Bacteriological Profile in Infected Orthopedic Implants

Geetha Kaipa[1], Kiran Babu Reddem[2], Babul Reddy[3], K Sameera[4]

[1]Assistant Professor, Department of Microbiology, Maheshwara Medical College and Hospital, Isnapur, Patancheruvu, Telangana
[2]Assistant Professor, Department of Pediatrics, Maheshwara Medical College and Hospital, Isnapur, Patancheruvu, Telangana
[3]Associate Professor, Department of Orthopedics, TRR Medical College and Hospital, Inole, Hyderabad, Telangana
[4]Professor and HOD, Department of Biochemistry, Alluri Sitarama Raju Academy of Medical Sciences, Eluru, Andhra Pradesh

Abstract

Aim: To study and characterize the bacterial pathogens and its current antibiotic sensitivity and resistance patterns in patients with orthopedic implant related infections.

Materials and Methods: The present study on bacteriological profile and common bacterial infections in Post-operative cases with special reference to implants was carried out in department of microbiological combined with orthopedics department in tertiary care hospital. A total 428 patients who underwent orthopedic surgery with implant were recruited in the study. Informed consent was taken from the patients. Samples received in the central laboratory of the hospital were also included in the study.

Results: In 77 culture positive samples, 35 samples are Gram positive cocci infection. 42 patients have Gram negative bacilli infection. Staphylococcus aureus is the most common organism isolated in culture. Gram positive organism percentage of infection is 45%, Gram negative organism percentage of infection is 54%. In Gram positive organism MRSA was isolated in culture of 17 patients (22%), MSSA was isolated in culture of 6 patients (7%), MR CoNS was isolated in culture for 5 samples (6%), CoNS was isolated in Culture for 5 samples (6%), Enterococci was isolated in culture for 2 samples (3%). In MRSA 82% of isolates are sensitive to Doxycycline, 58% were sensitive to Gentamicin, 47 were sensitive to Co-trimoxazole and 35% were sensitive to Erythromycin. All the isolates of MRSA were sensitive to vancomycin and linezolid. In Gram negative organism Pseudomonas is the most common organisms isolated in culture followed by Klebsiella, Proteus, E.coli, Citrobacter and Acinetobacter. In Pseudomonas all isolates were sensitive to Amikacin, Gentamicin, Ceftazidime. 81% were sensitive to Ciprofloxacin, 36% were sensitive to Cefotaxime. In Proteus 87% of the isolates were sensitive to Amikacin, 63% were sensitive to Gentamicin, 87% were sensitive to Ciprofloxacin, and 38% were sensitive to Cefotaxime. In E.coli 87% were sensitive to Amikacin, 75% were sensitive to Gentamicin, 63% were sensitive to Cefotaxim, 65% were sensitive to Ciprofloxacin, and 37% of the isolates are sensitive to Co-trimoxazole.

Conclusions: Early intervention by proper selection of antibiotics according to culture and sensitivity will reduce the infection rate and hospital stay.

Keywords: Gram positive cocci, Gram negative bacilli, Staphylococcus aureus, Ciprofloxacin.

Introduction

In modern era Implant surgery has become one of the commonest Orthopaedic operation. Fracture fixation is not possible without an Implant. Implant helps to fix the fractures and alleviating the sufferings of many patients. But post –operative infection is a devastating complication.[1] Treatment of infected orthopaedic implants requires microbiological diagnosis of the causative organism infection can extend the patients hospital stay and cost of treatment along with increasing the morbidity [2,3]. Nutritional status of the patient along with comorbidities like diabetes, smoking, hypertension, compound fractures increases the risk of infection [4]. Orthopedic infection spread by direct contact, adjacent site (lymphatic spread), penetrating injury. Post-operative infections can be classified as early if it occurs within two weeks and characterized by local hyperthermia, erythema and pus discharge, Delayed if it occurs between two to ten weeks and presents with persistent pain signs,

erythema, swelling, pus discharge. Late If it occurs ten weeks after index surgery and characterized by consolidation, pseudoarthrosis, sequestrum formation and soft tissue calcification.[5] In both delayed and late infections periosteal reaction can be seen radiologically infection rate can be reduced with proper aseptic precautions, by early microbiological diagnosis, early intervention in treatment with appropriate antibiotics. Common micro-organisms causing infections are Staphylococcus aureus, Coagulase Negative Staphylococcus (CONS), Methicillin Resistant Coagulase Negative Staphylococcus (MR-CONS), Enterococci, Escherichia coli, Klebsiella, Proteus mirabilis and pseudomonas. [6,7,8]

We conducted this study to find out the prevalence of bacterial infection in Orthopaedic Implant surgeries, to isolate and identify the bacteriological agents and to perform the antimicrobial sensitivity pattern of the bacterial isolates.

Aim

To study and characterize the bacterial pathogens and its current antibiotic sensitivity and resistance patterns in patients with orthopedic implant related infections

Objectives

To isolate the bacteria in implant infective patients who underwent orthopedic surgeries with implants. To do the phenotypic testing of the isolates for antimicrobial susceptibility testing, drug resistant pattern of the isolates, molecular characterization of the common bacterial isolate by polymerase chain reaction , biofilm formation of the isolated organisms and estimate the serum procalcitonin levels in orthopedic infections

Materials and Methods

The present study on bacteriological profile and common bacterial infections in Post-operative cases with special reference to implants was carried out in department of microbiological combined with orthopedics department in tertiary care hospital. The study is carried out from October 2018 to September 2021. A total 428 patients who underwent orthopedic surgery with implant were recruited in the study. Informed consent was taken from the patients. Samples received in the central laboratory of the hospital were also included in the study

Inclusion Criteria

1. Patients who underwent orthopedic surgeries with implants
2. Patients who gave their informed consent

Exclusion Criteria

Patients not willing to participate in the study.

Pus sample was collected with aseptic precautions and evaluated for bacterial profile by gram stain, culture followed by antimicrobial susceptibility testing by phenotypic and molecular methods (PCR), Blood sample was collected under aseptic precautions for estimation of procalcitonin

Microbiological evaluation of the Samples was done by performing gram stain and inoculation on to the culture media, Pus specimen is mainly inoculated on to the Blood agar and MacConkey agar by doing intermittent heating for obtaining the isolated colonies. The culture plate is incubated at 37^0c for 12 to 18hrs. Smear was prepared on the clean glass slide and gram stain was done to identify the organism. Obtaining the isolated colonies is the prerequisite for performing the biochemical reactions, Catalase, oxidase, indole, citrate, coagulase, urease, triple sugar iron agar, bile esculin are used in identification of the orthopedic implant associated infections. Antibiotic susceptibility testing was done by using Modified Kirby-bauer susceptibility test, screening for oxacillin mediated resistance and E test (Epsilometer test) for vancomycin was done for Staphylococcus aureus. The current Clinical laboratory standard Institute (CLSI) guidelines are used for Screening of extended spectrum beta of lactamases(ESBL) by using Modified Kirby-bauer susceptibility test, Gram negative bacteria which were resistant to third generation cephalosporins Ceftriaxone, Cefotaxime, Ceftazidime, Cefpodoxime were screened for ESBL production

Combined disk test

It depends on comparing the inhibition zones around disks containing an indicator Cephalosporin with and without Clavulanic acid. As per CLSI guidelines, 10 µg of Clavulanic acid is added to each of a Cefotaxime (30 µg) and a Ceftriaxone (30 µg) disk. If ESBL is produced, the zone diameters given by the disks with Clavulanic acid are > 5mm larger than those without the inhibitor. ESBL production is positive if the zones given by the disks with clavulanic acid> 5mm larger than those without clavulanic acid.

Molecular Identification of Antimicrobial Resistance Gene Helini Biomolecules, Helini custom antibiotic resistance gene assay the real time PCR kit

Biofilm demonstration is by done by using congored agar, the bacterial colony strains obtained were inoculated in congo red agar and it is incubated for 24-48 hrs at 37 degree Celsius. Isolates forming biofilm will produce black coloured colonies on congo red agar.

Sample Rejection criteria

1. Inadequate sample

2. Sample collected from improper technique like pus sent in syringes or collected by using gauge piece 3. Time delay between sample collection and transport to the laboratory

Results

A total number of 150 samples were taken from patient's who underwent Orthopaedic Implant surgeries and culture and sensitivity test was done. Among those patients 77 are culture positive and 73 are culture negative.

The prevalence of orthopedic implant infections in our study was found to be 18%, among them 45% were gram positive and 54% gram negative infections.

Table 1: Number of culture positive and negative samples

Parameters	No of Samples	Percentage
No of culture positive samples	35	45%
No of culture Negative samples	42	54%
Total no of samples	150	

Out of 77 culture positive samples 69 are open fractures and majority were grade two and three (Gustillo Anderson) and 8 were closed fractures. Among the positive samples 59 were early infections, 14 were delayed and 4 were late infections.

Among the culture positive male patients are associated more commonly with Orthopaedic Implant infections.

Table 2: Male and female patient's sample

Total	Male	Female
77	63	14

Out of 77 culture positive samples, 63 patients are male, 14 patients are female. 150 Implant associated infection patients, 0-10 years of age is 5 and Percentage of this age group is 3%. 11-20 years of age is 12 and percentage of this age group is 8%. 21-30 years of age is 25 and percentage of this age group is 17%. 31-40 years of age is 44 and percentage of this age group is 29%. 41-50 years of age is 34 and percentage of this age group is 23%. 51-60 years of age is 18 and percentage of this age group is 12%. 61-70 years of age is 6 and percentage of this age group is 4%. 71-80 years of age is 2 and percentage of this age group is 1%. 81-90 years of age is 4 and percentage of this age group is 3%. Out of 4 patients in 81-90 years of age group, one patient had head of femur fracture, one patient had shaft of femur fracture, and two patients had both bone leg fractures.

Table 3: Age wise distribution of implant associated infections:

Age in years	No of patients samples tested	Percentage
0-10	5	3%
11-20	12	8%
21-30	25	17%
31-40	44	29%
41- 50	34	23%
51- 60	18	12%
61-70	6	4%
71 -80	2	1%
81- 90	4	3%
Total	150	100%

The most common risk factor for Implant associated infections is Diabetes mellitus, Smoking and alcohol. Patients having anemia, hypertension are predisposed to Orthopedic Implant associated infection. In 77 culture positive samples 27 patient have Diabetes mellitus, 20 patient have H/o smoking and alcohol, 4 patients have anemia, 16 patient have hypertension, 10 patients have both Diabetes mellitus and Hypertension.

Table 4: Types of implant

Type of implants	No of patients
Intramedullary Nail	92
Plating	46
K wire	12

Intramedullary nail, plating, K-wires are used to join the fractures

In 77 culture positive samples, 35 samples are Gram positive cocci infection. 42 patients have Gram negative bacilli infection. Staphylococcus aureus is the most common organism isolated in culture followed by pseudomonas, Klebsiella, proteus, E.coli, Citrobacter, MR CoNS, CoNS, Enterococci, MSSA, Acinetobacter.

Table 5: No of gram positive organisms and gram negative organisms in the isolate

Organisms	No of patients	Percentage
Gram positive organism	35	45%
Gram Negative organism	42	54%
Total	77	51%

Gram positive organism percentage of infection is 45%, Gram negative organism percentage of infection is 54%. In Gram positive organism MRSA was isolated in culture of 17 patients (22%), MSSA was isolated in culture of 6 patients (7%), MR CoNS was isolated in culture for 5 samples (6%), CoNS was isolated in Culture for 5 samples (6%), Enterococci was isolated in culture for 2 samples (3%)

Table 6: Spectrum of micro-organism in implant associated infection

Microorganisms grown in culture	No of patients	Percentage
MRSA	17	22%
MSSA	6	7%
MRCONS	5	5%
CONS	5	5%
Enterococci	2	3 %
E.coli	5	6%
Klebsiella	9	11%
Proteus	7	9%
Pseudomonas	16	20%
Acinetobacter	1	1%
Citrobacter	4	5%
Total	77	100%

In MRSA 82% of isolates are sensitive to Doxycycline, 58 % were sensitive to Gentamicin, 47 were sensitive to Co-trimoxazole and 35% were sensitive to Erythromycin. All the isolates of MRSA were sensitive to vancomycin and linezolid.

Table 7: Percentage of drug sensitivity

MRSA	Percentage of Drug sensitivity
Doxycycline	82%
Gentamicin	58%
Cotrimoxazole	47%
Erythromycin	35%
Vancomycin	100%

In Gram negative organism Pseudomonas is the most common organisms isolated in culture followed by Klebsiella, Proteus, E.coli, Citrobacter and Acinetobacter. In Pseudomonas all isolates were sensitive to Amikacin, Gentamicin, Ceftazidime. 81% were sensitive to Ciprofloxacin, 36% were sensitive to Cefotaxim. All the isolates were resistant to, Co-trimoxazole and Ampicllin. In Klebsiella all isolates were sensitive to Amikacin, 87% were Sensitive to Gentamicin, 63% were sensitive to Cefotaxim and 87% of the isolates were sensitive to Ciprofloxacin. All isolates are resistant to Ampicllin.

Table 8: Percentage of drug sensitivity

Pseudomonas	Percentage of drug sensitivity
Amikacin	100%
Gentamicin	100%
Ceftazidime	100%
Ciprofloxacin	36%

In Proteus 87% of the isolates were sensitive to Amikacin, 63% were sensitive to Gentamicin, 87% were sensitive to Ciprofloxacin, and 38 % were sensitive to Cefotaxime.

In E.coli 87% were sensitive to Amikacin, 75% were sensitive to Gentamicin, 63% were sensitive to Cefotaxim, 65% were sensitive to Ciprofloxacin, and 37% of the isolates are sensitive to Co-trimoxazole. All isolates were sensitive to Piperacillin – Tazobactam. Acinetobacter is sensitive to Amikacin, Doxycycline.

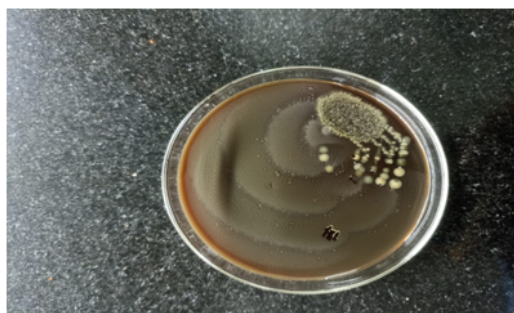**Figure 1: Patient with orthopaedic implant associated infection after surgery**

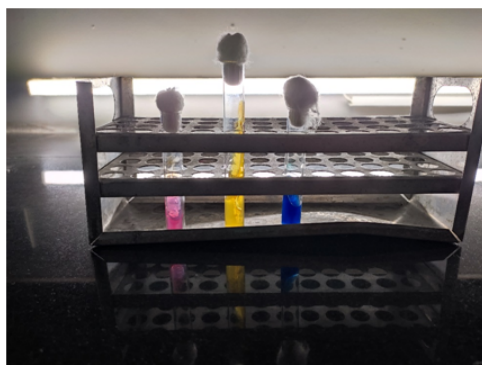**Figure 2: Proteus isolated from implant associated infections**

Figure 3: Biochemical reactions of klebsiella isolated from culture positive patients

Figure 4: Staphylococcus aureus (beta haemolytic colonies)

Discussion

The Bacteriological Profile and common bacterial infection in orthopedic surgeries with special reference to Implants - study is conducted in Microbiology Department combined with Orthopaedic Department. Implant associated infection in Orthopaedic surgery interferes with wound healing and prolong the duration of hospital stay for patients. Microorganism isolated in culture often changes in drug resistant pattern. So culture

Kaipa *et al.* International Journal of Current Pharmaceutical Review and Research

and sensitivity for each and every Orthopedic Implant infected patient is essential.[9,10]

Fractures are more common in Males than Females. Reason for increased number of fracture due to drunken drive, usage of mobile phones while driving and not following the traffic rules. Female has decreased number of fracture due to restricted activity. Implant associated Infection also more in Males than Females. And 89% were open fractures predominantly grade 2 and 3. Our study was comparable to Angappan Perumal et al. [11].

In open fracture Implant associated infection is more due to wound contamination. Skin injury allows the entry of microorganism into soft tissues and bones.

Among 77 infected patients 35% had Diabetes mellites which leads to hyperglycemia and interferes with neutrophil chemotaxis and phagocytic mechanism which results in poor wound healing. This study correlates with Ta Wei Kevin Kok et al.[12]

In this study we found the 15% prevalence of implant associated infection which was similar to that of, F. pirisi, L. pennestri. Vigano, et al.[13]

Among the culture positives Gram positive cocci Staphylococcus aureus (29%) out of which MRSA were 22% and MSSA were 7%. is the most common organism which is isolated in culture, followed by Pseudomonas (20%), Klebsiella (11%), Proteus (9%), E.coli (6%). It indicates that nosocomial pathogen is Staphylococcus aureusthe least common organism isolated is Acinetobacter.

Phenotyping of Methicillin resistant Staphylococcus aureus is done by using Cefoxitin test according to CLSI guidelines. Genotyping in done by PCR. Among 17 isolates 2 sample tested and detected for mec A gene.

In 77 culture positive samples procalcitonin level is checked for 10 samples and it is found to be within normal limits. Reinforcing the fact that these infections usually do not lead to sepsis.

Prevention of implant associated infection by following strict aseptic precautions and judicious use of antibiotics, proper control of comorbid conditions specially diabetes

Conclusion

Treating the Orthopedic Implant associated infection is a great challenge. Improper administration of antibiotics will not cure the infection and prolongs the hospital stay, there by increases the morbidity and great economic burden to the patient.

So early intervention by proper selection of antibiotics according to culture and sensitivity will reduce the infection rate and hospital stay. Males

are having higher incidence accidents and Orthopaedic Implant associated infection than females. Patients are having increased rate of Implant associated infection in open fractures than closed fractures. Early infection is more than delayed infection.

Patients are having increased number infection rate in emergency surgery than elective surgery. Patients with co-morbidity has the higher rate of Orthopaedic Implant associated infection. MRSA is the commonest isolate in this study. To control Orthopaedic Implant associated infections, screening and proper treatment of MRSA is essential.

MRSA is the most common organism isolated in culture. Screening of MRSA carrier in hospital worker, pre-operatively in patients will reduce the infection rate. Adequate Intra–operative precautions and post-operative measures should be taken to prevent MRSA infection.

Limitation of the Study

Screening for MRSA nasal carriers in hospital workers and patients was not done in this present study. Detection and treatment of MRSA carriers will reduce the orthopedic associated infections.

References

1. Al-Mayahi M, Betz M, Müller DA, Stern R, Tahintzi P, Bernard L, Hoffmeyer P, Suvà D, Uçkay I. Remission rate of implant-related infections following revision surgery after fractures. Int Orthop. 2013 Nov;37(11):2253-8.

2. Drago L, Clerici P, Morelli I, Ashok J, Benzakour T, Bozhkova S, Alizadeh C, Del Sel H, Sharma HK, Peel T, Mattina R, Romanò CL. The World Association against Infection in Orthopaedics and Trauma (WAIOT) procedures for Microbiological Sampling and Processing for Periprosthetic Joint Infections (PJIs) and other Implant-Related Infections. J Clin Med. 2019 Jun 28;8(7):933.

3. Rares Mircea Birlutiu˙ Victoria Birlutiu, Manuela Mihalache, Cosmin Mihalache, Razvan Silviu Cismasiu: Diagnosis and management of Orthopaedic Implant related infections; a comprehensive review of the literature. Birlutiu et al. Review Article - Biomedical Research. 2017; 28: 11.

4. Moriarty TF, Kuehl R, Coenye T, Metsemakers WJ, Morgenstern M, Schwarz EM, Riool M, Zaat SAJ, Khana N, Kates SL, Richards RG. Orthopaedic device-related infection: current and future interventions for improved prevention and treatment. EFORT Open Rev. 2017 Mar 13;1(4):89-99.

5. Ribeiro M, Monteiro FJ, Ferraz MP. Infection of orthopedic implants with emphasis on

bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter. 2012 Oct-Dec;2(4):176-94.

6. Benazir S, Nazir U, Nazir S, Shafi A, Bashir L, Angmo D, et al.Orthopaedic implant infections: interplay of associated factors. Int J Adv Med. 2019;6:357-64.

7. Quirino, A.; Marascio, N.; Scarlata, G.G.M.; Cicino, C.; Pavia, G.; Pantanella, M.; Carlisi, G.; Mercurio, M.; Familiari, F.; Rotundo, S.; et al. Orthopedic Device-Related Infections Due to Emerging Pathogens Diagnosed by a Combination of Microbiological Approaches: Case Series and Literature Review. Diagnostics 2022; 12: 3224.

8. Rouhi, Gholamreza & Amani Hamedani, Mohsen: A brief introduction into orthopaedic implants: screws, plates, and nails. 2012.

9. Parvizi J, Della Valle CJ. AAOS Clinical Practice Guideline: diagnosis and treatment of periprosthetic joint infections of the hip and knee. J Am Acad Orthop Surg. 2010 Dec; 18(12):771-2.

10. Doshi P, Gopalan H, Sprague S, Pradhan C, Kulkarni S, Bhandari M. Incidence of infection following internal fixation of open and closed tibia fractures in India (INFINITI): a multi-centre observational cohort study. BMC Musculoskelet Disord. 2017 Apr 14;18(1):156.

11. Perumal A, Ashok Kumar C, Sheila Doris T, A study on the microbial profile of orthopedic implant infections and its risk factors in a tertiary care hospital. Indian J Microbiol Res 2016;3(4):412-418

12. Kok, Ta & Agrawal, Nikunj & Sathappan, S.S. & Chen, Kelven. Risk Factors for Early Implant-Related Surgical Site Infection. Journal of Orthopaedic Surgery. 2016: 24. 72-76. 10.

13. Pirisi L, Pennestrì F, Viganò M, et al. Prevalence and burden of orthopaedic implantable-device infections in Italy: a hospital-based national study. BMC Infect Dis. 2020; 20(1): 337.