Bacterial Aetiological Agents in Infected Orthopaedic Implants - A Cross Sectional Study from Andhra Medical College, Visakhapatnam

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ABSTRACT

BACKGROUND

Orthopaedic implant site infection is one of the major problems of surgical site infection associated with high morbidity and mortality. As implants are commonly used in orthopaedic procedures and especially when preceded by trauma, orthopaedic procedures are more prone for surgical site infections. We wanted to study the aerobic bacterial aetiology of infected orthopaedic implants.

METHODS

This was a cross sectional study carried out over a period of one and half years. The study group comprised of 100 patients who had undergone orthopaedic prosthetic implant surgeries and presented with signs and symptoms of infections. The demographic data were recorded, type of surgery, the time of infection during postoperative period and risk factors were noted. Serous / purulent discharge adjacent to infected implants were processed in the laboratory as per the standard protocol.

RESULTS

Among the 100 samples studied, 79 % were culture positive and 21 % culture sterile. 55.7 % were Gram-positive cocci isolated in pure, 29.1 % were Gram-negative bacilli (GNB) isolated in pure and 15.2 % were a mixture of Gram-positive cocci and Gram-negative bacilli. *Staphylococcus aureus* 45 (80.4 %) was the predominant isolate followed by Coagulase Negative staphylococci 11(19.6 %). Among the GNB (35), the predominant isolate was *Pseudomonas aeruginosa* 10 (28.6 %) followed by *Klebsiella pneumoniae* 8 (22.9 %). Infections occurred during the early post-operative period in 63 % cases. Methicillin resistant *Staphylococcus aureus* were 53.4 % and 35 % were extended spectrum beta lactamases (ESBL) among Enterobacteriaceae strains.

CONCLUSIONS

Orthopaedic implant site infections are common during early post-operative period. Methicillin resistant staphylococcus and ESBL strains were high. This study of aerobic bacterial analysis and their current antibiogram of orthopaedic implant infections would greatly help the orthopaedic surgeons in selecting appropriate antibiotics for prophylaxis as well as better management of patients.

KEYWORDS

Orthopaedic Implants, MRSA, ESBL, Orthopaedic Implant Infections, Biofilm

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BACKGROUND

Prevention of surgical site infection in orthopaedic surgery and bone trauma is very important and is different from non-orthopaedic surgical sites. It is characterized by low inoculum for implant infections; pathogenicity of coagulase-negative staphylococci, possible haematogenous origin; and the requirement of extended surveillance of patients after discharge. Orthopaedic implant site infection is one of the major problems of surgical site infection associated with high morbidity and mortality. 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 microbiological contamination and infection.[1] The incidence of orthopaedic implant related infections has reduced to less than 1 - 2 % in institutions with highly trained surgeons.[2] However, it remains a diagnostic, therapeutic and cost related problem.

It is said that overall 5 % of internal fixation devices get infected, where the incidence of infection after internal fixation of closed fractures is generally lower (0.5 - 1 %), whereas for internal fixation of open fractures, the incidence is still higher and may exceed 30 %.[3] Infection is a major problem in orthopaedics leading to implant failure. It is a challenging task to treat orthopaedic implant infections that may lead to implant replacement and, in severe cases, may result in amputation and mortality.[4] Sources of infectious bacteria include the environment of the operating room, surgical equipment, clothing worn by medical and paramedical staff, resident bacteria on the patient's skin and bacteria already residing in the patient's body.[5]

Implant-associated infections are the result of bacterial adhesion to an implant surface and subsequent biofilm formation at the implantation site.[6] Formation of biofilm takes place in several stages, starting with rapid surface attachment, followed by multi-layered bacterial cell proliferation and intercellular adhesion in an extracellular polysaccharide matrix. Staphylococcus comprises up to two-thirds of all the pathogens in orthopaedic implant infections and they are the principal causative agents of two major types of infection affecting bone, septic arthritis and osteomyelitis, which involve the inflammatory destruction of joint and bone; these infections are difficult to treat because of the ability of the organisms to form small colonies and to grow into biofilms. Many staphylococcus strains, particularly *S. epidermidis* and some *S. aureus* strains, produce biofilm.[7,8] We wanted to study the aerobic bacterial aetiology of infected orthopaedic implants.

METHODS

This was a cross sectional study carried out in the Department of Microbiology, Andhra Medical College, Visakhapatnam for over a period of one and half years from January 2015 to June 2016. The study group comprised of 100 patients who had undergone orthopaedic prosthetic implant surgeries and presented with signs and symptoms of infections. The demographic data like age, gender, duration and type of surgery, the time of infection during post-operative period and risk factors were noted. History of prophylactic antibiotic usage was also recorded.

Inclusion Criteria

- Patients with clean fractures who had undergone surgery with implants were included.
- Patients with orthopaedic diseases who had undergone implant surgery were also included in the study.

Exclusion Criteria

- Patients with known uncontrolled diabetes.
- Patients with open multiple fractures.
- Clinically diagnosed malignancies.

Sample Collection

Samples were collected from serous / purulent discharge adjacent to infected implant and tissue, using two sterile cotton swabs under aseptic conditions. Pus was collected with sterile disposable syringe. The samples were processed in the laboratory as per the standard protocol by inoculating on Blood agar, MacConkey agar and incubated overnight at 37^0 C aerobically. Pathogenic organisms were isolated and identified by conventional biochemical tests. The antibiotic susceptibility testing was performed by Kirby- Bauer disc diffusion method as per CLSI guidelines.[9] Commercially available antibiotic discs (Hi-media) were used.

Control Strains

E. coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* 25923. Cefoxitin 30 mcg disks were used to detect methicillin resistant *Staphylococcus aureus* strains. The MH agar plates with growth suspension were incubated at 35°C for 24 hours and the zone size of < 21 mm was considered methicillin resistant. For coagulase negative staphylococci, zone size < 24 mm was considered as methicillin resistant. Phenotypic confirmatory combination disc diffusion test was done to detect ESBL production among Enterobacteriaceae species.

Statistical Analysis

The sample size was calculated on the basis of A.D. Khosravi et al. study for detecting a significant difference.

P = 73

Z = 9%

$N = 4 \times 73 \times 27 / 81 = 99$

Sample size was calculated as 100 patients

Numerical variables are presented as Mean + SD whereas categorical variables are presented as number of cases and percentages. Statistical comparisons were carried out using independent student's t - test for numerical variables and chi-square test or Fischer's exact test for categorical variables as appropriate. A P - value of < 0.05 was considered statistically significant.

RESULTS

A total of 100 samples were tested of which 69 (69 %) were males and 31 (31 %) were from female patients and the male to female ratio was 2.2:1. The peak incidence was seen in the age group of 21 to 30 years (44.9 %) in males and 41 - 50 years (48.4 %) in females. Among the 100 samples 79 (79 %) were culture positive and 21 (21 %) were culture sterile. In the present study among the 79 culture positives, 44 (55.7 %) were Gram-positive cocci isolated in pure, 23 (29.1 %) were Gram-negative bacilli isolated in pure and 12 (15.2 %) were a mixture of gram-positive cocci and Gram-negative bacilli. A total of 56 Gram-positive cocci (44 pure + 12 mixture) and 35 Gram-negative bacilli (23 pure + 12 mixture) were isolated.

Distribution of Pure Isolates of Gram-Positive Cocci (N = 44)

There were 33 (75 %) isolates of *Staphylococcus aureus* and 11 (25 %) pure isolates of Coagulase Negative staphylococcus. Among the Gram-positive cocci (56), *Staphylococcus aureus* 45 (80.4 %) was the predominant isolate followed by Coagulase Negative staphylococci 11(19.6 %). Among the Gram-negative bacilli (35), the predominant isolate was *Pseudomonas aeruginosa* 10 (28.6 %) followed by Klebsiella pneumoniae 8 (22.9 %), *Proteus mirabilis* 5 (14.2 %), *Klebsiella oxytoca* 4 (11.4 %), Acinetobacter species 4 (11.4 %), *Escherichia coli* 3 (8.6 %) and *Stenotrophomonas maltophilia* 1 (2.9 %). Out of the 79 culture positives, 53 (67 %) of the infections occurred within 6 months after surgery, 22 (27.9 %) between 6 to 24 months and 4 (5.1 %) of infections after 24 months postoperatively.

Isolate	Total	Percent (%)
Pseudomonas aeruginosa	7	30.4 %
Acinetobacter species	4	17.4 %
Klebsiella pneumoniae	4	17.4 %
Proteus mirabilis	3	13.0 %
Klebsiella oxytoca	2	8.7 %
Escherichia coli	2	8.7 %
Stenotrophomonas maltophilia	1	4.4 %
Total	**23**	**100 %**
Distribution of mixed isolates (N = 12)		
Klebsiella pneumoniae + Staphylococcus aureus	4	33.3 %
Klebsiella oxytoca + Staphylococcus aureus	2	16.6 %
Pseudomonas aeruginosa + Staphylococcus aureus	3	25 %
Proteus mirabilis + Staphylococcus aureus	2	16.6 %
Escherichia coli + Staphylococcus aureus	1	8.3 %
Total	**12**	**100 %**

Table 1. Distribution of Pure Isolates of Bacilli (N = 23)

Distribution of Isolates (N = 53) <6 Months Duration Post-Surgery

Staphylococcus aureus was the most predominant isolate in infections occurring in < 6 months' duration after surgery both in pure and mixed isolates.

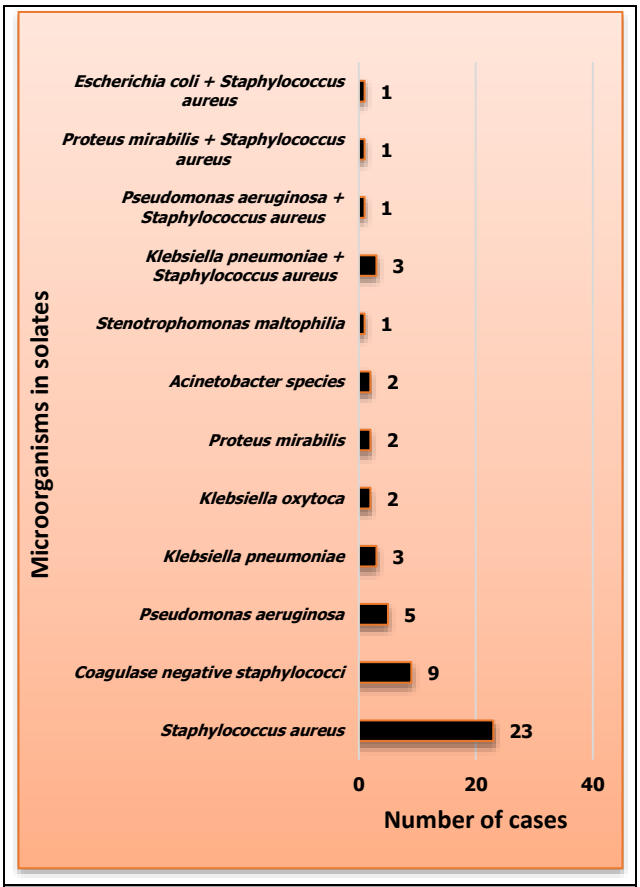

Figure 1. Distribution of Isolates in Infections in Less than Six Months Post-Surgery

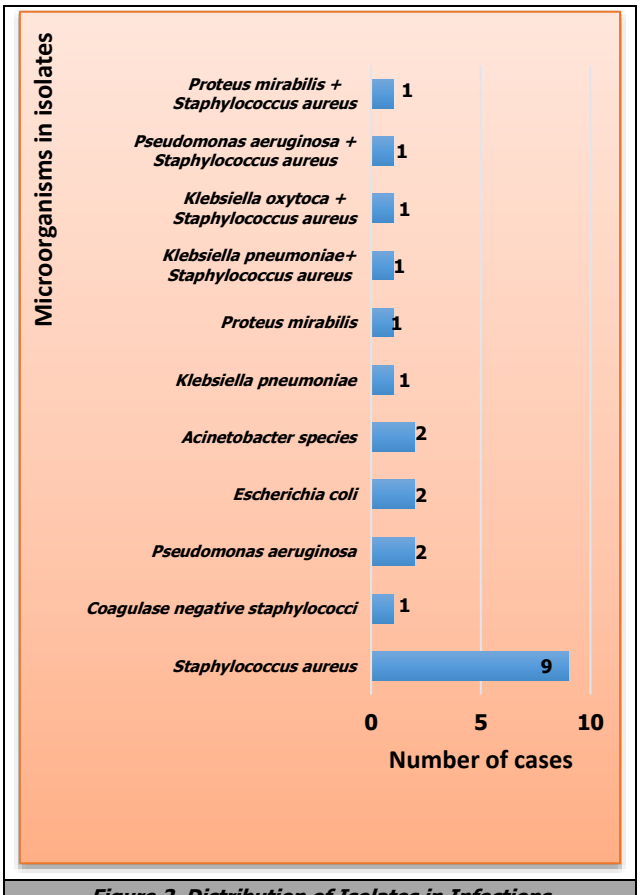

Figure 2. Distribution of Isolates in Infections in 6 to 24 Months Duration Post-Surgery

Distribution of Isolates (N = 22) in 6-24 Months Duration of Post-Surgery

Among the infections occurring in 6 to 24 months' duration after surgery, *Staphylococcus aureus* showed predominance both among pure and mixed isolates.

Distribution of Isolates (N = 04) in > 24 Months Duration Post-Surgery

Among the infections occurring after > 24 months' duration after surgery, *Staphylococcus aureus* showed predominance both among pure and mixed isolates. Implant surgeries were done predominantly for femur 20 (25.48 %) followed by radius / ulna and tibia 15(18.9 %) each followed by humerus 11 (13.9 %), hip 10 (12.7 %) and knee 4 (5.06 %) and scapula 4 (5.06 %).

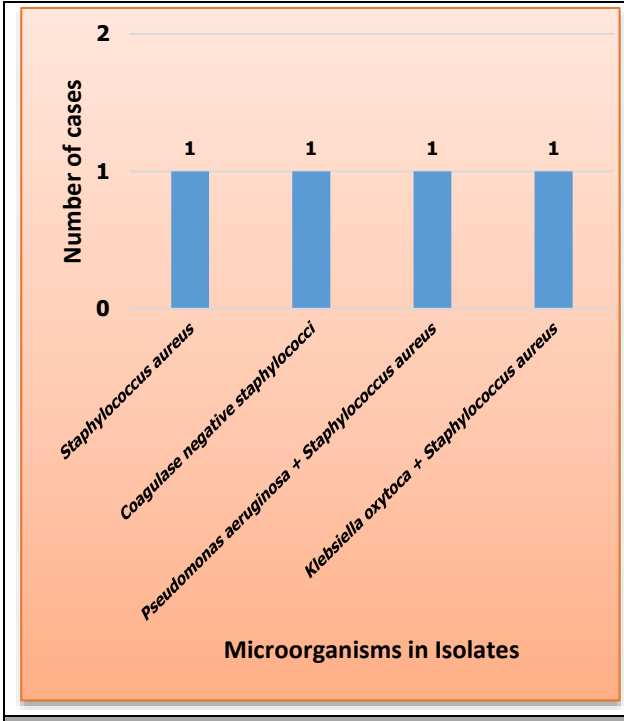

Figure 3. Distribution of Isolates in Infections after 24 Months Duration Post-Surgery

Resistance Pattern in Patients on Prophylactic Antibiotics

A total of 79 cases showed resistance as follows: Among prophylactic drugs used 29 (78.3 %) cases showed resistance to ceftriaxone, 21 (61.7 %) to cefotaxime and 2 (25 %) showed resistance to piperacillin + tazobactam. Among the Gram-positive cocci, *Staphylococcus aureus* was 100 % sensitive to teicoplanin followed by linezolid (95.5 %) and others. Coagulase Negative staphylococcus was 100 % sensitive to linezolid, teicoplanin followed by others. Out of 45 *Staphylococcus aureus* isolates 24 (53.4 %) were MRSA strains. Most of the GNB were sensitive to piperacillin tazobactam followed by ceftazidime clavulanate and meropenem. pseudomonas species were 90 % sensitive to colistin. Acinetobacter species were 100 % sensitive to tigecycline. Out of 20 Enterobacteriaceae isolates, 7 (35 %) were ESBL producers.

Isolate	LZ	TEI	VA	CX	AZM	CTX	CTR	AMC	CAZ	LE
Staphylococcus aureus n=45	43 (95.5%)	45 (100%)	43 (95.5%)	21 (46.6%)	31 (68.8%)	14 (31.1%)	17 (37.7%)	18 (40%)	15 (33.3%)	35 (77.7%)
CONS N = 11	11 (100%)	11 (100%)	10(90.9 %)	5 (45.5%)	8 (72.7%)	4 (36.3%)	6 (54.5%)	4 (36.3%)	5 (45.5%)	8 (72.7%)

Table 2. Antibiotic Susceptibility Pattern of Gram-Positive Cocci N = 56 (44 + 12)

LZ- linezolid, TEI-teicoplanin, VA- vancomycin, CX- cefoxitin, AZM- azithromycin, CTX- cefotaxime, CTR- ceftriaxone, AMC- amoxyclav, CAZ- ceftazidime, LE-levofloxacin.

Isolate	PIT	CAC	CAZ	LE	MRP	CTX	CIP	TOB	AK	AZM	CL
Klebsiella pneumoniae N = 8	6(75 %)	6(75 %)	3(37.5 %)	3(37.5 %)	4(50 %)	0(0 %)	2(25 %)	2(25 %)	3(37.5 %)	112.5 %	NA
Klebsiella oxytoca N = 4	3(75 %)	3(75 %)	1(25 %)	2(50 %)	2(50 %)	0	1(25 %)	1(25 %)	1(25 %)	1(25 %)	NA
Pseudomonas sps N = 10	10(100 %)	8(80 %)	3(30 %)	6(60 %)	8(80 %)	2(20 %)	2(20 %)	2(20 %)	2(20 %)	2(20 %)	9(90 %)
Proteus mirabilis N = 5	4(80 %)	3(60 %)	2(40 %)	1(20 %)	1(20 %)	0	0	0	1(20 %)	0	NA
Acinetobacter spp N = 4	4(100 %)	4(100 %)	1(25 %)	0 ()	2(50 %)	0	1(25 %)	0	0	0	NA
Escherichia coli N = 3	3(100 %)	2(66.6 %)	1(33.3 %)	0()	2(66.6 %)	1(33.3 %)	0	0	0	1(33.3 %)	NA
Stenotrophomonas maltophilia N = 1	1(100 %)	1(100 %)	1(100 %)	0	1(100 %)	0	0	0	1(100 %)	0	NA

Table 3. Antibiotic Susceptibility Pattern of Gram-Negative Bacilli N = 35 (23 + 12)

Note: PIT- piperacillin tazobactam, CAC- ceftazidime clavulanate, CAZ- ceftazidime, LE- levofloxacin, MRP- meropenem, CTX- cefotaxime, CIP- ciprofloxacin, TOB – tobramycin, AK- amikacin, AZM- azithromycin, TGC- tigecycline, CL- colistin, NA-Not Applied

DISCUSSION

Implant related infections continue to pose a problem for orthopaedicians. Post-operative surgical wound infections are another important risk factors with locally introduced infections as a result of wound sepsis which is contagious to the prosthesis. The organisms forming part of normal cutaneous flora can be transmitted from improperly decontaminated skin into the traumatized bone or soft tissue during the operative manipulation. In addition, many intrinsic and extrinsic risk factors could be involved in the pathogenesis of orthopaedic device related infections (ODRIs). The intrinsic factors include the age, nutritional status, obesity, nosocomial infections, a long post-operative stay and corticosteroid therapy. The diagnosis and treatment of the infections are complicated by the formation of a bacterial biofilm and an increase in the number of multidrug resistant bacteria stresses the value of an adequate diagnosis, leading to a proper therapy of these

patients. The treatment of orthopaedic device related infections (ODRIs) most frequently includes long term antimicrobial treatments and the removal of implants.

Despite several efforts to find medical therapies to treat biofilm infections, the physical removal of an infected medical device is often necessary, thus carrying an additional economic burden. There is great interest in finding methods or strategies to inhibit biofilm formation. Various strategies have been proposed to achieve this on medical devices, including the use of antibiotics, development of new anti-adhesive medical surfaces and coating medical devices with several different compounds, including antibiotics.

Applying antimicrobial agents is an easy and frequently used way to control biofilms. However, many antimicrobial agents that are effective against planktonic bacterial cells turnout to be ineffective against the same bacteria when growing in a biofilm. Combined use of multiple antimicrobial agents with different chemical mechanisms and modes of action may be a strategy to improve the performance of these antimicrobial agents and circumvent bacterial adaptation. However, the unformidable resistance of biofilm to conventional antibiotic therapy has prompted a great deal of research on synthetic surfaces and coatings that resist bacterial colonization.

Understanding of the interaction between microorganisms, the implant and host may improve current approach to the diagnosis and treatment of implant associated infections.

In the present study, of the total samples 69 % were from males and 31 % were from females which correlated with the study of Khosravi D et al. 10 who reported 68.5 % males and 31.5 % females, Onche et al.[11] who reported 65.7 % males and 34.2 % females, Mehsen JT et al.[12] 69.7 % males and 30.3 % females, Khan MS et al.[13] 64.4 % males and 35.5 % females, Vishwajith et al.[14] 75.5 % males and 24.5 % females, Roopashree S et al.[15] reported 86.95 % males and 13.04 % females and Gomez J et al.[16] who reported 33.6 % males and 60.9 % females.

In the present study, 44.9 % were from the age group of 21 to 30 years among males and 48.4 % were from 41 to 50 years' age group among females. Khosravi D et al.[10] reported 33.7 % between the age group of 31 and 40 years and Roopashree S et al.[15] reported 26.08 % between 41 and 50 years. Out of the total samples, 79 % were culture positive in the present study which correlated with the study by Fernandes A et al.[17] who observed 84 % such cases, Roopashree S et al.[15] who reported 73.01 %, Gomez J et al.[16] 60 %, Vishwajith et al.[14] reported a higher culture positivity of 94.8 %, Khosravi D et al.[10] 93.9 % and Satyachandrika V et al.[18] 90 %. Mehsen JT et al.[12] reported a low culture positivity of 12.12 %, Onche et al.[11] 7.5 % and Khan et al.[13] observed 5.76 % cases.

In the present study, 55.7 % were Gram-positive cocci which correlated with Satyachandrika V et al.[18] 55.6 %, Khan MS et al.[13] 50 %, Vishwajith et al.[14] 48.69 %, Gomez J et al.[16] 60.6 % and Fernandes A et al. 61 %, Mehsen JT et al.[12] reported a slightly lower incidence of 43.75 %.

Gram-negative bacilli isolated in the present study were 29.1 % and correlated with Gomez J et al. study 33.3 % and Fernandes A et al. 38 % whereas Satyachandrika V et al. reported 44.4 %, Mehsen JT et al. 50 %, Khan MS et al. 50 %, Vishwajith et al. 51.3 % and Agarwal AC et al.[19] reported a higher incidence of 74.7 %.

Mixed isolates were 15.2 % in the present study which correlated with Vishwajith et al. who reported 21.42 % whereas Fernandes A et al. reported a higher incidence of 35.7 %, Onche et al. and Khosravi D et al. reported a lower incidence of 5.9 % and 2 % respectively.

Staphylococcus aureus was the predominant isolate 49.48 % in the present study which correlated with Khan MS et al. 50 %, Vishwajith et al. 48.69 %, Onche et al. 44 %, Jameel Tahseen Mehsen et al. 43.75 %, Fernandes A et al. 42 %, Satyachandrika V et al. 33.3 % whereas Roopashree S et al. reported a higher incidence of 65.21 % and Agarwal AC et al. and Khosravi D et al. reported a lower incidence of 21.62 % and 21.94 % respectively.

Coagulase negative staphylococci were 12 % in the present study which correlated with Vishwajith et al. 16 %, Roopashree S et al. 8.69 % and Fernandes A et al. 7 % where as Satyachandrika V et al. reported a higher incidence of 22.2 %. *Pseudomonas aeruginosa* were 28.6 % in the present study which correlated with Agarwal AC et al. and Vishwajith et al. who reported 26 % and 25.4 % respectively whereas Onche et al. Fernandes A et al. Satyachandrika V et al. Roopashree S et al. Mehsen JT et al. reported 8.3 %, 8 %, 6.7 %, 6.52 % and 6.25 % respectively. Hsieh PH et al. reported a higher incidence of 39.6 %.

Klebsiella pneumoniae were 22.9 % in the present study which correlated with Khosravi D et al. who reported 16.7 %, Khan MS 16.6 %, Vishwajith et al. 15.3 % and Hsieh PH et al. 15 % where as Mehsen JT et al. reported 12.5 %, Fernandes A et al. 9 %, Onche et al. 8.3 % and Roopashree S et al. 6.52 %.

Proteus species were 14.2 % in the present study which correlated with Mehsen JT et al. 18.75 % and Onche et al. 11 % whereas Fernandes A et al. Khosravi D et al. Satyachandrika V et al. Vishwajith et al. reported 8 %, 5.8 %, 4.4 % and 3.3 % respectively.

Acinetobacter were 11.4 % in the present study whereas Satyachandrika V et al. reported 4.4 %, Khosravi D et al. 4.5 % and Vishwajith et al. 3.3 %. *Escherichia coli* were 8.6 % in the present study which correlated with Roopashree S et al. who reported 8.69 %, Onche et al. 11 %, Mehsen JT et al. 12.5 %, Khosravi D et al. 14.8 % whereas a higher incidence was reported by Agarwal AC et al. [19] 34.4 %, Vishwajith et al. 22 %, Hsieh PH et al. 19 % and Satyachandrika V et al.[18] 17.8 %.

In the present study early infections occurred in 67 %, delayed infection in 27.9 % and late infection in 5.1 % which correlated with studies of Khosravi D et al. who reported 72.9 % early, 22.6 % delayed and 4.5 % late infections and Roopashree S et al. reported 54 % early and 26 % delayed infections and a higher incidence of 19 % in late infections. Fernandes A et al.[17] reported a lower incidence of 26 % in early, 18 % in delayed and a higher incidence of 56 % in late infections.

Staphylococcus aureus was the predominant isolate in early and delayed infections in the present study which correlated with findings of Khosravi D et al. and Fernandes et al.[17] Most commonly affected implant site in the present

study was femur 25.48 % which correlated with Fernandes A et al.[17] who reported 26 % and Vishwajith et al. 24.5 %.

Tibial implants were infected in 18.9 % in the present study which correlated with Fernandes A et al.[17] who reported 16 % whereas Vishwajith et al. reported a higher incidence of 57.1 %. Radius and ulna implants were infected in 18.9 % in the present study which correlates with Fernandes A et al. who reported 16 % whereas Vishwajith et al. reported a lower incidence of 4 %. Humerus implants were infected in 13.9 % in the present study whereas Fernandes A et al.[17] and Vishwajith et al. reported 8 % each. In the present study, *Staphylococcus aureus* were 100 % sensitive to teicoplanin followed by linezolid, vancomycin and fluoroquinolones which correlated with Vishwajith et al.[14] Fernandes A et al.[17] Khosravi D et al.[10] Roopashree S et al.[15] and Satyachandrika V et al.[19] reported 100 % sensitivity to linezolid and vancomycin.

In the present study Gram-negative bacilli were sensitive to piperacillin tazobactum, Ceftazidime, Clavulanic acid and meropenem which correlated with Vishwajith et al.[14] who reported that Gram-negative bacilli were sensitive to cefotaxime, gentamycin, ciprofloxacin, amikacin and piperacillin tazobactam. Khosravi D et al.[10] reported sensitivities to imepenem and ciprofloxacin. Fernandes A et al.[17] reported sensitivities to carbapenems and fluoroquinolones.

In the present study, among *Staphylococcus aureus* strains, 53.4 % were Methicillin resistant which correlated with Vishwajith et al.[14] who reported 50 % and Satyachandrika V et al.[19] 66.6 % whereas Roopashree S et al.[15] reported a lower incidence of 13.33 %.

Among the Enterobacteriaceae isolates, 35 % were Extended Spectrum β Lactamase (ESBL) producers in the present study whereas Satyachandrika V et al.[19] reported a higher incidence of 60 % in their study.

Thus, the results of the present study are in accordance with other studies in the literature mentioned above. From a research perspective, the results from this study will help the clinicians for proper management and to institute proper antibiotic therapy in these cases.

CONCLUSIONS

Infections are more common during the early post-operative period which may be due to intraoperative or immediate postoperative source. MRSA accounted for 53 % among *Staphylococcus aureus* and 35 % were ESBL among Enterobacteriaceae strains indicating that there is increased emergence of drug resistant strains. This study of aerobic bacterial analysis and their current antibiogram of orthopaedic implant infections would greatly help the orthopaedic surgeons in selecting appropriate antibiotics for prophylaxis as well as better management of patients.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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