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RESEARCH ARTICLE

PROSPECTIVE STUDY OF MICROBIOLOGY OF BIOFILM IN ORTHOPAEDIC IMPLANT SURGERY

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Abstract

Background: Infection remains a major obstacle leading to implant failure, increased morbidity, and mortality. Implant-related infections continue to pose a problem for orthopaedicians. 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 isolate and identify the Bacteriological agent forming Biofilm in orthopaedic implant infection and their antibiotic sensitivity.

Methods: In a prospective study, 100 patients who underwent orthopaedic implant removal surgery from Dec 2019 – to Sept 2021 were enrolled; 82 patients' culture reports came out positive.

Results: Of the 100 samples, 82(82%) were culture positive, and 18(18%) were culture negative. The femur was the most commonly affected bone in both males (median age–37.1yrs) and females (median age–41.3 yrs). *Pseudomonas Aeruginosa* was the organism that was most commonly isolated, followed by *Staphylococcus Aureus* and *E.coli*. No anaerobes were isolated. Aggressive antibiotic therapy was often found inadequate to eliminate the infections caused by the biofilm-forming organism. Conservative surgical treatment was associated with treatment failures.

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

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Introduction:-

Globally, an increasing number of people are facing bone and joint problem, that are due to aging or accidents. Both elective and emergency orthopaedic surgeries involve cutting, drilling, or instrumenting the bone either for joint replacement or for realignment of long bones. So, a breach in the sterile procedure may happen, which enhances the microbial infection at the surgical site. Orthopaedic implants are placed in situ for a longer duration of time – often for a period of 1-3 years, imposing a risk of infection. Such Implants Associated Infection‖ may result in implant failure, sometimes leading to amputation and death.

Implant surgery is main-stream in orthopaedic operations for successfully alleviating the pain and improving the mobility in damaged joints. Infections associated with fracture fixation can occur exogenously in cases of open trauma, during insertion of the fixation device, or during disturbed wound healing [1-3]. Stainless steel implants are associated with significantly greater infection rates than titanium implants[4,5].A possible reason for this could be that soft tissue reaction to steel implants is the formation of a fibrous capsule, enclosing a liquid-filled void [6,4]. Bacteria can spread and multiply freely in this non-vascularized space, which is also less accessible to the host defense mechanisms. Prevention of initial bacterial adhesion is of utmost importance since mature biofilm is very difficult to treat.

In Implant associated infection, the organisms adhere to the implant's surface and some isolates may interact with the host proteins to form BIOFILMS. This leads to the chronicity of infections and sometimes to the development of resistance to antimicrobial therapy. The most common organisms that are isolated among the Implant infections are *Staphylococcus aureus*, and a few gram-negative bacilli like *(Pseudomonas Aeruginosa, E.coli, Klebsiella sp., Proteus sp*.).These organisms have the ability for biofilm formation.

Infections are devastating complications after such surgery. In the past century, the incidence of infection has drastically reduced due to the modern theatre facilities and the aseptic measures. Yet they still stance a problem in the developing countries, with high morbidities and substantial costs. Combative therapeutic options such as prolonged and high-end antibiotics, additional surgeries, and prolonged rehabilitation are associated with complications that require prolonged hospitalization with a possibility of a renewed disability. The health care costs of the revision surgeries are high and the chances of infection are higher than with primary surgeries, thus burdening both the patients and the treating hospitals.

Material And Methods:-

The Aim of the study was to find out the Microbiology of biofilm in orthopaedic implant infection and their sensitivity.

The study was conducted at The Department of Orthopaedics & The Department of Microbiology at Netaji Subhash Chandra Bose Medical College And Hospital, Jabalpur (M.P).A total of 100 samples of post-operative orthopaedic implant surgery infections included.

Technique –

Infected tissue material and pus sample was taken in a vial from the interface of plate and bone and kept in a sterile vial mixed with normal saline (Fig 1)

Fig 1:- Biofilm in sterile vial mixed with normal saline.

Biofilm Taken Out From Implant Removal Surgeries

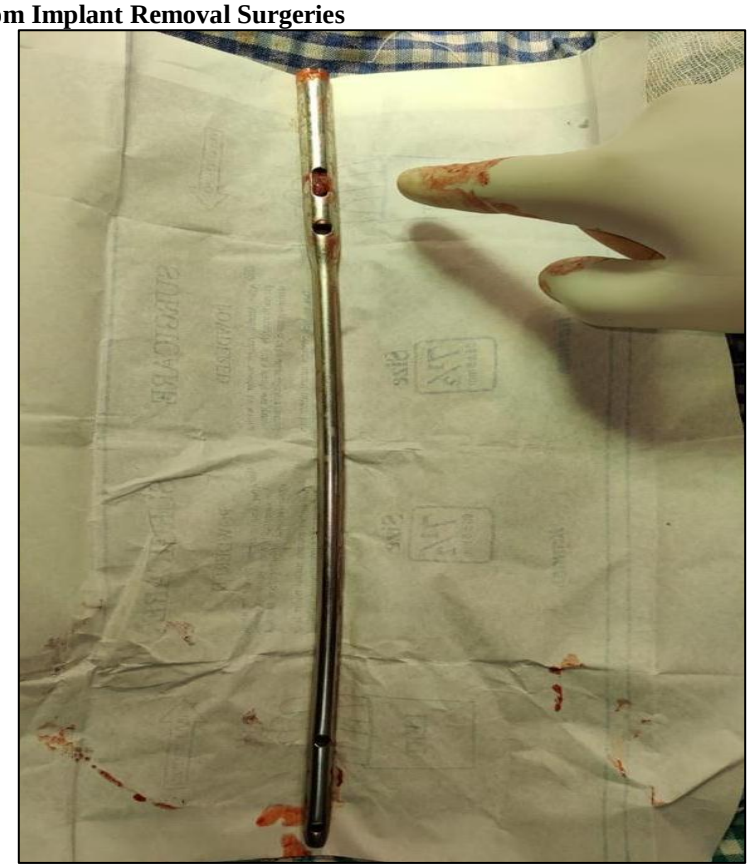

Fig 2:- Femur interlocking nail with Biofilm in the screw hole.

Fig 3:- Intra op. image of Raft plate Removal showing Biofilm adherent at screw insertion site.

In plating Biofilm adherent to bone outer surface and plate inner surface and in nail biofilm adherent to screw hole was taken out with all aseptic precautions and kept in a sterile vial mixed with normal saline.

Processing Of Samples:

The sample was inoculated into the Nutrient agar plate, Blood agar plate, and Mac Conkey agar plate and incubated at 37 degrees Celsius for 24 hours. After that sample was processed for –

1. Gram staining
2. AFB staining
3. 10% KOH Mounting
4. Antibiotics sensitivity

Result:-

1. The mean age of subjects in the study was 36.4±11.9 years, the range being 12-62 years. Most common age group of my study was between 21-40 years corresponding to about 47% of total study subject , 2nd most common being those between 41-60 years age accounting for 35% of total study subjects.
2. Of the 100 subjects taken in our study, 69 were male, corresponding for 69% of the study individuals.
3. Out of 100 patients, 16 patients were Smokers; 13 out of 100 (13%) had history of tobacco chewing, 40 out of 100 study individuals had no risk factor.
4. In 100 study subject, 67 (67%) had closed type fracture pattern, 33 (33%) had open grade fracture pattern.
5. In 100 study subject, closed reduction, open reduction and MIPPO technique were used for fracture fixation in 50 (50%), 45 (45%) & 5 (5%) respectively.
6. Of the 100 study subjects, interlocking nail implant 40 (40%) and 36 (36%) plate were removed during surgery.
7. In our study, femur was most commonly infected bone 46(46%) in lower limb while bones of forearm were most commonly infected 10(10%) in upper limb.
8. Of the 100 study subject, stainless steel implants were used in 93 (93%) cases, while Titanium implant were used in 7 (7%) cases
9. In 100 study subjects, the early, delayed and late onset infections occurred in 38 (38%), 30 (33%) and 32(32%) patients respectively.

10. In our study subject, it is inferred that the most of the isolates from orthopaedic implant infections are *Pseudomonas aeruginosa* 36 (43.9%), *Staphylococcus aureus* 24 (30%), *E.coli* 18 (21.9%).
11. In our study clinically Biofilm seen in 97 cases out of 100 implant removal surgery.
12. In 100 implant removal study subjects, 89 89%) had union at fracture site and 11 (11%) had non-union at fracture site.
13. In our study subject, Biofilm are collected under strict aseptic precautions and processed in the diagnostic microbiological laboratory in which 82 (82%) were culture positive and rest were culture negative.
14. In our study subject, *Pseudomonas aeruginosa* are 100 % sensitive to Gentamycin, Imipenem, Levofloxacin, 84% sensitive to Piperacillin+Tazobactam and 50% sensitive to ceftazidime. *Klebsiella pneumonia* is 100% sensitive to Levofloxacin, Cefuroxime and Piperacillin+Tazobactam, 75 % sensitive to Cefepime an Imipenem. *Escherichia coli* are 100% sensitive to cefuroxime, 80% sensitive to Levofloxacin, Imipenem and Piperacillin+Tazobactam. *Staphylococcus aureus* are 100% sensitive to Amoxyclav, Vancomycin, Gentamycin, Doxycycline , 57% sensitive to Azithromycin and Clindamycin.
15. In our study subject, at first follow up after implant removal clinical sign of infection absent in 81(81%) case, 10(10%) cases lost to follow up at 1st follow up time while in 9 (9%) cases sign of infection seen.
16. In our study, at 2nd follow up after implant removal, clinical sign of infection absent in 94 (94%) cases , 4 (4%) cases were lost to follow up at 2nd follow up time, while in 2 (2%) cases signs of infection were seen.

Tables And Graph

Table No.1:- Frequency of study subjects.

Age group	**Frequency**	**Percent**
0-20	15	15
21-40	47	47
41-60	35	35
>60	3	3

Table No. 2:- Frequency Of Bone Affected.

Bone	Frequency	Percent
Femur	46	46
Tibia	29	29
Humerus	8	8
Forearm	10	10
Patella	5	5
Bi-malleoli (Tibia, Fibula)	2	2

Table No. 3:- Infection After Primary Surgery.

Onset	Frequency	Percent
Early (< 3 Weeks)	38	38
Delayed (3-10 Weeks)	30	30
Late (>10 Weeks)	32	32

Table No. 4:- Bacteriological Profile.

Bacteria	Fequency	Percent
Staphylococcus aureus	24	24
E. Coli	18	18
Pseudomonas aeruginosa	36	36
Klebsiella	4	4
No Growth	18	18

Table No.5:- Antibiotics Sensitivity.

Antibiotic disc	Disc content	*Pseudomonas aeruginosa*, n=36		*Klebsiellapneumoniae* n=4		*Staph Aureus* n=24		*Escherichia coli* n=18	
		Isolate	%	isolate	%	isolate	%	isolate	%
Gentamycin	30 µg	36	100 %	-	-	24	100 %	-	-
Levofloxacin	5 µg	36	100 %	4	100%	-	-	14	80%
Cefepime	30 µg	32	89%	3	75%	-	-	10	60%
Cefuroxime	30 µg	24	68%	4	100%	-	-	18	100 %
Imipenam	10 µg	36	100 %	3	75%	-	-	14	80%
Piperacillin+Tazobactam	100 / 10 µg	30	84%	4	100%	-	-	14	80%
Vancomycin		-	-	-	-	24	100 %	-	-
Doxycyclin	30 µg	-	-	-	-	24	100 %	10	60%
Clindamycin	2µg	-	-	-	-	13	57%	-	-
Azithromycin	15 µg	-	-	-	-	13	57%	-	-

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 which stresses the value of an adequate diagnosis, leading to a proper therapy for these patients.

Among the orthopaedic implant infections, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common organisms isolated by using standard microbiological protocols. These organisms are capable of producing Biofilms.

In this study, 100 samples were collected from the patients with the clinical features suggestive of implant-associated infections and processed by using a standard microbiological profile. Of these 100 samples, 82% (82) of the samples showed culture positivity, and 18% (18) samples were culture negative. A study conducted by **Sujata Prasad et al**(7) showed a growth rate of > 80 % and **Marta Ribeiro et al**(8) reported a growth rate of around 80%. The antimicrobial therapy should be stopped at least 2 weeks prior to the tissue sampling for anaerobic cultures. But most of our patients were already on an empirical antimicrobial therapy or they had a history of antimicrobial treatment in the recent past. This could possibly explain why less number of cultures tested positive in our study.

In our study, *Pseudomonas aeruginosa* was isolated from 43.9% of the cases, followed by *Staphylococcus* 30%, *E. coli* 21.9%, and *klebsiella* 4.8%. No organisms were isolated in 18 cases (18%). The present study advanced a different

opinion from various other studies in which Staphylococci was reported as the most prevalent organism by Jain et al (39.27%), **Khan et al** (50%), **Lidgren et al** (67%), and **Tago et al** (67.30%), (9,10,11,12)

In our study, among the organisms isolated, 87.5% are Gram-negative bacilli and 12.5% are gram-positive cocci. While in **Dorotateterycz** (13) where the gram-positive organism accounts for 76% and **Morrad Mohammed et al**(14) gram-negative bacilli accounts for 23%.

In our study incidence of Early, Delayed, and Late infection in patients was 38%, 30%, and 32% respectively. **Khan et al and Thool et al** reported equal numbers of early, delayed, and late infections. (10,15)**Raf et al, Lidgren et al** found more delayed infections (16,11), and **Jain et al** found more early infections followed by late infections(9).

In our study, among closed-type fractures, 36 (53.7%) plate fixation developed SSI. While overall in all types of fracture, Interlocking nail fixation (40%), Plate fixation (36%) developed SSI. **Nazri et al and Khan et al** found more infection with plates (17,10). Maybe it was due to the softer tissue exposure and compromise of sterility with plate fixation which generally needed an open procedure.

In our study, Interlocking nail fixation was in majority to develop SSI because it was even used for open grade type 1 fracture fixation, which would be a possible reason for implant infection.

In our study, Femur (n=46, 46%) was the most commonly infected bone, and in lower limb fractures 75 (75%) developed SSI. Similar results were reported by **Khosravi et al, Kumar et al, Nazri et al** and **Raahave** found more post-operative infection rates in the lower extremity (18,19,17,20), and **Lidgren et al** found higher infection rates after operation in the hip region(11) **Bubbar** compared different regions but found no difference (21).

Out of 100 cases that were studied, 33(33%) were open fractures with 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.

In our study, males had a preponderance of Osteo-articular Infections (69%), who were mainly young adults in association with Road Traffic Accidents. These were mainly open fractures with soft tissue damage, hematoma formation, and wound contamination, which led to the spread of the bacteria to the bone.

In 100 implant removal study subjects, 89(89%) had a union at the fracture site, out of which 5 developed osteomyelitis as a complication, and 11 (11%) had non-union at the fracture site.

In our study, Pseudomonas aeruginosa was 100 % sensitive to Gentamycin, Imipenem, Levofloxacin , 84% sensitive to Piperacillin+Tazobactam and 89% sensitive to cefepime. Klebsiella pneumonia is 100% sensitive to Levofloxacin,Cefuroxime, and Piperacillin+Tazobactam,75 % sensitive to cefepime imipenem. Escherichia coli were 100% sensitive to cefuroxime, 80% sensitive to Levofloxacin, Imipenem and Piperacillin+Tazobactam . Staphylococcus aureus was 100% sensitive to Amoxyclav, Vancomycin, Gentamycin, and Doxycycline, and 57% sensitive to Azithromycin and Clindamycin. **Sujatha et al**(7) showed in their study that Pseudomonas aeruginosa was 100 % sensitive to Amikacin, Imipenem, Meropenem,66% sensitive to Ciprofloxacin, and 50% sensitive to Ceftazidime. Klebsiella pneumonia is 100% sensitive to Imipenem, Meropenem, and Piperacillin+Tazobactam,50% sensitive to cefotaxime, and 25% sensitive to amikacin. Escherichia coli are 100% sensitive to amikacin, cefotaxime, Imipenem, Meropenem, and Piperacillin+Tazobactam. **Borgohain Munin et al**(22) study shows that most of the Staphylococci aureus were resistant to Penicillin, and most of them were sensitive to Linezolid, Tetracyclines, and Vancomycin. Pseudomonas showed good sensitivity towards Colistin, Tigecycline, and most resistance to Cephalosporins, Amikacin, and Gentamycin. Imipenem, Meropenem, and Piperacillin+Tazobactum showed moderate sensitivity. Klebsiella was found to show good sensitivity to Colistin, Tigecycline, Minocycline, and Levofloxacin. Meropenem and Imipenem showed moderate sensitivity. Klebsiella species showed an antibiogram pattern almost similar to Pseudomonas.

Conclusion:-

Pseudomonas aeruginosa is the most common organism followed by *Staphylococcus aureus* and *E Coli*, which is encountered among the orthopaedic implant infections at Netaji Subhash Chandra Bose Medical College, Jabalpur.

Pseudomonas aeruginosa, *E. coli*, and *Klebsiella* are sensitive To Imipenem, Gentamycin, Levofloxacin, and Cefuroxime while *Staphylococcus aureus* is sensitive to Amoxyclav, Vancomycin, Gentamycin, and Doxycycline.

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