ORIGINAL ARTICLE

Detection of Biofilm Production and Antibiotic Resistance Pattern in Clinical Isolates from Orthopaedic Implants Associated Infections

R Shakthi[1], D Venkatesha[2]

ABSTRACT

Introduction: Orthopaedic Implant associated infection is the major problem which leads to implant failure and economic burden to the patient. Implant-associated infections are due to the formation of biofilm at the implantation site leading to multidrug resistant organism. **Aim:** This study was done to evaluate the causative organisms, their antibiotic sensitivity pattern and their ability to form biofilm over the Implants used in Orthopaedic surgeries. **Material and methods:** This study was carried out in the department of Microbiology, Adichunchanagiri institute of Medical sciences from September 2019 to August 2022. Swabs from 100 patients who had undergone orthopaedic implant or prosthetic surgery and presented with signs and symptoms of infection were collected and processed as per standard procedures. All the isolates were subjected to detect biofilm production by Congo red agar method, MRSA detection by Cefoxitin disc diffusion test, vancomycin resistant *Enterococcus* by using Vancomycin (Minimum inhibitory concentration) MIC strips and Extended spectrum β lactamases (ESBL) production by phenotypic confirmatory combined-disc test. **Results:** Out of the 100 samples processed, culture positivity was observed in 72 specimens. Among them, *Staphylococcus aureus* 19(26%) was the predominant isolate and 44 (61%) are biofilm producers. 17 (89%) were MRSA (Methicillin resistance *Staphylococcus aureus*) strains and 15(37%) were ESBL producing strains. **Conclusion:** The appropriate pre- and post- operative care should be taken to prevent orthopaedic implant associated infections. *Staphylococcus* spp was the commonest isolate and its ability to produce biofilm indicates the need for an appropriate antibiotic policy and screening for MRSA carriers to reduce the infection.

KEY WORDS: Orthopaedic implant, Infection, Biofilm, MRSA, ESBL.

Introduction

In modern era, Prosthetic replacements and Implant surgery has become one of the commonest Orthopaedic operation which alleviates the pain and improve the mobility in damaged joints. But post-operative infection is a devastating complication[1]. Detection of infection after Prosthetic replacements and Implant surgery is a challenge for management and prevention of complications[2]. Infection can extend the patients' hospital stay, cost of treatment, increased mortality and morbidity, increased risk of readmission and debridement[3,4]. The prevalence of orthopaedic implant site infection reported in India is about 2.6%.[5] These infections are classified in to three stages, i.e., early (less than two weeks), delayed (2 to 10 weeks) and late (more than 10 weeks) infection.[6]

The sources of the pathogens can be either endogenous or exogenous, but most of them are from the endogenous bacterial flora under certain favourable conditions[7,8]. The common micro-organisms causing infections are *Staphylococcus aureus*, coagulase negative *Staphylococcus* spp (CONS), *Enterococci*,

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[1]Associate Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G.Nagara, Mandya, Karnataka, India, [2]Professor and Head , Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G.Nagara, Mandya, Karnataka, India

Address for correspondence:
R Shakthi, Associate Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G.Nagara, Mandya, Karnataka, India. E-mail: drshakthir@bgsaims.edu.in

Escherichia coli, *Klebsiella*, *Proteus mirabilis* and *Pseudomonas*[9]. Implant-associated infections are the result of adhesion of bacteria to implant surface and subsequent biofilm formation at the implantation site. Once biofilm is formed it is difficult by host defence and ordinary antimicrobial therapy to remove it[10].

These days, most of the bacterial isolates are showing resistance to the major first line drugs. However, each hospital has different microbial flora and show different antibiotic susceptibility pattern so that empirical antibiotic therapy. Hence, this study was conducted to evaluate the prevalence of causative organisms, their antibiotic sensitivity pattern and their ability to form biofilm over the implants used in Orthopaedic surgeries.

Material and Methods

The present study was conducted in the department of Microbiology, Adichunchanagiri institute of Medical sciences for a period of 3 years from September 2019 to August 2022. Sample size was calculated using prevalence study formula $n=Z^2P(1-P)/d^2$, where n = Sample size, Z = statistic for a level of confidence (1.96 for 95% confidence level), P = Expected prevalence or proportion, and d = Precision.[11]

The study group comprised a total of 100 patients who had undergone orthopaedic implant or prosthetic surgery and presented with signs and symptoms of infection. Informed written consent from patients and ethical clearance from the institution were obtained for the study. The patients particular were recorded on a prescribed proforma which included name, age, sex, diagnosis, comorbidity, smoking history, nutritional status, type of implant as variables.

All patients were given first generation cephalosporins as a standard prophylactic intravenous antibiotic in the operating room. Patients were observed for postoperative wound infection till discharge. The follow up was done up to three months according to a protocol, first visit after two weeks and subsequent visits on monthly basis.

The diagnosis of infection was based clinical observations. Orthopaedician collected the samples from the discharges which were adjacent to the infected implant and tissue, using sterile cotton swabs or a sterile disposable syringe and was immediately transported to the laboratory for culture and antibiotic sensitivity testing. All the samples were subjected to Gram staining and ZN staining. For isolation of aerobic bacteria, the sample was inoculated onto blood agar and MacConkey agar and incubated at 37 °C for 24 to 48 hours. For isolation of anaerobic bacteria, the sample was inoculated in Robertson's cooked meat broth.[12]

All positive cultures were processed for the identification of isolates, antibiotic susceptibility tests and biofilm detection.

Figure 1: Blood agar showing bacterial growth

Figure 2: MacConkey agar plate showing positive and negative culture of specimen

Antibiotic susceptibility testing was carried out by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute guidelines (CLSI), with *Staphylococcus aureus* American type culture collection (ATCC) 25923 and *Escherichia coli* ATCC 25922 as control strains[13]. All the confirmed *S. aureus* isolates and coagulase negative *Staphylococcus* spp. (CONS) strains were screened for methicillin resistance using Cefoxitin (30μg) disc (HiMedia Lab. Pvt Ltd), *Enterococcus* isolates were tested for vancomycin sensitivity by using Vancomycin MIC strip (HiMedia Lab. Pvt Ltd). All Gram negative organisms were screened for ESBL production by phenotypic confirmatory combined-disc test[12,14].

Congo red method was used for the detection of biofilm with *S. epidermidis* ATCC 35984 and *S.*

epidermidis ATCC12228 as positive and negative controls respectively. Isolates were considered as strongly positive if there is a black colony with dry crystalline consistency.[12,14]

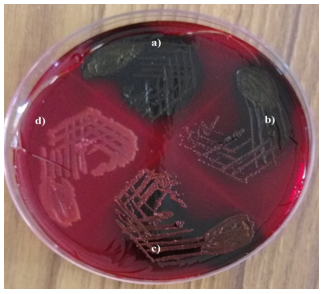

Figure 3: Biofilm film detection by Congo red agar method: a) Strong biofilm producer; b) Moderate biofilm producer; c) Weak biofilm producer; d) Non biofilm producer

Statistical Analysis

Statistical analysis was done using Microsoft excel and Statistical package for social sciences (SPSS) - 14 software. Data were calculated and interpreted as percentage.

Results

Among 100 Patients, majority 84 (84%) were male and 16 (16%) were female. Majority of patients (25%) were of 31-40-year age group followed by 41-50 year (22%).

Table 1: Age and gender distribution of cases

Age (Years)	Male (%)	Female (%)	Total Number (%)
<10	0	0	0
10-20	5	3	8
21-30	12	3	15
31-40	19	6	25
41-50	20	2	22
51-60	17	1	18
≥61	11	1	12

Out of 100 patients investigated, 62 (62%) were with intra-medullary nail, 32 (32%) with plate and 6 (6%) with K-wire.

Among 100 patients, 72 (72%) were culture positive and 28 (28%) were culture negative.

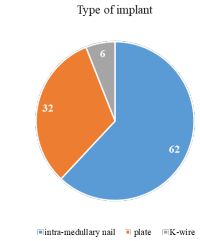

Figure 4: Types of implant

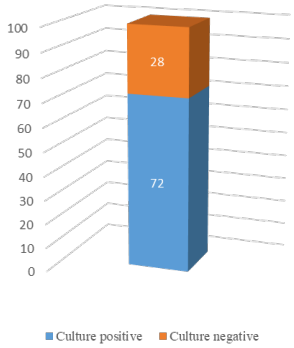

Figure 5: Number of Culture positive and negative of samples

Out of 72 culture positive patients, 31 specimens yield Gram positive cocci and 41 specimens yield Gram negative bacilli. *Staphylococcus aureus* 17 (26%) is the most common organism isolated in culture followed by *Pseudomonas, Klebsiella, Proteus, E. coli, Citrobacter*.

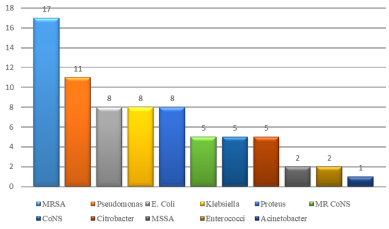

Figure 6: Spectrum of microorganism in implant associated infection

Among 72 isolates, 44 (61%) are biofilm producers and 28 (39%) were non-biofilm producers. Out of 44 biofilm producers, *S. aureus* 13 (30%) is the most common.

All Gram-positive isolates were susceptible to linezolid, vancomycin and Teicoplanin. Most of the Gram-negative isolates showed maximum suscepti-

Table 2: Antibiotic susceptibility pattern of Gram positive cocci

	Antibiotics								
Organisms	**AMC (%)**	**GEN (%)**	**COT (%)**	**CIP (%)**	**LZ (%)**	**PIT (%)**	**TEI (%)**	**VA (%)**	**HLG (%)**
MRSA (n=17)	0	10 (59)	08 (47)	12 (71)	17(100)	8 (47)	17(100)	17(100)	-
MSSA (n=2)	2(100)	2 (100)	01 (50)	1(50)	2 (100)	2 (100)	2 (100)	2 (100)	-
CONS (n=5)	3 (60)	3 (60)	03 (60)	3 (60)	5 (100)	4 (80)	5 (100)	5 (100)	-
MRCONS (n=5)	0	2 (40)	02 (40)	3 (60)	5 (100)	3 (60)	5 (100)	5 (100)	-
Enterococci (n=2)	2 (100)	-	-	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)

Table 3: Antibiotic susceptibility pattern of Gram negative bacilli

	Antibiotics							
Organisms	**AMC (%)**	**CAZ (%)**	**CFM (%)**	**COT (%)**	**CIP (%)**	**G (%)**	**PIT (%)**	**IPM (%)**
Pseudomonas (n=11)	5 (45)	9 (82)	9 (82)	5 (45)	8 (73)	8 (72)	9 (82)	10 (91)
E. coli (n=8)	3 (38)	5 (63)	7 (88)	4 (50)	4 (50)	6 (75)	6 (75)	7 (88)
Klebsiella (n=8)	4 (50)	3 (38)	5 (63)	3 (38)	4 (50)	5 (63)	7 (88)	7 (88)
Proteus (n=8)	2 (25)	4 (50)	4 (50)	2 (25)	4 (50)	6 (75)	6 (75)	8 (100)
Citrobacter (n=5)	2 (40)	3 (60)	2 (40)	3 (60)	3 (60)	3 (60)	4 (80)	4 (80)
Acinetobacter (n=1)	0	1(100)	1 (100)	0	1 (100)	1(100)	1 (100)	1 (100)

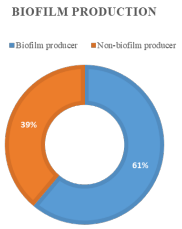

Figure 7: Number of organisms producing biofilm

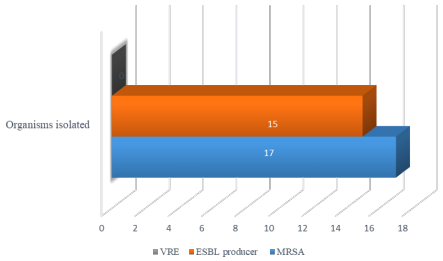

Figure 8: Resistance pattern among isolates

bility to Imipenem and piperacillin-tazobactam.

Out of 19 *S. aureus* isolates, 17 (89%) were MRSA strains. Out of 41 Gram- negative bacilli, 15 (37%) were ESBL producing strain with *Klebsiella* 6 (40%) being major organism followed by *Pseudomonas* 4 (26%) and *E. coli* 3 (20%). All Enterococcal isolates were sensitive to Vancomycin, no VRE (Vancomycin resistant *Enterococci*) was isolated.

Discussion

Despite great advance in antimicrobial therapy, Orthopaedic implant site infection are the major cause of treatment failure and morbidity in patients. Implant-related infections continue to pose a problem for the Orthopaedician.

In this study, the prevalence of implant site infection from clinically suspected case was 72% (Figure 2), which is less compared to studies of Suneet T, et al. (77.98%), Anisha Fernandez, et al. (84%) and more compared to Gomez et al. (60%)[15–17]. Many studies conducted in India reported prevalence rate between 60-95%, this variation could be due to misdiagnosing and under-reporting of cases[15].

In the present study, majority of patients were from 31- 40-year age group, which is in concordance with other studies[15,18], this may be due to high activity of the individuals for their daily routine work and have high risk to sustain injury and road traffic accidents.

In our study, among culture positive isolates, majority were Gram negative bacilli (41%) compared to Gram positive cocci (31%), these finding is similar to the studies of Suneet T, et al. (60% & 38.80%) and Khosravi, et al. (64.5% &33.5%)[15,19]. *Staphylococcus aureus* (26%) was the most common organism isolated in culture followed by *Pseudomonas* (24%), which correlates with the studies of Khosravi, et al. (28.35%), Suneet T, et al. (28.35%) and Jain, et al. (26.6%)[15,19,20]. Many studies have reported *Klebsiella* has second most common isolate, this variation in different isolates could be due to different nosocomial pathogens present in operating theatre of hospitals.[20–22]

In this study, isolated organisms showed resistance to common used antibiotics. Most of the *S. aureus* were sensitive to linezolid, vancomycin, Teicoplanin and Pipericillin-tazobactum. CoNS were highly sensitive to linezolid, vancomycin, Teicoplanin (100%) followed by Pipericillin-tazobactum (80%), whereas *Enterococci* showed 100% susceptibility to most of the antibiotics. Among *S. aureus*, the high level resistance to commonly used drugs may be due to presence of MRSA (89%) and biofilm production (30%). Higher prevalence of MRSA has been reported by Jain, et al. (40%) and Satya Chandrika V, et al. (64%)[20,23]. Hence, vancomycin, linezolid, Teicoplanin can be used in the regimen for treatment of orthopaedic implant infection, especially in case of MRSA and VRE. In case of Gram negative organisms, majority were susceptible to Imipenem and piperacillin-tazobactam and resistance to common used antibiotics, this may be due to the presence of ESBL and biofilm production.

In our study, among 72 isolates, 44 (61%) showed biofilm production. *S. aureus* 13(30%) was the most common isolate. Majority of *S. aureus* were MRSA strains whereas, among Gram negative bacilli 15 (37%) were ESBL producers and resistant to commonly used antibiotics. The prevalence of ESBL is1-3 similar to the study of Anisha Fernandes, et al. (31.7%)[16]. Our study showed increase in prevalence of Gram negative organism infection with multidrug resistance. This high level of resistance could be due to production of biofilm and it leads to long term antimicrobial therapy, prolonged stay in hospital, increased cost, morbidity, treatment failure and removal or replacement of implants and prosthesis.

There are few limitations of this study. Even though the study period is for 3 years, we have followed up postoperative patients for only 3 months, but in implant surgeries infection can develop 1 year long after surgery and demographic characteristics of hospital population may be changed.

Conclusion

Treating the Orthopaedic implant associated infection is a great challenge. Improper administration of antibiotics will lead to antibiotic resistance, hinder the cure and prolongs the hospital stay, there by increases the morbidity and economic burden to the patient.

It is high time that all clinical laboratories should start detecting the resistant profile routinely. So early intervention by proper selection of antibiotics according to culture and sensitivity plays a key role. MRSA is the commonest isolate in our study. So prevention of Orthopaedic Implant associated infection, screening of MRSA carrier in hospital worker, pre-operatively in patients and adequate intra-operative and post-operative measures should be taken to prevent MRSA infection.

A strict adherence to the antibiotic policy of the institution and multidisciplinary cooperation involving the orthopaedic surgeon and Microbiologists will reduce the incidence of orthopaedic implants site infection.

References

1. Al-Mayahi M, Betz M, Müller DA, Stern R, Tahintzi P, Bernard L, et al. Remission rate of implant-related infections following revision surgery after fractures. International Orthopaedics . 2013;37:2253–2258. Available from: https://doi.org/10.1007/s00264-013-2092-1.
2. Drago L, Clerici P, Morelli I, Ashok J, Benzakour T, Bozhkova S, et al. 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. Journal of Clinical Medicine. 2019;8(7):1–16. Available from: https://doi.org/10.3390/jcm8070933.
3. Najjar Y, Saleh M. Orthopedic Surgical Site Infection: Incidence, Predisposing factors, and Prevention. International Journal Of Medical Science And Clinical Invention. 2017;4(2):2651–2661. Available from: http://dx.doi.org/10.18535/ijmsci/v4i2.04.
4. Shah MQ, Zardad MS, Khan A, Ahmed S, Awan AS, Mohammad T. Surgical site infection in orthopedic implants and its common bacteria with their sensitivities to antibiotics, in open reduction internal fixation. Journal of Ayub Medi-

cal College Abbottabad. 2017;29(1):50–53. Available from: https://jamc.ayubmed.edu.pk/jamc/index.php/jamc/article/view/900/864.
5. Rajkumari N, Gupta AK, Mathur P, Trikha V, Sharma V, Farooque K, et al. Outcomes of surgical site infections in orthopedic trauma surgeries in a tertiary care centre in India. Journal of Postgraduate Medicine. 2014;60(3):254–259. Available from: https://doi.org/10.4103/0022-3859.138731.
6. Brewer GE. Operative surgery at city hospital with a preliminary report on the study of wound infection;vol. 5. New York, USA. D. Appleton and Company. 1990. Available from: http://resource.nlm.nih.gov/101752578.
7. Hadadi A, Zehtab MJ, Babagolzadeh H, Ashraf H. Contributing Risk Factors for Orthopedic Device Related Infections in Sina Hospital, Tehran, Iran. 2011;13(2):117–122. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3371915/.
8. Asres GS, Legese MH, Woldearegay GM. Prevalence of Multidrug Resistant Bacteria in Postoperative Wound Infections at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Archives of Medicine. 2017;9(4):1–9.
9. Lakshminarayana SA, Kumar DCS, Prakash R, Sangeetha S. Bacteriological Profile of Orthopedic Patients in a Tertiary Care Hospital, Bengaluru. International Journal of Science and Research. 2015;4(6):1504–1508. Available from: https://www.ijsr.net/archive/v4i6/SUB155550.pdf.
10. Campoccia D, Montanaro L, Arciola CR. The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials. 2006;27(11):2331–2339. Available from: https://doi.org/10.1016/j.biomaterials.2005.11.044.
11. Daniel WW, Cross CL. Biostatistics: A foundation for analysis in the health sciences. 10th ed. New York, USA. John Wiley & Sons. 2013.
12. Collee JG, Miles RS, Watt B. Tests for the Identification of Bacteria . In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Practical Medical Microbiology. Churchill, Livingstone, New York, USA. Mackie & McCartney. 1996;p. 131–151.
13. Performance Standards for Antimicrobial susceptibility testing. 31st ed. USA. Clinical and Laboratory Standard Institute. 2021. Available from: https://www.treata.academy/wp-content/uploads/2021/03/CLSI-31-2021.pdf.
14. Forbes BA, Sahm DF, Weissfeld AS. Overview of bacterial identification methods and strategies. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby, Missouri, USA. Elsevier. 2007.
15. Tandon S, Pathak A, Mishra SK, Vijayvargiya M. Incidence and Risk Factors for Early Surgical Site Infection in Elective Orthopaedic Implant Surgeries: A Prospective Study. Journal of Evolution of Medical and Dental Sciences. 2015;4(15):2525–2531. Available from: https://doi.org/10.14260/jemds/2015/364.
16. Fernandes A, Das M. The Microbiological Profiles of Infected Prosthetic Implants with an Emphasis on the Organisms which form Biofilm. Journal of Clinical and Research. 2013;7(2):219–223. Available from: https://doi.org/10.7860/JCDR/2013/4533.2732.
17. Gómez J, Rodríguez M, Baños V, Martínez L, Claver MA, Ruiz J, et al. Orthopedic implant infection: prognostic factors and influence of long-term antibiotic treatment on evolution. Prospective study, 1992-1999. Enfermedades Infecciosas y Microbiología Clínica. 2003;21(5):232–236. Available from: https://doi.org/10.1016/s0213-005x(03)72928-0.
18. Shree SR, Prathab AG. Characterization of Aerobic Bacteriological isolates from Orthopedic Implant Site Infections with Special Reference to Biofilm Formation in a Teritiary care Hospital. Journal of Evolution of Medical and Dental Sciences. 2015;4(33):5634–5642. Available from: https://doi.org/10.14260/jemds/2015/825.
19. Khosravi AD, Ahmadi F, Salmanzade S, Dashtbozor A, Montazeri EA. Study of Bacteria Isolated from Orthopedic Implant Infections and their Antimicrobial Susceptibility Pattern. Research Journal of Microbiology. 2009;4(4):158–163. Available from: https://doi.org/10.3923/jm.2009.158.163.
20. Jain BK, Banerjee M. Surgical site infections and its risk factors in orthopedics: A prospective study in teaching hospital of central India. International Journal of Research in Medical Sciences. 2013;2(1):110–113. Available from: https://ijorim.com/siteadmin/article_issue/1368097582Ijorim24.pdf.
21. Classen DC, Kvans RS, Pestotnik SL, Horn SD, Meneove RL, Burke JP. The Timing of Prophylactic Administration of Antibiotics and the Risk of Surgical-Wound Infection. New England Journal of Medicine. 1992;326(5):281–286. Available from: https://doi.org/10.1056/NEJM199201303260501.
22. Efem SEE, Aja A, Inyang U. Surgical wound infection rate in Calabar University Teaching Hospital, Calabar. West African Journal of Medicine. 1991;(SuppI A):367–375.
23. Chandrika V, Kirani KRL. Bacteriological Spectrum of Post-Operative Orthopedic Implant Infections and Their Antibiogram. Journal of Krishna Institute of Medical Sciences University. 2016;5(1):20–26.

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