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Analysis of Biofilm Formation and its Correlation with Antibiotic Susceptibility Pattern of Uropathogens

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Abstract: ***One the most commonly acquired bacterial infection is Urinary tract infection (UTI). It has become major health problem with the advent of increasing resistance to commonly prescribed antibiotics. Biofilm formation accounts as a major causative factor for antibiotic resistance by uropathogenes. The purpose of this study was to detect biofilm formation by uropathogenes isolated from UTIs, their antibiotic susceptibility pattern. The isolated bacteria were tested for biofilm production by congo red agar (CRA) method. Antibiotic susceptibility pattern of uropathogens was done by Kirby-Bauer disc diffusion method. Out of 100 cultures positive cases 41 (41%) isolates were biofilm producers. The maximum biofilm production was seen in Enterococcus spp 28 (68.3%), 9 (21.95%) of E. coli isolates showed biofilm formation followed by 3 (7.32%) of K. pneumoniae and 1 (2.43%)of Pseudomonas isolates. There were no biofilm producing isolates of Enterobacter spp., Acinetobacterspp. and P. mirabilis found in the present study. The antibiotic resistance was higher among biofilm producers to commonly used antibiotics as compared to non-biofilm producers. It was concluded that the ability of slime production and biofilm formation of uropathogenic strains had significant role in antibiotic resistance. Biofilm formation is the major virulence determinant of uropathogens, so it is necessary to screen all urinary isolates for biofilm production.***

Keywords: Biofilm, Uropathogens, Antibiotic susceptibility, congo red agar, UTI

1. Introduction

Urinary tract infection (UTI) is the commonly acquired bacterial infection. Cases are being found with less response to treatment and higher recurrence rate attributed to increasing antibiotic resistance. This poses serious health problem [1]**.** Antibiotic resistance of uropathogens has been known to be increasing worldwide. Improper use of antibiotics and biofilm production are being the important causes of resistance [2]. Biofilms are an assembly of microbial cells which can be formed by single or a mixture of bacterial species that are irreversibly associated with a surface and enclosed in a matrix of polysaccharide materials that allow the growth and survival in hostile environments. [3, 4] Biofilms confer advantages to the biofilm forming bacteria such as protection from antimicrobial agents, exchange of nutrients, metabolites, and/or genetic exchange from proximity to other organisms. [5] Biofilms allow limited penetration of antibiotics and also the cell multiplication of organisms occurs at slower rate inside the biofilms. This may contribute to the development of chronic infections [6, 7]. Both Gram positive and Gram negative bacteria have capacity to synthesize biofilms. Bacteria with common tendency for biofilm production include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Enterococcus faecalis Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa [*8].

The objectives of this study were:

1) To determine the biofilmformation capability of uropathogens by congo red agar method.
2) Comparing antimicrobial susceptibility pattern of biofilm producing and non biofilmproducing uropathogens.

2. Material and methods

The study was carried out in the Departmentof Microbiology at R.C.S.M. Government medical college and CPR Hospital Kolhapur during the period of June 2018 to November 2018.

All the urine samples that were sent to Microbiology laboratory for bacterial culture were examined for presence of pus cells by wet mount and Gram stained smear.Then samples were inoculated on culture media following semiquantitative method. Only those isolates with presence of Pus cells on primary smear and having significant colony count were considered for the study. Then the isolates were identified on the basis of the colony morphology, Gram's staining and standard biochemical tests [9].

Bacterial susceptibility to anti‑microbial agents was determined by Kirby–Bauer disk diffusion method on Mueller‑Hinton agar as per CLSI 2018 guidelines.

Biofilm detection was done by using Congo Red agar method. Total 100 isolates were studied for detection of biofilm production.

***Congo Red agar method (*11)**

The suspensions of the strain to be tested were inoculated into plate which contained a specially prepared solid medium- Brain Heart Infusionbroth (BHI) which was supplemented with 5%sucrose and Congo Red. The medium

was composed of BHI (37gms/l), sucrose (50 gms/l), agar No.1 (10 gms/l) and the Congo Red stain (0.8 gms/l). Congo Red was prepared as a concentrated aqueous solution and it inoculatedand incubated aerobically for 24-48 hours at37°C. Positive result was indicated by blackcolonies with a dry crystalline consistency, nonbiofilm producing strains developed red colonies.

3. Results

A total of 100 isolates from urine specimens were analyzed for biofilm formation and antibiotic susceptibility. Of these, 32 urine specimens were from catheterized patients and 68 were mid‑stream urine specimens. 55of the participants in the study were females and 45 were males.

Maximum patients belonged to the age group of more than 50 years (32) followed by the age group between 21 and 30 years (28).

Table 1: Age Distribution of patients

Age group	Total no.
< 20	12
21-30	28
31-40	10
41-50	08
>50	32

Symtomatic UTI, catheterization, and a prolonged duration of catheterization (≥7 days), uncontrolled DM, presence of stenting correlated significantly with increased propensity of microorganisms to form biofilms in the urinary tract. Gram‑negative organisms were the predominant isolates from the urine specimens accounting for 72% of the isolates, while Gram‑positive isolates were 28%. *E. coli* was isolated from37 urine specimens followed by and *Enterococcus* spp (28) and Klebsiella pneumoniae (15) Table2.

Table 2: Percentage distribution of uropathogens in UTI patients

Organism	Isolates	Biofilm Producers	Percentage (%)
Escherichia coli	37	09	24.32
Enterococcus species	28	28	100
Klebsiella pneumoniae	15	03	20
Enterobacter species	10	00	00
Proteus mirabilis	04	00	00
Acinetobacter species	03	00	00
Pseudomonas species	03	01	33.33
Total	100	41	100

Biofilm formation was seen in 41 (41%) isolates. The maximum biofilm production was seen in *Enterococcus* spp 28 (68.3%), 9 (21.95%) of *E. coli* isolates showed biofilm formation followed by 3 (7.32%) of *K. pneumoniae and 1 (2.43%) of Pseudomonas* isolates. There were no biofilm‑producing isolates of Enterobacter spp., *Acinetobacter* spp and *P. mirabilis* found in the present study [Table 3].

Table 3: Distribution of biofilm producing organisms

Sr. No.	Name of Organism	Total No.	Percentage (%)
1	Enterocoocus species	28	68.30
2	Escherichia coli	09	21.95
3	Klebsiella pneumoniae	03	07.32
4	Pseudomonas species	01	02.43

All Enterococcus isolates were biofilm producers and showed higher resistance pattern with susceptibility only to Vancomycin (100%), Linezolid (100%) and Nitrofurantoin (70%).

Amongst Gram Negative bacteria Biofilm producers showed resistance to first line agents with good susceptility to Imipenem and Meropenem and Nitrofurantoinonly (Table4).

Table 4: Resistance pattern of Gram negative bacteria (n=72)

No.	Antibiotic	Biofilm producing Gram-negative Organisms (n=13) %		Non-biofilm producing Gram-negative organisms (n=59)%	
1	Meropenem	12	92.31	59	100
2	Imipenem	11	84.62	59	100
3	Piperacillin-tazobactam	00	00	45	76.27
4	Nitrofurantoin	09	69.23	48	81.36
5	Ampicillin	00	00	12	20.34
6	Amoxycillin-clavulinic acid	00	00	39	66.11
7	Ceftazidime	00	00	32	54.24
8	Cefotaxime	00	00	27	45.76
9	Ceftriaxone	00	00	25	42.37
10	Tobramycin	04	30.77	48	81.36
11	Ciprofloxacin	00	00	35	59.32

4. Discussion

Amongst all infectious diseases, urinary tract infections (UTIs) represent one of the most common diseases in both developed and developing countries causing a large number of morbidity in different age groups [12]. It has been noticed that the etiological characteristics of UTI and their antibiotic resistance patterns may vary in different geographic locations [13]. Therefore it is essential to study local etiological agents and their antibiotic susceptibility patterns for appropriate treatment and eradication of UTIs. Biofilm production by pathogenic bacteria of the urinary tract may further complicate treatment options by showing high level of resistance to antibiotics [14].

Biofilms play a significant role in colonization during infection, providing an opportunity for the bacteria to develop drug resistance. Biofilm forming bacteria are encased in a well-hydrated matrix composed of secreted exopolymeric substances, proteins and nucleic acids from deadlysed cells that affords protection against host immune clearance and antibiotic therapy (**15, 16).**

In present study, a total of 100 isolates from urine specimens were analyzed for biofilm formation and antibiotic susceptibility. Of these, 32 urine specimens were from catheterized patients and 68 were mid‑stream urine specimens.

Of these, 55 of the isolates in the study were from female patients while 45 were from male patients thus giving slight female preponderence. This finding was similar to observations by Sao et al with UTI being more common in female patient (56.66%) in comparison to male patient (43.33)[17].

Maximum patients belonged to the age group of more than 50 years (32) followed by the age group between 21 and 30 years (28). Tayal et al also noted similar findings[18].

Gram-negative organisms were the predominant isolates from the urine specimens accounting for 72% of the isolates, while Gram-positive isolates were 28%. *E. coli* was isolated from37 urine specimens followed by and *Enterococcus* spp (28) and Klebsiella pneumoniae (15) Table2.

Panda PS et al also noted similar findings with (88.3%) were gram negative and 35 (11.7%) were gram positive organisms and E. coli was the commonest organism[19].

Results found by Tayal et al were also similar. They reported Gram-negative organisms as the predominant isolates from the urine specimens accounting for 89% (122), while Gram-positive organisms were 11% (15)[18].

Biofilm formation was seen in 41 (41%) isolates. The maximum biofilm production was seen in *Enterococcus* spp 28 (3%), 9 (21.95%) of *E. coli* isolates showed biofilm formation followed by 3 (7.32%) of *K. pneumoniae and 1 (2.43%)of Pseudomonas* isolates. There were no biofilm-producing isolates of Enterobacter spp., *Acinetobacter* spp. and *P. mirabilis* found in the present study [Table 3].

These findings correlated with Panda PS et al. where biofilm formation was detected in 137/300 (45.6%) isolates. All the biofilm producing organisms were found to be multi drug resistant (MDR).While most common biofilm producer was E. coli[19].

Tayal et al observed lower prevalence of biofilm formation in 37 (27%) isolates. The maximum biofilm production was seen in *Enterococcus* spp. (71%). 26% of *E. coli* isolates showed biofilm formation followed by 18% of *K. pneumoniae* isolates[18].
Biofilm-producing urinary isolates demonstrated higher antibiotic resistance pattern as compared to their nonbiofilm- producing counterparts. It was found that, most effective antibiotics against Gram negative bacteria were imipenem and meropenem (Table4).

All Enterococcus isolates were biofilm producers and showed higher resistance pattern giving Vancomycin and Linezolid as only drugs of choice.

5. Conclusion

From this study we have concluded that the ability of biofilm formation of uropathogenic strains had significant role in antibiotic resistance. Biofilm formation is the major virulence determinant of uropathogen, so it is necessary to screen all urinary isolates for biofilm production.

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