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Original Article

Microbiology Section

The Microbiological Profiles of Infected Prosthetic Implants with an Emphasis on the Organisms which Form Biofilms

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ABSTRACT

Background: In spite of the decreasing incidence of orthopaedic device related infections to 1%, nowadays, device-related infections still remain a diagnostic, therapeutic and cost -related problem.

Aims and Objective: To record the common causative organisms and the contributing risk factors for orthopaedic device-related infections in a tertiary care teaching hospital.

Methods: In a prospective study, fifty patients who underwent orthopaedic device implantation from Jan 2009 – June 2010 were enrolled; among them, 42 patients were complicated with infections. The demography, microbiological data, treatment and the outcome of each patient were recorded.

Statistical Analysis: The data was analyzed in terms of frequency and percentage.

Results: Of the 50 samples, 42(84%) were culture positive, while 8(16%) were cultures negative. The femur was the most commonly affected bone in both males (median age–37.1yrs) and females (median age–41.3 yrs). *Staphylococcus aureus* was the organism which was most commonly isolated and which caused biofilms, followed by non-fermenting, gram negative bacilli and *Klebsiella spp*. We reported the first case till date in the literature of *Candida krusei* PJI, to the best of our knowledge. No anaerobes were isolated. Tissue trauma, open fractures, post-operative surgical site infections and Diabetes mellitus were found to be the important risk factors. The biofilm forming organisms were commonly associated with polymicrobial infections and even an aggressive antibiotic therapy was often inadequate to eliminate the infections. A conservative surgical treatment was associated with treatment failures. Implant removal or replacement was required in most of the cases to eradicate the infection.

Conclusion: The most common bacteria which were isolated included *Staphylococcus aureus,* followed by *Pseudomonas aeruginosa* and *Klebsiella*. A majority of them are resistant to the commonly used antibiotics, leading to treatment failures which necessitated an implant removal.

Key Words: Prosthetic joint infection, Implant surgery, Biofilm

INTRODUCTION

A prosthetic replacement and an implant surgery is commonplace in orthopaedic operations for successfully alleviating the pain and improving the mobility in damaged joints.

Hundreds of thousands of patients undergo joint replacement surgeries each year, worldwide and millions of people have an in-dwelling prosthetic articulation [1]. Prosthetic Joint Infections (PJIs) are devastating complications which follow such surgery. In the past century, the incidence of PJIs has drastically reduced due to the modern theatre facilities and the aseptic measures. Yet they still pose a problem in the developing countries, with high morbidities and substantial costs.

Clinically and economically, emphasis should be laid on the prevention of such infections. When microorganisms seed on a foreign body, they proliferate and undergo a phenotypic alteration to develop a biofilm. Biofilms resist the antibiotic penetration, thus requiring the dose to be increased several fold. Aggressive therapeutic options such as prolonged and high-end antibiotics, additional surgeries and a prolonged rehabilitation are associated with complications which require a prolonged hospitalization with a possibility of a renewed disability. The health care costs of the revision surgeries are high and the chances of an infection are higher than with primary surgeries, thus burdening both the patients and the treating hospitals.

This study was aimed at assessing the risk factors which were associated with orthopaedic implant infections and at evaluating the causative organisms, their antibiotic sensitivities and their abilities in forming biofilms.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Microbiology, at a tertiary care teaching hospital, for a period of one and a half years, from January 2009 – June 2011. The patients who had undergone prosthesis or implant surgeries, who presented with the signs and symptoms of infections which were confirmed by laboratory and other investigations, were included in the study. This study was carried out after obtaining the institutional ethics committee's approval and informed consents from the patients. The demographic data like age, sex, the duration and the type of surgery, the time of the infection and the risk factors were noted.

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. The tissues were collected in Thioglycollate broth medium and Robertson's cooked meat medium and they were immediately transferred to the microbiology laboratory and were incubated at 37°C for 24 hours, in order to enrich the bacterial cells. If the implant was removed, it was collected in glucose broth

and incubated at 37°C for 24 hours. Gram staining and acid fast staining of all the samples were done. Subcultures were made on Sheep blood agar for the anaerobic bacteria, on duplicate blood agar plates, one for the aerobic and one for the anaerobic bacteria and on MacConkey's agar, Sabouraud's Dextrose Agar (SDA) and on the Lowenstein-Jensen medium. The media was incubated under different conditions – at 37°C for 5 days in an anaerobic chamber (Sheep Blood Agar), at 37°C in the presence of 10% CO_2 in a candle jar for 24 hours (Blood agar), at 37°C (Blood agar and MacConkey's agar), at 28°C and 37°C (SDA) and at 37°C for 30 days (LJ medium). The isolates were identified by using standard microbiological procedures [2] and they were tested for their antimicrobial susceptibilities by the Kirby Bauer method according to the guidelines of the Clinical and Laboratory Standards Institute [3].

The detection of the bacterial biofilm formation was done by the Tissue Culture Plate Method (TCP) and the Tube Method (TM) as have been described in detail by Mathur *et al.* [4]

The statistical analysis was done in terms of percentage and frequency.

RESULTS

Among the total 50 patients who were investigated in the present study, 42(84%) had positive cultures, while in 8(16%), the cultures were negative. The most prevalent isolated bacteria was *S. aureus.* The only fungus which was isolated was *C.krusei*. No anaerobes were isolated [Table/Fig-1].

The most commonly affected sites in decreasing order were the femur (26%), tibia (16%) , bimalleolar (16%) and the humerus (8%), followed by the radius, the ulna and the tarsal bones. There was a male preponderance (76%) with an average age of 37.1 years. Young adults were commonly affected in association with Road Traffic Accidents. and 24% were females, with an average age of 41.25 years.

Based on Trampuz's and Zimmerli's [5] classification, 26% had early, 18% had delayed and 56% had late infections. There was a surge in the infection rate after 10 weeks [Table/Fig-2]. [Table/Fig-3] shows the prevalence of the affected sites in relationship with the onset of the symptoms. Out of 50 cases which were studied, 30(22.5%) were open fractures with extensive tissue damage, which led to infections. These patients developed an early onset of the infections as compared to the late onset which was seen in closed injuries. Open fractures/tissue damage (22.5%), and post operative surgical infections (16.18%) were the major risk factors. Others included old age; hypertension (8.09 %), Diabetes, immunosupression (6.62%), malnutrition and obesity (5.15%).The risk factors and the onset of infection have been highlighted in [Table/Fig-4]. No significant difference was noticed between the onset of the infection and the type of operative procedure [Table/Fig-5].

Among the positive cultures, 35.7% (15) showed a mixed culture of more than 2 organisms. The highest number of isolates from a single culture was three. Among the 63 isolates, 20 (31.7%) were ESBL producers and 8(12.7%) were MRSA, which resulted in treatment difficulties.

In the modified TCP method, among the total number of 64 isolates which were tested for biofilm formation, 18 (28.1 %) were strong biofilm producers, 3(4.7%) were moderate producers and 43 (67.2%) isolates were considered to be non or weak biofilm producers. The TM showed a good correlation with the TCP assay only for the strong biofilm forming isolates and total of 15 (23.4%) isolates were picked up as strong. 11 (17.2%) were moderate and 37 (57.8%) were weak/absent biofilm producers. In our study, 18 isolates showed biofilm production by the Tissue Culture Plate Method [Table/Fig-6].

Organism isolated	Number (Percentage) N-64
S. aureus	21 (33%)
Methicillin resistant S.aureus	8 (12%)
Enterococcus	6 (9%)
Coagulase negative Staphylococcus	3 (5%)
Methicillin resistant coagulase negative Staphylococcus	1 (2%)
Pseudomanas aeruginosa	5 (8%)
Acinetobacter	5 (8%)
Proteus	3 (5%)
E.coli	1 (2%)
Klebsiella	6 (9%)
Citrobacter	4 (6%)
Candida krusei	1 (2%)

[Table/Fig-1]: Microbiology of organisms isolated

Organism	Early < 2 weeks	Delayed 2- 10 weeks	Late > 10 weeks	Total
S.aureus	6	3	20	29
Enterococcus spp	1	1	4	6
Coagulase negative Staphylococcus	1	1	2	4
Pseudomonas spp	1	0	4	5
Acinetobacter spp	2	0	3	5
Proteus	0	1	2	3
Escherichia coli	0	1	0	1
Klebsiella spp	1	1	4	6
Citrobacter spp	1	0	3	4
Candida krusei	0	1	0	1

[Table/Fig-2]: Prevalence of organisms isolated in relation to onset of infection

Site affected	Early < 2 weeks	Delayed 2-10 weeks	Late > 10 weeks	Total
Femur	1	3	9	13
Tibia	2	2	4	8
Fibula	1	0	2	3
BB LL	2	0	0	2
Humerus	1	1	2	4
Radius	0	0	1	1
Ulna	0	0	1	1
BB UL	0	1	3	4
Bimalleolar	4	1	3	8
Foot	1	0	2	3
Spine	1	0	1	2
Knee	0	0	2	2

[Table/Fig-3]: Prevalence of site affected in relation to onset of symptoms

Risk Factor	Early < 2 weeks	Delayed 2- 10 weeks	Late > 10 weeks	Total	%
Old age	1	4	6	11	8.09
Diabetus Mellitus	2	2	5	9	6.62
Anaemia	2	3	5	10	7.35
Tissue damage/ Open fracture	11	5	14	30	22.05
Immunosuppression	1	3	5	9	6.62
Concurrent UTI	2	1	3	6	4.41
Renal failure & Hemodialys is	1	0	3	4	2.94
Post operative surgical infect ion	6	4	12	22	16.18
Chronic osteomyelitis	0	0	4	4	2.94
Malnutrition	2	1	4	7	5.15
Obesity	2	3	2	7	5.15
Hypertension	1	3	7	11	8.09

[Table/Fig-4]: Risk factors associated with PJI.

	Early < 2 weeks	Delayed 2- 10 weeks	Late > 10 weeks	Total
Extramedullary internal Fixation	6	4	12	22
Intramedullary internal Fixation	7	3	10	20
Total hip replacement	0	2	4	6
Total knee replacement	0	0	2	2

[Table/Fig-5]: Relation between operative procedures and time of onset of infection

Organism	Number (Percentage) N- 18
Methicillin resistant S.aureus	6(38%)
Staphylococcus aureus	5(28%)
Acinetobacter Spp	3(17%)
Klebsiella Spp	2(11%)
Enterococcus	1(5.6%)
Candida krusei	1(5.6%)

[Table/Fig-6]: Prevalence of biofilm producing organisms.

Three isolates which showed biofilm formation were isolated repeatedly in spite of an extensive antimicrobial therapy and the infection was resolved only following the implant removal. These isolates belonged to a polymicrobial infection. Two of these isolates were *Klebsiella* spp., out of which one was multi drug resistant and sensitive only to piperacillin/tazobactam and the carpabenems. The 3rd isolate was MRSA. Among the remaining cases, the implant was removed or replaced in 11 patients and hence there was no repeated isolation. Four cases did not have recurrent infections in spite of leaving the implant in situ.

DISCUSSION

Implant related infections continue to pose a problem for the orthopaedicians. The diagnosis and the treatment of these 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.

However, the organisms which have adhered to the prosthesis are occasionally impossible to detect by the common bacterial cultures. Various sampling techniques which include direct swabs, periprosthetic fluid sampling and sampling from the implant after sonication, have been described. Esteban *et al.,* [6] reported an increase in the sensitivity from 84.2-94.7% with sonication techniques over the conventional periprosthetic tissue culture. Gomez *et al.,* [7] reported only 60% culture positivity. Zimmerli *et al.,* [8] and Khosravi *et al.,* [9] published positive culture rates of 89% and 93.9% respectively. We noticed an 84% culture positivity from three samples which we collected in our study.

The frequency of the aetiological agents varies among the published reports [10,11]. In the present study, aerobic gram positives cocci accounted for 60.9% (39) cases and aerobic gram negatives accounted for 37.5% (24) cases. *S. aureus* was the most common isolate, followed by non fermenting gram negative bacilli and *Klebsiella* spp. This strongly supports an intra operative contamination and we assumed that these were the main nosocomial pathogens in the operating room. The present findings were in agreement with those of a study which was done by Khosravi *et al.,* [9] *which* reported that *S. aureus* as the most prevalent isolate, followed by *Klesiella ozaenae* and *P. aeruginosa* among the gram negative bacilli. Though the infections which are caused by fungi and mycobacteria are rare [1], we reported the first case till date in the literature of *C. krusei* PJI. No anaerobes were isolated, though anaerobes play a significant role in the pathogenesis of PJI occurring at >24 months following implant insertions, especially when there is an extra medullary internal fixation device [9]. However, none of the patients in the present study presented with PJI after 18 months. Additionally, the antimicrobial therapy should be stopped at least 2 weeks prior to the tissue sampling for anaerobic cultures [12]. But most of our patients were already on an empirical therapy or they had a history of an antimicrobial treatment in the recent past. This could possibly explain why no anaerobes were isolated in our study.

The antimicrobial susceptibility testing revealed the high rate of antimicrobial resistance in *Methicillin resistant Staphylococci* and *Acinetobacter* isolates. *Staphylococcus,* which was the major isolate, showed a high sensitivity to vancomycin. Most of the gram negative isolates showed sensitivity to the carbapenems and the fluroquinolones. Most of our patients were treated with 3rd generation cephalosporins and gatifloxacin initially and then the therapy was altered, if required, based on the antibiotic susceptibility profile. We suggest a combination of vancomycin with imipenem or gatifloxacin for the treatment coverage of a majority of isolated bacteria, based on our findings.

The treatment of Orthopaedic Device Related Infections (ODRIs) most frequently includes long-term antimicrobial treatments and the removal of the implants. In our study, the devices were removed in 50% of the cases, while the rest of the patients were treated with intravenous antibiotics and multiple wound debridement. A recent evidence from observational trials [12,13] and one randomized clinical trial [14] indicated that a subset of patients can be successfully treated by debridement and a long-term antimicrobial therapy with the retention of the implant. It has been stated that the patients who are eligible for such a treatment must meet the following criteria: an acute infection with its signs and symptoms lasting for <14–28 days, an unambiguous diagnosis which is based on the

histopathology and the microbiology, a stable implant and a good quality of bone stock, and the susceptibility of the micro organism to an effective, orally available, antimicrobial agent [15].

Based on our findings, the onset of PJI was >10 weeks following the implant surgery in 56% of the cases. Similar findings have been reported by Guilieri *et al.* [16] PJIs are usually acquired during implant surgeries and are caused by less virulent organisms or by the haematogenous route from remote infections [8]. Six patients in our study developed PJIs through the haematogenous route, as was confirmed by the bacteraemia. The risk for haematogenous infections in our study was higher in the hip prostheses than in the knee prostheses [8].

In the present study, males had a preponderance for Prosthetic Osteoarticular Infections (76%), who are mainly young adults in association with Road Traffic Accidents. These fractures were mainly open with extensive soft tissue damage, haematoma formation and wound contamination, which led to the spread of the bacteria to the bone. Femoral and tibial fractures were the most commonly affected bone fractures. Conversely, Prosthetic Joint Infections were commonly seen to affect the femur in elderly women with a history of a fall. We noticed that 84.6% of the early infections were associated with soft tissue and periosteal damages, open fractures and contaminated wounds.

Post operative surgical infections are another important risk factor with locally introduced infections as a result of wound sepsis which is contiguous to the prosthesis. We found that 59% of the PJI associated Surgical Site Infections (SSI) were caused by *Staphylococcus Spp.* Among them, 50% of the coagulase negative Staphylococci which were isolated were associated with post operative surgical infections. These organisms form part of the normal cutaneous flora and they 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 ODRIs. The intrinsic factors included the age, nutritional status, obesity, additional nosocomial infections, a long preoperative stay and corticosteroid therapy [17]. In our study, advanced age was responsible for the infections (although it was not shown to be an independent contributing factor), as was reported in other studies, as well [17, 18]. The elderly patients often had several risk factors like hypertension, cardiac abnormalities, Diabetes mellitus and other immunosuppressive conditions.

The reactivation of the latent foci of chronic osteomyelitis which is caused by tissue disruption, which is associated with an implant surgery, may occur infrequently [1]. In the present study, four cases with *Staphylococcal* chronic osteomyelitis developed late onset PJIs. The patients with renal failure, who were on haemodialysis, showed late onset PJIs with gram negative bacilli in 100% of the cases. *Klebsiella S*pp. was the most common isolate in this clinical setting. Excess or under nutrition showed predisposition for PJIs due to an altered cellular and humoral immunity, as well as the difficulty in the mobilization and the subsequent development of bed sores and surgical wound contaminations in the obese.

Biofilms are microbial communities which are encased within a polysaccharide rich extracellular matrix on the surfaces of these devices. They are associated with an enhanced resistance against most of the antimicrobial agents, leading to treatment failures. In our study, 18 isolates showed biofilm production by the Tissue Culture Plate method. The most common organism which produced a biofilm was *Staphylococcus aureus*. The biofilm producing organisms were associated with therapeutic failures and the infection was resolved only on implant removal. In the patients who received a conservative surgical treatment, the biofilm producing isolates were repeatedly isolated; these isolates were polymicrobial resistant to antimicrobials.

Various methods have been described to detect the biofilm production. These methods include the Tissue Culture Plate (TCP) method, the Tube Method (TM), the Congo Red Agar (CRA) method, bioluminescent assays and light or fluorescence microscopy of the extracellular polysaccharide (slime), which facilitate the examination. We compared the tube method and the tissue culture plate method. The tube method correlates well with the TCP test for the strongly biofilm producing isolates, but it was difficult to discriminate between the weak and the biofilm negative isolates due to the high variability in observed results. In the present study, TCP showed values of 28.1%, 4.7% and 67.2%, whereas TM showed values of 23.4%, 17.2% and 57.8% for the strong, moderate and the weak biofilm producers respectively. We conclude in agreement with the previous reports, that the tube test cannot be recommended as a general screening test to identify the biofilm producing isolates.

CONCLUSION

The results of this study emphasize the need to account for local factors while assessing the risk for ODRIs . The appropriate pre and postoperative wound care for the dirty wounds, especially when external fixators are used and in patients who are in a poor condition, should be done with more caution. Staphylococcus spp are the commonest isolates and their ability to produce biofilms stresses the need for an appropriate antibiotic policy to put in place to eradicate the infection. As the studies in India and other developing countries are few, more studies are required in this area. The ODRIs lay lot of strain on the health services and the economy of the society, which necessitates further studies to determine the causative micro organisms, their antibiotic susceptibilities, and the associated risk factors, in order to institute timely and effective preventive measures or an appropriate and aggressive treatment, for reducing the costs and for improving the quality of life. However, larger studies with bigger sample sizes are required to attain these goals.

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