterns of *Enterococcus faecalis* is summarized in table 4. One [0.7%] strain was isolated, which was sensitive to amoxicillin clavulanate, cefuroxime, ceftriaxone, ciprofloxacin it was resistant to ceftazidime, imipenem and ceftazidime.

Table 4: Antimicrobial susceptibility patterns of gram positive bacteria

Bacterial species	Antibiotic									
	P	CRO	CTX	CIP	CLO	AMO	IMP	CFU	CFT	AUG
<i>S. aureus</i> [79]	S	41 [51.9%]	39[49.4%]	37[46.%]	37[46.8%]	34 [43.0%]	30 [38.0%]	29 [36.7%]	7 [8.9%]	–
	R	38 [48.1%]	40[50.6%]	42[53.%]	42[53.2%]	45 [57.0%]	49 [62.0%]	50 [63.3%]	72[91.1%]	–
<i>Beta hemolytic streptococcus</i> [5]	S	4[80%]	–	–	4 [80%]	5 [100%]	4 [80%]	2 [40%]	–	–
	R	0[0%]	–	–	1[20%]	0[0%]	1[20%]	3 [60%]	–	–
<i>Coagulase negative Staphylococcus</i> [5]	S	2 [40%]	–	–	1 [20%]	3 [60%]	3 [60%]	3 [60%]	–	–
	R	3 [60%]	–	–	4[80%]	2 [40%]	2 [40%]	2 [40%]	–	–
<i>Enterococcus faecalis</i> [1]	S	1 [100%]	0[0%]	1[100%]	–	–	0[0%]	1 [100%]	0[0%]	1 [100%]
	R	0[0%]	1 [100%]	0[0%]	–	–	1 [100%]	0[0%]	1 [100%]	0[0%]
Total [90]	S	48 [53.9%]	39 48.75[%]	38 [47.5%]	42 [47.2%]	42 [47.2%]	37 [41.1%]	35 [38.9%]	7 [8.8%]	1 [100%]
	R	41 [46.1%]	41 [51.3%]	42 [52.5%]	47 [52.8%]	47 [52.8%]	53 [58.9%]	55 [61.1%]	73 [91.2%]	0 [0.0%]

Key; CRO = Ceftriaxone,CTX= Cefoxitim,CIP = Ciprofloxacin,CLO = Cloxacillin,AMO = Amoxicillin, IMP =Imipenem,CFU = Cefuroxime,CFT= Ceftazidime,AUG = Augmentin,S = Susceptible,R = Resistant

5.4.2. Antimicrobial susceptibility of gram negative bacteria

The antimicrobial susceptibility patterns for *Escherichia coli* are summarized in table 5. Out of 2 [1.5%] isolates, highest sensitivity was seen with ciprofloxacin 1 [50%] and resistance was seen with amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem and ceftazidime.

The antimicrobial susceptibility of *Klebsiellais* summarized in table 5. Out of 3[2.2%] isolates, highest sensitivity was seen with ciprofloxacin [100%], amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem, cefoxitin and ceftazidime showed equal sensitivity [66.7%].

The antimicrobial sensitivity patterns of *Proteus Mirabilis* are summarized in table 5. Highest sensitivity was seen with amoxicillin clavulanate, ceftriaxone, imipenem and cefoxitin[78.6%], followed by ciprofloxacin and cefuroxime with ceftazidime showing the highest resistance [64.3%].

The antimicrobial susceptibility patterns of *Non lactose fermenters* are summarized in table 5. Out of 2[1.5%] strains isolated, highest sensitivity was seen with imipenem and ciprofloxacin [100%], and cefoxitim, 50%. Absolute resistance was seen with amoxicillin clavulanate, cefuroxime, ceftriaxone, ceftazidime.

The antimicrobial susceptibility patterns of *Pseudomonas aeruginosais* summarized in table5. Out of twenty six strains isolated, highest sensitivity was seen with ciprofloxacin [92.3%], followed by imipenem [76.9%]. Highest resistance to ceftriaxone [92.3%] was seen followed by ceftazidime [53.8%].

Table 5: Antimicrobial susceptibility patterns of gram negative bacteria

Bacterial species	Antibiotic							
	P	CRO	CTX	CIP	AMO	IMP	CFU	CFT
<i>Escherichia coli</i> [2]	S	0 [0%]	-	1 [50%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
	R	2 [100%]	-	1 [50%]	2 [100%]	2 [100%]	2 [100%]	2 [100%]
<i>Klebsiella spp</i> [3]	S	2[66.7%]	2[66.7%]	3[100%]	2[66.7%]	2[66.7%]	2[66.7%]	2[66.7%]
	R	1 [33.3%]	1 [33.3%]	0 [0%]	1 [33.3%]	1 [33.3%]	1 [33.3%]	1 [33.3%]
<i>Proteus mirabilis</i> [14]	S	11[78.6%]	11[78.6%]	10[71.4%]	11 [78.6%]	11[78.6%]	8 [57.1%]	5 [35.7%]
	R	3 [21.4%]	3 [21.4%]	4[28.6]	3 [21.4%]	3[21.4%]	6 [42.9%]	9 [64.3%]
<i>Non-lactose fermenters</i> [2]	S	0 [0%]	1 [50%]	2[100%]	0 [0%]	2 [100%]	0 [0%]	0 [0%]
	R	2 [100%]	1 [50%]	0 [0%]	2 [100%]	0 [0%]	2 [100%]	2 [100%]
<i>Pseudomonas aeruginosa</i> [26]	S	2 [7.7%]	-	24[92.%]	-	20[76.9%]	-	12[46.2%]
	R	24[92.3%]	-	2[7.7%]	-	6 [23.1%]	-	14[53.8%]
Total [47]	S	15 [31.9%]	14 [73.7%]	40 [85.1%]	13 [61.9%]	35 [74.5%]	10 [47.6%]	19 [40.4%]
	R	32 [68.1%]	5 [26.3%]	7 [14.9%]	8[38.1%]	12 [25.5%]	11 [52.4%]	28 [59.6%]

Key; CRO = Ceftriaxone, CTX= Cefoxitim,CIP = Ciprofloxacin,AMO = Amoxicillin,IMP =Imipenem,CFU = Cefuroxime, CFT= Ceftazidime, S = Susceptible,R = Resistant

5.5. Antibiotic prescription patterns in the pediatric surgical wards

The antibiotics and number of prescribed used to treat surgical wound infection are summarized in Table 6 and 7. Out of the 150 participants, 23 [15.3%] were not prescribed antibiotics. Most patients [41.3%] had a single antibiotic prescribed. The remaining patients received either two or three antibiotics. Eighty six [57.3%] patients received ceftriaxone and 43 [28.7%] received cefuroxime. The other commonly prescribed antibiotics were: flucloxacillin, amoxicillin clavulanate and metronidazole. Less common prescribed antibiotics were meropenem, gentamicin, ciprofloxacin, clindamycin, amikacin, ampiclox, erythromycin, and tetracycline.

Table 6: Number of antibiotics prescribed per patient

Number of antibiotics per patient	Frequency [n]	Percent [%]
1	62	41.3
2	48	32.0
3	17	11.3
None	23	15.3
Total	150	99.9

Table 7: Antibiotics prescribed

Antibiotic	Frequency[n]	Percent [%]
Ceftriaxone	86	57.3
Cefuroxime	43	28.7
Flucloxacillin	28	18.7
Amoxicillin clavulanate	19	12.7
Metronidazole	15	10.0
Meropenem	7	4.7
Gentamicin	3	2.0
Ciprofloxacin	2	1.3
Clindamycin	2	1.3
Amikacin	1	0.7
Ampiclox	1	0.7
Erythromycin	1	0.7
Tetracycline	1	0.7

Table 8: Multiple antibiotic resistance pattern for gram positive bacteria

Isolated gram positive bacteria	R0	R1	R2	R3	R4
<i>S. aureus</i> [79]	27(34.2%)	23(29.1%)	16(20.3 %)	7(8.9%)	6(7.6%)
<i>Beta hemolytic streptococcus</i> [5]	2(40%)	1(20%)	1(20)	1(20)	0
<i>Coagulase negative Staphylococcus</i> [5]	0	2(40%)	1(20%)	0	0

R0- no resistant for any antibiotic ,**R1**-resistant for 1 antibiotic, **R2**- resistant for 2 antibiotics,**R3**- resistant for 3 antibiotics, **R4**-resistant for 4 antibiotics.

Table 9: Multiple antibiotic resistance pattern for gram negative bacteria

Isolated gram negative bacteria	R0	R1	R2	R3	R4
<i>Escherichia coli</i> [2]	0	0	2(100%)	0	0
<i>Klebsiella pp</i> [3]	0	1(33%)	2(66.7%)	0	0
<i>Proteus mirabilis</i> [14]	0	6(42.90)	8(57.1)	0	0
<i>Pseudomonas aeruginosa</i> [26]	24(92.3%)	1(3.8%)	1(3.8%)	0	0

R0- no resistant for any antibiotic ,**R1**-resistant for 1 antibiotic, **R2**- resistant for 2 antibiotics,**R3**- resistant for 3 antibiotics, **R4**-resistant for 4 antibiotics.

6. Discussion

Wound infections are common across all age groups and they cause disease burden both to the patient and the health services [1]. Approximately half of the participants were below 5 years of age and the proportions were almost equal for both sexes. Sixty two percent of the participants were primary school children the rest were preschool children. Burns and accidents were the most common causes of wounds and most cases were found within the burns wards and orthopaedic wards.

The prevalence of wounds that were infected was at 82%, which is higher compared to other studies [13, 14, 15, 16], but similar to others [18]. Despite use of antibiotic prophylaxis, the prevalence still remains high. Factors that could play a role in persistent wound infection are incorrect choice and dose of drugs. *Staphylococcus aureus* was the most common, followed by *Pseudomonas aeruginosa*, *Proteus mirabilis*, Coagulase negative *Staphylococcus*, Beta hemolytic *Streptococcus*, *Klebsiella* spp, Non lactose fermenters, and *Enterococcus faecalis*. This finding is consistent with other studies [23, 25, 47]. *Staphylococcus aureus* was predominant and 50.6% of the isolates were MRSA which is similar to other findings [11, 12, 23, 24]. Polymicrobial wound infection which has shown increasing prevalence, growing resistance to antimicrobial agents, and ability to delay wound healing were seen. Wounds are always colonized by aerobic and anaerobic bacterial and fungal strains mostly belonging to the microbiota of the surrounding skin and external environment [66, 67]. *Staphylococcus aureus* is the most prevalent in wound infections.

There are increased *Staphylococcus aureus* antibiotic resistant strains mostly β -lactam resistant strains such as MRSA [47]. In this study the prevalence was at 52.7%, highest sensitivity was seen with ceftriaxone 51.9%. Cefoxitin, cloxacillin, ciprofloxacin, augmentin, imipenem cefuroxime and ceftazidime showed sensitivity below 50% which is consistent with other findings [23]. Forty [50.6%] of the cultures showed resistance against cefoxitin and therefore they contained MRSA, which is consistent with other findings [23, 47]. Resistance mechanisms include enzymatic inactivation of the antibiotic by penicillinase, alteration of the target with decreased affinity for the antibiotic e.g. penicillin binding protein 29 of MRSA and D-ala-Dlac of peptidoglycan precursors of vancomycin-resistant strains and trapping of the antibiotic for vancomycin and efflux pumps for fluoroquinolones [69]. Resistance to methicillin is conferred by the *mecA* gene, which codes for an altered penicillin binding protein that has a lower affinity for binding β -lactams [68].

The most prevalent BHS are the *Streptococcus pyogenes* and *Streptococcus epidermidis*[23]. Highest sensitivity was seen with amoxicillin clavulanate, which is consistent with a study carried out at Kenya National Hospital, [29]. It was equally sensitive to ceftriaxone, imipenem, cloxacillin and cefuroxime, consistent with another study [48]. Highest resistance to cefuroxime was seen.

Escherichia coli showed highest sensitivity with ciprofloxacin, comparable to other studies [29, 49, 51]. Absolute resistance was seen with amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem and ceftazidime, similar to other findings [50]. *E. coli* is a facultative gram negative anaerobe commonly found in the gastrointestinal tract, due to frequent exposure to antimicrobials, resistance has emerged over time. Resistance is due to either reduced affinity of existing PBP components or the acquisition of a supplementary β -lactaminsensitive PBP.

The most prevalent *Klebsiella* spp are *Klebsiella pneumonia* and *Klebsiella oxytoca*[71]. It accounts for 8% of all hospital acquired infections. The isolates, showed highest sensitivity to ciprofloxacin but above average sensitivity to augmentin, cefuroxime, ceftriaxone, imipenem, ceftazidime and ceftazidime which is consistent with other studies [52,53,54], in which *Klebsiella* spp species were found to be sensitive to penicillin, cephalosporins, quinolones, trimethoprim and cotrimoxazole. The use of broad spectrum antibiotics has led to development of multidrug resistant strains that produce extended-spectrum beta-lactamase [71].

Proteus mirabilis causes 90% of the *Proteus* infections and is considered a community acquired infection [73]. Highest sensitivity to amoxicillin clavulanate, ceftriaxone, imipenem and ceftazidime was seen, but highest resistance to ciprofloxacin, cefuroxime and ceftazidime [64.3%]. This is comparable to findings in other studies [29, 56, 57]; however there is reported steady decrease in ciprofloxacin susceptibility due to excessive use of fluoroquinolones. Twenty six strains of *Pseudomonas aeruginosa* were exposed to four antibiotics. Highest sensitivity was seen with ciprofloxacin, followed by imipenem. Highest resistance to ceftriaxone followed by ceftazidime was seen. Studies have shown that most *Pseudomonas aeruginosa* isolates are sensitive to piperacillin, ceftazidime and imipenem [23, 58, 59]. The decreased sensitivity to these drugs is due to antibiotic overuse and inappropriate use. Ciprofloxacin showed high in vitro activity. *Pseudomonas aeruginosa* resistance arises from the combination of unusually restricted outer-membrane permeability and secondary resistance mechanisms [69]. Derepression of chromosomal AmpC cephalosporinase; production of plasmid or integron-mediated β -lactamases from different molecular classes, diminished outer membrane permeability, over expression of active efflux systems with wide

substrate profiles; synthesis of aminoglycoside-modifying enzymes and structural alterations of topoisomerases II and IV determining quinolone resistance. These mechanisms are often present simultaneously, thereby conferring multi resistant phenotypes to the most frequently used Anti-pseudomonas antibiotics [70]. NLF represented 1.5% of the strains isolated. This includes all NLF bacteria other than *Pseudomonas aeruginosa*. Highest sensitivity to imipenem and ciprofloxacin was seen, followed by ceftazidime. Absolute resistance to amoxicillin clavulanate, cefuroxime, ceftriaxone and ceftazidime was seen. CoNS showed highest sensitivity to amoxicillin clavulanate, cefuroxime and imipenem. A resistance of 60% to ceftriaxone was seen and cloxacillin exhibited least sensitivity. Studies have found that CoNS is increasingly recognized as a clinically significant agent and resistance to semisynthetic penicillins has been observed in 80% of cases. CoNS are ubiquitous in nature and when exposed to medical devices, they attach on the surface via van der Waal's forces, hydrophobic interactions, and polarity ultimately forming a thick biofilm which reduces the organism's susceptibility to specific antimicrobials [72].

Enterococci cause frequent surgical site infections and the strains isolated were sensitive to amoxicillin clavulanate, cefuroxime, ceftriaxone and ciprofloxacin. The organism was resistant to ceftazidime, imipenem and ceftazidime. This finding is comparable to similar studies, in which high sensitivity was observed for penicillins [64, 65].

Inappropriate use of antibiotics can lead to development of resistance to antibiotics [10]. Most patients had a single antibiotic prescribed. The remaining patients received either two or three antibiotics. The prescriptions, however, were based on expert opinion other than culture and sensitivity for most patients. Even though most patients were on several antibiotics, the prevalence of wound infection remains high. Selection of antibiotics should be based on infecting organism, tissue penetration ability, low toxicity and absence of allergies [16]. The other commonly prescribed antibiotics were: flucloxacillin, amoxicillin clavulanate and metronidazole. Less common prescribed antibiotics were meropenem, gentamicin, ciprofloxacin, clindamycin, amikacin, ampiclox, erythromycin, and tetracycline. The use of penicillins, beta lactamase inhibitors, macrolides, second generation and third generation cephalosporins was comparable to a study done in Ireland. However, broad spectrum antibiotics were used in combination to target a wider coverage of the potentially synergistic aerobic and anaerobic microorganisms [29, 44].

7. Conclusion

Wound infection rate remains high and antibiotic resistance is steadily increasing. Most of the patients had been admitted for at least 8days. This suggests that most infections were hospital acquired. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the main causes of infections. This is because *Staphylococcus aureus* is the main flora on the skin and in the environment while *Pseudomonas aeruginosa*'s versatile characteristics allow it to be found in a variety of conditions and places. Antibiotics were widely used however this correlated negatively with the rate of wound infection due to wrong choice of drug and dosage.

8. Recommendation

- Due to high resistance of the organisms to antibiotics, sensitivity tests should be regularly carried out to enhance rational use of antibiotics and antibiotic choice should be made based on the sensitivity patterns. The prescribed antibiotics should have the dose and duration clearly indicated and upon administration, it should be clearly marked on the patient's treatment sheet.
- Treatment guidelines for use of antibiotics should be formulated based on the hospital formulary and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics.
- Prolonged hospitalization should be avoided to reduce the risk of hospital acquired infections.

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10. Appendices

Annex I: Participants information sheet and informed consent English version

1. Participant information sheet

Title: Antimicrobial susceptibility pattern of common bacterial that cause wound infection in the paediatric surgical patients at Yekatit 12 Hospitals, Addis Ababa, Ethiopia

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please feel free to ask.

Introduction

I am a student at Addis Ababa University of, pursuing a Master of medical laboratory in diagnostic and public health microbiology. The purpose of this study is to identify the cause of wound infection for appropriate management in the pediatric surgical wards at the yekatit 12 hospital. I therefore request you to allow me to get a wound swab from the child and answer the questions provided. The specimen will be analyzed in the laboratory to determine the cause of the infection and drugs that can be used to treat it. I will sponsor the study.

Your participation is voluntary

This consent form gives information about the study. Once you understand and agree to take part, you will be requested to sign or put your mark on this form. Refusal to participate in the study will not affect the treatment that is being given to you in the hospital and once in the study, you can withdraw if you wish to.

Risk and discomfort

There is no risk involved in taking the wound swab except for a slight touch of the cotton swab that will be felt at the point of swabbing.

Benefits

The clinicians will give you information on wound infection prevention and the results obtained will be used to effectively manage the wound infection.

Confidentiality

All efforts will be made to keep your personal information confidential. After the study the data will be destroyed.

Enquiries or questions

For any questions, inquiries or research related injury, please contact: Principal investigator Tofik Mahmud

Principal investigator addressee

Department of Medical Laboratory Sciences, school of allied health sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

Mobile: - 0911 -83-33-73

tofikmahmud@yahoo.com

2. Informed consent form

Statement of consent and signatures

I have been informed about the study plans to Antimicrobial susceptibility pattern of common bacterial that cause wound infection in the paediatric surgical patients at Yekatit 12 Hospitals, Addis Ababa, Ethiopia.

The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my wound specimen for the mentioned study. I agreed that the specimen would be tested for bacterial identification and antibiotic pattern.

So, I, the undersigned, have fully agreed to participate in the study “**Antimicrobial susceptibility pattern of common bacteria isolated from wound infection in the paediatric surgical patients at Yekatit 12 Hospitals, Addis Ababa, Ethiopia.**”

Participant name/code: _____ Signature: _____

Date: _____

For those who can't read the information

Advisor nurse name.....

Signature

Date

3. Information sheet for parents/ guardian (Assent form information sheet)

Title: Antimicrobial susceptibility pattern of common bacteria isolated from wound infection in the paediatric surgical patients at Yekatit 12 Hospitals, Addis Ababa, Ethiopia

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please feel free to ask.

Introduction

I am a student at Addis Ababa University of, pursuing a Master of medical laboratory in diagnostic and public health microbiology. The purpose of this study is to identify the cause of wound infection for appropriate management in the pediatric surgical wards at the yekatit 12 hospital. I therefore request you to allow me to get a wound swab from the child and answer the questions provided. The specimen will be analyzed in the laboratory to determine the cause of the infection and drugs that can be used to treat it. I will sponsor the study.

Your participation is voluntary

This consent form gives information about the study. Once you understand and agree to take part, you will be requested to sign or put your mark on this form. Refusal to participate in the study will not affect the treatment that is being given to you in the hospital and once in the study, you can withdraw if you wish to.

Risk and discomfort

There is no risk involved in taking the wound swab except for a slight touch of the cotton swab that will be felt at the point of swabbing.

Benefits

The clinicians will give you information on wound infection prevention and the results obtained will be used to effectively manage the wound infection.

Confidentiality

All efforts will be made to keep your personal information confidential. After the study the data will be destroyed.

Enquiries or questions

For any questions, inquiries or research related injury, please contact: Principal investigator Tofik Mahmud

Principal investigator addressee

Department of Medical Laboratory Sciences, school of allied health sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

Mobile: - 0911 -83-33-73

tofikmahmud@yahoo.com

4. Informed assent form [children 7-12 years]

I am Tofik Mahmud from Addis Ababa University. I am doing a study to test the sensitivity of bacteria that cause wound infection to different antibiotics at the paediatric surgical wards in Yekatit 12 hospital. We are asking you to take part in the research study because your parent recommended you for this study. For this research, we will ask you some questions then take a wound swab for laboratory testing. We will keep all your answers private, and will not show them to [parent[s]/guardian, friends or teacher. Only people working on the study will see them.

Risk and discomfort

There is no risk involved in taking the wound swab except for a slight touch of the cotton swab that will be felt at the point of swabbing.

Benefits

The clinicians will give you information on wound infection prevention and the results obtained will be used to effectively manage the wound infection.

You should know that:

- You do not have to be in this study if you do not want to. You won't get into any trouble with parent/guardian, your doctor, the school or me if you say no.
- You may stop being in the study at any time. [If there is a question you don't want to answer, just leave it]
- Your parent[s]/guardian[s] were asked if it is OK for you to be in this study. Even if they say it's OK, it is still your choice whether or not to take part.
- You can ask any questions you have, now or later. If you think of a question later, you or your parents can contact the following researchers or institution;

Sign this form only if you:

- Have understood what you will be doing for this study,
- Have had all your questions answered,
- Have talked to your parent[s]/legal guardian about this project, and
- Agree to take part in this research

Child's NameSign.....Date.....

Name of parent[s] or Legal guardian[s].....

Sign.....Date.....

Witness explaining study.....

SignatureDate.....

2. Informed consent form (የሰምምነት ማረጋገጫ ፊርማ)

የሰምምነት ማረጋገጫ ፊርማ

ይህንን ቅጽ አንብቤ ዋለሁ ወይም ተነባልኛል። ይህንን መረጃ ከሚመለከቱ አካል ጋር ተወያይቼ በታለሁ።

የነበሩኝ ጥያቄዎች በሙሉ መልስ ስለተሰጠኝ አሁን ሃሳብ ተከትሎ ክልተኛ ረድቻለሁ።

እዚህ ጥናት ላይ ተሳታፊ የሚሆነው በፈቃደኝነት እንደሆነ ተረድቻለሁ።

ይህንን ቅጽ በመፈረም የጥናቱ ስራ ላይ ተሳታፊ እንደሆኔ ሙብ ተቆንመው ሆኖ ሌሎች ለሌሎች ሰምም ትኩረት ማድረግ አለብኝ።

የተሳታፊው ስም _____ ፊርማ _____

ቀን _____

የምስክርስም _____ ፊርማ _____

ቀን _____

4. Informed assent form (የሰምምነት ማረጋገጫ ፊርማ)

ስለሕፃን ሰምምነት ቅጽ [ከ7-12 ዓመታት መካከል] ላሉ

እኔ ቶፊክ መሀምድ እባላለሁ የመጣሁት ከአዲስ አበባ ዩኒቨርሲቲ ነው።

በየካቲት 12 ሆስፒታል በፔዲያትሪክ ስኬት ስር ሕፃናት ሕክምና ክፍል ውስጥ ለተለያዩ ቁስሎች መነሻ የሆኑት ባክቴሪያዎች ለተለያዩ መድኃኒቶች የሚያሳዩ መቀያየር ባህሪ ያላቸውን ለማጥናት ለማወቅ ጥናት እያደረግኩ ነው።

በዚህ ጥናት ላይ እንድትሳተፍ የምንጠይቀው ወላጆች ህጻንዎን ጠይቅ ህሳብ ሳቸውን ስለሰጡ ነን። ለዚህ ጥናት የተለያዩት ያቋዎችን እንጠይቅሃለን።

ከዛም የቁስል ህንፃ ሙና የምንወስድ ይሆናል። ለምትሰጡን/ ለምትሰጡን መልሶች በሙሉ ሚስጥራዊነቱን እንጠብቃለን። ሚስጥራዊነቱን በመጠበቅ

[ለወላጆችህ/ አሳዳጊዎችህ ጓደኞችን ወይም ለመምህራኖችህ አናሳይም] በጥናቱ ላይ የሚሰሩ ሰዎች በቻ የሚሰሩት ይሆናል።

የሚያስከትለው ስጋት እና የሚከሰተው አለመመቻት የቁስል ህንፃ ሙና በመንወስድ በትሰዓት ትንሽ ሚስጥራዊ ስህተት መምደብ ሊጠይቅህ ይችላል። ለምንም ዓይነት ስጋቶች እያስከትልም።

ጠቀሜታዎች

ሐኪሙ በንክኪ የሚተላለፉ ቁስሎች መከላከያ መረጃ የሚሰጥ ህእና የዚህ ጥናት ውጤት ደግሞ በንክኪ የሚከሰት ቁስል በውጤታማነት ለማዳን ጥቅም ላይ የሚውል ይሆናል።

ማወቅ ያለብህ ነገሮች፡

ፍላጎት ከሌለህ በዚህ ጥናት ላይ መሳተፍ አይኖርብህም። ላለመሳተፍ ከሌለህ ስምዎንም አይነት ግርካዎችህ/ አሳዳጊዎችህ፤

ከሐኪም ህወይም ከትምህርት ቤት ህወይም ከአኔ የሚደርስብህ አይሆንም።

በዚህ ጥናት ውስጥ ከተሳተፍ ከበኋላ በየትኛውም ጊዜ ማቋረጥ ትችላለህ። [ምሳሌ ስጦት የማትፈልገው ጥያቄ ካለህ ተወው]

ወላጆችህ/ አሳዳጊዎችን እንተ በዚህ ጥናት ላይ መሳተፍ የምትችል ከሆነ ጠይቀው ነበር።

ምንም እንኳን እነሱ በጥናቱ ላይ እንድትሳተፍ ፈቃደኛ ቢሆኑም አሁንም ጥናቱ ላይ ለመሳተፍ ወይም ላለመሳተፍ ምርጫው የአንተ ይሆናል።

አሁንም ሆነ በኋላ ላይ የትኛውም ዓይነት ጥያቄዎች ካሉ ህመጠ የቅትችላለህ።

በኋላ ላይ ጥያቄ የሚኒር ህክምና ወይም ወላጆችህ ሊያነጋግሩ ህክፈሉን የሚከተሉትን አጥኚዎች ወይም ኢንሱቲትዩቲቭ ንግግር ይችላሉ።

ይህንን ቅጽ የምትፈርመው፡

ይህንን ጥናት ለማካሄድ አስፈላጊነቱን ከተረዳህ

ጥያቄዎችህ በሙሉ ምላሽ ካገኙ

ከወላጆችህ/ ከህጋዊ አሳዳጊዎችህ ጋር ስለ ጉዳዩ ከተወያየህ በት

እናም በዚህ ጥናት ላይ ለመሳተፍ ከተስማማህ

የሕግ-ስም: _____ ፊርማ _____

የወላጅ-ወይም-ህጋዊ-አሳዳጊ-ስም _____

ፊርማ _____ ቀን _____

ይህንን ጥናት የሚያስረዳም ስክር

ፊርማ _____ ቀን _____

Annex III: Questioner (English and Amharic version)

5. Questionnaire English version

Code [Initials].....

Date.....

1. Gender - Male Female

2. Age in years - 0-5 5-10 10-13

3. Is the child in school- Yes No

4. Ward.....

5. Surgical procedure performed.....

6. Duration of hospital stay [from date of admission].....

7. Duration of wound.....

8. Other co-morbidities- No Yes

If above is yes specify.....

9. What is the cause of the wound?

Surgical Burns Bites [insect, animal or snake] accident others [specify].....

10. Antibiotics prescribed

.....

.....

1. Questionnaire Amharic version(የአማራጭመጠይቅ)

መለያ ቁጥር

ቀን.....

1. ጾታ ወንድ ሴት
2. እድሜ 0-5 5-10 10-15
3. ትምህርት የጀመረ/ች አዎ የለም
4. ታካሚ የተኛበት ክፍል
5. የቁሰሉ ህክምና አይነት
6. ሆስፒታል የቆዩበት ጊዜ (ከገቡበት ጊዜ ጀምሮ)
7. የቁሰሉ ቆይታ
8. ቁሰሉ ያስከተለው በሽታ አለ አዎ የለም
አዎ ከሆነ ይገለጽ
9. የቁሰሉ ምክኒያት ቀዶ ህምና ቃጠሎ ንክሻ(ትነኝ፣ እንሰሳት፣ እባብ) አደጋ ሌላ ካለ(ይገለጽ).....
10. የታዘዘ መድሃኒት

Annex IV: Laboratory protocol

1. Identified micro-organisms

Gram positive bacteria

Bacteria	Isolate [tick where applicable]
<i>Staphylococcus</i> spp	
<i>Streptococcus pyogenes</i>	
<i>Enterococcus</i>	
<i>Acinetobacterbaumanii</i>	
<i>Alcaligenesspp</i>	

Gram negative bacteria

Bacteria	Isolate [tick where applicable]
<i>Pseudomonas aeruginosa</i>	
<i>Escherichia coli</i>	
<i>Klebsiellaspp</i>	
<i>Proteus</i> spp	

2. Antimicrobial sensitivity testing- Disc diffusion method.

A disc of blotting paper is impregnated with known volume and appropriate concentration of an antimicrobial. The disc is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc. To ensure reproducibility and comparability of results, the modified Kirby-Bauer diffusion technique will be used.

Modified Kirby-Bauer susceptibility testing technique

A sterile medium will be prepared according to the manufacturer's instructions. The PH of the medium will be set at 7.2-7.4. The media will be poured into a 90mm sterile petri dish to a depth of 4mm [about 25ml per plate]. This will be done on a level surface so that the depth of the medium is uniform. NB If the media is too thin the inhibition zone will be falsely large and if too thick the zones will be falsely small. Each new batch of agar will be controlled using *E. faecalis* [ATCC 29212 or 33186] and cotrimoxazole disc. The zone of inhibition should be 20mm or more in diameter. The plates will be stored at 2-8°C in sealed plastic bags. For use the plates will be dried with their lids slightly raised in 35-37°C incubator for about 30minutes. About one hour before use, the working stock of the discs will be allowed to warm to room temperature, protected from direct sunlight.

Method

- 1] Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4ml of sterile physiological saline or nutrient broth.
- 2] In a good light match the turbidity of the suspension to the turbidity of the 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 rotating and pressing 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°C to ensure even distribution.
- 4] With the petri dish lid in place, allow 3-5 minutes [no longer than 15minutes] for the surface of the agar to dry.
- 5] Using sterile forceps, needle mounted in a holder, or multidisc dispenser, place appropriate antimicrobial discs, evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs will be applied on each petri dish. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved in one place.
- 6] Within 30minutes of applying the discs, invert the plate and incubate it aerobically at 35°C for 16-18 hours.
- 7] After overnight incubation, examine the control and the 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.

Interpretation of zone sizes

Using the interpretative chart, the zones of each antimicrobial will be interpreted reporting each organism as Resistant, Intermediate susceptible, Susceptible.

Antibiotic sensitivity table

Bacteria.....

Antibiotic	Resistant	Intermediate	Sensitive
Amoxicillin			
Clavulanate			
Cefuroxime			
Ceftriaxone			
Imipenem			
Ciprofloxacin			
Cefoxitin			
Cloxacillin			
Ceftazidime			

3. Characterizations

3.1 Gram stain

1. This will be used to differentiate Gram positive [appears purple] and Gram negative [appears pink] bacteria. The following steps will be followed.
2. Fixing the dried smear by passing over a flame three times.
3. The fixed smear will be covered with crystal violet for 30-60 seconds.
4. The stain will be rapidly washed with clean water.
5. All the water will be tipped off and the smear covered with grams iodine.
6. The iodine will be washed with clean water.
7. The smear will be decolorized rapidly [in a few seconds] with acetone alcohol, then washed with clean water.
8. The smear will be covered with neutral red stain for two minutes.
9. The stain will then be washed off with clean water.
10. The back of the slide will be wiped clear and placed in a draining rack for the smear to air dry.
11. The smear will then be examined microscopically first with 40x objective to check the staining and see the distribution of materials and then in oil immersion objective to look for bacteria and cells.

3.2 Biochemical

1. Indole test

This will be used to identify enterobacteria. Most strains of enterobacteria break down the amino acid tryptophan with the release of indole.

Method

Using a sterile straight wire, 5ml of sterile medium will be inoculated with test organism. An indole paper strip will be placed in the neck of the tube and stopper put. Incubation will be done at 35-37°C overnight.

Indole production will be exhibited by reddening of the lower part of the strip.

2. Motility

Spreading of turbidity throughout the medium will be a positive proof.

3. Catalase test

Will be used to differentiate the bacteria that produce the enzyme catalase such as *staphylococci* from non-catalase producing bacteria such as *streptococci*.

Method

- i. 2-3ml of hydrogen peroxide solution will be poured into a test tube.
- ii. Using a wooden stick or a glass rod several colonies of the test organism will be removed and immersed in the hydrogen peroxide solution.
- iii. Active bubbling indicates a positive catalase test.

4. Coagulase test

This test will be used to identify *Staphylococcus aureus* which produces coagulase. Both tube test and slide test will be employed.

Method

Slide test [detects bound coagulase]

- i. A drop of distilled water will be placed on each end of a slide or on two separate slides.
- ii. A colony of the test organism will be emulsified in each of the drops to make two thick suspensions.
- iii. A loop full [not more than] will be added to one of the suspensions and mixed gently.
- iv. Clumping of the organisms will occur within 10 seconds if the organism is *Staphylococcus aureus*.
- v. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Tube test [detects free coagulase]

- i. Plasma will be diluted in the ratio of 1:10.
- ii. Three small test tubes will be available and labeled; test organism, positive control and negative control.
- iii. 0.5ml of the diluted plasma will be pipetted into each tube.
- iv. Five drops [about 0.1ml] of the test organism will be added into the labeled positive and 5 drops of the *Staphylococcus aureus* culture will be added to the tube labeled positive and 5 drops of sterile broth in the tube labeled negative.
- v. The tubes will be incubated at 35-37°C after mixing gently. Clotting will occur after one hour, if no clotting occurs after one hour examination will be repeated after every 30 minutes for up to 6 hours.
- vi. Clotting is indicative of *Staphylococcus aureus*.

5. Oxidase test

This test will be used to identify *Pseudomonas* spp.

Method

- 1] A piece of filter paper will be placed in a petri dish and soaked with 2-3 drops of freshly prepared oxidase reagents.
- 2] Using a piece of stick or glass rod, a colony of the test organism will then be smeared on the filter paper.
- 3] Development of blue- purple colour within a few seconds indicates positive oxidase test.

6. Voges-proskauer [v-p] test.

This test will be used to identify *Klebsiella* spp.

Method

- i. 2ml of sterile glucose phosphate peptone water will be inoculated with the test organism and incubated at 35-37°C for 48 hours.
- ii. A small amount of creatinine will be added and mixed well.
- iii. 3ml of sodium hydroxide will be added and mixed well.
- iv. The bottle cap will be removed and left for one hour at room temperature.
- v. Development of pink colour will be indicative of *Klebsiella pneumoniae*.

7. Urease test.

This test will be used to identify *Proteus* spp

Method.

- i. A straight wire will be used to inoculate a tube of MIU with a colony of the test organism.
- ii. An indole paper strip will be placed in the neck of the tube above the medium. The tube will be stoppered and incubated at 35-37°C overnight.
- iii. Production of urease will change the colour of the paper strip to pink.

8. Bacitracin test

This test will be used to identify *Streptococcus pyogenes*.

Method

- i. Bacitracin disk will be placed on a culture plate inoculated with the organism and incubated at 35-37°C overnight.
- ii. A zone of inhibition around the disc will be indicative of *Streptococcus pyogenes*.