Antimicrobial resistance in surgical infections: experience from open tibia fracture-related infection intraoperative tissue specimens

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Background
Antimicrobial resistance in surgical infections is known to have significant morbidity and mortality in extreme cases on top of increased costs in medical expenditure. This study aimed to analyze antimicrobial resistance patterns of microbes in open tibia fracture-related infection (FRI) using intraoperative tissue samples.
Methods
Between January and June 2022, a descriptive cross-sectional observational study was conducted involving patients aged 18 and above with open tibial shaft fractures. Microbiological culture and sensitivity of three intraoperative deep tissue and bone samples taken by sterile techniques using separate instruments in patients with infection. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method along with biomechanical tests for microbial identification.
Results
Fifty-seven deep tissue samples were taken from 19 patients with FRI who underwent surgery during the study period were analyzed. Almost all (96.5%) intraoperative tissue samples were culture-positive identifying 73 bacterial isolates out of which 57.9% were polymicrobial. The most frequent isolated bacteria were Gram-negative bacteria 56 (76.7%) with a predominance of Proteus spp 13 (17.8%). Methicilin Resistance Staphyaureus comprised 12 (16.4%) of the isolates whereas Inducible Clindamycin resistance was identified in 8 (57.1%) of Staphyloccus aureus isolates. More than half of the isolates 30 (53.6%) comprised of Extended Spectrum Beta Lactamase producing bacteria. There was high resistance to Cephalosporins and Amoxyclavulanic acid as the efficacy was low ranging from 28.2, 27.8, and 30.0% for cefotaxime, ceftazidime and amoxicillin/clavulanic acid, respectively. Almost all Gram-negative bacteria 47 (83.9%) had multidrug resistance pattern except for Pseudomonas aureginosa that had 77.8 to 88.9% susceptibility to all tested antibiotics.
Conclusion
High resistance to cephalosporins in patients with delayed or late infection in open tibia FRI is alarming and further studies to establish causes are indicated.

Keywords:
antimicrobial resistance, culture and sensitivity, fracture -related infection, open tibia fractures, surgical infections, tissue specimen

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Background

Fracture-related infection (FRI) is a challenging complication after surgical fracture treatment. Consequences include reoperations, prolonged treatment with antibiotics, prolonged immobilization, inability to participate in social and work-related activities, increased medical costs, loss of function, and even amputation [1,2]. Identification of an organism and determination of antibiotic resistance patterns are crucial to a successful outcome in the management of osteomyelitis.

Despite the alarming antimicrobial resistance and morbidity related to FRI globally, antimicrobial therapeutic practices are still based on a Surgeon's discretion from clinical experience, without local microbiological evidence of the pattern of infecting organisms. Microbiological studies are crucial in view of continuous change of microbial ecology to determine the bacterial spectrum of the infections. An antibiogram can then be provided to help determine the best agents to use for prophylactic and therapeutic

treatments. The current study is the first in this setting involving bone or tissue biopsies in microbiological evaluation and forms the stepping stone for further studies in order to formulate a local antibiotic policy when managing open FRI. The aim was to describe the spectrum of bacterial cultural growth in open tibial FRI through bone or tissue biopsies and the antibiotic sensitivity pattern.

Silago *et al*. found 85.1% of the isolates from patients with chronic osteomyelitis grew S.aureus and of which 28.6% were confirmed to methicillin resistant S. aureus (MRSA). Majority of the isolates were gram positive, 81.3%. In that study, the most frequent isolated bacteria isolate was S. aureus by 69.2% [3]. Increase in third generation cephalosporins antimicrobial resistance is reflected in the recent systematic review by Lester *et al*. where E coli had 18.4% resistance to Ceftriaxone as compared with 54.4% in Klebsiella pneumoniae. Nontyphoidal salmonellae had a 1.9% resistance to third generation cephalosporin [4].

In another study by Roth *et al*.; *E.Coli* and Klebsiella pneumoniae were highly resistant to all antibiotics except amikacin and carpenems. E coli had susceptibilities of 42.4% to amoxycillin/clavulanic acid and 41.4% to ceftriaxone where as Klebsiella pneumoniae had susceptibilities of 20.7% to amoxicillin/clavulanic acid and 15.6% to ceftriaxone. There was overall high resistance to third generation to cephalosporins indicating high rates of beta lactamase production [5] A similar trend of antimicrobial resistance has been demonstrated in multiple studies [6–11].

Patients and methods

This was a descriptive cross-sectional observational study that involved intraoperative deep tissue specimen from skeletally matured patients aged 18 and above, with FRI. The study was carried out between January and June 2022. Samples for microbiological cultures were obtained following a standardized protocol. Antibiotics were avoided during or before the sampling, preferably for at least 2 weeks. Preoperative antibiotic prophylaxes were withheld until all culture samples were obtained. Three deep tissue or fluid samples were collected intraoperatively at the area of suspected FRI. Superficial or skin tissue or fluid samples, swabs, and sinus tracts were not considered for bacterial identification due to their low sensitivity.

Each sample was obtained sample was obtained with separate sterile surgical instruments using nontouch technique and transported at room temperature in separate sterile culture containers to the microbiology laboratory using a Tryptic Soy Broth for further processing. Tissue specimens were processed in the Microbiology laboratory. Specimens were received in Tryptic Soy Broth transport media and immediately incubated at 37°C for 18–24 h. The specimen was then cultured into blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18–24 h. Preliminary identification of bacterial isolates was based on colonial morphology and Gram Staining characteristics. Isolates were subcultured on Nutrient Agar (Oxoid, UK) and incubated aerobically at 37°C for 18–24 h to get pure isolates for conventional biochemical tests. The identification tests for Gram negative bacteria included oxidase, carbohydrate utilization, Indole production, Urease, Citrate utilization and hydrogen sulphide gas production. Gram-positive bacteria were identified using Catalase, Coagulase, and Stapharex tests.

Antimicrobial susceptibility testing was performed using the Kirby Bauer disk diffusion method. The inoculum was adjusted to 0.5 McFarland standard's turbidity and swabbed onto the surface of a Muller–Hinton Agar plate. The plates with antibiotic discs were incubated aerobically at 37°C for 18–24 h. The drugs tested for Gram-negative bacteria were in the following concentrations: amoxicillin-clavulanic acid (AMC) (30 μg), Levofloxacin (LEV) (5 μg), gentamicin (CN) (10 μg), Meropenem (MEM) (10 μg), Piperacillin/-tazobactam, Cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), amikacin (AK) (30 μg). Gram-positive bacteria were tested against Penicillin G (P) (10units), Levofloxacin (LEV) (5 μg), gentamicin (CN) (10 μg) Cefoxitin (FOX) (30 μg) erythromycin (E) (15 μg) and Clindamycin (DA) (2 μg). Diameters of the zone of inhibition around the disc were measured using a graduated caliper in millimeters, and interpreted as sensitive, intermediate, and resistant according to the CLSI guideline (CLSI, 2022). Reference strains: *P. aeruginosa* (ATCC-27853), *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as quality control throughout the study for culture and antimicrobial susceptibility testing[12].

Data management and analysis

Data entry from Research Electronic data capture (REDCAP) software [13] and exported to SPSS program (SPPS, Armonk, New York, USA) version 24 and analyzed accordingly. Continuous data were summarized by means, standard deviations and comparisons made through Z or *t* tests. Categorical variables were summarized in frequency tables and comparison made through χ^2 or Fischer's exact test. A *P* value of less than 0.05 was considered significant and *P* value less than 0.2 was selected to run factors into multivariable analysis from binary analysis.

Results

In the 57 tissue specimens collected, 55 (96.5%) were culture positive, and 73 bacterial isolates were identified. From the cultured samples, 33 (57.9%) samples yielded polymicrobial isolates, and 56 (76.7%) isolates comprised of Gram-negative bacteria. *Staphylococci* were the only Gram-positive bacteria isolated in which 17 (23.3%) isolates comprised of 14 (19.2%) *Staphylococcus aureus* and 3 (4.1%) isolates were Coagulase negative Staphylococci (CoNS). Methicilin Resistant Staphyloccoccal aureus (MRSA) comprised of 12 (16.4%) of all bacterial isolates. However, MRSA comprised of 12 (85.7%) of all Staphyloccoccus aureus, Extended Spectrum Beta Lactamase (ESBL) producing bacteria comprised of 30 (53.6%) of all Gram-negative bacteria and Inducible Clindamycin Resistance (ICR) identified by a D test comprised of 8 (57.1%) of all Staphyloccus aureus isolates. Unidentified Gram-negative Rods consisted of 10 (13.7%) of all bacterial isolates.

The antibiotic susceptibility testing was carried out to establish the antibiogram of isolated microbes in these open tibial shaft fracture-related infection. Among *Staphylococcal aureus* isolates there was about 78.6% susceptibility on Clindamycin as opposed to high resistance pattern in the rest of antibiotics of which there was 100% resistance to Penicillin G (Table 1).

There was overall high resistance to most antibiotics specifically Cephalosporins (cefotaxime and Ceftazidime) and amoxyclav. The bacterial susceptibility was in the range of 0–44.4% except for Serratia marcescens which was 100% sensitive. The susceptibility of Gram-negative bacteria to Ceftazidime ranged from 0 to 61.5% with most bacteria except for Pseudomonas that was 77.8% susceptible. Almost all Gram-negative bacterial isolates exhibited high resistance to one or more antibiotics simulating the Multi drug resistance nature of these isolates except for Pseudomonas aeruginosa that had a 77.8–88.9% susceptibility to all tested antibiotics (Table 2).

There was overall good susceptibility to Meropenem, Clindamycin, Amikacin, Piperacillin-Tazobactam and Gentamicin in all bacterial isolates. High resistance was noted with Penicillin G, Cefoxitin, Cefotaxime and Erythromycin (Table 3).

Discussion

The current study identified strikingly high rate of MRSA identified among *Staphylococcal aureus* isolates at 85.7% contrary to what was found in previous studies [3–5,10,11] infection implying ecological change in the bacterial population that is propagating antimicrobial resistance. High rates of Staphylococcal resistance as exemplified by MRSA is clinically alarming as staphylococcal bacteria are some of the commonest pathogens implicated in FRI where prolonged morbidities and amputations may result in rare instances. The current study also found 57.1% rate of inducible Clindamycin resistance carried, higher than the rate found by Manyahi *et al*. [10]. More than half of the isolates (53.6%) comprised of Extended Spectrum Beta Lactamases (ESBL) bacteria another culminating factor to antimicrobial resistance and this is consistent to studies conducted in Zambia [5] and a systematic review by Lester *et al*. [4] that have found increasing rates of ESBL organisms which complicate the antimicrobial resistance and may contribute in increasing morbidity and mortality related to infection. Isolation of rare bacteria Citrobacter freundii in musculoskeletal infection is consistent to a study by Silago *et al*. that similary involved tissue and bone sampling [3].

Antibiotic susceptibility tested among Staphylococcus aureus found a 100% resistance to Penicillin G and very low efficacy of erythromycin, gentamicin, and levofloxacin except for good efficacy with Clindamycin at 78.6% in the present study. Penicillin resistance is consistent with previous studies [5,6,10,11] demonstrating increasing resistance. Although relatively lower compared with previous studies Clindamycin efficacy is similar to what was found Manyahi *et al*. [10] demonstrating a rising rate of Inducible Clindamycin Resistance.

The present study also identified an increasing trend of Multidrug Resistant among the Gram-negative bacteria that had an overall rate of 80.3% among Gram-negatives, far higher than what was found in previous studies [4–6]. The current study found a high rate of resistance of Gram-negative infections to Cephalosporins and Amoxycillin/clavulanic acid with average efficacy of 28.2, 27.8, and 30.0% for cefotaxime, ceftazidime, and amoxyclav, respectively. These efficacy rates are lower than those identified in Kenya, in a study by Sitati *et al*. [14] emulating an increasing

Table 1 Antibiotic susceptibility pattern of gram-positive bacteria from Open Tibial Shaft fracture- related infection intraoperative tissue samples: percentage of bacterial susceptibility

Bacteria	Number of Isolates	Levofloxacin	Gentamicin	Penicillin G	Clindamycin	Cefoxitin	Erythromycin
Staphylococcus aureus	14	28.6%	35.7%	0	78.6%	2.7%	28.6%

Table 2 Antibiotic susceptibility pattern of Gram-negative Bacteria from Open Tibial Shaft fracture-related infection intraoperative tissue samples: percentage of bacterial susceptibility

Bacteria	Number of Isolates	Levofloxacin	Gentamicin	Amikacin	Meropenem	Cefotaxime	Ceftazidime	Amoxyclav	Piperacillin-Tazobactam
Pseudomonas aureginosa	9	77.8%	88.9%	77.8%	88.9%	NA	77.8%	NA	77.8%
Proteus spp	13	61.5%	76.9%	76.9%	100%	38.5%	61.5%	46.2%	76.9%
Enterobacter spp	3	33.3%	0	100%	66.7%	0	0	0	66.7%
Klebsiella pneumoniae	8	75%	75%	75%	100%	12.5%	25%	37.5%	50%
E.Coli	9	66.7%	100%	44.4%	100%	44.4%	55.6%	66.7%	88.9%
Serattia marcescens	1	100%	100%	100%	100%	100%	0	0	100%
Citrobacter spp	1	0	0	0	100%	0	0	0	0
Providencia spp	2	100%	100%	100%	100%	0	50%	50%	100%
Unidentified gram-negative rods	10	70%	60%	40%	100%	30%	30%	40%	40%

resistance pattern to these commonly prescribed antibiotics in the study setting. Moremi *et al*. also found similar resistance pattern of Gram-negative bacteria to Cephalosporins and Amoxycillin Clavulanic acid [15] Enterobacter spp, Klebsiella pneumoniae, E.coli and Citrobacter freundii were found to be resistant to almost all antibiotics tested except Meropenem and Amikacin as reflected in the recent study in by Roth *et al*. [5].

The present study found efficacy of Piperacillin-Tazobactam to Gram-negative bacteria to average at 66.7% nearly twice the rate of resistance found by Moremi *et al*. where resistance to Piperacillin-Tazobactam was found to be 35.5% [15] Pseudomonas aureginosa was susceptible to all tested antibiotics and more sensitive to Meropenem and Gentamicin, similar to findings by Manyahi *et al*. [10].

Gentamicin efficacy to Gram negative bacteria was also found to be lower at 66.6%, compared with higher rate found by Sudduth *et al*. [16]. Effective antibiotics for Gram-negative bacteria as found in the current study are Meropenem, Amikacin, Gentamicin, Piperacillin-Tazobactam, and Levofloxacin in decreasing order of preference.

The susceptibility pattern of the bacterial isolates in delayed and Late FRI may have implications when choosing empirical treatment before definite microbiological diagnosis. Meropenem, Clindamycin, Amikacin, Piperacillin-Tazobactam and Gentamicin are the antibiotics of choice in that order of preference as found in the present study. However use of antibiotics in empirical treatment should be guided by classification of FRI (Early, delayed or late) and local micro biodata.

Limitation

Sonication of extracted implants was not carried out due to lack of appropriate containers and transport media, which could increase the bacterial isolates. Anerobic culture and molecular identification of the bacteria were also not done due to limited laboratory facilities. Antibiotic sensitivity other antimicrobials like Vancomycin and Linezolid was not carried out dues to limited resources.

Conclusion

Methicillin Resistance Staph. aureus, Inducible Clindamycin Resistance and Extended Spectrum Beta-Lactamase producing bacteria represent the challenging bacterial community that fuels antimicrobial resistance. High resistance to cephalosporins in patients with

Table 3 Overall antibiotic susceptibility Pattern to bacterial isolates from Open tibia fracture-related Infection

Antibiotic	Susceptible isolates	Proportion of Susceptible bacteria (%)
Levofloxacin	40 (*n*=70)	57.1
Gentamicin	45 (*n*=70)	64.3
Amikacin	37 (*n*=56)	66.1
Meropenem	54 (*n*=56)	96.4
Cefotaxime	13 (*n*=47)	27.7
Amoxycillin-Clavulanic acid	19 (*n*=47)	40.4
Ceftazidime	28 (*n*=56)	50.0
Piperacillin-Tazobactam	37 (*n*=56)	66.1
Penicillin G	0 (*n*=14)	0
Clindamycin	11 (*n*=14)	78.6
Cefoxitin	2 (*n*=14)	14.3
Erythromycin	4 (*n*=14)	28.6

delayed or late infection in open tibia fracture-related infections is alarming and further studies to establish causes are indicated.

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Ethical Clearance

Ethical clearance was sought and cleared from MUHAS and MOI ethical committees.

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Conflicts of interest

No conflict of interest to be declared.

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