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Comparison of antimicrobial susceptibility pattern between biofilm and non-biofilm forming uropathogens isolated from community acquired urinary tract infections

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ABSTRACT

Introduction: Urinary tract infections (UTIs) are the most commonly encountered bacterial infections in healthcare with a spectrum of presentation ranging from benign symptoms of dysuria to life-threatening pyelonephritis.
Aim: To isolate and identify the uropathogens causing community-acquired urinary tract infections and to compare antimicrobial susceptibility pattern between biofilm and non-biofilm forming uropathogens
Materials and Methods: Midstream urine samples collected from 100 patients attending the OPD were processed as per standard protocol. Antimicrobial susceptibility was performed for the uropathogens. Biofilm production among the uropathogens was tested by the Tissue culture plate method.
Results: Out of the 100 patients with clinically suspected UTI significant growth of bacteria was seen in 47%. E.Coli (40%) being the commonest uropathogen isolated and found to be sensitive to Ak (80%) followed by Nitro (63%). Klebsiella spp and Pseudomonas exhibit a stronger biofilm-producing property. Antimicrobial resistance was seen to be higher among biofilm producing uropathogens.
Conclusion: There is a direct correlation between biofilm production and resistance to antimicrobials among the uropathogens comparing to non-biofilm producers.

1. Introduction

Urinary tract infections (UTIs) are the second most common infections in community practice. Worldwide, UTIs prevalence was estimated to be around 150 million persons per year.[1] Prevalence of UTI is common in females than males, 40%-50% of whom suffer at least one clinical episode during their lifetime.[2]

UTIs are caused by the microbial invasion of the urinary tract, that extends from the renal cortex of the kidney to the urethral meatus either upper(pyelonephritis or pyelitis) or lower(cystitis or urethritis) part.[3]

Upper UTI: Fever with chills, nausea, vomiting, flank pain, frequency, urgency, dysuria and urinary incontinence in case of children. Lower UTI: Frequent and painful micturition, suprapubic pain, terminal hematuria and strangury.[4]

Epidemiologically, it is subdivided into the community and hospital-acquired UTIs. Acute community-acquired UTIs are common and approximately 25% of the patients with an episode of UTI later develop recurrent UTI.[5]

Community-acquired UTIs are those with signs and symptoms of UTI which develop in patients visiting the OPD or inpatients within 48 hours of admission. The current management of these infections is empirical without urine culture/susceptibility testing. Alike many community-acquired infections, the antimicrobial resistance in community-acquired UTI is increasing.[5]

Uncomplicated UTI: It is usually seen in young women without any underlying urinary tract or systemic disease. Complicated UTI: This occurs in the presence of obstruction, indwelling catheterization, calculi, neurolog-

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ical abnormalities, vesicoureteric, analgesic nephropathy, renal scarring, and impaired renal function.

Escherichia coli (uropathogenic E.Coli – UPEC) is the most common cause of all forms of UTIs. Other causes include Klebsiella, Proteus, Staphylococcus saprophyticus, Enterococci, etc.[6] UPEC forms multicellular communities known as biofilms on the surface of catheter materials and within bladder epithelial cells. Biofilm formation impedes the treatment of UTIs by protecting encased bacteria from both the host immune response and antimicrobial therapy.[6]

Irrational use of drugs in community-acquired UTIs has led to the development of antimicrobial resistance which is seen often in bacteria producing biofilms.

As limited studies are available on biofilm-producing uropathogens isolated from community-acquired UTIs, the present study is focused to compare and assess the antimicrobial susceptibility pattern among uropathogens (biofilm-forming and non-biofilm forming) causing community-acquired UTI.

2. Aim

To isolate and identify the uropathogens causing community-acquired urinary tract infections and to compare antimicrobial susceptibility pattern between biofilm and non-biofilm forming uropathogens.

3. Materials and Methods

This cross-sectional study was conducted at the Institute of Microbiology in collaboration with patients attending OPD of the Tertiary care Hospital, Chennai from August to September 2019 in 100 patients with symptoms of urinary tract infection.

3.1. Inclusion criteria

Patients aged more than 18 years of both sexes attending OPD with signs and symptoms of UTI such as increased frequency and urgency of micturition, dysuria, burning micturition, fever. Patients with signs and symptoms of UTI attending OPD and inpatient developing UTI within 48hrs of admission in the hospital.

3.2. Exclusion criteria

Patients with catheter-associated urinary tract infections and patients developing UTI after 48 hrs of admission in the hospital.

3.3. Methodology

Under aseptic precautions after giving proper instructions to the patients about the collection of urine, clean catch mid-stream urine sample was collected in universal leak proof sterile container. The samples were immediately transported to the laboratory and processed immediately. Macroscopically, the specimens were observed for color and turbidity

A direct microscopic examination was done. Semi-quantitative urine culture by the standard calibrated bacteriological loop. Isolated pathogens were tested for biofilm production by the tissue culture plate method. The antimicrobial resistance pattern is determined using the Kirby-Bauer disc diffusion method. Antimicrobial susceptibility testing was done for both biofilm-producing and non-biofilm producing bacterial isolates by Kirby – Bauer disk diffusion method as per CLSI guidelines and the resistant pattern was analyzed for both the categories.

Statistical differences were analyzed using a conventional chi-squared test and a P value$<$0.05 is considered significant. The data obtained were analyzed using SPSS statistical software.

4. Results

This cross-sectional study was focused on community-acquired UTI was conducted in the Institute of Microbiology in collaboration with patients attending OPD of the Govt. General Hospital, Chennai. 100 patients with symptoms & signs of urinary tract infection attending OPD were included. Females seem to be more affected (62%) with UTI. Urine Cultures tested positive showed significant growth in 47 (47%) of cases. E.Coli (40%) being the commonest uropathogen isolated. Other uropathogens were K.oxytoca (32%), K.Pneumonia (9%) Pseudomonas (13%), and Enterococci (6%). E.Coli and Klebsiella were found to be sensitive to Ak(80%,73%) respectively followed by Nitro(63%,67%). Maximum resistance was observed for Ampicillin (90% each), Cotrimoxazole (68%, 86%) Norfloxacin (64%,76%) among both E.coli and Klebsiella. High level of resistance to Ampicillin, Cotrimoxazole, and Norfloxacin. The biofilm-producing organisms were tested by the tissue culture plate method. Biofilm production was more among female patients (71%). Klebsiella spp and Pseudomonas exhibit a stronger biofilm-producing property. Antimicrobial resistance was seen to be higher among biofilm producing uropathogens. Among the biofilm producers, K.oxytoca exhibited higher resistance particularly against ampicillin (53%), norfloxacin (46%) and cotrimoxazole (53%) and pseudomonas exhibit higher resistance against norfloxacin, ciprofloxacin, and ceftazidime each (33%) respectively (p-value 0.005).

5. Discussion

In the present study, 100 patients suspected of having signs and symptoms of urinary tract infection were studied.

Out of 100 patients, significant growth of bacteria was seen in 47%. The study by Nisha Majeed et al. in the year 2016 reported culture positivity as 56 cases (66%) in common Community-acquired UTI cases correlating well

Table 1: E.Coli

Antibiotics	Biofilm forming		Non biofilm forming		P value
	Sensitivity	Resistance	Sensitivity	Resistance	
AK	31.00%	15.00%	47.00%	5.00%	0.018*
GEN	21.00%	26.00%	21.00%	21.00%	0.404
COT	15.00%	31.00%	15.00%	26.00%	0.434
NIT	26.00%	21.00%	36.00%	15.00%	0.269
NOR	15.00%	26.00%	15.00%	21.00%	0.404
CTX	10.00%	42.00%	15.00%	36.00%	0.384
AMP	0.00%	42.00%	5.00%	37.00%	0.470

Table 2: K.Oxytoca

Antibiotics	Biofilm forming		Non biofilm forming		P-value
	Sensitivity	Resistance	Sensitivity	Resistance	
AK	26.00%	26.00%	46.00%	0.00%	<0.0001*
GEN	26.00%	26.00%	33.00%	13.00%	0.020*
COT	26.00%	53.00%	13.00%	33.00%	0.004*
NIT	26.00%	26.00%	40.00%	13.00%	0.020*
NOR	6.00%	46.00%	6.00%	40.00%	0.391
CTX	6.00%	46.00%	20.00%	26.00%	0.003*
AMP	0.00%	53.00%	0.00%	46.00%	0.322

Table 3: K.Pneumonia

Antibiotics	Biofilm forming		Non biofilm forming		P-value
	Sensitivity	Resistance	Sensitivity	Resistance	
AK	25.00%	25.00%	50.00%	18.00%	0.228
GEN	25.00%	25.00%	50.00%	17.00%	0.165
COT	25.00%	25.00%	25.00%	16.00%	0.115
NIT	25.00%	25.00%	50.00%	18.00%	0.228
NOR	25.00%	25.00%	25.00%	18.00%	0.228
CTX	0.00%	25.00%	25.00%	19.00%	0.306
AMP	0.00%	25.00%	0.00%	16.00%	0.115

Table 4: Pseudomonas

Antibiotics	Biofilm forming		Non biofilm forming		P-value
	Sensitivity	Resistance	Sensitivity	Resistance	
AK	33.00%	16.00%	50.00%	0.00%	<0.0001*
GEN	33.00%	16.00%	33.00%	11.00%	0.301
NOR	16.00%	33.00%	16.00%	16.00%	0.005*
CAZ	16.00%	33.00%	33.00%	16.00%	0.005*
PT	50.00%	0.00%	50.00%	0.00%	n/a
CIP	16.00%	33.00%	33.00%	16.00%	0.005*

Table 5: Enterococcus

Antibiotics	Biofilm forming		Non biofilm forming		P value
	Sensitivity	Resistance	Sensitivity	Resistance	
VAN	100.00%	0.00%	100.00%	0.00%	n/a
LZ	100.00%	0.00%	100.00%	0.00%	n/a
HLG	0.00%	33.00%	33.00%	28.00%	0.443
TET	0.00%	33.00%	0.00%	27.00%	0.355
NT	33.00%	0.00%	66.00%	0.00%	n/a
NOR	0.00%	33.00%	0.00%	26.00%	0.278
AMP	33.00%	0.00%	66.00%	0.00%	n/a
CIP	0.00%	33.00%	0.00%	28.00%	0.443

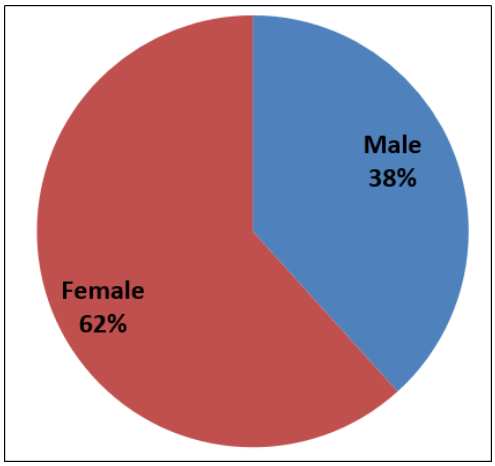

Fig. 1: Distribution of gender

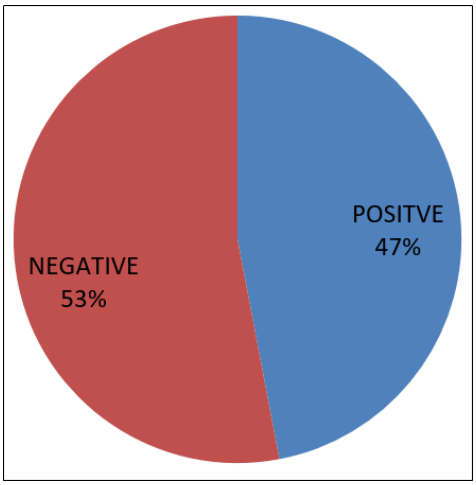

Fig. 2: Distribution of results of urine culture

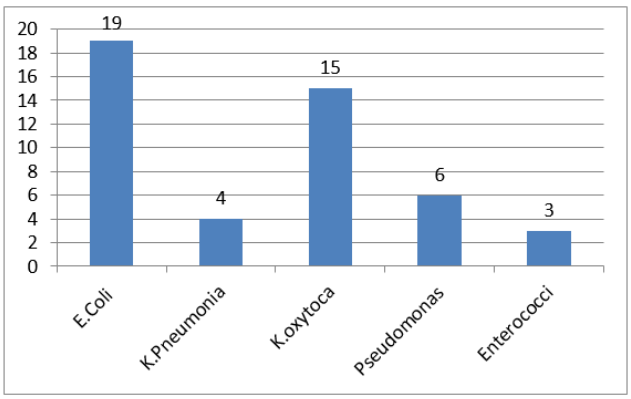

Fig. 3: Distribution of uropathogens isolated

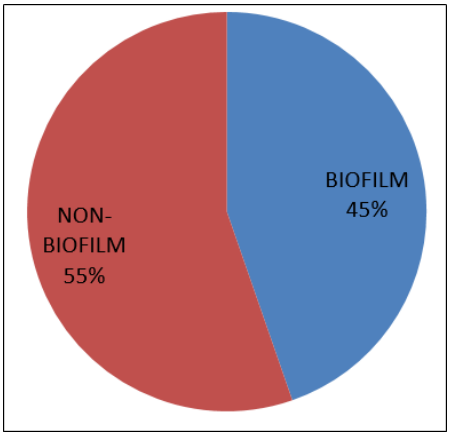

Fig. 4: Distribution of Biofilm and non-biofilm uropathogens

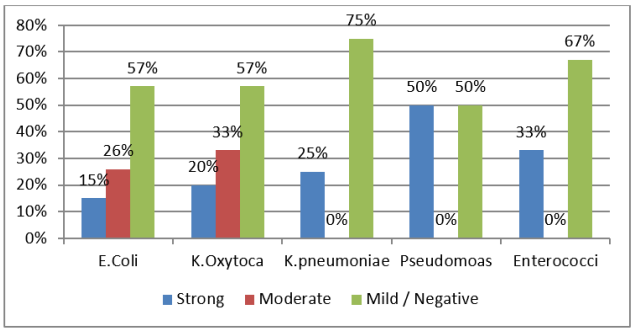

Fig. 5: Biofilmproducers among the uropathogens

with our study.[7]

In the present study, females (62%) were more affected by UTI than males (38%). Similar findings were reported by Dharmishta et al., Linhares et al. and Nisha Majeed et al. Men are usually less prone to UTI as compared to females owing to a longer course of the urethra and bacteriostatic properties of prostatic secretions.[7–9]

In the present study among the uropathogens E.coli (40%) was the most common organism causing UTI. Nisha Majeed et al and Mohammad Ak et al. reported 54.4% and 61% respectively. Intestinal flora is a common source of UTI.[7–10]

In our study frequency and distribution of different uropathogens isolated were E.coli (40%), Klebsiella oxytoca (32%), Klebsiella pneumonia (9%), Pseudomonas (13%) and Enterococcus (6%).

A similar distribution of pathogens was seen in the study by Nisha et al.[7] E.coli (54%), Klebsiella spp (45%), Pseudomonas, (3%) Enterococcus (5%) in outpatients cases. The drug sensitivity pattern of uropathogens in our study is as follows: E.Coli and Klebsiella were found to be sensitive to Ak (80%,73%) respectively followed by Nitro (63%, 67%).

Maximum resistance was observed for Ampicillin (90% each), Cotrimoxazole(68%, 86%) Norfloxacin (64%, 76%) among both E.coli and Klebsiella.

High levels of resistance to Ampicillin, Cotrimoxazole, and Norfloxacin can be explained by the longtime period for which these drugs have been available and in use for UTI.

Nisha et al.[7] also reported E.coli showed 70% resistance to Ampicillin, Norfloxacin each and Cotrimoxazole – 80%. Similar findings were observed in the study conducted by Mohammad Ak et al.,[10] Norfloxacin (69%), Ak (51%), cotrimoxazole (76%). Hence the pattern of drug resistance which is more consistent with the previous data on community based studies.

But resistance rates vary from country to country. Uropathogens isolated from the USA showed only 39.1% and 18.6%% for Ampicillin and Cotrimoxazole respectively.

In the present study, biofilm-producing organisms were tested by the tissue culture plate method. Among the uropathogen, strong biofilm producers are Pseudomonas (50%) followed by Klebsiella spp (45%).

In the present study, the antimicrobial resistance was seen to be higher among biofilm producing uropathogens. K.oxytoca exhibited higher resistance particularly against ampicillin (53%) norfloxacin (46%) and cotrimoxazole (53%) and pseudomonas exhibit higher resistance against norfloxacin (33%). A similar pattern of resistance was seen in the study conducted by V Bardoloi et al.[11]

6. Conclusion

UTIs seem to occur more commonly in females. E.Coli and K.Oxytoca are the commonest organisms isolated from UTI patients. K.Pneumoniae and Pseudomonas are involved in strong biofilm production. They exhibit a higher resistance against drugs like norfloxacin, ampicillin, and cotrimoxazole. There seems to be a direct correlation between biofilm production and resistance to antimicrobials. Apart from this, biofilms have also lead to a higher incidence of recurrence and other complications.

7. Source of Funding

None.

8. Conflict of Interest

None.

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