Section: General Medicine

Original Research Article

DETECTION OF BIOFILM PRODUCTION FROM UROPATHOGENIC BACTERIA AND THEIR IMPACT ON ANTIBIOTIC RESISTANCE PROFILE IN A TERTIARY CARE CENTER

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Abstract

Background: Bacterial biofilms are serious global health concern due to their multidrug resistant. Biofilm are complex community of millions of adherent bacterial cells embedded within a self-produced matrix of extracellular polymeric substance, it has ability to tolerate antibiotics and host defence system therefore it contributes to persistent chronic infections. Aim & Objectives: The aim of this study was to evaluate biofilm formation by uropathogens and to determine the association of biofilm formation with drug resistance. **Materials and Methods:** A total of 150 urine samples were collected over a period of seven months.The detection of biofilm production was done by Congo Red Agar Method (CRAM) and Tube Adherence Method (TAM). Antibiotic susceptibility testing for urinary isolates done by kirby Baeur disk diffusion method. **Results:** Escherichia coli was the commonest isolate identified. Of the 150 clinical isolates 38 (26%) were positive for biofilm production by Congo Red Agar Method (CRAM) and 31 (21%) by Tube Adherence Method (TAM). Higher antibiotic resistance was observed among biofilm producers than among non-biofilm producer. **Conclusion:** Escherichia coli was the most common isolate in the urine sample. Congo Red Agar Method (CRAM) was found to be more sensitive than the Tube Adherence Method (TAM). Biofilm producers were found to be multidrug resistant than non-biofilm producers.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most important causes of morbidity affecting people of all ages, including young women, children, and the elderly. Some studies say that approximately 40% of women have had a UTI at some point in their lives.[1] Uropathogens can produce biofilm in the urinary tract, also on medical devices like catheters etc which in turn helps in the formation of a dormant reservoir. Re-activation of bacteria from dormant reservoirs is one of the leading causes of recurrent UTI.[2]

Biofilm is a group of bacterial cells that stick to each other on a surface and are embedded within a layer (the slime layer) of a self-produced matrix of extracellular polymeric substances called glycocalyx.[3]

The cells within biofilm are subjected to variable environmental conditions including decreased oxygen tension and the presence of key nutrients, and so they are differ from bacteria living on the surface. This leads to different phenotypes, gene expression, etc that help the bacteria survive in unfavourable circumstances.[4]

Microorganisms developing in such environment are intrinsically more resistant to antibiotics, so these type of infections are difficult to treat. Such infections may require a higher concentration of antibiotics, as the concentration of bacteria can increase upto a thousand folds.[5]

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These multi Drug Resistant (MDR) organisms are now a major community health problem, so it is important for us to determine the causes for MDR. Therefore, our study focuses on biofilm production which is one of the mechanism by which drug resistant uropathogens acquire resistance . In our study, we screened UTI cases for etiological agents and strains isolated were tested for biofilm production by the Tube Adherence Method (TAM) and Congo Red Agar Method (CRAM). Finally, we correlated our findings of biofilm production with those of drug resistance pattern of uropathogens.

MATERIALS AND METHODS

This prospective analytical study was carried out in the department of microbiology from December 2021 to June 2022. after obtaining institutional ethical committee clearance and informed written consent from patients.

A total of 150 isolates were collected from patients admitted to our hospital with symptoms of UTI for at least two days. Patients of all age groups and of both sexes were included in the present study. Midstream urine samples were obtained after a proper anogenital toilet. Samples were inoculated in blood agar and Mac Conkey's agar with a calibrated loop to determine Colony Forming Units (CFU). Patients with significant bacteriuria were included in the present study. Organisms were identified on the basis of their growth characteristics, gram staining and biochemical tests as per the standard recommended procedures.[6]

AST was carried out by the Kirby Bauer disc diffusion method on Muller Hinton Agar as per Clinical and Laboratory Standard Institute guidelines7. The detection of biofilm production was done by using two methods, namely Tube Adherence Method (TAM) and the Congo Red Agar Method (CRAM)).

1. Tube Adherence Method: The test organism was inoculated in Brain Heart Infusion (BHI) broth and incubated for 48 hours at 37ºC. Tube was then decanted and stained with a 1% crystal violet solution. Tubes were washed with distilled water three times and dried. The presence of a layer of stained material adhered to the inner wall of the tube was considered a positive result. The presence of a stained ring only at the liquid air interface was considered negative[8] (Fig. 1).
2. Congo Red Agar Method: BHI broth supplemented with 5% sucrose and congo red was used for CRAM. The medium was composed of BHI (37gm/litre), sucrose (50gm/litre), agar agar (10mg/litre) and congo red stain (0.8gm/litre). An aqueous solution of congo red was autoclaved separately and used for media preparation. Plates were inoculated and incubated aerobically for 24 to 48 hours at 37ºC. Positive results were noted by observing the formation of black colonies with dry crystalline consistency, whereas weak biofilm producers usually remained pink. The darkening of colonies in the absence of dry crystalline consistency indicated an intermediate result[9] (Fig. 2).

Statistical Analysis

All the tests mentioned above were performed in duplicate. Staphylococcus epidermidis ATCC 12228 was taken as a negative control strain and Staphylococcus epidermidis ATCC 31484 was taken as a positive control strain.

Statistical analysis was carried out using paired and unpaired't' test. p values <0.05 were considered significant.

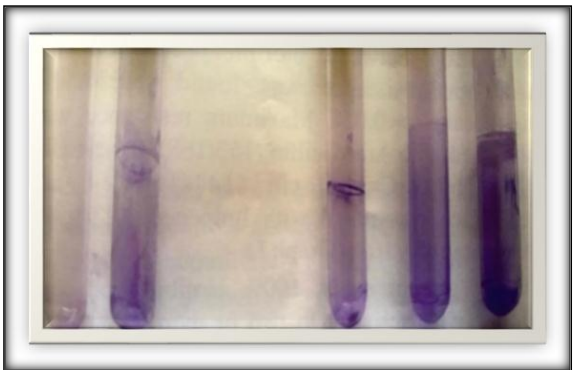

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Figure 1: Tube Adherence Method for biofilm detection: The left side of the image shows positive and negative controls. The right side shows negative test and positive tests.

Figure 2: Congo Red Agar Method for Biofilm Detection, Positive: Black Colonies

RESULTS

A total 150 isolates were identified by conventional methods. Biochemical identification of uropathogens up to species level revealed, E. Coli to be the predominantly isolated pathogen (49%), followed by Klebsiella pneumoniae (24%). (Table: 1).

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Table 1: Distribution of Urinary isolates positive for biofilm formation

ORGANISMS	BIOFILM PRODUCERS (BY CRAM)	NON BIOFILM PRODUCERS	TOTAL
Escherichia coli	17	56	73 (49 %)
Klebsiella pneumoniae	9	27	36 (24 %)
Pseudomonas aeruginosa	7	14	21 (14%)
Citrobacter	1	8	9 (6%)
Acinetobacter	1	2	3 (2%)
Enterobacter	3	5	8 (5%)
Total	38(25.3%)	112(74.6%)	150(100%)

By CRAM ,112 (74.6%) of the strains were non biofilm producers and 38 (25.3%) were biofilm producers .However, by TAM 31(21%) isolates were found to be biofilm producers, whereas 119 (79.3%) were non biofilm producers.(Table 2).

Table 2: Results of Biofilm Production by Two Methods

NUMBER OF ISOLATES	CRAM	TAM
25	+	+
13	+	-
6	-	+
106	-	-
150	38(26%)	31(21%)

Sensitivity and specificity of CRAM were found to be 81% and 86% respectively, when compared to TCPM. Biofilm producing strains showed relatively high drug resistance against all the antibiotics tested as compared to non biofilm producing strains. The correlation between biofilm production and antibiotic resistance was found to be statistically significant ($p<0.05$). Maximum resistance was observed with Amoxicillin (153/168), Ampicillin (153/168), Cephalexin (141/168), Least resistance was noted with Imipenem (22/168) and Amikacin (54/168) (Table 3).

Table 3: Antibiotics Resistance Pattern of Biofilm Positive Isolates and Biofilm Negative Isolates

ANITIBIOTICS	BIOFILM POSITIVE ISOLATES	BIOFILM NEGATIVE ISOLATES
Amikacin (AK)	26 (68%)	40 (35%)
Ciprofloxacin (CIP)	28 (74%)	52 (47%)
Cotrimoxazole (COT)	30 (78%)	32 (29%)
Gentamicin (GEN)	25 (65%)	27 (27%)
Meropenem (MRP)	5 (13%)	6 (6%)
Piperacillin tazobactam (PIT)	8 (21%)	8 (8%)
Nitrofurantoin (NIT)	7 (18%)	4 (4%)
Cefoperazone sulbactam (CFS)	8 (21%)	10 (9%)

Table 4: Antibiotic Susceptibility Pattern of Biofilm Producing E. Coli Isolate

E. coli isolates	Sensitive	Resistant
Amikacin (AK)	7(4%)	10(59%)
Ciprofloxacin (CIP)	5(29%)	12(71%)
Cotrimoxazole (COT)	6(35%)	11(75%)
Gentamicin (GEN)	9(53%)	8(57%)
Meropenem (MRP)	15(89%)	3(11%)
Piperacillin tazobactam (PIT)	12(70%)	5(30%)

In the present study, 100% of biofilm producing strains were resistant to two or more number of antibiotics and were considered MDR phenotypes10. Among these, the maximum number of isolates were resistant to five or more number of the antibiotics used. In contrast, resistance was much lower in non-biofilm producing strains. Of the total 112 non biofilm producer strains 86 (76%) strains showed resistance to two or more antibiotics, whereas 26 (24%) non biofilm producing strains were sensitive to all antibiotics used.

DISCUSSION

UTI is a major public health problem in developing countries and it is one of the most commonly encountered clinical conditions.The present study showed E. coli was the most frequently isolated pathogen, followed by Klebsiella pneumoniae. Both are known to be responsible for a high percentage of UTIs and cause symptomatic UTIs.[11] These bacteria have multiple virulence factors, including biofilm formation, to establish themselves in the urinary tract. Biofilm formation helps the organisms survive in adverse conditions, even in the presence of antibacterial agents.

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Of 150 isolates, 38(25.33%) were in vitro biofilm producers. A total of 25.33 % of biofilm producers were detected by CRAM, which clearly indicates that CRAM is more sensitive to detect biofilm production as compared to TAM. TAM and CRAM can both be used for general screening methods for the detection of biofilm production in the laboratory. Significant biofilm production was exhibited by E. coli (44.73%), followed by Klebsiella pneumoniae (23.68%) .

Our results agree with previous studies, where E. coli and Klebsiella were found to be predominant biofilm producers in the catheterised as well as non-catheterised UTI patients.[12,13,16]

Biofilm producing strains showed relatively high drug resistance against all antibiotics tested as compared to non-biofilm-producing strains.The maximum number of strains showed resistance to 2-3 numbers of antibiotics tested. This is a worrisome trend and UTI caused by resistant strains pose a challenge for clinicians to treat the patients. It has been observed that biofilm production is often associated with long-term persistence of organisms in urinary tract. Dramatically increased resistance to antibiotics makes the situation more complicated14.

In the present study, a strong correlation was noted between biofilm production and resistance to multiple antibiotics, where 100% biofilm producing strains were MDR phenotypes (resistant to two or more antibiotics).[10] This was in accordance with other studies.[5,15]

The proximity of cells within a biofilm can facilitate exchange of plasmids, which shows poor response to antibiotic therapy may contribute to the spread of antibiotic resistant traits.[2]

An increased expression of efflux pump is another mechanism explained earlier for the development of antibiotic resistance among biofilm-producing bacteria. Trapping of antibiotics in the hexo-polysaccharides matrix, the ability of bacteria to escape from the host immune system when coated with biofilm, quorum sensing, altered metabolism and decreased growth rate may all be responsible for the development of antibiotic resistance among them.[5] These organisms may not be resistant to antibiotics initially but develop resistant when associated with biofilm. The detection of biofilm-producing bacteria in UTI changes the treatment plan. Blocking biofilm production by uropathogens in vivo provides alternative methods of therapy, which in turn will reduce the use of antibiotics. This will ultimately result in the prevention of the development of multidrug resistance among the uropathogens.

CONCLUSION

We conclude that E. coli was the most frequent isolate, whereas Klebsiella pneumoniae shows a high percentage of biofilm production. The Congo Red Agar Method (CRAM) was found to be more sensitive method than Tube Adherence Method (TAM) for screening. Highly significant correlation (100%) that Biofilm producers were found to be multidrug resistant than non-biofilm producers.

Biofilm production can be considered as one of the important virulent mechanisms of clinically significant UTI.

REFERENCES

1. M. Kunin, Urinary tract infections in females Clinical Infectious Diseases, 1994,vol. 18, no. 1(1–12).
2. Soto SM. Importance of biofilms in urinary tract infections: new therapeutic approaches Adv Biol 2014; 2014:543974.
3. Apurba S. Sastry, Essentials of Medical Microbiology New Delhi:Jaypee Brothers ,3rd edition, 2021.
4. Oli AK, Raju S, Rajeshwari, Nagaveni S and Kelmani CR Biofilm formation by multidrug-resistant Enterococcus faecalis (MDEF) originated in clinical samples. J Microbiol Biotech Res 2012; 2(2): 284-88.
5. Stewart PS. Mechanisms of antibiotic resistance in biofilms Int J Med Microbiol 2002; 292(2):107-13.
6. Koneman E, Allen SD, Janda WM, Schreckenberger PC Color atlas and textbook of diagnostic microbiology, 5th ed., San Fransisco, Lippincott 2006: 624-71.
7. (2012).Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement CLSI document M100- S22.Wayne, PA: Clinical and Laboratory Standards Institute.
8. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A Detection of biofilm formation among the clinical isolates of Staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol. 2006 Jan;24(1):25-9. doi: 10.4103/0255-0857.19890. PMID: 16505551.
9. Freeman DJ, Falkiner FR, Keane CT A New method for detecting slime production by coagulase negative staphylococci J Clin Pathol 1989; 42(8): 872-74.
10. Levy SB. Factors impacting on the problem of antibiotic resistance. J Antimicrob Chemother 2002; 49(1): 25-30.
11. Hwang-Soo Joo, Michael Otto. Molecular basis of in vivo biofilm formation by bacterial pathogens. Chem Biol 2012;19(12): 1503-13.
12. Anbarasu Priyadharshini, Mangaiyarkarasi T, Balasubramaniam.R, Dhandapany Senthil Pragash and Gopal. R. Biofilm production and antibiotic resistance among uropathogens causing bacteriuria in diabetic individuals. Sch J App Med Sci 2014; 2(2A):568-71.
13. Subramanian P1, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antibiotic resistance pattern of biofilm forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J 2012; 5(7):344-48.
14. Atray D, Atray M. Correlation between biofilm production and antibiotic resistance pattern in uropathogenic Escherichia coli in a tertiary care hospital in Southern Rajasthan, India. Int J Curr Microbiol App Sci 2015; 4(7): 640-46.
15. Awasthi TR, Pant ND, Dahal PR. Prevalence of multidrug resistant bacteria in causing community acquired urinary tract infection among the patients attending outpatient department of Seti Zonal Hospital, Dhangadi, Nepal. Nepal J Biotechnol 2015; 3(1): 55-9.
16. Sultan, A. M. and Nabiel, Y. Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: Which one could be better?Africa.Afr. J. Cln. Exper. Microbiol 2019; 20 (1): 60-66.

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