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ORIGINAL RESEARCH

Prevalence of bacterial isolates causing surgical site infection with an emphasis on their biofilm forming capacity

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ABSTRACT

Background: Biofilm forming bacteria are the cause of many chronic and recurrent infections and are believed to be main factor for the development of non-healing wounds by inducing chronic inflammation. Therefore, early detection ofbiofilm producing bacterial infection along with their antimicrobial susceptibility pattern is needed for initiation of appropriate therapy. A prospective study was planned to detectbiofilm production in surgical site wound isolates and to compare antimicrobial susceptibility pattern of biofilm producing and biofilm non-producing isolates. **Method:** The present study was conducted for six months during which 170 pus isolates obtained from patients having surgical site wounds admitted in various wards in NSCB medical college, Jabalpur (M.P.) were studied. The organism was identified using standard microbiological procedures and AST was done by Kirby-Bauer disc diffusion method in accordance with CLSI guidelines 2023. Biofilm production was detected by Modified Tissue Culture Plate (MTCP) method. **Result and Discussion:** A total of 170 isolates were studied which included *Staphylococcus aureus* (n = 25), Coagulase negative *Staphylococcus* (n=21), *Pseudomonas aeruginosa* (n=44),*Escherichia coli* (n=20), *Acinetobacterbaumannii* (n=20), *Klebsiella*spp. (n=15), *Citrobacter*spp. (n=13), *Proteus* spp. (n=08),*Enterobacter*spp. (n=04). Out of these, 63% isolates showed biofilm production. Also, multidrug resistance (MDR) was observed in 78% of the biofilm producing isolates and 40% of non-biofilm producing strains. The association of biofilm formation and multi drug resistance was found to be statistically significant. **Conclusion:** This study showed significantly high rate of biofilm formation in surgical site infection wound isolates. Also, it was observed that MDR strains were more commonly biofilm producers. Timely identification of these bacterial biofilms in surgical wounds will change the course of treatment thus helping in better patient management.

Keywords: Surgical site infections, biofilm, antimicrobial resistance, modified Tissue culture plate method

INTRODUCTION

Surgical site infections (SSI) are among the most common hospital acquired infections that are associated with significant mortality and morbidity worldwide.[1]The chance of developing SSIafter surgery is determined by the extent of devitalized tissue, presence of excessive dead space or hematoma, pathogenicity of the organism present and size of bacterial inoculum.[2]Based on the extent of tissue infected, SSIs are classified into incisional wounds, which can be superficial or deep, and organ/space SSIs. Superficial and deep incisional SSI involves skin, subcutaneous tissue, fascial and muscle layers at incision site whereas organ/space SSI involves infection in any part of the anatomy in organs and spaces other than the incision, which was opened or manipulated during operation.[3]Postoperative wound infections are mostly caused by patient's own endogenous flora, but can also be caused from exogenous sources.[2]The Centre for Disease Control and Prevention guidelines for the prevention of SSI has recognized *Staphylococcus aureus*, Coagulase negative *Staphylococcus* (CONS), *Enterococcus* spp.,*Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*and *Enterobacter*spp. as most frequently isolated pathogens.[4]Recent studies suggest that biofilm- producing organisms play a significant role in persistent skin and soft tissue wound infections in the postoperative surgical patient population. This is mainly done by inducing chronic inflammation and delaying wound healing.[5]

The term 'Biofilm' is made up of two words 'bio' and 'film'. A film is a thin layer and bio indicates living component of the film. Biofilmare defined

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asmicrobially derived sessile community characterized by cells that are irreversibly attached to biotic/abiotic surface and are embedded in a matrix of extracellular polymeric substances (EPS). Bacterial cells forming biofilmhave an altered phenotype with respect to growth rate and gene transcription. They also exhibit multidrug resistance.[6,7]Biofilms are formed on various environmental abiotic and biotic surfaces. In human body, bacteria are present in various sites where they colonize primarily in the form of biofilms. These include both pathogenic and non-pathogenic flora of skin, oropharynx, nose and intestine.[6]These are also associated with human infections like native valve endocarditis, otitis media, chronic bacterial prostatitis, cystic fibrosis, periodontitis and chronic wounds. They are also found in indwelling biomedical devices like prosthetic heart valves, central venous catheters, orthopedic implants, contact lenses and intrauterine devices.[6,8]

Biofilm producing bacteria cause disease by many mechanisms like detachment of cells from biofilm and gaining access tothe blood stream, endotoxin production, host immune system evasion and gaining antimicrobial resistance through plasmid exchange. Even in immune-competent individuals biofilm infections are difficult to resolve by host defense mechanism as bacteria embedded in exopolysaccharide decrease and delay penetration of antibodies and thus escape the effect of host humoral immune system in response to infection. Also, for the same reason these bacteria in biofilm are less susceptible to antibiotics than their planktonic forms. Thus, antibiotic therapy alone fails to clear biofilms related infections making them serious health issue. Further, it helps in the spread of antibiotic resistant traits in nosocomial pathogens by increasing mutation rates and by exchanging genes responsible for antibiotic resistance.[9,10]Therefore, determining the species present and their relative contributions to biofilms is of great clinical importance. The present study was carried out to investigate the capacity of various bacterial isolates from surgical site wound infections to produce biofilms alongwith their antibiotic sensitivity pattern.

MATERIAL AND METHODS

A prospective study was conducted in the Department of Microbiology, NSCB medical college, Jabalpur (M.P.) over a period of six monthsfrom July to December 2023, after due approval from institutional ethics committee. The pus samples from patients having surgical site infection as per CDC criteria were collected and sentto microbiology laboratory during this period. These sampleswere then processed by standard conventional microbiological techniques.[11-14]

Collection and processing of samples

Skin was cleaned by 2% chlorhexidine and 70% alcohol. Pus was either aspirated in syringe or collected on sterile swab and sent to laboratory. Pus samples received werethen subjected to microscopy and culture. Cultures were performed on blood agar and Mac-Conkey agar plates. Inoculated media were examined for growth after overnight incubation at 37^0C. The evaluation of colony morphology on the plating media was done and the subsequent identification was carried out as per standard microbiological protocol.[11-13]

The antimicrobial susceptibility testing will be performed by Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) as per current Clinical and Laboratory Standard Institute (CLSI) guidelines.[15] The antimicrobial drugs tested for Gram positive organisms were Erythromycin (15µg), Penicillin (10units), Cefoxitin (30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Linezolid (30µg), Doxycycline (30µg), Clindamycin(2µg), Vancomycin (30µg) and Amoxicillin/clavulanic acid (20µg/10µg). Antibiotics for Gram negative organisms were gentamicin (10µg), netilmicin (30µg), amikacin (30µg), piperacillin/tazobactam (100µg/10µg), ciprofloxacin (5µg), imipenem (10µg), meropenem (10µg), cefepime (30µg), ceftazidime (30µg) and aztreonam (30µg). An isolate was considered as Multi-Drug Resistant (MDR) if it was resistant to at least three classes of antimicrobial agents.[16]

Detection of biofilm production – modified Tissue Culture Plate Method (mTCP)[17]

Test organism was inoculated in brain heart infusion (BHI) broth supplemented with 2% sucrose dispensed in glass test tubes. These were incubated overnight at 37˚C. Next day, inoculated broth was diluted in the ratio of 1:100 with fresh broth. 200µL of this diluted culture broth was later added to 96 well- flat bottom, non-adherent, polystyrene tissue culture plates. Inoculated tissue culture plates were further incubated for 24 hours at 37˚C. After incubation, the contents of the wells were removed and wells were washed five times with 0.2 mL of phosphate buffered saline to remove planktonic bacterial forms. Adhered biofilms were treated with 2% sodium acetate for 30 minutes and stained with crystal violet (0.1% w/v) for half an hour. Excess stain was rinsed off with distilled water. Further, 160µL of 33% glacial acetic acid was added into the microwells. After 15 min, OD was taken by an automated micro-ELISA reader at wavelength of 570nm. These OD values were considered as an index of bacterial adhesion and biofilm formation. Biofilm formation was considered as weak/no biofilm formation if OD value was less than 0.266, moderate if OD value was between 0.266-0.532 and strong when OD value was greater than 0.532.

Statistical Analysis

The data was collected on Microsoft Excel spread sheet and doubly checked forerrors. High and moderate biofilm production was considered positive and weak/none biofilm production was considered negative. All the data was analyzed using SPSS

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software. Association of two or more set of variables was analyzed using Chi-square test. A 'p'value <0.05 was considered as statistically significant.

RESULTS

A total of 150non-repetitive samples from post-operative wound infections were included in the study. Male (58%) were predominant as compared to females (42%). Majority of organisms were isolated from 21-30 years age group (44%) followed by 31-40 years age group (20%). This can be due to high outdoor activities in this group of people. Out of total sample, monomicrobial infection were seen in 101 samples (67%) while 34 (23%) showed multi-microbial infection and 15 (10%) samples were culture negative. Total number of isolates obtained were 170. Out of these, 124 were Gram negative bacteria (73%) while 46 were Gram positive (27%). *P. aeruginosa* was the most frequently isolated Gram-negative organism with isolation rate of 25.9% followed by *E. coli* and *Acinetobacter*spp. (both had isolation rate of 11.8%). Organism wise profile of these isolates is shown in table 1.

In the present study, detailed antibiotic resistance pattern in all bacterial isolates that included biofilm producers as well as non-producers were studied. The antibiotic panel was in accordance with CLSI guidelines M100, 33rd edition. *Staphylococcus. aureus*and CONS showed 0% resistance to vancomycin. Linezolid was also found highly effective, with 8% resistance in *S. aureus* and 4% resistance in CONS. Table 2 shows the antibiotic resistance pattern of Gram-positive bacterial isolates in detail.

Antibiogram of the Gram-negative isolates revealed high resistance to routinely administered antibiotics like ciprofloxacin, co-trimoxazole, gentamicin, ceftazidime and doxycycline while carbepenems were found to be the most effective class of antimicrobials. Detailed antibiogram is shown in table 3. Overall prevalence of multidrug resistant strains noted was 64% which was remarkably high.

Modified tissue culture plate method was used for identifying biofilm production. Out of 170 isolates, 107 (63%) showed biofilm production. Biofilm formation among Gram positive cocci was seen more in CONS (72%) as compared to *S. aureus* (56%). Among the Gram-negative organisms, biofilm production was more prevalent in *P. aeruginosa* (75%) followed by *Klebsiella*spp. (66%). Table 4 shows the magnitude of biofilm production among individual bacterial isolates by modified tissue culture plate method (MTCP).

Further, association of biofilm formation and antimicrobial resistance was studied and it was found that multi drug resistant isolates showed significantly higher rate of biofilm formation. P value of the association was found to be statistically significant. This is shown in table 5.

Table 1: Distribution of various bacterial isolates.

Organism	SSI	
	N	%
S. aureus	25	14.7
Coagulase negative *Staphylococcus* (CONS)	21	12.3
P. aeruginosa	44	25.9
***Acinetobacter*spp.**	20	11.8
***Klebsiella*spp.**	15	8.8
E. coli	20	11.8
***Citrobacter*spp.**	13	7.6
***Proteus* spp.**	8	4.7
***Enterobacter*spp.**	4	2.3
Total	170	100

Table 2: Antibiotic resistance pattern of Gram-positive bacterial isolates (n = 46)

Antibiotics	*S. aureus* (n=25)		CONS (n=21)	
	N	%	n	%
Erythromycin	11	44	08	38
Penicillin	23	92	18	85
Cefoxitin	15	60	12	57
Trimethoprime- sulfamethoxazole	17	68	11	52
Clindamycin	08	32	07	33
Cephalexin	17	68	12	57
Amoxicillin- clavulanate	15	60	13	62
Doxycycline	07	28	6	28
Linezolid	02	08	01	04
Vancomycin	0	00	0	00

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Table 3: Antibiotic resistance pattern of Gram-negative bacterial isolates (n=124)

Antibiotics	*Klebsiella*spp. (n= 15)		*E.coli* (n=20)		*Citrobacter*spp. (n=13)		*Proteus* spp. (n=8)		*Enterobacter* spp. (n=4)		P. aeruginosa (n=44)		Acinetobacter spp. (n=20)	
	n	%	n	%	n	%	n	%	n	%	N	%	n	%
Gentamicin	6	40	10	50	5	38	3	38	2	50	30	68	6	30
Amikacin	5	33	6	30	4	31	3	38	1	25	29	66	9	45
Amoxicillin-clavulanate	9	60	13	65	9	69	4	50	2	50	NA	NA	10	50
Piperacillin-tazobactam	6	40	7	35	4	31	2	25	1	25	14	32	11	55
Ciprofloxacin	8	53	15	75	8	61	5	62	02	50	34	77	12	60
Meropenem	4	26	2	10	3	23	1	12	0	0	11	25	4	20
Imipenem	3	20	1	05	2	15	0	0	0	0	10	22	3	15
Trimethoprime-sulfamethoxazole	10	67	14	70	7	53	4	50	02	50	NA	NA	17	85
Ceftazidime	5	33	8	40	5	38	5	62	2	50	18	41	21	97
Doxycycline	11	73	11	55	9	69	NA	NA	3	75	NA	NA	13	65

Table 4: Detection of biofilm formation by modified tissue culture plate method (MTCP)

Organism	Number	Number of biofilm forming isolates	Percentage (%)
S. aureus	25	15	60
CONS	21	15	71
Pseudomonas aeruginosa	44	33	75
***Acinetobacter*spp.**	20	09	45
***Klebsiella*spp.**	15	10	66
E. coli	20	11	55
***Citrobacter*spp.**	13	07	54
***Proteus* spp.**	08	05	62
***Enterobacter*spp.**	04	02	50
Total	170	107	63

Table 5: Comparison of multidrug resistant organisms among biofilm forming (BF) and non-biofilm forming (NBF) isolates

Organism	Number of BF isolates	BF MDR		Number of NBF isolates	NBF MDR		'p' value
		n	%		N	%	
St. aureus	15	12	80	10	4	40	<0.05
CONS	15	10	67	6	2	33	<0.05
P. aeruginosa	33	27	82	11	6	54	<0.05
***Acinetobacter*spp.**	09	07	78	11	5	45	<0.05
***Klebsiella*spp.**	10	08	80	05	02	40	<0.05
Escherichia coli	11	08	73	09	02	22	<0.05
***Citrobacter* spp.**	07	05	71	06	02	33	<0.05
***Proteus* spp.**	05	05	100	03	01	33	<0.05
***Enterobacter* spp.**	02	02	100	02	01	50	<0.05
Total	107	84	78.5	63	25	39.6	**<0.05**

DISCUSSION

In the present study, rate of isolation of Gram-negative bacilli was 73% while Gram positive cocci were only 27%. In a study by Alharbi et al, 66% of the isolates were gram negative while 34% isolates were gram positive cocci.[18] Mostafa et al found gram-negative bacteria were accounting for 67% cases of SSI while remaining were gram positive bacilli.[19] Several other studies also found Gram negative bacilli to be predominant.[20-23] In our study, bacteriological profile of SSI included *P. aeruginosa* as the most frequently isolated gram organism with a isolation rate of 25.9% followed by *S. aureus* (14.7%), CONS (12.3%), *E. coli* and *Acinetobacter*spp. (both had isolation rate of 11.8%), *Klebsiella*spp. (8.8%), *Citrobacter*spp. (7.6%), *Proteus* spp. (4.7%) and *Enterobacter*spp. (2.3%). Alharbi et al also isolated *P. aeruginosa* (29%)and *St. aureus* (29%) as most common isolate followed by *E. coli* (16.13%), *K. pneumonia* (12.10%), *P. vulgaris* (9.68%), *St. epidermidis* (5.65) and *Streptococcus pyogenes* (3.23%).[18] In another study by Hosimin et al, most commonly isolated organism was *St. aureus* (24.5%) followed by *P. aeruginosa* (20.4%), *Proteus* spp.

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(16.3%) and *Klebsiella*spp. (14.3%).[20] Out of the 123 pus samples studied by Sanchez et al, *Klebsiella*spp. (31.7%) was most frequently isolated Gram-negative bacteria followed by *Acinetobacter*spp. (25.2%) and *P. aeruginosa* (23.6%) while *St. aureus* accounted for 11.4% of cases.[21] The variation in the results of the present study may be due to difference in the bacteriological profile of different geographical areas. In the current study modified tissue culture plate method was used for identifying biofilm production. Overall rate of biofilm production was 63%. Shakthi R et al reported 61% bacterial isolates positive for biofilm production.[23] Mostafa et all reported very high rate of biofilm production (77%) in their study.[19] Carlos et al found biofilm production rate to be 61%.[21] Dowd et al in their study found 66% isolates to be biofilm producers.[24]Roopashree et al studied orthopedic SSIs and reported 72% isolates as biofilm producers.[25] All the studies reported biofilm production rate to be significantly high.

In the present study, Gram positive cocci showed high resistance to penicillin, cefoxitin, trimethoprim–sulfamethoxazole and erythromycin while all of them showed effective sensitivity to vancomycin, linezolid and doxycycline. The antibiogram of Gram-positive organisms in the study by Saffanah et al showed that organisms were highly resistant to penicillin and erythromycin, while showed high sensitivity to doxycycline and linezolid.[26] Shakthi et al in their study reported most of the *Staphylococcus* spp. were sensitive to linezolid, vancomycin, Teicoplanin and Pipericillin-tazobactum.[23]Roopa Shree et al also noted high grade of resistance to amoxicillin, erythromycin, cephalexin and cefoxitin. Vancomycin and linezolid were found effective against all the isolates.[25] Other researchers also studied susceptibility pattern of Gram-positive organisms and reported majority isolates showed resistance against penicillin, cephalexin, erythromycin, clindamycin, amoxycillin-clavulanate, cefoxitin and cotrimoxazole. No resistance was observed towards vancomycin and linezolid.[18-21]

In the present study, the Gram-negative bacteria were mostly resistant to cephalosporins, doxycycline, co-trimoxazole, ciprofloxacin and amoxicillin–clavulanate. Among the aminoglycosides, high resistance was seen against gentamicin as compared to amikacin. Carbapenems were most effective class of antimicrobials with overall activity more than 90%.

Sanchez et al in their study reported Gram negative isolates showed high resistance against ceftraixone, levofloxacin, ciprofloxacin, ceftazidime and co-trimoxazole.[21]Shakthi et al also reported majority of Gram-negative bacilli showing resistance against fluoroquinolones, ceftazidime, ceftriaxone, co-trimoxazole and gentamicin.[23]

It was observed that susceptibility tocephalosporins,fluoroquinolones, tetracyclines and co-trimoxazole has decreased significantly. These are frequently used antimicrobials worldwide both as empirical and definitive therapy for treating variety of infections and high resistance may be due to indigent use of these drugs. This demands generation of region wise hospital antibiogram based upon the antimicrobial drug sensitivity of local bacterial isolates. This could be helpful in prescribing effective empirical therapy along with preventing dissemination of antimicrobial resistant strains in the community as well as in the hospital.

The current study showed a comparison of the drug resistance pattern of the biofilm forming (BF) and the non-biofilm forming (NBF) organisms and it revealed that there was statistically significant difference in the prevalence rate of multi drug resistance among the biofilm forming and non-biofilm forming bacteria ($p<0.05$). Other authors also noted similar association.[21,23]Increasing burden of biofilmproduction and drug resistance among theroutine clinical isolates is alarming as thisleads to persistent chronic infections whichpose significant challenge to clinicians for managing such infections.

CONCLUSION

Biofilms induce delay in wound healing and significantly increase the risk of infection. In spite of various guidelines and infection prevention protocols being followed in health care settings, SSIs remain an important cause of morbidity, prolonged hospital stay and death. In order to prevent these infections, appropriate antibiotic prophylaxis, following aseptic protocolsand infection prevention/control measuresintra operatively and during post operative care are important measures. In fact, pre-operative skin preparation appears to be single most important measure that reduces risk of biofilm formation by removing both normal and pathogenic flora. With the evidence from our study, we suggest that biofilm detection should be included as routine diagnostic procedure to predict the emergence of biofilm producing isolates at the earliest. This will help in better patient management by modifying the therapy. Also measures to minimize biofilm development should be taken to reduce the risk of such infections.

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REFERENCES

1. Hrynyshyn, A.; Simões, M.; Borges, A. Biofilms in Surgical Site Infections: Recent Advances and Novel Prevention and Eradication Strategies. Antibiotics 2022, 11, 69. https://doi.org/10.3390/antibiotics11010069.
2. Young, P.Y.; Khadaroo, R.G. Surgical Site Infections. Surg. Clin. N. Am. 2014, 94, 1245–1264.
3. Singhal H, Kaur K. Wound infection clinical presentation. Medscape Medical News from WebMD. Available at

DOI: 10.69605/ijlbpr_13.6.18

http://emedicine.medscape.com/article/188988-clinical. 2015
4. Centers for Disease Control and Prevention (CDC); National Healthcare Safety Network (NHSN). Available online: https://www.cdc.gov/nhsn/psc/ssi/index.html
5. Wolcott R, Cutting KF, Dowd SE. Surgical site infections: biofilms, dehiscence and delayed healing. Wounds UK. 2008;4(4):108-113.
6. Attinger C, Wolcott R. Clinically Addressing Biofilm in Chronic Wounds. Advances in wound care. 2012;1(3):127-132.
7. Percival, S.L. Importance of Biofilm Formation in Surgical Infection. Br. J. Surg. 2017, 104, e85–e94.
8. Stoodley LH, Stoodley P, Kathju S, Høiby N, Moser C, Costerton JW, Moter A et al. Towards diagnostic guidelines for biofilm-associated infections. Immunol Med Microbiol. 2012;65:127–145.
9. Donlan RM and Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin. Microbiol Rev 2002;15(2):167-193.
10. Zhao G, Usui ML, Lippman SI, James AG, Stewart PS, Fleckman P et al. Biofilms and Inflammation in Chronic Wounds. Adv Wound Care. 2007;2(7):389-399.
11. Surgical Site Infection (SSI) Event: Center for Disease Control. 2010. http://www.cdc.gov/nhsn/PDFs/pscManual/9pscSSIcurrent.pdf?agree=yes&next=Accept. Accessed on S eptember 30 2023
12. Colle JG, Marr W. Specimen collection, culture containers and media. In: Colle JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14thed. New York: Churchill Livingstone; 1996: 95-111.
13. Colle JG, Miles RB, Watt B. Tests for identification of bacteria. In: Colle JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14thed. New York: Churchill Livingstone; 1996: 131-149.
14. Duguid JP. Staining Methods. In: Collee JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14thed. New York: Churchill Livingstone; 1996: 793-812.
15. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility test: Twenty forth information supplement. CLSI document M100-S33. Wayne, PA: CLSI;2023.
16. cdc.gov.in[Internet]. Management of MDRO's in healthcare settings. www.cdc.gov/hicpac/mdro/mdro-toc.pdf
17. Saxena S, Banerjee G, Garg R, Singh M. Comparative study of biofilm formation in *Pseudomonas aeruginosa* isolates from patients of lower respiratory tract infection. J ClinDiagn Res. 2014;8(5):9-11.
18. Alharbi SA, Zayed ME. Antibacterial susceptibility of bacteria isolated from burns and wounds of cancer patients. J Saudi ChemSociet. 2014;18:3–11.
19. Mostafa I. EL-Amir, AsmaaGaber R, Mohamed Yousef A, Tunis Gamal, and Mohamed Ali El-Feky, "Detection of Biofilm in Surgical Site Infection by Microtiter Plate Method and Its Correlation with Icad, Andicaain Staphylococcus Spp." American Journal of Microbiological Research, vol. 8, no. 1 (2020): 19-23. doi: 10.12691/ajmr-8-1-3.
20. Hosimin K, Prabakaran G. Studies on isolation and characterization of some wound infection causing bacteria. Int J Cur Adv Res. 2012;11(2):26-31.
21. Sanchez CJ, Mende K, Beckius ML, Akers KS, Romano DR, Wenke JC, et al. Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infectious Diseases 2013,13:47.
22. Khairy GA, Kambal AM, Al-Dohayan AA, Al-Shehri MY, Zubaidi AM, et al. (2011) Surgical site infection in a teaching hospital: A prospective study. J T U Med Sc 6: 114-120.
23. Shakthi R, Venkatesha D. Detection of Biofilm Production and Antibiotic Resistance Pattern in Clinical Isolates from Orthopaedic Implants Associated Infections. J Med Sci Health 2023; 9(2):157-162.
24. Wolcott RD, Gontcharova V, Sun Y, Zischakau A, Dowd SE. Bacterial diversity in surgical site infections: not just aerobic cocci any more. J of Wound Care. 2009;1
25. Roopa Shree S, A. G. Prathab. "Characterisation 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; Vol. 4, Issue 33, April 23; Page: 5634-5642, DOI: 10.14260/jemds/2015/825
26. Nurul Indah Saffanah, DiniAgustina, Dan IkaRahmawatiSutejo. Postoperative Orthopedic Surgical Site Infection Antibiogram of dr. Soebandi Hospital, Jember in 2019. JPSCR: Journal of Pharmaceutical Science and Clinical Research, 2020, 02, 110-120, DOI: 10.20961/jpscr.v5i2.41947