Section: Microbiology

Original Research Article

ETIOLOGICAL AGENTS OF ACUTE OSTEOMYELITIS WITH SPECIAL REFERENCE TO THE ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND ASSOCIATION OF PANTON VALENTINE LEUCOCIDIN GENE AMONG STAPHYLOCOCCUS AUREUS ISOLATES

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ABSTRACT

Background: Osteomyelitis is a severe bone infection that poses a significant challenge in medical practice due to its chronicity, recurrence, and antibiotic resistance. Staphylococcus aureus is the primary etiological agent, with the Panton-Valentine Leucocidin (PVL) toxin contributing to its virulence and severity. This study aims to isolate aetiological agents of acte osteomyelitis, assess the prevalence of S. aureus in osteomyelitis cases, analyze its antibiotic susceptibility patterns, and determine the association of the PVL gene. **Materials and Methods:** A total of 75 osteomyelitis samples were collected from clinically diagnosed patients at a tertiary care hospital and processed using standard microbiological techniques, including culture, biochemical testing, and polymerase chain reaction (PCR) for PVL gene detection. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, with results interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. **Result:** The findings reveal a high prevalence of methicillin-resistant S. aureus (MRSA), significant resistance to beta-lactam antibiotics, and an association of PVL-positive strains with more severe disease manifestations. **Conclusion:** The study highlights the necessity for early molecular diagnosis, stringent antimicrobial stewardship, and novel therapeutic approaches to manage osteomyelitis effectively.

INTRODUCTION

Osteomyelitis is defined as an infection-induced inflammation of the bone, often involving the marrow, cortex, periosteum, and surrounding soft tissues.[1] It can be categorized as acute, subacute, or chronic based on duration, and as hematogenous or contiguous in terms of pathogenesis. The global incidence of osteomyelitis is estimated at 21.8 cases per 100,000 person-years, with a significantly higher burden among immunocompromised individuals and those with diabetes mellitus.[2] Staphylococcus aureus is the predominant causative agent, implicated in 80–90% of pyogenic osteomyelitis cases. Its virulence is enhanced by multiple factors, including surface adhesins, biofilm formation, and toxin production.[3] Among these, Panton-Valentine Leucocidin (PVL) is a critical virulence determinant that induces leukocyte lysis, tissue necrosis, and exacerbation of infection4. PVL-positive S. aureus strains have been associated with severe osteomyelitis, increased thrombosis risk, and multi-organ dysfunction. PVL in S. aureus has been shown to be an epidemiological biomarker of severe S. aureus infections in several studies.[5] The increasing prevalence of MRSA and vancomycin-resistant strains necessitates a thorough understanding of S. aureus epidemiology, resistance patterns, and virulence mechanisms to develop effective treatment strategies.

This study aims to determine the prevalence of S. aureus in osteomyelitis, assess its antibiotic susceptibility patterns, and evaluate the presence of the PVL gene to establish its correlation with disease severity.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Government Stanley Medical College, Chennai, from April 2019 to March 2020, after getting approval from Institutional Ethics

Committee. It was a hospital-based, observational study focusing on osteomyelitis cases. Patients of all ages and genders with clinically and radiologically confirmed osteomyelitis were included in this study. 75 non-consecutive sequestrum and pus samples were collected from patients who were clinically confirmed as osteomyelitis. Blood samples were also obtained in cases suspected of hematogenous osteomyelitis. Samples were transported under sterile conditions and processed immediately in the microbiology laboratory. Gram staining and Ziehl-Neelsen staining were performed.

Samples were inoculated onto Blood Agar, MacConkey Agar Sequestrum samples were additionally inoculated in Brain Heart Infusion (BHI) broth for enrichment and incubated at 37ºC for 18-24 hours. Colonies were identified using standard biochemical tests.[3,6]

Antibiotic Susceptibility Testing was done by Kirby-Bauer Disk Diffusion method as per CLSI 2019 guidelines,[7] using Penicillin, Cefoxitin, Erythromycin, Clindamycin, Vancomycin, Linezolid, Rifampicin, Ciprofloxacin, Gentamicin, and Cotrimoxazole. Isolates resistant to cefoxitin disk were subjected to Vancomycin MIC testing using Broth Microdilution and E-test6. Molecular Detection of PVL Gene was done by conventional PCR.

RESULTS

The bones of the lower limb were most commonly involved affecting 56% of the test population; of these 31% was isolated from Tibia. This was followed by femur and bones of the foot contributing 12% each. Trauma, particularly accidents was the most common predisposing factor to osteomyelitis in this study accounting to 61.33% of cases. This was followed by the post-implant cases with 9.33%. hematogenous spread and infections spreading from contiguous sites accounting to 8% each. Out of the 75 samples tested culture positivity was around 82.67% and culture negatives were 17.33%. The sensitivity is 82%. It has a positive predictive value of 100% where P value is < 0.05 with 95% confidence interval. Out of the total samples tested, monomicrobial growth was observed in 80.65%, and polymicrobial growth in 19.35%.

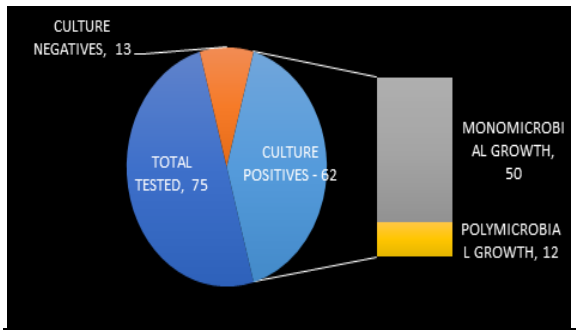

Figure 1

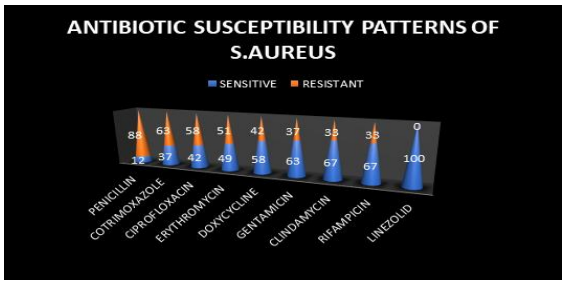

Figure 2

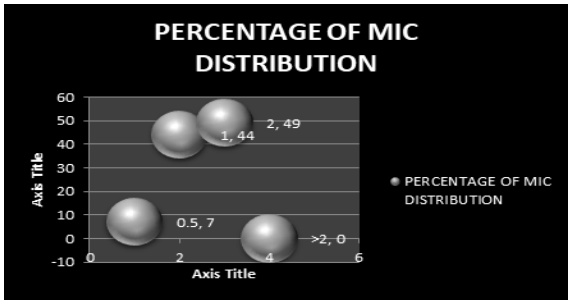

Figure 3

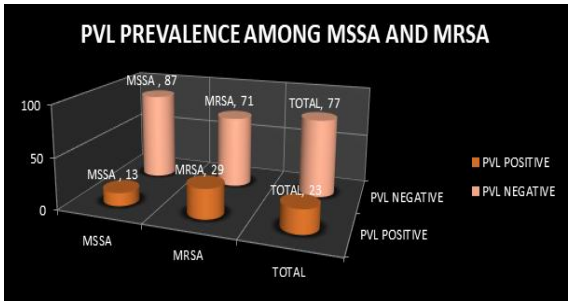

Figure 4:

Table 1: Distribution of Isolates causing Osteomyelitis (n=74).

S.No	Organism	Number of Organisms	Percentage
1	Staphylococcus aureus	43	58.11
2	Pseudomonas aeruginosa	8	10.81
3	Staphylococcus epidermidis	5	6.76
4	Klebsiella pneumoniae	5	6.76
5	Acinetobactor baumanii	3	4.05
6	Streptococcus pyogenes	3	4.05
7	Proteus mirabilis	3	4.05
8	Klebsiella oxytoca	2	2.70
9	Escherichia coli	1	1.35
10	Proteus vulgaris	1	1.35
11	Total isolates	74	100

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Table 2: Distribution of MRSA among Staphylococcus aureus Isolates (n=43).

Parameter	Frequency (n=43)	Percentage (%)
MRSA cases	28	65
MSSA cases	15	35

All the 43 isolates were found to be sensitive to Vancomycin (100%) by Vancomycin screen agar and this was further confirmed by broth microdilution method and E test.

Table 3: Prevalence of inducible clindamycin resistance among MRSA and MSSA isolates(n=43).

Isolate	Clindamycin Sensitive N(%)	Clindamycin Resistant N(%)	Inducible Clindamycin Resistance N
MSSA(15)	15(100)	NIL	NIL
MRSA(28)	14(50)	14(50)	11(39.29%)
Total(43)	29(67.44)	14(32.55)	11(25.58%)

Table 4: Distribution of PVL Gene Among S. aureus Isolates

Parameter	Count (n=49)	Percentage (%)
PVL-positive	18	37
PVL-negative	31	63

Table 5: Association of PVL Gene with Clinical Severity

Clinical Presentation	PVL-positive (n=18)	PVL-negative (n=31)
Abscess formation	12	8
Bone necrosis	9	4
Septicemia	5	2
Multi-organ involvement	4	1

DISCUSSION

The inclusion of multiple tables provides a detailed insight into the prevalence, antibiotic susceptibility, and molecular characteristics of S. aureus in osteomyelitis cases. The high prevalence of MRSA (48%) [Table 1] in this study aligns with findings from similar studies in India and globally. For instance, a study by Gadepalli et al. (2019) reported an MRSA prevalence of 45% in bone infections in North India, while a multicentric study conducted in the United States by Kourbatova et al. (2020) found MRSA rates between 40-60%. The high fluoroquinolone resistance (53%) observed in this study is concerning, as fluoroquinolones are often used as first-line agents in osteomyelitis treatment. Similar findings were reported by Shallcross et al. (2020), who documented fluoroquinolone resistance rates exceeding 50% in MRSA-related osteomyelitis. This suggests that empirical fluoroquinolone use should be reconsidered, and susceptibility testing should guide antibiotic selection. The emergence of vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA) is an alarming finding, with one MRSA isolate showing a MIC ≥2.0 µg/mL. Similar trends have been observed in studies by Howden et al. (2010), emphasizing the need for alternative therapeutic approaches. PVL-positive isolates accounted for 37% of S. aureus cases, a finding that is in line with research by Vandenesch et al. (2003), which linked PVL presence to more severe osteomyelitis presentations and aligns with the findings of Shallcross et al. (2020), who reported similar associations with increased disease severity Notably, PVL-positive isolates exhibited a significantly higher incidence of abscess formation (66.7%), bone necrosis (50%), and multi-organ involvement (22%),suggesting a strong correlation between PVL toxin and severe osteomyelitis. This is consistent with research by Vandenesch et al. (2003), who identified PVL as a major virulence factor in osteomyelitis and soft tissue infections. Similar trends were observed in research by Bocchini et al. (2006), who reported that PVL-positive strains caused more aggressive and necrotizing infections compared to PVL-negative strains. When comparing resistance patterns between MRSA and MSSA isolates, it is evident that MRSA exhibits significantly higher resistance to multiple antibiotics, particularly beta-lactams, macrolides, and fluoroquinolones. This further stresses the importance of judicious antibiotic use and the implementation of antibiotic stewardship programs to mitigate resistance development Vancomycin MIC distribution indicated that one MRSA isolate (2%) had an MIC ≥2.0 µg/mL, suggesting vancomycin-intermediate S. aureus (VISA) emergence. This is consistent with findings from Howden et al. (2010), who reported increasing VISA prevalence in chronic osteomyelitis cases. Given the rise in vancomycin-resistant strains, alternative treatment strategies such as daptomycin and linezolid should be considered. A global perspective on PVL prevalence suggests variation among geographic regions. Studies from Europe (Vandenesch et al., 2003) reported lower PVL prevalence (5-10%) compared to African and Middle Eastern regions, where prevalence rates of 40-75% have been noted (Shittu et al., 2019). This variation suggests possible regional differences in circulating S. aureus strains, antibiotic practices, and infection control measures. Overall, this study reinforces the importance of molecular diagnostics for PVL gene detection and routine antimicrobial susceptibility testing in osteomyelitis cases. The

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increasing prevalence of MRSA and vancomycin resistance highlights the urgent need for alternative therapeutic strategies, including anti-virulence therapies, monoclonal antibodies targeting PVL, and host-directed immunotherapies.

CONCLUSION

Osteomyelitis remains a formidable clinical challenge due to its complex pathophysiology, high recurrence rates, and increasing antibiotic resistance. This study underscores the significant role of trauma, particularly from accidents, as a leading predisposing factor, highlighting the urgent need for improved injury prevention and early intervention strategies. A proactive approach—including rapid clinical suspicion, culture-based diagnosis, and targeted antibiotic therapy—is essential for optimizing patient outcomes. The high prevalence of Staphylococcus aureus, including methicillin-resistant strains, limits treatment options and raises concerns about antibiotic stewardship. The emergence of vancomycin resistance further complicates management, necessitating the exploration of alternative therapeutics. While molecular detection of virulence factors such as the PVL gene could enhance prognostic accuracy, its widespread implementation remains constrained by cost and resource limitations. Future research should focus on innovative treatment modalities, such as therapies targeting bacterial virulence factors, biofilm disruption techniques, and immune-modulating interventions. Until such advancements become clinically viable, a multidisciplinary approach emphasizing rational antibiotic use, stringent infection control measures, and robust surveillance programs will be critical in preventing an impending antibiotic resistance crisis.

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