Microbiology of unresolved bone infection: is it recurrence or recalcitrance?

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Abstract

Background

Bone infections are dreaded complications and remain a challenge for orthopaedic surgeons. Unresolved bone infection is caused by a broad spectrum of microorganisms; however, a few microorganisms persist from the initial infection. There are no published series reporting the microbiology in unresolved bone infection in low- or middle-income countries. This study aims to review the recurrence and recalcitrance of microorganisms in unresolved bone infection in the South African setting.

Methods

A single-centre retrospective cohort study reviews patients who underwent revision eradication surgery for unresolved bone infection between June 2016 and March 2023. Recalcitrance of bone infection was defined as the persistence of the same species of pathogen isolated at the time of index eradication surgery for bone infection. Recurrence was defined as a change in pathogen profile at the time of revision infection eradication surgery.

Results

Eleven patients had unresolved bone infections following eradication surgery. There were eight males and three females, with a mean age of 43.27 years (± 12.64 SD, range 25–58). The anatomical sites most frequently affected were the tibia (6/11, 55%) and femur (3/11, 27%). There is no statistically significant difference in the number of single species yielded ($p = 0.586$), polymicrobial species yielded ($p = 1.0$) or negative yield ($p = 0.635$) at secondary surgery. There is no similarity in the distribution of microorganisms at index surgery and secondary surgery. The causative pathogen for cases of unresolved bone infection had a higher probability to be different from the initial isolate obtained at the time of index surgery (73%), representing recurrence rather than persistence of the original infection.

Conclusion

This study reported a higher probability of encountering different species of microorganisms at secondary surgery as opposed to the recurrence of similar species. The culture obtained at index surgery is not a reliable predictor of microorganisms involved in unresolved bone infection. Therefore, new deep specimens are always required to determine the causative microorganism of unresolved bone infection.

Level of evidence: 4

Keywords: unresolved bone infection, osteomyelitis, fracture-related infection, recurrence, recalcitrance

Introduction

Bone infection is a dreaded complication following orthopaedic trauma surgery, and remains a challenge to orthopaedic surgeons.[1,2] Unresolved bone infection is defined as the persistence or re-emergence of bone infection after index infection eradication surgery.[2,3] Additionally, the term 'treatment failure' can be synonymous with 'unresolved bone infection'. The term 'treatment failure' has been described by multiple studies; however, variability in the definition depends on the clinical context.[4-6] Lu et al. defined treatment failure as the recurrence in infection or amputation.[6] Tsang et al., on the other hand, defined treatment failure as the inability to achieve either fracture union or the resolution of infection.[4]

Bone infection has been a persistent concern and an age-old problem impacting individuals across all age spectrums.[7] Patients with unresolved bone infections endure multiple surgeries, pain and suffering, long hospital stays, and increased costs of care. Marais et al. reported the significant burden faced by developing countries, such as South Africa, concerning the prevalence of osteomyelitis.[1] This is a consequence of several factors, including increased incidence of trauma, immunodeficiency diseases, systemic and local host compromises (renal failure, hepatic failure, diabetes mellitus), and malnutrition.[1] Laney et al. illustrated a very

high prevalence of trauma in South Africa, predominantly attributed to road traffic accidents (both motor vehicle accidents [MVA] and pedestrian vehicle accidents [PVA]), followed by interpersonal violence.[8] In South Africa, the road accident fatality rate surpasses any other World Health Organization (WHO) country.[1,9,10] The challenge of high disease prevalence is further exacerbated by the financial implications associated with treating chronic osteomyelitis. In Southern Africa, where the burden of disease is considerably greater, the high cost of treatment poses a substantial challenge to our resource-restricted healthcare system.

Bone infection can re-occur months to years after the index operation, leading to unresolved bone infection.[11] Studies have reviewed the rate of microbial recurrence and recalcitrance in bone infection and reviewed the microorganisms involved with their respective antibiogram.[12-16] In a large cohort study, Young et al. found similarities in the distribution of microorganisms at index surgery compared to secondary surgery.[12] These studies help guide the selection of empirical antibiotic regimens; however, there remains scant evidence in the context of unresolved bone infection or treatment failure. The clinical question in these cases is whether unresolved bone infection is caused by persistence of the initial microorganisms (recalcitrance) or a new microorganism (recurrence).

To the knowledge of the authors, there are no published series reporting the microbiology in unresolved bone infection in low- or middle-income countries. This study aims to review the microbiology and antibiogram data of all patients who underwent revision infection eradication surgery for unresolved bone infection in a South African population.

Methods

Prior to the commencement of the study, ethical approval was obtained from the relevant ethical review board (N22/01/007). This single-centre retrospective cohort study was performed at a tertiary-level bone infection and limb reconstruction unit. All patients who underwent revision infection eradication surgery for unresolved bone infection between June 2016 and March 2023 were analysed.

The diagnosis of fracture-related infections (FRI) was made according to the international consensus definition proposed by Metsemakers et al., and modified by Govaert et al. in 2019.[2,3] Chronic osteomyelitis was defined as bone infection with associated bone necrosis for a minimum duration of ten days, where the pathogens were thought to have persisted either intracellularly or interstitially in biofilm or persistent states. Unresolved infection following the index eradication surgery was defined as the persistence or re-emergence of confirmatory or suggestive signs, as described by the aforementioned diagnostic criteria.[2,3] Patient demographics, comorbidities, aetiology of infection, site of infection, Cierny-Mader (C&M) staging, microbiology culture results, and antibiogram of isolates were recorded.

All patients in this cohort underwent revision infection eradication surgery with deep tissue sampling collected intraoperatively. Deep tissue sampling was conducted during both index eradication and secondary surgery. Deep tissue samples were gathered for culture and sensitivity (MC&S) testing by the hospital's microbiology service. Molecular diagnostic techniques were not used in this study.

In this study, *recalcitrance* was defined as 'the persistence of the same species of pathogen isolated at the time of index eradication surgery for bone infection'. *Recurrence* was defined as 'a change in the isolated pathogen profile at the time of revision infection eradication surgery from the index procedure'. Similar microorganisms were defined as the same species isolated at index and secondary surgery. Different microorganisms were defined as a change in species at the time of secondary surgery.

Statistical analysis was performed using SPSS v25 (IBM Corp, Armonk, NY, USA). Parametric data are reported as mean and standard deviation (SD) with 95% confidence intervals (CI) where appropriate. Non-parametric data are described with median, interquartile range (IQR) and range. Categorical data are described as frequencies and/or counts, with 95% CI where appropriate. Fisher's exact test was used to detect significant differences between categorical data. A p-value of less than 0.05 ($p < 0.05$) was used as the threshold for statistical significance in this study.

Results

Between June 2016 and March 2023, 252 patients underwent single-stage eradication surgery for bone infection. These patients had a minimum of six months of follow-up after index eradication surgery. Of the 252 patients, 223 patients had resolution of bone infection, one patient died after surgery, and 17 patients were lost to follow-up. The remaining 11 patients had unresolved bone infections following initial eradication surgery. There were eight males and three females, with a mean age of 43.27 years (± 12.64 SD, range 25–58 years). The patients' demographics, comorbidities, aetiology, C&M stage, and site of infection are shown in *Table I*. The primary aetiology in the majority of cases was open fracture (8/11, 73%). The anatomical sites most frequently affected were the tibia (6/11, 55%) and femur (3/11, 27%). The median time between surgeries was 281.63 days (IQR 11.2–552.05 days, range 42–914 days).

Microbiology culture results at index surgery were compared to culture results from samples obtained at the time of secondary surgery. At the time of the index surgery, 3/11 (27%) cultures were single species, whereas 6/11 (55%) of cultures were involved in polymicrobial growth, and only 2/11 (18%) were culture negative. At the time of secondary surgery following unresolved bone infection,

Table I: The demographics of the patients with unresolved bone infection

Demographics	n = 11
Male sex (%, n)	8 (73%)
Female sex (%, n)	3 (27%)
Comorbidities:	
Age (mean, IQR)	47.0 (28–56)
Smoking (%, n)	6 (55%)
Diabetes (%, n)	1 (9%)
HIV (%, n)	0 (0%)
Epidemiology (%, n)	
Fracture-related-infection	10 (91%)
Osteomyelitis	1 (9%)
Injury type/aetiology (%, n)	
Open fracture	8 (73%)
Closed fracture	1 (9%)
Gunshot wound	1 (9%)
C&M stage	
1	1 (9%)
2	0 (0%)
3	4 (36%)
4	6 (55%)
Anatomy	
Tibia	6 (55%)
Humerus	3 (27%)
Ankle	1 (9%)
Calcaneus	1 (9%)

Data reported as mean, median (interquartile range) or as frequencies with counts in parenthesis

Table II: The comparison of microorganisms cultured at index surgery compared to secondary surgery for unresolved bone infection

Index operation (n = 15)		Secondary surgery (n = 11)			
Microorganism found in culture at index operation		Same microorganism at secondary surgery n (%)	Similar species and similar antibiogram at secondary surgery n (%)	Different microorganism at secondary surgery n (%)	Cultured negative at secondary surgery n (%)
Staphylococcus aureus	2/15 (13%)	1/11 (9%)	0/11 (0%)	1/11 (9%)	1/11 (9%)
Staphylococcus epidermidis	2/15 (13%)	0/11 (0%)	0/11 (0%)	3/11 (27%)	0/11 (0%)
Pseudomonas aeruginosa	2/15 (13%)	0/11 (0%)	0/11 (0%)	3/11 (27%)	0/11 (0%)
Enterobacter cloacae	2/15 (13%)	0/11 (0%)	0/11 (0%)	2/11 (18%)	0/11 (0%)
Morganella morganii	1/15 (7%)	1/11 (9%)	1/11 (9%)	1/11 (9%)	0/11 (0%)
Proteus hauseri	1/15 (7%)	1/11 (9%)	1/11 (9%)	1/11 (9%)	0/11 (0%)
Proteus mirabilis	1/15 (7%)	0/11 (0%)	0/11 (0%)	2/11 (18%)	0/11 (0%)
Moxarella group	1/15 (7%)	0/11 (0%)	0/11 (0%)	0/11 (0%)	1/11 (9%)
Streptococcus pyogenes	1/15 (7%)	0/11 (0%)	0/11 (0%)	0/11 (0%)	1/11 (9%)
Streptococcus pseudintermedius	1/15 (7%)	0/11 (0%)	0/11 (0%)	0/11 (0%)	1/11 (9%)
Negative culture	2/15 (13%)	N/A	N/A	0/11 (0%)	2/11 (18%)
Aeromonas hydrophila	1/15 (7%)	No additional sampling	No additional sampling	No additional sampling	No additional sampling

Data reported as frequencies with counts in parenthesis

1/11 (9%) cultures yielded a single microbial culture, whereas 5/11 (46%) cultures were involved in polymicrobial growth, 4/11 (36%) cultures yielded culture-negative results, and 1/11 (9%) had no available culture results at secondary surgery.

There was no statistically significant difference in the probability of a monomicrobial positive culture at index surgery compared to secondary surgery (index surgery 3/11 [27%] vs secondary surgery 1/11 [9%]) (p = 0.586). Furthermore, there was no statistically significant difference in the probability of positive polymicrobial cultures at index surgery compared to secondary surgery (index surgery 6/11 [55%] vs secondary surgery 5/11 [46%]) (p = 1.0). In addition, although there was an increase, there was no statistically significant difference in the amount of culture-negative infection at index surgery compared to secondary surgery (index surgery 2/11 [18%] vs secondary surgery 4/11 [36%]) (p = 0.635).

The number of microorganisms isolated at index surgery (n = 15) was compared to the number of microorganisms isolated at secondary surgery (n = 11) for unresolved bone infection (p = 0.225). For each microorganism isolated at index surgery, the number of the same species, different species or negative culture yielded at secondary surgery was documented (*Table II*). At secondary surgery, only 3/11 (27%) of microorganisms isolated were similar to microorganisms isolated at index surgery, and 8/11 (73%) microorganisms isolated at secondary surgery were different. There was no statistically significant difference in the number of similar microorganisms isolated at secondary surgery compared to different microorganisms isolated at secondary surgery (p = 0.086).

A heatmap (*Figure 1*) illustrates the incongruence between microbiological results at index surgery compared to secondary surgery. The most commonly isolated pathogen at both index and secondary surgery was *Staphylococcus aureus* (index surgery 2/15, 13% and secondary surgery 2/11, 18%). However, in 8/11 (73%) cases, the causative pathogen was not isolated at the time of secondary surgery (*Figure 1*).

A total of 3/11 (27%) microorganisms isolated at secondary surgery met the threshold for potential persistence. *Proteus hauseri* and *Morganella morganii* indicated the highest probability of persistence (1/1, 100% and 1/1, 100%, respectively). However, the probability of *S. aureus* remaining the persistent causative organism is 50% (1/2). Any conclusions regarding the probability of persistence of these organisms must be approached with caution due to the small sample size of this study.

In the present study, 4/26 (15%) isolates (methicillin-resistant *S. aureus, Acinetobacter baumannii*, and *M. morganii)* were found to exhibit multidrug resistance. Furthermore, 5/26 (19%) isolates (*Enterobacter cloacae* [4/5] and *M. morganii* [1/5]) demonstrated extended-spectrum beta-lactamase resistance patterns.

Discussion

In international literature, the most common trend is the persistence of similar microorganisms in unresolved bone infection, representing recalcitrance rather than recurrence of infection with different microorganisms.[12] A broad range of microorganisms was noted upon culture in the treatment of unresolved bone infection.[12] International literature supports the practice of administration of broad empiric antibiotics after sample collection in patients with unresolved bone infection while pending new culture results and administering targeted antibiotic treatment depending on the results.[12] This cohort was compared to international literature, more specifically to the studies of Young et al. and Wang et al., which are similar study designs to our cohort.[12,17]

Young et al. reported single species isolated in 60% and multiple species isolated in 28.8% during index surgery.[12] They documented a negative culture rate of 11.2% at index surgery.[12] Additionally, Young et al. identified the most commonly cultured microorganisms at index surgery as *S. aureus,* and *Enterobacterales* followed by *S. epidermidis*.[12] At the time of secondary surgery, Young et al. reported single species isolated in 60% and multiple species in 17.6%.[12] They documented a negative culture rate of 22.4% at secondary surgery. Young et al. identified the most commonly cultured microorganisms at secondary surgery as *S. aureus* and *Enterobacterales*.[12] They reported a consistent distribution of microorganisms at index surgery and secondary surgery.[12] Young et al. reported an equal probability of recalcitrance or recurrence, with 38.4% of microorganisms cultured at secondary

surgery being similar and 39.2% being different when compared to index surgery.[12] They found the highest persistence rate in *S. aureus* (46.3%), coagulase-negative staphylococci (50%) and *Pseudomonas* species (50%). Young et al. also reported 61.11% of persistent microorganisms had similar antibiograms at secondary surgery compared to index surgery.[12]

Wang et al. reported a higher probability of recurrence of unresolved bone infection, with 27.8% of microorganisms being similar species isolated at secondary surgery compared to index surgery.[17] Wang et al. identified the most commonly cultured microorganism as *S. aureus, and Enterobacterales* followed by *S. epidermidis*.[17] Wang et al. reported that *P. aeruginosa* and *S. aureus* were more likely to persist, with *P. aeruginosa* (28%) being twice as likely to persist compared to *S. aureus* (10.85%).[17]

In our series, we observed different frequencies of monomicrobial and polymicrobial culture in both index and secondary surgery compared to Young et al. [12] However, we found similar negative culture rates in both index and secondary surgery compared to the study of Young et al. [12] In our series, the frequencies of both single and polymicrobial growth decreased slightly from index to secondary surgery, while the rate of negative culture yields increased. In this cohort, the most commonly isolated microorganism in both index and secondary surgery was *S. aureus*, which is not in keeping with the studies of Young et al. and Wang et al.[12,17] In this cohort, we found no similarity in the distribution between index and secondary surgery, which is not in keeping with Young et al.[12] The present study's findings are not in keeping with the recalcitrance and recurrence rate found by Young et al. [12] However, this study is in keeping with the findings of Wang et al. who reported a higher rate of recurrence with different microorganisms. [17] In this cohort, the causative microorganism of unresolved bone infection was most likely to be different from the initial isolate obtained at the time of index surgery (73%), representing recurrence of infection rather than persistence of the original infection. In this cohort, we found a similar persistence rate in *S. aureus* (1/2, 50%) compared to Young et al.[12] Compared to Young et al., similar distribution is seen in this cohort: 2/3 (67%) of patients yielded similar microorganisms and similar antibiograms.[12]

The literature indicates an increasing trend of increased antimicrobial resistance in microorganisms cultured in bone infection.[18] However, there is no conclusive evidence of resistance associated with prolonged exposure to antimicrobials in bone infection.[19] Dudareva et al. reported that 15% of microorganisms cultured in osteomyelitis are a result of multidrug-resistant

Figure 1. Heatmap illustrating the relationship between microorganism cultured at index surgery and secondary surgery

			Isolated pathogens at secondary surgery																	
			Gram-negative bacteria								Gram-positive bacteria									
			Negative culture	*Acinetobacter baumannii*	*Aeromonas hydrophila*	*E. coli*	*Enterobacter cloacae*	*Moraxella* group	*Morganella morganii*	*Proteus hauseri*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus agalactiae*	*Streptococcus pseudintermedius*	*Streptococcus pyogenes*	No additional sampling
Isolated pathogens at index surgery		Negative culture																	
	Gram-negative bacteria	*Aeromonas hydrophila*																	N/A
		Enterobacter cloacae																	N/A
		Moraxella group																	
		Morganella morganii																	
		Proteus hauseri																	
		Proteus mirabilis																	
		Pseudomonas aeruginosa																	
	Gram-positive bacteria	*Staphylococcus aureus*																	
		Staphylococcus epidermidis																	
		Streptococcus pseudintermedius																	
		Streptococcus pyogenes																	

X- and Y-axis are grouped according to Gram stain and then arranged in alphabetical order.

Colour key: >1 1 (similar pathogen) 1 (different pathogen)

pathogens, which aligns with the present study's findings, where 4/32 (13%) cultures in both index and secondary surgery indicated multidrug-resistant microorganisms.[20]

The literature provides possible reasons for microbial recalcitrance, including *S. aureus* adherence to previously altered bone resulting in unresolved bone infection, and the immune evasion strategies or mechanisms of *S. aureus*.[14,21,22] *S. aureus* possesses the capacity to form staphylococcal abscess communities (SAC) at the centre of the lesion, generating a fibrin-based pseudocapsule.[23] The pseudocapsule shields *S. aureus* from host immune cells, resulting in microbial recalcitrance.[23] Invasion and colonisation of the osteocyte lacunocanalicular network (OLCN) is a recent discovery for a new mechanism of immune evasion.[22,24,25] De Mesy Bentley et al. have demonstrated that *S. aureus* colonise and proliferate within the submicron canaliculi of bone, deforming diameters as small as 0.2 mm to facilitate invasion and proliferation into the submicron canaliculi.[24] The hypothesis is made that *S. aureus* might possess a novel invasion mechanism that allows for asymmetric cell division into and through a canaliculus.[22,24,25] Another potential mechanism of *S. aureus* recalcitrance is long-term intracellular infection. *S. aureus*, traditionally seen as an extracellular pathogen, can survive and proliferate within leukocytes during infection.[22] The literature reports internalisation of *S. aureus* by non-professional phagocytes, such as epithelial, endothelial, keratinocyte and fibroblast cells.[26-28] Once invaded within the host cells, *S. aureus* can evade apoptosis by host-immune cells.[22,29]

The pathophysiology of recurrence in bone infection with a different microorganism is not well described, as most literature focuses on the pathophysiology of recalcitrance in bone infection. Theories for microbial recurrence at secondary surgery include direct contamination during primary surgery or multiple surgeries thereafter, implant-associated infections during index surgery, and immunosuppression.[17,30,31]

In addition to the current literature, the study proposes an additional possibility or consideration for alteration in culture results (microorganism recurrence). Insufficient sampling technique at index surgery may result in low yield or inadequate culture results guiding the choice of antimicrobial therapy, allowing for the persistence of unrepresented microorganisms at the fracture site.

The main limitation of this study is the limited sample size, which might compromise the quality of the study design. This study investigated bone infection cases of FRI and osteomyelitis; no cases of periprosthetic bone infection (PJI) were investigated in this study.

Conclusion

In a South African sample, culture obtained at index surgery was not a reliable predictor of microorganisms involved in unresolved bone infection. This study concludes that a broad range of microorganisms is found in secondary surgery compared to index surgery. There was a higher probability of encountering different species of microorganisms at secondary surgery as opposed to the recurrence of similar species. Attention should be given to the antibiogram of the new causative organism to treat microorganisms with directed narrow-spectrum antibiotics. Therefore, attention should be addressed not only to the recalcitrance of microorganisms but also to the recurrence of microorganisms at secondary surgery. Future research is encouraged to provide evidence for the observed recurrence of different microorganisms at secondary surgery.

Ethics statement

The authors declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.
Prior to commencement of the study, ethical approval was obtained from the following ethical review board: Stellenbosch University Health Research Ethics Committee (N22/01/007).
This article does not contain any studies with human or animal subjects. For this study formal consent was not required.

Declaration

The authors declare authorship of this article and that they have followed sound scientific research practice. This research is original and does not transgress plagiarism policies.

Author contributions

AJvR: study conceptualisation, data collection, manuscript preparation, approval of final manuscript
SJT: manuscript preparation, approval of final manuscript
RGV: manuscript preparation, approval of final manuscript
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