ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA THAT CAUSE WOUND SEPSIS IN THE PAEDIATRIC SURGICAL PATIENTS AT KENYATTA NATIONAL HOSPITAL

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A dissertation submitted in partial fulfillment of the requirements for the award of Master of Pharmacy in Clinical Pharmacy by the University of Nairobi.

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DECLARATION

This dissertation is my original work and to the best of my knowledge, it has not been presented in any other institution for the award of a master's degree or any other academic purposes.

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DEDICATION

I dedicate these research findings to the Kenyatta National Hospital, the clinicians working in the paediatric surgical wards and the microbiologists.

ABSTRACT

Background: Wound infections contribute significantly to morbidity and mortality in surgical patients. A number of factors contribute to wound infection; however microorganisms are the major causes with bacteria being the most prevalent. Determination of local bacterial sensitivity patterns to antibiotics is important in providing a guide for antibiotic selection and appropriate management.

Objectives: The main objective of this study was to identify the bacteria that cause wound infections and their antimicrobial sensitivity patterns in the paediatric surgical wards at Kenyatta National Hospital.

Methodology: A cross- sectional design was used and the target population was children below the age of 13 years admitted in the surgical wards. The study was carried out for a period of two months, from mid April 2014 to mid June 2014. Data was collected using a questionnaire, specimens from the infected wounds were collected using sterile swabs and analyzed in the microbiology laboratory. Data was analyzed using the statistical software, SPSS version 20.

Results: The prevalence of wound infection was 82%. *Staphylococcus aureus* was the most prevalent followed by *Pseudomonas aeruginosa*, *Proteus* spp, coagulase negative staphylococcus, Beta hemolytic streptococcus, *Klebsiella* spp, Non lactose fermenters, and *Enterococcus faecalis*. Patients who had mixed infections were 8.67% of the total participants. *Staphylococcus aureus* was highly sensitive to ceftriaxone but resistant to ceftazidime. MRSA formed 50.6% of the *Staphylococcus aureus* isolates. BHS 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 NLF 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. *Enterococcus faecalis* was sensitive to amoxicillin clavulanate, cefuroxime, ceftriaxone and ciprofloxacin but resistant to cefoxitim, imipenem and ceftazidime.

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.

Recommendations: 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, ability to penetrate tissue, low toxicity and no allergic reactions. 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.

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LIST OF ACRONYMS

BHS- Beta hemolytic S*treptococcus*

CoNS- Coagulase negative *Staphylococcus*

HAI-Hospital Acquired Infections

ICU- Intensive Care Unit

IQR- Interquartile range

KNH- Kenyatta National Hospital

MIU- Motility Indole Urea

MRSA- Methicillin Resistant *Staphylococcus aureus*

MSSA-Methicillin Susceptible *Staphylococcus aureus*

NLF- Non lactose fermenters

PBP- Penicillin binding protein

SPP- Species

SSI- Surgical Site Infection

UON- University of Nairobi

LIST OF TABLES

CHAPTER ONE: INTRODUCTION

1.1 Background

A wound results following disruption of the skin which can be intentional or accidental (1). 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.

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 pre-operative 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.

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 immunosuppressants (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.

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 Problem Statement

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 pediatric patients at Kenyatta National Hospital. Inappropriate antimicrobial use is associated with increased resistance (10). It was therefore important to identify the causative organisms and determine the antimicrobial sensitivity patterns to help reduce infections and ensure appropriate use of antimicrobials.

1.3 Purpose of the study

Wound infection is a common problem in children, proper management with appropriate antibiotics is therefore important to reduce morbidity that may arise. This study aimed to determine the aetiology and antimicrobial sensitivity patterns of bacteria that cause wound infections in the surgical paediatric patients at KNH. Antibiotic misuse and overuse can lead to resistance which necessitated the need for the study.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to identify the bacteria that cause wound sepsis and their sensitivity patterns in the paediatric surgical wards at the Kenyatta National Hospital.

1.4. 2 Specific objectives
The specific objectives were:

1) To determine the prevalence of wound infection in the paediatric surgical wards.
2) To identify the bacteria that cause wound infection.
3) To determine the antimicrobial sensitivity patterns of the isolated bacteria
4) To find out the antibiotics used in management of wounds in the paediatric surgical wards

1.5 Research Questions
The research sought to answer the following questions:

1) What is the prevalence of wound infection in surgical paediatric patients?
2) What bacteria causes wound sepsis in the paediatric surgical wards?
3) What are the antibiotic sensitivity patterns of the isolated bacteria?
4) Which antibiotics are used in management of septic wounds in the paediatric surgical wards?

1.6 Significance

The findings of the study was to help in choosing appropriate antibiotics by considering the sensitivity patterns that were observed, hence appropriate management of the infected wounds. This would result in a cost effective therapy for the patient and reduced financial burden of hospitalization.

1.7 Limitations

Wound infections are caused by a number of microorganisms which include bacteria, fungi and viruses. This study was only able to address sepsis caused by aerobic bacteria. The study

was not able to address the factors that contribute to resistance patterns that were observed. It was also not able to identify the specific types of BHS and NLF due to in availability of the specific reagents for identification.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter 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.

2.2 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, 16). With the introduction of antibiotic prophylaxis, the infection rates have greatly reduced. 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 caeserian section (20). 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). A study done in Kenya found the prevalence of wound infection among women who had undergone caeserian section to be 19% (22).

No study has been done in the surgical paediatric patients, therefore, there is need to determine the rate of wound infection among the surgical paediatric patients at the Kenyatta National Hospital.

2.3 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 co*li 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 pneumonia* 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 Caeserian 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 *Enterobateriaciae* (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 wards showed the prevalent bacteria in SSI are: *Staphylococcus aureus, Enterobateriaceae*, *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 paediatric surgical patients at the Kenyatta National Hospital, to identify the bacteria that cause both SSIs and chronic wound infections.

2.4 Antimicrobial susceptibility of bacteria causing wound infection

Most *Pseudomonas aeruginosa* isolates are sensitive to piperacillin, ceftazidime, and imipenem, a gradual emergence of resistance to -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 -lactams, cephalosporins, -lactam– -lactamase inhibitor combinations, and carbapenems.

Enterococci, causes frequent surgical wound infections and almost all of the *Enterococcus faecalis* isolates are susceptible in vitro to glycopeptides 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 defence (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 freundi, Serratia* spp *and acinetobacter baumanii* (29). Amoxicillin- clavulanic had significant activity against *Enterobacteriaceae, Streptococcus pyogenes, Streptococcus faecalis* except *Klebsiella* which showed activity of 44%. Piperacillin, ticarcillin and tarzobactam 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 *Enterobacteriacea* but resistance was seen with *Pseudomonas* spp. Ciprofloxacin had good activity against Enterobacteriacea, 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 cefoxitin (56, 57).

In a study on antimicrobial sensitivity of *Pseudomonas aeruginosa*, high resistance was observed with piperacillin, ticarcillin, ceftazime, 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).

2.5 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 rate 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/ salbactum, 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, co-amoxiclav and metronidazole were mostly prescribed.

A study carried out at the Kenyatta National Hospital found that the antibiotics were mostly used for wound infections: flucloxacillin, gentamicin, ceftriaxone, cefuroxime, augmentin, amoxicillin, ciprofloxacin, ceftazidime, cloxacillin, antituberculous drugs, chloramphenicol and erythromycin (29).

2.6 Summary

Wound infections are caused mostly by *Staphylococcus aureus, Streptococcus pyogenes, enterococci, Pseudomonas aeruginosa*, *Escherichia co*li and *Streptococcus epidermidis* (23). The infection can be either by a single or multiple organisms. The antibiotic susceptibility patterns for each bacterium differ therefore for effective and appropriate management of bacterial wound infections it is important to identify the causative bacteria and its sensitivity patterns.

CHAPTER THREE: METHODOLOGY

3.1 Introduction
This chapter describes the study design and methodology in details.

3.2 Study design
A cross sectional study was adopted. Wound swabs were taken from the patient for laboratory testing and the parent or guardian of the patient was interviewed to obtain demographic data. The patient records were evaluated to obtain information on the medication. The study was carried out for a period of two months from mid April 2014 to mid June 2014.

3.3 Area of study
The study was carried out at Kenyatta National Hospital, Nairobi County. It was founded in 1901 with a bed capacity of 40 which has increased to about 1800. It is located along Hospital Road, Upper Hill, Nairobi. Within the KNH complex there is University of Nairobi's College of Health Sciences, Kenya Medical Training College, Kenya Medical Research Institute and National Laboratory Service (Ministry of Health). It is the largest referral hospital in East and Central Africa. The hospital has 50 wards, 22 outpatient clinics, 24 specialized theatres and an Accident & Emergency department. The surgical wards are located on the fourth, fifth and sixth floors with the paediatric surgical wards being wards 4A, 4D and 6B. The hospital serves patients with surgical conditions from all over the country; however, little research has been done on antibiotic sensitivity patterns in the surgical paediatric patients, at the hospital which makes it a suitable study site. The study was carried out in wards 4A, 4D and 6B, which is the general paediatric surgical ward, burns ward and paediatric orthopedics wards respectively. The specimens collected were analyzed at the microbiology laboratory located in the Department of Medical Microbiology of the University of Nairobi.

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

3.4.1 Inclusion criteria
Children thirteen years old and below who had 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 included.

3.4.2 Exclusion criteria

All children above thirteen years old and those, whose parents or guardian did not consent to participate in the study.

3.5 Ethical Considerations

The protocol containing informed consent form was submitted to the Ethics and Review Committee KNH/UON and the Department of Pharmaceutics and Pharmacy Practice for approval and clearance. Permission was also sought from the head of the Department of surgery before collecting data. Equal attention and participation opportunity was given to all willing participants. Informed consent was obtained from the guardians or parents before the study by explaining to them what the study was all about, voluntary participation, the risks and benefits, the cost and confidentiality of the information collected contacts for enquiries and finally have them sign the consent form (Appendix4). No inducement was given to the participant. Confidentiality was maintained on all information and data collected by keeping it under lock and key. At the conclusion of the study all the raw data collected was destroyed by shredding and burning the data collection tools.

3.6 Sampling

3.6.1 Sampling Technique

Participants were recruited by simple random sampling, whereby a coin was tossed for every patient who consented and those who got tails were chosen to participate in the study. This process was not repeated for those patients who had failed for the first time; however, the new patients were given a chance to participate through the same process until a sample size of 150 was achieved. This method was used to ensure external validity of the study findings.

3.6.2 Sample size

The prevalence of wound infection in paediatric patients as shown by a study done at Bethany Kids Kijabe Hospital, Kenya was 6.8% (75). This was scaled up to 11%. At 95% confidence interval, the sample size was calculated using a formula by Fishers (73).

$$N = \frac{Z^2 pq}{d^2}$$

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

Thus;

N=$\underline{1.96^2 x 0.11 x 0.89}$

$\quad$ 0.05^2

$\quad$ =150.4

$\quad$ =150

3.7 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 helped 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 1). 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 by research assistants in microbiology laboratory (Appendix2) (74). The information on sensitivity patterns was filled in the questionnaire after the culture and sensitivity patterns were available. The antibiotics that were tested include; amoxicillin clavulanic acid, cefuroxime, ceftriaxone, imipenem, ciprofloxacin, cefoxitin, cloxacillin and ceftazidime. All data was kept under lock and key, with accessibility limited to the researcher only.

3.8 Pilot Study or Pre-Testing

The questionnaire was piloted in the study area before the data collection. The researcher and the research assistants selected ten patients from the study site and the data collected using the questionnaire. The samples collected were taken to the microbiology laboratory for analysis within one hour after collection. Standard procedures were followed in the isolation

and identification of the microorganisms as well as determination of the sensitivity patterns. Any ambiguity was addressed before commencing the main study.

3.9 Validity

Internal validity was ensured by using a comprehensive questionnaire and following standard procedures in the laboratory during isolation and identification of microorganisms and in determining the antimicrobial sensitivity patterns. This was enhanced by having the procedures performed by qualified personnel and using appropriate biochemical tests and sensitivity discs.

External validity was ensured through random sampling and using an appropriate sample size.

3.10 Reliability

Reliability was ensured at the piloting stage. The questionnaire was administered to a selected number of patients and their responses compared for consistency. At the same time the specimens taken to the laboratory were analyzed in the same way.

3.11 Specimen collection and analysis

The researcher or research assistants collected a specimen from each wound using sterile cotton swabs after interviewing the parent or guardian. It was collected before wound cleaning, labeled and delivered to the laboratory within one hour for bacteriological examination. Precautions were taken to avoid cross contamination at all stages.

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 inoculated plates were labeled and incubated at $37^{\circ}C$ for 24 hours for the organisms to grow. Identification of the bacteria was done using the recommended standard procedures (Appendix 2) (74).

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 categorized as resistant or sensitive (Appendix 2) (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 whereby the information on the prescribed antibiotics was obtained.

The data collected was analyzed using the statistical software SPSS version 20. All variables were subjected to descriptive and inferential statistics.

CHAPTER FOUR: RESULTS

4.1 Introduction

This chapter contains the results obtained on the baseline characteristics, the prevalence of wound infection, the antimicrobial sensitivity tests and the antibiotics used in the paediatric surgical wards at KNH.

4.2 Socio-demographic characteristics

There were 150 children admitted at KNH paediatric surgical units who were recruited in the study. The mean age of the participants was 5.3 years (standard deviation; 0.4). 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 orthopaedic wards.

Table 1: Baseline characteristics

	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
Orthopaedic 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	4	2.7

4.3 Duration of wound and hospitalization

The median (IQR) durations of hospitalization and wound healing among paediatric surgical patients at KNH 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

	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

4.4 Prevalence of bacteria causing 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 due to one organism. *Staphylococcus aureus* was the most common,(52.7%), followed by *Pseudomonas aeruginosa*, *Proteus mirabilis,* 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: Prevalence of bacteria causing wound infection

Strain	Frequency (n)	percentage
Staphylococcus aureus	79	(52.7%)
Pseudomonas aeruginosa	26	(17.3%)
Proteus mirabilis	14	(9.3%)
BHS	5	(3.3%)
CoNS	5	(3.3%)
Klebsiella spp	3	(2.2%)
Escherichia coli	2	(1.3%)
NLF	2	(1.3%)
Enterococcus faecalis	1	(0.7%)
No growth	27	(18%)

4.5 Antimicrobial susceptibility patterns

4.5.1 Antimicrobial susceptibility of *Staphylococcus Aureus*

The antimicrobial sensitivity patterns for *Staphylococcus aureus* is summarized in table 4. Out of the 79 (52.9%) isolates, highest sensitivity was seen with ceftriaxone, 41 (51.9%), cefoxitin 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. Fourty (50.6%) of the cultures showed resistance against cefoxitim and therefore they contained MRSA.

Table 4*:* Antimicrobial susceptibility of *Staphylococcus aureus*

Antibiotic	Susceptible		Resistant	
Ceftriaxone	41	(51.9%)	38	(48.1%)
Cefoxitim	39	(49.4%)	40	(50.6%)
Ciprofloxacin	37	(46.8%)	42	(53.2%)
Cloxacillin	37	(46.8%)	42	(53.2%)
Amoxicillin clavulanate	34	(43.0%)	45	(57.0%)
Imipenem	30	(38.0%)	49	(62.0%)
Cefuroxime	29	(36.7%)	50	(63.3%)
Ceftazidime	7	(8.9%)	72	(91.1%)

4.5.2 Antimicrobial susceptibilityof Beta hemolytic *streptococcus*

The sensitivity pattern of Beta Hemolytic Streptococcus is summarized in table 5. 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.

Table 5: Antimicrobial sensitivity of Beta hemolytic *Streptococcus*

Antibiotic	Susceptible		Resistant	
Amoxicillin clavulanate	5	(100%)	0	(0%)
Ceftriaxone	4	(80%)	1	(20%)
Imipenem	4	(80%)	1	(20%)
Cloxacillin	4	(80%)	1	(20%)
Cefuroxime	2	(40%)	3	(60%)

4.5.3 Antimicrobial susceptibility of *Escherichia coli*

The antimicrobial susceptibility patterns for *Escherichia coli* are summarized in table 6. 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.

Table 6: Antimicrobial susceptibility of *Escherichia coli*

Antibiotic	Susceptible		Resistant	
Ciprofloxacin	1	(50%)	1	(50%)
Amoxicillin clavulanate	0	(0%)	2	(100%)
Cefuroxime	0	(0%)	2	(100%)
Ceftriaxone	0	(0%)	2	(100%)
Imipenem	0	(0%)	2	(100%)
Ceftazidime	0	(0%)	2	(100%)

4.5.4 Antimicrobial susceptibility of *Klebsiella* spp

The antimicrobial susceptibility of *Klebsiella* is summarized in table 7. Out of 3(2.2%) isolates, highest sensitivity was seen with ciprofloxacin (100%), amoxicillin clavulanate, cefuroxime, cefriaxone, imipenem, cefoxitin and ceftazidime showed equal sensitivity (66.7%).

Table 7: Antimicrobial susceptibility of *Klebsiella* spp

Antibiotic	Susceptible		Resistant	
Ciprofloxacin	3	(100%)	0	
Amoxicillin clavulanate	2	(66.7%)	1	(33.3%)
Cefuroxime	2	(66.7%)	1	(33.3%)
Ceftriaxone	2	(66.7%)	1	(33.3%)
Imipenem	2	(66.7%)	1	(33.3%)
Cefoxitim	2	(66.7%)	1	(33.3%)
Ceftazidime	2	(66.7%)	1	(33.3%)

4.5.5 Antimicrobial susceptibility of *Proteus mirabilis*

The antimicrobial sensitivity patterns of *Proteus Mirabilis* are summarized in table 8. 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%).

Table 8: Antimicrobial susceptibility of *Proteus mirabilis*

Antibiotic	Susceptible		Resistant	
Amoxicillin clavulanate	11	(78.6%)	3	(21.4%)
Ceftriaxone	11	(78.6%)	3	(21.4%)
Imipenem	11	(78.6%)	3	(21.4%)
Cefoxitim	11	(78.6%)	3	(21.4%)
Ciprofloxacin	10	(71.4%)	4	(28.6%)
Cefuroxime	8	(57.1%)	6	(42.9%)
Ceftazidime	5	(35.7%)	9	(64.3%)

4.5.6 Antimicrobial susceptibility of Non lactose fermenters

The antimicrobial susceptibility patterns of Non lactose fermenters are summarized in table 9. 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.

Table 9: Antimicrobial susceptibility of Non lactose fermenters

Antibiotic	Susceptible		Resistant	
Imipenem	2	(100%)	0	(0%)
Ciprofloxacin	2	(100%)	0	(0%)
Cefoxitim	1	(50%)	1	(50%)
Amoxicillin clavulanate	0	(0%)	2	(100%)
Cefuroxime	0	(0%)	2	(100%)
Ceftriaxone	0	(0%)	2	(100%)
Ceftazidime	0	(0%)	2	(100%)

4.5.7 Antimicrobial susceptibility of *Pseudomonas aeruginosa*

The antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* is summarized in table 10. 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 10: Antimicrobial susceptibility of *Pseudomonas aeruginosa*

Antibiotic	Susceptible	Resistant
Ciprofloxacin	24 (92.3%)	2 (7.7%)
Imipenem	20 (76.9%)	6 (23.1%)
Ceftazidime	12 (46.2%)	14 (53.8%)
Ceftriaxone	2 (7.7%)	24 (92.3%)

4.5.8 Antimicrobial susceptibility of Coagulase negative *Staphylococcus*

The antimicrobial susceptibility patterns of coagulase negative *staphylococcus* are summarized in table 11. 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.

Table 11: Antimicrobial susceptibility of Coagulase negative *Staphylococcus*

Antibiotic	Susceptible	Resistant
Amoxicillin clavulanate	3 (60%)	2 (40%)
Cefuroxime	3 (60%)	2 (40%)
Imipenem	3 (60%)	2 (40%)
Ceftriaxone	2 (40%)	3 (60%)
Cloxacillin	1 (20%)	4 (80%)

4.5.9 Antimicrobial susceptibility of *Enterococcus faecalis*

The antimicrobial susceptibility patterns of *Enterococcus faecalis* is summarized in table 12. One (0.7%) strain was isolated, which was sensitive to amoxicillin clavulanate, cefuroxime, ceftriaxone, ciprofloxacin it was resistant to cefoxitim, imipenem and ceftazidime.

Table 12: Antimicrobial susceptibility of *Enterococcus faecalis*

Antibiotic	Susceptible		Resistant	
Augmentin	1	(100%)	0	(0%)
Cefuroxime	1	(100%)	0	(0%
Ceftriaxone	1	(100%)	0	(0%)
Ciprofloxacin	1	(100%)	0	(0%)
Cefoxitim	0	(0%)	1	(100%)
Imipenem	0	(0%)	1	(100%)
Ceftazidime	0	(0%)	1	(100%)

4.6 Antibiotic prescription patterns in the paediatric surgical wards

The antibiotics used to treat surgical wound infection are summarized in Table 11. 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 13: Number of antibiotics prescribed per patient

Number of antibiotics per patient	Frequency (n)	Percent (%)
1	62	41.3
2	18	12.0
3	17	11.3
None	23	15.3

Table 14: 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
Ciprofloxacillin	2	1.3
Clindamycin	2	1.3
Amikacin	1	0.7
Ampiclox	1	0.7
Erythromycin	1	0.7
Tetracycline	1	0.7

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMENDATIONS

5.1 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 -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 cefoxitim 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-D-lac 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 -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 KNH, (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 -lactam-insensitive 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, cefriaxone,imipenem, cefoxitin 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 cefoxitin 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 -lactamases from different molecular classes, diminished outer membrane permeability, overexpression 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 multiresistant phenotypes to the most frequently used antipseudomonal 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 cefoxitim. 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 umbiquitous 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 cefoxitim, 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).

5.2 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.

5.3 Recommendations

5.3.1 Recommendations for policy and Practice

1. 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, ability to penetrate tissue, low toxicity and no allergic reactions. 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.

2. 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.

3. Prolonged hospitalization should be avoided to reduce the risk of hospital acquired infections.

5.3.2 Recommendations for research

1. Research should be carried to evaluate whether the recommended standard procedures are followed when managing wounds.

2. Research should be carried out to assess the extent of microbial contamination of the hospital environment which includes; the patient beds, water sinks, floor, walls and staff dresses. This will help in coming up with measures that will help in reducing wound infections.

3. In this study we were not able to carry out the necessary tests to identify the specific BHS, NLF and CoNS this should be done to identify the prevalence of the specific bacteria.

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Appendix 1: Questionnaire

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

…………………………………………………………………………………………..

………………………………………………………………………………………….

…………………………………………………………………………………………..

…………………………………………………………………………………………...

…………………………………………………………………………………………...

11. Identified micro-organisms

   (a)Gram positive bacteria

Bacteria	Isolate (tick where applicable)
Staphylococcus spp	
Streptococcus pyogenes	
Enterococcus	
Acinetobacter baumanii	
Alcaligenes spp	

   (b)Gram negative bacteria

Bacteria	Isolate (tick where applicable)
Pseudomonas aeruginosa	
Escherichia coli	
Klebsiella spp	
Proteus spp	

12. Antibiotic sensitivity

   Bacteria……………………………………………………………………………

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

Bacteria……………………………………………………………………………….

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

Bacteria …………………………………………………………………………

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

Bacteria …………………………………………………………………………………

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

Appendix 2: Identification tests

Gram stain

i.   This will be used to differentiate Gram positive (appears purple) and Gram negative (appears pink) bacteria. The following steps will be followed.

ii.   Fixing the dried smear by passing over a flame three times.

iii.   The fixed smear will be covered with crystal violet for 30-60 seconds.

iv.   The stain will be rapidly washed with clean water.

v.   All the water will be tipped off and the smear covered with grams iodine.

vi.   The iodine will be washed with clean water.

vii.   The smear will be decolorized rapidly (in a few seconds) with acetone alcohol, then washed with clean water.

viii.   The smear will be covered with neutral red stain for two minutes.

ix.   The stain will then be washed off with clean water.

x.   The back of the slide will be wiped clear and placed in a draining rack for the smear to air dry.

xi.   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.

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.

Motility

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

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 0f 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.

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 availed 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 5drops 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 35-37C after mixing gently. Clotting will occur after on hour, if no clotting occurs after one hour examination will be repeated after every 30minutes for up to 6hours.

vi. Clotting is indicative of *Staphylococcus aureus.*

Oxidase test

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

Method

1) Apiece 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.

4)

Voges-proskeur (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 48hours.

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.

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.

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.

Appendix 3: 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 co-trimoxazole 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.

Appendix 4: Consent form.

Principal investigator

Dr. Linet Kanaga Elamenya, Department of Pharmaceutics and Pharmacy Practice, UON.

Tel 0724883203.

Introduction.

I am a student at the University of Nairobi, pursuing a Master of Pharmacy in Clinical Pharmacy. The purpose of this study is to identify the cause of wound infection for appropriate management in the pediatric surgical wards at the Kenyatta National 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.

Cost

You will not incur any costs in the laboratory analysis.

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:

Dr. Linet K. Elamenya, mobile number 0724883203.

The secretariat, Kenyatta National Hospital Ethics and Review Committee, telephone number 02-726300

Statement of Consent and Signatures

I have read this form or had it read to me. I have discussed the information with those concerned and all my questions have been answered and so I understand it well. I understand that participation in the study is voluntary. By signing this form I do compromise on my rights as a research participant.

Participant's   Name……………………………………..   Sign…………………
Date…………

Witness   Name……………………………………..   Sign……………………..
Date…………

University of Nairobi/KNH Ethics and Review Committee

Kenyatta National Hospital

P.O. Box 20723-00200

Nairobi.

Tel: 2726300 Ext 4354

Copy to Investigators

Study Subjects

Appendix 5: Child assent form (7-12 years)

I am Dr. Linet Elamenya from the University of Nairobi. I am doing a study to test the sensitivity of bacteria that cause wound infection to different antibiotics at the paediatric surgical wards in Kenyatta National 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 Name …………..………………….Sign……………..Date…………………..

Name of Parent(s) or Legal

Guardian(s)………………………………………………………

Sign……………………………………………Date…………………………………………

….

Witness explaining study…………………………………………………………………..

Signature ………………………...........Date………………………………………………….

Enquiries or questions.

For any questions, inquiries or research related injury, please contact:

 Dr. Linet K. Elamenya, mobile number 0724883203.

The secretariat,

Kenyatta National Hospital and University of Nairobi/KNH Ethics and Review Committee

Kenyatta National Hospital

P.O. Box 20723-00200

Nairobi.

Tel: 2726300 Ext 4354

Copy to Investigators

Study Subjects

Appendix 6: Ethics Approval Letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/62   Link:www.uonbi.ac.ke/activities/KNHUoN   12th March 2014

Dr. Elamenya Linet Kanaga
Dept.of Pharmaceutics & Pharmacy Practice
School of Pharmacy
University of Nairobi

Dear Dr. Kanaga

RESEARCH PROPOSAL: AETIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA THAT CAUSE
WOUND SEPSIS IN THE PAEDIATRIC SURGICAL PATIENTS AT KENYATTA NATIONAL HOSPITAL (P6/01/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed
and approved your above proposal. The approval periods are 12th March 2014 to 11th March 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN
   ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events
   whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of
   notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study
   participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72
   hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period.
   (Attach a comprehensive progress report to support the renewal).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research
   Committee for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study
   This information will form part of the data base that will be consulted in future when processing related
   research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.uonbi.ac.ke/activities/KNHUoN.

Protect to Discover

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Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c.   The Chair, KNH/UoN-ERC
          The Deputy Director CS, KNH
          The Principal, College of Health Sciences, UoN
          The Dean, School of Pharmacy, UoN
          The Chairman, Dept. of Pharmaceutics & Pharmacy Practice, UoN
          The Assistant Director, Health Information, KNH
          Supervisors: Dr. Peter N. Karimi, Dr. Nasser Nyamweya

Protect to Discover