



## IN VITRO BIOFILM FORMATION BY UROPATHOGENIC ESCHERICHIA COLI AND ITS PATTERN OF ANTIMICROBIAL SUSCEPTIBILITY

### Microbiology

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### ABSTRACT

**BACKGROUND-** The most common cause of urinary tract infection is Escherichia coli, though other bacteria or fungi may rarely be the cause. Recurrent UTI's that are predominantly caused by uropathogenic Escherichia coli's forms biofilm.

#### OBJECTIVES-

1. To determine the biofilm formation by E.coli.
2. To evaluate the antibiotic susceptibility testing.

**MATERIAL AND METHODS-** The present prospective study was carried out from December 2014 to May 2015 in the Department of Microbiology of SMS medical college, Jaipur. A total of 100 consecutive, nonrepetitive E. coli isolates were subjected to biofilm formation study by Christensen's tube adherence method. All the isolates were also subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method in accordance with the Clinical Laboratory Standard Institute 2013) guidelines and followed inclusion and exclusion criteria. Epi-info software was used for data analysis.

**RESULTS-** Out of the 100 E. coli isolates studied, 62 (62%) were positive for biofilm formation. Overall drug resistance was found to be very high among both biofilm as well as non-biofilm forming isolates indicating excessive drug resistance among both community and hospital organisms.

**CONCLUSION-** The results of present study indicated a need for continued surveillance of antimicrobial resistance and greater understanding of the nature of biofilm producing uropathogens causing UTI, so as to increase positive outcomes of clinical interventions and the development of novel and more effective treatments for the problematic diseases.

### KEYWORDS

Antimicrobial resistance, biofilm, E.coli, urinary tract infection

### INTRODUCTION-

About 150 million people develop a urinary tract infection (UTI) in a given year.<sup>1</sup> UTI is the most common hospital acquired infection, which accounts for almost 40% of all nosocomial infections.<sup>(2-4)</sup> Mostly they are associated with urinary catheters, a commonly used device among hospitalized patients. Strains of uropathogenic E. coli (UPEC) are the primary cause of urinary tract infections, including both cystitis and pyelonephritis. It is believed that a primary reservoir of UPEC isolates is within the human intestinal tract, as the isolate responsible for a UTI in a given individual often matches rectal isolates from that same person.<sup>5</sup> These extraintestinal organisms form intracellular bacterial communities, thereby posing biofilm-like properties within the bladder epithelium<sup>(6-8)</sup> or on the indwelling devices such as catheters.

Biofilms are defined as assemblage of microbial cells that are irreversibly associated with a surface and enclosed in a matrix primarily of polysaccharides.<sup>9</sup> Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections. Microbes form a biofilm in response to various different factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics.<sup>10</sup> So, the rationale behind the study was to detect (INVITRO) biofilm of UPEC's and antimicrobial susceptibility pattern among UTI patients.

### MATERIAL AND METHODS-

The present study was a prospective cross-sectional analysis conducted over period of 6 months from December 2014 to May 2015. Ethical clearance was obtained by the Institutional Ethical and Research Committee.

**Collection of Urine Sample:** A total of 100 consecutive non-duplicate clinical isolates of E. coli from urine specimens from outpatient's department (OPD) and inpatients department (IPD) of both the genders were collected from department of Microbiology, SMS medical college, Jaipur. The samples were studied for biofilm production and were analyzed for the correlation between susceptibility pattern of biofilm production and  $\beta$ -lactam antibiotics in ESBL producers.

### Methodology-

**Detection of Biofilm Formation:** All the 100 E.coli isolates were subjected to biofilm production. Numbers of tests are available to test biofilm production in E. coli. The methods include Tissue Culture Plate method, Tube method (TM) and Congo Red Agar (CRA) methods. Identification of the isolates was done based on cultural characteristics and reactions in standard biochemical tests.<sup>11</sup>

**Antibiotic Susceptibility Testing:** Antibiotic susceptibility test was performed using a disc diffusion method on Mueller Hinton Agar according to Bauer Kirby et al., 1966, discs of antibiotics used were: Amikacin, Cefotaxime, Imipenem, Gentamycin, Norfloxacin, Amoxicillin, Ceftriaxone and Ticarcillin. Interpretation of results were done in accordance with the Clinical Laboratory Standards Institute (CLSI)-2013 and CLSI-2014 guidelines.<sup>12</sup>

### Data Analysis

The recorded observations were entered in Epi data software and the collected data was consolidated on Microsoft Excel sheets and further analyzed in Epi info 7.1.3.0 version. The results were expressed as proportions and percentages. Chi-square test was used for qualitative variables to find association and P value <0.05 was considered statistically significant.

### RESULTS-

**Table 1- Drug resistant and Biofilm formation of various E.coli isolates**

Drug resistant mechanism	Hospital							
	IPD (59)				OPD (41)			
	Male (35)		Females (24)		Males (22)		Females(19)	
B+	B-	B+	B-	B+	B-	B+	B-	
ESBL	6	5	7	5	7	4	6	3
Carbapenemase	2	1	2	0	0	0	0	1
Esbl+ Carbapenemase	4	4	3	1	0	0	0	1
NONE	10	3	1	5	9	2	5	3

B+= Biofilm positive, B-= Biofilm negative

As per table 1- biofilm production based on the differences in gender and hospitalization status and the various drug resistance mechanisms

exhibited by the isolates have been mentioned A total of 41 and 59 E. coli isolates from OPD and IPD while 57 and 43 isolates from male and female patients, respectively were considered for biofilm study. During the study, 62 out of 100 (62%) biofilm forming isolates and 38 out of 100 (38%) non-biofilm forming isolates showed at least one type of drug resistance mechanism (ESBL or carbapenemase production or both).

**Table 2-Antibiotic Resistance Profile (%) of E.coli isolates**

	FO	FI	MI	MO
Antibiotic tested	Biofilm +	Biofilm-	Biofilm +	Biofilm-
Amikacin	6.25	0	13.6	15.3
Ampicillin	81.2	66.6	77.2	84.6
Aztreonam	62.5	66.6	72.7	92.3
Cefazolin	62.5	66.6	77.7	92.3
Cefotax ime	62.5	66.6	72.7	92.3
Ceftazidime	62.5	66.6	72.7	92.3
Cefepime	56.2	66.6	72.7	92.3
Cefoxitin	25	50	38	50
Cotrimoxazole	50	66.6	45	77.7
Colistin	6.2	16.6	0	0
Gentamicin	15.3	0	15.7	10
Imipenem	12.5	16.6	28.5	38.4
Meropenem	50	50	66.6	84.6
Nitrofurantoin	18.7	0	14.2	38.4
Norfloxacin	56.2	66.6	73.6	75
Piperacillin	75	66.6	80.9	69.2
Piperacillin/tazobactam	6.2	0	20	25
Tetracycline	68.7	83.3	60	76.9
Ticarcillin	75	60	73.6	76.9

**Table 2** shows males and females antibiotic resistance profile in IPD and OPD resistance noticed may be due to the production of antibiotic hydrolyzing enzymes such as beta-lactamases that accumulate within glycocalyx and produce concentration gradients thereby protecting the underlying cells. (FO- FEMALE OPD, FI- FEMALE IPD, MI- MALES IPD, MO-MALES OPD)

## DISCUSSION & CONCLUSION-

In the present study, uropathogenic E. coli has been selected for biofilm study. Both 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 this study. They may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infection. This review deals with the different strategies regarding E. coli as a pathogen and the virulence traits of its pathotypes highlighting the species as a commensal, opportunistic and specialized pathogen.<sup>13</sup>

The biofilm formation is two-step process in which the bacteria first adhere to a surface mediated by capsular antigen or flagellar antigen, followed by multiplication to form a multi-layered biofilm, which is associated with production of exopolysaccharide matrix.

Detecting the ability of E. coli isolates to produce biofilm, test was done by using Christensen tube method (Christensen et al.,1982). As, it has been recommended by several authors in their studies as a reliable and more accurate qualitative tool for determining biofilm formation by clinical isolates. A mechanism of adherence to the intestinal epithelium has been suggested for ETEC in-vivo, where fimbriae are responsible for initial adhesion and Capsular exopolysaccharide are responsible for the formation of primarily microcolonies in which bacteria multiply.<sup>14</sup> The ability of bacteria to form biofilms helps them to survive hostile conditions within host and is considered to be responsible for chronic or persistent infections.

In vitro susceptibility tests performed by several authors have shown considerable increase in resistance of biofilm to killing. Several mechanisms have been proposed to explain this high resistance of biofilms, including restricted penetration of antimicrobial agents into biofilms, slow growth owing to nutrient limitation, expression of genes involved in the general stress response, and emergence of a biofilm-specific phenotype. As a result, according to our study all IPD and OPD isolates of both the gender groups were resistant to beta-lactams. Another finding was, Biofilm producer isolates were less susceptible to

antimicrobial agents than the nonbiofilm producers, especially with reference to beta lactams and aminoglycosides in both IPD gender groups and OPD male group. Biofilm organisms were more susceptible to fluorquinolones and carbapenems, especially in both OPD gender groups and IPD female groups. Nonbiofilm forming E. coli isolates were found to be more resistant as compared to biofilm forming isolates in all our study groups except IPD male groups.

UTI patients infected with these strains cannot be treated with beta lactam antimicrobial agent and mono bactams. Amikacin and imipenem are found to be alternative treatment for ESBL producer. Multidrug resistance and ESBL is a common problem in hospital which emphasizes the need for judicious use of producing extended-spectrum b-lactamases antimicrobial agent and their continuous in vitro (ESBLs) in the community.

**Conflict of Interest-** None declared

**Source of Funding-** None

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