Antibiotic susceptibility patterns of bacteria isolates in post-operative wound infections among patients attending Mama Lucy Kibaki Hospital, Kenya

Johnstone Amulioto (jamulioto@gmail.com)  
Kenyatta University School of Medicine  https://orcid.org/0000-0002-2906-3780

Margaret W. Muturi  
Kenyatta University School of Medicine

Scholastica Mathenge  
Kenyatta University School of Medicine

Gideon M. Mutua  
Mama Lucy Kibaki Hospital

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Abstract

Background Surgical site infections accounts for high mortality rates, morbidity and elevated costs of treatment for surgical patients. In recent years, surgical site infections attributed to antibiotic resistant bacteria has been on the rise globally. To effectively formulate a comprehensive treatment protocol for surgical site infection, there is need for knowing the likely causative agents and their antibiograms. In this regard, this study sought to determine the prevalence and antibiotic susceptibility patterns of bacteria isolates from post-operative wound infections among patients attending Mama Lucy Kibaki Hospital.

Methods A cross-sectional descriptive study was carried out between October 2018 and March 2019. The study included patients of all age group with surgical site infections following general, obstetrics and gynecological surgeries. Pus swabs were aseptically obtained from 58 consented patients with clinical evidence of surgical site infections. Gram stain, culture, biochemical tests and antibiotic susceptibility tests were done for each pus swab. Antibiotic susceptibility testing was done using Kirby bauer disc diffusion method. Data was analysed using a statistical package for the social sciences (SPSS) version 20.

Results An isolation rate of 94.8% was observed. Staphylococcus aureus 28.2% was the preponderant isolate followed by Escherichia coli 15.4% with Methicillin resistant Staphylococcus aureus accounting for 65.4% (n=17) of the total Staphylococcus species . Chloramphenicol was the most sensitive drug to all the bacteria isolates. Ampicillin and Amoxycillin recorded resistance rates >90% against positive and gram negative bacteria respectively. Klebsiella pneumoniae 81.3% and E.coli 74% were the most resistant bacteria.

Conclusion Majority of the drugs were resistant to gram negative rods, therefore there is need for continuous monitoring to determine the susceptibility patterns of the most common surgical site infections bacteria isolates which are found in the hospitals.

Introduction

Surgical sites infections (SSI) are a worldwide problem in the field of surgery accounting for increased deaths, morbidity and elevated healthcare costs in surgical patients [1][2]. The rates of these infections worldwide vary greatly, with most studies observing incidence rates of between 2.6%-58% [3][4][5]. The implicated pathogens vary from one setting to the other, with several researchers observing Staphylococcus aureus, Pseudomonas aeruginosa (39.13%), Escherichia coli and Klebsiella pneumoniae (25.5%) as the predominant SSI bacteria isolates [6][7][8][9].

An increasing proportion of these infections are due to multi-drug resistant bacteria and this has complicated the treatment of SSIs [10]. This resistance has been attributed to the irrational use of antimicrobial agents which has exerted selective pressure on bacteria leading to the emergence of resistant bacterial strains [11]. Moreover, the decline in antibiotic development pipeline has further complicated the treatment of SSIs [12]. Successful treatment of these infections depends on accurate
identification of etiologic agents and their antibiogram profiles. However, there is a paucity of clinical microbiological data especially in developing countries which has hampered the management of these infections [13].

In Kenya, the situation is the same with regard to the paucity of this data. Hence, this study was done to determine the prevalence and antibiotic susceptibility profiles of bacteria isolates in post-operative wound infections among patients attending Mama Lucy Kibaki Hospital. The results are intended to help clinicians when formulating a comprehensive treatment protocol for SSIs. Pre-operative surgical prophylaxis also rely on local susceptibility profiles of bacteria.

**Methods**

**Study design and setting**

This was a cross-sectional descriptive study carried out at Mama Lucy Kibaki Hospital for a period of 6 months, between October 2018 and March 2019. Mama Lucy Kibaki Hospital is a level 5 public health facility located in the eastern part of Nairobi city, Kenya. This study included patients of all age group with surgical site infections following general, obstetrics and gynecological surgeries. The patients were drawn from the surgical wards, maternity wards and pediatric ward. Those patients who did not consent were excluded.

**Sampling**

Patients with surgical site infection were identified by surgeons during routine ward rounds and the clinical information documented in patient file. The same information was relayed to the principal investigator through the clinicians. Eligible patients were taken through the research process and informed consents obtained. A serialized structured questionnaire was used to collect additional patient clinical data.

**Sample collection**

The pus swabs were aseptically obtained from 58 patients with clinical evidence of surgical site infection (drainage from incision) within the research period. After the wound immediate surface exudates and contaminants were cleansed off with sterile moistened gauze and normal saline solution. The pus was collected using a moistened sterile cotton swab from the deep viable tissues of the wound by Levine method [14]. The collected swab was then placed in a tube with Stuart transport medium and the replaced cap tightened. Two pus swabs were collected from each patient, one for gram stain and the
other for culture. All the swabs were carefully labelled and transported immediately to Kenyatta university laboratory in appropriate leak proof specimen transport bags.

**Bacterial culture and identification**

Upon reaching the laboratory, all the pus swabs were carefully checked and their details entered into the investigators booklet. Smears of the pus swabs were prepared, gram stained and examined microscopically. The other swabs from each patient were inoculated on MacConkey agar and blood agar and the inoculated plates incubated at 35-37°C for 24 hours. After incubation, the plates were examined for growth. Colony characteristics such as swarming growth and hemolysis on blood agar, changes in physical appearance in differential media and enzymatic activities of the organisms were used for preliminary bacterial identification. Biochemical tests such as catalase, coagulase, urease, indole, methyl red, voges proskauer and citrate (IMViC) tests were performed for the identification of the various bacteria.

**Antibiotic susceptibility testing**

Antibiotic susceptibility testing was performed on Mueller Hinton agar using various antibiotics by Kirby bauer disc diffusion method as per standards prescribed in bacteriology [15]. Using a sterile swab, Mueller Hinton agar surface was uniformly coated with the suspension of the test organism matching 0.5 McFarland turbidity standard. Following incubation of test plates and controls at 35°C for 24 hours, the plates were examined for confluent growth. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains. The zones of inhibitions were determined using breakpoints provided by Clinical and Laboratory Standards Institute and British society for antimicrobial chemotherapy [16][17]. The following antibiotics were tested; Chloramphenicol (50ug), Vancomycin (30ug), Amoxycillin (30ug), Doxycycline (30ug), Ciprofloxacín (30ug), Cefepime (30ug), Ceftriaxone (30ug), Amikacin (30ug), Gentamicin (30ug), Oxacillin (1ug), Cotrimoxazole (25ug), Azithromycin (15ug) and Ampicillin (10ug). These antibiotics were selected based on the availability and prescription frequency of these drugs in the study area.

**Data analysis**

Data was entered in excel, cleaned and the information exported to IBM statistical package for the social sciences version 20. Descriptive statistics such as mean and percentages were determined. Pearson chi-square test was used to establish the association of social demographic data and the different bacteria isolates. P value of < 0.05 was considered to indicate statistically significant differences. Finally, the results were presented using tables.
Results

A total of 58 cases of surgical site infection were observed at the facility between October 2018 and March 2019. Of these 19 (32.8%) were males while 39 (67.2%) were females with pediatric patients accounting for 3 (5.2%) of the total cases. The patients’ mean age was 31.12 years, with the youngest and eldest patient being 7 and 61 years respectively.

A total of 78 bacteria were isolated from the culture positive swabs, with monomicrobial and polymicrobial growth occurring in 60.3% (35/58) and 34.5% (20/58) of the swabs respectively. Whereas only 5.2% (3/58) of the swabs were culture negative. *Staphylococcus aureus* 28.2% (n=22) was the prevalent isolate followed by *E.coli* 15.4% (n=12), *Acinetobacter species* 14.1% (n=11), *Pseudomonas aeruginosa* 9.0% (n=7), *Enterobacter species* 9.0% (n=7), *Bacillus species* 9.0% (n=7), *Coagulase negative staphylococci* 5.1% (n=4), *Proteus species* 5.1% (n=4), *Klebsiella pneumoniae* 2.6% (n=2), *Morganella morganii* 1.3% (n=1) and *Citrobacter freundii* 1.3% (n=1) (see additional file 1).

Most of the bacteria were recovered from the abdomen 60.3% and the lower limbs 29.5%, with the arms and other body sites accounting for 3.8% and 6.4% of the total bacteria respectively (Table 1). Majority of these isolates were gram negative rods 57.7% (45) with gram positive bacteria accounting for 42.3% (33) of the total bacteria. This number was high in female patients 73.1% (57) than male patients 26.9% (21) but the difference was not statistically significant (p=0.136). Chloramphenicol 90.9% (n=30/33), Vancomycin 87.9% (n=29/33) and Doxycycline 75.8% (n=25/33) were highly sensitive to gram positive bacteria. However Azithromycin 36.4% (n=12/33) and Ampicillin (3.0%) (n=1/33) had the lowest sensitivity rates for gram positive bacteria (Table 2).

Cefepime, Gentamicin and Amikacin showed 100% resistance to *Acinetobacter species* and *Klebsiella pneumoniae* respectively. Chloramphenicol 53.3% (n=24/45) was the only drug that had the highest sensitivity for gram negative rods. Multi-drug resistance (resistance to ≥ 4 antibiotics) was observed with *Klebsiella pneumoniae* and *E.coli* (Table 3). Among gram positive bacteria, *Methicillin resistant Staphylococcus aureus* (MRSA) accounted for 65.4% (n=17/26) of the total *Staphylococcus species* (Table 2).

Table 1 Proportions of bacteria isolated from different wound sites
<table>
<thead>
<tr>
<th>Wound site</th>
<th>Gram positive bacteria N (%)</th>
<th>Gram negative bacteria N (%)</th>
<th>Total isolates N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen</td>
<td>24 (51.1)</td>
<td>23 (48.9)</td>
<td>47 (60.3)</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>7 (30.4)</td>
<td>16 (69.6)</td>
<td>23 (29.5)</td>
</tr>
<tr>
<td>Arms</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Other body regions</td>
<td>1 (20.0)</td>
<td>4 (80.0)</td>
<td>5 (6.4)</td>
</tr>
</tbody>
</table>

Table 2: Antibiotic susceptibility pattern of gram positive bacteria

<table>
<thead>
<tr>
<th>Types of bacteria isolates</th>
<th>Tested antibiotics</th>
<th>30ug (%)</th>
<th>30ug (%)</th>
<th>50ug (%)</th>
<th>30ug (%)</th>
<th>10ug (%)</th>
<th>15ug (%)</th>
<th>1ug (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>S</td>
<td>90.9</td>
<td>77.3</td>
<td>95.5</td>
<td>86.4</td>
<td>4.5</td>
<td>50.0</td>
<td>27.3</td>
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<tr>
<td></td>
<td>I</td>
<td>0.0</td>
<td>9.1</td>
<td>0.0</td>
<td>9.1</td>
<td>0.0</td>
<td>4.5</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>9.1</td>
<td>13.6</td>
<td>4.5</td>
<td>4.5</td>
<td>95.5</td>
<td>45.5</td>
<td>59.1</td>
</tr>
<tr>
<td>Coagulase negative staphylococci</td>
<td>S</td>
<td>75.0</td>
<td>100.0</td>
<td>100.0</td>
<td>50.0</td>
<td>0.0</td>
<td>25.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>25.0</td>
<td>0.0</td>
<td>0.0</td>
<td>25.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>R</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>25.0</td>
<td>100.0</td>
<td>75.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Bacillus species</td>
<td>S</td>
<td>85.7</td>
<td>28.6</td>
<td>71.4</td>
<td>57.1</td>
<td>0.0</td>
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<tr>
<td></td>
<td>I</td>
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<td>42.9</td>
<td>14.3</td>
<td>14.3</td>
<td>0.0</td>
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<tr>
<td></td>
<td>R</td>
<td>14.3</td>
<td>28.6</td>
<td>14.3</td>
<td>28.6</td>
<td>100.0</td>
<td>85.7</td>
<td>-</td>
</tr>
</tbody>
</table>

VA=vancomycin, CIP=ciprofloxacin, C=chloramphenicol, DO=doxycycline, AX=ampicillin, AZM=azithromycin, OX=oxacillin, S=sensitive, I=intermediately sensitive, R=resistant
<table>
<thead>
<tr>
<th>Types of bacteria isolates</th>
<th>Tested antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (50ug %)</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Proteus species</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>R</td>
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<tr>
<td>E.coli</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>I</td>
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<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>S</td>
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<tr>
<td></td>
<td>I</td>
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<tr>
<td></td>
<td>R</td>
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<tr>
<td>Enterobacter species</td>
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<td></td>
<td>I</td>
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<td></td>
<td>R</td>
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<td>Acinetobacter species</td>
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<td></td>
<td>I</td>
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<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
</tbody>
</table>
Discussion

The present work observed monomicrobial growth in 60.3% (35/58) of the swabs that were cultured, with polymicrobial and culture negative growth occurring in 34.5% (20/58) and 5.2% (3/58) of the swabs respectively. These results were comparable to findings from a study carried out in Nigeria which observed single growth in 48 (75%) samples, with polymicrobial growth occurring in 16 (25%) samples [18]. Both of these studies observed a high isolation rate of bacteria, except that in the present work three swabs failed to yield any growth which could be attributed to antibiotic use prior to sample collection.

This project managed to isolate a total of 78 bacteria with *Staphylococcus aureus* as the preponderant bacteria. These results were in harmony with a similar study conducted at one of the national hospital in the country [19]. This predominance of *Staphylococcus aureus* observed at the current setting was because this bacteria is a skin flora, therefore its presence in surgical wounds could be as a result of endogenous contamination of the surgical site during patient skin incision. Exogenous sources such as contaminated hospital surfaces or equipments and the hands of healthcare providers could also account for *Staphylococcus aureus* preponderance [20].

However, findings by this work were inconsistent with studies by Namiduru et al [21], Kokate et al [22] and PatelDisha et al [23] which identified *E.coli* (32.8%), *Pseudomonas aeruginosa* and *Klebsiella species* as the leading causes of surgical site infection. The difference could be attributed to the invasive nature of the procedures performed by hospitals. This is because, patients undergoing procedures involving the gastro-intestinal tract may be at a risk of developing SSI as a result of endogenous contamination of the surgical site with enteric rods during surgery.

The project also saw the predominance of gram negative bacteria over gram positive bacteria which was analogous with findings from a study by Tuon et al [24]. But these results were incongruous with discoveries by a separate research worker who observed the preponderance of gram positive bacteria over gram negative bacteria [25]. The differences in the types of bacteria isolates can be attributed to the different microbiota present in different hospital environments. In addition, the increase in resistance rates of gram negative rods to antibiotics could account for the high prevalence of gram negative rods at the current setting.

The high resistance rates of Amoxycillin and Ampicillin recorded by the present work were in harmony with observations by another worker [26]. Findings on the resistance rates of gram negative rods were comparable to the results obtained from a study in Ethiopia [27]. Additionally, MRSA rate of 65.4% observed in the present setting was in harmony with results from a study conducted in Uganda that reported a MRSA rate of 65.9% [28]. These current occurrences were attributed to lack of effective surveillance programs for SSI etiologies and their antiograms. One of the limitation was the design of
this study which could not allow for follow-up of patients which meant that some of the cases were never captured.

Conclusions

The current work observed that *Staphylococcus aureus* was the leading cause of post-operative wound infection among patients attending the hospital. High resistance rates were observed among gram negative bacteria, with only chloramphenicol as the most sensitive drug to all the isolates. Therefore, there is need for continuous monitoring to determine the susceptibility patterns of the most common bacteria isolates which are found in the hospitals. The current results are intended to assist clinicians during empirical therapy and also in monitoring bacteria resistance trends across institutions.

Abbreviations

SSI: surgical site infection; KUERC: Kenyatta University Ethics Review Committee; IBM: International Business Machines Corporation; MRSA: *Methicillin resistance Staphylococcus aureus*; ATCC: American type culture collection.

Declarations

Ethical approval and consent to participate

Ethical approval was given by Kenyatta University Ethics Review Committee (KUERC) reference no PKU/700/1772. Informed consents were obtained by signing of informed consent forms by adults. For participants below 18 years, parents or legal guardians would sign the consent forms on their behalf. All the records pertaining to patients were kept anonymous.

Availability of data and Materials

The datasets supporting the conclusions of this article are included within the article and its additional file.
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Consent for publication
Not applicable

Competing Interests
The authors declare that they have no competing interests

Author’s contributions
JA was involved in the conception and study design, acquisition of data, interpretation of data and drafting of the manuscript. MM and SM supervised the project and reviewed the draft. GM helped during acquisition of clinical data at the hospital. All the authors read and approved the final manuscript.

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