

Microbiological profile and antimicrobial susceptibility pattern in orthopaedic implant associated infections

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Received: May 2025, Accepted: August 2026

ABSTRACT

Background and Objectives: Postoperative wound infection is a serious complication in presence of prosthetic implantation (infectivity rate 10.4%). Implant infection is particularly high in developing countries. Hence there is a need to know the clinical presentation, common organisms and their susceptibility pattern in a particular region.

Materials and Methods: The patients who had undergone orthopaedic prosthesis or implant surgeries and presented with signs and symptoms of infection were included in this study. Pus, tissue, fluid samples obtained were streaked in Blood agar and MacConkey agar plates and incubated at 37°C, two Sabouraud Dextrose agar slants incubated at 28°C and 37°C and Gram staining and Acid-fast staining performed. Antimicrobial susceptibility testing was performed for all bacterial isolates. Data collection was done using a proforma and analysis of data was done and expressed as frequency and percentage.

Results: A total of 149 samples obtained from patients with Orthopaedic implant associated infections were processed. Of these 131 (87.9%) were culture positive. *Staphylococcus aureus* was the most common pathogen, followed by *Pseudomonas* spp. *Escherichia coli* and *Staphylococcus* other than *S. aureus*. Ten (7.6%) cases had multiple organisms and 2 fungi were also isolated (1.5%). Among the *S. aureus*, MSSA was the commonest and 42 (30.2%) isolates were multidrug resistant organisms.

Conclusion: Infection is the major cause of delayed healing in patients with Orthopaedic implants. Early identification and aggressive treatment with the appropriate antibiotics are necessary to decrease infection rate, reduce hospital stay, prevent biofilm formation, decrease the need for Implant removal or replacement, and prevent disability in the patients.

Keywords: Biofilm; *Staphylococcus aureus*; Implant-associated infections; Antimicrobial susceptibility; Multidrug resistance

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INTRODUCTION

Orthopaedic implant refers to medical devices that are intended to replace a bone, joint or cartilage that is either damaged or deformed. These include prosthetic joints and various fracture fixation devices including internal fixators like k-wires, dynamic hip screws, plates, intra medullary nails and external fixators (1, 2). They are made up of a variety of metals like stainless steel, cobalt-chromium, titanium, polyethylene, polymethyl methacrylate (PMMA) and ceramics (3). With advancing technologies and better post-operative care, the incidence of implant failure has decreased. But increase in number of patients requiring an orthopaedic implant has led to the increase in the absolute number of patients with implant failure. Infection following fracture fixation remains an important complication of orthopaedic surgery. The incidence of fracture-related infection is approximately 1-2% following fixation of closed fractures but may reach 30% or more in open fractures, depending on the severity of soft-tissue injury and other clinical factors. In India, a multicentre prospective cohort study reported an overall infection incidence of 2.9% following internal fixation of tibial fractures, with rates of 1.6% in closed fractures and 8.0% in open fractures (4). Periprosthetic infection rate varies from 0.5% to 2.2% in patients who are undergoing primary arthroplasty and it may be as high as 20% after revision surgery (5). A major risk factor for these infections is the extent of soft tissue and periosteal damage associated with fracture and subsequent surgery. Biofilm formation is one of the main factors causing difficulty in treating orthopaedic implant infections as the bacteria present in biofilms are resistant to treatment with antibiotics (6). This study was conducted to detect the microbiological profile, clinical characteristics, antimicrobial sensitivity pattern and biofilm forming capacity of orthopaedic implant infections in our study area.

MATERIALS AND METHODS

This is a prospective observational study conducted in the Department of Microbiology and Orthopaedics in a Tertiary care Hospital, Pondicherry, from the period of November 2020 to November 2022, conducted after obtaining IHEC approval (IHEC NO: AV/IEC/2020/167). The study population included 149

patients who underwent orthopaedic implant and prosthesis surgery and presented with clinical features of infection.

Tissue, pus or synovial fluid was collected from clinically suspected patients with implant or prosthetic joint infection under strict aseptic precautions and transported immediately to the microbiology laboratory for processing.

All the samples were processed according to standard microbiological procedures. The specimens were first streaked in Blood agar, MacConkey agar and Sabouraud Dextrose Agar (SDA) (2 tubes). Then two smears were prepared and Gram staining and acid-fast staining performed. The direct smear was examined for the presence of pus cells. The samples which had moderate or plenty of pus cells were taken into the study. The Blood agar and MacConkey agar plates were incubated for 37°C for 24 to 48 hrs. The growth obtained was subjected to biochemical tests to identify the organism. The biochemical tests used for bacterial identification included catalase test, slide and tube coagulase test, mannitol fermentation, oxidase test, citrate utilization, urease test, triple sugar iron (TSI) reaction, oxidative/fermentative (OF) test, indole/MR/VP tests, carbohydrate fermentation tests, and nitrate reduction test, depending on the organism. Identification was done following standard microbiological protocols and conventional biochemical reactions. Simultaneously antibiotic sensitivity testing performed using Kirby Bauer disc diffusion method in Mueller Hinton agar following CLSI guidelines. Quality control for antimicrobial susceptibility testing was performed using standard ATCC strains: *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The antibiotics tested for AST included: ampicillin, penicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, chloramphenicol, ciprofloxacin, cotrimoxazole, erythromycin, gentamicin, high-level gentamicin (for Enterococci), vancomycin, linezolid, amikacin, piperacillin-tazobactam, meropenem, aztreonam, levofloxacin, polymyxin-B, colistin, and fosfomycin as per organism requirement.

ESBL detection was performed using the phenotypic double-disc synergy test and confirmed by the CLSI phenotypic confirmatory test using ceftazidime/ceftazidime-clavulanate.

MBL detection was done by the EDTA-imipenem combined disc test demonstrating synergy.

The biofilm forming capacity of the organisms

were identified using test tube method. A loopful of the isolate colony was added to a sterile test tube with 2 ml brain heart infusion broth with 1% glucose and kept for overnight incubation. The tubes were decanted and washed twice with Phosphate buffer saline and dried. The tubes were then stained with 0.1% crystal violet for 30 minutes and excess stain was washed with distilled water and the tubes were air dried and observed for visible film lining the sides and bottom of the tubes. It was scored as weak, moderate or strong biofilm production.

Orthopaedic implant infections were classified based on time of symptom onset:

- Early infection – occurring < 3 weeks after surgery
- Delayed infection – occurring between 3 and 10 weeks
- Late infection – occurring > 10 weeks after surgery for prosthetic joint infections: early < 3 months, delayed 3 months–2 years, late > 2 years.

Data collection was done using a predesigned proforma and data analysed as frequencies and percentage.

Ethical approval. This research has been approved by the authors affiliated institute.

The informed consent was obtained from all patients and relevant persons (such as parents or legal guardians) for the publication of this study. Additionally, all data included in the manuscript have been anonymized to ensure the confidentiality of participants. No identifiable information has been disclosed.

RESULTS

Of the patients who presented with symptoms suggestive of Orthopaedic implant associated infections, 149 patients with significant pus cells in direct smear examination were included in our study. The number of male patients (119 (79.9%)) who presented with infection was more compared to female patients (30 (20.1%)). Similarly, patients belonging to middle age group were more compared to the extremes of age. Forty (26.8%) of the 149 cases belonged to 41-50 years age group, followed by 36 (24.2%) cases in 31-40 years age group. Only 4 cases (2.7%) were present in 0-10 years and >80 years range.

Fig. 1 demonstrates the age-group wise distribution in percentage and Fig. 2 shows the gender distribution of patients.

Lower limb (103 (69.1%)) was most commonly in-

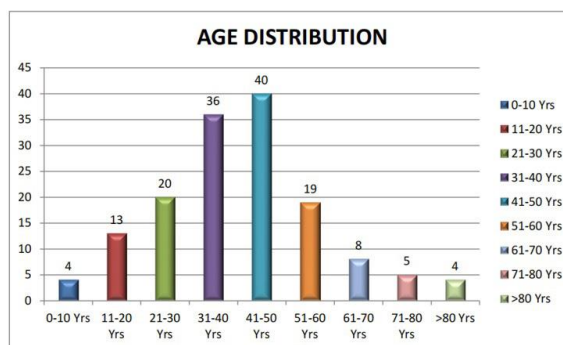


Fig. 1. Chart showing the age distribution of cases

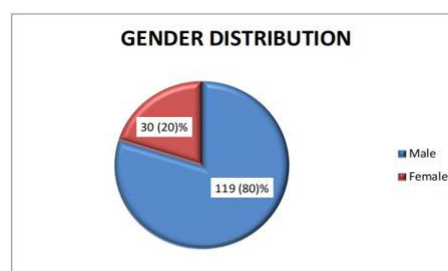


Fig. 2. Pie chart showing the gender distribution of patients with infected implants

involved as compared to upper limb (43 (28.9%)) or other sites. Among the 149 implant infection cases studied, 140 were internal fixators, 4 external fixators and 5 prosthetic joints. The patients mostly presented with early onset infection in our study as in Table 1.

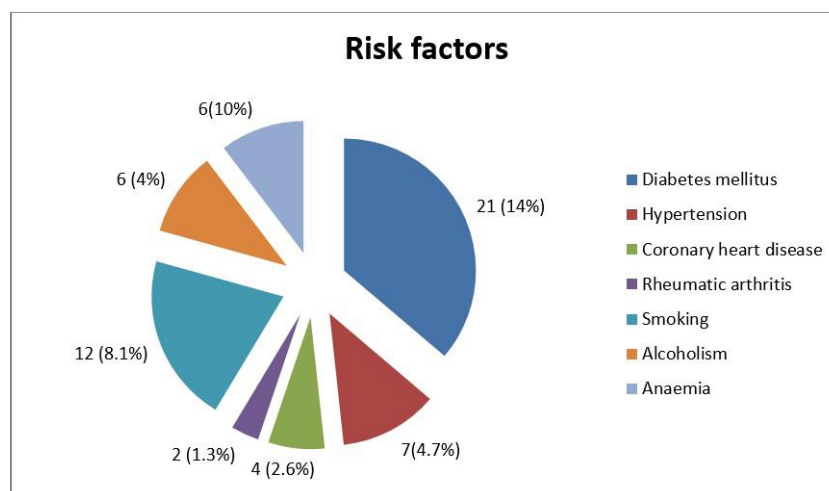
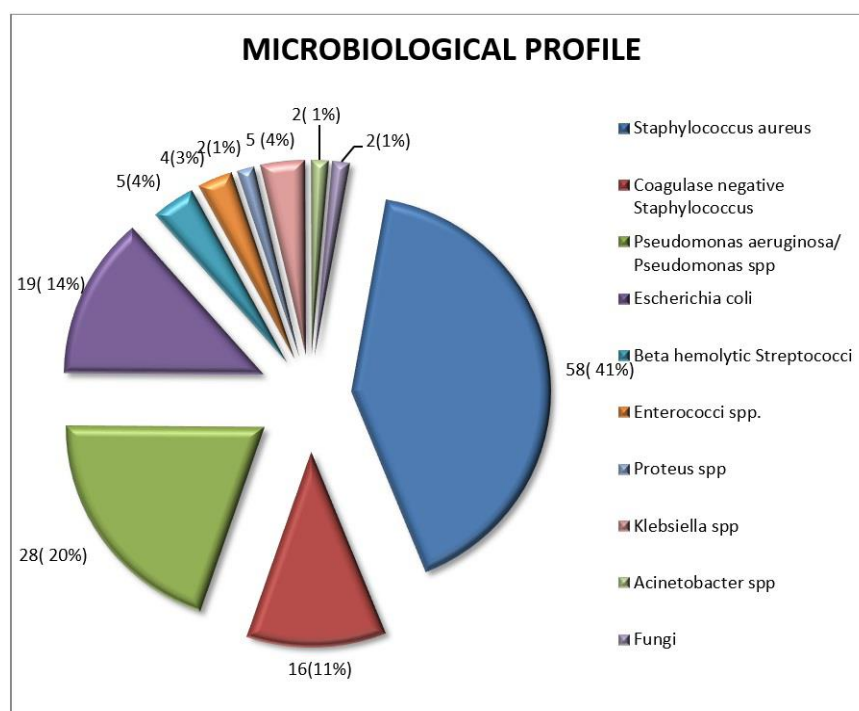
Among the 149 cases, 108 (72.5%) were cases operated for open fracture and 41 (27.5%) cases for closed fracture. One thirty-one samples were culture positive, of which 121 (92.8%) monomicrobial and 10 (7.2%) polymicrobial infection and 18 cases were culture negative. Diabetes mellitus was the most common factor associated as in Fig. 3.

S. aureus was most common isolate in our study both in monomicrobial and polymicrobial infections. *P. aeruginosa* was the second most common organism isolated and the most common gram-negative isolate. The microbiological profile obtained is as in Fig. 4. The microbiological profile of the organisms observed in each stage of infection is as in Table 2.

Antimicrobial sensitivity testing was performed. Among the *S. aureus* isolated 47 (81%) were Methicillin Sensitive *S. aureus* (MSSA) and 11 (19%) were Methicillin Resistant *S. aureus* (MRSA). The antimicrobial susceptibility pattern for different isolates obtained is as in Tables 3 and 4.

Table 1. Onset of infection

Onset	Internal fixator		External fixator		Prosthetic joints	
	Number	Percentage	Number	Percentage	Number	Percentage
Early	93	66.4	4	100	2	40
Delayed	27	19.3	0	0	1	20
Late	20	14.3	0	0	2	40
Total	140	100	4	100	5	100

**Fig. 3.** Risk factors associated with implant infection**Fig. 4.** Microbiological profile

Of the 26 samples tested for biofilm production, there were 6, 9 and 11 strong, moderate and weak/no biofilm producers respectively. Implant removal was done in 28 (19%) cases. The remaining cases were treated with aggressive wound debridement and antibiotic loaded cement beads or stimulation beads with antibiotics.

DISCUSSION

Implant-associated infections are a major cause of morbidity, prolonged immobilisation, and extended

Table 2. microbiological profile based on onset of infection

		Early	Late	Delayed
<i>Staphylococcus aureus</i>	1	37	8	13
SOSA	2	8	7	0
<i>Pseudomonas aeruginosa</i>	3	23	2	2
<i>Escherichia coli</i>	4	18	1	0
Beta- hemolytic streptococci	5	4	0	1
<i>Enterococci</i>	6	2	2	
<i>Proteus</i> spp.	7	1	0	1
<i>Klebsiella</i> spp.	8	4	1	
<i>Acinetobacter</i> spp.	9	1		1
No growth	10	9	6	4

hospital stay in patients with fracture fixation devices and prosthetic joints. In our study, males were more commonly affected, a trend also reported by Latha T et al. (7), Alelign D et al. (8) and Corrigan RA et al. (9). Most patients belonged to the 31-50-year age group, which corresponds with earlier studies indicating increased susceptibility in the middle-aged population due to higher trauma exposure and associated comorbidities (7, 9). Open fractures formed a significant proportion of infected cases, similar to the findings of Oliviera PR et al., who also reported higher infection rates in open injuries (10). Implant infections may occur via perioperative, hematogenous, or contiguous routes (3) and are categorised as early, delayed, or late based on standard postoperative time intervals for fracture fixation devices (1, 2) and prosthetic joints.

The findings of our study are comparable to several recent reports from India and abroad. Studies by Latha et al., Alelign et al., and Corrigan et al. have also shown *S. aureus* as the predominant pathogen in implant-associated infections. Similarly, polymicrobial infection rates in our study align with observations from Manning et al..

Early-onset infection was more common in our study, accounting for 99 cases (66.4%), followed by delayed-onset infection in 28 cases (18.8%) and late-onset infection in 22 cases (14.8%). This pre-

Table 3. Antimicrobial susceptibility testing of gram-positive isolates

Antibiotic	<i>Staphylococcus aureus</i> (58 isolates) Sensitive (%)	SOSA (16 isolates) Sensitive (%)	<i>Enterococci</i> spp. (4 isolates) Sensitive (%)	Beta haemolytic <i>Streptococci</i> (5 isolates) Sensitive (%)
Ampicillin	-	-	2 (50)	5 (100)
Penicillin	-	-	2 (50)	5 (100)
Bacitracin	-	-	0 (0)	5 (100)
Amikacin	54 (93)	14 (88)	-	-
Amoxiclav	47 (81)	9 (56)	-	-
Cefoxitin	47 (81)	9 (56)	-	-
Cefotaxim	47 (81)	9 (56)	-	5 (100)
Ceftriaxone	47 (81)	9 (56)	-	-
Chloramphenicol	52 (90)	9 (56)	1 (25)	0 (0)
Ciprofloxacin	34 (59)	9 (56)	2 (50)	4 (80)
Cotrimoxazole	46 (79)	7 (44)	1 (25)	2 (40)
Erythromycin	33 (57)	5 (31)	0 (0)	2 (40)
Gentamycin	47 (81)	12 (75)	-	-
HLG	-	-	4 (100)	-
Linezolid	1 (100)	2 (100)	4 (100)	5 (100)
Vancomycin	1 (100)	2 (100)	4 (100)	5 (100)

Table 4. Antimicrobial susceptibility of Gram-negative isolates

Antibiotic	<i>Pseudomonas</i> spp (28 isolates) Sensitive (%)	<i>Escherichia coli</i> (19 isolates) Sensitive (%)	<i>Klebsiella</i> spp (5 isolates) Sensitive (%)	<i>Proteus</i> spp (2 isolates) Sensitive (%)	<i>Acinetobacter</i> spp (2 isolates) Sensitive (%)
Amikacin	20 (71)	16 (84)	5 (100)	2 (100)	0 (0)
Amoxiclav	-	16 (84)	4 (80)	0 (0)	0 (0)
Cotrimoxazole	-	12 (67)	5 (100)	1 (50)	0 (0)
Ciprofloxacin	22 (79)	16 (84)	5 (100)	1 (50)	0 (0)
Cefotaxime	16 (57)	15 (79)	5 (100)	1 (50)	0 (0)
Gentamicin	16 (57)	11 (58)	5 (100)	1 (50)	0 (0)
Piperacillin/tazobactam	22 (79)	18 (95)	5 (100)	2 (100)	0 (0)
Meropenem	20 (71)	18 (95)	5 (100)	2 (100)	0 (0)
Colistin	1 (33)	1 (100)	-	-	0 (0)
Polymyxin B	3 (100)	1 (100)	-	-	0 (0)
Levofloxacin	3 (100)	-	-	-	0 (0)
Aztreonam	2 (66.7)	-	-	-	0 (0)
Ofloxacin	0 (0)	-	-	-	0 (0)
Fosfomycin	-	-	-	-	0 (0)

dominance of early-onset infection is consistent with previous studies reporting a higher proportion of infections during the early postoperative period (11-13). Lower limb 103 (69.1%) was more commonly involved, tibia being the most common bone affected. This is similar to study by Corrigan RA et al., in which 47% tibia was affected (9). Stainless steel implant being the common material of implant used in our hospital setting, the high number of cases with stainless steel implants cannot be correlated with the increased incidence in it. Several conditions have been recognised to significantly increase the risk of post-operative infection such as Rheumatoid arthritis, Diabetes mellitus (DM), Sickle cell anaemia, psoriasis, renal failure, immunosuppression due to prior renal or liver transplant, malnourishment, obesity, concurrent UTI (bladder retention in post operation), malignancy etc. Diabetes mellitus was the risk factor most frequently found in our study participants. The pathogenesis of Diabetes in causing wound infection in implant surgeries is mainly due to the hyperglycaemic state that impairs neutrophil chemotaxis and phagocytosis, resulting in weakened antibacterial defence and impaired wound healing. Suboptimal glucose control peri-operatively is associated with increase in the length of the hospital stay, so optimising blood sugar level perioperatively becomes essential to decrease the postoperative adverse outcome.

Out of the 149 samples that were processed only

131 (87.9%) were culture positive, whereas 18 (12.1%) were culture negative. Culture negativity among the symptomatic cases may be explained due to the presence of anaerobic organisms, highly fastidious organisms, prior antimicrobial therapy etc. Of the 131 culture positive isolates 121 (92.8%) were monomicrobial, ten (7.2%) were polymicrobial. This is similar to study by Alelign D et al. with 4 of 72 (5.5%) culture positive samples being polymicrobial (8). A study by Manning et al had higher rate (21.3%) of polymicrobial infection (14).

The two fungi that were isolated included *Candida albicans* and *Rhodotorula* spp. *Candida* is the most common fungal pathogen isolated in association with orthopedic implant infections. A multi-institutional study by Khalid Azzam et al. shows *Candida* species as the most common fungi isolated (15) and a case of infection by *Rhodotorula minuta* in patient with two stage hip replacement was reported by Cerikcio glu et al. (16).

Of the 139 bacterial isolates 83 (59.7%) were Gram-positive, whereas 56 (40.3%) were Gram-negative. *S. aureus* was the most common organism isolated constituting to 41.1% (58 cases) of the total bacterial isolated. This is comparable to many other studies, including those by Alelign D. et al., Corrigan R.A. et al., Latha T. et al., Jain A. et al., and Elifranji Z.O. et al., in which *S. aureus* was the most common pathogen isolated (7-9, 17, 18). The high prevalence of *S. aureus* may be due to endogenous infection

from colonization of the adjoining skin or exogenous sources from the environment, surgical instruments, or contamination from healthcare workers. The most common Gram-negative isolate in our study was *Pseudomonas* spp. (19.9%). This is similar to the study by Latha T. et al., which had *Pseudomonas* spp. as the most common Gram-negative isolate, accounting for 26.3%, followed by *E. coli* (16.7%) (7).

Eleven (19%) of the 58 *S. aureus* isolates were methicillin-resistant (MRSA). A higher proportion of MRSA was seen in other studies conducted by Alelign D. et al., Latha T. et al., Jain A. et al., and Elifranji Z.O. et al., with MRSA around 57.9%, 57.3%, 40.5%, and 30.4%, respectively (7, 8, 17, 18). This may be due to the low prevalence of methicillin resistance in our region, better antimicrobial stewardship programs, and low nasal carriage. Seventeen (29.3%) of the *S. aureus* isolates were multidrug-resistant (MDR). Among the *S. aureus* increased resistance was found against ciprofloxacin, erythromycin, cotrimoxazole and gentamycin. All patients checked for Vancomycin and Linezolid sensitivity were sensitive. Among the 16 *Staphylococcus* other than *S. aureus* (SOSA) isolated 9 (56.2%) were methicillin sensitive (MSSOSA) and 7 (43.8%) were Methicillin Resistant (MRSOSA). 43.7% of the SOSA isolated were MDR. It also showed increased resistance to erythromycin (68.8%), cotrimoxazole (56.2%), ciprofloxacin and chloramphenicol (33.8%). All the *Enterococci* and β - haemolytic *Streptococci* were sensitive to vancomycin, linezolid and High-level gentamycin. VRE were not isolated in our study. In the study by Perumal A. et al., only 84.61% of the enterococci were susceptible to vancomycin (11), whereas in the study by Jain A. et al., 12.5% of *Enterococcus faecium* isolates were resistant to vancomycin (17). Among the 28 isolates of *Pseudomonas* spp., 8 were found to be resistant to meropenem, and 6 were resistant to piperacillin-tazobactam. Seven of the 28 isolates were MDR. Three isolates were resistant to all except one drug; therefore, susceptibility testing for second-line drugs was performed, and they were found to be susceptible to polymyxin B and fosfomicin. In the study by Elifranji Z.O. et al., susceptibility to piperacillin-tazobactam was only 66.7%, and susceptibility to carbapenems was only 47% (18). *E. coli* was the most common Enterobacterales isolate, followed by *Klebsiella* spp. and *Proteus* spp. All isolates were susceptible to meropenem and piperacillin-tazobactam, except one *E. coli* isolate, which

was a metallo-beta-lactamase (MBL) producer. In the study by Alelign D. et al., *E. coli* showed only 57.1% susceptibility to meropenem (8). The study by Jain A. et al. showed 77.2% susceptibility to imipenem, and 42.8% of the *E. coli* isolates were ESBL producers (17). In the study by Kumar S. et al., three *E. coli*, three *P. aeruginosa*, and six *Klebsiella* spp. were MBL producers (4). As carbapenems are the mainstay of treatment for ESBL (extended-spectrum beta-lactamase)-producing organisms, resistance to carbapenems causes difficulty in treatment, prolongs hospital stay, and increases morbidity.

Moreover, the treatment options available for MBL producers are limited and are associated with specific toxicities; hence, their presence is a matter of concern. Additionally, newer drugs for the treatment of carbapenem resistance, such as plazomicin, cefiderocol, meropenem-vaborbactam, and imipenem-relebactam, are not available in India. Both *Acinetobacter* spp. isolates in our study were resistant to all first-line and second-line antibiotics tested. The treatment options for CRAB (carbapenem-resistant *Acinetobacter baumannii*) include high-dose sulbactam, ampicillin-sulbactam, polymyxins, minocycline, trimethoprim-sulfamethoxazole, and aminoglycosides, if susceptible. Of the 15 moderate and strong biofilm producers, only 4 (26%) isolates were MDR, which is similar to the 29.8% MDR isolates in our study. This does not indicate any increase in drug resistance in biofilm producers. According to a study by Coraça-Huber et al., antibiotic resistance is not directly related to the capacity of the strains to form biofilms in vitro (19). The study also stated that organisms that were inherently less tolerant to multiple antibiotics were able to produce strong biofilm in vivo. Of the 149 cases included in the study, implant removal was performed in 28 (19%). The remaining cases were treated with aggressive wound debridement and implantation of antibiotic-loaded cement beads or stimulation beads with antibiotics.

Indian studies by Jain et al., Chetia et al., Thahir et al., and Dave et al. similarly highlight the predominance of *S. aureus* and increasing AMR trends, supporting our regional findings and emphasizing the need for region-specific antimicrobial policies.

Recent advancements in managing implant-associated infections include silver, copper, zinc and gallium-coated implants, titanium nanotube-based antibiotic delivery, peptide-coated surfaces, phage

or lysin-loaded hydrogels, and enzyme-based bio-film disruption techniques such as dispersin-B and DNase.

Limitations of the study. The incidence rate of infection for patients who underwent orthopedic implant surgery could not be determined due to the limited follow-up period and the shorter duration of the study. Additionally, prior use of antibiotics for prophylaxis and empirical therapy may have altered the microbiological profile and susceptibility pattern. Organisms that are obligate anaerobes could not be identified due to the unavailability of anaerobic culture methods, and only conventional methods were used for identification, thereby limiting the identification of rare and unculturable organisms.

CONCLUSION

Infections related to orthopedic implants, including fracture fixation devices and prosthetic joints, present a challenge both for diagnosis and treatment. They prolong hospital stay, affect patients psychologically, and increase morbidity, thus imposing an economic burden on the patient. Hence, timely diagnosis and proper management of orthopedic device-related infections are needed. The presence of methicillin-resistant *S. aureus* and carbapenem-resistant organisms such as *E. coli* (CRE), *P. aeruginosa* (CRPA), and *Acinetobacter* spp. (CRAB) in our study are a matter of concern, since the antibiotics given for empirical treatment in our hospital (cefotaxime and cefoperazone-sulbactam) are not effective against them. Hence, a proper antibiotic policy based on the local antibiogram needs to be devised and strictly adhered to. Additionally, novel methods to avoid implant infection and biofilm formation, including the use of various antibacterial metals/alloys, need to be emphasized. This, along with strict aseptic measures followed during, before, and after surgery, can decrease the infection rate among patients with orthopedic implant-associated infections.

ACKNOWLEDGEMENTS

The authors are thankful to Vinayaka Missions Research Foundation (Deemed to be University) for providing the necessary facilities for this study.

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