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Original Research Article

Biofilm formation in uropathogenic Escherichia coli isolates and its association with extended spectrum betalactamase production and drug resistance

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Abstract: The objective of this study is to determine the association of biofilm formation, extended spectrum beta lactamase [ESBL] production and antibiotic susceptibility pattern among uropathogenic Escherichia coli [UPEC] isolates. The present study was conducted on 137 UPEC isolates (counts > 10^5cfu/ml) from UTI cases. In all these isolates biofilm formation was detected by microtitre plate [MTP] method, ESBL production by combined disc diffusion technique and antibiotic susceptibility testing [AST] by Kirby- Bauer disk diffusion method. The data was analysed by using Medcalc software package. Chi-square test was applied. Among 137 UPEC isolates, 48 (35%) were biofilm producers [BFP's]and 89 (65%) were biofilm non producers [BFNP's] by MTP method. Of the total number of137 isolates, the highest number of strains were susceptible to amikacin followed by gentamicin, nitrofurantoin, cefepime.Among 137 isolates, ESBL producers were 28 (75%) of which 21 (75%) were BFP's also, which makes a total of 49 (35%) (ESBL+Biofilm) and BFNP's were 7[25%]. ESBL non-producing E.coliwere109 (80%), of which 32 (29.3%) were BFP's and 77 (70.6%) were BFNP's. The ability of biofilm formation was significantly higher in ESBL producing strains than that of ESBL non-producing strains ($p<0.05$). The ability of biofilm formation was found to be higher among ESBL producing stains of E. coli. Higher resistance rate was noted among biofilm producers to almost all the antimicrobial agents except few than non-biofilm producers.

Keywords: Uropathogenic *Escherichia coli*, Biofilm, Extended spectrum betalactamase, Drug resistance.

INTRODUCTION

Urinary tract infection [UTI] is one of the major nosocomial infections commonly caused by Escherichia coli which accounts for 90% of community acquired and 50% of hospital acquired UTI's. [1]. Uropathogenic Escherichia coli [UPEC] have several virulent determinants which enable them to colonize the bladder mucosa and injure it, leading to inflammatory changes and overcome the host immune regulators [2]. Biofilm formation and ESBL production are few of them.

Biofilm film is a mechanism exhibited by several microbes to servive in unfavourable conditions. The bacterial biofilm is a structured community of bacterial cells enclosed in polymeric matrix and adherent to a surface [3]. Biofilm producing uropathogenic bacteria may be responsible for many recurrent UTI's [4] and are highly resistant to antibiotic treatment [3]. The emergence and worldwide rapid increase in prevalence of ESBL producing bacteria that are multidrug resistant pose treatment problem resulting in high morbidity, high mortality and increased healthcare costs [5].

Objectives

This study was conducted to know the biofilm formation in UPEC. Further we have determined the correlation between biofilm formation and drug resistance with commonly used antibiotics along with ESBL production in UPEC.

MATERIALS AND METHODS

The present study was conducted on 137 uropathogenic E.coli isolates recovered from UTI cases attending the OPD at NRIGH, Chinakakani.Mid stream

urine samples collected from 520 patients (outpatients and in patients) suspected of UTI were cultured by semi-quantitative culture technique [6]. Urine samples were processed immediately and the bacterial isolates were identified by using microbiological techniques which include morphological appearance of the colonies staining reactions and biochemical properties. Plates which were culture positive for E.coli with a count more than 10^5cfu/ml were proceeded for ESBL detection, biofilm production and antibiotic sensitivity testing.

Detection of biofilm producers [BFP's]:

Biofilm detection was done by microtitre plate method (MTP) and standard tube method (TM).

Microtitre Plate Method:

The ability of microorganisms to form biofilm on abiotic surfaces is detected by growing them in an MTP, which is then detected quantitatively by spectrophotometer using an ELISA reader[7].

Tube Method

A quantitative assessment of biofilm was determined by inoculating brain heart infusion broth with 2% sucrose with loopful of bacterial growth from overnight culture plates incubated for 24hrs at 37^0C. Tubes were decanted and washed with physiological buffered saline [PBS] and dried tubes were stained with crystal violet 0.1%. Excess stain was removed and tubes were washed with deionizer water. Tubes were then dried in inverted position and observed for biofilm formation. Bio film formation was considered positive when visible film lined the wall and bottom of the tube.

Detection of ESBL producers:

All the 137 E.coli isolates were subjected for the detection of ESBL production. The phenotypic confirmation of the ESBL producing stains was performed by combined disk method as per CLSI guidelines [8,9].

Antibiotic susceptibility test(AST):

AST of biofilm producing bacteria was done on Muller Hinton agar using the following antibiotic discs [10] Norfloxacin, Nitrofurantion, Cefizoxime, Ceftazidime, Amoxyclavulanic acid, Cotrimoxazole, Ampicillin, Amikacin, Imipenem, Tetracycline. All the antibiotic discs were obtained from the Himedia. E.coli ATCC 25922 was used as positive control and diffusion technique according to CLSI guidelines.

RESULT

Out of 520 mid stream urine samples, 137(26.3%) showed significant growth (>10^5cfu/ml). Among 137 E.coli isolates, 48 (35%) were BFP's and 89 (65%) were biofilm non- producers [BFNP's] by MTP method. By TM 44(32%) were BFP's and 93 (67.8%) were BFNP's. (Table 1).Out of 137 E.coli isolates, 28 (20.4%) were found to be ESBL producers and 109 (80%) were ESBL non- producers. (Table 1)

Table-1: Biofilm and ESBL distribution of isolates

Biofilm producers	48 (35%)
Biofilm non producers	89 (65%)
Total	137 (26.3%)
ESBL producers	28(20.4%)
ESBL non producers	109(80%)
Total	137 (26.3%)

Association of ESBL production and biofilm formation among E.coli isolates.

Out of 28 ESBL producing pathogenic strains of E.coli, 21 (75%) were BFP's and 7[25%] were BFNP's whereas among109 ESBL non-producing E.coli, 32 (29.3%) were BFP's and 77 (70.6%) were BFNP's. Both ESBL and biofilm producers were 49[36%].The ability of biofilm formation was found to be significantly high in ESBL producing strains of E.coli than that of ESBL non-producing E.coli strains ($P<0.05$).

Table-2: Biofilm and ESBL distribution of isolates

ESBL producers No = 28 (20.4%)	
Biofilm producers	Biofilm non producers
21 (75%)	7(25%)
Non ESBL producers No = 109	
Biofilm producers	Biofilm non producers
32(29.3%)	77(70.6%)

Antibiotic susceptibility pattern of the uropathogenic E.coli isolated.

Of total 137 E.coli isolates the highest number of strains were susceptible to nitrofurantoin followed by, gentamicin, amikacin, ceftriaxone and cefepime. Similarly least number of the strains were susceptible to amoxicillin and cephalexin.

Antibiotic resistance pattern of E.coli among BFP's and BFNP's:

The antibiotic resistance among biofilm producing *E.coli* was found significantly higher than that of biofilm non producing *E.coli* ($p<0.05$). The correlation between biofilm production and antibiotic resistance was found statistically significant ($p<0.05$) in most of the antibiotics (ciprofloxacin, ofloxacin, norfloxacin, amoxyclav, gentimicin, cotrimoxazole, cephalexin, cefixime, ceftazidime, cefotaxime, ceftriaxone and cefepime) but the correlation was not found to be signifiicant in case of amikacin and nitrofurantoin.

Table-3: Antibiotic resistance pattern of E.coli among biofilm producers and non producers along with the antibiotic susceptibility pattern of all the E. coli isolates

Antibiotics	Resistance pattern		Total resistant No(%)	Total susceptible No(%)
	Biofilm producers n = 48 (35%)	Biofilm non-producers n = 89 (65%)		
Ciprofloxacin	36(75%)	40(45%)	76(55%)	61(45%)
Ofloxacin	33(68%)	35(39%)	68(50%)	69(50%)
Norfloxacin	36(75%)	40(45%)	76(55%)	61(45%)
Gentamicin	25(52%)	23(26%)	48(35%)	89(65%)
Amikacin	9(19%)	45(5%)	54(40%)	83(60%)
Cotrimoxazole	33(68%)	37(42%)	70(51%)	67(49%)
Amoxicillin	44(92%)	77(87%)	121(88%)	16(12%)
Cephalexin	45(94%)	84(74%)	129(94%)	8(6%)
Cefixime	44(91%)	52(59%)	96(70%)	41(30%)
Ceftazidime	36(76%)	36(40%)	72(52%)	65(48%)
Cefotaxime	36(76%)	31(35%)	67(49%)	70(51%)
Ceftriaxome	32(67%)	25(28%)	57(42%)	80(58%)
Cefiepime	31(65%)	29(33%)	60(44%)	77(56%)
Nitrofurantoia	13(27%)	25(28%)	38(28%)	99(72%)

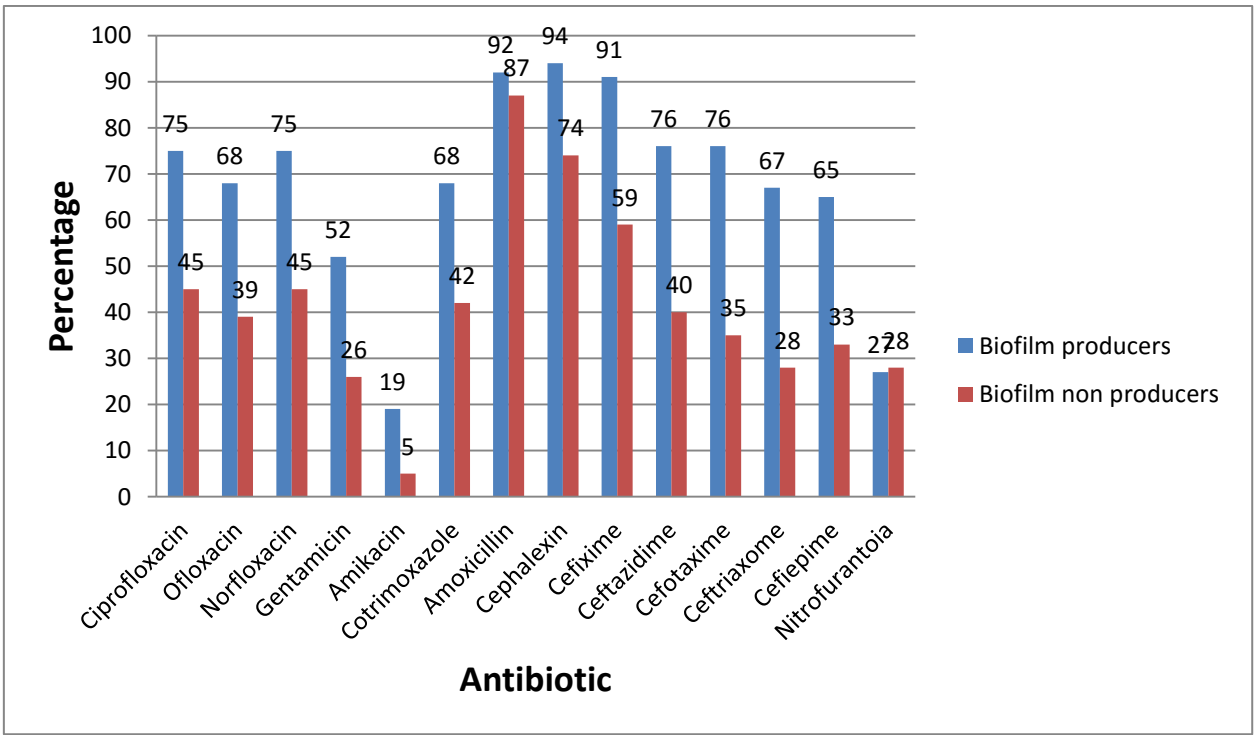

Fig-1: Antibiotic resistance pattern of biofilm and nonbiofilm producers uro pathogenic E.coli

Data analysis:

Medcalc software package was used for statistical analysis. Chi-square test was applied.

DISCUSSION

Biofilm-associated infections have a major deleterious impact on artificial implants and it often serves as source of recurrent infections [11]. These are reported to affect 90% of indwelling stents in patients [12]. In the urinary tract, bacterial biofilms develop on bladder mucosa-producing persistent and recurrent UTI's, chronic cystitis, prostatitis, etc[13]. It has been reported that 10-15% of patients undergoing short-term catheterization develop UTI.

Biofilms-associated bacterial infections are difficult to eradicate using antibiotics. The matrix may also be involved in the protection of the bacteria against toxic molecules such as antimicrobials, hydroxyl radicals, andsuperoxide anions. The biofilm matrix could also inhibit wash out of enzymes, nutrients, or even signaling molecules that could then accumulate locally and create more favorable microenvironments within the biofilms [14].

There are various methods for biofilm detection [15,16,17]. From the total of 137 UPEC isolated, 48(35%) of them showed biofilm formation by the MTP method and 44 [32%] by TM, thus showing MTP method is superior to TM in our study.

Among 137 clinical isolates, 28 (20.4%) were ESBL producers of which 21 (75%) were biofilm producers also and 7 (25%) were BFNP. The biofilm forming ability was found to be significantly high in ESBL positive strains of UPEC than that of ESBL negative strains ($P<0.05$). This is in accordance with other studies. [18-24].

ESBL's are enzymes that are responsible for resistance of bacteria towards third generation cephalosporins and monobactams[25]. Most of the plasmids responsible for ESBL production carry genes encoding resistance to other drugs also [26]. Due to frequent presence of cross-resistance to several other classes of antibiotics (like aminoglycosides and fluoroquinolones) in ESBL-producing organisms, the treatment of the infections by these bacteria are often present as the therapeutic challenges [25]. Further higher ability of the ESBL producing organisms to form biofilm makes the treatment even more difficult, increasing the mortality and severity of the infections [24]. Microlides (erythromycin, clarithromycin, and azithromycin) are known to have antibiofilm activity against biofilm producing organisms by inhibiting a key component of the biofilm, alginate. And several studies have recommended, the combined therapy (being macrolides one of the first antibiotics chosen) as the treatment of choice in infections caused by biofilm producing organisms[3].

In this study, the antibiotic resistance of biofilm producing E.coli was found significantly higher than that of biofilm nonproducing E.coli ($p<0.05$). The association between biofilm production and antibiotic reistance was found to be statistically significant ($p<0.05$) except in case of amikacin, nitrofurantoin and cephalexin. Microorganisms growing in a biofilm are intrinsically resistant to many antibiotics increasing the antibiotic resistance up to 1000 folds and high antimicrobial concentrations are required to inactivate organisms growing in a biofilm [27,28]. This may be because of the insufficient concentration of the antibiotics reaching some areas of the biofilms and metabolic inactiveness (along with the presence of active antibiotic degradation mechanisms contributing to halt the accumulation of the drugs up to as effective concentration) of the bacteria located at the base of the biofilms [3].

CONCLUSION

The ability of biofilm formation was found higher among ESBL producing strains of E.coli. There was higher resistance rate among biofilm producing E.coli isolates to almost all the antimicrobial agents except a few. According to our antimicrobial susceptibility pattern for E.coli, to start preliminary treatment we recommend to use of amikacin or nitrofurantoin.

By this study we conclude that biofilm forming and ESBL producing microorganisms showed high resistance rate to almost all the antimicrobial agents except a few. For the treatment of renal infection, choice of antibiotic should be selected on basis of the urine culture and sensitivity report.

Limitations of the study: Due to lack of easy availability of the advanced laboratory and due to lack of the funds we could not confirm the ESBL and biofilm producing organisms by using molecular technology.

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Conflict of interest: None

Ethical approval: Approved by the institutional ethical committee

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