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Study on Biofilm Production by Uropathogenic *Escherichia coli* Isolated from Tertiary Health Care Centers of *Vidarbha* and its Correlation with Antibiotic Susceptibility

N.A. Ghanwate and P.V. Thakare

Department of Microbiology, Sant Gadge Baba Amravati University, Amravati - 444 602, India.

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The chronic nature of some urinary tract infections (UTI) is being attributed to the ability of *E. coli* to form a biofilm. Bacteria growing within a biofilm lose their sensitivity to antibiotic quickly, thus resulting in persistent infections. The uropathogenic *E. coli* isolated from 200 samples were tested for antimicrobial susceptibility by disc diffusion method. *In vitro* quantitative estimation of biofilm formation by the isolates was determined by tube method. Comparison of two different media, for biofilm formation i.e. Luria Bertani broth(LB) and Brain Heart Infusion(BHI) broth with and without 2% sucrose were studied. From 77 *E. coli* isolates, 40 were positive for *in vitro* biofilm production. Among them 11 were classified as strong biofilm producers and 29 as moderate. It was found that 52% of biofilm producing *E. coli* were resistant to amoxicillin, 49% to cotrimoxazole, 41% to norfloxacin, 40 % to gentamycin and nalidixic acid, 35% to chloramphenicol and 31% to ciprofloxacin. The percentage of resistance in the nonbiofilm producing *E. coli* isolates ranged between 6-31%. Comparison of two media showed that the percentage of biofilm formation was more in LB than in BHI broth. There was a significant correlation between biofilm production and antibiotic resistance. The present study demonstrated that uropathogenic *E.coli* have high propensity to form biofilm that renders it resistant to conventional antimicrobial therapy.

Key Words: *E. coli*, Biofilm, UTI, Antibiotic resistance.

Urinary tract infection is one of the most important causes of morbidity and mortality [1] with respect to antibiotic resistance and high recurrence rates. Uropathogenic *E. coli* forms intracellular bacterial communities with many biofilm like properties within the bladder epithelium. These intracellular biofilm like pods allow bacteria to outlast a strong host immune response to establish a dormant reservoir of pathogens inside the bladder cells. Re-emergence of bacteria from this reservoir might be the source of recurrent infections [2]. *E. coli* is the most frequent urinary pathogen isolated from 50% - 90% of all uncomplicated urinary tract infection[1] which has been reported to be able to form intracellular biofilm like aggregates inside bladder cells making the bacteria hard to reach by both host defence mechanisms and antibiotics[3]. In the biofilm stage, a phenotypic change occurs in which the bacteria require generally much higher concentration of antibiotics to inhibit their growth[4]. Bacteria growing within a biofilm lose their sensitivity to antibiotics. Thus biofilms results in persistent infections that

* To whom all correspondence should be addressed.
Mob.: +91-9922445913
E-mail:nirajghanwate@gmail.com

cannot be resolved with standard antibiotic treatments. The present study was aimed for *in vitro* qualitative estimation of biofilm production in *E. coli* isolated from urinary tract infection and its correlation with antibiotic resistance. Simultaneously two media, LB and BHI broth with and without 2% sucrose were compared to determine the frequency of biofilm formation.

MATERIALS AND METHODS

Clinical isolates

A total of 200 clean catch midstream urine samples of patients were collected in sterile containers. A measured amount of urine i.e. 0.01 ml was inoculated by using calibrated loop method on Hi-crome UTI agar (Hi-media). After incubation, culture growth with cfu $>10^5$ was considered as significant bacteriuria. From the colonies on Hi-crome agar, *E. coli* was identified. The isolated uropathogenic *E. coli* (UPEC) were tested for biofilm formation and antibiotic sensitivity test.

Antibiotic sensitivity test

E. coli isolates were tested for antimicrobial susceptibility by Kirby Bauer Disc Diffusion method on Muller Hinton agar, according to norms of Clinical Laboratory Standard Institute[5]. Antibiotics used and their concentration in mg included – co-trimoxazole [25)] amoxicillin [10], gentamicin [10], norfloxacin[10], nalidixic acid[30], ciprofloxacin[5], chloramphenicol[30], and nitrofurantoin[30] (Hi-media, Mumbai).

Detection of biofilm formation

In vitro quantitative assessment of biofilm formation was determined by tube method as previously described by Christensen *et al* [6]. To compare the frequency of biofilm formation, two types of media i.e. Luria Bertani (LB) broth and Brain Heart Infusion (BHI) broth, (Hi-media) with and without 2% sucrose was used. Broth(10ml) was taken in test tubes and inoculated with loopful of UPEC isolates from overnight culture plates and incubated at 37ºC for 24 h. After incubation broth was decanted and washed with phosphate buffer

Table 1. MAR index of antibiotics against isolated uropathogenic *E. coli*

Antibiotics	% of Resistant	% of Sensitive	MAR index
Co- trimoxazole	80.51	19.49	0.1
Amoxicillin	77.92	22.08	0.097
Gentamicin	57.14	42.86	0.071
Norfloxacin	57.14	42.86	0.071
Nalidixic acid	51.94	48.06	0.064
Chloramphenicol	45.45	54.55	0.056
Ciprofloxacin	42.82	57.18	0.053
Nitrofurantoin	14.28	85.72	0.017

Table 2. Antibiotic susceptibility results (%) of the biofilm producing *E. coli* isolates

Antibiotics	Resistance of all Isolates (n=77)	Biofilm positive (n=40)	Biofilm negative (n=37)
Co- trimoxazole	80.51	49.35	31.16
Amoxicillin	77.92	51.94	25.97
Gentamycin	57.14	40.25	16.88
Norfloxacin	57.14	41.55	15.58
Nalidixic acid	51.94	40.25	11.68
Ciprofloxacin	42.82	31.16	11.68
Chloramphenicol	45.45	35	10.38
Nitrofurantoin	14.28	7.79	6.49

saline (pH 7.3) to remove loosely bound bacteria and dried. Dried tubes were stained with crystal violet (1%) stain. Excess stain was removed and tubes were washed with deionized water. Then the tubes were dried in inverted position and observed for biofilm formation.

Biofilm formation was considered positive if a visible film lined the wall and bottom of the tube. Tubes were examined and the amount of biofilm formation was scored as 0 – absent, 2 – moderate or 3 – strong.

RESULTS

A total of 200 urine samples were analyzed during the period of study. Out of 200 urine sample 150 samples had significant bacteriuria with the count more than 10^5 cfu/ml, 38 samples had no significant count and remaining 12 samples showed no growth. Out of 150 urine samples with significant count, *E.coli* was isolated in 51%, *Klebsiella pneumoniae* in 38%, *Enterococcus faecalis* 6% followed by *Staphylococcus* in 4% and *Micrococcus* in 1%.

A total of 77 clinical isolates of *E.coli* were obtained from 150 significant samples. All these 77 isolated *E. coli* were resistant to commonly used antibiotics such as co-trimoxazole[62], amoxicillin[60], gentamicin[44], norfloxacin[44], nalidixic acid[40], ciprofloxacin[33], chloramphenicol[35] and nitrofurantoin [11]. The MAR indices for antibiotics are given in Table 1. The antibiotic susceptibility results of UPEC are shown in Fig. 1.

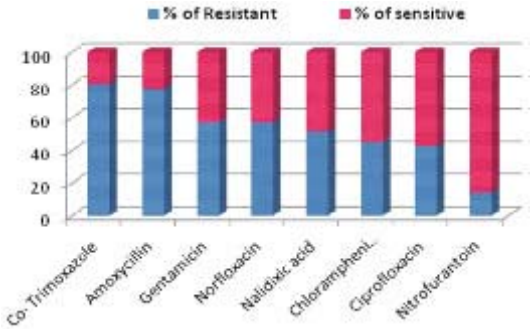

Fig 1. Antibiotics response of uropathogenic *E. coli*

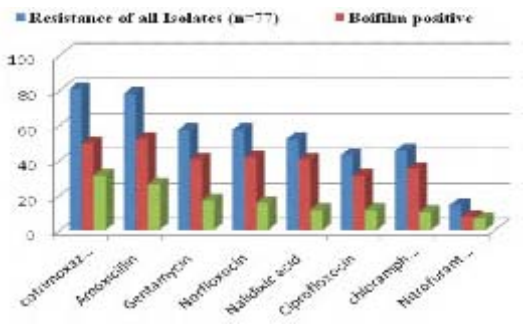

Fig 2. Antibiotic susceptibility results (%) of biofilm producing *E. coli*

Fig 3. Biofilm production by Test tube method 1-weak, 2, 3- moderate, 4-strong.

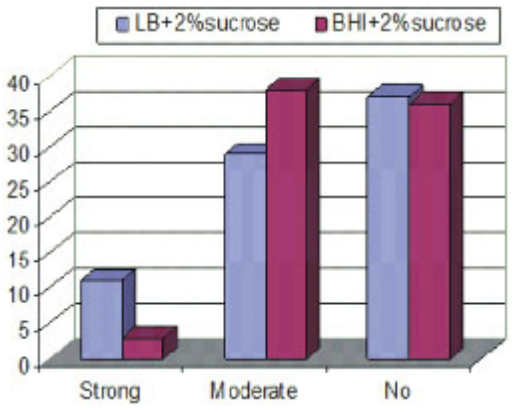

Fig 4. Comparison of LB and BHI for biofilm formation

Quantitative tube method of biofilm screening was performed for 77 isolates of *E. coli* out of which 40 isolates were positive for biofilm production. Some of the isolates showed positive results in tube method are shown in fig 3. In the quantitative assay for the biofilm production, the 11 isolates were classified as strong biofilm producing and 29 moderate biofilm producing. The overall percentage of resistance observed among all the *E. coli* isolates including biofilm producers and non biofilm producers for 8 antibiotics tested is given in Table 2. Fig 2. clearly indicates that the percentage of resistance among biofilm producing UPEC in more than the non biofilm producing UPEC.

In comparison of two media for biofilm formation, it was observed that out of 77 isolates of *E. coli*, 11 isolates were able to form strong biofilm in LB, while only 3 isolates were able to form strong biofilm in BHI. 29 isolates showed moderate biofilm production in LB, while in BHI 38 isolates were moderate biofilm producers Fig 4. In BHI medium, the majority of isolates exhibited a poor ability to form biofilms, while a larger number of strains were able to form strong biofilms in LB. It was noted that there was no biofilm production in media without sucrose.

DISCUSSION

The present study demonstrated that *E. coli* is the most frequent isolate of urinary tract infection, have high propensity to form biofilm and poor antibiotic sensitivity. Biofilm production in *E. coli* may promote colonization and lead to increased rate of urinary tract infections. Such infections may be difficult to treat as they exhibit multidrug resistance. In our study production of biofilm among uropathogenic *E. coli* was found to be 52% and the antibiotic sensitivity of the biofilm producers ranged from 7- 49% and 6 - 31% for non biofilm producers. The percentage of resistance in biofilm producing UPEC was more. In the study conducted by Sharma *et al*., in 2009 [7], the significant production of biofilm was 54% in uropathogenic *E. coli* and the antibiotic sensitivity of the biofilm producing isolates ranged from 16-57% and for non biofilm producers it was 35-78%. The study conducted by Matija *et al*.,[10] demonstrated that 53% strains of UPEC were biofilm producing. There was a significant correlation between biofilm production and resistance to multiple antibiotics such as ampicillin, cotrimoxazole, nalidixic acid and norfloxacin as also demonstrated by Suman *et al*., in 2005[2]. Resistance of biofilm forming UPEC to nalidixic acid was demonstrated by Solo *et al*.[9].

The tube method for the *in vitro* quantitative and qualitative estimation of biofilm production is easy and the LB media used for the biofilm production have more frequency to form biofilm than the BHI, while 2% sucrose enhance the biofilm formation in both medium. Mathur *et al*.,[8] documented an increase in biofilm formation by addition of 2% sucrose to BHI broth. There was a significant increase in biofilm production from 4.6% to 46%. We conclude from this analysis that *E.coli* isolates exhibits wide spectrum of biofilm forming capabilities in each of the tested medium.

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