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Occurrence of Post-Surgical Wound Infections, Bacterial Isolates, and their Susceptibility to Commonly Used Antibiotics at Kenyatta National Hospital, Kenya

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Abstract: ***Surgical site infections are now the most common and costly of all hospital-acquired infections (HAIs), accounting for 20% of all HAIs. This was a cross-sectional study to determine the occurrence of SSI, the common causative microbes and their antibacterial susceptibility pattern. Sixty-two specimens from various types of surgical wounds were processed by standard methods and antimicrobial susceptibility testing done by disc diffusion method. Recovered bacteria were Staphylococcusaureus (29.6%), Escherichiacoli (22.2%), Pseudomonasaeruginosa (12.9%), Enterococcusfaecalis and Enterobacterspp (7.4%), Klebsiellaspp (5.6%), Streptococcuspyogenes (3.7%) and Acinetobacterspp (1.9%).62.5% of the S. aureus were Methicillin-resistant MRSA, while 75% of E. faecalis were resistant to Vancomycin. Gram-negative rods were highly resistant to Ceftriaxone. Control and prevention measures should be put into place as strategy to minimize the incidence of SSIs and the spread of resistance isolates.***

Keywords: Surgical site infection, antimicrobial resistance, antibiotics, Kenya

1. Background

Surgical site infection (SSI) rates in low-and middle-income countries (LMICs) range from 8 to 30% of procedures, making them the most frequent healthcare-acquired infection (HAI) with substantial morbidity, mortality, and economic impacts (Mehtar et al., 2020) . SSIs impact many aspects of the patient journey including, increased pain and reduction in mobility, delayed wound healing, increased use of antibiotics, a common need for additional surgery, increased length of hospital stay, and increased mortality (Harrop et al., 2012) . Most wound infections are caused by contamination during surgery with the patient’s own micro-organisms (Townsend et al., 2016) . They may be superficial and self-limiting, involving the skin only, or they may be deeper and life-threatening. SSIs are classified by the Centers for Disease Control and Prevention (CDC) as superficial incisional, deep incisional and organ/space infections (Heal et al., 2016)

To treat SSI, antibiotics are commonly used, however, the rise in antibiotic resistance (ABR) is an impediment to wound healing (Harrop et al., 2012) . Overuse and misuse of antibiotics in LMICs is a probable contributing factor to ABR development (Manenzhe et al., 2015) . The small amount of data available from LMICs raises a real concern about the dynamic spread of multi-drug-and extensively-drug resistant bacteria, especially Gram-negatives, in settings where the availability of appropriate treatments is absent.

2. Literature Review

Arya et al. (2005) in a prospective survey of SSI conducted in India (Arya *etal*., 2000) that evaluated 516 bacteria isolates from pus samples collected from post operative wounds found *Staphylococcusaureus* to be the most frequent isolate bacteria followed by *Escherichiacoli*, *Pseudomonasaeruginosa*, *KlebsiellaPneumonia* and others. She noted majority of the isolates as being resistant to ampicillin-clavulanic acid, ampicillin, cephalexin, cefuroxime, cefotaxime, fluoroquinolone and cotrimoxazole.

Another study by Shamsuzzaman et al. (2003) to evaluate antimicrobial susceptibility pattern among the most common bacteria associated with post operative wound infection, also reported *S. aureus* as the most common isolates. In addition, they reported most of the isolates as being resistant to ampicilin and amoxicillin. Moreover, 1.8% of *S. saureus* isolates were methicilin resistant (MRSA) while most of the gram-negative bacteria isolated such as *E. coli*, *Klebsiellaspp*, and *Pseudomonasaeruginosa* were sensitive to quinoles and amino glycosides but resistant to cephalosporins (Shamsuzzaman et al., 2003) . Antimicrobial resistance has become a serious worldwide public health problem and global strategies of interventions to slow the emergence and reduce the spread of antimicrobial resistant microorganisms have been and continue to be formulated (Bratzler & Houck, 2004) .

Problem definition

SSIs are the 2nd leading nosocomial infectious with a prevalence of 2.6% globally. SSI prevalence ranges between 4.3% to 21% in Nigeria and 8% to 11% in Kenya. There are limited published data on the prevalence of bacteria isolates causing Surgical Site Infections and their antibiotic susceptibility patterns at the surgical wards of Kenyatta National Hospital to help guide antimicrobial prophylaxis and patient care. The study of surgical site infections is important to give the trend of bacteria causing SSI in KNH. With increasing emergence of drug resistances in some pathogenic bacteria there is needed to determine the bacteria causing SSIs and evaluate their antibiotic patterns. The results of this study could help formulate an policy on SSIs control. As such, this study aimed at determining the occurrence of post-surgical infections in KNH, their bacteria spectrum and their susceptibility to the various commonly used antibiotics

3. Methodology

This was a cross sectional study conducted at the Kenyatta National hospital (KNH) using a population sample of 62 male and female adult patients admitted at the surgical ward after undergoing surgery. The participants were purposively recruited into the study. KNH is the largest referral and teaching hospital in Kenya and has a wide catchment.

To be included in this study one had to be an adult (>18 years), had undergone surgery at KNH, had an identifiable superficial SSI with the following signs: a purulent exudate draining from surgical site, swelling erythema, pain, local temperature and peri wound cellulitis or the surgeon's diagnosis of infection after the surgical period and consented to participate in the study. Immunocompromised patients and those on long term treatment with corticosteroids were excluded.

Specimen Collection

Participant clinical data and other demographic data were collected. Pus from the surgical wounds were collected using sterile cotton swabs and immediately transported to the laboratory at the Department of Medical microbiology of the University of Nairobi (UoN) for bacteriological studies.

Once in the laboratory, each participant sample was inoculated onto two blood agar (BA) and one MacConkey (MAC) plate. One BA plate was incubated under 5%-10% carbon dioxide (CO_2) in a candle jar, the second BA plate and MAC plate were incubated aerobically at 37°C for 24 hours. After 24 hours, the plates were examined for growth, those with no growth were re-incubated for extra 24 hours to enable the growth of slow growing bacteria. Plates that had presence of growth were classified on whether they had a mixed or pure growth. Isolates were identified by their colonial morphological characteristics and the effect they had on the media. A colony was picked and a slide smear made followed by gram staining for microscopic examination. Convectional biochemical tests: e. g., catalase, coagulase, indole, urease, and oxidase, Methyl red, vogues proskauer, citrate utilization test, motility test, carbohydrate fermentation tests were done for identification and were identified according to Bargey's manual and manual of clinical microbiology (Holt 2003). For gram-negative rods, they were inoculated in Triple Sugar Iron agar and in analytical profile index (API 20E) for identification.

Antibiotic susceptibility testing

Identified bacteria were cultured on Mueller Hinton agar while *Streptococcuspyogenes* isolates were cultured on BA for antimicrobial susceptibility testing. Kirby Bauer disk diffusion method was used for the susceptibility testing. Quality control was assured by adding *Staphylococciaureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as control.

Staphylococcusaureus isolates were also subjected to methicillin susceptibility testing using Oxacillin discs.

Data Analysis

Participant data was checked for completeness, consistency, and accuracy. It was then coded and analyzed using the statistical package for social sciences (SPSS) version 19. The results were presented in text, graphs, tables and charts.

Ethical Considerations

This study was approved by the ethics research committee of the KNH-UoN. Signed inform consent was required from study participants prior to participating in the study.

4. Results

Out of the 62 pus samples that were collected, bacterial isolates were recovered from seven in ten samples (n=45, 72.6%) while approximately three in ten samples (n=17, 27.4%) had no bacterial growth. From the 45 samples that had growth, 36 had pure cultures while nine had mixed colonies. In total, 54 different bacterial species were isolated.

The most occurring bacterial isolate was *Staphylococcusaureus* (n-16) 29.6% followed by *Escherichiacoli* (n-12) 22.2%, Pseudomonas *aeruginosa* (n-7) 12.9%, *Enterobacter spp* (n-4) 7.4%, *E. faecalis* (n-4) 7.4%, *Proteus. spp* (n-4) 7.4%, Klebsiella *spp* (n-3) 5.6%, and *S. pyogens* (n-2) 3.7%. The least occurring bacterial isolates were *Acinetobacterspp* (n-1) 1.9% and S. *epidermidis* (n-1) 1.9%.

There were more male patients (n-37) compared to females (n-25) participants. Among the males, the most isolated pathogen was *S. saureus* (20%) followed by *E. coli* (16%), while the least isolated organism was *Klebsiellaspp* and *S. epidermidis* (2%). In females the most isolated pathogen was also *S. aureus* but with a less percent of (8%) followed by *E. coli* and *E. faecalis* (5%), *Klebsiellaspp* and *S. pyogenes* (4%) and the least isolated was *Proteusspp* and *Acinetobacterspp* (2%).

Antimicrobial susceptibility patterns

Antibiotics susceptibility pattern of Gram Negatives Isolates

Antimicrobial susceptibility testing was done on four isolates*E. coli*, Proteus spp, *P. aeruginosa*, and Klebsiella spp. *E. coli* showed high resistance to ceftriaxone (91.7%). *P. aeruginosa* isolates showed high resistance to Amikacin (71.4%) with one isolate (14.3%) showing resistance to Meropenem while 2 (42.9%) were resistance to piperacillin n = 2 (42.9%). *Klebsiella* spp isolates had high resistance to Ampicillin and Augmentin (66.7%). *Proteus spp* showed high resistance to Ceftazidime and Ceftriazone (66.7%). This is summarized in table1.

Table 1: Antibiotics susceptibility pattern of Gram Negatives Isolates showing resistance results

ANTIBIOTIC	E. coli %	Proteus spp %	P. aeruginosa %	Klebsiella spp %
Amikacin	41.7	25.0	71.4	33.3
Ampicillin	83.3	33.3	-	66.7
Augmentin	50.0	50.0	-	66.7
Ceftazidime	41.7	33.3	-	33.3

Ceftriazone	91.7	66.7	-	33.3
Cefotaxime	66.7	-	-	-
Cefuroxime	25.0	25	-	-
Ciprofloxacin	33.3	-	-	33.3
Piperacillin	-	-	42.9	
Meropenem	-	-	14.3	0.0
Timentin	-	-	57.1	-
Cefepime	-	-	85.7	-

KEY; %-percentage

Antibiotics susceptibility pattern of Gram-positive Isolates showing resistance

In cases of *Staphylococcusaureus* 56.3% of the isolates were found resistant to Methicillin (Oxacillin), this isolate also showed high resistant to Augmentin (62.5%), Erythromycin (56.3%). In the case of *S. epidermidis* the 1 isolate isolated was found to be resistant to Erythromycin and Cotrimoxazole. Out of the 4 *Enterococcusfaecalis* isolates only 1 had resistance to Erythromycin and Cotrimoxazole (25%) and 3 of the isolates were Vancomycin (75%) resistant as shown in Table 2.

Staphylococcusaureus retained 43.8% sensitivity to Methicillin while *S. epidermidis* was sensitive to Augmentin, Cephalexin and Cotrimoxazole. *Enterococcus faecalis* was found to be highly sensitive to Augmentin and Erythromycin (75%) respectively.

Table 2: Antibiotic sensitivity pattern of Gram positive cocci showing resistance

ANTIBIOTIC	*S. aureus* %	*S. epidermidis* %	*S. pyogenes* %	*E. faecalis* %
Ampicillin	56.3	0	0	50
Augmentin	62.5	0	50	75
Erythromycin	56.2	100	0	25
Cephalexin	37.5	0	50	50
Cotrimoxazole	50	100	50	50
Vancomycin	31.3	0	50	75
Oxacillin	56.3	-	-	-

KEY; %-percentage

Frequency and Percentage of Bacteria Isolated from Various Operation Sites

In the operation of incision and drainage *Saureus* was the most isolated n-3 (18.8%) and in laparotomy *Proteusspp* 75% and *E. Faecalis* 75% respectively was the most isolated. The 1 isolate of *S. epidermidis* was also isolated from laparotomy.

Table 3: Frequency and Percentage of Bacteria Isolated from Various Operation Sites

Type of surgery	*E. coli*	*Proteus spp*	*Klebsiella spp*	*Pseudomonas spp*	*Enterobacter spp*	*Acinetobacter spp*	*S. aureus*	*E. faecalis*	*S. pyogenes*	*S. epidermidis*
ID	2 (16.7)	0	1 (33.3)	0	0	0	3 (18.8)	1 (25)	1 (50)	0
HERN	1 (8.3)	0	0	1 (14.3)	0	0	0	0	0	0
APPEND	2 (16.7)	0	0	1 (14.3)	2 (50)	0	2 (12.5)	0	0	0
ORIF	0	1 (25)	0	2 (28.6)	1 (25)	1 (100)	1 (6.3)	0	1 (50)	0
LAP	5 (41.7)	3 (75)	0	3 (42.9)	1 (25)	0	6 (37.5)	3 (75)	0	1 (100)
CORR SUR	0	0	0	0	0	0	0	0	0	0
OTHERS	2 (16.7)	0	2 (66.7)	0	0	0	4 (25)	0	0	0
TOTAL	12	4	3	7	4	1	16	4	2	1

KEY; ID-Incision & Drainage, HERN-Heriorropathy, APPEND-Appendectomy, ORIF-Open reduction internal fixation. LAP-Laparotomy, OTHERS-Breast surgery, corrective surgery

5. Discussion

The risk of infection after surgery depends upon the factors including the type and length of surgical procedure; age, underlying conditions and previous history of the patient: skill of the surgeon; diligence with which infection control procedures are applied and the type and timing of pre-operative antibiotic prophylaxis.

In this study, 54 microbes were isolated from the 45 samples that grew bacteria. The most isolated pathogen was *Staphylococcusaureus* (n-16, 29.6%), followed by *Escherichiacoli (n-12, 22.2%) and Pseudomonas aeruginosa (*n-7, 12.9%). These findings agree with Arya *et al.* (2000) who found the same trend. Similarly, Shamsuzzaman et al. (2003) identified *Staphylococcusaureus* as the most common causative bacteria agent of surgical site Infections.

The high prevalence of *Staphylococcu aureus* infection observed in this study could be because it is endogenous source of infections. Nasal carriage of *S. aureus* is an important risk factor for infection of SSIs as the organism is a normal flora in the nostrils. Infection with this organism may also be due to contamination from the environment such as surgical instruments. It is noted that with disruption of the natural skin barrier *S. aureus*which is a common bacterium on surface can easily find its way into Surgical sites (Brown, 1990) .

The isolation of coliforms; *Escherichiacoli* (22.2%), *Proteusspp* (7.4%) and *Enterobacterspp* (7.4%) could be as a result of contamination of the surgical site with the patients endogenous flora as they are normal flora of the GIT (Anguzu & Olila, 2007) .

Majority of gram-negative isolates showed high resistance toceftriaxone as shown by *E. coli* (91.7%), and *Proteus* spp (66.7%); to Ampicillin by *Ecoli (*83.3%), *and Klebsiella spp* (66.5%); to Augmentin by *Klebsiella spp* (66.7%), and *Proteus spp* (50%). These findings agree with the findings of Amrita *et al.* (2010). Resistance to Ceftriaxone and Ampicillin could be explained by they having been used as prophylaxis preoperatively hence increasing their use in patients. Over-use of antibiotics has been shown to contributes to organisms developing resistance (Seppälä et al., 1992). *Pseudomonasaeruginosa* isolates had high resistance to Amikacin (71.4%), Cefepime (85.7%) and Piperacillin (42.9%).

Methicillin resistance *Staphylococcusaureus* were at 56.3% of isolates. *E. faecalis* isolates showed high resistance to Vancomycin (75%). This resistance pattern for both *S.* aureus and *E. faecalis* confirmed that MRSA and VRE clinical isolates are important causes of hospital acquired surgical site infections. Our results on MSRA resistance is similar to the study done in Mulago hospital by Kironde et al. (2001) . The resistance observed in *Staphylococcusaureus* could also be attributed to irrational use of antibiotics for conditions that may not clinically indicate their use, over the counter sell of antibiotics in pharmacies without prescription by authorised practitioners, some new drug and formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them (Kironde et al., 2001). *Staphylococcusaureus* was isolated across the various anatomical sites e. g. thigh (18.8%), knee (25%), cheek and chest (6.3%). Thismay be due to surface contamination by organisms on the skin and environment causing nosocomial infections (Kironde et al., 2001) .

6. Conclusions

We should clearly understand and identify SSIs as a problem and devise a system to track, analyse and monitor these in our hospitals. In view of such highly resistance isolates causing SSIs in our hospital it will be very difficult to treat these cases, so the only hope lies within prevention of such infections. There is also need to be careful in selection of antibiotic regime in SSIs cases to minimize incoming magnitude of drug resistance among bacteria.

7. Future Scope

There is need for continuous surveillance genotypic characterization of clinical isolates and their antimicrobial susceptibility patterns. Surgical site Infections need to be prevented by the surgeons' personnel, operation environment should be clean and aseptic and modern operating techniques should be employed. Patients should be educated on importance of hygiene after undergoing surgery to minimize contamination of the surgical sites. Since a high proportion of samples had positive cultures, infections control is recommended as a strategy to minimize speedy resistance organisms' future studies should be extended to include cultures under anaerobic conditions to establish presence of other bacteria that require such environment for growth. There is need to develop National surveillance of antibiotic resistant organisms.

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