

THE COMPARISON OF MICROBIOLOGIC PATTERN IN CHRONIC OSTEOMYELITIS OVER 5 YEARS PERIOD, HAYATABAD MEDICAL COMPLEX, PESHAWAR

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INTRODUCTION

Osteomyelitis is an inflammatory state of bones caused predominantly by an infection.¹ Osteomyelitis can be caused by trauma, surgical contamination, vascular insufficiency, or acute hematogenous osteomyelitis, which is more common in pediatric age groups.² Traumatic osteomyelitis accounts for approx. 80% pathogenesis of osteomyelitis.³ Osteomyelitis can be divided into acute and chronic types. Acute osteomyelitis can be defined as a recent bone infection with a systemic inflammatory response, while chronic osteomyelitis is characterized by symptoms of 6 weeks to 3 months.^{4,5} The presence of fever, chronic pain, erythema, swelling & tenderness around affected bone, impaired wound healing & persistent sinus tracts are the

ABSTRACT

OBJECTIVES

This study aimed to quantify the changes in microbiological patterns associated with chronic osteomyelitis over five years. It specifically focused on infections caused by multi-drug resistant (MDR) bacteria and the susceptibility of antimicrobial treatments in the Department of Orthopedic and Spine at HMC, Peshawar.

METHODOLOGY

This cross-sectional study was conducted in the Department of Orthopedic & Spine Surgery Hayatabad Medical Complex Peshawar, Pakistan, from 1st August 2023 to 31st July 2024. The sample size was 133. A non-probability consecutive sampling technique was used for sampling. All patients fulfilling the inclusion criteria were included in our study. Patient's age (< 45 years or > 45 years) & gender (men/women) were our demographic variables, while the presence of chronic osteomyelitis was our research variable. Data was analyzed using IBM-SPSS-V.25.

RESULTS

Out of 134 patients in the study, 70(52.2%) were males & 64(47.8%) were females. Among patients in Group A, i.e., from 11th Nov 2018 to 1st Nov 2019, the most prevalent microbe causing osteomyelitis was Pseudomonas aeruginosa, 29%, followed by MRSA found in 26.3%. Among patients in Group B, i.e., 1st Nov 2023 to 1st Nov 2024, the most prevalent microbe causing osteomyelitis was Methicillin Sensitive Staph aureus (MSSA) 40.2% followed by Pseudomonas aeruginosa 13.8%. MRSA was isolated from 2 cases. Among instances of various osteomyelitis, E-Coli & Pseudomonas aeruginosa were the most resistant microbes to multiple antibiotics.

CONCLUSION

The evolving antibiotic resistance to various microbes has made it mandatory to perform cultures of infected bone & to use antibiotics that are sensitive to specific organisms. Further, in our setup, there has been a decline in several MRSA cases in 5-year period causing osteomyelitis. Pseudomonas aeruginosa & E-Coli are associated with multi-drug-resistant Chronic Osteomyelitis.

KEYWORDS: Osteomyelitis, Bone, Antibiotics, Infections

clinical features associated with chronic osteomyelitis.⁶ Cierny and Mader developed a classification system for chronic osteomyelitis, which combines both the stages of the anatomic disease, i.e., Medullary, Superficial, localized & diffuse, and physiologic state of host, i.e., Normal host, compromised host (systemic, locally or both) & a host in which treatment is worse than the disease (unfit for surgery).^{7,8} In former times, experts usually recommended parenteral antibiotics for the treatment of osteomyelitis as penetration of antibiotics to bone is low. Traditionally, IV therapy was recommended for 4-6 weeks, followed by an oral route for weeks to months, while currently, Surgical debridement along with a Local antibiotic delivery system, followed by IV therapy for an initial 2 weeks, is recommended to achieve maximum serum

concentration of antibiotic. The duration of antibiotic treatment shows that regimens less than 4 weeks are highly prone to failure & therapies longer than 6 weeks also don't improve the outcome. So, antibiotic regimens are used for 6 weeks.^{9,10} Cierny-Mader devised a 2-stage treatment of osteomyelitis. In the first stage, adequate debridement, wash & antibiotic-loaded cemented beads having Vancomycin & Gentamicin are mostly placed along with the obliteration of dead space.¹¹ In the second stage, after 4-6 weeks, beads are removed & replaced with a cancellous bone graft.¹² The antibiotics can also be locally transferred by antibiotic-coated nails, spacers & antibiotic-coated ILN.¹³ Staph aureus is The most common organism causing osteomyelitis, accounting for approx. 75% of the cases & among these, 50% are caused by MRSA. Other organisms causing osteomyelitis include Streptococcal species, E-Coli, Pseudomonas & Enterococcal species.^{14,15} Dudareva et al., from Oxford, UK, conducted a study on two cohorts of patients having chronic osteomyelitis.¹⁶ Their research found that from 2001-2004, Staph aureus was involved in 21.7% of cases of osteomyelitis, among which MRSA caused 9.6% of cases. Enterobacteriaceae was involved in 16.3% of cases, while pseudomonas was approximately 5.4%. Ten years later, from 2013 to 2017, it was found that Staph aureus is involved in 33.2% of cases, with 4.3% MRSA positive. Enterobacteriaceae was involved in 23.3%, while pseudomonas in 7.3% of cases. Pozo et al. from Spain, in a study, found that the tibia was the most commonly involved bone in non-union & the most frequently isolated bacteria were Staphylococcus aureus, i.e., 58.5% of positive cultures.¹⁷ Singh et al. from Bihar, India in their study found that out of 132 patients having osteomyelitis, 43.9% isolates were Staphylococcus aureus positive (32.7% were MRSA positive & 67.2% were MSSA positive), Coagulase negative Staphylococcus aureus (CONS) was 9.8%, Enterococcus in 8.3%, E-coli in 18.9%, Klebsiella in 11.3%, Pseudomonas aeruginosa in 3.7% & Proteus mirabilis in 5.3%.¹⁸ Unawareness about the microbiology pattern over five years, antibiotic resistance & most common micro-organisms causing osteomyelitis in the Orthopedic unit, HMC, Peshawar, was our research problem. Our research will provide important information about the most common organisms involved in osteomyelitis & as a result, can help in the initiation of empirical therapy. It will also guide surgeons regarding antibiotic resistance & the microbes' sensitivity to various antibiotics.

METHODOLOGY

This cross-sectional study was conducted in the Department of Orthopedic & Spine Surgery Hayatabad

Medical Complex Peshawar, Pakistan, from 1st August 2023 to 31st July 2024. Approval for the study was taken from the Hospital Ethical Committee & informed consent was taken from patients or attendants. The sample size was calculated using the Rao soft sample size calculator. The confidence interval is 95%, the anticipated proportion is 9.6% & margin of error is 5%. The sample size was 133. A non-probability consecutive sampling technique was used for sampling. All adult patients aged >20 years with a diagnosis of chronic osteomyelitis were included in the study. Patients having a history of tuberculosis, autoimmune diseases such as Rheumatoid arthritis or SLE & cancer patients, which can affect bone health & healing, were excluded from the study. Chronic osteomyelitis was diagnosed using clinical and radiographic findings. The diagnosis of chronic osteomyelitis was based on clinical assessment and the presence of sequestra or sinus tracts on X-rays or CT scans & a positive culture of sequestrum or sinus tracts. After receiving approval from the Hospital's ethical committee, a study was conducted. All patients fulfilling the inclusion criteria were included in our study. Patient's age (< 45 years or > 45 years) & gender (men/women) were our demographic variables, while the presence of chronic osteomyelitis was our research variable. Patients were divided into two groups: Group A had confirmed diagnosis of chronic osteomyelitis from 11th Nov 2018 to 1st Nov 2019 & Group B had confirmed diagnosis of chronic osteomyelitis from 1st Nov 2023 to 1st Nov 2024. Deep bone samples were taken from the infection site under strict aseptic measures to detect chronic osteomyelitis. Up to 10 samples were taken from each patient from abnormal tissues, including dead bone, granulation tissue & pus from the medullary cavity. All collection procedures were performed under the supervision of an Associate professor & a Professor in Orthopedics, having at least 15 years of post-fellowship experience & a trained staff nurse. All samples, i.e., deep infected tissues or pus, were sent for Culture and Sensitivity from the same laboratory. Gram staining & culture were used to detect the bacteria causing chronic osteomyelitis. The Kirby-Bauer disk diffusion technique was used to detect antibiotic susceptibility. Data was analyzed using IBM-SPSS-V.25. Mean± S.D was evaluated for numerical variables, i.e., age, gender & duration of illness. Categorical variables like gender, age groups & duration of illness were assessed by counts & percentages. Data is presented in the form of tables and diagrams.

RESULTS

Out of 134 patients in the study, 70(52.2%) were males & 64(47.8%) were females. 82(61.1%) patients were

>45 years and 52(38.8%) were <45years old. The average duration of osteomyelitis was 6 weeks. Among patients having chronic osteomyelitis, 14.8% of patients had diabetes mellitus & 5.2% of patients were hypertensive. Among patients in Group A, i.e., from 11th Nov 2018 to 1st Nov 2019, the most prevalent microbe causing osteomyelitis was Pseudomonas aeruginosa, 29% (21/72). MRSA was found in 26.3% (19/72), E-Coli was obtained from 22.2% (16/72) isolates, MSSA was found in 15.2% (11/72), Klebsiella pneumonia 4.1% (3/72), Actinobacteria & Aeromonas hydrophilia were found in one patient. Among patients in Group B, i.e., from 1st Nov 2023 to 1st Nov 2024, the most prevalent microbe causing osteomyelitis was Methicillin Sensitive Staph aureus (MSSA) 40.2% (29/72). Pseudomonas aeruginosa was found in 13.8% (10/72), E-coli 12.5% (9/72), Klebsiella pneumonia 11.1% (8/72), Streptococcus pyogenes 6.9% (5/72), Coagulase negative Staph aureus 5.5% (4/72), Enterobacter species 4.1% (3/72), one case of Citrobacter specie and bacillus cereus was found. MRSA was isolated from 2 cases. Among instances of various osteomyelitis, E-Coli & Pseudomonas aeruginosa were the microbes most resistant to multiple antibiotics. The chi-square test of independence was used to find the association between the most prevalent microbes in both groups, i.e., Group A & Group B, in causing chronic osteomyelitis & the results were statistically significant, i.e., p-value<0.05 showing increased prevalence of Methicillin sensitive staph aureus in causing chronic osteomyelitis in group A as compared to group B.

Table 1: Chi-square test of independence to find association between most prevalent microbes in Groups A & B

		MS SA	Pseudomonas Aeruginosa	Total	Chi-square value	P-Value
Group A	Observed	29	10	32	11.42	0.00725
	Expected	20	15.5	35.5		
Group B	Observed	11	21	39		
	Expected	20	15.5	35.5		
Total		80	62	142		

Table 2: Comparison of microbiological pattern in chronic osteomyelitis patients over 5 years

Micro-Organism	Group A	Group B
Methicillin Sensitive Staph aureus (MSSA)	29(40.2%)	11(15.2%)
Methicillin Resistant Staph aureus (MRSA)	2(2.7%)	19(26.3%)
Pseudomonas Aeruginosa	10(13.8%)	21(29%)
Escherichia-Coli	9(12.5%)	16(22.2%)
Klebsiella Pneumonia	8(11.1%)	3(4.1%)
Streptococcus pyogenes	5(6.9%)	None

Table 3: Antibiotics and their sensitivity/resistance pattern in various microbes in Group B

Antibiotics	MS SA (n=29)		Pseudomonas Aeruginosa (n=10)		E-Coli (n=9)		MRSA (n=2)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Vancomycin	18	11	--	10	--	9	02	09
Piperacillin-Tazobactam	--	--	1	9	4	5	--	9
Ceftazidime avibactam	--	--	1	9	1	8	--	9
Oxacillin	8	21	--	10	--	9	--	9
Linezolid	27	2	--	10	--	9	--	9
Doxycycline	25	4	--	10	6	3	--	9
Fusidic acid	25	4	--	10	--	9	--	9
Fosfomycin	24	5	--	10	--	9	--	9
Cloramphenicol	--	--	--	10	4	5	--	9
Amikacin	--	--	--	10	3	6	--	9
Gentamicin	--	--	3	7	6	3	--	9
Cefepime	--	--	2	8	3	6	--	9
Meropenem	--	--	4	6	--	9	--	9
	--	--	4	6	6	3	--	9

Table 4: Antibiotics and their sensitivity/resistance pattern in various microbes in Group B

Antibiotics	Bacillus cereus (n=04)		Klebsiella Pneumonia (n=8)		Coagulase negative Staph aureus (n=4)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Vancomycin	04	--	--	10	02	2
Piperacillin-Tazobactam	--	4	--	08	--	--
Oxacillin	--	04	--	08	02	02
Linezolid	01	03	--	08	04	--
Doxycycline	--	4	01	7	03	01
Fusidic acid	--	4	--	8	--	--
Clindamycin	01	03	--	8	03	01
Cefepime	--	04	--	8	--	--
Meropenem	01	03	2	06	--	--
Fosfomycin	--	04	07	01	--	--
Ceftazidime avibactam	--	--	04	04	--	--

DISCUSSION

Chronic osteomyelitis is a prolonged, lasting infection of bone & bone marrow. Chronic osteomyelitis is well known for being resistant & requires aggressive surgical debridement in addition to antibiotic therapy.¹ The dead bone and implant are the favorite sites for bacteria to adhere to & result in biofilm formation.¹⁹ Chronic osteomyelitis may require antibiotic therapy for months to years & as a result, microbe identification is necessary for long-term treatment.²⁰ Injudicious antibiotic use has led to antibiotic resistance; hence,

culture & continuous monitoring are necessary for treating chronic osteomyelitis.²¹ Dudareva et al., in their study, found that Multidrug resistance pathogens associated with osteomyelitis were found in 17.1% of infections in the 2001-2004 cohort & were found in 15.2% of cases of infection from the 2013-2017 cohort.¹⁶ Their study found that a combination of glycopeptide, i.e., Vancomycin & aminoglycoside, i.e., Gentamicin, has the lowest resistance, with 58.8% of infections susceptible to these antibiotics' combinations. In our study, it was found that among patients in Group A, i.e., 11th Nov 2018 to 1st Nov 2019, the most prevalent microbe causing osteomyelitis was *Pseudomonas aeruginosa* 29%, followed by MRSA was found in 26.3%, E-Coli 22.2%, MSSA 15.2%, *Klebsiella pneumonia* 4.1%. Among patients in Group B i.e., 1st Nov 2023 to 1st Nov 2024, the most prevalent microbe causing osteomyelitis was Methicillin Sensitive *Staph aureus* (MSSA) 40.2% followed by *Pseudomonas aeruginosa* 13.8%, E-coli 12.5%, *Klebsiella pneumonia* 11.1%, *Streptococcus pyogenes* 6.9%, Coagulase negative *Staph aureus* 5.5%, Enterobacter species 4.1% & MRSA was isolated from 2 cases. Among instances of osteomyelitis, E-Coli & *Pseudomonas aeruginosa* were the most resistant microbes to multiple antibiotics. Similar to our study, Dudareva et al. from Oxford, UK, found that *Staph aureus* was involved in 21.7% of cases of osteomyelitis, among which MRSA caused 9.6% of cases. Similarly, Pozo et al. from Spain found that the most commonly isolated bacteria were *Staphylococcus aureus*, i.e., 58.5% of positive cultures. Singh et al. from Bihar, India, in a study, found that out of 132 patients having osteomyelitis, 43.9% isolates were *Staphylococcus aureus* positive (32.7% were MRSA positive & 67.2% were MSSA positive), E-coli in 18.9%.^{16,17,18} Antibiotic resistance is one of the main concerns in treating osteomyelitis patients. Our study found that MRSA, E-Coli & *Pseudomonas aeruginosa* were most resistant to multiple antibiotic regimens. Similar to our research, Jerzy et al. found that approx. 83% of *Staph aureus* were resistant to Methicillin & 67% were resistant to Ceftazidime. Gram-negative bacteria were resistant to multiple drugs, i.e., E-Coli was sensitive to only Ceftazidime, while *Pseudomonas aeruginosa* was sensitive to Ceftazidime & Ciprofloxacin. Similar to our study, Zhang et al. from China found that the resistance of *Pseudomonas aeruginosa* strains to Cefotaxime, cefuroxime, cefazolin & cefoxitin was nearly 100%. E-Coli resistance to Ciprofloxacin was 44.4%.^{22,23}

LIMITATIONS

The study is limited by its cross-sectional design, preventing assessment of the progression of root

resorption over time. Being a single-center study, the findings may not be generalizable to other populations. Radiographic limitations, including the lack of three-dimensional imaging like CBCT, may result in diagnostic inaccuracies. Observer bias in radiographic interpretation could also affect reliability. Additionally, the study does not include histological confirmation, and potential confounding factors such as orthodontic treatment, trauma, or systemic conditions may not be fully accounted for.

CONCLUSIONS

The treatment of chronic osteomyelitis, in addition to aggressive surgical debridement, requires prolonged antibiotic therapy. Due to evolving antibiotic resistance to various microbes, it is mandatory to perform a culture of infected bone & to use antibiotics that are sensitive to specific organisms. Further, in our setup, there has been a decline in several MRSA cases in 5-year period causing osteomyelitis. *Pseudomonas aeruginosa* & E-Coli are associated with multi-drug-resistant Chronic Osteomyelitis.

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