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ORIGINAL ARTICLE

CHARACTERISATION OF AEROBIC BACTERIOLOGICAL ISOLATES FROM ORTHOPEDIC IMPLANT SITE INFECTIONS WITH SPECIAL REFERENCE TO BIOFILM FORMATION IN A TERITIARY CARE HOSPITAL

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ABSTRACT: INTRODUCTION: Orthopedic implant site infection is one of the major constituents 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, orthopedic trauma surgery is at grave risk of microbiological contamination. Often isolates causing these infections are associated with biofilm formation resulting in increased antibiotic resistance. **OBJECTIVES:** To determine the aerobic bacteriological profile with their antibiotic susceptibility patterns from pus samples of patients with orthopedic implant site infections. To determine the potential of these isolates to produce biofilm. **MATERIALS AND METHODS:** Pus samples were collected and sent to the laboratory from patients with suspected implant infections over a period of 6 months were processed according to CLSI guidelines. Biofilm detection was done using Congo red Agar (CRA) method, tube method and Tissue culture plate method. **RESULTS:** Of the 46 culture positive cases out of 63, most common isolate was Staphylococcus aureus 30(65.21%) followed by Coagulase negative Staphylococcus 4(8.69%), Escherichia coli 4(8.69%), Klebsiella species 3(6.52%), Pseudomonas species 3(6.52%), enterococcus species 2(4.32%). 13.33% of S. aureus was Methicillin resistant (MRSA), 100% of gram negative bacilli were ESBL and AmpC producers, 27.27% of gram negative bacilli were resistant to Imepenem and Meropenem, 1 vancomycin resistant enterococci was isolated. 72% of the isolates were biofilm producers by Congo Red Agar method, 76% by Tube method, and 84% by tissue culture plate method. **CONCLUSIONS:** Staphylococcus aureus (MSSA) is the most common organism causing orthopedic implant site infection. Gram negative isolates exhibit multidrug resistance patterns. Significant numbers of isolates causing implant infections are biofilm producers. Antibiotic preference should be individualized depending on local sensitivity pattern of the hospital.

KEYWORDS: Orthopedic implant, infections, bacteriological profile, antibiotic sensitivity, biofilm. Formation.

INTRODUCTION: Orthopedic implant site infection is one of the major constituent 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, orthopedic trauma surgery is at grave risk of microbiological contamination and infection.[1] Though the incidence of orthopedic implant related infection has been reduced to less than 1%, it remains a diagnostic, therapeutic and cost related problem.[2]

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]

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Major risk factor for development of orthopedic implant site infection depends upon the extent of damage which has occurred to the soft tissues and periosteum following fracture, because devascularised bone and necrotic tissue are ideal for multiplication of bacteria as there is no access for the immune host defenses to reach the infection site, since there is damage to the periosteal blood supply and lack of perfusion of the soft tissues.[4] It also leads to delayed healing of fracture. Other risk factors include the patient's co morbid conditions like Diabetes Mellitus, Rheumatoid Arthritis, Sickle cell anemia, malnourishment, obesity, immuosupression due to prior renal or liver transplantation, presence of infectious foci in the body like UTI.[5]

Source of infection can be endogenous or exogenous; patient can acquire infection from other patient or a hospital staff, environmental sources like air, water, food, medication, equipment /instrumentation, solid linen and hospital waste postoperatively.[6]

Pathogenesis of orthopedic implant site infections involves interaction between the host, the implant and the microorganisms. When the microorganism comes in contact with the implant which is devoid of microcirculation it proliferates and undergoes a phenotypic alteration to form a biofilm. These microorganisms survive within the biofilm causing a difficulty in delivery of antibiotics, the dosage of which has to be increased several folds as these biofilms resist antibiotic penetration.[7]

Any delay or inefficiency in the treatment of these infections will lead to significant morbidity in terms of pain, loss of function and need for further surgery and antibiotics.[1] Ultimately it leads to prolonged hospitalization.

Hence, one should have knowledge on the microbiological profile and their antibiotic susceptibility patterns for aggressive treatment and management and to prevent complications. Data from other hospitals cannot be used as the sensitivity pattern differs from hospital to hospital.

This study was done in view of evaluating the causative organism, their antimicrobial susceptibility patterns and their potential to form 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 6 months from January 2014 to June 2014. A total of 63 patients who had undergone an implant surgery and presented with signs and symptoms of infections were included in the study.

This study was conducted after obtaining the ethical clearance from the institution ethical committee and informed consent from the patients. The demographic data like age, sex, type of surgery, time of infection, co morbidities were noted.

The samples for bacterial examination were obtained from the discharge adjacent to the infected implant and tissue, by using a sterile cotton swab or a sterile disposable syringe. These samples were immediately transferred to the microbiology laboratory in appropriate sterile containers depending on the type of sample.

All specimens were processed for Gram stain, Acid fast stain (in case of aspirate) and aerobic bacterial culture by inoculating the specimen on Blood Agar, MacConkey Agar and Thioglycollate medium and incubated at 37°C for 24hours.

The isolates were identified by standard biochemical tests and Antibiotic susceptibility testing was done according to CLSI guidelines 2014 using Kirby Bauer disk diffusion method.[8]

Biofilm production of these isolates were tested using three methods, Congo Red Agar (CRA) method (Figure1), Tube method (Figure 2) and Tissue culture plate method (Figure 3) as described by Afreenish Hassan et al.[9]

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RESULTS: Among the 63 patients who were investigated in the present study, 46(73.01%) showed positive culture, while 17(26.98%) had negative culture. 4 out of 63 patients had double growth with two organisms. So total of 50 organisms were isolated from 46 positive cases. Staphylococcus aureus 30(65.21%) was the most frequent isolate obtained followed by Coagulase negative Staphylococcus (CONS) 4(8.69%), Klebsiella species 3(6.52%), pseudomonas species 3(6.52%), Enterococcus species 2(4.32%) and Proteus species 2(4.32%).

The double growth included combination like Eschericia coli and Proteus mirabilis, Citrobacter freundii and Enterococcus faecalis, Proteus mirabilis and Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus. (Table1).

ANTIBIOTIC RESISTANT PATTERNS: 26(86.66%) out of 30 isolates of S. aureus were Methicillin Sensitive Staphylococcus aureus (MSSA), while 4(13.3%) were Methiclin Resistant Staphylococcus aureus (MRSA). Out of 34 Gram positive cocci (S. aureus and CONS) 100% of them were sensitive to Vancomycin and Teicoplanin.

Out of 11 gram negative bacilli (Enterobacteraciae), 100% of them were ESBL and AmpC producers by screening methods. 3(27.27%) out of 11 isolates were MBL producers by screening method. (Resistant to both Imepenem and Meropenem).

Out of 2 Enterococcus obtained, 1 isolate was resistant to Vancomycin (VRE) and high level Gentamycin (HLG).

Out of 3 Pseudomonas obtained, all 3 of them were sensitive to Amikacin, Aztreonam and Imepenem. 2 out of 3 isolates were resistant to ceftazidime. (Table2).

BIOFILM PRODUCTION: 36(72%) out of 50 isolates were biofilm producers by Congo Red Agar (CRA) method, 38(76%) were biofilm producers by Tube method (TM) and 42(84%) by Tissue Culture Plate (TCP) method. (Table 3).

AGE DISTRIBUTION: The most common age group in this study was between 41- 50 years (26.08%), followed by 31- 40 years (19.56%), and 21-30 years (15.21%). (Table 4).

GENDER DISTRIBUTION: 40(86.95%) patients out of 46 were males and 6(13.04%) were females.

TYPE OF THE BONE INVOLVED: The most common bone involved in the study was femur in 16 pts (34.74%) followed by tibia in 13(28.26%), fibula, ulna and LL BB 4 pts (8.69%) each. (Figure 4).

TYPE OF WOUND: Among 46 patients 31(67.391%) had clean wounds, 7(15.2117%) had clean-contaminated wounds, 6(13.043%) had contaminated and 2(4.347%) dirty/infected wound. (Figure 5).

CO MORBID CONDITIONS: The most common co morbid condition found in this study was old age in 10(21. 73%), followed by DM in 7(15.217%) and HTN in 5(10.869%) pts. (Figure 6).

DURATION OF PRESENTATION: Wounds were classified based on Trampuz's and Zimmerli's classification.[10] 25(54.347%) out of 46 presented with signs of infections within 2weeks (early)

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following operation, 12(26.086%) presented between 2 weeks to 10weeks (Delayed) and 9(19.565%) after 10 weeks (late) of operation.

DISCUSSION: Orthopedic implant site infections continue to be a diagnostic and therapeutic challenge. It is much more complicated by the formation of biofilm leading to burden in antibiotic selection and prolonged antimicrobial therapy due to emergence of multidrug resistant pathogens. Collection of sample for the diagnosis has utmost importance as it has influence on culture positivity. Different methods of sample collection include direct swabs, periprosthetic fluid sampling and sampling the implant after sonication.[11] It is said that with sonication the culture positivity rate can be increased from 84.2% to 94.7%. In our study the culture positivity was found to be 73.01% which is less when compared to other studies where Anisha Fernandez et al[2] reported 84% and Khosravi et al,[4] Vishwajith et al[1] reported the culture positivity of 93.9% and 94.89% respectively. However Gomez et al[12] reported even lesser positivity of 60%. Most of the samples in our study were direct swabs which could have contributed to the low positivity rate.

From our study it was found that the most common organism causing orthopedic implant site infection is Staphylococcus aureus which correlates with most of the other similar studies like Anisha Fernandez et al,[2] Khosravi et al[4] and Vishwajith et al.[1]

The second most common organism isolated was CONS and Eschericia coli. CONS is a normal skin commensal which could have reached the surgical site due to improper disinfection of the skin during surgery or it might be due to improper collection of sample for the diagnosis. The pattern of organism obtained suggest the role of nosocomial pathogens which were present in the operating room or in the post-operative wards where patients are monitored along with regular dressings at frequent intervals. One of the drawback of the study was not to culture for anaerobic organisms which can also cause implant site infections mostly beyond 24 months of the surgery,[4] however no patient in the present study presented after that duration. Most of the patients had history of antibiotic treatment in the recent past which is again a factor against isolation of anaerobes.[13]

The antimicrobial susceptibility patterns revealed a high level multidrug resistance in the gram negative isolates which were sensitive only to Amikacin, Imepenem and Meropenem. As a prophylaxis, Cephalosporins were given in our hospital, to which all the gram negative isolates were resistant. So by this study we suggest the use of Amikacin in patients with normal renal parameters or Imepenem if the patient has a renal system compromise for the treatment. Most of the gram positive cocci including S. aureus and CONS were sensitive to methicillin; hence we suggest the use of cloxacillin rather than using higher antibiotics like Vancomycin and Linezolid. Whenever a orthopedic implant site infection is suspected patient can be started on a combination of Imepenem and Cloxacillin for covering both Gram positive cocci and Gram negative bacilli till the sensitivity pattern is available.

In our study 25(54.34%) of the patients presented with early infection, similar finding was seen in the study by Khosravi et al[4] where 72.9% of patients presented with early infection. This finding suggests that the implant site infections are usually acquired during surgery by a less virulent organism. It can also be acquired by hematogenous route from remote infections. In our study 2 patients had hematogenous spread as confirmed by positive blood culture. Both the blood culture positive patients had Eschericia coli in the blood stream probably due to the presence of concurrent UTI at the time of surgery.

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In this study male preponderance with 86.95% was seen for the development of the implant site infection, where more than 50% of the infections followed surgeries for 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. Old age was the common risk factor found in our study followed by Diabetes Mellitus and Hypertension. Though old age alone cannot be considered as a single risk factor, these patients had other co morbidities like DM, HTN and immunosupression.

In our study Biofilm production was done using three methods CRA, TM and TCP. There was no significant difference between CRA and TM in detecting the production of biofilm. Like in other studies TCP was the most sensitive in detecting weak, moderate and strong biofilm producers. 84% of the isolates were biofilm producers by TCP among which S. aureus was the predominant pathogen probably because it was the most common organism isolated in the culture. This bioflim production probably explains the longer duration of antimicrobial therapy and longer hospital stay in our patients leading to increased morbidity.

CONCLUSION: From this study we can conclude that orthopedic implant site infection is a diagnostic and therapeutic challenge which can pose a serious threat to the patient leading to high morbidity. Emphasis has to be given to assessment of the risk factors, type of wound, pre and post antibiotic prophylaxis for the effective prevention of implant site infections. Staphylococcus aureus (MSSA) is the most common organism causing orthopedic implant site infections. Local sensitivity pattern is required for the selection of antibiotics in treatment of the same. These organisms often have the potential to produce biofilm. Universal precautions have to be more strictly adhered to in OTs and post-operative wards with primary importance given to hand washing. More studies with bigger sample size and longer period of study are required for proper assessment of data.

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Figure 1: Congo Red Agar Method

Figure 2: Tube Method

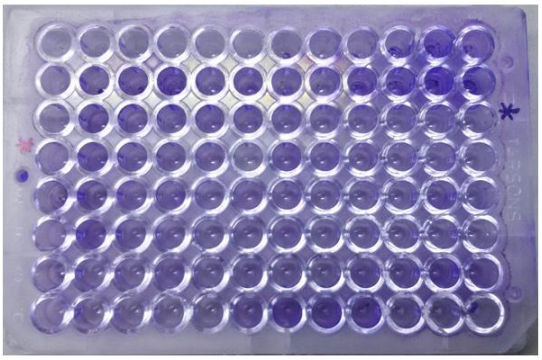

Figure 3: Tissue Culture Method

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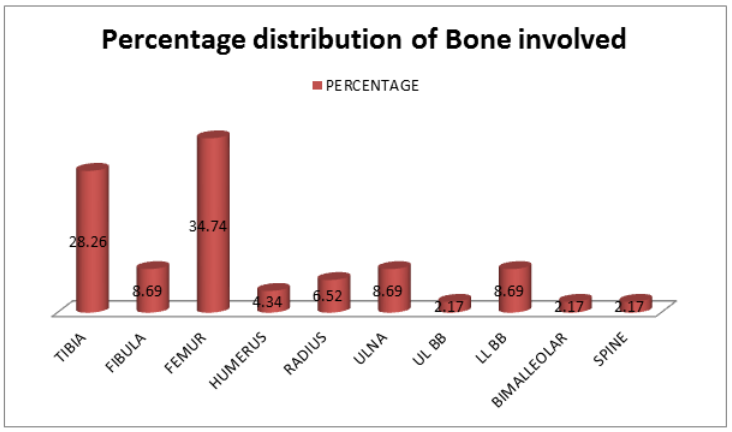

Figure 4: Percentage distribution of type of wound

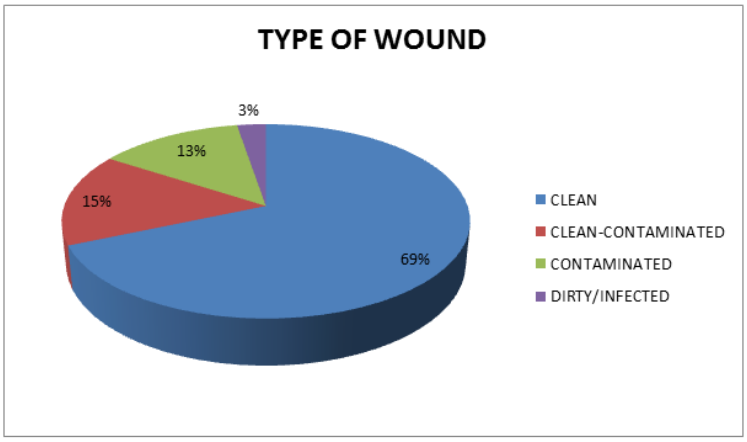

Figure 5: Percentage distribution of bone involved

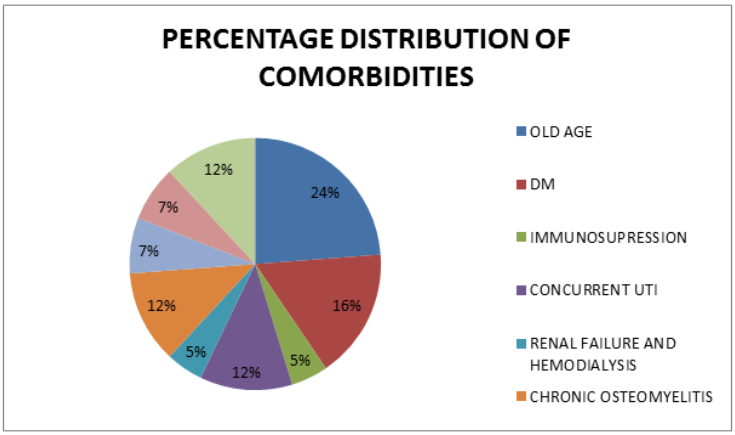

Figure 6: Percentage distribution of comorbidities

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ORGANISM	PERCENTAGE OF PATIENTS, N=46
Staphylococcus aureus	30(65.21%)
CONS	4(8.695%)
Enterococcus species	2(4.347%)
Eschericia coli	4(8.695%)
Proteus species	2(4.347%)
Klebsiella species	3(6.521%)
Pseudomonas species	3(6.521%)
Citrobacter species	2(4.347%)

Table 1: Percentage distribution of organisms causing infection

Antibiotics	GPC (n=34)	GNB (n=11)
Penicillin	29(85.29%)	-
Augumentin	22(64.70%)	-
Amikacin	-	4(36.36%)
Gentamicin	14(41.17%)	10(71.42%)
Cefotaxime	-	11(100%)
Cefoxitin	4(11.76%)	11(100%)
Ceftazidime	-	11(100%)
Ceftrioxone	-	11(100%)
Ciprofloxacin	20(58.82%)	9(81.81%)
Linezolid	0	-
Teicoplanin	0	-
Imepenem	-	3(27.27%)
Meropenem	-	3(27.27%)

Table 2: Percentage distribution of Antibiotic Resistant Patterns

METHODS	PERCENTAGE OF ISOLATES, N=50
Congo Red Agar method	36(72%)
Tube method	38(76%)
Tissue culture method	42(84%)

Table 3: Percentage distribution of isolates producing biofilm by 3 different methods

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AGE GROUPS (YEARS)	NO. OF PATIENTS, n=46
< 10	0
10-20	4
21-30	7
31-40	9
41-50	12
51-60	4
61-70	3
71-80	5
81-90	1
>90	1

Table 4: Age distribution of patients

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