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BIOFILM PRODUCTION AMONG UROPATHOGENS AND THEIR ANTIBIOGRAM

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ABSTRACT

Urinary tract infection (UTI) is the most commonly acquired bacterial infection. Antibiotic resistance of uropathogens has been known to increase worldwide and biofilm production being the prime cause. The objective of this study was to detect the production of biofilm by uropathogens isolated from UTIs and their antimicrobial susceptibility pattern. A total 570 urine samples from clinically suspected UTI patients were processed by standard microbiological procedures. The isolated uropathogens were tested to biofilm production by Congo Red agar method and tube adherence method. Antimicrobial susceptibility testing of uropathogens was done by Kirby – Bauer disc diffusion method. Out of 272 cultures positive cases, *Eshcherichia coli* was the commonest uropathogen isolated followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antimicrobial susceptibility pattern of biofilm producers showed high resistance to commonly used antibiotics. The present study showed significant correlation between biofilm production and antibiotic resistance, so it is necessary to screen all isolates for biofilm production.

KEYWORDS - : Biofilm, Uropathogens, Tube adherence method, Congo Red agar method

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INTRODUCTION

Urinary tract infection (UTI) is defined as the colonization of microbes in any part of the urinary tract [1]. UTI poses serious health threat because of antibiotic resistance and high recurrence rate. *Escherichia coli* is the most frequently isolated microorganism in UTI[2]. Urinary tract infections account for an estimated 25% to 40% of nosocomial infections[3]. Biofilms are the microbial communities of the surface-attached cells, which are embedded in a self produced extracellular polymeric matrix[4]. Both, Gram positive and Gram negative bacteria have the capability to produce biofilm. Biofilm formation allows the strains to persist for long time in the genitourinary tract and interfere with bacterial eradication[1].The microbial biofilms pose a serious health problem as the microorganisms in the biofilm are difficult to treat with antimicrobial agents. The decreased susceptibility to antimicrobial agents within biofilms arises from multiple factors such as decreased diffusion of antimicrobial agents, reduced bacterial growth rates and local alteration of microenvironment that may impair activity of antimicrobial agent[5]. Furthermore, proximity of cells within a biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance[4]. So, the present study was carried out to know the antibiotic susceptibility pattern of biofilm producing uropathogens.

AIMS and OBJECTIVE

- To study the frequency of biofilm production among uropathogens.
- To study the antimicrobial susceptibility pattern of biofilm producing uropathogens.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology at S.R.T.R Govt. Medical College, Ambajogai during the period of June 2012 to May 2013. The mid stream urine samples were collected in sterile container from 570 patients, suspected to have a urinary tract infection and transported immediately to the laboratory. The samples were inoculated onto Blood agar, Mac Conkey's agar and incubated for 24 hours at 37°C. The identification of the isolate was done on the basis of the colony morphology, Gram's staining and standard biochemical test[6]. Antibiotic susceptibility was performed by using the Kirby-Bauer disc diffusion method as per the CLSI guidelines[7]. Biofilm detection was done by using tube method and Congo Red agar method.

- ***Tube adherence method (TA method)[8] -***

This is a qualitative method for biofilm detection. The suspension of the strain to be tested was poured into a glass tube which contains Brain Heart Infusion broth and incubated at 35° C for a period of 2 days. Then the supernatant was discarded and the glass tube stained with 0.1% safranin solution, washed with distilled water three times and dried. A positive result is defined as the presence of a layer of the stained material which adhered to the inner wall or bottom of the tube. The exclusive observation of a stained ring at the liquid-air interface was considered as negative. (Fig:1)

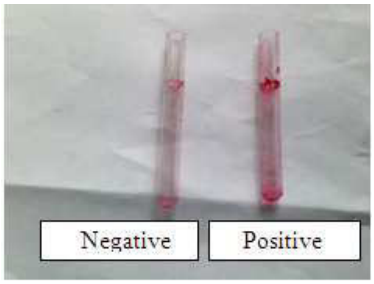

Figure1
Tube adherence method

- ***Congo Red agar (CRA) method*** [9] **-**

The suspension of the tested strains were inoculated into plate which contained a specially prepared solid medium- Brain Heart Infusion broth (BHI) which was supplemented with 5% sucrose and Congo Red. The medium was composed of BHI (37gms/l), sucrose (50 gms/l), agar No.1 (10 gms/l) and the Congo Red stain (0.8 gms/l). Congo Red was prepared as a concentrated aqueous solution and it was autoclaved at 121°C for 15 minutes, separately from the other medium constituents and it was then added when agar had cooled to 55°C. The plates were inoculated and incubated aerobically for 24-48 hours at 37°C. Positive result was indicated by black colonies with a dry crystalline consistency. (Fig: 2)

Figure2
Congo Red agar method

Biofilm production by any of these two methods was taken as a positive and statistical analysis was done by Z test.

RESULTS

Out of 570 urine samples processed in the present study, 272 (47.71%) were culture positive and remaining 298 (52.29%) were culture negative. *E. coli* (48.90%) was the predominant isolate followed by *Staphylococcus aureus* (17.65%) and *Klebsiella pneumoniae* (13.24%). (Table No. 1)

Table 1
Distribution of uropathogens obtained from Urine samples (n=272)

Bacterial isolate	Number	Percentage (%)
Escherichia coli	133	48.90%
Staphylococcus aureus	48	17.65%
Klebsiella pneumoniae	36	13.24%
Pseudomonas aeruginosa	29	10.66%
Proteus spp.	16	5.88 %
Coagulase negative Staphylococci	10	3.68%

Out of 272 isolates, 122 (44.85 %) were positive for biofilm production. Among these, major biofilm producing isolate was *E. coli* followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* (Table No. 2)

Table 2
Distribution of biofilm producing uropathogens

Bacterial isolate	Biofilm producer	Non-biofilm producer	Total
Escherichia coli	80(60.15%)	53(39.85%)	133
Staphylococcus aureus	19(39.58%)	29(60.42%)	48
Klebsiella pneumoniae	13(36.11%)	23(63.89%)	36
Coagulase negative Staphylococci	2(20%)	8(80%)	10
Proteus spp.	3(18.75 %)	13(81.25%)	16
Pseudomonas aeruginosa	5(17.24%)	24(82.76%)	29
Total	122(44.85%)	150(55.15%)	272

A total of 98 (36.02%) isolates were positive for biofilm production by both methods. Biofilm production by tube method and Congo Red agar method was seen in 112 (41.17%) and 108 (39.70%) isolates respectively (Table No.3).The difference in biofilm production by these two method was found to be statistically non significant (SEP, Z= -0.31, P>0.05).

Table 3
Results of biofilm production by tube method and Congo Red agar method (n=272)

Total no. of isolates	Tube method	Congo Red agar method
98	+	+
150	-	-
10	-	+
14	+	-

The antibiotic resistance was more common among biofilm producing *Staphylococcus aureus* as compared to non-biofilm producers with exception of norfloxacin and nitrofurantoin. All *Staphylococcus aureus* were 100 % sensitive to vancomycin (Table No. 4)

Table 4
Antibiotic resistance pattern of biofilm producing Staphylococcus aureus in comparison with non-biofilm producer

Antimicrobial agent	Biofilm producer S. aureus (n=19)	Non-biofilm producer S. aureus (n=29)	P value
Cefoxitin	17(89.47%)	16(55.17%)	<0.05
Cotrimoxazole	16(84.21%)	18(62.07%)	<0.05
Ciprofloxacin	15(78.95%)	14(48.28%)	<0.05
Gentamicin	13(68.42%)	11(37.93%)	<0.05
Tetracycline	12(63.16%)	9(31.03%)	<0.05
Lomifloxacin	11(57.89%)	6(20.69%)	<0.05
Norfloxacin	11(57.89%)	9(31.03%)	>0.05
Nitrofurantoin	5(26.32%)	2(6.90%)	>0.05
Vancomycin	0(0%)	0(0%)	-

Gram negative biofilm producers were more resistant to various antibiotics as compared to non-biofilm producers. All Gram negative organisms were 100 % sensitive to imipenem and meropenem and 100 % resistant to ampicillin. (Table No. 5)

Table 5
Antibiotic resistance pattern of gram negative biofilm producers in comparison with non- biofilm producers

Antimicrobial agent	Biofilm producer (n=101)	Non-biofilm producer (n=113)	P value
Amoxicillin clavulanic acid	95(94.06%)	82(72.57%)	<0.05
Cotrimoxazole	78(77.23%)	65(57.52%)	<0.05
Lomifloxacin	83(82.18)	67(59.29%)	<0.05
Ampicillin	101(100%)	113(100%)	-
Cefotaxim	74(73.27%)	47(41.59%)	<0.05
Ciprofloxacin	71(70.30%)	38(33.63%)	<0.05
Norfloxacin	57(56.44%)	31(27.43%)	<0.05
Gentamicin	54(53.47%)	27(23.89%)	<0.05
Nitrofurantoin	37(36.63%)	13(11.50%)	<0.05
Imipenem	0(0 %)	0(0 %)	-
Meropenem	0(0 %)	0(0 %)	-

DISCUSSION

Urinary tract infections are serious health threat with respect to antibiotic resistance and biofilm production being prime cause for antibiotic resistance. In our study, *E. coli* (48.85%) was the most frequently isolated uropathogen. This finding was in close association with other studies [10, 11]. In the current study, biofilm production was seen in 44.85% of uropathogens. A similar study showed 54% of biofilm production by uropathogens from UTI [12]. A significant production of biofilm was seen in 80(60.15%) isolates of *E. coli*, followed by *Staphylococcus aureus* 19(39.58%) and *Klebsiella pneumoniae* 13(36.11%). This observation was in accordance with other study [13]. The detection of the biofilm production by Congo Red agar method and tube adherence method were 39.70% and 41.17 % respectively. In contrast to other studies, difference between detection rates of biofilm production by two methods was statistically non significant [14, 15].

Antibiotic resistance was higher among biofilm producers to commonly used antibiotics as compared to non biofilm producers. This may be because bacterial biofilms are often associated with long term persistence of organism in various environments, decreased bacterial growth rate in a biofilm, decreased diffusion of antibiotics in biofilm so they display dramatically increased resistance to antibiotics[5]. The present study showed most effective antibiotic against Gram negative bacteria were imipenem and meropenem and vancomycin was most effective against Gram positive bacteria.

CONCLUSION

The present study showed significant correlation between biofilm production and drug resistance. So it is necessary to screen all urinary isolates for biofilm production. This will help our clinician in prescribing an appropriate antibiotic against urinary tract infection.

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