Study of bacterial profile and virulence characteristics including antimicrobial resistance in isolates from patients with Implants

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Abstract:

Background: Infection is a major problem in orthopaedic implantations leading to implant failure. Implanted foreign bodies are highly susceptible to bacterial and fungal infection **Aim:** To identify the bacterial agents causing implant infections and the resistant pattern of isolates in patients with implants. The study also is aimed to analyse the virulence characteristics of isolated bacterial agents **Materials and methods:** Under strict aseptic precautions samples - Pus or Fragments of excised tissue removed at wound. The isolates were identified by using standard microbiological procedures Gram staining of smears and biochemical tests relevant to the isolates were done. **Result & Discussion:** In our study,

implant infections showed culture positive in 61.7% of acute infections and 25% in delayed infection and 13.3% in late infection. Out of 137 culture positive cases, 131 (95.7%) were monomicrobial and 6 (4.3%) were polymicrobial. *Staphylococcus aureus* (31.3%) was the most common isolate and resistance pattern of Gram positive organisms was penicillin(81.1%), cotrimoxazole (84%), Erythromycin(47%). The resistance pattern of Gram negative organisms was cefotaxime (64.3%), ceftazidime (57.6%).

Keywords: *Implant, Staphylococcus aureus, Gram positive organism, Gram negative organism*

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Introduction

Orthopaedic implants are mainly used for bone fixation and joint replacement[(1)].Orthopaedic devices are increasingly used for fracture fixation, including intramedullary nails, external-fixation pins, plates, and screws[(2)].Theaim of joint replacement is alleviation of pain and improvement of function. With increasing life expectancy, increasingly more patients suffer from osteoarthritis and therefore need joint replacement[(3)].

Infection is a major problem in orthopaedic implantations leading to implant failure. In the UK and USA, about 800000 joint arthroplasties are done annually, with projections to greater than 4 million by 2030[(4)]. The incidence of infection following elective orthopaedic surgery is in the range of 0.7% to 4.2%, while incidence can be much higher in trauma cases where infection rates range from 1% of closed fractures to more than 30% in open fractures[(5)]. It is a challenging task to treat orthopaedic implant infections which may lead to implant replacement and in severe cases, amputation and even mortality[(6)].

Implant-associated infections are caused by microorganisms growing in biofilms, which live attached to a surface in a highly hydrated extracellular matrix. Within biofilms, microorganisms develop into organized and complex communities with structural and functional heterogeneity resembling multi-cellularorganisms.

Release of cell-to-cell signalling molecules (quorum sensing) allows microorganisms in a biofilm to respond in concert by changing their gene expression involved in biofilm differentiation. Depletion of metabolic substances causes microbes to enter into a stationary state, rendering them up to 1000 times more resistant to most antimicrobial agents than their planktonic (free-living) counterparts[(7)].

Implanted foreign bodies are highly susceptible to bacterial and fungal infection. This is due to locally compromised host defense [(8-11)]. The risk of infection after internal fixation is between 0.4% and up to 16.1% according to the type of fracture (closed or varying degrees of open infection) [(12,13)].

Aim & objective

To identify the bacterial agents causing implant infections and the resistant pattern of isolates in patients with implants. The study also is aimed to analyse the virulence characteristics of isolated bacterial agents

Materials and methods

This prospective study was conducted in the Institute of Microbiology, Madras Medical College, Chennai. The study was conducted for a period of one year from March 2018 to February 2019. Ethical clearance was obtained from Institutional Ethics Committee prior to the conduct of the study and informed consent was obtained from all patients included in the study. This study was carried out in 180 cases with implant infections.

Inclusion criteria:

Patients with orthopaedic implants, cardiovascular implants and dental implants with clinical signs of infection within 90 days of surgery will be included.

Specimen collection:

Under strict aseptic precautions samples - Pus or Fragments of excised tissue removed at wound or from infected sinuses were collected and transported to the laboratory immediately. The samples for the bacteriological examination were obtained from the secretions which were adjacent to the infected implant and tissue, by

using a sterile cotton swab and a sterile disposable syringe. Other specimens collected are blood and removed implants.

Identification of isolates:

The isolates were identified by using standard microbiological procedures Gram staining of smears and biochemical tests relevant to the isolates.

Antimicrobial susceptibility testing:

The antimicrobial susceptibility testing of the isolates are done by modified Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton Agar (MHA).

Detection of biofilm formation:

The detection of the bacterial biofilm formation by the isolates was done by the Tube adherence Method[14].

Direct Gram's Stain

The smear was prepared for microscopic examination of pus cells and bacteria, by sterile pus culture swab on the surface of a clean and dry sterile glass slide. The smear was allowed to dry and then heat fixed gently. Gram staining of the smear was done method. Stained smear was examined under low power of microscope for the pus cell. Smear was seen under oil immersion method in (100x)for the presence of gram positive or gram negative bacteria, their morphology and arrangements of the organisms.

Culture of Aerobic organisms:

The wound swab was inoculated on to Nutrient agar plate, 5% sheepblood agar, Mac Conkey agar plate and by bacteriological loop streak culture was done and plates were incubated at 37ºC for 24 hours.

Biochemical tests:

Biochemical test such as Catalase, Modified oxidase and oxidase, Indole test, Citrate test, Triple sugar iron test, Urease test, Mannitol salt agar test, Slide and Tube coagulase test, Bile esculin test were done using standard methods. In case of Gram

negative isolates, motility by hanging drop method, Nitrate reduction test, Lysine, Arginine, Ornithine (LAO) test, Oxidation or fermentation pattern of organism was done by Hugh & Leifson method.

Results

Table 1: Total number of implant infection cases (n=180)

Type of implant	Number ofcases	Percentage
Orthopaedic implants	175	97.2%
Dental implants	3	1.7%
Cardiac implants	2	1.1%
Total	180	100%

Table 2.Distribution of risk factors (n=180)

Risk factors	No of cases (n=180)	Percentage
Diabetes mellitus	74	41.1%
Smoking	36	20%
Alcoholism	19	10.5%
Anaemia	3	1.7%
Dm/alcoholism	3	1.7%
Dm/smoking	6	3.3%
Dm/smoking/alcoholism	1	0.5%
Smoking/alcoholism	8	4.4%
Steroids	2	1.1%
Nil	28	15.5%
Total	180	100%

Table 3.Distribution of culture results (n=180)

	No of cases (n=180)	Percentage
Total no of culturepositive	137	76.1%
Total no of culture negative	43	23.9%
Total no of cases	180	100%

Table 4.Bacterial isolates of implant infection (n=137)

Bacterial isolate	No of cases(n=137)	Percentage
Staphylococcus aureus	43	31.3%
Staphylococcus epidermidis	22	16%
Pseudomonas aeruginosa	21	15.3%

Klebsiella pneumoniae	14	10.2%
Proteus mirabilis	13	9.4%
Proteus vulgaris	5	3.6%
Acinetobacter spp	9	6.5%
Escherichia coli	4	2.9%
Polymicrobial	6	4.3%
Total	137	100%

Table 5: Antibiotic susceptibility pattern of gram positive organisms

Antibiotics	***Staphylococcus aureus* (n=43)**		***Staphylococcus epidermidis* (n=22)**	
Amikacin	31	72%	15	68.1%
Ciprofloxacin	24	55.8%	11	50%
Trimethoprim-sulphamethoxazole	7	16.2%	9	40.9%
Clindamycin	28	65.1%	15	68.1%
Erythromycin	23	53.4%	13	59%
Penicillin	8	18.6%	5	22.7%
Cefoxitin	24	55.8%	-	-
Linezolid	43	100%	22	100%
Vancomycin(MIC)	43	100%	22	100%

In this study the most common gram positive bacteria isolated in this study was *Staphylococcus aureus* 43(31.3%) which showed 100% sensitivity to Linezolid and vancomycin(MIC).Cefoxitin sensitivity was 55.8% .Drugs such as Amikacin, Ciprofloxacin and Erythromycin showed 72%,56% and 53% sensitivity respectively. *Staphylococcus epidermidis* 22(16%) showed 100% sensitivity to Linezolid and vancomycin(MIC). Drugs such as Amikacin, Erythromycin and Ciprofloxacin showed 68%, 59%, and 50% sensitivity respectively.

Table 6: Antibiotic susceptibility pattern of gram negative bacilli

Antibiotics	***Pseudomonas aeruginosa (21)***		***Klebsiella pneumoniae (14)***		***E.coli(4)***		***Proteus Mirabilis(13***		***Proteus vulgaris(5)***		***Acinetobacterspp(9)***	
Amikacin	16	76.1%	11	78.5%	2	50%	8	61.5%	3	60%	5	55.5%
Gentamicin	18	85.7%	8	57.1%	3	75%	7	53.8%	4	80%	3	33.3%
Cotrimoxazole	-		5	35.7%	1	25%	6	46.1%	2	40%	3	33.3%

Ceftazidime	9	42.8%	5	35.7%	3	75%	5	38.4%	2	40%	2	22.2%
Cefotaxime	-		3	21.4%	2	50%	-		-		-	
Cefotaxime-clavulanic acid	-		5	35.7%	2	50%	-		-		-	
Ciprofloxacin	13	61.9%	8	57.1%	2	50%	8	61.5%	1	20%	3	33.3%
Tetracycline	-		8	57.1%	2	50%	-		-		-	
Piperacillin-tazobactam	15	71.4%	9	64.2%	2	50%	9	69.2%	2	40%	6	66.7%
Imipenem	21	100%	11	78.5%	4	100%	13	100%	5	100%	8	88.9%

In this study all the Gram negative bacilli showed 100% sensitive to Imipenem except *Klebsiella pneumoniae* and *Acinetobacter spp* showed 78.5% and 88.9% respectively. All the Gram negative bacilli showed 60-80% sensitive toAmikacin, 50-60% sensitive to Ciprofloxacin and 60-70% sensitive to Pipercillin- Tazobactam.

Table 7.Percentage of MSSA and MRSA (n=43)

Staphylococcus aureus **Isolates (n=43)**	**MSSA percentage**		**MRSA percentage**	
43	24	55.9%	19	44.1%

Table 8. Percentage of ESBL producers (n=18)

Gram negative E-bacilli (n=18)	**ESBL producers**	**Percentage of ESBL producers**
Escherichia coli(4)	2	50%
Klebsiella Pneumoniae (14)	8	57.4%

Table 9.Biofilm producing organisms (n=41)

Isolates	**Biofilm Producers**	**Percentage**
Staphylococcus aureus (n=43)	19	44.1%

Staphylococcus epidermidis(n=22)	12	54.5%
Pseudomonas aeruginosa(n=21)	7	33.3%
Klebsiella pneumoniae(n=14)	3	21.4%

Discussion

This prospective study was conducted on patients with implant Infections with orthopaedic implants, dental implants and cardiac implants in Rajiv Gandhi Government General Hospital, Chennai. Among 2812 patients who underwent orthopaedic implant surgeries for bone fractures and joint replacement, dental implant surgeries, cardiac implant surgeries during the study period, 180 patients developed implant infection and they were included in this study.

In this study, out of 180 cases who developed implant infection 175 patients belonged to orthopaedic implant infection, 3 patients belonged to dental implant infection and 2 patients belonged to cardiac implant infection (Table 1)

Of the 2812 patients who underwent orthopaedic implant surgeries for bone fractures and joint replacement, dental implant surgeries, cardiac implant surgeries during the study period, 180 patients developed implant infection (6.4%) , which correlates with the study by Angappan perumal *et al.*, (2016)[15]. Among 180 cases, 137(76.1%) have been culture positive and 43(23.9%) cases were culture negative, which correlates with the study by Trupti B .Naik *et al* (2016)[16].

In this study, out of 180 cases,24(13.3%) patients with open interlocking nail in femur, 25(13.8%) patients with open interlocking nail in tibia,19(10.5%) patients with dynamic compression plate in femur, 17(9.4%) patients with dynamic compression plate in tibia,15(8.3%) patents with closed interlocking nail

in femur,12(6.%) patients with closed interlocking nail in tibia, 3(1.67%) patients with dental implants and 2(1.1%) patients with cardiac implants developed implant infection, which correlates with the study by Angappan perumal *et al.*, (2016)[15].

In this study out of 180 infected cases, 160 (88.9%) patients were male and

20(11.1%) were female. Higher incidence in male may be attributed tomore prone for trauma as they travel outside frequently for work such as industries, construction sites, etc., This correlates with the study by Angappan Perumal *et al.,* (2016)[15] and Al-Mulhim *et al.*, (2014)[17].

In this study, higher percentage of implant infection were noted in the age group between 20-60 years. This higher percentage of implantinfection in this age group was due to working population age group and more young adults were affected in road traffic accidents due to rash driving and drunken driving. This higher incidence of infection in this age group correlates with study by Angappan Perumal *et al.,* (2016)[15].

In our study Diabetes mellitus(41.1%) was considered as an important risk factor for implant infection (Table:2) ,which correlates to the study Angappan Perumal *et al* (2016)[15] , Ta Kevin Kok *et al* (2016)[18] .Next to Diabetes mellitus, smoking(20%) and alcoholism(10.5%) were common risk factor incausing implant infection.

The pathogenesis of Diabetes mellitus in causing implant infection is mainly due to the hyperglycaemic state that impairs neutrophil chemotaxis and phagocytosis, resulting in weakened antibacterial defense and impaired wound healing. Suboptimal glucose control peri-operatively is also associated with increase in length of the hospital stay, so optimizing blood sugar level peri-operatively was essential to decrease the postoperative infections.

Out of 180 cases, 116 (65%) had undergone emergency surgery and 64(35.6%) cases had undergone elective surgery. Higher incidence of implant infection in emergency surgery in this study correlates with the study of Ta Kevin Kok *et al* (2016)[18].

Higher rate of implant infection in emergency surgery was due to following factors-less preparation time for surgery, contamination at surgical site, inadequate preoperative optimization of co-morbidities like Diabetes mellitus, Anemia, etc. and not giving any prophylactic antibiotics.

In this study, implant infections showed culture positive in 61.7% of early/acute infections and 25% in delayed infection and 13.3% in late infection in the present

study, which correlates with the study by A.D. Koshravi *et al.*, 2009 (73%,22.6%, and 5% respectively)[17].

Early infection has high prevalence because trauma and fracture fixation using metallic implants may produce functional and structural damage to the localhost tissue resulting in impaired cellular and humoral immune response.

In this study out of 137 culture positive cases, 131 (95.7%) were monomicrobial and 6 (4.3%) were polymicrobial (Table 3). Study by Trisha N.Peel *et al* (2012) showed Poly microbial infection (36%) frequently involved combination of Gram negative and Gram positive organisms. Biofilm forming organisms are commonly associated with poymicrobial infections. The incidence of implant infections is relatively higher than other studies. Higher percentage of infection could be due to Pre-operative soft tissue damage due to trauma is the risk factor for developing implant infection and emergency surgery also has been attributed to development of implant tinfection.

In this study, out of 137 culture positive cases,*Staphylococcus aureus* was the common pathogen isolated 43 (31.3%), *Staphylococcus epidermidis* 22 (16%), *Pseudomonas aeruginosa* 21(15.3%), *Klebsiella pneumoniae* 14 (10.2%), *Proteus mirabilis* 13 (9.4%), *Acinetobacter spp*. 9(6.5%), *Proteus vulgaris* 5 (3.6%), *Escherichia coli* 4 (2.9%), and Polymicrobial infection 6(4.3%) (Table:4). This correlates with the study of Lakshmi narayana *et al.*, (2013)[19], Roopashree *et al*, (2015)[20].

In this study the most common gram positive bacteria isolated in this study was *Staphylococcus aureus* 43(31.3%) which showed 100% sensitivity to Linezolid and vancomycin (MIC).Cefoxitin sensitivity was 55.8%. Drugs such as Amikacin, Ciprofloxacin and Erythromycin showed 72%, 56% and 53% sensitivity respectively.

Staphylococcus epidermidis 22 (16%) showed 100% sensitivity to Linezolid and vancomycin (MIC). Drugs such as Amikacin, Erythromycin and Ciprofloxacin

showed 68%, 59%, and 50% sensitivity respectively (Table:5). In this study among 66 isolates of gram negative bacilli,21 *Pseudomonas aeruginosa species*, 14 *Klebsiella pneumoniae species*, 13 *Proteus mirabilis species*, 5 *Proteus vulgaris species*, 9 *Acinetobacter spp*, 4 *Escherichia coli* species were isolated.

All the gram negative bacilli showed 100% sensitive to Imipenem except *Klebsiella pneumoniae* and *Acinetobacter spp* showed 78.5% and 88.9% respectively. All the gram negative bacilli showed 60-80% sensitive to Amikacin, 50-60% sensitive to Ciprofloxacin and 60-70% sensitive to Pipercillin- Tazobactam (Table:6). This correlates with the study of Anirudh dash and Ravi kant das *et al* (2015)[21]

In this study MRSA isolated was 19(44.1%) and MSSA 24(55.9%) (Table:7).This correlates with the study by Goel et al (2013)[9] and study by Satya Chandrika V *et al* (2016)[22].In this Study among ESBL producers, 2(50%) were *Escherichia coli*,8(57.4%) were *Klebsiella pneumoniae*.(Table:8).The study by Sonawane *et al* (2010)[23] shows 71.72% of ESBL producers.

In this Study 19(44.1%) *Staphylococcus aureus*, 12(54.5%) *Staphylococcus epidermidis*, 7(33.3%) *Pseudomonas aeruginosa*, 3(21.4%) *Klebsiella pneumoniae* were biofilm producers in implant infection (Table:9).

Rifampicin is important in treatment of implant infection as an anti-*Staphylococcal* biofilm antibiotic. Rifampicin is administered along with beta- lactam antibiotic or combined with quinolones like Ciprofloxacin or Levofloxacin to prevent drug resistance.Study by T.Fintan Moriarty *et al.,* (2016) showed in case of quinolone resistance, Rifampicin can be administered along with Fusidic acid, Cotrimoxazole, Linezolid, Clindamycin.

In this study *Staphylococcus aureus* (31.3%) was the most common isolate and resistance pattern of Gram positive organisms was penicillin(81.1%), cotrimoxazole (84%), Erythromycin(47%). The resistance pattern of Gram negative organisms was cefotaxime (64.3%), ceftazidime (57.6%). This correlates with study by Sonawane *et al* (2010)[23] and Jain *et al* (2014)[23].

Conclusion

Various biomaterials used in orthopaedic surgery show different susceptibilities to infection, because adhesion of infecting bacteria is controlled by biomaterial surface properties, like hydrophobicity and roughness. Controlling the hydrophobic properties of materials surfaces is likewise a new way to influence bacterial interaction with the surface and must be taken into account when developing newer and novel anti-infective biomaterials. Since bacterial adhesion is a much complex process affected by many factors, such as bacterial and material properties and environment, further studies are required to understand the mechanisms of bacterial adhesion and implant infection, and to provide adequate methodologies and antimicrobial agents for prevention.

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