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Identification, Antibiotic Susceptibility Patterns and Biofilm Detection of Isolates in Orthopaedic Implant Infections

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Authors' contributions

This work was carried out in collaboration between all authors. Author SB designed the study, performed the statistical analysis, wrote the protocol, and wrote the final draft of the manuscript. Author DKK supervised the conduct of the study. Authors SK and NAM managed the experimental process. Authors AB, SN and LB managed the literature searches for the study. Author UN managed the analyses. All authors read and approved the final manuscript.

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ABSTRACT

Background: As orthopaedic implants are being increasingly used, managing the implant-associated infections has become a challenge. The aim of this study was to evaluate the bacteriological profile with antibiotic susceptibility patterns and biofilm detection in orthopaedic implant-associated infections.
Study Design: Cross-sectional prospective.
Place and Duration of Study: The study was conducted in the department of Microbiology and Orthopaedics, Sher-i-Kashmir Institute of Medical Sciences (J&K) India, a tertiary care institute from August 2014 to February 2016.
Methods: The study was conducted on 100 patients having orthopaedic implant infections.

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Demography and patient parameters were recorded. Microbiological workup by microscopy, culture, antimicrobial susceptibility testing and biofilm detection was conducted as well.
Results: 100 cases were analysed out of which 86 cases revealed a positive culture and 14 cases revealed a negative culture. From these culture positive cases, 11 cases were observed as polymicrobial and a total of 97 isolates were recovered. 53 (54.6%) isolates were Gram-positive cocci and 44 (45.4%) were Gram-negative bacilli. The predominant Gram-positive organism observed was *Staphylococcus aureus*. Among the Gram-negatives, *Citrobacter* spp. was more prevalent, followed by *Acinetobacter* spp. 37(38.1%) isolates were found to be multidrug resistant. Gram-positive organisms demonstrated highest susceptibility to Linezolid (100.0%) where as Gram-negative isolates were highly sensitive to Imipenem(88.6%) and Polymyxin-B(93.2%) but showed high resistance towards Cephalosporins. 15.5% of the isolates were strong producers of biofilm. *Staphylococcus aureus* was the predominant biofilm producer and 57% biofilm producing organisms were multidrug resistant.
Conclusion: Orthopaedic implant–associated infection puts a great financial burden on patients as well as on hospital resources and leads to increased morbidity. Appropriate microbiological interventions will help in reducing the magnitude of the problem.

Keywords: Implant; infection; biofilm; bacteriological profile.

1. INTRODUCTION

Implantation of medical devices is an important and essential component in current medical practice [1]. Each year millions of patients improve their quality of life through surgical procedures involving implanted medical devices [2]. The use of orthopaedic implants has revolutionized treatment of bone fractures and non-infectious joint arthritis [3].

Orthopaedic implant-associated infections are a frightening disaster, both for the patient and the surgeon as it is associated with high rate of morbidity and medical costs [4]. These infections are typically caused by microorganisms growing in biofilms [5]. Biofilm bacteria can survive upto 1500(typically 100 to 250) times the amount of an antibiotic needed to kill the same bacteria growing in a liquid culture [6]. Current diagnosis of Orthopaedic implant-associated infections include a combination of clinical, laboratory, histopathological, microbiological and imaging studies [7].

The identification of the causative pathogens is of paramount importance as it allows installation of appropriate antibiotics to target the pathogen, minimizing unnecessary antibiotic usage, decreasing the incidence of drug toxicity and understanding their potential for biofilm production [8].

This study was conducted to detect the bacteriological profile and drug susceptibility patterns of orthopaedic implant infections in the patients attending SKIMS hospital, which inturn guided clinicians in proper management of such patients with effective antimicrobials and timely institution of infection control practices in the hospital.

2. MATERIALS AND METHODS

2.1 Study Design and Period

This cross-sectional study was conducted in the Department of Microbiology and the Department of Orthopaedics, Sher-i-Kashmir Institute of Medical Sciences(SKIMS), a tertiary care institute in the state of Jammu and Kashmir, India from August 2014 to February 2016.

2.2 Ethical Clearance

Ethical clearance was obtained from the Institute′s ethical clearance committee.

2.3 Sample Size

This study was conducted on the 100 orthopaedic implant patients of any age group with a clinically suspected infection.

2.4 Inclusion Criteria

Patient criteria- Patients who underwent orthopaedic implant surgery with clinical features of infection were included. The signs and symptoms depended upon whether the infection was early [fever, local persisting pain, erythema, wound healing disturbance, hematoma], delayed [increasing joint pain, loosening of implant, sinuses] or late [sinuses, aseptic loosening, sepsis].[7,9-11]

2.5 Type of Specimens

The types of specimens included Orthopaedic Implants [rod, screws, plates, wire, prosthesis], Pus [from sinus tract or persistent wound drainage over the implant site], Joint aspirates and tissue specimens.

2.6 Collection, Transport, and Culture of Specimens

- Implants were collected and transported to the microbiology laboratory in Brain Heart Infusion (BHI) broth.
- Pus was taken from the infected site with the help of two sterile cotton swabs or sterile disposable syringes.
- Joint aspirates were taken from the infected joint space with the help of a sterile disposable syringe.
- Tissue specimens were collected in BHI broth.

All the samples were processed within two hours of collection.

2.7 Procedures

Gram staining and microscopy was done for routine bacteriological identification [12]. The specimens (pus, aspirate, swabs) were inoculated on plates of blood agar as well as MacConkey agar and were incubated at 37ºC aerobically for 24 hours. Implants and tissue specimens were incubated in BHI broth at 37ºC aerobically for 24 hours and then sub-cultured on blood agar and MacConkey agar. Proper media controls were also set up in the form of uninoculated broth, blood, and MacConkey agar.

Organisms were identified by standard microbiological procedures including various biochemical tests [12]. Optimal culture sensitivity and specificity was achieved if two or more samples were considered to be positive for the same organism. All the isolates were subjected to antimicrobial susceptibility tests on Muller-Hinton agar by Kirby Bauer disc diffusion method based on CLSI guidelines [13]. The antibiotics tested for Gram-positive isolates were Amikacin (30 µg), Ampicillin–sulbactam (10/10µg), Azithromycin(15 µg), Cloxacillin (10 µg), Ciprofloxacin (5 µg),Clindamycin (2 µg), Co-trimoxazole(Trimethoprim/Sulphamethoxazole)(1 .25/23.75 µg), Erythromycin(15 µg), Gentamicin(10µg), Levofloxacin (5 µg), Linezolid (15 µg), Teicoplanin (30 µg), Tetracycline (30 µg), and Vancomycin (30 µg).

For Gram-negative isolates, antibiotics tested for susceptibility were Amikacin (30 µg), Cefepime (30 µg), Cefoperazone/Sulbactam (75/10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Co-Trimoxazole (Trimethoprim/Sulphamethoxazole1.25/23.75µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Piperacillin/Tazobactam (100/10 µg), Polymyxin –B (300 units) and Ticarcillin/Clavulanic acid (75/10 µg). Carbenicillin (100 µg) and Tobramycin (10 µg) were specifically used for *Pseudomonas* spp. All the media, reagents and antibiotic discs were procured from Hi-Media Laboratories, Mumbai, India.

Biofilm detection was done by Tissue Culture Plate method described by Christensen et al [14]. Isolates were plated in triplicates on tissue culture plates and interpreted by Stepanovic Method [15] as detailed in Table 1.

Table 1. Interpretation of biofilm production

Average OD value	Biofilm production
≤ ODc / ODc< ~ ≤ 2x Odcs	Non/weak
2x ODc< ~ ≤ 4x Odc	Moderate
> 4x Odc	Strong

Optical density cut-off value (ODc) = average OD of negative control + 3x standard deviation (SD) of negative control.

2.8 Statistical Analysis

Data was analyzed in SPSS 12.0 by students independent T-test and/ chi-square test. The results obtained were discussed on 5% level of significance i.e. P-value < 0.05 was considered significant.

3. RESULTS

3.1 Age and Gender Distribution

A total of 100 patients from whom samples were collected comprised of 79 males and 21 females. Patients were mostly in the age group of 20-39 years [Table 2].

Table 2. Gender wise distribution of age groups

Age group (in years)	Male	Female
	N (%)	N (%)
0-19	14 (17.72%)	2 (9.52%)
20-39	34 (43.04%)	8 (38.10%)
40-59	23 (29.11%)	5 (23.81%)
60 or above	8 (10.13%)	6 (28.57%)

3.2 Site and Specimen Distribution

The most common affected site was Femur (34%), followed by Tibia (27%) [Table3]. Pus (55%) was the most frequent sample used for analysis (Fig. 1).

Table 3. The distribution of affected sites

Site affected	N=100 (%)
Femur	34 (%)
Tibia	27 (%)
Humerus	11 (%)
Knee	5 (%)
Hip	4 (%)
BBUL (both bones upper limbs)	2(%)
BBLL (both bones lower limbs)	3(%)
Metatarsals	3(%)
Fibula	2(%)
Distal Radius	2(%)
Calcanium	2(%)
Ulna	2(%)
Patella	1(%)
Acetabulum	1(%)
Spine	1(%)

3.3 Microscopy and Culture

Gram staining demonstrated sensitivity and specificity of 14.63% and 77.7% [Table 4]. The culture positivity rate was 86% (N=86) and a total of 97 isolates were recovered [Table 5]. In11 culture positive cases, culture showed growth of more than one microorganism (polymicrobial).

3.4 Isolates identified

Of the 97 isolates, 53 (54.6%) were aerobic Gram-positive cocci and 44 (45.4%) were aerobic Gram-negative bacilli. The most prevalent organism was *Staphylococcus aureus*[37.1% (N=36)]. Among the Gram negatives, *Citrobacter*[16.5% (N=16)] was the most common organism isolated [Table 6].The most prevalent combination was that of *Staphylococcus* with *Citrobacter* spp. which were isolated in 5 of the 11 polymicrobial cultures [Table 7]. 37 (38.1%) isolates were multidrug resistant (MDR). [Table 8].

3.5 Antibiotic Susceptibility Patterns

Gram-positive organisms showed the highest susceptibility to Vancomycin, Teicoplanin, and Linezolid. 52.8% of these were also susceptible to Amikacin [Table 9]. Gram-negative isolates were highly sensitive to Imipenem (88.6%) and Polymyxin-B(93.2%),however, they were found resistant to most of the cephalosporins and showed moderate susceptibility (56.8%) towards Amikacin and Quinolones [Table 10].

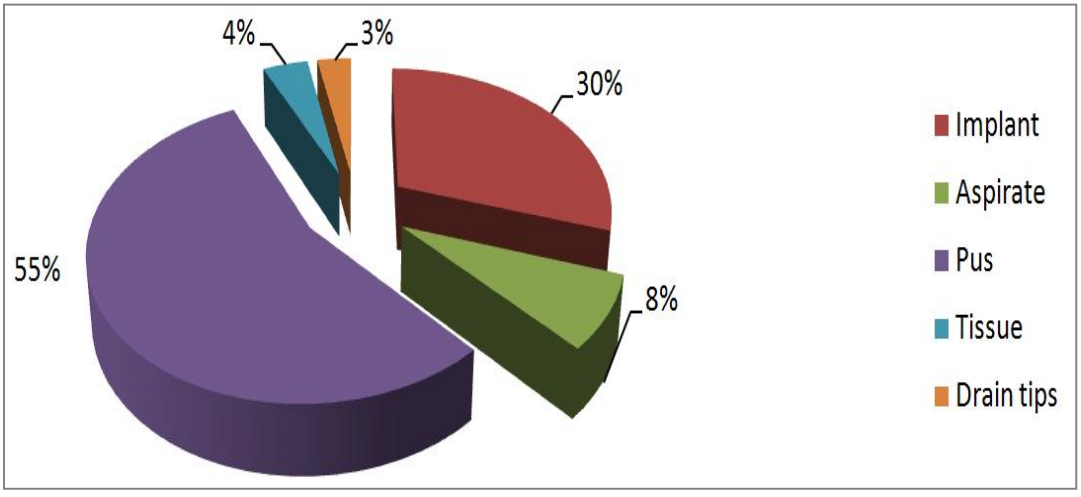


Fig. 1. Distribution of the specimen received

Table 4. Summarized results of effectiveness of gram stain in diagnosis of Orthopaedic implant infections

Positive	Negative	
True Positive (N=12)	False Negative(N=70)	Sensitivity (14.63%)
False Positive(N=4)	True Negative(N=14)	Specificity (77.7%)
Positive predictive value (75 %)	Negative predictive value (16.6 %)	

Table 5. Culture reports of tested samples

Culture Report	N=100 (%)
Culture positives	86 (%)
Culture negatives	14 (%)

Table 6. The distribution of the organisms recovered

Total isolates recovered	N=97 (%)
Gram Positive	53(54.6%)
Gram Negative	44 (45.4%)
Gram-positive organisms	**N=53 (%)**
MRSA	18 (18.6%)
MSSA	18 (18.6%)
CoNS	10 (10.3%)
Enterococcus spp.	7 (7.22%)
Gram-negative organisms	**N=44 (%)**
Citrobacter spp.	16 (16.5%)
Acinetobacter spp.	9 (9.28%)
Escherichia coli	8 (8.25%)
Klebsiella spp.	4 (4.12%)
Enterobacter spp.	1 (1.03%)
Pseudomonas spp.	3 (3.09%)
Serratia spp.	3 (3.09%)

3.6 Biofilm Detection

Tissue culture plate method for biofilms detected 15 (15.5%) isolates as strong biofilm producers and 4 (4.1%) as moderate biofilm producers [Picture 1]. MRSA (N=6) was the major biofilm producer[Table 11]. 29.73% (N=11) biofilm producers were multidrug resistant (MDR) [Table 12].

4. DISCUSSION

Orthopaedic implants are mainly used for bone fixation and joint replacement [16]. Implants being foreign bodies compromise the local host defence mechanisms and become highly susceptible to microbial infections [17,18]. Microorganisms rapidly adhere to the implant and resist elimination, which leads to the most disastrous consequences [19]. Numerous challenges associated with these infections include the need for multiple surgeries, long periods of disability for the patient, and occasionally sub-optimal outcomes [20,21].

Table 7. Combinations in Polymicrobial cultures

Organism 1	Organism 2	Total combinations
CoNS	*Citrobacter*	1
Enterococcus	*Acinetobacter*	1
MRSA	*Citrobacter*	2
MRSA	*Klebsiella*	1
MSSA	*Citrobacter*	2
MSSA	*Escherichia Coli*	1
MSSA	*CoNS*	1
MSSA	*Serratia*	1
MSSA	*Enterococcus*	1
Total		11

Due to ineffective means of definitive diagnosis, inappropriate use of antibiotics and hence drug resistance as well as the ability of microorganisms to evade the host response through biofilm formation, treatment of orthopaedic implant infections is often inadequate, leading to chronic infections and other complications [22]. Thus there is a need for proper diagnosis and management of such infections, this being the focus of our study and keeping in view that such a study has not been conducted in our settings.

There was a male preponderance [N=42 (42%)] for the cases that reported to us and commonly affected young adults in the age group of 20-39 years as this age group is more prone to road traffic injuries, sports injuries and other industrial accidents. The same patterns have been seen in a study by Peden M.[23] Lower extremity fractures were more common in our patient

Table 8. The proportional distribution of MDR isolates

Organism	N	MDR	% of MDR
Staphylococcus aureus	36	18	50.0%
Citrobacter spp.	16	6	37.5%
Acinetobacter spp.	9	4	44.4%
CoNS	10	3	30.0%
Enterococcus spp.	7	3	42.9%
Escherichia coli	8	1	12.5%
Klebsiella spp.	4	1	25.0%
Serratia spp.	3	1	33.3%
Enterobacter spp	1	0	0
Pseudomonas spp.	3	0	0
Total	97	37	38.1%

Table 9. Antibiotic sensitivity of gram-positive organisms (N=53)

Antibiotic	Sensitive	%	Resistant	%
Cloxacillin	27	50.90%	26	49.10%
Ampicillin + Sulbactam	26	49.06%	27	50.94%
Amikacin	28	52.80%	25	47.20%
Gentamicin	30	56.60%	23	43.40%
Ciprofloxacin	21	39.60%	32	60.40%
Levofloxacin	31	58.50%	22	41.50%
Erythromycin	26	49.10%	27	50.90%
Azithromycin	21	39.60%	32	60.40%
Clindamycin	22	41.50%	31	58.50%
Vancomycin	51	96.20%	2	3.80%
Teicoplanin	52	98.10%	1	1.90%
Linezolid	53	100.00%	0	0.00%
Tetracycline	35	66.00%	18	34.00%
Co-trimoxazole	8	15.10%	45	84.90%

Table 10. Antibiotic sensitivity of gram-negative isolates(N=44)

Antibiotic	Sensitive	%	Resistant	%
Piperacillin+Tazobactam	13	29.5%	31	70.5%
Ticarcillin+Clavulanic acid	14	31.8%	30	68.2%
Amikacin	25	56.8%	19	43.2%
Gentamicin	19	43.2%	25	56.8%
Ciprofloxacin	25	56.8%	19	43.2%
Levofloxacin	25	56.8%	19	43.2%
Ceftriaxone	12	27.3%	32	72.7%
Ceftazadime	14	31.8%	30	68.2%
Cefepime	12	27.3%	32	72.7%
Cefaperazone-Sulbactam	13	29.5%	31	70.5%
Imipenem	39	88.6%	5	11.4%
Polymyxin-B	41	93.2%	3	6.8%
Co-trimoxazole	22	50.0%	22	50.0%
Carbencillin	2	66.7%	1	33.3%
Tobramycin	1	33.3%	2	66.7%

Table 11. Prevalence of biofilm producing organisms

Organism	Strong N (%)	Moderate N (%)	Weak N (%)
MRSA	5(27.8%)	1(5.6%)	12 (66.7%)
MSSA	4 (22.2%)	1 (5.6%)	13 (72.2%)
CoNS	3 (30.0%)	0 (0.0%)	7 (70.0%)
Acinetobacter	1 (11.1%)	0 (0.0%)	8 (88.9%)
Klebseilla	0 (0.0%)	0 (0.0%)	4 (100.0%)
Enterococcus	1 (14.3%)	1 (14.3%)	5 (71.4%)
Citrobacter	0 (0.0%)	1 (6.3%)	15 (93.8%)
Pseudomonas	1 (33.3%)	0 (0.0%)	2 (66.7%)
E. coli	0 (0.0%)	0 (0.0%)	8 (100.0%)
Enterobacter	0 (0.0%)	0 (0.0%)	1 (100.0%)
Serratia	0 (0.0%)	0 (0.0%)	3 (100.0%)

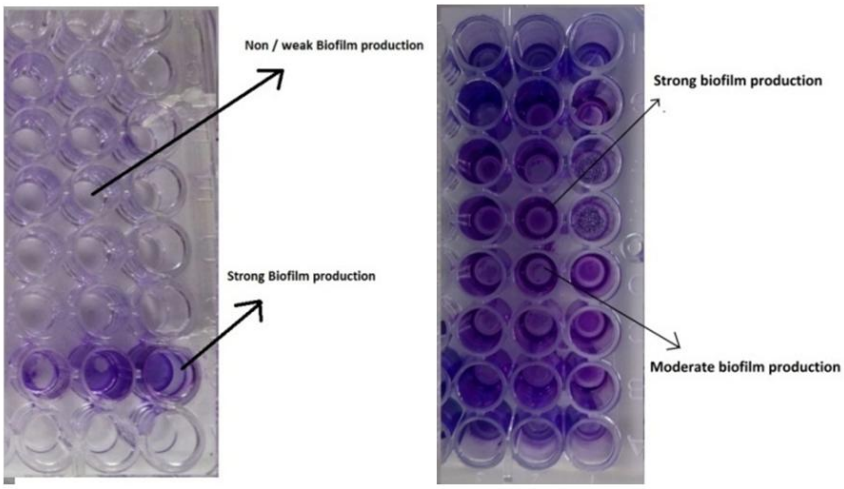
population. In case of road traffic accidents (RTA's), these injuries occur due to the interaction of gravitational force and the impact absorption by the lower limbs at the time of accidents. Similarly, Singh R. et al. reported lower limbs (38%) as the major site involved in fractures [24].

Gram staining of pus or joint aspirate is generally performed as a part of the microbiological assessment of suspected orthopaedic implant infections, especially in peri-prosthetic infections. However, its effectiveness is questionable. In our study, the sensitivity and specificity of Gram staining was 14.36% and 77.7% respectively

Table 12. Biofilms in relation to multidrug resistance

MDR	Biofilm Producers (Strong + Moderate) N (%)	Non/ weak Biofilm producers N (%)
MDR +	11(29.73%)	26(70.27%)
MRD -	8(13.33%)	52(86.67%)
Total	19	78

The P-value is 0 .048. The result is significant at P< .05.



Picture 1. Representative results of biofilm production showing weak, strong and moderate biofilm production in the wells of tissue culture plate

which is comparable to the study of Della Valle CJ et al. who reported 14.7% sensitivity of Gram staining in diagnosing peri-prosthetic infections [25]. Another study by Banit et al. found a comparatively higher sensitivity of 44% for the test [26]. Although it might be considered effective to perform Gram stains because they can be carried out quickly, at a fairly low-cost with minimal technical expertise, this test is poor at diagnosing orthopaedic implant infections.

In our study, the culture positivity was 86% and 14% of the samples were culture negative. Similar results have been obtained by Zimmerli et al. when reporting the culture positivity of 89% in patients with suspected prosthetic joint infections [27]. Vishwajith et al. in their study reported a high culture yield of 94.89% [28]. However, Gomez et al. reported a lesser culture positivity of 60% [29]. Negative results on culture also create a real challenge in the diagnosis of orthopaedic implant infections. The reasons for such culture negativity can be the administration of antibiotics prior to obtaining culture samples and presence of more fastidious organisms. Different studies have indicated that there is a significant proportion (5%-34%) of infections in which the culture is negative, which is comparable to our study.[30-32]

The most prevalent organisms isolated were aerobic Gram-positive cocci [N= 53(54.6%)] followed by aerobic Gram-negative bacilli [N=44 (45.4%)]. These results are comparable to the study by Gomez et al. who also reported aerobic Gram-positive cocci (60.6%) as the most prevalent organism[29]. However, Khosravi et al. in their study found aerobic Gram-negative bacilli as the most prevalent microorganisms (64.5%).[33] *Staphylococcus aureus* [N=36 (37.1 %)] was the most common Gram- positive isolate comprising of MSSA (methicillin-sensitive *Staphylococcus aureus*) [N=18 (18.6%)] and MRSA (methicillin-resistant *Staphylococcus aureus*) [N=18 (18.6%)] followed by CoNS (coagulase-negative *Staphylococcus*) [N=10 (10.3%)] and *Enterococcus* [N=7 (7.2%)].*Staphylococcus aureus* also has been reported as the most common bacteria isolated from orthopaedic implants in a study by Shah MQ et al. [34] Khan MS et al. detected *Staphylococcus aureus* as the commonest organism in their study constituting 50% of the total isolated organisms.[35] Pulido L. et al. isolated methicillin-resistant *Staphylococcus aureus* (MRSA) (19%) and methicillin-sensitive *Staphylococcus aureus* (MSSA) (19%) as the more frequent Gram-positive isolates in the peri-prosthetic infections.[36]Trisha Peel et al.

isolated *Enterococcus* in 10% of all Prosthetic joint infections(PJI´s) which is comparable to our study [37].

Staphylococcus aureus is an important pathogen isolated in such infections. Patients, as well as healthy people, can carry this organism in their nasal passages. Healthcare workers colonized by *Staphylococcus aureus* act as a medium in transmitting this organism to patients. Hospital supplies like bed linen, instruments and dressings have also been found to act as reservoirs of these organisms. Dutta et al. in their study found 45.2% of hospitalized patients and 6.6% of hospital staff as carriers of *Staphylococcus aureus* [38].

In our study, *Citrobacter* spp. (16.5%) was the most prevalent Gram-negative organism followed by *Acinetobacter* spp. (9.28%).*Citrobacter* spp. has not often been reported as a common pathogen in orthopaedic implant infections, however, it has been isolated in small percentages from these infections in studies by Vanderhooft et al. and Kaufman et al.[39,40] *Citrobacter* spp. are generally considered to be environmental contaminants or harmless inhabitants in intestinal tracts of man. However, with poor host defences or other factors favouring their establishment in tissues, serious infections may result by this organism.[41] The outbreaks of *Acinetobacter* infections, occurring mostly in intensive care units are of much concern. Silva R. et al have reported 14.9% *Acinetobacter baumannii* isolates while describing the bacterial profile of orthopaedic implant-associated Gram-negative infections [42]. Our study shows that infections due to *Acinetobacter* spp. account for 9.28% of the total isolates recovered and measures are needed to be taken in order to control these infections.

The pattern of organisms isolated in our study reflects the nosocomial origin of implant infections and possibly suggests an intra or peri-operative contamination of the wounds by these nosocomial pathogens present in the operating room or in the postoperative wards.

Staphylococcus with *Citrobacter* spp. was the most frequent combination in polymicrobial infections(12.7%) in our study which again suggests the role of nosocomial aetiology of these infections. Steckelberg J.M et al. reported 12% polymicrobial infections in PJI's [43]. However Peel TN et al. reported a higher percentage (36%) of polymicrobial infections in their study [44].

Infections are a frequent cause of implant failure and the mainstay of treatment is the administration of appropriate antibiotics with or without the removal of implants. The antimicrobial susceptibility testing of the organisms isolated in our study showed variable results. A high percentage of drug resistance among the isolates was observed. Most of the Gram-positive isolates showed an excellent sensitivity to the second line antibiotics like Vancomycin, Linezolid and Teicoplanin. They were also susceptible to drugs like Cloxacillin, Levofloxacin and Amino-glycosides (Amikacin and Gentamicin).Similar pattern was shown in a study by Satya Chandrika V. et al. where Gram-positive cocci were mostly sensitive to Vancomycin and Linezolid [45].

Gram-negative organisms also showed variable resistance patterns in our study. Most of these isolates showed resistance to Cephalosporins which are used as first-line prophylactic drugs in patients undergoing orthopaedic implant insertion in our hospital. Highest sensitivity of 88.64% and 93.18% was seen with Imipenem and Polymyxin-B respectively. Roopa Shree et al. in their study have also reported Cephalosporin resistance by Gram-negative organisms isolated from orthopedic implant site infections [46].

There has been a remarkable increase of antibiotic-resistant bacterial strains, which has made antibiotic choices for infection control increasingly limited and more expensive [47]. As our set of patients have reported infections inspite of being on pre and postoperative prophylactic antibiotic therapy, the use of higher-end antibiotics is to be considered as the mainstay of treatment of such cases. Further, as this was not the study of the actual incidence of postoperative infections of orthopaedic implants, so commenting on the efficacy of first line drugs in preventing these infections will be difficult. As there were organism specific sensitivity patterns in our cases, it is strongly suggested that initiation of treatment with the second line drugs should be preceded by a proper microbiological investigation. However, in life-threatening situations like sepsis in such patients, Imipenem in combination with Linezolid or Vancomycin could be given. Such measures will be a step forward to proper antibiotic stewardship.

The alarming emergence of the resistant strains may lead to increased morbidity and mortality [48]. In this study, 38.1% of total isolates recovered were multidrug resistant(MDR). Similar findings were seen by Westrich G.H et al. who reported the total percentage of MDR organisms as 42.5% among the infected arthroplasty patients [49]. The rapid and ongoing spread of antimicrobial-resistant bacteria throughout health-care institutions is considered a critical medical and public health issue. This is preventable and can be controlled by adhering to infection control practices, primary among them being hand sanitization. Other infection control measures include regulating traffic in the wards and operation theatres, screening of the staff as well as the patients for the nasal carriage of these organisms particularly MRSA and active prophylaxis against any nasal reservoir of infection. Properly engineered operation theatres with the vertical laminar flow and air filters along with use of body exhaust suits can decrease the chances of infection. Such practices have shown to decrease the incidence of these infections significantly as reported by De Lucas- Villarrubia J.C et al. [50].

Biofilms play a pivotal role in healthcare-associated infections (HAIs), especially those related to the implantation of medical devices. A worrying feature of biofilm-based infections is represented by the higher antibiotic resistance of bacterial cells growing as biofilms as compared with planktonic cells [51]. Biofilm-associated bacterial infections are difficult to eradicate using antibiotics. It has been observed that biofilm makes the micro-colonies impermeable to antibiotics, hydroxyl radicals, and superoxide anions which bind at the outer surface of the matrix layer of the biofilm [52]. In our study, 19.6% organisms were shown to produce biofilms and *Staphylococcus aureus* was the most predominant biofilm producer followed by CoNS. Out of these 19 biofilm producers,11 were multidrug resistant and this was statistically significant. A positive correlation between biofilm formation and multiple drug resistance has also been reported in different studies by Badave GK et al. and Bala M et al. while studying *Acinetobacter baumannii* isolates [53,54]. Similarly Babapour E et al. showed a significant correlation between power of biofilm formation and antibiotic resistance in their study when amongst 115 isolates with moderate or strong biofilm forming trait, 106 (94.23%) were MDR [55]. However, Qi L et al. in their study commented that there are individual differences among the isolates and the increase in the resistance of some antibiotics occurred independently of the quantity of biofilm produced [56].

5. CONCLUSION

Though vast in scope, as parameters from two inherently related departments (Orthopaedics and Microbiology) in the management of Orthopaedic implant infections, this research served as the first step in a continuous exercise in better understanding and managing the cases of orthopaedic implant infections presenting to our hospital. The dynamics of the relationships between different variables will require further study over longer timelines as well as focus to increase the knowledge related to our settings. During this study, it became obvious that institution-specific factors were operational and would require institution-specific interventions to yield results which are reproducible. Further studies need to be undertaken to refine the microbiological as well as the orthopaedic management of the infections associated with orthopaedic implants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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