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COMPARISON OF ANTIMICROBIAL RESISTANCE PATTERN IN BIOFILM PRODUCER AND NON-BIOFILM PRODUCER *ESCHERICHIA COLI* FROM PATIENTS WITH UTI.

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ABSTRACT **Background:** *Escherichia coli* (*E.coli*) is the most common isolated organism associated with Urinary tract infection(UTI). *Escherichia coli* form intracellular bacterial communities with biofilm-like properties within the bladder epithelium. Biofilm formation is one of the most important virulence factors exhibited by *Escherichia coli* among other virulence factors. Biofilm can restrict the diffusion of substances and the binding of antimicrobials enclosing them in an extracellular biochemical matrix. **Objective: -** The study was aimed to detect biofilm production by Uropathogenic *Escherichia coli* (UPEC) and to compare the antimicrobial resistance pattern of biofilm producing and non biofilm producing isolates from patients with UTI. **Material & Methods**: After obtaining institutional ethical approval, a total of 300 Uropathogenic *Escherichia coli* isolated from with UTI from July 2019 to December 2019 at the National Institute of Medical Science and Research (NIMS) Hospital, Jaipur, Rajasthan were tested for in vitro biofilm production by three different methods (Tube method, Congo red agar method and Tissue culture Plate method). All isolates were tested for resistance to 15 different antibiotics by Kirby Bauer disc diffusion method. **Result:** Out of 300 Uropathogenic *Escherichia coli* isolates 55.7% of the isolates were found to be biofilm producers by TCP method, biofilm detection by TM showed to 62.7 of isolates with biofilm producers. Biofilm producing UPEC showed significantly higher degree of resistance to most/all of the antibiotics compare to Non Biofilm producing Uropathogenic *Escherichia coli*. However among biofilm producers low resistance was observed to few higher antibiotics such as Meropenem 24.6%, Imipenem 38.3%, Nitrofurantoin 41.3% and Piperacillin/Tazobactam 53.9%. **Conclusion**: Biofilm producer *Escherichia coli* is responsible for high drugs resistance in comparison to non-biofilm producer.

KEYWORDS : Biofilm, Resistance, E.coli

INTRODUCTION

Urinary tract infection (UTI) is one of the most common microbial infection affecting persons of all ages groups. All females are at higher risk of cystitis due to the short length from the anus to urethra and urethral openings to the bladder [1]. Recurrent UTI remains a serious health problem in many women despite the broad array of successful antimicrobial treatment [2].

The risk of developing urinary tract infection significantly increases with the use of indwelling devices such as catheters and urethral stents or sphincters [3]. Bacterial virulence factors exhibited by Uropathogenic *Escherichia coli* has led to recurrent and relapse UTIs [4]. *Escherichia coli* is one of the most common isolated organism associated with Urinary tract infection. *Escherichia coli* form intracellular bacterial communities with biofilm-like properties within the bladder epithelium [5].

Biofilm formation by *Escherichia coli* is one most important virulence factors exhibited among various other virulence factors [6]. Biofilms make the organism to be more virulent, resist the host immune response and lead to the evolution of antibacterial drug resistance by enclosing them in an extracellular biochemical matrix [7]. Biofilms have a role in up to 60 % of human infections and they are very difficult to be eradicated with antimicrobial therapy. In vitro susceptibility tests have shown a considerable increase in the resistance of biofilm cells to killing [8]. Biofilm can restrict the diffusion of substances and the binding of antimicrobials. This will provide effective resistance for biofilm cells against large molecules such as antimicrobial proteins lysozyme and complement. According to the National Institutes of Health, ≥60% of all microbial infections are caused by biofilms [5].

Increasing antimicrobial resistance is a great concern to ensure appropriate treatment. In this context, the present study was aimed to detect biofilm production by Uropathogenic *Escherichia coli* and to compare the antimicrobial resistance pattern of biofilm producing and non biofilm producing isolates from patients with UTI.

MATERIALS AND METHOD:

After obtaining institutional ethical approval, a total of 300 Uropathogenic *Escherichia coli* isolated from patients with UTI from July 2019 to December 2019 at the National Institute of Medical Science and Research (NIMS) Hospital, Jaipur, Rajasthan were tested for Biofilm production by three different methods (Tube method, Congo red agar method, and Tissue culture Plate method) and Antibiotic susceptibility testing by Kirby Bauer disc diffusion method.

Antibiotic Susceptibility Test: All the isolates were subjected to determine the resistance pattern using is different antibiotics by Kirby Bauer disc diffusion method, following CLSI guidelines [9]. The following antimicrobial drugs were tested. Ampicillin (10 µg), Cefuroxime (30 µg), Cefpodoxime (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Amoxyclav (30 µg), Gentamicin (30 µg), Norfloxacin (10 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Co-trimoxazole (25 µg), Piperacillin/ Tazobactam (100/10 µg), Imipenem (10 µg), and Meropenem (10 µg).

METHODS FOR DETECTED BIOFILM PRODUCTION

Congo red agar method (CRA): Congo red agar prepared by brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. Microorganisms were inoculated on the CRA plate and incubated at 37°C for 24– 48 hours. A positive result was indicated by black colonies with a dry crystalline consistency [10].

Tube method (TM): Microorganisms were inoculated in 10 ml of BHI broth with 2% sucrose in a test tube and incubated at 37°C for 18-24 hours. After incubation broth full test tube was decanted and washed with PBS (pH 7.3) and left it for drying then stained with crystal violet (0.1%) for half 30 minutes. After 30 minutes excess stain removed out then allow to dried and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Tubes were examined, and the amount of biofilm formation was scored as absent, moderate, or strong [11].

Tissue culture plate method (TCP): This method is considered as a Gold Standard Method in detection of Biofilm. Microorganisms were inoculated in 10 ml of BHI broth with 2% sucrose in a test tube and incubated at 37°C for 18-24 hours. The broth with visible turbidity was diluted to 1 in 100 with fresh broth. Individual wells of flat-bottom

polystyrene plates were filled with 0.2 ml of the diluted broth, and broth without microorganism served as a control to check sterility and nonspecific binding of the medium. These plates were incubated for 24 hours at 37°C. After incubation, the content of the well was gently removed and washed 4 times with 0.2 ml of phosphate buffer saline (PBS pH 7.2) to remove free-floating "planktonic" bacteria. Biofilms formed by adherent "sessile" organisms in the plate were fixed with sodium acetate (2%) for half an hour and stained with crystal violet (0.1%) for half an hour. Excess stain was rinsed off by thoroughly washing with deionized water and plates were kept for drying. Adherent bacterial cells usually formed a biofilm on all side wells and were uniformly stained with crystal violet. Optical densities (OD) of stained adherent bacteria were determined with a micro Enzyme-Linked Immunosorbent Assay auto reader at a wavelength of 570 nm (OD 570 nm) and were graded as per Christensen et al.[Table 1]. These OD values were considered as an index of bacteria adhering to the surface and forming biofilms [12].

Table 1: Classification of bacterial adherence by TCP method Mean OD values Adherence Biofilm formation.

Mean OD values	Adherence	Biofilm formation
<0.120	None	None/weak
0.120-0.240	Moderate	Moderate
≥0.240	Strong	High

Statistical analysis was performed with the SPSS, Trial version 23. The qualitative data were expressed in proportion and percentages. The difference in proportion was analyzed by using the chi-square test. Probability P.value <0.05 was considered statistically significant.

RESULT: Out of 300 Uropathogenic *Escherichia coli* isolates 55.7% of the isolates were found to be biofilm producers by TCP method. Tissue Culture Plate method is considered as a Gold Standard Method in detection of Biofilm. In our study 133 (44.3%) Uropathogenic *Escherichia coli* isolates were Non- Biofilm Producer by Tissue culture Plate Method. Figure-1.

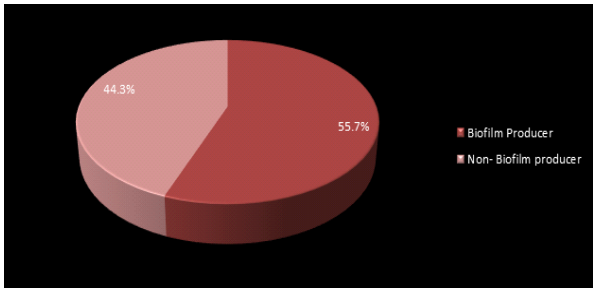

Figure-1. Distribution of Biofilm Producer and Non- Biofilm producer by Tissue culture Plate Method.

Out of total isolates, 62.7%, 45.7%, and 55.7% were positive for biofilm productions by the Tube method (TM), Congo red agar (CRA), and Tissue culture plate (TCP) method respectively. Figure-2.

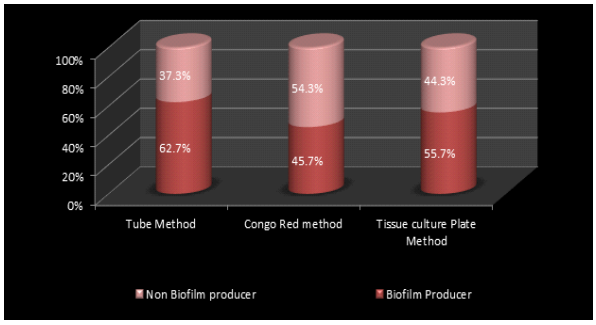

Figure-2. Comparison of Biofilm Producer and Non- Biofilm producer by Three Method.

Biofilm producing Uropathogenic Escherichia coli showed significantly higher degree of resistance to most/all of the antibiotics compare to Non Biofilm producing Uropathogenic *Escherichia coli*. However among biofilm producers low resistance was observed to few higher antibiotics such as Imipenem 38.3%, Meropenem 24.6%, Piperacillin/Tazobactam 53.9% and Nitrofurantoin 41.3%. Shown in Table 2. The antibiotic resistance among biofilm producing *Escherichia coli* was found to be higher than that non-biofilm with a p=0.01(<.05) which is statistically significant.

Table-2. Statistical analysis of the resistance pattern of *Escherichia coli* isolates in Biofilm production and Non- Biofilm Producer by the Tissue Culture Plate Method. Indicates statistically significant P.value ≤.05.

Antibiotics	Biofilm Producer N= 167	Non- Biofilm Producer N= 133
Ampicillin	92.8%	78.2%
Cefuroxime	89.8%	75.2%
Cefpodoxime	88.6%	74.4%
Ceftazidime	82.6%	68.4%
Ceftriaxone	89.2%	72.9%
Cefepime	86.8%	59.4%
Amoxyclav	85.6%	72.2%
Gentamicin	63.5%	42.9%
Norfloxacin	83.8%	69.9%
Nitrofurantoin	41.3%	12.8%
Nalidixic acid	95.8%	78.2%
Co-trimoxazole	76.6%	57.1%
Piperacillin/ Tazobactam	53.9%	31.6%
Imipenem	38.3%	18.0%
Meropenem	24.6%	5.3%

DISCUSSION

Escherichia coli is the predominant causative agent of both symptomatic and asymptomatic UTIs, which accounts for more than 80% of the infections [13-14] Biofilm producing bacteria are responsible for many recalcitrant infections and are difficult to eradicate. Biofilm production in *Escherichia coli* promotes colonization and leads to increased UTI. Such infections may be difficult to treat as they exhibit multiple drug resistance.

In the present study biofilm production rate was 62.7%, 45.7%, and 55.7% by Tube method (TM), Congo red agar (CRA), and Tissue culture plate (TCP) method respectively. Similar study conducted by Rashmi M. Karigoudar, *et al* [15] reported 55%, 49%, and 69% were positive for biofilm productions by Tube method, Congo Red Agar, and Tissue Culture Plate method respectively. These findings are very similar to the findings of the present study. The findings of other studies conducted by many authors also support the findings of the present study. Saroj *et al*. [16] showed 69% isolates as biofilm producers by TM and TCP methods. The study conducted by Sevanan *et al*. [17]., Congo red method showed 59.4% strains to be biofilm producer Significant production of biofilm was seen in 67.5% isolates of E. coli in a study conducted by Sharma *et al*. [18] by TCP method .

Biofilm producing isolates showed the highest resistance to the antibiotics compared to non-biofilm producing isolates. In our study we found the biofilm producers maximum resistance was seen to antimicrobial such as Nalidixic acid 95.8% followed by Ampicillin 92.8%, Cefuroxime 89.8% and Ceftriaxone 89.2%. A study conducted by Kulkarni Ramesh Sudheendra, *et al* [2] Biofilm producers demonstrated resistance to Ampicillin 87.36% followed by Cefuroxime 81.58%, Amoxyclav 77.61%, Ceftriaxone 54.6%, and Cefepime 64.98%. and Aravindhan. G *et al* maximum resistance was seen Ampicillin 87%, Norfloxacin 85%, Ceftazidime 81%, and Gentamicin 69%. Similar study conducted by Sevanan *et al* [17] showed that resistance was seen to be maximum with Ampicillin (59.3%).

In our finding we get the Minimum resistance was seen to antimicrobial such as Meropenem 24.6% followed by Imipenem 38.3% and Nitrofurantoin 241.3% is shown in Table 2. The antibiotic resistance among biofilm producing *Escherichia coli* was found to be higher than that non-biofilm with a p=0.01(<.05) which is statistically significant. Significant association was observed between biofilm formation and multidrug resistance which was proved to be statistically significant regarding antibiotics.

In another Similar study by Aravindhan.G *et al* [19] showed the minimum resistance to Imipenem 22%, Nitrofurantoin 38%. In another nearby study conducted by Rashmi M. Karigoudar, *et al* [15] showed resistance rate of 42%, 11%, and 24.6% for Piperacillin/tazobactam, Imipenem, Nitrofurantoin respectively. The

findings of the present study are very near to the findings of this study. In the present study, the correlation between biofilm producer and non biofilm producer with antibiotic resistance was found statistically significant with for antibiotics.

CONCLUSION

India is known as the capital of drug resistance among countries. Antimicrobial resistance is the major health problem among biofilm producers as well as non-biofilm producer microorganisms. We concluded in this study that biofilm producer *Escherichia coli* is responsible for high drug resistance in comparison to non-biofilm producer *Escherichia coli*. Biofilm producer *Escherichia coli* showed more resistance to potent drugs like as Nalidixic acid, Ampicillin, Cefuroxime and Ceftriaxone. which are in use to treat severe urinary tract infections caused by Uropathogenic *Escherichia coli*. Further study should be conducted with a large sample size or community-based. Biofilm detection in *Escherichia coli* causing UTI should be carried out in regular practice which is essential to improve the efficacy of treatment or to outline an effective treatment in the management of UTI.

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