VOLUME-8, ISSUE-10, OCTOBER-2019 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

COMPARISON OF BIOFILM DETECTION METHODS IN THE FAMILY ENTEROBACTERICEAE

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ABSTRACT

Bio films are one of the important causes of wound infection and therefore their management requires an understanding of mechanism of biofilm production by bacteria. By definition, biofilms are matrix-enclosed aggregates of bacteria that are immobilized on surfaces or at interfaces in the ecosystems in which they are known to predominate .Tissue Culture Plate (TCP) method and Modified Tissue Culture Plate (MTCP) method were studied.

AIM: 1) To detect bio film production in pus isolates by TCP method and MTCP method;

2) To compare Antimicrobial Susceptibility Testing (AST) of biofilm producing and non biofilm producing isolates.

METHODOLOGY AND MATERIALS: The organisms was identified using standard microbiological procedures and AST was done by Kirby-Bauer disc diffusion method with the CLSI guidelines 2019.

Biofilm production was detected using TCP and MTCP methods.

RESULT: A total of 180 isolates were obtained out of 240 pus samples. Bacteriological profile includes Klebsiella spp. (n=46), Escherichia coli (n=52), Citrobacter spp. (n=30), Proteus spp. (n=40), Enterobacter spp. (n=12). Out of these, 58.8% isolates showed bio film production by TCP and 70% by MTCP method. The rate of biofilm detection by two methods was found to be statistically significant (p-value =0.004). In our study, biofilm production as per standard TCP method were more prevalent in Klebsiella spp. (35%) followed by Proteus spp. (20%), Enterobacter spp. (5%), E. coli (30%) and Citrobacter spp. (16%). The magnitude of bio film production by individual bacterial spp. The magnitude of biofilm production by individual bacterial spp is depicted .

CONCLUSION: MTCP method was found to be more accurate than TCP for biofilm detection and quantification.

KEYWORDS :

INTRODUCTION

Microorganism associated with biofilm formation usually have tendency to delay healing and show increased resistance to antimicrobial drugs which results in chronic infection. It increases morbidity of patient as well as of treatment.

Biofilm are one of the important causes of wound infection and therefore their management requires an understanding of mechanism of biofilm production by bacteria. Gram negative aerobes and anaerobic bacteria which are the part of respiratory, gastrointestinal, gastrourinnary flora are also important aetiological agents of wound infection[1].

Approx. 65% of all human microbial infections involve biofilms. These include native value endocarditis, cystic fibrosis and chronic wounds. They found in contact lens, intrauterine devices and orthopedics implants [2].Biofilm production is a multistage process involving growth and exopolysaccharides production followed by gradual maturation and all dispersion some of those methods include TCP method, Tube method, Congo red agar, flow cell method, confocal laser scanning microscopy, Calgary biofilm device and molecular methods for identifying genes responsible for Exopolysaccharide synthesis and bacterial adhesion [3,6]. For biofilm detection several methods been used among them. Among this TCP is considered as a gold standard phenotypic method for biofilm detection. In this method bacterial adherence spectrophotometrically. [4]

Though TCP is considered as a standard method. It will also have some difficulty for biofilm detection. Therefore a modified method known as MTCP was produced and a comparison of MTCP with TCP method was done.

SAMPLE COLLECTION AND PROCESSING

The prospective study was conducted at the Department of Microbiology, Saveetha Medical College, Chennai after taking ethical approval. The study was carried out for duration of 2 months

The Pus samples were collected using syringe or sterile swab from the microbiology laboratory . The Pus samples obtained fro y8m various wound and ulcers non-repetitively. No other organism other than *Enterobactericeae* were included in this study. The isolates were studied microscopically and cultured on blood agar and macconkey agar plates .They were further examined for the morphological characteristics gramstaining and biochemical reactions as per the standard microbiological protocol for the identification of the organisms.The isolates were processed for antibiotic susceptibility testing by kirby bauer disk diffusion methodas per bauer disk diffusion method as per the CLSI guidelines(2019). The antimicrobial drugs tazobactam/ pipracillin, ciprofloxacin, imipenem, meropenem, trimethoprim/ sulfamethoxazole, ceftazidime,doxycycline was interpreted.

BIOFILM PRODUCTION METHODS

Tissue Culture Plate :

The primary inoculants are then inoculated in Brain Heart Infusion (BHI) with 2% sucrose prepared in diluted ratio of 1:100 and loaded into a well flat bottom microtitre plate[4].

Plates are covered and incubated for 37°c for 24 hrs in aerobic condition and washed four times with phosphate buffer saline. Then the wells are decanted and stained with crystal violet for 30 mins, and then it was washed with distilled water and the optical densities was determined by a automated micro ELISA reader at wavelength of 570 nm.

Modified Tissue Culture Plate :

Standard TCP method was modified slightly by adding 33% glacial acetic acid to the microwells .After 15 mins OD values

was taken by automated micro ELISA reader at a wavelength of 570 nm. These OD values were considered as index of bacterial adhesion and biofilm formation.

STATISTICAL ANALYSIS

This method was analyzed by **CHI-SQUARE TEST.**

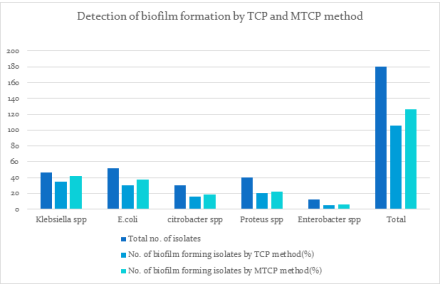
RESULT

A total of 180 isolates were obtained out of 240 pus samples. Bacteriological profile includes *Klebsiella* spp. (n=46), *Escherichia coli* (n=52), *Citrobacter* spp. (n=30), *Proteus* spp. (n=40), *Enterobacter* spp. (n=12). Out of these, 58.8% isolates showed biofilm production by TCP and 70% by MTCP method. The rate of biofilm detection by two methods was found to be statistically significant (p-value =0.004).

In our study, biofilm production as per standard TCP method was more prevalent in *Klebsiella* spp. (35%) followed by *Proteus* spp. (20%), *Enterobacter* spp. (5%), *E. coli* (30%) and *Citrobacter* spp. (16%). The magnitude of biofilm production by individual bacterial spp is depicted in (fig/Table-2)

(Fig/Table-1) Grading of biofilm formation by TCP and MTCP method

Biofilm formation	TCP		MTCP	
	N	%	N	%
Strong	69	38.3	74	41.1
Moderate	37	20.5	52	28.8
Weak/None	74	41.1	54	30
Total	**180**	**100**	**180**	**100**



(Fig/Table-2)

Antibiogram of the 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. High resistance by biofilm forming isolates was observed against ceftazidime (88%) followed by doxycycline (85%), co-trimoxazole (79%), gentamicin (60%), amoxyclav (69%), ciprofloxacin (66%), amikacin (58%), piperacillin+tazobactum (39%), meropenem (29%) and imipenem (18%).Antibiotic resistance pattern of bofilmm forming and non biofilm forming isolates is depicted in (TABLE-3)

(Fig/Table -3) Antibiotic resistance pattern of biofilm forming (BF)and non biofilm forming(NBF) isolates

Antibiotics	Resistance in BF isolates	Resistance in NBF isolates
	%	%
Gentamicin	60	33
Amikacin	58	28
Amoxycilin-Clavulanate	69	22
Piperacillin-tazobactum	39	8
Ciprofloxacin	66	40
Meropenem	29	0
Imipenem	18	0
Co-trimoxazole	79	41
Ceftazidine	88	61
Doxycycline	85	41

(Fig/Table-4)Comparision of multi drug resistance among biofilm forming(BF) and non biofilm forming(NBF) isolates

Organism	No. of BF isolates	BF MDR		No. of NBF isolates	NBF MDR		'p' value
		N	%		N	%	
Klebsiella spp	35	25	71.4	11	5	45.4	0.001
E.Coli	30	14	46.6	22	5	22.7	0.003
Citrobacter spp	16	11	68.7	14	3	21.4	0.001
Proteus spp	20	12	60	20	2	10	0.007
Entrobacter spp	05	05	100	07	1	14.2	0.03
Total	106	67	68	74	15	21	0.00

DISCUSSION

Biofilm producers in the wounds has great relevance in wound managements .Early identification of biofilm producing strains will aid in the selection of appropriate antibiotics which also plays a major role in the prevention to relapsing of infections.The current study showed that *klebseilla spp* was the most commonly isolated microorganism followed by *Escherchia coli*.A study by subramanian p et al, showed that the most commonly isolated organism was *klebseilla pneumoniae* follwed by *Escherchia coli* whereas the present study revealed that the most commonly isolated organism is *klebseilla spp* (35%) followed by *proteus spp*(20%).

A study conducted by fatima s et al and zubair m et al revealed that the most predominantly isolated organism was *Escherchia coli*. whereas the present study revealed that the most commonly isolated organism is *klebseilla spp* (35%) followed by *proteus spp*(20%).

Fatima s et al also concluded saying that the rate of biofilm production was higher in E.coli followed by proteus spp, *klebsiella spp,citrobacter* and other *enterobacter spp*. whereas the present study revealed that the most commonly isolated organism is *klebseilla spp* (35%) followed by *proteus spp(20%)*.

In another study conducted by Subramanian P et al.,resistance pattern of biofilm positive isolates showed 62%, 20%, 74%, 60%, 03%, and 03% resistance to gentamicin, amikacin, ceftriaxone, ciprofloxacin, piperacillin-tazobactum and imipenem respectively as compared to 29%, 11.6%, 37.7%, 24.6%, 1.4%, and 2.9% resistance shown by biofilm non-producers for the same antibiotics .In this study resistance pattern of biofilm isolates showed 60% , 58%, 69% ,39% ,66%, 29% ,18% 79%, 88%, 85%, resistance to gentamicin, amikacin, amoxicilin, piperacilin,ciprofloxacin, meropenem, imipenem,cotrimaxazole, ceftazidine , docycycline.Fatima S et al., also compared rate of biofilm production and drug susceptibility pattern of gram negative isolates. They observed that 69% biofilm producing isolates were MDR while only 41.5% non-biofilm producers were MDR . Similarly, Zubair M et al., also found biofilm producing isolates to show high degree of resistance for routinely administered antibiotics [20].

In this study, two phenotypic methods for detecting biofilm formation were used and their results were compared to find out most appropriate method for demonstrating biofilm formation. Out of the 160 isolates, the TCP method could detect biofilm in 84 isolates (52.5%)and The MTCP method detected biofilm in 105 isolates (65.6%). The study showed that the MTCP method is more sensitive than TCP method (p-value < 0.05). Stepanovic´ et al., evaluated 30 clinical isolates of staphylococci for biofilm formation. In their study, TCP method

identified 73.3% isolates as biofilm producer while MTCP method detected 83.3% isolates to be biofilm producer.They compared the results of both the methods and found the difference to be statistically significant . Another investigator Babapour E et al., studied 156 clinical isolates of Acinetobacter spp.And on comparison, the rate of biofilm formation was 66.7% and 73.7% by TCP and MTCP methods respectively.He concluded that the MTCP method is more accurate than TCP method in evaluating biofilm formation. The result of this study is in accordance with the above mentioned studies.

CONCLUSION

High rate of biofilm formation shown by the members of Enterobacteriaceae suggests it to be one of the important mechanisms of anti microbial resistance. MTCP method is better than TCP method for biofilm detection and quantification. This is a simple, reliable accurate method and can be utilized for biofilm screening.

REFERENCES

[1] Singhal H, Kaur K. Wound infection clinical presentation. Medscape Medical News from Web MD. 2015
[2] Sharma M, Yadav A. Biofilms: microbes and disease. Braz J Infect Dis. 2008;12(6):526-30.
[3] Wolcott R, Costerton JW, Raoult D, Cutler SJ. The polymicrobial nature of biofilm infection. Clin Microbiol Infect. 2013;19(2):107-12.
[4] Christensen GD, Simpson WA, Younger JA, Baddour LM, Barret FF, Melton DM, et al. Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of Staphylococci to medical devices. J Clin Microbiol. 1985;22(6):996-1006.
[5] Brook I, Frazier EH. Aerobic and anaerobic bacteriology of wounds and cutaneous abscesses. Arch Surg. 1990;125(11):1445-51.
[6] Bose S, Ghosh AK. Biofilms: a challenge to medical science. J Clin Diag Res. 2011;5(1):127-30
[7] 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: Churchill Livingstone. 1996:131-49.
[8] Duguid JP. Staining Methods. In: Collee JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. Churchill Livingstone. 1996:793-812
[9] CLSI. Performance standards for antimicrobial disc susceptibility testing. 26th ed. CLSI supplement M100-S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
[10] Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011;15:305-11.
[11] J.G Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. New York: Churchill Livingstone. 1996: 95-111.