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BIOFILM FORMATION BY UROPATHOGENIC ESCHERICHIA COLI AND ITS ANTIBIOTIC RESISTANCE PATTERN IN TERTIARY CARE CENTRE

Medical Science

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ABSTRACT

Urinary tract infections (UTI) is a serious health problem with respect to antibiotic resistance and biofilm formation being the prime cause for antibiotic resistance. The biofilms produced by *E.coli* may be difficult to treat as they exhibit multidrug resistance. The present study is aimed to perform invitro detection of biofilm formation among *E.coli* strains from urine cultures. A total number of 180 *E.coli* strains were isolated from urine samples suffering from UTI. The samples were processed using standard phenotypic identification techniques and tested for biofilm production by three methods congo red agar(CRA), tube method(TM) and tissue culture plate method(TCP). Among 180 *E.coli i*solates, 80 and 100 strains were collected from catheterized and non-catheterized patients respectively. In biofilm production 96, 97 and 119 were positive for biofilm productions by Congo red agar (CRA), Tube method(TM) and Tissue culture plate (TCP) method respectively. Among the biofilm producers maximum resistance was seen to cephalexin 168(93%), ampicillin 158(87%), norfloxacin 154(85%), ciprofloxacin154(85%),ceftazidime with clavulinic acid 146 (81%),gentamicin125(69%), piperacillin tazobactam 81(45%), cefoperazone with sulbactam 79(43%). The antibiotic resistance among biofilm producing *E.coli* was found to be higher than that of non-biofilm with a p=0.01(<0.05)which is statistically significant. A greater understanding of the nature of biofilm producing *E.coli* in UTIs will help in the development of new and more effective treatment.

KEYWORDS

Escherichia Coli, Antibiotic Resistance, Biofilm.

INTRODUCTION:

The most common bacterial infections are urinary tract infections(UTI). Women are of greater risk than male of developing urinary tract infection , mostly it involves the lower urinary tract. All women are at greater risk to cystitis due to the short distance from anus to urethra and urethral openings to bladder[1]. Bacteria enters through the urinary tract and multiplies in the bladder. *Escherichia coli* causes cystitis, sexual intercourse may also lead to cystitis. *E.coli* is a type of bacteria that normally lives in the intestine. UTI is a serious health problem with respect to antibiotic resistance and biofilm formation being the prime cause for antibiotic resistance.

According to national institutes of health, biofilms are responsible for >60%of all microbial infections[4]. The biofilms produced by *E.coli* may be difficult to treat as they exhibit multidrug resistance. Biofilm prevalence among uropathogenic. *E.coli* ranges from 60%-70%.Biofilm formation also increase the risk of recurrent UTI. This can be reduced by appropriate treatment with antibiotics like Fluroquinolone, cephalosporin and aminoglycosides.

The present study is aimed to perform invitro detection of biofilm formation among *E.coli* strains from urine cultures by three different methods and to correlate the biofilm production with antibiotic resistance pattern.

MATERIALS AND METHOD:

The present study was conducted in the Department Of Microbiology at Saveetha medical college, Chennai, India.

The ethical clearance has been obtained for the study using conventional methods, a total number of 180 *E.coli* strains were isolated from urine samples suffering from UTI .

The samples were tested for biofilm production by three methods and then performed antibiotic susceptibility testing by Kirby-Bauer diffusion method.

EXAMINATION OF URINE:

Urine samples were collected and processed using the following standard techniques:

Gram staining was performed for all the samples collected. Plating of the samples were done by semi quantitative method on MacConkey agar plate and cystine lactose electrolyte deficient agar medium and incubated at 37 C for 24 hour. Biochemical test were performed for all the samples according to the standard guidelines. Antibiotic susceptibility testing by Kirby Bauer disc diffusion method was done following CLSI guidelines[13].

METHODS FOR DETECTION OF BIOFILM

Congo red agar method(CRA):

Isolates were inoculated on Congo red agar plate and incubated aerobically for 24-48 hours at 37 C. The results are:

Strong Biofilm producers showed black colonies with a dry crystalline consistency.

Intermediate biofilm producers showed darkening of the colonies with the absence of a dry crystalline colonial morphology[10].

Weak biofilm producers remain pink,though occasional darkening at the center of colonies.

Tube method(TM):

The colonies from overnight culture plates are inoculated at trypticase soy broth with 1% glucose (10ml) and incubated for 24 hours at 37 C. Then tubes decanted and washed with phosphate buffer saline, dried and stained with crystal violet (0.1%). Tubes were washed to remove excess stain with deionized water and tubes were dried in the inverted position to observe for biofilm formation.

Positive results were production of visible film lining the wall and bottom of the tube.

Negative results were Ring formation at the liquid interface.

Tissue culture plate method(TCP):

Isolates from fresh agar plates were inoculated in trypticase soy broth and incubated for 24 hours at 37 C then diluted with fresh trypticase soy broth in 1 in 100 dilution. Tissue culture plate which is sterile polystyrene 96 well flat bottom is filled with 0.2ml aliquots of the diluted culture. The broth served as control to check sterility and non-specific binding of media. The TCP was incubated for 24 hours at 37 C. By tapping the plates, content of each well was gently remove

and washed 4 times with 0.2ml Phosphate buffered saline to remove free floating planktonic bacteria, Wells were stained with crystal violet. To remove excess stain wells were washed with deionized water and the tubes were dried. Optical density (OD) was determined at a wavelength of 570nm with micro ELISA auto reader. Experiment was repeated thrice and standard deviation was calculated. The mean OD value obtained from media control was deducted from all the test OD value.

Antibiotic Susceptibility Test:

Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method, following CLSI guidelines[13]. Gram-negative antibiotics - ampicillin, co-trimoxazole, nitrofurantoin, ciprofloxacin, amoxicillin-clavulanic acid, norfloxacin, gentamicin, piperacillin tazobactam, cephalexin, nalidixic acid, amikacin, cefoperazone with sulbactam, ceftazidime with clavulanic acid, and imipenem were used along with controls.

RESULT:

Among 180 *Escherichia coli i*solates, 80 and 100 strains were collected from catheterized and non-catheterized patients respectively and all the *isolates* were subjected to biofilm production . In biofilm production 96, 97 and 119 were positive for biofilm productions by Congo red agar (CRA), Tube method(TM) and Tissue culture plate (TCP) method respectively. By all the three methods 119 strains were positive for biofilm producers and TCP was found to be very sensitive. Catheterized patients were maximum biofilm producers.

Among the 80 catheterized patients, 73(91.2%) and 7(8.75%) strains were biofilm producers and non-biofilm producers, detected by TCP method respectively. By TM method, 60(75%) were biofilm producers and 20(25%) were nonbiofilm producers. Biofilm producing strains were 59(73.7%) and non-biofilm producers were 21(26.2%) by CRA method.

In the 100 non catheterized patients, by TCP method 46(46%) strains were biofilm producers, 54(54%) non-biofilm producer, by TM method 37(37%) were biofilm producer and 63(63%) were non-biofilm producers. In CRA method 37(37%) were biofilm producers and 63(63%) were non-biofilm producers. As a total of 119 isolates, 61 *E.coli* strains were biofilm producers from catheterized and non-catheterized patients is shown in Fig/Table1. In both male and female between the age group of 20 to 40 years high numbers of *E.coli* strains were isolated. There is no significant comparison between both male and female shown in Fig/ Table2.

Overall resistance pattern of *E.coli* strains is shown in Fig /Table 3. Among the biofilm producers maximum resistance was seen to cephalexin 168(93%), ampicillin 158(87%), norfloxacin 154(85%), ciprofloxacin154(85%), ceftazidime with clavulinic acid 146 (81%), gentamicin 125(69%),piperacillin81(45%),cefoperazone with salbactam79(43%). Minimum resistance was seen to amikacin 71 (39%), co-trimoxazole 64(35%),imipenem 20(11%)followed by nitrofurantoin(21%) is shown in Fig/Table4.The antibiotic resistance among biofilm producing *E.coli* was found to be higher than that of non-biofilm with a p=0.01(<0.05)which is statistically significant.

DISCUSSION:

E.coli is a predominant species among facultative anaerobic bacteria of the gastrointestinal tract. Both of its frequent community lifestyle and the availability of a wide array of genetic tools contributed to establish *E.coli* as a relevant model organism for the study surface colonization. Several key factors, including different extracellular appendages are implicated in *E.coli* surface colonization and their expression and activity are finely regulated both in space and time to ensure productive events leading to mature biofilm formation.

Saroj et al. showed 69% isolates as biofilm producers by TM and TCP methods[5]. The study conducted by Sevanan et al., Congo red method showed 59.4% strains to be biofilm producer[4]. Significant production of biofilm was seen in 67.5% isolates of *E. coli* in a study conducted by Sharma et al. by TCP method[6].

In the present study, it showed that among 80 catheter associated UTI, 91.25% isolates produced biofilm by TCP method,75% isolates produced biofilm by TM method and 73% isolates produced biofilm by CRA method. Among 100 non catheterized patients 46% isolates produced biofilm by TCP method, 37 % isolates produced biofilm by TM method, 37% isolates produced biofilm by CRA. Similarly, among the 80 catheterised patients, 8.75% strains were non bio-film producer by TCP method, 25%strains were nonbiofilm producers by TM method and 27% were nonbiofilm producers by CRA method. Among 100 non catheterized patients, 54% isolates were nonbiofilm producers by TCP method, 63 % isolates were nonbiofilm producers by TM method and 63% isolates were nonbiofilm producers by CRA.

In a study conducted by Poovendran et al., all biofilm forming strains were maximum resistance to amoxyclav (100%), followed by chloramphenicol (100%), gentamicin(86%), cefotaxime (86%), ceftazidime (84%), cotrimoxazole(83%), piperacillin with tazobactam (83%), and amikacin (70%). Resistance to co-trimoxazole, tetracycline and ampicilin were comparatively higher among biofilm producer than nonbiofilm producer and there was a significant correlation between biofilm production and resistance to multiply antibiotics.

The study conducted by Sevanan et al. showed that resistance was seen to be maximum with erythromycin(90.6%), amikacin(71.9%), cotrimoxazole (65.6%), ampicillin(59.3%),meropenem(56.3%), chloramphenicol(56.3%), tobramycin(53.1%) and gentamycin(50.0%), respectively. In the present study, the correlation between biofilm producer and non biofilm producer with antibiotic resistance was found statistically significant with P=0.01 for antibiotics ampicillin, norflaxacin, ciproflaxacin, cephalosporin.

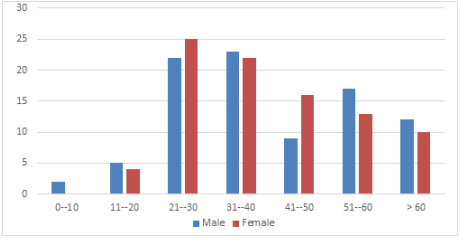
CONCLUSION:

A greater understanding of the nature of biofilm producing *E.coli* in UTIs will help in the development of new and more effective treatment. Therefore, there is a need to find out a suitable method for detection of biofilm formation among *E.coli* .From the present study we have concluded that TCP method is more qualitative and reliable method to detect biofilm producing *E.coli*. Biofilm forming microorganisms showed resistance to multiple drugs.

TABLES AND CHARTS

Fig\Table-1: Biofilm and Non-Biofilm forming *E.coli* isolates by different methods and its correlation with catheterized/non-catheterized.

Escherichia coli Isolates(180)	Tissue Culture Plate (TCP)		Tube Method (TM)		Congo Red Agar Method (CRA)	
	Biofilm Producers (%)	Non biofilm Producers (%)	Biofilm Producers (%)	Non biofilm Producers (%)	Biofilm Producers (%)	Non biofilm Producers (%)
Catheterized (80)	73(91.26%)	7 (8.15%)	60 (75%)	20 (25%)	59(73.75%)	21(26.25%)
Non Catheterized (100)	46 (46%)	54 (54%)	37 (37%)	63 (63%)	37 (37%)	63(63%)
Total (180)	119	61	97	83	96	84



Fig\ Table-2: Age sex wise distribution of Escherichia coli isolates

Antibiotics	Sensitive (%)	Resistance (%)
Ampicillin	22(12%)	158(87%)
Co-trimoxazole	116 (64%)	64 (37%)
Norfloxacin	26 (14.4%)	154 (85%)
Ciprofloxacin	26 (14.4%)	154 (85%)
Gentamicin	55 (31%)	125 (69%)
Amikacin	107(59.4%)	71 (39%)
Cephalexin	12 (6.7%)	168 (93%)
Cefoperazone with sulbactam	101 (56%)	79 (43%)

Piperacillin / tazobactam	99 (55%)	81 (45%)
Ofloxacin	24 (13.3%)	156 (85%)
Imipenem	160 (88.9%)	20 (11%)
Nitrofurantoin	140 (77.8%)	40 (21%)
Ceftazidime/clavulanic acid	34 (18.9%)	146 (81%)

Fig\Table-3: Antibiotic Susceptibility Pattern Of *E.Coli* isolates

Antibiotics	Biofilm producers (n=119), n(%)		Nonbiofilm producers(n=61),n(%)	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	29(24.3%)	90(75.6%)	10(16.4%)	51(84%)
Co-trimoxazole	57(47.9%)	62(52%)	21(34.4%)	40(65.6%)
Norfloxacin	20(16.8%)	99(83%)	9(14.8%)	52(85.2%)
Ciprofloxacin	20(16.8%)	99(83%)	9(14.8%)	52(85.2%)
Gentamicin	40(33.6%)	79(66%)	26(42.6%)	35(57.4%)
Amikacin	62(52%)	57(47.9%)	20(32.8%)	41(67.2%)
Cephalexin	10(8.4%)	109(91.6%	1(1.6%)	60(98.4%)
Cefoperozone with sulbactam	50(42%)	69(58%)	23(37.7%)	38(62.3%)
Piperacillin	77(64.7%)	42(35.3%)	19(31%)	42(68.9%)
Oflaxacin	31(26%)	88(74%)	9(14.8%)	52(85.2%)
Imipenem	93(62%)	26(22%)	50(82%)	11(18%)
Nitroflurantoin	74(62%)	45(38%)	40(65.6%)	21(34.4%)
Ceftazidime/ Claculanic acid	25(21%)	96(80.7%)	12(19.7%)	49(80.3%)

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