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Bacteriological profile of orthopaedic implant site infection and their antimicrobial susceptibility pattern in a tertiary care hospital

[1]Santosh Kumar, [2]Dharitri Mohapatra, [3]Sasmita Khatua, [4]Nirupama Chayani

[1]Consultant, Microbiologist, Dy. Medical Superintendent (Central Hospital), Brajarajnagar, Jharsuguda MCL, Coal India Limited, Odisha, India
[2]Associate Professor, Department of Microbiology, SCB Medical College, Cuttuck, Odisha, India
[3]Assistant Professor, Department of Microbiology, SCB Medical College, Cuttuck, Odisha, India
[4]Professor and HOD, Department of Microbiology, SCB Medical College, Cuttuck, Odisha, India

Corresponding Author:
Dr. Dharitri Mohapatra (dr.dharitri@rediffmail.com)

Abstract

Orthopaedic implant site infection is one of the major surgical site infection in orthopaedic surgery with high morbidity and mortality. Due to the use of implants, which are foreign to the body, orthopaedic trauma surgery is at the grave risk of microbial contamination. Overall 5% of the internal fixation devises get infected. The incidence of infection after internal fixation of closed fractures is generally lower (0.5-1%), where as in case of internal fixation of open fractures, the incidence is still higher and may exceed 30%. The prevalence of orthopaedic implant site infection reported in India is about 2.6%.

Methodology: This total of study has been carried out in the Department of Microbiology, in collaboration with the Department of Orthopaedic, SCBMCH, Cuttack for a period of 2 years. Total of 159 pus sample collected aseptically were processed in the laboratory and bacterial isolation and identification were done by standard microbiological procedures. Isolates were subjected for antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method according to CLSI guideline. All the isolates were subjected to detect biofilm production by Congo red agar method, ESBL production by phenotypic confirmatory combined-disc test, MRSA detection by cefoxitin disc diffusion test, MBL production by IMP-EDTA (Imipenem-Ethylenediaminetetracetic acid) combined disc method and Phenotypic detection of vancomycin resistant Enterococcus by Vancomycin agar screen method.

Results: Out of 159 clinically diagnosed cases 124(77.9%) were culture positive out of Which 114(91.1%) were monomicrobial and 10(8.07%) were polymicrobial. Among 134 pathogens isolated 81(60.4%) were Gram positive and 52(38.8%) were gram negative. Only one acid fast bacilli was isolated. Staphylococcus aureus 38(28.5%) was the most commonly isolated pathogen followed by Klebsiella spp.21 (15.6%). Most of the Gram positive isolates were susceptible to linezolid, vancomycin & tigecycline whereas gram negative isolates showed maximum susceptibility to cefoperazone sulbactum and piperacellin tazobactam combination of antimicrobials. *Klebsiella* spp. 12(14.8%) was the most common ESBL producers followed by *E. colli* 9(11.11%). Among the MBL producers *Klebciella* spp.

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6(7.4%) was most common organism. Out of 134 isolated pathogen 82(61.6%) were biofilm producer and staph. aureus 24(18.05%) being the commonest. Out of 3 isolates of Enterococci 1(33.3%) was VRE.

Conclusion: Our study revealed high rates of antimicrobial resistance in our Hospital. Strict adherence to antimicrobial policy and multidisciplinary approach involving orthopaedic surgeons and infectious diseases specialists and microbiologists will reduce the incidence of orthopaedic implant site infection.

Keywords: OISI, biofilm, MRSA, ESBL, MBL, VRE

Introduction

Orthopaedic implant site infection is one of the major surgical site infection associated with high morbidity and mortality. Orthopaedic trauma surgery is always at a risk of infection due to use of implants for open reduction and internal fixation. Since the implants used are foreign to the body, there is always a risk of microbial contamination and infection. The endogenous or exogenous microorganisms that enter the wound during surgery are responsible for those infections. Major risk factor for development of orthopaedic implant site infection depends upon the exlent of damage to the soft tissues and periosteum following fracture Pathogenesis of orthopaedic implant site infection involves interaction between the host, the implant is devoid of microcirculation the microorganisms proliferates and undergoes phenotypic alterations to form a bio film. These microorganisms survive within the biofilm causing a difficulty in delivery of antibiotics. Any delay in the treatment of these infection will lead to significant morbidity and prolong hospitalisation. Hence one should have knowledge on the microbiological profile and their antibiotic susceptibility pattern for aggressive treatment and to prevent complication. As the data varies from hospital to hospital the study was done in view of evaluating the causative organisms, their antimicrobial susceptibility pattern and their potential to form biofilms.

The orthopaedic implants and prosthetic surgery has become one of the commonest orthopaedic operation, because of the success of this procedure in restoring function of fractured bones and prostheses for load bearing joints like hip, knee etc. This is the major procedure to alleviate pain and to improve mobility in people with fractured bone and damaged joints [1]. Moreover, the number of elderly and trauma patients requiring internal fixation or joint replacement devices is steadily increasing due to increased life expectancy and urbanisation. Due to the use of implants for open reduction and internal fixation, which are foreign to the body, orthopaedic trauma surgery is at grave risk of microbial contamination and infection [1]. Open or compound fractures are fractures that communicate with the outside environment through skin wounds and pose increased risk of infection [2].

Though the incidence of orthopaedic implant related infection has been reduced to less than 1%, it remains a diagnostic, therapeutic and cost related problem [3]. It is said that overall 5% of internal fixation devices get infected, whereas the incidence of infection after internal fixation of closed fractures is generally lower (0.5% -2%). In case of internal fixation of open fractures, the incidence is still higher and may exceed 30% [4, 5, 6, 7, 8]. The prevalence of orthopaedic implant site infection reported in India is about 2.6% [9]. In this study, we have isolated the organisms at orthopaedic implant associated infection and evaluated their drug sensitivity patterns. Also correlated with the various risk factors associated with orthopaedic implant site infection.

Materials and Methods

The study was conducted in the department of Microbiology S.C.B Medical College, Cuttack

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from for a period of two years from September-2016 to August- 2018. The study group comprised of a total of 159 patients who had undergone orthopaedic implant or prosthetic surgery and presented with signs and symptoms of infection. The institutional ethical committee approval and inform concents of the patients were obtained. The demographic data like age, sex, duration and the type of implant and the risk factors were noted.

The samples collected from the secretions present adjacent to the infected implant and tissues by using sterile swab or sterile syringe, and immediately transferred to Microbiology laboratory. All the samples were subjected to Gram staining and ZN staining. Swab were inoculated onto 5% sheep blood agar, Mac Conkey agar and BHI broth. The plates were incubated at 37^{o} C for 24-48 hrs. and examined for growth of bacteria. All positive cultures were identified by their *characteristic* colony morphology, Gram staining and confirmed by standard biochemical tests. Samples which were positive for AFB were inoculated on LJ medium. Subcultures done from BHI broth if no growth was observed. Anti-microbial susceptibility testing done on Muller Hilton Agar by Kirby Bauer's disc diffusion method as per CLSI guidelines. All the confirmed staph aureus isolates and coagulase negative staphylococcus spp. (CONS) strains were screened for methicellin Resistance based on disc diffusion methods using Cefoxitin (30µg) disc obtained from Himedia Lab. Pvt Ltd (fig-1). Biofilm production were tested using Congo Red Agar method (fig-2). MBL production by IMP-EDTA (Imipenem-Ethylenediaminetetraacetic acid) combined disc method, ESBL production by phenotypic confirmatory combined-disc test, and phenotypic detection of vancomycin resistant Enterococcus by Vancomycin agar screen method [10-14].

Results

Out of 159 clinically diagnosed cases, 124 (77.98%) were culture positive and 35 (22.02%) were culture negative. Majority of the patients 68 (42.76%) belonged to the age group of 31-40 year followed by age group of 41-50 year 39 (24.52%). Among the culture positive cases, majority 56 (35.22%) were in the age group of 31-40 year followed by the age group of 41-50 year 30 (18.86%) (Table 1).

Table 1: Culture positive and negative of samples with respect to age groups.

Age groups (years)	Positive	Negative	Total
<10	0 (0%)	0(0%)	0(0%)
10-20	2(1.26%)	1(0.62%)	3(1.88%)
21-30	23(14.46%)	7(4.40%)	30(18.86%)
31-40	56(35.22%)	12(7.54%)	68(42.76%)
41-50	30(18.86%)	9(5.66%)	39(24.52%)
51-60	9(5.66%)	4(2.51%)	13(8.17%)
61-70	4(2.51%)	2(1.26%)	6(3.77%)
71-80	0 (0%)	0(0%)	0(0%)
Total	124(77.98%)	35(22.02%)	159(100%)

Out of 159 cases, males were 105 (66.04%) and females were 54 (33.96%). Among all culture positive cases of orthopaedic implant site infection, 81 (50.95%) were male, whereas 43 (27.04%) were female (p value 0.87; chi-square (Ψ^2) value 0.024). Male to female ratio was foun to be 1.8:1. Among all suspected cases of infection, most common implant used was plates in long bones 85 (53.46%), followed by Kuntscher nails 42 (26.41%) and most common implant infected was plates in long bones 85 (53.46%), followed by Kuntscher nails 42 (26.41%). Knee prostheses has lower infection rate as compared to other, but there is no significant difference (Pearson chi-square (Ψ^2) value 1.93; df = 5; p value= 0.859) of occurrence of infection is found among the orthopaedic implants (Table 2). Out of 124

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culture positive sample, monomicrobial infection were observed in 114 (91.13%) cases while 10 (8.07%) showed polymicrobial infection. There was 10 (8.07%) polymicrobial growth with two isolates among all culture positive cases.

Table 2: Number of implants used and which revealed infections.

Implants/Prostheses	No. of Implants/Prostheses used	No. of Implants/Prostheses infected
Plates in long bones	85(53.46%)	66(53.22%)
Kuntscher nails	42(26.41%)	34(27.43%)
Cannulated screws	7(4.39%)	6(4.84%)
Cerclage wire	4(2.51%)	3(3.22%)
Austin-moore endoprostheses	10(6.29%)	8(6.45%)
Knee prostheses	11(6.98%)	7(5.64%)
Total	159(100%)	124(100%)

Including the 10 pathogens from polymicrobial growth, total 134 pathogens have been isolated from 124 culture positive sample. Out of 134 pathogens, Gram negative pathogens were 81 (60.44%) and Gram-positive pathogens were 52 (38.80%). There was only one (0.74%) isolate of acid fast bacilli (AFB). The most common isolated pathogen was Staphylococcus aureus 38 (28.35%), followed by Klebsiella spp. 21 (15.68%) and Escherichia coli 16 (11.95%). There was only one (0.74%) isolate of Mycobacterium tuberculosis (Table 3).

Table 3: Number of organism isolated from implant cite of orthopaedic wound infections.

Organisms	Number	%
Staphylococcus aureus	38	28.35%
Coagulase negative Staphylococcus	11	8.20%
Enterococcus spp.	3	2.23%
Klebsiella spp	21	15.68%
Pseudomonas aeruginosa	13	9.71%
Escherichia coli	16	11.95%
Acinetobacter spp.	11	8.20%
Citrobacter spp.	9	6.71%
Enterobacter spp.	7	5.23%
M. tuberculosis	1	0.74%
Proteus spp.	4	2.98%
Total	134	100%

Majority 69 (51.49%) of the patients was in the early post-operative period. Staphylococcus aureus 38 (28.35%) was the most common isolated pathogen in the early 21 (15.67%), delayed 13 (9.70%) and late 4 (2.98%) post-operative period (Table 4).

Table 4: Organisms isolated from the cite of implant infections with respect to the duration.

Organisms	Early (<2 weeks)	Delayed (2-10 weeks)	Late (>10 weeks)	Total
S. aureus	21(15.67%)	13(9.70%)	4(2.98%)	38(28.35%)
CoNS	5(3.73%)	4(2.98%)	2(1.49%)	11(8.21%)
Enterococcus spp.	1(0.75%)	2(1.49%)	0(0%)	3(2.24%)
Klebsiella spp.	11(8.21%)	8(5.97%)	2(1.49%)	21(15.67%)
P. aeruginosa	6(4.48%)	5(3.73%)	2(1.49%)	13(9.70%)
E.Coli	9(6.72%)	6(4.48%)	1(0.75%)	16(11.94%)

Acinetobacter spp.	6(4.48%)	4(2.98%)	1(0.75%)	11(8.21%)
Citrobacter spp.	5(3.73%)	3(2.24%)	1(0.75%)	9(6.71%)
Enterobacter spp.	3(2.24%)	3(2.24%)	1(0.75%)	7(5.22%)
Proteus spp.	2(1.49%)	2(1.49%)	0(0%)	4(2.98%)
M. tuberculosis	0(0%)	1(0.75%)	0(0%)	1(0.74%)
TOTAL	69(51.49%)	51(38.06%)	14(10.45%)	134(100%)

Most of the Gram-positive isolates were more susceptible to linezolid, vancomycin and tigecycline. The Gram-negative isolates showed maximum susceptibility to imipenem (IPM), cefoperazone-sulbactam (CFS) and piperacillin-tazobactam (PIT) combination of antimicrobial agents (Table 5&6).

Table 5: ABST patterns of gram positive bacteria

Gram positive cocci	AMC	GEN	CFS	COT	CIP	LZ	PIT	TEI	VA
S. aureus (n=38)	56	60	74	52	46	92	88	100	100
CoNS (n=11)	58	54	68	56	42	100	87	100	100
Enterocuccus spp.(n=3)	33	33	33	33	33	66	100	100	66

Table 6: ABST patterns of gram negative bacteria

Gram negative bacilli	AMC	CAZ	CFS	COT	CFM	CIP	GEN	PIT	IPM
Klebsiella spp. (N=21)	47	29	76	48	63	58	62	80	86
Pseudomonas aeruginosa (N=13)	69	48	77	58	52	60	67	76	88
Acinetobacter spp. (N=11)	36	62	80	62	63	62	64	74	92
E. coli (N=16)	36	62	87	52	56	38	77	82	90
Citrobacter spp. (N=9)	44	57	79	72	56	57	65	82	94
Enterobacter spp. (N=7)	45	70	86	74	71	62	73	87	96
Proteus spp. (N=4)	25	50	75	25	50	50	75	75	100

Out of 134 isolated pathogens, 82 (61.61%) were biofilm producer and 52 (38.35%) were non-biofilm producer. Among biofilm producer, Staphylococcus aureus was most common 24 (18.05%) followed by Klebsiella spp. 13 (9.78%), Escherichea coli 10 (7.52%). By applying chi-square (Ψ^2) test, p value is found to be 0.99, which indicate insignificant association (Table 7). Out of total 38 isolates of *Staphylococcus aureus*, MRSA strain 11 (28.95%) were detected**.**

Table 7: Number of organisms producing biofilm

Organisms	Biofilm producer	Non-Biofilm producer	Total
Staphylococcus aureus	24 (18.05%)	14 (10.52%)	38 (28.57%)
CoNS	7 (5.28%)	4 (3.00%)	11 (8.28%)
Enterococcus spp.	2 (1.50%)	1 (0.75%)	3 (2.26%)
Klebsiella spp.	13 (9.78%)	8 (6.01%)	21 (15.79%)
Pseudomonas aeruginosa	8 (6.01%)	5 (3.76%)	13 (9.77%)
E. Coli	10 (7.52%)	6 (4.51%)	16 (12.03%)
Acinetobacter spp.	7 (5.28%)	4 (3.00%)	11 (8.28%)
Citrobacter spp.	5 (3.76%)	4 (3.00%)	9 (6.76%)
Enterobacter app	4 (3.00%)	3 (2.26%)	7 (5.26%)
Proteus spp.	2 (1.50%)	2 (1.50%)	4 (3.00%)
Total	82 (61.65%)	51 (38.35%)	133 (100%)

Out of 81 Gram negative isolates, ESBL (Extended spectrum β-lactamase) producing strain were 39 (48.14%). *Klebsiella spp.* 12 (14.82%) was the most common ESBL producer, followed by *Escherichea coli* 9 (11.11%) and *Pseudomonas aeruginosa* 7 (8.64%). From this

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table it has been seen that there is no significant difference (Pearson chi-square (Ψ^2) value 5.314; p value 0.5) of occurrence of ESBL producing organism in different infected orthopaedic implant studied (Table 8).

Table 8: Number of organism producing ESBL

Organisms	ESBL producer	ESBL non-producer	Total
Klebsiella spp.	12(14.82%)	9(11.11%)	21(25.93%)
E. coli	9(11.11%)	7(8.64%)	16(19.75%)
P. aeruginosa	7(8.64%)	6(7.41%)	13(16.05%)
Acinetobacter spp.	5(6.13%)	6(7.41%)	11(13.58%)
Citrobacter spp.	3(3.70%)	6(7.41%)	9(11.11%)
Enterobacter spp.	1(1.23%)	6(7.41%)	7(8.64%)
Proteus spp.	2(2.47%)	2(2.47%)	4(4.94%)
Total	39 (48.14%)	42 (51.86%)	81(100%)

Out of total 81 Gram negative isolates, 17 (20.99%) were MBL producer. *Klebsiella* spp. 6 (7.41%) was most common MBL producer followed by *Pseudomonas aeruginosa* 3 (3.70%) and *Escherichea coli* 3 (3.70%). From this table it has been seen that there is no significant difference (Pearson chi-square (Ψ^2) value 2.123; df 6; p value 0.908) of occurrence of MBL producing organism in different infected orthopaedic implant studied (Table 9). Out of total Enterococcus faecalis (3) isolates, 2 (66.67%) were vancomycin sensitive while 1 (33.33%) was vancomycin resistant (VRE). Obesity (38.71%) was found to be most common risk factor followed by diabetes (29.84%) and hypertension (22.58%).

Table 9: Number of organisms producing MBL

Organisms	MBL producer	Non-MBL producer	Total
Klebsiella spp.	6(7.41%)	15(18.52%)	21(25.93%)
E. coli	3(3.70%)	13(16.05%)	16(19.75%)
P. aeruginosa	3(3.70%)	10(12.35%)	13(16.05%)
Acinetobacter spp.	2(2.47%)	9(11.11%)	11(13.58%)
Citrobacter spp.	2(2.47%)	7(8.64%)	9(11.11%)
Enterobacter spp.	1(1.23%)	6(7.41%)	7(8.64%)
Proteus spp.	0(0%)	4(4.94%)	4(4.94%)
Total	17 (20.99%)	64(79.01%)	81(100%)

Discussion

Orthopaedic implant surgery has become one of the commonest orthopaedic operation, because of the success of this procedure and aims is to alleviate pain, restoring function and to improve mobility [Goel, 2006] [15]. In spite of great advance in antimicrobial therapy, Orthopaedic implant site infection are the major cause of treatment failure and morbidity in patients. This present study was undertaken to review the spectrum of microorganisms, impact of implants on infection, biofilm formation and antimicrobial susceptibility and resistance pattern in orthopaedic implant site infection. This study was conducted in the Department of Microbiology, S.C.B. Medical and Hospital, Cuttack, from September 2015 to August 2017, in 159 clinically suspected cases of Orthopaedic implant site infection. The purpose of the study was to isolate the microorganisms causing orthopaedic implant site infections and to know their abilities to produce biofilm. In the current era of decreased susceptibility or antibiotic resistance, it was also very important to emphasize and estimate the resistance pattern with reference to MRSA, VRE, ESBL, MBL production.

In the present study, the prevalence of Orthopaedic implant site infection from clinically

suspected cases observed was 77.98% [Table-1]; which is less when compared to other studies where Anisha Fernandez, *et al*. (2013) reported 84%. Khosravi, *et al*. (2009) and Vishwajith, *et al*. reported the culture positivity of 93.9% and 94.89% respectively. In another study conducted by Zimmeli, *et al*. (2004) reported 89% culture positivity. However, Gomez, *et al*. (2003), who reported the prevalence 60%. Various other studies also have been conducted in different parts of the world including India, to find out the prevalence of Orthopaedic implant site infection and has been found to vary widely from 60% to 95%. This wide range of result is probably due to under reporting and misdiagnosis of cases.

In our study, majority of the patients belonged to the age group of 31-40 year (42.76%) followed by 41-50 years (24.52%) [Table 2]. Out of 124 culture positive cases, majority 56 (35.22%) were in the age group of 31-40 and 30 (18.86%) were in the age group 41-50 year. This is in accordance to the studies done by Roopa Shree S, *et al*. (2015), who also reported that the implant infection is commonly seen in the age group of 31-50 years. This may be because persons of these age groups mostly go out for their daily work and have increased risk to sustain Road Traffic Accidents (RTA).

In this study, out of 159 clinically suspected orthopaedic implant site infection cases, 105 (66.04%) were male and 54(33.96%) were female in total and among all laboratory confirmed orthopaedic implant site infection cases, 81 (50.95%) were male and 43(27.04%) were female (p value 0.87; chi-square value 0.024). Male to female ratio was found to be 1.8:1. [Table 3]. This is less than the study done by Roopa Shree S, *et al.* (2015), who reported 86.95% male and 13.04% female. It was observed that males were more than females, this may be because of the fact that most of the orthopaedic implant surgeries are followed in orthopaedic OTs due to road traffic accident where patients had fractures associated with extensive tissue damage, hematoma formation and wound contamination which was a risk factor for developing infection.

In the present study, out of total 159 implants which were used in clinically suspected cases of infected orthopaedic implants, plates in long bones i.e. 85 (53.46%) and Kuntscher nails i.e. 42 (26.41%) was the commonest orthopaedic implant device [Table 4]. A study conducted by Onche I, *et al*. (2004) also reported, plates in long bones (56.7%) and Kuntscher nails (23.6%) as most commonly used orthopaedic implant devices.

Out of 124 culture positive cases, monomicrobial infection were 114 (91.13%) while 10 (8.07%) were polymicrobial infection with two isolates [Table 5]. This observation was in accordance with the study conducted by Roopa Shree S, *et al*. (2015) and Onche I, *et al*. (2004) who observed the similar type of findings.

Our study showed that Gram negative isolates were more 81 (60.44%) than Gram positive isolates 52 (38.80%). One acid fast bacilli (0.74%) which was detected from the spine (L1-L2) [Table 6]. These findings of distribution were similar to the findings of Khosravi, *et al*. (2009), who reported Gram positive and Gram negative organism 33.5% and 64.5% respectively but contradicted to Gomez, *et al*. (2004), who reported 60.6% and 33.3% respectively, probably due to different nosocomial pathogens present in our operating rooms.

In this study, total 134 pathogens have been isolated from 124 culture positive cases, including the 10 polymicrobial isolates. *Staphylococcus aureus* 38 (28.35%) was the most commonly isolated pathogen from orthopaedic implant site infected cases, followed by CoNS 11 (8.20%) and *Enterococcus species* 3 (2.23%). Among Gram negative isolates, *Klebsiella species* 21 (15.68%), *Escherichia coli* 16 (11.95%), *Pseudomonas species* 13 (9.71%), *Acinetobacter species* 11 (8.20%), *Citrobacter species* 9 (6.71%), *Enterobacter species* 7 (5.23%) and *Proteus species* 4 (2.98%). One *Mycobacterium tuberculosis* (0.74%) was also isolated from spine (L1-L2) [Table 7]. *Staphylococcus aureus* (28.35%) was the predominant pathogens isolated followed by *Klebsiella spp.* (15.68%), which correlates with Khosravi, *et al*. (2009) who reported *Staphylococcus aureus* (22%) and *Klebsiella spp*. (16%). A similar study conducted by Jain, *et al.* (2014) and Khan MS, *et al.* (2008), have seen *Staphylococcus*

aureus (26.6%) and *Klebsiella species* (16.6%). Other studies like Anisha Fernandez, *et al.* (2013) and Lakshminarayana SA, *et al*. (2015), also reported the similar frequency of isolated pathogens. The different types of isolated bacteria and the relative rates of each isolation may vary from one hospital study to another [16-21]. There was 10 (8.07%) polymicrobial growth with two isolates among all culture positive cases [Table 8].

In our study, out of total 124 culture positive cases, majority 69 (51.49%) of the patients presented with early infection followed by delayed 51(38.06%) and late 14 (10.45%) [Table 9]. In study conducted by Roopa Shree S, *et al*. (2015) observed 54.35% of infection within 2 weeks (early) following operation, 26.01% presented between 2-10 weeks (delayed) and 19.56% after 10 weeks (late) of operation. Khosravi, *et al.* (2009), who reported onset of infection as 72.9% early, 22.6% delayed and 4.5% late in post-operative period. This high prevalence of early infection in this study, may be related to inadequate disinfection procedure to eliminate microorganisms from the environment and contamination of surgical instrument. Additionally, trauma and fracture fixation using metallic implants may produce structural and functional damage to the local host tissue causing devascularisation, malperfusion, disturbance of endothelial permeability, hypoxia, acidosis, haematoma, edema and increased intra-compartmental pressure. This may result in an impaired humoral and cellular immune competence (Valenziano, *et al*. 2002). On a local level it may decrease resistance to the pathogenic microbiological load with subsequent manifestation of infection in the traumatized tissue and put the patients at a higher risk of early infection.

The antimicrobial susceptibility pattern revealed a high level multidrug resistance in Gram positive and Gram negative isolates. Gram positive isolates were mostly sensitive to linezolid, vancomycin, teicoplanin and piperacillin-tazobactam combination. We have found in our study that most of the Gram positive bacteria showed decreased susceptibility to commonly used antibiotics. *Staphylococcus aureus* were mostly susceptible to linezolid (92%) and piperacillin-tazobactam combination (88%), cefoperazone-sulbactam combination (74%), vancomycin and teicoplanin each with 100% susceptibility. CoNS were mostly susceptible to piperacillin-tazobactam combination (87%) and linezolid, vancomycin, teicoplanin each were 100% susceptible. *Enterococcus spp* were 100% susceptible to piperacillin-tazobactam combination and teicoplanin [Table 10-A]. The high level of resistance pattern may be due to increased rate of biofilm production, MRSA and VRE. Hence, vancomycin, linezolid, piperacillin-tazobactam combination and teicoplanin can be considered as an important drug in the regimen for treatment of orthopaedic implant site infection, especially in setting with high resistance to other antibiotics.

Current study revealed that all Enterobacteriaceae isolates were most commonly susceptible to imipenem, followed by piperacillin-tazobactam and cefoperazone-sulbactam combination. Most of the Enterobacteriaceae showed decreased susceptibility to commonly used antimicrobial agents i.e. amoxy-clav, cotrimoxazole, ceftazidime [Table 10-B]. These high level of resistance pattern to the antibiotics was probably due to the increased ability of bacteria to produce biofilm, ESBL and MBL.

In our study, 82 (61.65%) isolates were biofilm producer among which *Staphylococcus aureus* 24 (18.05%) was predominant pathogen, followed by *Klebsiella spp.* 13 (9.78%), *Escherechia coli* 10 (7.52%), *Pseudomonas aeruginosa* 8 (6.01%), CoNS 7 (5.28%), *Acinetobacter spp.*7 (5.28%), *Citrobacter spp.* 5 (3.76%), *Enterobacter spp.* 4 (3.00%), *Enterococcus spp.* and *Proteus spp.* each with 2 (1.50%) [Table 11]. This biofilm production explains the longer duration of antimicrobial therapy and longer hospital stay in our patients leading to increased cost, morbidity, treatment failure, implant removal and revision surgeries. Roopa Shree S, *et al*. (2015), reported 72% biofilm producer in which Staphylococcus aureus was also the predominant pathogen. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted penetration by antibiotics

into biofilm, decreased growth rate and expression of resistance genes [Kim L, *et al*.].

Our study showed MRSA (28.95%) [Table 12] which is similar to the study conducted by Sonawane, *et al*. (2010) and Goel, *et al*. (2013) who reported MRSA 27.85% and 30% respectively. In other study, which is conducted by Jain, *et al*. (2014) and Satya Chandrika V, *et al*. (2014) also have observed MRSA 40% and 64% respectively, thus indicating the increasing prevalence of MRSA. The incidence of Methicillin Resistant Staphylococcus aureus (MRSA) in India ranges from 30-70%. The incidence of nosocomial infections which are caused by MRSA continues to increase; therefore, the importance of their detection, especially for treatment and epidemiological purposes [22].

In this study, three isolates of *Enterococcus faecalis* were isolated. Out of these, 1 (33.33%) were vancomycin resistant while 2 (66.67%) were sensitive to vancomycin by vancomycin agar screen method [Table 15]. This is in accordance with the study done by Vidyasagar K, (2012) who reported VRE positivity 29% and vancomycin sensitive Enterococci 71% by vancomycin agar screen method.

ESBLs are increasingly reported worldwide but emerged more during the last decades in species of Enterobacteriaceae and their prevalence reach alarming rates [23, 24, 25]. Infection caused by such pathogens often limit the therapeutic options and cause treatment failure [23, 24, 26]. Our study showed 39 (48.14%) ESBL producing pathogens, among which *Klebsiella spp.* 12 (14.82%) was predominant pathogen, followed by *Escherichea coli* 9 (11.11%), *Pseudomonas aeruginosa* 7 (8.64%), *Acinetobacter spp*. 5 (6.13%), *Citrobacter spp.* 3(3.70%), *Proteus spp.* 2 (2.47%), *Enterobacter spp.* 1 (1.23%) [Table 13]. In another study conducted by Anisha Fernandes, *et al*. (2013) who reported 31.7% while Chandrika V, *et al*. (2014) reported 60% ESBL producing Gram negative bacteria.

We have found 17 (20.99%) MBL producing isolates and *Klebsiella spp.* 6 (7.41%) was the most common. Other MBL producing isolates were *Escherichea coli* and *Pseudomonas aeruginosa* each with 3 (3.70%), *Acinetobacter spp.* and *Citrobacter spp.* each with 2(2.47%) and *Enterobacter spp.* 1(1.23%) [Table 14]. This is comparatively less, when compared with Roopa Shree S, *et al.* (2015), who reported 27.27% MBL producing Gram negative pathogens.

MBL producing isolates showing significant problem in hospitals, which is associated with higher morbidity and mortality. With increasing isolation of ESBL producing isolates in the hospital setting necessitating the use of carbapenems, the problem of MBL production is also increasing. Moreover, given the fact that MBLs will hydrolyse all classes of β-lactams and that we are several years away from the development of a safe therapeutic antibiotics; their continued spread would be a clinical disaster. Since these organisms also carry other multidrug resistance genes and the viable treatment option remains the potentially toxic polymyxin B and colistin [Livermore D M, 2000].

In the present study, different types of implant and prostheses were used in the orthopaedic implant patients. Plates in long bone 66 (54.07%) was most commonly used implant, followed by Kuntscher nails (26.41%), cannulated screw (4.39%), cerclage wire (1.88%), Austin-Moore endoprostheses (6.29%) and knee prostheses (6.98%)[Table 4]. Muhammad Salman, *et al*. (2014) reported culture positivity in tibial plating (52.94%), humerus plating (35.29%) and Austin-Moore prostheses (11.76%).

In our study, Obesity (38.71%) was the common risk factor associated with orthopaedic implant site infection followed by diabetes (29.84%). Other risk factors were hypertension (22.58%), alcohol (20.16%), smoking (21.77%), anaemia (18.55%) and old age (7.25%). Anaemia was mostly seen in female patients [Table 16]. Roopa Shree S, *et al.* (2015), mentioned the diabetes (15.21%), hypertension (10.87%), old age (21.73%) and Ta Wei Kevin Kok, *et al*. (2016) reported diabetes (40.4%) and smoking (54.5%) as risk factor commonly associated with orthopaedic implant site infection.

Conclusion

The adverse outcome of orthopaedic implant site infection can be associated with significant morbidity, revision surgery, high cost, and even mortality. The patient's functional status may also be adversely affected by an orthopaedic implant site infection. Considering the grave scenario of antibiotic resistance in our country, it is high time that all clinical laboratories start detecting the resistant profile routinely and accurately. Hence, the need is to provide timely accurate diagnosis and management of the patients and accordingly to bring out profound positive change. High rate of antimicrobial resistant were orbserved in our study. Many factors must be considered like previous antibiotic therapy, knowledge of most common positive organism causing infection orthopaedic implant and their antimicrobial sensitivity profile. A strict adherence to the antibiotic policy and multidisciplinary collaboration involving the orthopaedic surgeon, infection diseases specialist and Microbiologists will reduce the incidence of orthopaedic implants site infection.

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