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Microbiological epidemiology at Chris Hani Baragwanath Academic Hospital: Poly-microbial osteomyelitis analysis

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Microbiological epidemiology at Chris Hani Baragwanath Academic Hospital: Poly-microbial osteomyelitis analysis



Tiego Josiah Hlapolosa

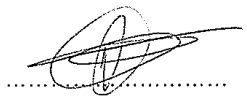
A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of

Master of Medicine

Johannesburg, 2023

Declaration

I Tiego Josiah Hlapolosa declare that this research report in the format of a “submissible” paper is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the division of Orthopaedic Surgery at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

03 October 2023

Dedication

To my wife and son, I wouldn't be where I am without the support structure and love that you continuously provide to me. Thank you

In memory of my parents

Mbuti Andries & Leah Koko Hlapolosa

Presentations and publications arising from the research project

South African Orthopaedic Association Annual Congress-Cape Town September 2023

Acknowledgements

I would like to pay tribute to my mentor and primary supervisor Dr Paul Kgagudi for all the support and guidance throughout this challenging journey. I would also like to pay credit to Dr Maxwell Jingo for all the expert guidance and direction from inception until the completion of this research report

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Nomenclature

CHBAH	Chris Hani Baragwanath Academic Hospital
CMN	Cephalomedullary nailing
ESBL	extended broad-spectrum beta-lactam
GnB	Gram-negative bacteria
HIV	Human Immunodeficiency Virus
IMN	Intramedullary nailing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MVA	Motor vehicle accident
OM	Osteomyelitis
ORIF	Open reduction and internal fixation
PVA	Pedestrian vehicle accident
RTAs	Road Traffic Accidents
TB	Tuberculosis
USA	United States of America

“Submissible” format of a paper

Microbiological epidemiology at Chris Hani Baragwanath Academic Hospital: Poly-microbial osteomyelitis analysis



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Abstract

Background

The majority of the patients seen and treated at our clinical setting present during chronic osteomyelitis stage, which is anecdotally likely to be poly-microbial. Adults with poly-microbial infection have a predilection for gram-negative bacteria and anaerobes, a scenario that hypothetically leads to a higher morbidity of poly-microbial osteomyelitis following high-energy trauma. Our study looks into the epidemiology of poly-microbial osteomyelitis treated at our Tumour and Infection unit.

Methods

This was a retrospective study of patients treated for osteomyelitis from 1st of June 2016 to 30th of May 2021. Medical records of eligible patients were retrieved for examination. Demographic data such as age, sex and race were recorded. Clinical presentation, and organisms cultured, including their anti-microbial sensitivities were documented.

Results

There were 63 participants in the study. Thirty-one (49.21%) participants had mono-microbial osteomyelitis with 32 (50.79%) participants having poly-microbial osteomyelitis. Majority of the poly-microbial patients presented with a draining sinus (68.75%) and most was located in the tibia (50%).

Multiple mixed pathogens (both gram-positive and gram-negative) were cultured in combination in our patients and this comprised 71.21% of the total bacteria cultured. *Staphylococcus Aureus* was the commonest bacteria (30%) isolated, followed by *Enterococcus faecalis* (12%). The

commonest gram-negative bacteriae cultured was *Enterococcus cloacae* (10%) followed by *Acinetobacter baumannii* at 7%. Most *enterobacteriacae* species were sensitive to Ertapenem and Ceftazidime.

Conclusion

A slightly higher incidence of poly-microbial osteomyelitis was found in our study than that reported in literature. Furthermore, our study demonstrated a wide variety of organisms found in poly-microbial osteomyelitis, with a large contribution made by gram-negative anaerobic rod-shaped bacteria. Cephalosporins were not shown to be valuable as broad-spectrum cover drugs, and most bacteria cultured were found to be insensitive to them. What we suggest is tailoring antibiotics to the specific cultured bacteria and sensitivity. Prompt management of patients with open fractures with early administration of intravenous antibiotics and adequate surgical management may lead to a reduction in the prevalence of chronic osteomyelitis.

Introduction

Osteomyelitis (OM) is an old age disease dating back to the times of Hippocrates.[1] The term is a fusion of two words *osteo* meaning “bone”, *myelitis* meaning “infection”. The disease is established following two clinical scenarios: (i) haematogenous spread and (ii) direct inoculation. In adults, 50% of cases are secondary to open fractures and surgery thereof, with the rest of the cases occurring due to haematogenous spread from a distant source.[2] A high volume of patients with open wounds and fractures from road traffic accidents (RTAs) as well as other trauma-related injuries are commonly seen in developing countries.[3]

Kremers *et al*. reported that OM demonstrated a predilection for males more than females, with more than 60% of cases accounted for by males.[2] These injuries are difficult to treat and subsequently leading to chronic infections in those cases.[3]

The gold standard for diagnosing OM is positive microbiological cultures from deep bone tissue biopsy samples obtained during surgery.[3] This approach has demonstrated high sensitivity and specificity for making a causative diagnosis. Blood cultures have high sensitivity only in acute forms of osteomyelitis and are usually not useful in the diagnosis of chronic bone infections.[3] Bernard et al. reported that surgical bone biopsy through the sinus tract had a sensitivity of 87%, a specificity of 79%, and a diagnostic accuracy of 82.5%. Bacteraemia occurred in nearly 20% of the patients presenting with bone infection.[4]Gram-positive organisms are the most commonly isolated bacteria in chronic OM, with *Staphylococcus aureus* (*S. aureus*) being the commonest bacteria isolated.[3] Gram-negative bacteria (GnB) are: (i) sharply increasing as reported in literature as causative agents in chronic OM, and (ii) usually drug-resistant e.g. extended broad-spectrum beta-lactam (ESBL) producing organisms. Therefore, GnB presents a challenge in the anti-microbial treatment/approach used.[5] Other studies have identified Methicillin-resistant

Staphylococcus aureus (MRSA) as a growing threat for hospital and community acquired infections. In 2016, MRSA infections were reported to account for almost 40% of implant-related infections in the United States of America (USA).[3] Locally, Mthethwa and Marais in their series reported a combination of *Enterobacteriaceae* species, followed by *Staphylococcus*, *Pseudomona*s and *Enterococcus* species in 45% of their cases of poly-microbial OM[1].

Successful surgical treatment in cases of poly-microbial OM requires aggressive and repeated surgical debridement; followed by a long course of intravenous antibiotics.[6] Anti-microbial therapies should be tailored to local sensitivity patterns and individual scenario. As a result, knowledge of institutional micro-organisms culture patterns is critical to successfully treat OM. In the South African context, we have an endemic problem of adult high-energy trauma with an overwhelming open fracture burden. On that basis, our study aimed to document and analyse the epidemiology of poly-microbial OM treated at our Tumour and Sepsis unit. Furthermore, our study seeked to propose antibiotic therapy relevant at our local clinical setting.

Objectives of the study were to: (i) document the incidence of poly-microbial OM, (ii) identify the commonest micro-organisms in poly-microbial OM and (iii) determine the antibiotic sensitivity profile in poly-microbial OM.

Methods

This was a retrospective study of patients treated for OM from 1st June 2016 to 30th May 2021. Medical records of 63 patients previously treated for OM at our Tumour and Sepsis unit, Chris Hani Baragwanath Academic Hospital (CHBAH) were retrieved for examination. Patient demographic data, i.e. age, race, gender and clinical aspects of the disease (site of infection, type of presentation and microbiological specimen from the national health laboratory services

(NHLS)) for each case were documented. All data were entered onto a Microsoft excel spreadsheet by the principal investigator.

Inclusion criteria:

- Patients 18 years of age and older
- Patients treated for poly-microbial osteomyelitis from 01 June 2016 – 30 May 2021
- Patients with complete laboratory results

Exclusion criteria:

- Patients visiting for re-treatment of osteomyelitis
- Patients suffering from TB and fungal infections

Data Analysis

All data were imported to Stata version 17 for analysis. Categorical variables were analysed using frequencies and percentages. Bar, Pie and column graphical representations were used to describe the distribution of categorical variables. To determine the incidence of poly-microbial OM and mono-microbial OM patients; frequency distributions were used. The normality assumption for continuous data was done using the Shapiro-Wilk test. Continuous variables were analysed using median and interquartile range (IQR) since the data were non-normal and the study sample size being small. Box and whisker plots were used to describe the distribution of the continuous data. The commonest organism in poly-microbial OM was described using the frequency distribution. The anti-biogram sensitivity profile in poly-microbial OM was reported in a Table using

frequencies and percentages. Association between pathogen classification and body classification was done using cross-tabulations and a Fisher's exact test was used to assess for the significant differences in the proportions. The level of significance was set at 5%.

Results

There were 63 participants recorded for our study duration and 31 (49.21%) had mono-microbial OM while 32 (50.79%) had poly-microbial OM. The majority of patients were males in both groups (n = 22, 68.75%) - poly-microbial; and (n = 20, 64.52%) - mono-microbial as shown in Figure 1.

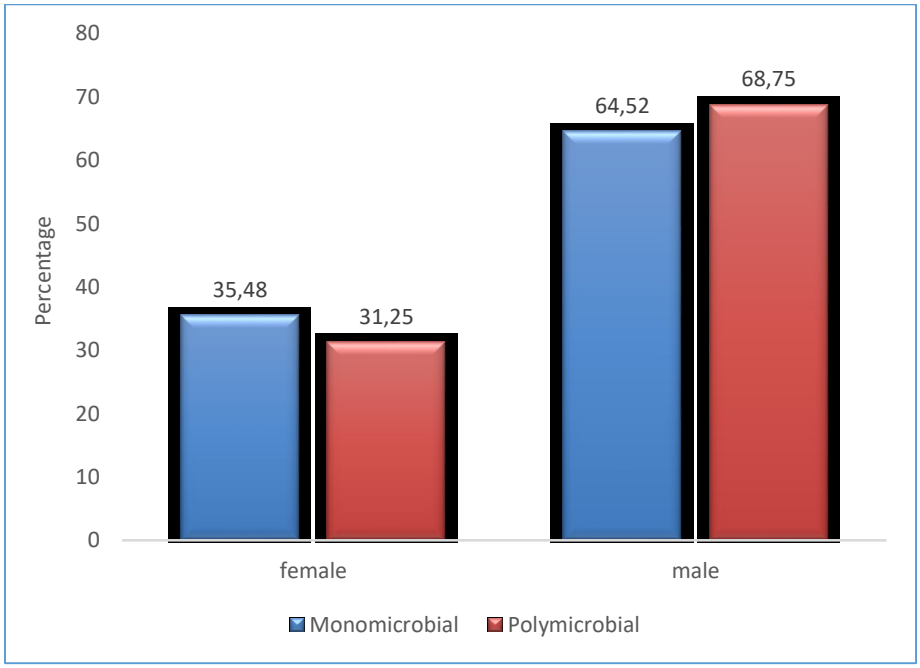


Figure 1. Participants gender composition in the study.

The age distribution is shown in Figure 2. The median age for patients with poly-microbial OM was 36 years (IQR = 31.5 – 56 years).

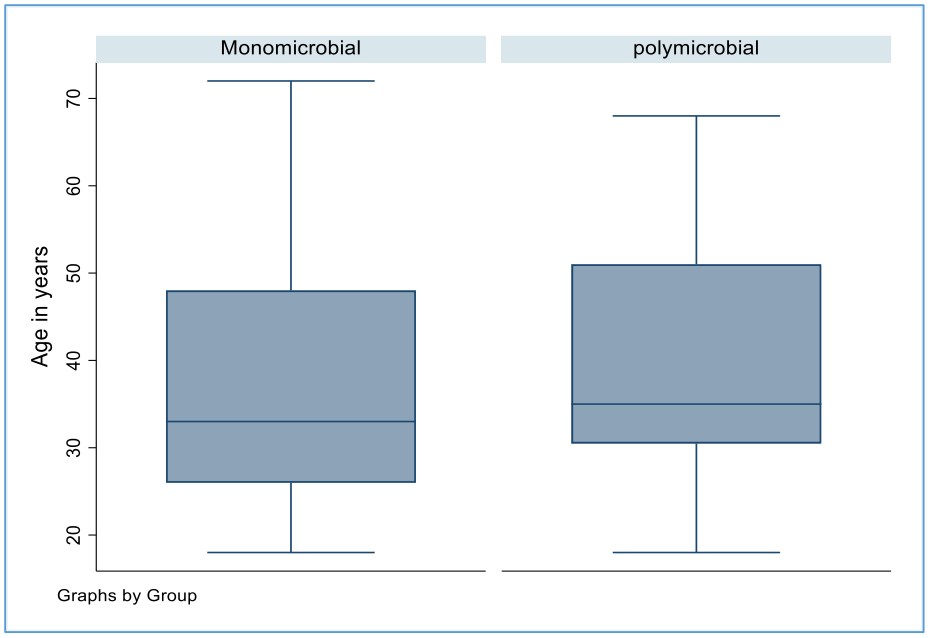


Figure 2. Age distribution per group.

The bone type affected was also assessed and the results are shown in Figure 3. The most common bone affected was the tibia, which accounted for 50% in poly-microbial OM patients. The femur was the second bone of predilection.

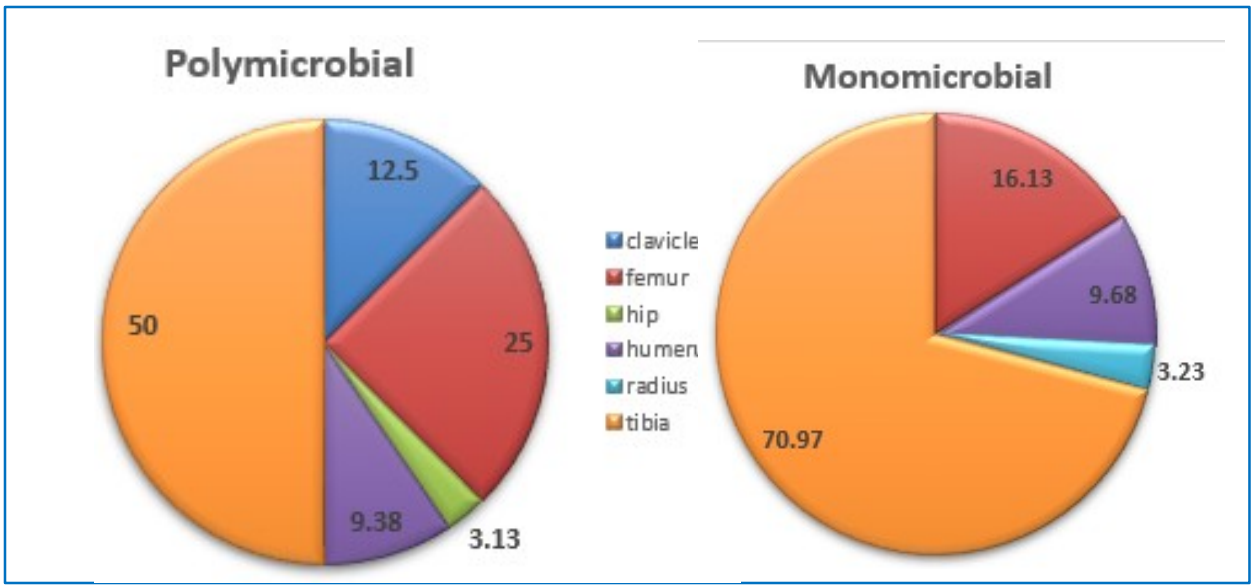
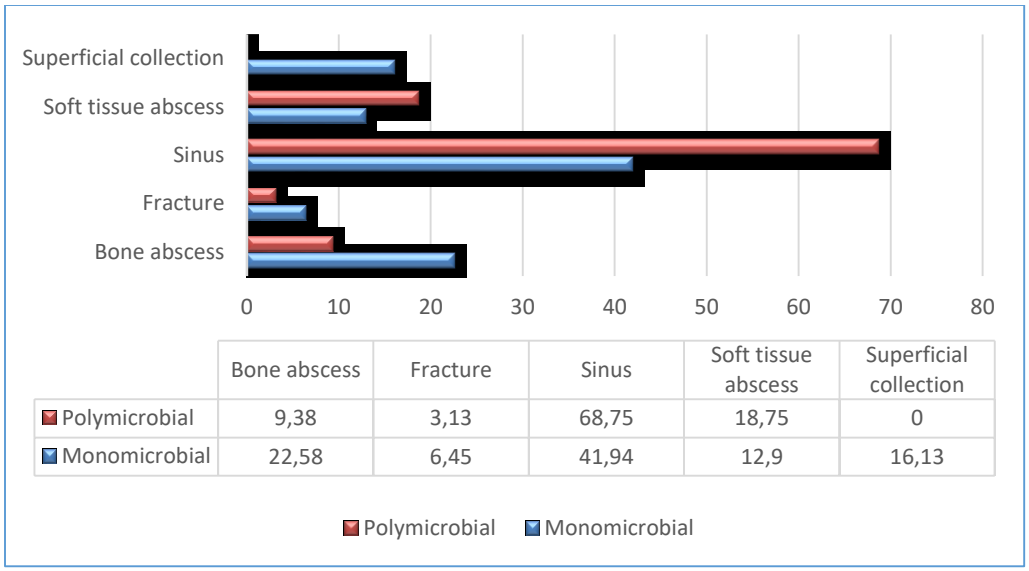


Figure 3. The bone distribution.

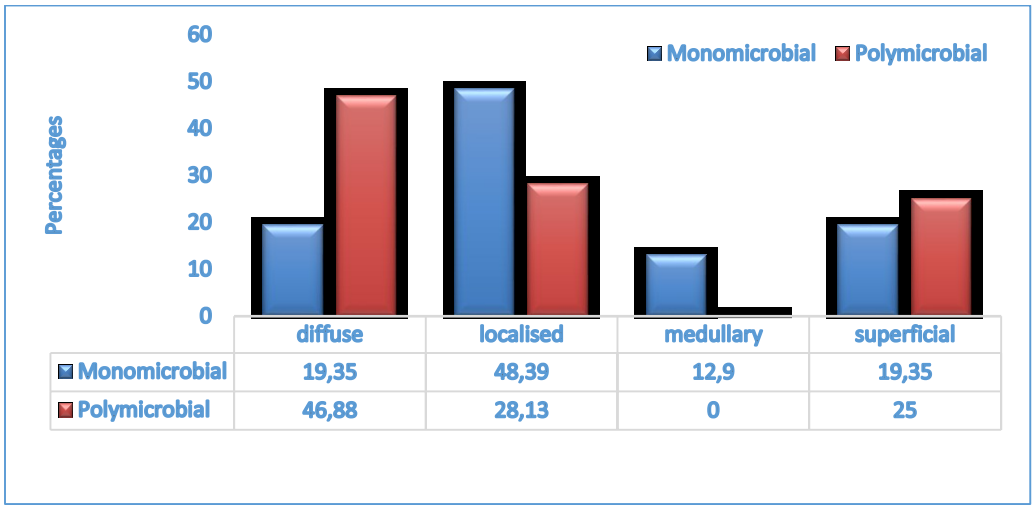
The type of presentation distribution between mono-microbial OM and poly-microbial OM groups is shown in Figure 4. Most patients had a sinus at presentation, 68.75% in the poly-microbial OM group and 41.94% in the mono-microbial OM group. Abscess formation was the second form of presentation, 22.58% of mono-microbial OM patients had a bone abscess while soft tissue abscess was the second mode of presentation in the poly-microbial OM group as it presented in 18.75% of the patients.



	Bone abscess	Fracture	Sinus	Soft tissue abscess	Superficial collection
Polymicrobial	9,38	3,13	68,75	18,75	0
Monomicrobial	22,58	6,45	41,94	12,9	16,13

Figure 4. Mode of presentation distribution.

The classification distribution is shown in Figure 5. In the mono-microbial OM group, most people had a localised (48.39%) presentation while in the poly-microbial OM group, majority (46.88%) were classified as diffuse.



	diffuse	localised	medullary	superficial
Monomicrobial	19,35	48,39	12,9	19,35
Polymicrobial	46,88	28,13	0	25

Figure 5. Classification distribution.

The number of pathogens isolated by individual frequency is shown in Figure 6. The poly-microbial OM group is defined as individuals with two or more micro-organisms isolated. Most of the poly-microbial OM patients had two pathogens (31.82%) isolated and 21.21% had four pathogens isolated individually.

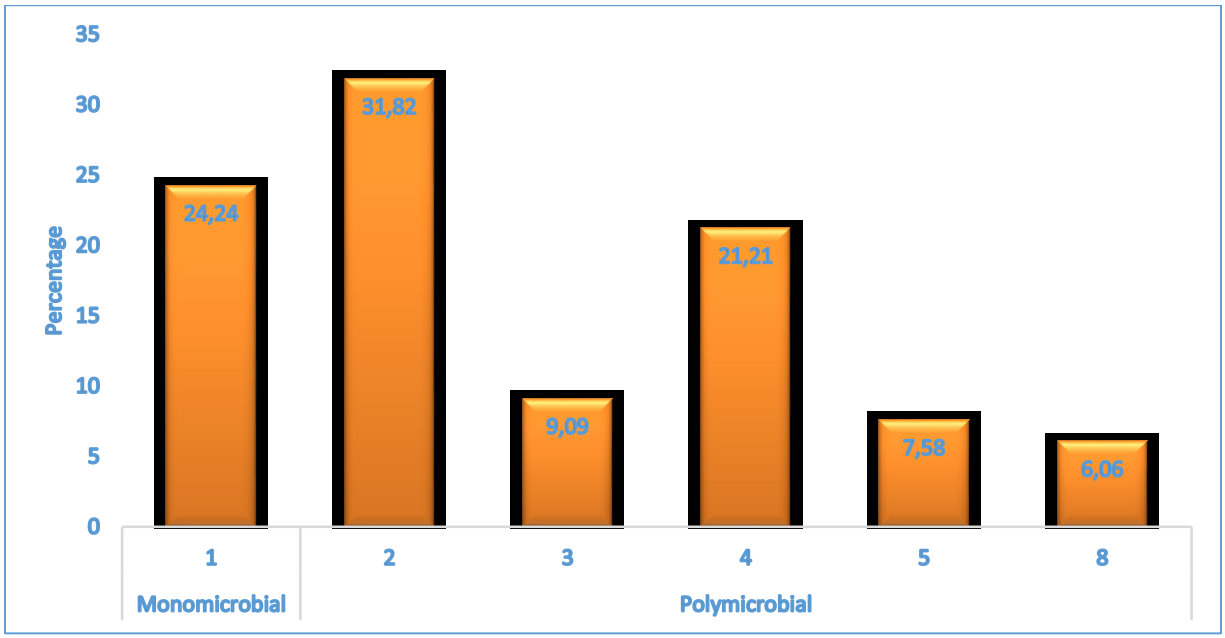


Figure 6. The number of pathogens isolated by frequency.

Table I shows the list of all the pathogens isolated and their frequency distribution in both the mono-microbial OM group and the poly-microbial OM group. The commonest pathogen was *S. aureus* identified in 28.79% of the patients followed by *Enterococcus faecalis* (*E. faecalis*) identified in 9.09% of the patients. *Enterobacter cloacae* (*E. cloacae*), *Acinetobacter baumannii* (*A. baumannii*), *Proteus mirabilis (P. mirabilis)* and *Pseudomonas aeruginosa* (*P. aeruginosa*) accounted for 7.58%, 6.82%, 6.82% and 5.3%, respectively. *Pseudomonas aeruginosa* (*P. aeruginosa*) accounted for 7.58%, 6.82%, 6.82% and 5.3%, respectively.

Table I: Bacteria cultured for mono-microbial and poly-microbial combined.

Bacteria cultured	**Mono-microbial n (%)**	**Poly-microbial n (%)**	**Frequency**	**Percentage (%)**
Staphylococcus aureus (*S. aureus*)	8(25.0)	30(30.0)	38	28.79
Enterococcus faecalis (*E. faecalis*)		12(12.0)	12	9.09
Enterobacter cloacae (*E. cloacae*)	4(12.5)	10(10.0)	10	7.58
Acinetobacter baumannii (*A. baumannii*)	2(6.625)	7(7.0)	9	6.82
Proteus mirabilis (*P. mirabilis*)	1(3.13)	8(8.0)	9	6.82
Pseudomonas aeruginosa (*P. aeruginosa*)	1(3.13)	6(6.0)	7	5.3
Methicillin-resistant *Staphylococcus aureus* (MRSA)	1(3.13)	4(4.0)	5	3.79
Enterococcus faecium (*E. faecium*)	3(9.38)	2(2.0)	5	3.79
Eschericia coli (*E. coli*)	1(3.13)	3(3.0)	4	3.03
Bacillus species	2	2(2.0)	3	2.27
Enterobacter species	2	1(1.0)	3	2.27
Klebsiella species	-----	3(3.0)	3	2.27
Citrobacter freundii	1(3.13)	1(1.0)	2	1.52
Enterococcus gallinarum	-----	2(2.0)	2	1.52

Klebsiela pneumoniae	1(3.13)	1(1.0)	2	1.52
Proteus vulgaris	-----	2(2.0)	2	1.52
Serratia liquefaciens	1(3.13)	1(1.0)	2	1.52
Serratia marcescens	-----	2(2.0)	2	1.52
Anaerobic gram-negative bacillus (AGNB)	1(3.13)	-----	1	0.76
Bacteroides capillosus (BACTA)	1(3.13)	-----	1	0.76
Bacteroides eggerthii	-----	1(1.0)	1	0.76
Bacteroides fragilis	-----	1(1.0)	1	0.76
Citrobacter koseni	-----	1(1.0)	1	0.76
Clostridium clostridioforme	-----	1(1.0)	1	0.76
Clostridium perfringes	-----	1(1.0)	1	0.76
Klebsiella oxytoca	-----	1(1.0)	1	0.76
Staph haemolyticus	1(3.13)	-----	1	0.76
Streptococcus pyogenes	-----	1(1.0)	1	0.76
Streptococcus viridans	-----	1(1.0)	1	0.76
Yersinia enterocolitis	-----	1(1.0)	1	0.76

Figure 7 shows the pathogen classification. Most of the patients (n = 94, 71.21%) had mixed pathogens (gram-negative and gram-positive), (n = 32, 24.24%) had gram-positive pathogens and (n = 6, 4.5%) had gram-negative pathogens.

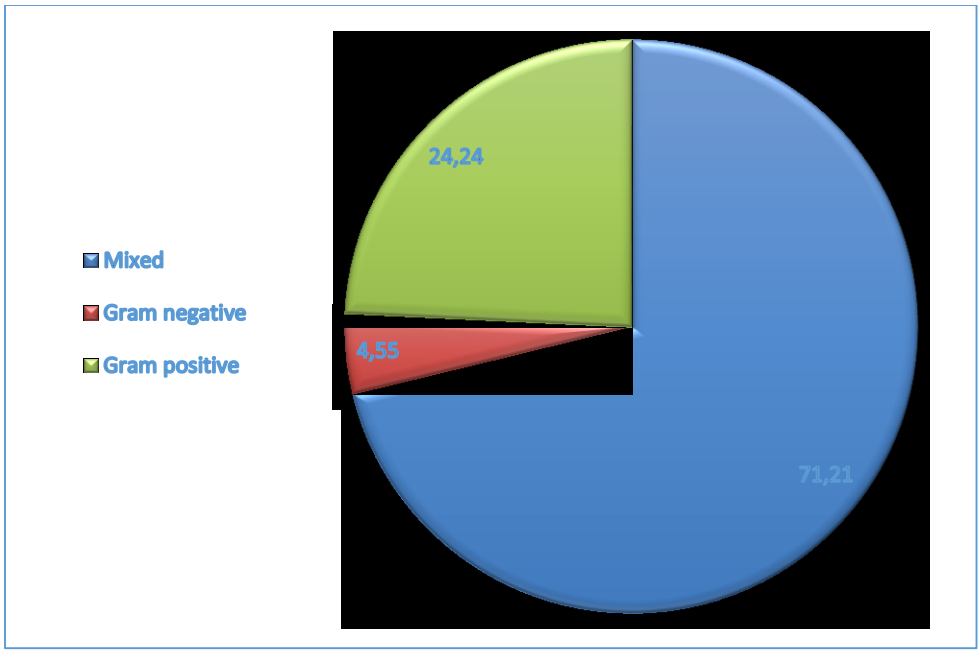


Figure 7. The pathogen classification.

The anti-microbial sensitivity pattern for each of the pathogens is shown in Table II. *S. aureus* was variably sensitive to Ampicillin (n = 8, 21.05%), Cloxacillin (n = 6, 15.79%), Vancomycin (n = 7, 18.42%) and Amoxicillin (n = 7, 18.42%). *E. faecalis* was predominantly sensitive to Ertapenem (n = 4, 33.33%). *E. cloacae* were mostly sensitive to Cloxacillin (n = 5, 50%). *A. baumannii* was sensitive to Colisitn (n = 6, 66.67%). All MRSA pathogens were sensitive to Vancomycin (n = 5, 100%).

Table III shows the anti-microbial sensitivity pattern exclusively for poly-microbial OM. *S. aureus* was sensitive to Cloxacillin (n = 13, 72, 22%). *E. faecalis* was sensitive to Ampicillin (n = 9, 90%) with *E. cloacae* sensitive to Ertapenem (n = 8, 80%). *P. Aeruginosa* was sensitive to Ceftazidime (n = 4, 66.67%).

Table IV shows the body classification and pathogens. Most of the pathogens were in the tibia. However, there was no association between pathogen classification and body classification, p-value > 0.05

Table II: Anti-microbial sensitivity patterns for both mono-microbial and poly-microbial OM.

Pathogens	***S. aureus***	***E. faecalis***	***E. cloacae***	***A. baumannii***	***P. mirabilis***	***P. aeruginosa***	***MRSA***	***E. faecium***	***E. coli***	***K. species***
	n = 38	**n = 12**	**n = 10**	**n = 9**	**n = 9**	**n = 7**	**n = 5**	**n = 5**	**n = 4**	**n = 3**
Antibiotics	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Cloxacillin	33(86.84)		1(10)	1(11.11)				1(20.0)		
Ampicillin	2(5.26)	11(91.67						1(20.0)		
Azithromycin	3(7.89)									
Clindamycin	2(5.26)									
Erythromycin	3(7.89)									
Ertapenem	4(10.53)		8(80)		2(22.22)			1(20.0)		
Meropenem			3(30)							
Imipenem			5(50)		2(22.22)					1(33.33)
Vancomycin	5(13.16)						5(100)	3(60.0)		

Colistin				6(66.67)						
Amoxicillin		11(91.67)						1(20.0)		
Piperacillin-Tazobactam				2(22.22)	2(22.22)				2(50.0)	2(66.67)
Amoxicillin Clavulanic acid		1(8.33)			4(44.44)				2(50.0)	
Cefepime			2(20)							
Ciprofloxacin			1(10)		1(11.11)					
Ceftazidime				1(11.11)		5(71.43)				
Cefotaxime										
Cefuroxime					1(11.11)					
Gentamycin										

Table III: Anti-microbial sensitivity patterns for poly-microbial OM.

Pathogens	***S. aureus***	***E. faecalis***	***E. cloacae***	***A. baumannii***	***P. mirabilis***	***P. aeruginosa***	***MRSA***	***E. faecium***	***E. coli***	***K. species***
	n = 18	**n = 10**	**n = 10**	**n = 9**	**n = 8**	**n = 6**	**n = 4**	**n = 4**	**n = 3**	**n = 3**
Antibiotics	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Cloxacillin	13(72.22)		1(10)	1(11.11)				1(25.0)		
Ampicillin	2(11.11)	9(90)						1(25.0)		
Azithromycin	2(11.11)									
Clindamycin	1(5.56)									
Erythromycin	1(5.56)									
Ertapenem	1(5.56)		8(80)		2(25.0)			1(25.0)		
Meropenem			3(30)							
Imipenem			5(50)		2(25.0))					1(33.33)
Vancomycin	1(5.56))							2(50.0)		

Colistin				6(66.67)						
Amoxicillin		9(90)						1(25.0)		
Piperacillin-Tazobactam				2(22.22)	1(12.5)				2(50.0)	2(66.67)
Amoxicillin-Clavulanic acid		1(10.0))			2(25.0)				2(50.0)	
Cefepime			2(20)							
Ciprofloxacin			1(10)		1(12.5)					
Ceftazidime				1(11.11)		4(66.67))				
Cefotaxime										
Cefuroxime					1(12.5)					
Gentamycin										

Table IV: Bacterial pathogens in relation to body classification.

	Mixed **n (%)**	**Gram-negative** **n (%)**	**Gram-positive** **n (%)**	**p-value**
Clavicle	6(6.38)	0	0	0.549
femur	24(25.53)	2(33.3)	6(18.75)	
hip	5(5.32)	0	0	
humerus	4(4.26)	0	4(12.5)	
radius	2(2.13)	0	1(3.13)	
tibia	53(56.38)	4(66.67)	21(65.63)	

Discussion

Chronic OM is a complex infection due to its varied aetiology and associated factors. It needs to be approached in a multi-disciplinary manner. Because OM requires antibiotic therapy for prolonged periods, this poses a major financial burden; therefore, epidemiological studies on the bacterial and anti-microbial sensitivity profile are valuable in guiding healthcare policy developers on developing appropriate antibiotic guidelines.

Our study demonstrated that a majority of patients with infected with poly-microbial OM (50.79%) were males (68.75%), with a median age of 36 years. This is in agreement with what Khalid *et al*. found in their study, where they reported 67.35% of patients out of 130 patients to be male.[7] The majority of our patients with poly-microbial OM were of African descent (87.5%). This reflects, demographically the population of Soweto, of which CHBAH is the main treating hospital.

The most common type of inoculation found in our study for mono-microbial OM was post-surgical (20.03%) following either tibial or femoral intramedullary nailing. For poly-microbial OM, surgery was only causal in 15.63% of that cohort. The above-statements cements trauma as the leading cause of chronic OM and theoretically poly-microbial OM. Neeharika *et al*. in their study reported that 45% of their cases of chronic OM resulted from trauma.[8] Mthethwa and Marais reported mixed poly-microbial infections to be 16.7% resulting from post-traumatic aetiology.[1] This is similar to the findings of our study. The tibia bone was the commonest affected lower limb bone in our study, accounting for 50% of poly-microbial infected patients. The femur bone was secondarily affected, accounting for 25% of poly-microbial infected patients. Our results are consistent with those of Salman *et al.*, where 12% of their patients presented with lower limb infections.[9]Jorge *et al*. in their study had 79.2% infections in the lower limbs.[10]

The majority of poly-microbial infected patients in our study presented clinically with a draining sinus (68.75%). Kubwimana *et al*. also discovered the hallmark clinical presentation to be a sinus discharging pus in 50 – 93% of their patients.[11] Our findings are clear and consistent with these global findings. Mthethwa and Marais in their study, published results that revealed bacterial pathogens in relation to the physiological status of the host/patient, in accordance with the Cierny and Mader staging classification system for chronic OM, with the majority of their patients being host Type B.[1] Our study focused on the anatomical component of the classification system, and showed that the majority of our poly-microbial infected patients presented with diffuse chronic OM according to Cierny and Mader's classification scheme (46.8%). This correlates to a type IV infection which has both medullary and cortical involvement.[12] The latter representing a severe spectrum component of the disease.

Sheehy *et al*., in their series found *S. aureus* to be the most commonly isolated organism regardless of aetiology (32%), and their poly-microbial OM infections accounted for 29% of

cases.[13] In our study, *S. aureus* was identified in 28.79% of patients as the commonest organism, and in agreement with Sheehy *et al*. This was followed by *E. faecalis* at 9.04% and *E. cloacae* at 7.58%. The high prevalence of *E. cloacae* in 10% of poly-microbial infected patients in our study group highlights the rise of gram-negative organisms in poly-microbial OM already highlighted in the literature.[14]

Peng *et al*. reported on the proportion and cases of gram-positive (53.85%) and gram-negative bacteria at 46.15% in their 84 patient study.[15] In our study, 24.24% of our patients cultured gram-positive organisms and 4.55% patients cultured gram-negative organisms. A high proportion of our patients cultured a mixed pathogen pattern at 71.21%. This is significantly higher than previously reported in both local and international literature. The latter can possibly be explained by the delays our patients experience before they are taken to theatre for their initial debridement post-injury. This coupled with the significant burden of open fractures in our context has serious implications on chronic OM and subsequently the poly-microbial type. Jorge *et al*. reported an increased number of post-traumatic poly-microbial infections in Gustilo-Andersen grade 3 type open fractures.[7] Kindsfater and Jonassen infections in 7% of their grade 2 and 3 open fracture patients debrided in < 5 hours, a percentage that rose to 38% for patients debrided ≥ 5 hours' post-injury.[16] This correlates well with our findings and it possibly explains our high number of mixed pathogen poly-microbial infections.

The commonest gram-positive organism cultured in our poly-microbial group was *S. aureus* at 30%, followed by *E. faecalis* at 12%. MRSA although dreaded was present in 4% of cases with *E. faecium* accounting for only 2% of our cohort. Gram-negative bacteria cultured in the poly-microbial group were *E. cloacae* (10%), *P. mirabilis* (8%), *A. baumannii* (7%) and *P. aeruginosa* in 6% of cases. This reflects an increase in the number of gram-negative bacteria prevalence in poly-microbial infections consistent with international trends already highlighted earlier.

The micro-organisms cultured in our group displayed a broad range of sensitivities to antibiotics. The majority of *S. aureus* were sensitive to Cloxacillin (72.22%). Jones *et al*. revealed 53% of *S. aureus* in their study being susceptible to Oxacillin.[17] This correlates well with our findings. *S. aureus* was also sensitive to Ampicillin, Azithromycin and Clindamycin in our study group. MRSA cultured (n = 5) was sensitive to Vancomycin in all our poly-microbial infected patients (100%). *E. faecalis* was sensitive to both Ampicillin and Amoxicillin (90%) and, Amoxicillin-Clavulanic acid in 10% of cases. *Enterobacteriaceae* species, *E. cloacae* and *P. mirabilis* were sensitive to Ertapenem, 80% and 25%, respectively. *P. aeruginosa* was mainly sensitive to Ceftazidime (66.67%) in our poly-microbial group.

A. baumannii was mostly sensitive to Colistin (66.67%) and Piperacillin-Tazobactam (22.22%). Mthethwa and Marais in 2017 found *A. baumannii* to be sensitive to Cloxacillin in 5 of 7 patients who cultured the bacteria.[1]The sensitivity differential displays the potential difficulty at our local setting when treating *A. baumannii*, with resistance to anti-microbials most likely due to geographical reasons. In future we can expand our study to explore the in-depth the resistance patterns that exist for organisms cultured. A further discussion around the impact of HIV on poly-microbial osteomyelitis in our context can further be explored, even though Mthethwa and Marais showed that HIV does not appear to alter the microbiology of chronic osteomyelitis, a different context may yield different outcomes.

Conclusion

The incidence of poly-microbial osteomyelitis in our study was found to be slightly higher than previously reported in other studies. In our study we had an incidence of 50.79% of poly-microbial OM, with a high incidence of mixed pathogens cultured (72.1%). The commonest micro-organisms in our poly-microbial OM group of patients were *S. Aureus* (30%). Our study found

that, the majority of *S. aureus* were sensitive to Cloxacillin (72.22%) and *A. baumannii* was mostly sensitive to Colistin (66.67%) and Piperacillin-Tazobactam (22.22%). Moreover, the proportion of mono-microbial OM patients in our study was 49.1%. We recommend treating surgeons to be aware of the importance of instituting early debridement in minimising the occurrence of poly-microbial OM. A wide variety of anti-microbial sensitivities noted for the different pathogens will hopefully sensitize and depict the burden of treatment posed by poly-microbial OM, especially in a high trauma burden context.

Ethics statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Prior to the commencement of the study, ethical approval was obtained from the Human Research Ethics Committee (Medical) of University of the University with reference number: M210842.

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

JTH: study conceptualisation, data capture, data analysis, manuscript preparation, revision and approval of final manuscript

PMK: study conceptualisation, manuscript revision and approval of final manuscript

MJ: study conceptualisation, manuscript revision and approval of final manuscript

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Appendices

Appendix A: Data Collection Sheet

Appendix B: Ethics Certificate

Appendix C: Hospital permission letter

Appendix A: Poly-microbial osteomyelitis data Collection Sheet

Study number	
Age	
Gender	
Race	
Comorbidities	
HIV status	
Type of inoculation	
Bone affected	
Type of presentation (superficial collection/ Soft tissue abscess/bone abscess/fracture/sinus)	
Classification (medullary/superficial/localised/diffuse)	
Mono- versus poly-microbial	
Number of organisms (2/3/4/5 organisms)	
Bacteria cultured	
Antibiotic sensitivity	

Appendix B: Ethics certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr TJ Hlapolosa

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210842

NAME: (Principal Investigator)	Dr TJ Hlapolosa
DEPARTMENT:	School of Clinical Medicine Department of Surgery Division of Orthopaedic Surgery Medical School University
PROJECT TITLE:	*Microbiological epidemiology at Chris Hani Baragwanath Academic Hospital: Poly-microbial osteomyelitis analysis*
DATE CONSIDERED:	2021/08/27
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr M Jingo
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/03/16

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **August** and therefore reports and re-certification will be due in the month of **August** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2022/04/15
Date

Appendix C: Hospital Permission letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 30 June 2021

TITLE OF PROJECT:

Microbiological epidemiology at Chris Hani Baragwanath Academic Hospital: Poly-microbial osteomyelitis analysis.

UNIVERSITY: Witwatersrand

Principal Investigator: Dr T J Hlapolosa

Department: Orthopaedic Surgery

Supervisor/s : Dr P M Kgagudi et.al.

Permission Head Department (where research conducted): Yes

NHRD No GP 2021 06 057

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

.....
Recommended
(On behalf of the MAC)
2021/06/30

.....
Approved/Not Approved
Hospital Management Date:
Date: 30/06/2021