Epidemiology, resistance characteristics, virulence determinants, and treatment outcomes of *Staphylococcus aureus* bone and joint infections: a one-year prospective study at a tertiary care hospital in India

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ABSTRACT

Purpose: The study was aimed to explore the differences between the *Staphylococcus aureus* osteosynthesis-associated infection (OAI) and non-implant related infections (NIRI) in terms of epidemiology, resistance characteristics, virulence determinants, treatment, risk factors, and outcome.

Methods: A prospective study was conducted from 2018 through 2019. The phenotypic and genotypic characterization of *S. aureus*, risk factors, treatment, and outcome were compared.

Results: A total of 60 patients were included. 50% had OAIs (70%) ($p = .045$). Overall, MRSA (OR 0.69; $p = .020$) and old age (OR 0.95; $p = 0.035$) were the important risk factors. Implanted patients presented with the features of chronic osteomyelitis (93.3%, $p = 0.01$). NIRI cases composed of only 66.7% of OM, and 55% of them were acute. OAI isolates were more frequently *luk* gene positives (50%) than isolates from the NIRI group (33.3%). Patients with OAI by *luk* positive isolates significantly had prolonged hospital stay ($p = 0.043$; OR-0.96, CI-0.91–1.0). Most of the NIRIs (60%) managed with antibiotics, but frequent surgical intervention (OR 10.68; $p = .024$) with prolonged systemic antibiotics (OR 1.07; $p = .029$) helped all OAIs to recover. Patients without implants were recovered in a higher number (83.3%).

Conclusion: Our study highlighted that the differences exist between the OAI and NIRI, specifically in terms of clinical features, distribution of *luk* genes, treatment approach, and outcome. Risk factors for both types of infection remained the same.

KEYWORDS

MRSA; osteosynthesis-associated infection; leukotoxins; outcome

1. Introduction

Bone and joint infections (BJIs), such as septic arthritis (SA), osteomyelitis (OM), and spondylodiscitis (SD), cause serious morbidity and pose significant management challenges. Severe bone destruction, very high recurrence rates, and the prolonged nature of these diseases, which can last for decades, are both physically and economically crippling for patients. The increasing number of orthopedic surgical procedures has provided additional opportunities for these infections to take hold. *Staphylococcus aureus* is the most common etiological agent of BJIs [1], and it causes 30–35% of all orthopedic implant-related infections (OIRIs) and over 50% of all skin and soft-tissue infections (SSTIs) and non-implant OM [2]. This notorious microbe possesses a large variety of virulence factors that contribute to BJIs [3]. One essential virulence factor is leukotoxin, and *S. aureus* strains can produce up to four different leukotoxins: γ -hemolysin (HlgACB), *LukSF/Pvl*, *LukAB/HG*, and *LukED*. These pore-forming leukotoxins are bi-component proteins that contribute to pathogenesis by inducing neutrophil lysis,

apoptosis, and tissue necrosis [4]. Most studies have focused on Panton-Valentine leukocidin (*pvl*) and its association with more than one type of invasive disease. In contrast, few studies have specifically focused on BJIs, although some have focused on a single BJI. Additionally, the other leukotoxins have been poorly studied. The present study was undertaken to explore whether there are relevant differences among the *S. aureus* isolates associated with osteosynthesis-associated infections (OAIs) and non-implant related infections (NIRIs) in terms of their epidemiology, resistance characteristics, virulence determinants, treatment, risk factors, and outcomes.

2. Materials and methods

A prospective observational study was conducted at a 2000-bed tertiary care hospital of Kasturba Medical College in Manipal from December 2018 to November 2019. The hospital caters to the hilly Udupi district, and other neighboring districts including Uttara Kannada, Shimoga, Davangere, Chikmagalur, Hassan,

and Mangalore. Intraoperative specimens, such as bone tissues, deep wound swabs, and sinus tract samples, were collected from patients with BJIs. Among the patients admitted to the Orthopedic Department of our hospital, only patients with *S. aureus* BJIs were included.

2.1. Identification and antimicrobial susceptibility testing (AST)

Specimens were cultured on 5% sheep blood agar and MacConkey agar. *S. aureus* was identified by colony characteristics, catalase testing, and the MALDI-TOF VITEK MS system. Antimicrobial susceptibility was evaluated using the VITEK 2 system (bioMérieux, Inc., Durham, NC) based on the broth microdilution minimum inhibitory concentration principle. Interpretation of the AST was made accordingly to Clinical and Laboratory Standard Institute (CLSI) guideline.

2.2. Detection of leukotoxin genes

S. aureus genomic DNA was extracted using the Qiagen, genomic (Germany) DNA extraction kit according to the manufacturer's protocol. Multiplex PCR was performed to detect the *lukSF* (pvl) and *lukED* genes, which encode different leukotoxins. The primers used were previously described by Post *et al.* [2]. DNA was amplified in a Bio-Rad PTC-0200 DNA Engine thermal cycler (California, United States). The cycling conditions consist of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 30 s, and elongation at 72°C for 1 min, with a final extension at 72°C for 5 min. The amplified products were visualized by electrophoresis in an agarose gel stained with ethidium bromide.

Several patient outcomes were analyzed, including infection recurrence, median hospital stay, post-infection sequelae, and mortality. All patients were followed-up for six months after the end of treatment for the initial infection.

2.3. Definitions used

a. **OAI:** Clinical and microbiological evidence of infection documented as proposed by Metsemakers *et al.* after surgical fixation of fractures with internally placed implants [5].

b. **Recurrence:** Clinical signs of infection with the same microorganism within the first month after the end of treatment for the first episode.

c. **Recovered:** Complete clinical and microbiological resolution of the former infection after the end of treatment.

2.4. Statistical analysis

Descriptive statistics, such as medians, interquartile ranges (IQRs), frequencies, and percentages, were used. Comparisons between continuous variables were performed using independent-sample *t*-tests, and comparisons between categorical variables were performed using chi-square or Mann Whitney U test. Relative risks (RRs), with corresponding 95% confidence intervals (95% CIs), were calculated to describe the strength and direction of the association. Univariate logistic regression analysis was performed to identify the risk factors that were associated with each outcome. A *p* value less than 0.05 was considered statistically significant.

3. Results

3.1. Patient demographics

A total of 69 patients with staphylococcal BJIs were included in the study. Nine patients lost to follow-up were excluded. Among the 60 included patients, 49 (81.7%, *p* = 0.01) were male, and the median age was 39.5 years (interquartile range [IQR], 25–53 years).

3.2. Clinical presentations

Of the 60 cases, 30 were OAIs, and the remaining 30 were NIRIs. Overall, osteomyelitis was the commonest clinical presentation (80%, *p* = 0.01). Implanted patients primarily developed late-onset infection with the features of OM (93.3%, 28/30; *p* = 0.00). They had an infection at a median of 12.9 weeks (IQR, 4.3–38.5 weeks) after the initial fixation of the implant. OM in this group was chronic. NIRIs were composed of SD (20%) and SA (13.33%) and 66.7% OM. Nineteen patients had comorbid diseases, and diabetes was the most common (68.4%, 13/19). (Table 1)

3.3. Microbial analysis of the infections and antimicrobial susceptibility profiles

Of the 60 enrolled BJI cases, MRSA accounted for 40 cases (66.7%, *p* = 0.014). It was the leading pathogen for 70% OAIs and 63.3% NIRIs (Table 1). Overall, 82.5% (33/48) of OM, 75% (3/4) of SD, and 50% (4/8) of SA cases were due to MRSA.

MRSA isolates were 100% sensitive to commonly used antibiotics, such as vancomycin, linezolid, and teicoplanin. Low susceptibility rate was seen for ciprofloxacin (9.3% & 5.3%; *p* = .012) and clindamycin (66.7% & 68.4%; *p* = .05) (Figure 1).

Table 1. Characteristics of OAI and NIRI

	OAI (%n = 30)	NIRI (%n = 30)	p value
Patient Demography			
Male sex	25 (83.3%)	5 (16.7%)	0.01
Male: Female	5:1	4:1	
Median Age (years)(IQR)	41 (29-53.8)	36 (24-49)	
Co-morbid illness	9 (30%)	10 (33.3%)	0.783
Clinical presentations & Microbiology			
Osteomyelitis	28 (93.3%)	20 (66.7%)	0.010
Septic arthritis	-	4 (13.33%)	0.040
Spondylodiscitis	2 (6.7)	6 (20%)	0.132
MRSA	21 (70%)	19 (63.3%)	0.045
MSSA	09 (30%)	11 (36.7%)	
Presence of <i>luk</i> genes	15 (50%)	10 (33.3%)	0.194
<i>Luk D/E</i>	11 (36.7%)	7 (23.3%)	0.264
<i>Pvl</i> gene	2 (6.7%)	2 (6.7%)	1.0
<i>Pvl+LukD/E</i>	2 (6.7%)	1 (3.3%)	0.557
Treatment			
Median duration of antibiotics (IQR) (weeks)	3.7 (2-4.5)	3 (2-5.4)	0.300
Median No. of surgical interventions	2	1	0.047
Surgical interventions			
Complete removal of implant	11 (36.7%)	-	
Debridement and implant retention	5 (16.7%)	-	
Conversion of fixation-One stage exchange	7 (23.3%)	-	
Conversion of fixation-Two stage exchange	7 (23.3%)	-	
Outcome			
Recurrence of infection	09(30%)	07 (25%)	0.375
Post infection sequelae	08 (26.7%)	4 (13.3%)	0.200
Recovered	21 (70%)	25 (83.3%)	0.226
Median length of hospital stay (IQR) (Days)	13.5 (7-26)	11.5 (7-20)	0.306

Figure 1. Antibiotic sensitivity of MRSA isolates from OAI & NIRI(%).

3.4. Leukotoxin (*luk*) genes were present in many BJI-related isolates

Of the 60 isolates, 25 (41.7%) carried *luk* genes. *LukED* was present in 18 (30%) isolates. Four of it (6.7%) carried *pvl* (6.7%), and 3 (5%) had both *lukED* and *pvl*. The carriage rates of *luk* genes among the isolates from OAIs and NIRIs was 50% (15/30) and 33.3% (10/30, $p = 0.197$; Table 1), respectively. Of the 40 MRSA isolates, 16 (40%) contained *luk* genes; 11 (27.5%) had *lukED*, 3 (7.5%) contained *pvl* (3/40), and 2 (5%) of them had both *lukED* and *pvl*. *Luk* gene-positive isolates were detected in 43.8% (21/48) of OM cases [*lukED* 33.3% (16); *pvl* 6.2% (3); *pvl+lukED* 4.2% (2)] and 37.5% (3/8) of SD cases [*lukDE* 25% (2) and *lukDE+pvl* 12.5% (1)]. Twenty five percentage of SA isolates (1/4) possessed only *pvl* leukotoxin gene. The *pvl* gene was more common in isolates from acute OM (66.7%, $p = 0.085$), and *lukED* was more prevalent in isolates from chronic OM (87.5%, $p = 0.23$).

3.5. Treatment

Patients with staphylococcal BJIs were treated with antibiotics for a median duration of 3 weeks (IQR, 2–5 weeks). There were no significant differences in treatment duration between patients with MRSA and MSSA infections. ($p = 0.21$, 3.8 weeks vs. 2.5 weeks) or between patients with OAIs and NIRIs ($p = 0.30$; 3.7 weeks vs. 3 weeks). MRSA infections were treated with vancomycin, clindamycin, linezolid, doxycycline, and co-trimoxazole. MSSA infections were treated with cefazolin, clindamycin, doxycycline, and co-trimoxazole. In two cases, rifampicin was used as a combination therapy.

A total of 56 (93.3%) patients underwent surgical intervention. The median number of surgical interventions was 1 (IQR 1–2). There were significant differences in surgical intervention frequency between patients with OAIs and NIRIs ($p = 0.04$) and MRSA and MSSA infections ($p = 0.012$). Sixty percentage of the NIRIs managed conservatively with antibiotics (18/30). Different surgical strategies were obtained for OAIs. (Figure 2) Analysis of infection types showed that OM required surgical intervention more frequently ($p = 0.019$) and significantly longer antibiotic therapy ($p = 0.07$, 4.4 weeks).

3.6. Outcomes

Of the 60 patients with BJIs, 46 (76.7%) achieved recovery from the infection. The overall recovery rate ranged from 67.5% for MRSA infections to 95% for MSSA infections, and the difference was statistically significant ($p = 0.015$). Patients with NIRIs (25/30, 83.3%) recovered at a higher rate than patients with OAIs (21/30, 70%).

Analysis of different predictors was performed by logistic regression to determine their contribution to the outcome of BJIs in terms of recovery (Table 2). Overall, the presence of MRSA (OR = 0.69; $p = 0.020$) and older age (>40 years; OR = 0.95; $p = 0.035$) significantly decreased the recovery rate. In contrast, increased surgical intervention (OR = 5.12; $p = 0.020$) significantly improved the outcome. We also separately analyzed implanted and non-implanted patients. Frequent surgical intervention (OR = 10.68; $p = 0.024$) and the duration of antibiotic administration (OR = 1.07; $p = 0.029$) significantly improved patient recovery. Different therapeutic management strategies influenced recovery rate of the OAIs (Figure 2). The presence of *luk* genes in the *S. aureus* isolates

Figure 2. Surgical management of OAIs and recovery rate (%).

Table 2. Univariate Analysis of various factors influenced the outcome of OAI (n=30) & NIRI (n=30)

	OAI (Recovered Vs Not recovered)		NIRI (Recovered Vs Not recovered)	
Age	0.94 (0.88-1.0)	0.049	0.97 (0.92-1.03)	0.373
Male Sex	0.47 (0.06-3.54)	0.466	1.0 (0.67-1.49)	0.746
MRSA	0.75 (0.51-1.09)	0.374	0.74 (0.56-0.96)	0.085
Presence of luk genes	2.0 (0.38-10.51)	0.413	0.44 (0.22-23.27)	0.497
Presence of co-morbid conditions	0.26 (0.26-1.42)	0.231	1.13 (0.83-1.52)	0.640
Number of surgical intervention	10.68 (1.37-83.52)	0.024	5.40 (0.69-41.74)	0.106
Duration of antibiotics	1.07 (1.01-1.13)	0.029	0.99 (0.95-1.04)	0.798

significantly contributed to a longer hospital stay ($p = 0.043$; OR: 0.96, 95%CI: 0.91–1.0) for OAIs.

Infection recurrence occurred in 14 patients (23.3%). Significantly more patients with recurrence had MRSA (13/14, 92.9%), than MSSA (1/14, 7.1%); $p = 0.023$. Of the 60 patients with BJIs, 12 (20%) had post-infection sequelae, including amputation (4/60, 6.7%) and functional abnormalities (8/60, 13.3%). Patients with MRSA BJIs had significantly more post-infection sequelae than patients with MSSA BJIs [27.5% (11/40) vs. 5% (1/20); $p = 0.041$].

4. Discussion

This study reports the resistance patterns, virulence determinants, treatments, risk factors, and outcomes for various *S. aureus* BJIs in a tertiary care hospital. A significant majority of the *S. aureus* BJIs were caused by MRSA (66.7%, $p = 0.014$). Epidemiological studies of *S. aureus* BJIs at different hospitals in different regions of India have shown variations in MRSA incidence, from 12–80% [6]. The 50% MRSA incidence rate reported in the southern part of India is similar to the rates reported in the United States (28–54%) [7,8]. In contrast, the incidence rates of MRSA were lower (less than 5%) in Northern European countries and (more than 25%) in Southern and Eastern European countries [9].

MRSA isolates were more frequently detected in OAIs (70%) than in NIRIs ($p = 0.045$). The reason could be due to the increased exposure of the patients to the hospital environment and carriage of methicillin-resistant strains. As noted above, patients with OAIs stayed at the hospital for a longer duration (13.5 days vs. 11.5 days). Infection was more frequently the reason for repeated hospital admissions (36.7% vs. 16.7%; OR = 1.06, 95% CI = 0.56–2.02.). All MRSA isolates were susceptible to vancomycin (100%) and linezolid (100%). Besides, systemic therapies, gentamicin antibiotic beads were used to manage OAIs (5/30, 16.7%). The majority of the MRSA strains isolated from OAIs (80%) were sensitive to gentamicin. A study by Neut *et al.* stated that the use of gentamicin beads might be

a risk factor for gentamicin resistance [10]. In our study, there was a significantly higher frequency of clindamycin and ciprofloxacin-resistant isolates. Therefore, leukotoxin-producing strains may be challenging to treat in the future due to high clindamycin resistance as it fails to inhibit the toxin production. .

Concerning leukotoxin production, the more isolates from OAI produced leukotoxin than strains from NIRI. Also, the rate of *lukED* gene positivity was higher among isolates from OAIs, whereas *pvl* was equally distributed among isolates from both types of infections. The *pvl* gene (6.7%) was much less common than *lukED*; however, it was more common than reported in another study [2]. The presence of both leukotoxins suggests that both have roles in the pathogenesis of *S. aureus* BJIs. However, *lukED* might play a more important role in OAIs than *pvl*. Campoccia *et al.* suggested that the *pvl* leukotoxin might not play a prominent role in the pathogenesis of OAIs [11]. *Pvl* toxin targets polymorphonuclear neutrophils (PMNs), monocytes, and macrophages; induces pores to release cytokines; and enhances the acute inflammatory response [12]. It explains the occurrence of more *pvl* gene-positive *S. aureus* isolates in acute infection, and they are related to greater pathogenicity. Likewise, the presence of more *lukED*-positive isolates in chronic infection tells that these genes may not be as virulent as *pvl*. Persistence of bacteria in chronic cases also explains the biofilm formation, which was not evaluated in this study.

The overall recovery rate in this study was 78.3%, and a higher percentage of patients with NIRIs recovered than patients with OAIs. A possible explanation for the poorer outcomes in implanted patients is the frequent presence of MRSA (70%). These isolates were the most frequent etiology of late-onset OAIs, when mature biofilms and sequestrum formation are usually present (median delay, 12.8 weeks). The treatment strategy for OAIs was debridement, antibiotics, and implant removal or conversion of fixation. Different pathways of management were decided based on clinical situation and radiological features [13,14,15]. The presence of *luk* genes or MRSA did not influence the therapeutic management of OAIs. Patients developed the infection after an average of 40 weeks of implantation had undergone for implant removal ($p = .019$) as healing of the fracture happened radiologically. Implants were retained if OAIs developed after 6 weeks (mean) before the expected fracture union ($p = 0.031$). Single and two-stage surgery was performed in 14 patients when infection developed after 18 weeks of implantation in an average. This strategy was implied due to the non-healing of the fracture and implant loosening. Most of the NIRIs (18/30, 60%) managed with antibiotics.

Infections caused by *luk*-positive MRSA isolates were associated with a longer hospital stays due to post-infection sequelae, which is an indicator of the severity of the disease and the high burden for the patients.

5. Conclusions

The findings of this study establish MRSA as a significant pathogen in both OAls and NIRIs. Overall, osteomyelitis was the most prevalent clinical feature. OAls occurred more than 10 weeks after implantation with the characteristics of chronic OM. The OAI group trended toward a higher incidence of luk positive *S. aureus* isolates compare to NIRI group. The presence of leukotoxin genes was associated with a significantly longer hospital stay for the patients having chronic OAls. Both groups were managed differently. Most of the NIRIs managed with antibiotics, but more number of surgical interventions were required along with antibiotics to handle OAls. Patients without implants were recovered in a higher number. Different modes of surgical strategies influenced the recovery rate of the OAls significantly. Poor recovery among OAI patients was mainly due to post-infection sequelae by MRSA.

Author contribution

BB was involved in study design, literature search, and manuscript preparation. CM conceptualised, evaluated and edited the manuscript. BB & TS were responsible for collection of samples, data analysis, statistical analysis, and interpretation of data. SBN & BMKS was involved in clinical & laboratory analysis, manuscript editing and language editing. All authors approved the final version of manuscript.

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Ethical approval

The study has received institutional ethics committee approval.

References

- [1] Schwotzer N, Wahl P, Fracheboud D, et al. Optimal culture incubation time in orthopedic device-associated infections: a retrospective analysis of prolonged 14-day incubation. *J Clin Microbiol*. 2014;52(1):61–66.
- [2] Post V, Wahl P, Uçkay I, et al. Phenotypic and genotypic characterisation of *Staphylococcus aureus* causing musculoskeletal infections. *Epub 2014 Apr 3 Int J Med Microbiol*. 2014;3045–6:565–576. .
- [3] Peacock SJ, Moore CE, Justice A, et al. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infect Immun*. 2002;70:4987–4996.
- [4] Wright JA, Nair SP. Interaction of Staphylococci with bone. *Int J Med Microbiol*. 2010;300(2–3):193–204. *Epub 2009 Nov 3*.
- [5] Metsemakers WJ, Morgenstern M, McNally MA, et al. Injury. *Int. J Care Injured*. 2018;49:505–510.
- [6] Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, and et al. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. *Indian J Med Res*. 2013 Feb;137(2):363–9. PMID: 23563381; PMCID: PMC3657861
- [7] Thimmappa L, Bhat A, Hande M, et al. MRSA prevalence and antimicrobial susceptibility pattern of orthopedic surgery patients of a tertiary care hospital, South India. *J Med Clin Sci*. 2014;3:11–14.
- [8] Dukic VM, Lauderdale DS, Wilder J, et al. Epidemics of community-associated methicillin-resistant *Staphylococcus aureus* in the United States: a meta-analysis. *PLoS One*. 2013;8(1):e52722.
- [9] Otter JA, French GL. Molecular epidemiology of community associated Methicillin-resistant *Staphylococcus aureus* in Europe. *Lancet Infect Dis*. 2010;10:227–239.
- [10] Neut D, van de Belt H, Stokroos I, et al. Biomaterial-associated infection of gentamicin-loaded PMMAbeads in orthopaedic revision surgery. *J Antimicrob Chemother*. 2001;47:885–891.
- [11] Campoccia D, Baldassarri L, Pirini V, et al. Molecular epidemiology of *Staphylococcus aureus* from implant orthopaedic infections: ribotypes, agr polymorphism, leukocidal toxins and antibiotic resistance. *Biomaterials*. 2008a;2930:4108–4116. *Epub 2008 Aug 3*.
- [12] Arciola CR, Baldassarri L, Eiff CV, et al. Prevalence of genes encoding for staphylococcal leukocidal toxins among clinical isolates of *Staphylococcus aureus* from implant orthopaedic infections. *Int J Artif Organs*. 2007;30:792–797.
- [13] Schmidt AH, Swiontkowski MF. Pathophysiology of infections after internal fixation of fractures. *J Am Acad Orthop Surg*. 2000;8(5):285–291.
- [14] Osmon DR, Berbari EF, Berendt AR, et al. Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America. *Clin Infect Dis*. 2013;56(1):1–10.
- [15] Rightmire E, Zurakowski D, Vrahas M. Acute infections after fracture repair: management with hardware in place. *Clin Orthop Relat Res*. 2008;4662:466–472. *Epub 2008 Jan 10*.