VOLUME-7, ISSUE-6, JUNE-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Microbiology

SCREENING OF BIOFILM PRODUCING BACTERIA FROM VARIOUS CLINICAL SAMPLES

Malasane P.R. Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola

Barate D. L.* Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola. *Corresponding Author

ABSTRACT Biofilm is a microbial community which is embedded in extra cellular matrix. The ability of microorganism to develop biofilm is an important virulence factor and they are main cause of many chronic infections. They are responsible for the emergence of multi drug resistant strains resulting in treatment failure. In the present study a total of 50 bacteria were isolated from various clinical samples viz. blood, urine and pus. Out of 50 isolates 35 isolates were found to be biofilm producers after screening by Tube method (TM) and Congo Red Agar (CRA) method. These isolates were identified by standard conventional methods, which reveals that the biofilm producers were belongs to *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa.*

KEYWORDS : Biofilm, Tube Method (tm), Congo Red Agar (cra).

Introduction:- Biofilms are microbial communities of the surface-attached cells, embedded in a self-produced extracellular polymeric matrix (Donlan and Costerton, 2002). Bacteria often exist as sessile communities in nature called biofilms that develop structures which are both physiologically and morphologically different from free living bacteria. Biofilms are a group of microorganism attached to a surface and covered by an exopolysaccharide matrix. Various changes occur during their transition from planktonic to a surface attached community. In response to certain environmental signals, new phenotypic characteristics develop in such bacteria (Toole *et al.,* 2000).

Many microorganism in the natural environment are organized in biofilm structure (Costerton, 1999). Biofilms can be muticellular communities of bacteria, immobilized by an extracellular polymeric matrix produced by the bacteria, which can be attached to various biotic and abiotic surfaces (Izano *et al.*, 2008). This three-demensional biofilm structure is made up in 85% by the extracellular matrix which comprises polysaccharides, proteins, enzymes, DNA, bacterial glyolipids, water and in 15 % by aggregates of microorganism cells (Costerton 1999). Biofilm development depend on many physical,chemical and biological factors (Bryers, 2008).

The ica gene codes for intracellular adhesion and may also code and is required for biofilm production (Donlan *et al.*, 2002). Within a biofilm, bacteria communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing.

There are various methods to detect biofilm production. These include the Tissue culture plate (TCP) (Christense *et al.*, 1995), Tube method (TM) (Christensen *et al.*, 1982), Congo Red Agar method (CRA) (Freeman *et al.,* 1989), bioluminescent assay (Donlan *et al.*, 2001), piezoelectric sensors (Aparna and Yadav, 2008), and fluorescent microscopic examination (Zufferey *et al.,* 1988), which could be used in a routine clinical laboratory, for determining their ability to form biofilm.

Both Gram-positive and Gram-negative bacteria have the capability to form biofilms. Bacteria commonly involved include *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridians*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Donlan, 2001).

The ability of a microorganism to develop biofilm is an important virulence factor and they are the main cause of many infections. They are responsible for the emergence of multidrug- resist ant strains resulting in treatment failure (Swarna *et al*., 2012). Thus the present study aimed to know the prevalence of biofilm producing bacteria from Akola city and to check the antibiotic susceptibility pattern of biofilm producers.

Material and Methods :-A total 70 clinical sample viz, blood, pus, urine were collected for the isolation of test organism. The clinical samples were collected from various hospitals and pathology laboratories of Akola city. The clinical sample were collected in sterilized vials and brought to the laboratory. The isolates were examined for biofilm formation by two different method i.e Tube method (TM) and Congo Red Agar (CRA) method.

A) Tube Method(TM) :-
Biofilm production was investigated by the tube adherence test proposed by Christensen *et al*., (1986). This is a qualitative method for biofilm detection. In this method, Trypticase soy broth with 1% glucose was inoculated with a loopfull of test organism from culture overnight culture on nutrient agar individually. Broths were incubated at 37 °C for 24 hours. The tubes were decanted and tube were washed with phosphate buffer saline (pH 7.3). The tubes were dried and stained with crystal violet. Excess stain was washed with deionized water. Tubes were kept in inverted position. In positive biofilm formation, a visible stained film was seen along the walls of the tube.

B)Congo Red Agar (CRA) Method :-
The method developed by freeman *et al*., (1989) was used in this study. This is also simple qualitative method to detect biofilm production by using Congo Red Agar.In this the medium composed of Brain heart infusion broth, sucrose and Congo Red dye was used. First Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes. Then it was added to autoclaved Brain heart infusion agar with sucrose. Plates were inoculated with test organism and incubated at 37°C for 24 hours. Black colonies with a dry crystalline consistency indicated biofilm production.

Results and Discussion:- In the present study a total of 70 clinical sample were collected from various hospital of Akola city which includes urine, blood & pus. From the clinical sample about 50 isolates were obtained in the form of pure culture after isolated on MacConkey agar. The 50 isolates primarily isolated were checked for the biofilm formation. From the Screening it was found that 35 (70%) isolates were able to produce biofilm either by TM or CRA method or by both. On the basis of visual observations the isolate was termed as strong, moderate, or weak biofilm producers.

It was found that by tube method 16 (45.71%) was detected as strong, 8 (22.85%) moderate and 11(31.42%) as weak biofilm producers. While by CAR method 21(60%) were detected as strong &

7 (20%) each were moderate & weak producers (Table 1). In the study, all the biofilm producers were checked for their antibiotic susceptibility pattern. It was found that many of them showed resistance to two or more antibiotics

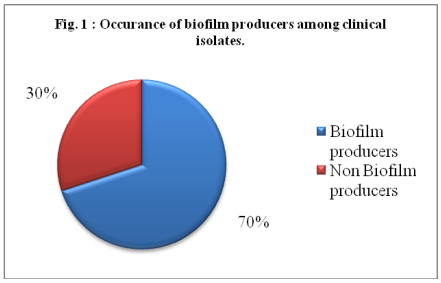
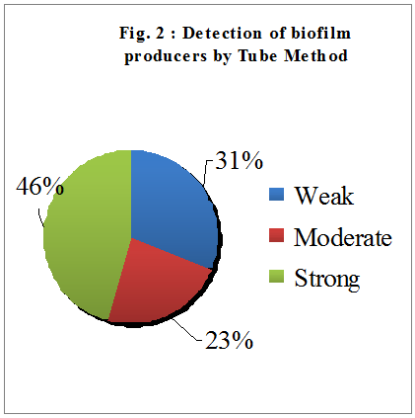
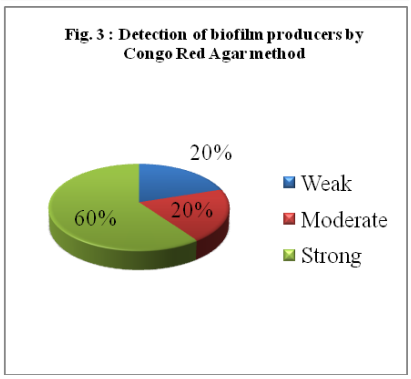
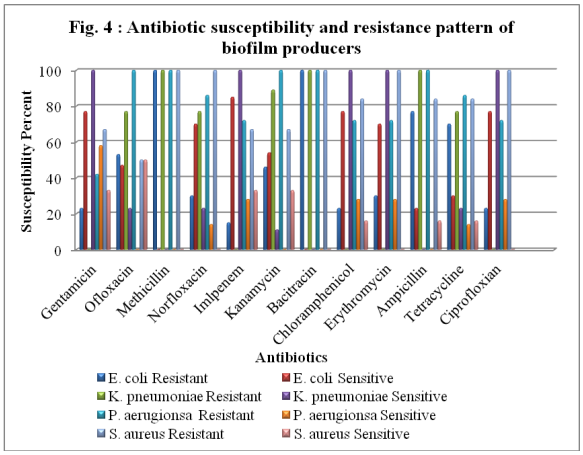


Table 1 :- Grading of biofilm producers by Tube method (TM) & Congo Red Agar (CRA) Method.

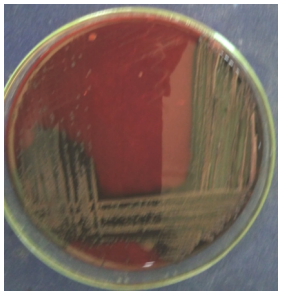
Biofilm Formation	Tube method (n=35)		Congo Red agar (n=35)	
	No.	%	No.	%
Weak	11	31.42%	7	20 %
Moderate	8	22.85%	7	20%
Strong	16	45.71%	21	60%







Screening of biofilm producers by Tube Method (TM)



Screening of biofilm producers by Congo Red Agar Method (CRA)

Discussion :-

Biofilm producing bacteria are responsible for many infections in hospital settings and community which makes difficult to eradicate then because of biofilm production. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotics into biofilm, decreased growth rate and expression of resistance genes. Thus, it is necessary to find out the biofilm producers among clinical isolates.

In the present study about 70% clinical isolates were found to produce biofilm (fig 1). Two methods were used to detect the production of biofilm among the isolates. It was found that about 45.71% strong biofilm producers were detected by Tube method & 60% were by Congo Red agar method (fig 2 & 3). This showed that Congo Red agar method detects more biofilm producers than Tube method. This finding is in concordance with studies of Bose *et al.,* (2009) who reported biofilm producers by Tube method 46% and by Congo Red agar method 60%. In another study Ruzika *et al.,* noted 53.7% biofilm formation by Tube method & 43.5% by Congo Red agar method, But De *et al.,* (2012) reported 50.67% of biofilm producers by Congo Red agar method which was only method adopted for their study and is comparable with the present results.

In the present study the isolates showing biofilm formation were identified as *E.coli*, *K. pneumoniae*, *P. aeruginosa* & *S. aureus*. These findings are consistent with that of Stickler *et al.,* (1996) who isolated *E. coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. This is in accordance with the results of Neeli *et al.,* (2006) and De *et al.,* (2012).

In the present study antibiotic susceptibility pattern of biofilm producers were also checked. It was found that many isolates showed multidrug resistance. This might be due to the difficulty in penetration of antibiotics due to biofilms. Similar findings are reported by Shubha *et al.,* (2012) who also reported muti drug resistance among the biofilm producers.

Conclusion :-The present study comes to conclusion that, high rate of biofilm production found among clinical isolates. CRA method detected more no of biofilm producers than Tube method (TM). *E. coli* was found to be prominent biofilm producers followed by *K. pneumoniae*, *P aeruginosa* & *S aureus*. Many isolates showed drug resistance so such information on the capacity of a clinical isolate to produce biofilm would help clinicians to device an appropriate treatment plan for the patient and can also help to prevent potentially fatal & persistent infections due to such bacteria.

References

1. Aparan M. S., Yadav S. (2008). Biofilms: microbes and disease. Braz. J. Infect. Dis., 12(6) : 526 - 30.
2. Bryers, J. D. (2008). Medical biofilms. Biotechnol. Bioeng.,100 : 1 - 18.
3. Bose S., Khodke M., Basak S., Mallick S. K. (2009) Detection Of Biofilm Producing Staphylococci : J. of Clinical and Diagnostic Research, 3(6) :1915-1920.
4. Christensen G. D., Simpson W. A., Bisno A. L., Beachey E. H. (1982). Adherence of slime producing strains of Staphylococcus epidermidis to smooth surfaces. Infect. Immun., 37 : 318 - 26.
5. Christensen G. D., Simpson W. A., Younger J. A. et al., (1995). Adherence of coagulas negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol., 22 : 996 - 1006.
6. Costerton, J. W., Stewart P. S., Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. Science, 284 : 1318 - 1322.

7. De Anuradha, Deshpande Dhanashree, Baveja Sujata M., Taklikar Shripad (2012). Detection of biofilm formation in bacteria from cases of urinary tract infections, septicemia, skin and soft tissue infections and post-operative infections by Congo Red Agar method e-jams,: 49.35.48.206.
8. Donlan R. M. (2001). Biofilms and device- associated infection. Emerg. Infect. Dis., 7(2) : 277 - 81.
9. Donlan R. M., Costerton J.W. (2002). Biofims: the survival mechanism of the clinically relevant microorganisms. Clin. Microbiol. Rev., 15 (2): 167 - 93.
10. Donlan R. M., Costerton W. (2002). Biofilms : Survival mechanisms of clinically relevant Microorganisms. Clinical microbiological review, 15(2) : 167 – 193.
11. Freeman J., Falkiner F. R., Keane C. T. (1989). New method for detecting slime producing by coagulase negative Staphylococci. J. Clin. Pathol., 42 : 872 - 4.
12. Izano, E.; Amarante, M.; Kher, W.; Kaplan, J. (2008). Differential roles of poly- N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Appl. Environ. Microbiol., 74 : 470 - 476.
13. Neeli, V. H., Parvathi T. and Balamurali P. K. (2016). Study of Biofilm Production and Anti-microbial susceptibility pattern of bacterial and fungal isolates from urinary catheters Int. J. Curr. Microbiol. App. Sci., 5(2): 415-424.
14. Ruzicka F et al. (2004). Biofilm detection and clinical significance of Staphylococcus epidermidis isolates. Folia Microbiol 49(5) : 596 – 600.
15. Shubha D. S., Banoo Sageera Shashidar, Fatima Farheen, Venkatesha D. (2012). Speciation and Antibiogram of Coagulase Negative Staphylococci (CONS) from various clinical specimens. IJPHRD, 3(1) : 91 – 95.
16. Stickler (1996). Bacterial biofilms and the encrustations of urethral catheter. Biofouling, 9(4) : 293-305.
17. Swarna S. R., Madhavan R., Gomathi S., Devaraj, Thamaraiselvi S. (2012). A study of biofilm on Diabetic foot ulcer. Int. J. Pharam. Bio. Sci., 4 :1809 - 14.
18. Thomas D. and Day F. (2007). Biofilm formation by plant associated bacteria. Annual Review of Microbiology, 61 : 401 - 422.
19. Toole G. O., Kaplan H. H., Kolter R. (2000). Biofilm formation as microbial development. Annual review of Microbiology, 54 : 49 – 79.
20. Zufferey J., Rime B., Francioli P., Bille J. (1988). Simple method for rapid diagnosis of catheter infection by direct Acridine orange staining of Catheter tips. J. Clin. Microbiol., 26 : 175 - 7.