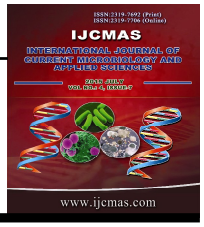
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 640-646

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 640-646**
http://www.ijcmas.com



Original Research Article

Correlation between Biofilm Production and Antibiotic Resistance Pattern in Uropathogenic *Escherichia coli* in Tertiary Care Hospital in Southern Rajasthan, India

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Keywords

Antibiotics, resistance Biofilm, Uropathogenic E. *coli*, Biofilm producers

A B S T R A C T

Biofilm formation has become a major factor in enhancing the antibiotic resistance in uropathogenic *E. coli*. In the present study, we observed biofilm production by two methods –Congo red agar method and Christenson's test tube method and compared the antibiotic resistance pattern between biofilm producers and non biofilm producers. Total of 112 uropathogenic *E. coli* were isolated out of which 84 isolates were biofilm producers. Antibiotic resistance pattern was compared between biofilm producers and non producers in Amikacin (45.2/28.5%), Gentamycin (57.1/35.7%), cefotaxime (92.8/64.2%), ceftazidime (100/64.2%), ceftriaxone (98.2/64.2%), ciprofloxacin (83.3/57.1%), doxyxycline (80.9/50%), tetracycline (90.4/57.1%), norfloxacin (92.8/64.2%) and nitrofurantoin (38/28.5%) and it was found to be significantly high in biofilm producers.

Introduction

Urinary tract infection is defined as presence of multiple organisms in the urinary tract through which urine flows from kidney via the bladder. Infection of the urinary tract is the second most common type of infection in the body (Ponnusamy and Nagappan, 2013).

Escherichia coli (*E. coli*) is one of the most prevalent pathogens among gram-negative bacteria, capable of causing complicated and uncomplicated UTI's (Ponnusamy *et al.*, 2012).

E. coli accounts for 70 to 95% of urinary tract infections. Bacteria follow ascending route of infections in 90% of urinary tract infections. These are primarily derived from fecal flora of the host, but haematogenous infections do occur (Golia *et al.*, 2012).

The use of available antibiotics has led to significant improvements in the management of UTIs; however, recurrent infections and an increasing resistance to conventional antibiotics have been observed (Lo *et al.*, 2014).

Biofilm plays a major role in virulence of bacteria. The possible relationship between bacteria persistence in the urinary tract and the presence of virulence factors (VFs) lead to biofilm formation like adhesins, toxins, lipo polysaccharides, iron acquisition, presence of capsule and serum resistance (Ponnusamy and Nagappan, 2013; Golia *et al.,* 2012). Biofilm formation enables single-cell organisms to assume a temporary multicellular lifestyle, in which "group behavior" facilitates survival in adverse environments.

What was once defined as the formation of a community of microorganisms attached to a surface has come to be recognized as a complex developmental process that is multifaceted and dynamic in nature.

The transition from planktonic growth to biofilm occurs in response to environmental changes, and involves multiple regulatory networks, which translate signals to concerted gene expression changes thereby mediating the spatial and temporal reorganization of the bacterial cell (Kostakioti *et al.*,2013).

Bacterial aggregation and subsequent biofilm maturation consists of reversible and irreversible stages and involves numerous conserved and/or species-specific factors. The first step involves the introduction of bacteria to a surface, a process which is at least in part stochastic, driven by Brownian motion and gravitational forces, and influenced by surrounding hydrodynamic forces (Kostakioti *et al.*,2013).

Within a niche, bacteria encounter attractive or repelling forces that vary depending on nutrient levels, pH, ionic strength, and temperature. Medium properties, along with bacterial cell-surface composition affect velocity and direction toward or away from the contact surface. Motile bacteria have a competitive advantage, utilizing flagella to overcome hydrodynamic and repulsive forces. The importance of flagellar motility for initial attachment has been documented for several pathogens, including *P. aeruginosa*, *Vibrio cholerae*, *Listeria monocytogenes*, and *E. coli* (Kostakioti *et al.,* 2013).

There are several advantages for microorganisms to form biofilms. They provide enclosed surface space which is occupied and can provide a degree of stability in the growth environment. They might have catalytic functions through the localizing cells in close proximity (Prasanna *et al.,* 2008).

Microbial biofilms have been associated with lot of persistent infections which respond poorly to antibiotic therapy and can withstand host immune response (Ponnusamy *et al.,* 2012, Ponnusamy and Nagappan, 2013).

This also helps in spread of antibiotic resistant traits in nosocomial infection (Ponnusamy and Nagappan, 2013).

In this study we screened 200 non repetitive clinical urinary isolates by conventional microbiological methods. Biofilm production was detected in each sample using two methods- Congo red agar method and Christenson's test tube method and further antibiotic susceptibility pattern was tested for various antibiotics. The main aim and objective of this study to evaluate biofilm production in various uropathogenic *E. coli* through two different methods and to correlate it with antibiotic resistance pattern.

Inclusion criteria-

Urine samples with pure cultures showing significant bacteruria (10^5 CFU/ml).

Exclusion criteria

Mixed cultures and asymptomatic bacteruria

Material and Method

200 urine samples fulfilling inclusion criteria were selected from urine samples in duration of July to December 2014.

Biofilm detection & antibiotic sensitivity was done by the following methods.

Methods for biofilm production--

Tube method (TM)

A qualitative assessment of biofilm formation was determined as described by Christensen *et al* (1982)

TSBglu (10mL) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes are washed with deionized water. Tubes were than dried in inverted position and observed for biofilm formation.

Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface is not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation is scored as positive or negetive. Experiments are performed in triplicate and repeated three times (Golia *et al.,* 2012).

Congo red Agar method (CRA)

Freeman *et al.* (1989) had described an alternative method of screening biofilm formation; which requires the use of a specially prepared solid medium -brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red.

The medium was composed of BHI (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L).

Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes, separately from other medium constituents and is then added when the agar had cooled to 55°C. Plates are inoculated and incubated aerobically for 24 to 48 hours at 37°C.

Positive result is indicated by black colonies with a dry crystalline consistency. A darkening of the colonies with the absence of a dry crystalline colonial morphology indicated an indeterminate result. Red or pink colored colonies indicate negative result (Mathur *et al.,* 2006).

Antibiotic susceptibility testing

It is done by Kirby Baeur disk diffusion method using the reference strain *E. coli* ATCC 29522 using commercially available discs from Himedia, Mumbai.

Colonies are inoculated into peptone water and turbidity is adjusted at 0.5 McFarland standard. Broth culture is spread on the plate to make lawn culture on Mueller Hilton agar.

Discs are applied on surface of agar and plates are incubated overnight at 30-35˚C in ambient air. Results are interpreted using CLSI guidelines (Koneman *et al.,* 2006).
Antibiotic resistance pattern was observed for following antibiotics: Amikacin, Gentamycin, Cefotaxime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Doxycycline, Tetracycline, Norfloxacin, Nitrofurantoin

Results and Discussion

Total 200 urine samples were processed with significant bacteruria. Out of 200 samples, 112 samples with *E. coli* isolates were observed.

The antibiotic resistance pattern of various *E. coli* isolates is tabulated below in table 1.

Biofilm production was observed in 84 (73%) isolates positive for both Christensons test tube method and Congo red agar method. 28 (27%) isolates were biofilm non producers.

Antibiotic susceptibility pattern of biofilm producers was observed as shown in table 2.

Antibiotic susceptibility pattern of biofilm non producers was observed as shown in chart 1.

Escherichia coli is the most frequent microorganism involved in urinary tract infection (UTI). Acute UTI caused by uropathogenic *E. coli* (UPEC) can lead to recurrent infection, which can be defined as either re-infection or relapse (Soto *et al.*, 2006).

In our study, we studied 200 samples of UTI out of which 112 were positive for *E. coli* isolates. Maximum resistance was observed in cephalosporins (86–91%) followed by norfloxacin (86%) and tetracycline (82%) while nitrofurantoin (36%) and amikacin (41%) were least resistant. Suman *et al.* (2007) reported similar resistance pattern to antibiotics in their study.

Biofilms have great significance for public health, because biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents. This susceptibility may be intrinsic (as a natural outcome of growth in the biofilm) or acquired (due to transfer of extrachromosomal elements to susceptible organisms in the biofilm).

In our study, 84 (73.3%) isolates were found to be biofilm producers which were highly resistant to antibiotics. Maximum resistance was observed with cephalosporins, norfloxacin followed by ciprofloxacin and tetracycline.

Least resistant drug observed was nitrofurantoin. In contrast, resistance was much less in non biofilm producer isolates as compared to biofilm production.

Golia *et al.* (2012) correlated biofilm production with antibiotic resistance and observed similar pattern however they observed 100% resistance to majority of drugs used including tetracycline and nitrofurantion.

Ponnusamy *et al.* (2012) also showed multidrug resistance in biofilm producing *E. coli* but they observed greater degree of resistance to amikacin (70%) and gentamycin (86%) as compared to our study.

The future expansion of this study can be done by genetic analysis of biofilm producing strains to observe the presence of *ica* gene.

In conclusion, the present scenario biofilm seems to play a major role in multidrug resistance in various organisms and this study also conclude that biofilm production plays an important role in antibiotic resistance in *E. coli* infection and thus different methods should be employed to avoid biofilm formation on various surfaces.

Table.1 Antibiotic susceptibility pattern of *E. coli* isolates

% of isolates	Sensitive	Resistant
Amikacin	59	41
Gentamycin	48	52
Cefotaxime	14	86
Ceftazidime	9	91
Ceftriaxone	12	88
Ciprofloxacin	23	77
Doxycycline	27	73
Tetracycline	18	82
Norfloxacin	14	86
Nitrofurantoin	64	36

Table.2 Antibiotic susceptibility pattern of no. of biofilm producer isolates

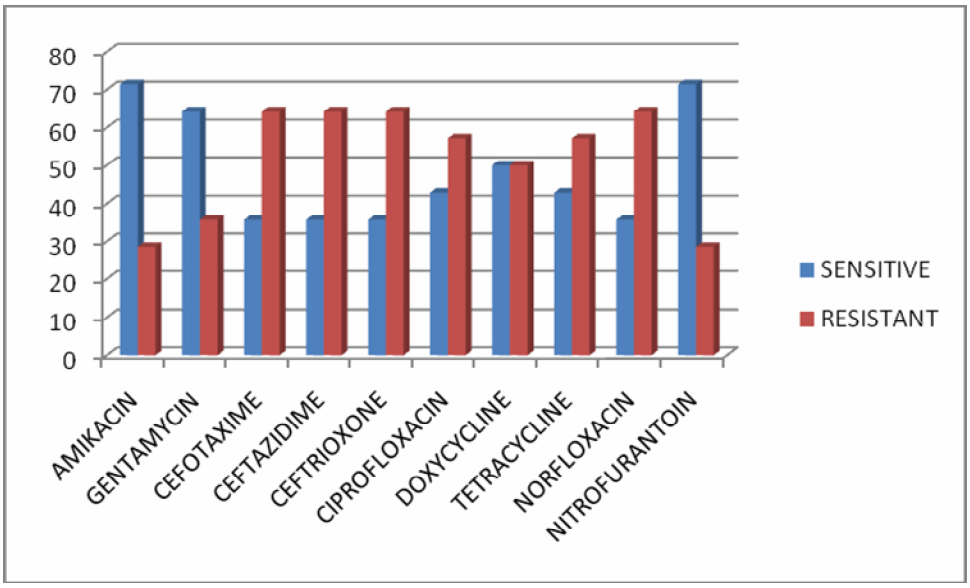
No. of biofilm producing isolates	Sensitive	Resistant
Amikacin	46	38
Gentamycin	36	48
Cefotaxime	06	78
Ceftazidime	00	84
Ceftriaxone	04	80
Ciprofloxacin	14	70
Doxycycline	16	68
Tetracycline	08	76
Norfloxacin	06	78
Nitrofurantoin	52	32

Total biofilm producers=84

Table.3 Correlation between resistance pattern of biofilm producers and non biofilm producers

No. of isolates resistant	Biofilm producer	Biofim non producer
Amikacin	38(45.2%)	08(28.5%)
Gentamycin	48(57.1%)	10(35.7%)
Cefotaxime	78(92.8%)	18(64.2%)
Ceftazidime	84(100%)	18(64.2%)
Ceftriaxone	80(98.2%)	18(64.2%)
Ciprofloxacin	70(83.3%)	16(57.1%)
Doxycycline	68(80.9%)	14(50%)
Tetracycline	76(90.4%)	16(57.1%)
Norfloxacin	78(92.8%)	18(64.2%)
Nitrofurantoin	32(38.01%)	08(28.5%)

Chart.1 Antibiotic susceptibility pattern in non biofilm producer isolates



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