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Research Article

Antimicrobial Susceptibility of Bacteria that cause Wound Sepsis in the Paediatric Surgical Patients at Kenyatta National Hospital

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Background: Despite improvement in the practice of medicine and attempts to provide aseptic conditions in the surgical wards, the incidence of wound infection is increasing. Management of wound infection remains a challenge in the surgical areas with the increasing resistance to antimicrobials. Local bacterial sensitivity data is therefore an important guide for antibiotic selection.

Objective: To determine the aetiology and antimicrobial sensitivity patterns of bacteria that cause wound sepsis in the paediatric surgical wards at the Kenyatta National Hospital.

Methodology: A cross- sectional study was carried out on 150 paediatric patients admitted in the surgical wards from mid April 2014 to mid June, 2014. The patients were selected by convenient sampling. Data was abstracted from patient files and specimens from the infected wounds were identified and analyzed for antibiotic susceptibility.

Results: The prevalence of wound infection was 82%. *Staphylococcus aureus* (52.7%) was the most prevalent infective agent followed by *Pseudomonas aeruginosa* (17.3%). *Staphylococcus aureus* was the most resistant organism with susceptibility of less than 50% to most drugs. About 50.6% of the Staphylococcus isolates were methicillin resistant. *Streptococcus* was less resistant with more than 80% susceptibility to all tested drugs except cefuroxime. *Escherichia coli* were sensitive to ciprofloxacin. All gram negative bacteria were highly sensitive to ciprofloxacin with the following susceptibilities: *Pseudomonas aeruginosa* (92.3%), *Proteus mirabilis* (71.4%) and others 100%. Imipenem which is a new and relatively expensive monobactam demonstrated reduced activity with the following susceptibilities: *Staphylococcus aureus* (38%), Streptococcus (80%) and all the gram negative bacteria (70%).

Conclusion: The most common causative agent was *Staphylococcus aureus* and less than 50% of the isolates were susceptible to all tested antibiotics.

Key words: Antibiotic, antibiotic resistance, antibiotic susceptibility, wound infection.

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1. Introduction

A wound results following disruption of the skin which can be intentional or accidental (Giacometti et al, 2000). Wound infections cause prolonged morbidity and mortality. 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 high cost of hospitalization.

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Early recognition of wound infection and appropriate management is important. Antibiotic therapy and surgery are the cornerstone measures in management. Wound infection can be caused by a single species of bacteria or multiple microorganisms. Surgical site infections are the second most common cause of nosocomial infections after urinary tract infections (Perencevic et al, 2003; Lissovey et al, 2009). Most surgical site infections occur in ambulatory patients after discharge from the hospital and are therefore beyond the hospital infection control surveillance programs (Perencevic et al, 2003).

A number of factors contribute to wound infection (Obuku et al, 2012). The predisposing factors include patient characteristics such as age, obesity, malnutrition, endocrine and metabolic disorders, smoking, hypoxia, anaemia, malignancies and immunosuppressants.

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.

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 (Straph et al, 2013). Chronic infection can cause septicemia or bone infection which can lead to death. Sepsis associated encephalopathy increases morbidity and mortality especially in patients within the intensive care unit (Maramattom 2007).

Despite improvement in the practice of medicine and attempts to provide aseptic conditions in the surgical wards, the incidence of wound infection is increasing. Management of wound infection remains a challenge in the surgical areas with the increasing resistance to antimicrobials (Anguzu et al, 2007).

At the Kenyatta National Hospital, little research has been done on antibiotic sensitivity patterns in the surgical pediatric patients. It is the largest referral hospital. Surgical paediatric patients are admitted in four different wards namely: burns ward, paediatric orthopaedic, surgical wards and burns unit. The burns unit admits patients with more than 60% burns, the burns wards admits patient with less severe burns.

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

2. Methodology

2.1 Study site and study design

It was a cross sectional study conducted on paediatric patients, admitted in the paediatric surgical wards at the Kenyatta National Hospital between mid April 2014 to mid June 2014. The wards included; the general paediatric surgical ward, burns ward, burns unit and paediatric orthopedics ward.

2.2 Study population and eligibility criteria

Children were included if they were aged thirteen years old and below and if their parents or guardian gave consent. They should either have been admitted with a wound or had a surgical wound for at least 72hours.

2.3 Sample size and sampling procedure

The sample size was calculated using Fishers formula (Mugenda and Mugenda, 2003). The prevalence of wound infection in paediatric patients was the main outcome of interest. The estimated prevalence of wound infection in the patients was 6% which was reported in a study carried out in Kenya (Wood et al, 2012), this was scaled up to 11%. Using an α=0.8, β =0.05, the calculated minimal sample size for the study was 138.

Participants were recruited by convenient sampling. Every patient who was admitted during the study period was approached and those who consented were given an opportunity to participate in the study. A total of 150 patients were recruited in the study.

2.4 Data collection

Guardians or parents were interviewed to obtain demographic data and the patient records were evaluated to obtain information on the medications taken by the patient.

Wound swabs were taken before wound cleaning, and submitted to the University of Nairobi laboratory within one hour for bacteriological examination. Precautions were taken to avoid cross contamination.

The organisms were inoculated on; Blood agar, Chocolate blood agar and MacConkey media from Oxoid company, United Kingdom. Inoculated plates were incubated at 37 °C for 24 hours. Identification of the bacteria was done by biochemical tests as described by Elmer et al, (2005). Kirby- Bauer Disc Diffusion sensitivity test was used to determine sensitivity patterns (Elmer and Paul, 2005).

Sensitivity to the following drugs was tested: amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem, ciprofloxacin, cefoxitin, cloxacillin and ceftazidime.

2.5 Data Analysis

The data was analyzed using the statistical software SPSS version 20. All variables were subjected to descriptive and inferential statistics. Chi-square test was used for inferential data analysis.

2.6 Ethical Considerations

Permission to carry out the research was granted by the Kenyatta National Hospital/University of Nairobi Ethics and Review Committee (KNH/UON ERC) as per the letter referenced KNH-ERC/A/62 dated 12th March 2014. Proxy informed consent was obtained from the guardians or parents; in addition, children above 7 years old assented. Confidentiality was maintained by using codes instead of patient names.

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3. Results

3.1 Participant recruitment

Four hundred and six patients were screened; two hundred and fifty six were excluded because of the reasons given in **Figure 1**.

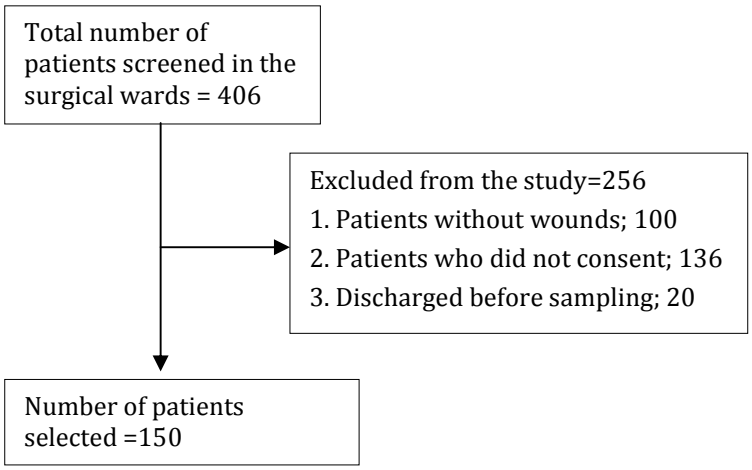

Figure 1: Summary of patient selection

3.2 Baseline characteristics of the study participants

The baseline characteristics of the study participants are summarized in **Table 1**. The mean age of the participants was 5.3 years (SD 0.4). Approximately one-half (51.3%) of the participants were below 5 years of age (**Table 1**).

There were 78 (52%) males, and 62% of the participants were primary school children. The rest were preschool children.

3.3 Causes of wounds

Burns (59.3%) and accidents (22.7%) were the most common causes of wounds. Majority of the cases were found in the burns (56%) and orthopaedic (24.7%) wards. There was a significance in the distribution of the causes of the wound by sex; boys were more likely to have surgical and accident wounds, (p=0.03).

School going children were more likely to suffer from burns, bites and accident wounds compared to those who were not going to school, (p<0.01). There was no significant association between co morbidity and cause of wound (p=0.23). Those who had a co morbidity had wounds for a comparatively longer duration (P<0.01).

3.4 Prevalence of identified bacteria

The distribution of different causative agents in various wards is summarized in **Table 2**. *Staphylococcus aureus* was the most prevalent in all the wards, burns unit (71.4%), burns ward (68.7%), surgical wards (34.8%) and orthopaedic ward (24.3%).

The prevalence of *Pseudomonas aeruginosa* was; burns wards (12.1%), orthopaedic ward (18.9%) and surgical ward (8.7%). *Proteus mirabilis* (6.67%) was the third in prevalence with the highest prevalence being in the burns ward. There was a significant difference in the distribution of bacteria in the various wards (P<0.01).

Table 1: Baseline characteristics of the study participants

Variable		Cause/Type of wound				Total n(%)
		Surgical n(%)	Burns n(%)	Bites n(%)	Accidents n(%)	
Age	0-4	7 (9.1)	52 (67.5)	1 (1.3)	17 (22.1)	77 (51.3)
	5-9	10 (20.4)	25 (51)	3 (6.1)	11 (22.4)	49 (32.7)
	10-13	6 (25)	12 (50)	0	6 (25)	24 (25)
Sex	Male	16 (20.5)	38 (48.7)	2 (2.6)	22 (28.2)	78 (52)
	Female	7 (9.7)	51 (70.8)	2 (2.8)	12 (16.7)	72 (48)
School	Yes	15 (16.1)	46 (49.5)	4 (4.3)	28 (30.1)	93 (62)
	No	18 (31.6)	43 (75.4)	0	6 (10.5)	57 (38)
Co morbidity	Yes	19 (13.8)	82 (59.4)	4 (2.9)	33 (23.9)	138 (92)
	No	4 (33.3)	7 (58.3)	0	1 (8.3)	12 (8)

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Table 2: Percentage prevalence of isolated bacteria

	Surgical ward n(%)	Orthopaedic n(%)	Burns unit n(%)	Burns ward n(%)	Total n(%)
Staphylococcus aureus	8 (34.8)	9 (24.2)	5 (71.4)	57 (68.2)	79 (52.7)
Enterococcus faecalis	0	0	0	1 (1.2)	4 (2.7)
BetaHemolytic Streptococcus	1 (4.4)	2 (5.4)	0	1 (1.2)	4 (2.6)
Pseudomonas aeruginosa	2 (8.7)	7 (18.9)	0	10 (12.1)	19 (12.7)
Escherichia coli	0	0	1 (14.3)	1 (1.2)	2 (1.3)
Klebsiella spp	0	1 (2.7)	0	0	1 (0.7)
Proteus mirabilis	0	2 (5.4)	0	8 (9.6)	10 (6.7)
NonLactose Fermenters	0	2 (5.4)	0	0	2 (1.3)
Coagulase Negative Staphylococcus	3 (13.0)	1 (2.7)	0	1 (1.2)	5 (3.3)
No growth	9 (39.13)	13 (35.14)	1 (14.29)	4 (4.82)	27 (18)
Totals	**23**	**37**	**7**	**83**	**150**

3.5 Antimicrobial susceptibility of the gram positive bacteria

The antimicrobial susceptibility of gram positive bacteria is summarized in **Table 3**.

Staphylococcus aureus isolates were (52.9%). The highest sensitivity was seen against ceftriaxone, 41(51.9%) and cefoxitin 39(49.4%), while highest resistance was seen against ceftazidime (91.1%). Forty (50.6%) of the cultures showed resistance against cefoxitim and therefore they contained Methicillin resistant *Staphylococcus aureus.*

Coagulase negative *Staphylococcus* showed highest sensitivity to amoxicillin- clavulanate, cefuroxime and imipenem at 60%. Resistance to ceftriaxone and cloxacillin was 60% and 80% respectively.

Beta hemolytic Streptococcus was sensitive to amoxicillin clavulanate, 100.0%, followed by ceftriaxone, imipenem and cloxacillin. Cefuroxime showed the highest resistance.

Staphylococcus was the most resistant organism with susceptibility of less than 50% to all antibiotics tested except ceftriaxone. Streptococcus was less resistant with more than 80% susceptibility to all antibiotics tested except cefuroxim.

Coagulase negative Staphylococcus was sensitive with more than 60% susceptibility to the antibiotics tested except ceftriaxone and cloxacillin.

3.6 Antimicrobial susceptibility of gram negative bacteria

The antimicrobial susceptibility of gram negative bacteria is summarized in **Table 4**. There were 19 isolates of *Pseudomonas aeruginosa*, they were subjected to susceptibility testing against four antibiotics which are recommended for its treatment. Highest sensitivity was seen with ciprofloxacin (92.3%), followed by imipenem (76.9%). *Pseudomonas aeruginosa* was highly resistant to cephalosporins.

Proteus spp showed highest sensitivity to amoxicillin clavulanate (78.6%). Highest resistance was seen against ceftazidime (64.3%). *Klebsiella* spp showed highest sensitivity to ciprofloxacin (100%). Equal sensitivity (66.7%), was seen with amoxicillin clavulanate, cefuroxime, cefriaxone, imipenem, cefoxitin and ceftazidime. *Escherichia coli* was only sensitive to ciprofloxacin**.**

Non lactose fermenters were highly sensitive to imipenem and ciprofloxacin (100%), followed by cefoxitim, (50%). These organisms showed absolute resistance to amoxicillin clavulanate, cefuroxime, ceftriaxone, ceftazidime.

All isolates of *Enterococcus* spp were sensitive to amoxicillin clavulanate, cefuroxime, ceftriaxone, and ciprofloxacin but resistant to cefoxitim, imipenem and ceftazidime.

All gram negative bacteria were sensitive to ciprofloxacin. All organisms showed marked resistance

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to imipenem which is a new monobactam and relatively expensive. Gram negative bacteria were highly susceptible to imipenem with a susceptibility of about 70%. Coagulase negative *staphylococcus* and beta haemolytic *streptococcus* showed susceptibility of above 60% to imipenem. *Staphylococcus aureus* showed high resistance to imipenem. It should only be used where the causative organism is known.

Cephalosporins; All gram negative bacteria identified in the study were susceptible to ceftriaxone except *Pseudomonas aeruginosa*. More than 50% of *Staphylococcus aureus* isolates were sensitive. It is preferred over the other cephalosporins and imipenem except for *Pseudomonas aeruginosa* which was resistant. Most organisms were resistant to ceftazidime, in most cases, less than 10% of the isolates were susceptible. Probably it should be removed from the formula. Cefoxitin and cefuroxime showed more than 50% activity against the gram negative bacteria. However, *Staphylococcus aureus* was highly resistant. *Proteus mirabilis* and *Klebsiella* spp showed high susceptibility above 60% to amoxicillin clavulanate. Coagulase negative *Staphylococcus* and beta haemolytic streptococcus were highly sensitive to amoxicillin clavulanate. However, *Staphylococcus aureus* was highly resistant.

Table 3*:* Antimicrobial susceptibility of gram positive bacteria

Antibiotic	***Staphylococcus aureus***		**Coagulase negative *Staphylococcus***		**Beta hemolytic *streptococcus***
Ceftriaxone	41	(51.9%)	2	(40%)	4 (80%)
Cefoxitim	39	(49.4%)	-		-
Ciprofloxacin	37	(46.8%)	-		-
Cloxacillin	37	(46.8%)	1	(20%)	4 (80%)
Amoxicillin clavulanate	34	(43.0%)	3	(60%)	5 (100%)
Imipenem	30	(38.0%)	3	(60%)	4 (80%)
Cefuroxime	29	(36.7%)	3	(60%)	2 (40%)
Ceftazidime	7	(8.9%)	-		-
Total isolates	**79**		**5**		**5**

Table 4: Antimicrobial susceptibility of gram negative bacteria

Antibiotic	***Pseudomonas aeruginosa* n(%)**	***Proteus mirabilis* n(%)**	***Klebsiella* spp n(%)**
Ciprofloxacin	24(92.3%)	10(71.4%)	3(100%)
Imipenem	20(76.9%)	11(78.6%)	2(66.7%)
Ceftazidime	12(46.2%)	5(35.7%)	2(66.7%)
Ceftriaxone	2(7.7%)	11(78.6%)	2(66.7%)
Amoxicillin clavulanate	-	11(78.6%)	2(66.7%)
Cefoxitim	-	11(78.6%)	2(66.7%)
Cefuroxime	-	8(57.1%)	2(66.7%)
Total isolates	**26**	**14**	**3**

4. Discussion

The culture and sensitivity results were compared with the antibiotics that were administered to the patients and shared with the clinicians for incorporation into the management of specific patients.

Staphylococcus aureus was most prevalent in the burns ward and the burns unit. *Pseudomonas aeruginosa* was the most prevalent in the orthopaedic wards. There were increased *Staphylococcus aureus* antibiotic resistant strains; mostly β-lactam resistant strains such as methicillin resistant *Staphylococcus aureus* (Kitara et al, 2011). Staphylcoccus *aureus* showed highest sensitivity to ceftriaxone. Sensitiviy to cefoxitin, cloxacillin, ciprofloxacin, amoxicillin clavulanate, imipenem cefuroxime and ceftazidime was below 50%, which is consistent with other findings (Giacometti et al,

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2000). Forty (50.6%) of the cultures showed resistance to cefoxitim and therefore contained Methicillin Resistant *Staphylococcus aureus*, which is consistent with other findings, (Giacometti et al, 2000; Kitara et al, 2011). Resistance mechanisms include enzymatic inactivation of the antibiotic by penicillinase, alteration of the target with decreased affinity for the antibiotic, trapping of the antibiotic and ejection through efflux pumps (Stratera and Yordanov, 2009). 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 (Hancock and Speert, 2000).

Coagulase negative *Staphylococcus* (CoNS), showed highest sensitivity to amoxicillin clavulanate, cefuroxime and imipenem. Sixty percent of the organisms were resistant to ceftriaxone while 80% to cloxacillin. Previous studies have reported 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 (Kelly, 2013).

Studies have shown that most *Pseudomonas aeruginosa* isolates are sensitive to piperacillin, ceftazidime and imipenem (Giacometti et al, 2000; Sivanmaliappan and Sevanan 2011; Saleh and Hatem 2013). The sensitivity to ceftazidime was 46.2%, with highest sensitivity to ciprofloxacin (92.3%), the decreased sensitivity to these drugs is due to antibiotic overuse and inappropriate use. *Pseudomonas aeruginosa* resistance arises from the combination of unusually restricted outer-membrane permeability and secondary resistance mechanisms (Strateva and Yerdanov, 2009). De-repression 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 multiresistant phenotypes to the most frequently used antipseudomonal antibiotics (Shahab, 2013).

Proteus mirabilis causes 90% of the Proteus infections and is considered a community acquired infection. It was not surprising therefore that it was more prevalent in the burns ward. Highest sensitivity was observed to amoxicillin- clavulanate, ceftriaxone and imipenem. Cefoxitin showed highest resistance followed by ciprofloxacin, cefuroxime and ceftazidime. This is comparable to other studies (Karimi et al, 2008; Kelly, 2013; Saleh and Hatem, 2013). There is a steady decrease in ciprofloxacin susceptibility due to excessive use of fluoroquinolones.

The most prevalent *Klebsiella* spp are *Klebsiella pneumoniae* and *Klebsiella oxytoca* (John and Harvin 2007). It accounts for eight percent of all hospital acquired infections. The isolates, showed highest sensitivity to ciprofloxacin and average sensitivity to amoxicillin-clavulanate, cefuroxime, cefriaxone, imipenem, cefoxitin and ceftazidime, which is consistent with other findings (Stock and Wiedemann 2001; Tansarli et al, 2013) . The use of broad spectrum antibiotics has led to development of multidrug resistant strains that produce extended-spectrum beta-lactamase (John and Harvin, 2007).

Escherichia coli showed highest sensitivity to ciprofloxacin which was comparable to other studies (Karimi et al, 2008; Drago et al, 2010; Kibre and Abera, 2011). Absolute resistance to amoxicillin- clavulanate, cefuroxime, ceftriaxone, imipenem and ceftazidime was seen, which was similar to other findings (Ibrahim et al, 2012). *Escherichia 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 either due to reduced affinity of existing protein binding penicillin components or the acquisition of a supplementary β-lactam-insensitive protein binding penicillin.

The most prevalent beta haemolytic *Streptococci* are the *Streptococcus pyogenes* and *Streptococcus epidermidis* (Giacometti et al, 2000). Highest sensitivity to amoxicillin clavulanate was seen, which is consistent with other findings, (Karimi et al, 2008; Denis and Stevens, 2014).

Enterococci faecalis cause frequent surgical site infections and the isolates 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 with penicillins (Rudy et al, 2004; Bertesteanu et al, 2014).

The study was only able to address sepsis caused by aerobic bacteria. A narrow range of antibiotics was tested.

Excessive use and misuse of antibiotics are attributable to inappropriate prescribing and lack of specific and timely microbiological diagnostic tests. Increased resistance adds to the burden of infections. Hospital acquired infections increase cost of healthcare, length of hospital stay and mortality. On the other hand, treatment of resistant strains increases the cost. In the study, patients with burns had highest prevalence of infected wounds. Staphylococcus was the most common aetiological agent and highly resistant to most antibiotics. Gram negative organisms were more susceptible to the antibiotics tested. Culture and sensitivity tests are therefore an important guide to antibiotic selection for each patient.

5. Conclusion

The prevalence of wound infection and the resistance to antibiotics was found to be high and measures are required to prevent further spread. The most common causative agent was *Staphylococcus aureus* and less than 50% of the isolates were susceptible to all tested antibiotics.

Conflict of Interest declaration

The authors declare no conflict of interest.

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