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A prospective study of bacterial isolates profile in infected open fractures

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The most common complication in open fractures is infection, which often escalates to sepsis, osteomyelitis, and amputations. The use of prophylactic antibiotics is one of the most effective strategies to prevent infection. The prevailing bacterial isolate patterns must guide the choice of antibiotics for both prophylactic and empiric therapy. This study aims to describe the bacterial isolate profiles in infected open fractures. A prospective cross-sectional study was carried out at Kenyatta National Hospital, Kenya, between October 2019 and January 2020. 66 infected open fractures were identified and pus swabs/infected tissue specimens taken for bacterial cultures. Other data were collected from patient interviews and their hospital records. Results revealed that the culture growth rate was 79%. Gram-negative isolates accounted for 73% while Gram-positive isolates were 27%. The most pre-dominant bacterial isolate was *Pseudomonas aeruginosa* (34%), followed by *Staphylococcus aureus* (27%), *Escherichia coli* (20%), *Proteus mirabilis* (16%) and *Klebsiella pneumoniae* (3%). There were more gram-negative than gram-positive bacterial isolates. The pre-dominant bacterial isolate was *P. aeruginosa* followed by *S. aureus*. The higher proportion of gram negative isolates is in variance with what is widely documented in the literature. The selection of antibiotics for both prophylaxis and empiric therapy should be tailored to the local patterns of bacterial isolates.

Key words: Bacteria isolates profile infected open fractures.

INTRODUCTION

Globally, the estimated incidence of long bone fractures is 11.5 per 100,000 people per year, occurs more in men than women, and has a bimodal age distribution, with the tibia being the most commonly affected bone (Courtbrown and Caesar, 2006). The incidence of these open fractures is high in our local setting due to unsafe modes of transport, particularly the upsurge in the use of motorcycles for public transport (Gachathi, 2016; Waithiru, 2015).

Surgical Site Infection (SSI) is one of the most common complications of open fractures. Infected open fracture wounds have historically been dreaded because of the debilitating effect on the patient. Up to the beginning of the 20th century, open fractures were often treated by

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prophylactic amputations to avoid the sequelae of infection, sepsis, and death (Pappe and Webb, 2008). Most studies report infection rates between 10 - 50% (Asif, 2011; Ioannis et al., 2014; Marcelo et al., 2017; Ondari et al., 2016). Earlier studies done at Kenyatta National Hospital have revealed higher rates than those observed globally. Asif (2011) reported an SSI rate of 50% in all open fractures, while Ondari et al. (2016) found a 28% infection rate in Gustilo II open fractures.

Many guidelines have been developed to reduce the rate of these infections (ACS/TQIP, 2019; British Orthopedic Association Standards for Trauma (BOAST), 2019; Hoff et al., 2011). The cardinal principles include antibiotic prophylaxis, debridement/ irrigation, fracture stabilization, and soft tissue coverage. Early administration of antibiotics and urgent surgical debridement are the most critical strategies in infection prevention. Determining the profile of microbial isolates from infected wounds and their sensitivity/resistance patterns is crucial in developing antibiotic protocols for prophylaxis and empirical therapy.

About 70% of all open fractures get contaminated at the time of injury, mainly by organisms from the patient's skin and the surrounding environment (Gustilo and Anderson, 1976). Further contamination occurs in the course of management at the hospital. A study done to determine if organisms isolated from wounds before initial debridement are similar to those causing infection after debridement found that the isolates were not similar and that infections were mainly caused by nosocomial microorganisms (Sitati et al., 2017). The profile of microbial isolates in infected open fractures may vary from one health institution to another and from time to time. Overall, the commonest isolate is *Staphilococus. aureus*. (Gusrilo and Anderson, 1976; Ashwin and Thomas, 2018).

This study was conducted at the Kenyatta National Hospital (KNH) Orthopedic Department. KNH is the largest national teaching and referral hospital in the East African region with over 1800 bed capacity. It is situated along Hospital Road, Upper Hill area in Nairobi about 5km from the city center. It receives the highest number of trauma patients from its vast catchment area. The high number of trauma cases makes it possible to achieve a good sample size within a reasonable period with results that would better represent the situation in the surrounding referring facilities. The objective of the study was to determine the profile of bacterial isolates in infected open fractures.

MATERIALS AND METHODS

This study was conducted in the Orthopedics wards at Kenyatta National Hospital between October 2019 and January 2020. It was a prospective descriptive cross-sectional study of patients aged between 18 and 75 years with infected open appendicular fractures following initial debridement. The ASEPSIS Score was used to determine infection -scores of 21 and above (Wilson et al., 1986). We excluded patients on chemotherapy and long-term steroids as well as those with diabetes mellitus.

Wound assessment for infection was done on the 3rd day following initial debridement. Non- infected wounds were re-assessed five days later. The sample size was 66 patients, which had been calculated using the Fisher's formula; base on the prevalence of Sitati et al. (2016) in the same hospital, which revealed an infection rate of 58.9%. The first participant was randomly selected. Consecutive sampling was then applied where all subsequent patients with infected open fractures following initial debridement and fitting the criteria were included until the sample size was achieved. Superficial infection was defined as involvement of only skin and subcutaneous tissue with no fluctuation in deep tissue or deep tissue dehiscence beyond the fascia. Deep infection was defined as fluctuation on palpation or purulent discharge from deep tissue layers below the fascia.

Pus swabs were taken from infected wounds using the Levine Method (Gardner et al., 2006). Infected tissue specimens were taken from patients with deep infections during their repeat debridement in theatre as well as during bed-side wound dressing. The Specimens were submitted to the microbiology laboratory within one hour of collection for microscopy, culture and sensitivity. They were cultured within one hour after delivery to the laboratory. Sheep Blood Agar was used for culture, incubated at 35 to 38 degrees Celsius for 18 h followed by further 18 h of sensitivity testing if growth was obtained. Bacterial identification was based on both gram-stain features and colony morphology.

Patients were interviewed and their hospital records checked to obtain demographic data, mechanism of injury, fracture characteristics and wound grading. A questionnaire was utilized to collect the data, which was entered into SPSS version 22 for analysis. Descriptive analysis was done for demographic and other baseline characteristics, while associations were analyzed using chi-square tests. The study was approved by the hospital/university ethics board and all patients gave informed consent.

RESULTS

Baseline characteristics

A total of 66 patients with infected open fracture wounds were recruited into the study. There were 57 (86) males and 9 (14%) females, a ratio of 6.3:1. The minimum age was 19 years while the maximum was 59 years; a range of 40 years. The median age was 36 and the mean age was 36.38 years.

Most of the injuries (59%) resulted from motorcycle accidents followed by motor vehicle accidents at 24%. The commonest site of open fractures was Tibia/fibula shaft followed by tibia plafond and foot/ankle at 30, 20 and 14% respectively. Most of the infected open fractures were Gustilo grade II (48%) followed by Gustilo IIIA (33%), and only 2 patients had Gustilo I fractures. 35% of the patients had their initial wound debridement done within 24 h, while 47 and 18% had their initial debridement done between 24 – 48 h and after 48 h respectively.

Culture growth

Figure 1 show that the Proportion of specimens with

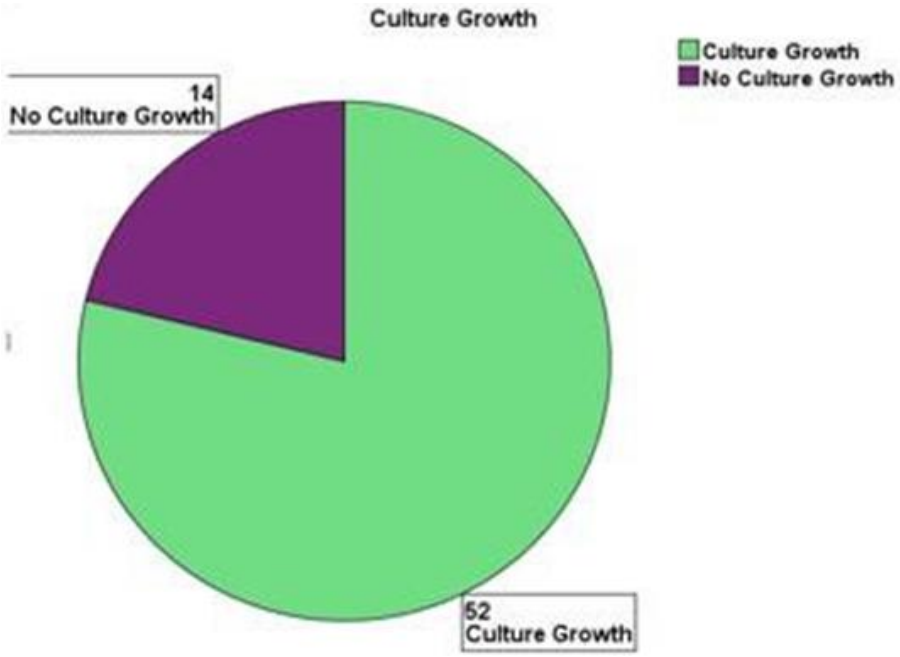

Figure 1. Proportion of specimens with culture growth.

Table 1. Cross tabulation of delayed debridement and type of bacteria cultured.

Delayed Debridement × Gram Stain Cross-tabulation			Gram Stain		Total
			Gram Positive	Gram Negative	
Delayed Debridement	Within 24 h	Count	7	16	23
		Expected Count	6.3	16.7	23.0
	Beyond 24 h	Count	11	32	43
		Expected Count	11.7	31.3	43.0
Total		Count	18	48	66
		Expected Count	18.0	48.0	66.0

The actual counts are similar to the expected counts when no association exists.

culture growth and there was culture growth in 52 specimens (79%) and no culture growth in 14 specimens (21%).

There was a 79% (n = 52) culture growth rate out of the 66 specimens. 81% (n = 42) had single bacterial isolates while 21% (n = 10) had 2 isolates each, thus the total number of isolates studied were 62.

Bacterial isolates

There were 45 (73%) gram negative and 17 (27%) gram positive bacterial isolates. Gram positive bacteria comprised of *S. aureus* while the Gram negatives included *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus Mirabilis and Klebsiella pneumoniae*.

Effect of delayed initial debridement on type of bacteria cultured

Table 1 shows cross tabulation of delayed debridement and type of bacteria cultured. Here the actual counts are similar to the expected counts when no association exists. Also, Table 2 reveals Chi-square tests for association between delayed debridement and type of bacteria cultured. Both tables indicate that there was no statistically significant association between delayed initial debridement and the type of bacteria cultured (P=0.673).

Proportion of specific bacterial isolates

Specific bacterial isolates proportion is described in

Table 2. Chi-square tests for association between delayed debridement and type of bacteria cultured.

Chi-Square Tests	Value	df	Asym Sig.1 sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.178[a]	1	0.673		
Continuity Correction[b]	0.017	1	0.895		
Likelihood Ratio	0.176	1	0.675		
Fisher's Exact Test				0.774	0.442
Linear-by-Linear Association	0.175	1	0.675		
No. of Valid Cases	66				

Chi square value is 0.178, with P value of 0.673. The level of statistical significance was set at 0.05.

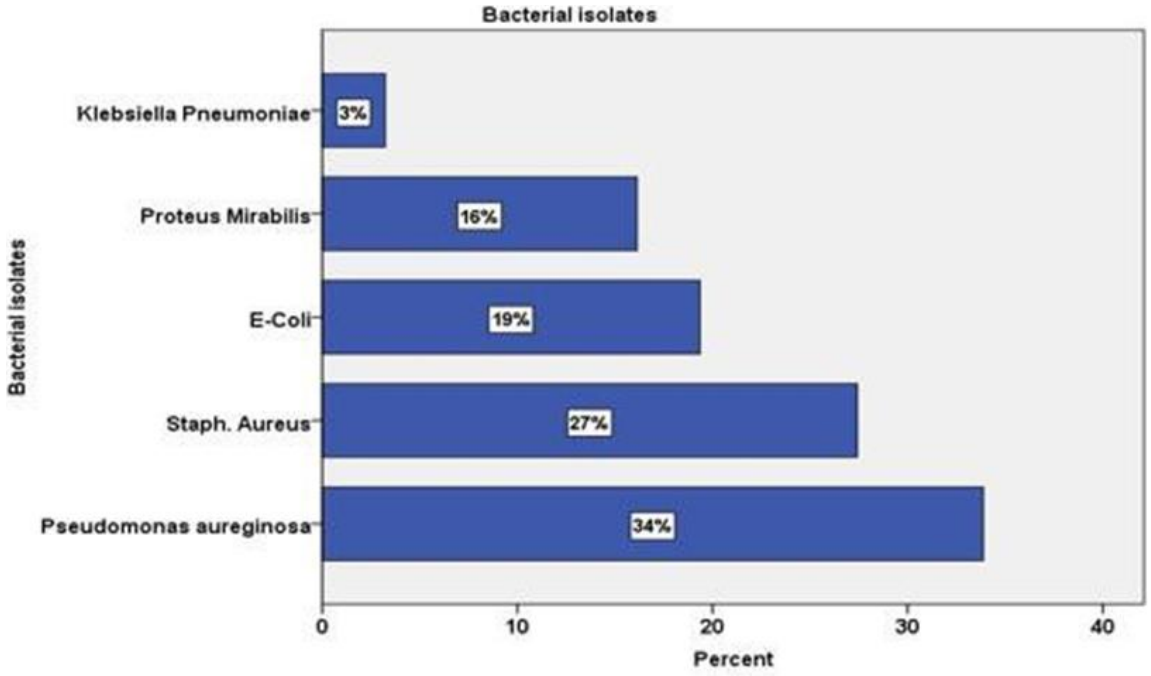

Figure 2. Bacterial isolates cultured.

Figure 2. The most pre-dominant bacterial isolate was *P aeruginosa* (34%), followed by *S. aureus* (27%), *E. coli* (20%), *P. mirabilis* (16%) and *K. pneumoniae* (3%).

DISCUSSION

The baseline characteristics concerning gender and age were similar or close to what was observed in other local studies (Gachathi, 2016; Waithiru, 2015; Ondari et al., 2016). The youthful male preponderance is consistent with the expected gender roles in the local society where males are mostly the bread winners, thus more likely to be involved in risky activities in the transport sector and other industries.

Most of the injuries resulted from motorcycle accidents (59%), followed by motor vehicle accidents (24%). Similar findings were noted by Gachathi in Eldoret: 67% of all injuries resulted from road traffic accidents, among which 65 were from motorcycle accidents while 33% resulted from motor vehicle accidents (Gachathi 2016). Most of the open fractures were Gustilo II (48%) and Gustilo IIIA (33%). The small number of infected Gustilo I fractures (3%) reflects the known minimal infection rate in this group (Gustilo and Anderson, 1976).

35% of the patients had their initial wound debridement done within 24 h as recommended by current literature. Many studies have shown no increase in infection rates when debridement is not done within the historical 6 h period (Reuss and Cole, 2007; Harley et al., 2002). The current consensus is to debride wounds within 24 h on a semi-emergency theatre list, mostly at day time when optimum operating room equipment and personnel are accessible, except for wounds with vascular injury, gross

contamination or compartment syndrome whose intervention should be emergent (BOAST, 2017; ACS/TQIP, 2019). There were patients who had their initial debridement done between 24 – 48 h (47%) and after 48 h (18%). The main reasons for the delays were; late presentation mainly from referring hospitals, lack of blood and blood products, patients being too sick for operations and needing further stabilization by specialists in other departments, and theatre space being taken over by more dire emergencies.

The high culture growth rate achieved (79 %) was attributed to the Levine technique for pus swab specimen collection. In a study comparing three different methods, Levine technique had the highest accuracy with a sensitivity of 90%. The mean concordance between swab specimens obtained using Levine's technique and tissue specimens was 78% (Gardner et al., 2006).

The overall proportion of gram negative bacterial isolates (73%) was higher than that of gram positive isolates (27%). The most pre-dominant bacterial isolate overall *was P. aeruginosa* (34%), followed by *S. aureus* (27%), *E. coli* (20%), *P. mirabilis* (16%) and *K. pneumoniae* (3%). These findings were similar to a few studies found in the literature, which found an overall higher proportion of gram negative bacteria. Among the gram positive bacteria, these three studies found *S. aureus* to be the most predominant (Ako-Nai et al., 2009; Al-Saadi et al., 2018; Nobert et al., 2016). Overall, the most common bacteria isolated from open fractures, as demonstrated in most studies is *S. aureus* (Ashwin and Thomas, 2018; Gustilo and Anderson, 1976).

Local studies at our hospital by Ondari et al. (2016) and Sitati et al. (2017) showed a higher overall proportion of gram positive than gram negative isolates, with *S. aureus* being the most predominant. Our results were different from these two studies. The two studies were quite similar to our study with respect to the setting, population and methodology, with the main difference being the study period. This shows how the pattern of bacterial isolates in a given health institution may vary from time to time, and it is also possible that there was an outbreak of pseudomonas infection in the wards during the period of this study. With delays in initial debridement, it is also possible that the wounds were already contaminated with pseudomonas bacteria prior to initial debridement. However, there was no statistical correlation between delayed initial debridement and the gram-stain characteristics of isolated bacteria (P value = 0.673). The mixing of patients who are awaiting debridement with long-stay patients may also contribute to this possible contamination.

Conclusion

There was a higher proportion of gram negative (73%) than gram positive (27%) bacterial isolates in infected open fracture wounds in our setting. The most common isolates were *P. aeruginosa*, followed by *S. aureus, E. coli, P. mirabilis and K. pneumoniae*. The higher proportion of *Pseudomonas* infection is different from what is documented in the literature and thus points to possible cross-contamination in the wards.

RECOMMENDATIONS

1) Patients awaiting initial debridement should be admitted to a separate trauma ward to minimize the chances of cross-contamination.
2) Surveillance for bacterial isolate profiles in infected wounds should be enhanced to inform a regularly updated prescription protocol for prophylactic/ empiric antibiotic.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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