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Biofilm Formation and Antibiotic Susceptibility Profile of Uropathogens: A Study

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Abstract

Introduction: Urinary tract infection (UTI) is one of the commonest causes of infections in humans. The causative organisms of UTI may be bacteria, fungi and viruses. 95% of UTI are caused by bacteria. The uropathogens commonly form biofilm causing persistence of infection.

Aim: To study the biofilm formation and antibiotic susceptibility profile of uropathogens.

Material & Methods: 200 bacterial strains isolated from urine samples were included in the study. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method according to CLSI Guidelines. Biofilm formation was detected by Tube nmethod.

Results: 77.5% strains were Gram negative bacilli and rest were Gram positive cocci. E.coli was the commonest isolate. All (100%) Gram positive cocci were resistant to Penicillin. 29% of Gram negative bacilli & 46.7% of Gram positive cocci were strong biofilm producers.

Conclusion: The emperical treatment of UTI must be stopped. Antibiotic Susceptibility testing and detection of Biofilm producing strains for uropathogens should be done routinely.

Introduction

Urinary tract infection (UTI) is one of the commonest causes of infections, just after upper respiratory tract infection (URI) in humans. [1] Approximately 10% of people suffer from UTI during their life time. [2] UTIs can be caused by diferent microorganisms such as bacteria, fungi and viruses.

95% of UTIs are caused by bacteria. *Escherechia coli* is the commonest cause of UTI and accounts for more than 80% of UTI cases. Urinary tract infections are defined as

presence of bacteria $\geq 10^5$ CFU/ml (colony forming unit/ml) of urine which is also called significant bacteruria. Amongst the fungi, Candida species are most commonly the causative agent to cause UTI. The patients may be symptomatic or asymptomatic. If left untreated, it can cause cystitis, urethritis and pyelonephritis etc. The resultant blood stream infection may lead to bacteriaemia and other serious complications. [3] The predisposing factors are female sex, phimosis, vesicoureteral reflux, catheterized patients. In most of the cases, UTI is traeated empirically. Antibiotic resistance is a major public health problem worldwide. The uropathogens also develop resistance to commonly used antibiotics in recent years. Moreover, uropathogens can form biofilm in urinary tract and over the catheters and cause persistence of infection, recurrent UTI and antibiotic resistance very commonly.[4] Biofilms can be defined as sessile communities of microbial cells irreversibly attached to a surface or interface or to each other which are embedded in a self produced matrix of extracellular polymeric biomolecules and are physiologically different from planktonic cells with respect to growth rate and gene transcription. [5] Various studies have reported that 80% of all human infections are due to biofilm formation. [6]

Hence, the present study was undertaken with the following objectives-----

- To study the antibiotic sensitivity profile of uropathogens isolated in the department of Microbiology.
- To detect the incidence of Multidrug resistant(MDR), Extensively drug resistant (XDR) and Pandrug resistant (PDR) uropathogens.
- To detect phenotypically the incidence of biofilm forming uropathogens.

Material and Methods

Setting: The present study has been conducted in the department of Microbiology with approval from Institutional Ethics Committee. It was a short term cross-sectional experimental study.

Sample size: The sample size has been calculated as per the formula [7]-

Sample size (ss) $=Z^2 \times (p) \times ((1-p)/c^2$

Where Z=Z value (1.96 for 95% confidence level)

p= prevalence %, expressed in decimal: 0.2 will be used

c= margin of error, 0.06 will be used

The minimum calculated sample size was approximately 171. A total number of 200 uropathogens was studied during the study period.

Selection criteria: 200 bacterial strains isolated from urine samples and characterized by conventional tests e.g. Gram staining, motility and biochemical tests, only were

included in the study. Urine samples will be received from Indoor Patient Departments (IPD) and Out Patient Departments (OPD) of our hospital which is a tertiary care hospital in a rural setup.

Antibiotic susceptibility test: All 200 bacterial strains were subjected to antibiotic susceptibility test using Mueller Hinton agar (MHA) plate with the commercially available antibiotic discs by Kirby Bauer disc diffusion method [8] according to Clinical Laboratory Standard Institute (CLSI) Guidelines. [9] Using sterile swabs, lawn culture of the test strain (turbidity adjusted to 0.5 Mc Farland standard) was done on Mueller Hinton agar (MHA) plate. With all aseptic precautions the antibiotic disc were put on that inoculated MHA plate. Six antibiotic discs were put on 90 mm diameter MHA plate. The antibiotic discs for Gram negative bacteria were put up for Amikacin (AK-30µg), Ciprofloxacin (CIP-5 µg), (Nitrofurantoin (300 µg), Tetracycline (TE-30µg), Ceftazidime (CAZ-30µg), Netilmicin (NET-30µg), Meropenem (MRP-10 µg), Aztronam (AT-30 µg), Piperacillin (PI-100 µg), Tigecycline (TGC-15 µg) and Colistin (CL-10 µg), as per CLSI Guidelines.[8] For Gram positive bacteria Penicillin (P-10 units), Ciprofloxacin (CIP-5 µg), Gatifloxacin (5-µg), Tetracycline (TE-30µg), Erythromycin (E-15 µg), Nitrofurantoin (NIT-300 µg), Vancomycin (VA-10 µg), Linezolid (LZ-30µg) were used as per CLSI Guidelines.[9] Detection of Extended Spectrum β-lactamase (ESBL) producing bacterial strains were detected by Combine disc method using Ceftazidime (CAZ-30µg) and Ceftazidime/Clavulanic acid (CAC) discs as per CLSI Guidelines. [9] Detection of AmpC β-lactamases producing strains were done by using Cefoxitin (CX-30µg) disc and Cefoxitin/ Cloxacillin disc. [10] Metallo β-lactamase producing strains were detected by using Imipenem and Imipenem/ EDTA discs. [111] Methicillin Resistant *Staphylooccus aureus* (MRSA) strains were detected by Cefoxitin (CX-30µg) disc and High Level Aminoglycoside Resistance (HLAR) for *Enterococci* were detected by using High level Streptomycin (HLS-300 µg) and High Level Gentamicin (HLG-120 µg) disc.[8]

The Multidrug resistant (MDR), Extensively drug resistant (XDR) and Pandrug resistant (PDR) bacterial strains were detected as per definition given by European Society for Disease Control (ECDC) and Center for Disease Control and prevention (CDC), Atlanta. [12] MDR is defined as acquired resistance to at least one agent in 3 or more antimicrobial categories. XDR is defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories. PDR is defined as nonsusceptibility to all agents in all antimicrobial categories. Detection of Biofilm formation was done by Tube method. [13] 10 ml Trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test strain

grown overnight on nutrient agar. The tubes were incubated at 37^0 C for 24 hours. The growth along with the broth was decanted. Then the tubes were washed with Phosphate buffer saline (pH 7.3).The excess stain was washed with deionized water and the tubes were dried. In positive biofilm producing strains a visible stained film can be seen lining the wall and bottom of the tube. The tests were interpreted as Strong, Moderate, Weak and Absent for biofilm production.

Observation and Results

A total number of 200 strains isolated from different clinical samples and characterized by conventional tests were included in the study.

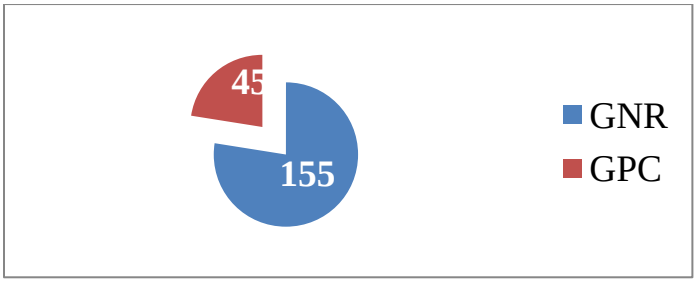

Figure 1: Isolation of Gram negative bacilli and Gram positive cocci (n=200)

Figure 1 shows the isolation of Gram negative bacilli and Gram positive cocci isdolated from urine samples. A total number of 200 bacterial uropathogens were studied. Out of which 155 (77.5%) strains were Gram negative bacilli, and 45 (22.5%) were Gram positive cocci. Amongst 45 Gram positive cocci 13(29.9%) were Coagulase positive *Staphylococcus* and 32 (71.1%) were *Enterococcus faecalis.* Out of 155 Gram negative bacilli, 64 (41.3%) were *E.coli*, 41 (26.5%) were *Klebsiellas pneumoniae*, 40 (25.8%) were *Pseudomonas aeruginosa*, 6 (3.9%) were *Acinetobacter baumanii complex*, 3 (1.9%) were *Proteus mirabilis* and 1 (0.6%) was *Citrobacter fruendi*.

Table 1: Antibiotic susceptibility profile of Gram positive cocci studied (n=45)

Antibiotics	Sensitive No. Percentage	
Penicillin	0	-
Ciprofloxacin	16	35.6
Gatifloxacin	21	46.7
Tetracycline	17	37.8
Erythromycin	10	22.2
Nitrofurantoin	32	71.1
Vancomycin	45	100
Linezolid	45	100

Table1shows the antibiotic susceptibility profile of Gram positive cocci studied. All 45 (100%) Gram positive cocci were sensitive to Vancomycin and Linezolid. The

sensitivity was observed to Nitrofurantoin (71.1%) followed by Gattifloxacin 21 (46.7% strains). Penicillin resistance was observed among all (100%) Gram positive cocci studied.

Amongst the 13 coagulase positive Staphylococcus, 6 (46.2%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) . Among 32 *Enterococcus faecalis* strains, 23 (71.9%) were High Level Aminoglycoside Resistant (HLAR).

Table 2: Antibiotic susceptibility profile of Gram negative bacilli studied (n= 155)

Antibiotics	Sensitive No. Percentage	
Amikacin	49	31.6
Ciprofloxacin	61	39.4
Nitrofurantoin	77	49.7
Tetracycline	33	21.3
Ceftazidime	91	58.7
Netilmycin	80	51.6
Meropenem	103	66.5
Aztreronam	57	38.8
Piperacillin	93	60
Tigecycline*	110	95.7
Colistin**	152	100

Tigecycline* was not tested for 40 Pseudomonas aeruginosa strains as *P.aeruginosa* is intrinsically resistant to Tigecycline. Hnce, only 115 strains were studied for Tigecycline.

Colistin** was not tested for 3 *Proteus mirabilis* strains as Proteus sp. is intrinsically resistant to Colistin. .Hence, only 152 srrains were studied for Colistin.

Table 2 shows the antibiotic susceptibility profile of 155 Gram negatiove bacilli studied. All 152 (100%) strains were sensitive to Colistin, followed by Tigecycline (95.7%) and Meropenem (66.5%) . 33 (21.3%) strains were only ESBL producers, 19 (12.3%) strains were only AmpC β-lactamase producer and 13(8.4%) were only metallo β-lactamase producers (MBL). 20 (12.9%) strains produced ESBL plus AmpC β-lactamases, 12 (7.7%) strains produced AmpC β-lactamases plus MBL, 9 (5.8%) strains produced ESBL plus MBL and 7 (4.5%) strains produced all the 3 β-lactamases i.e. ESBL plus AmpC β-lactamases plus MBL. Hence, in the present study, a total number of 113 (72.9%) strains were newer β-lactamases

producer and only 42 (27.1%) strains did not produce β-lactamases.

Table 3: Isolation of MDR, XDR and PDR strains (n=200)

Bacterial strains	MDR	XDR	PDR
GNR(155)	83	19	0
Coagulase +ve *Staphylococcus* (13)	4	1	0
Enterococcus faecalis (32)	24	2	0
Total	111	22	0

Table 3 shows isolation of MDR, XDR and PDR strains out of 200 uro[pathogens studied. In the present study, a total number of 111 (55.5%) multidrug resistant (MDR) and 22 (11 %) extensively drug resistant (XDR) strains were isolated. No pandrug resistant (PDR) strain was isolated. Out of 155 Gram negative bacilli studied, 83 (53.5%) were MDR and 19 (12.3%) were XDR.

Amongst 13 Coagulase +ve *Staphylococcus* strains studied 4 (30.8%) were MDR and 1 (7.7 %) were XDR. Similarly out of 32 *Enterococcus faecalis* strains studied 24 (75 %) were MDR and 2 (6.3 %) were XDR.

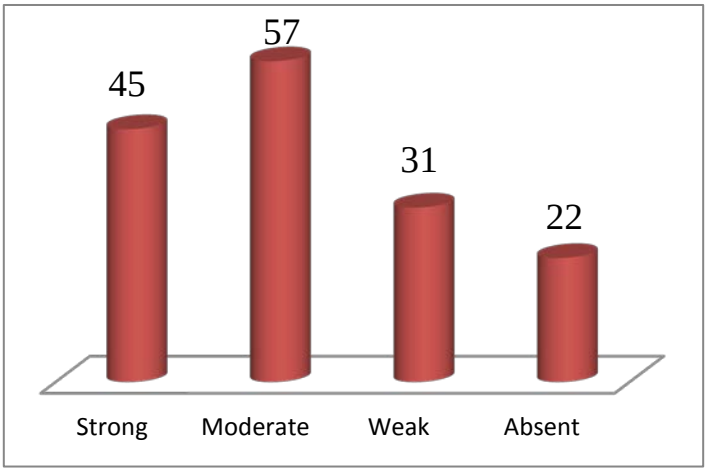

Figure 2: Production of different grades of biofilm by Gram negative bacilli studied. (n=155)

Figure 2 shows production of different grades of biofilm by 155 Gram negative bacilli studied. The biofilm production wsa detected by tube nethod. 45 (29 %) strains were strong, 57 (36.8 %) were moderate and 31 (20 %) were weak biofilm producers. 22 (14.2%) strains were biofilm nonproducers. Out of 64 E.coli strains studied, 25 (39.1%) were strong biofilm producers. Out of 45 strong biofilm producing Gram negative bacilli strains, 31 (68.9 %) were MDR and 14 (31.1%) were XDR strains.

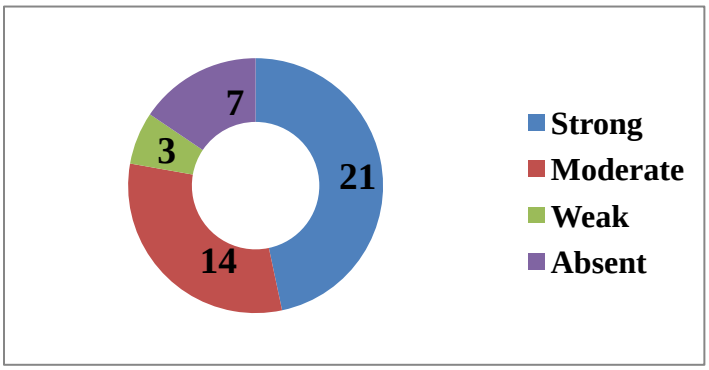

Figure 3: Production of different grades of biofilm by Gram positive cocci (n=45)

Figure 3 shows production of different grades of biofilm by 45 Gram positive cocci studied. 21 (46.7%) strains were strong, 14 (31.1%) were moderate and 3(6.7 %) were weak biofilm producers. 7 (15.6%) strains were biofilm nonproducers. Out of 32 Enterococcus faecalis, 19 (59.4 %) strains were strong biofilm producers.

Discussion

Urinary tract infections are the most common infections in clinical practice.[14] UTI are also the commonest infection in acute and long term care hospitalised patients. [15]

Urinary tract infections are serious health problems caused usually by antibiotic resistant organisms . UTI becomes chronic as the organisms tend to produce biofilm. Biofilms play an important role in colonization of urethral mucosa and urinary catheter. Within the biofilms the bacteria are encased in a extracellular matrix that gives protection against host's immune system, antibiotic therapy and disinfectants. [16,17]

The commonest bacteria causing UTI is E.coli, both in community as well as in the hospital. In the present study, E.coli was the commonest uropathogens (41.3%) followed by Klebsiella pneumoniae (26.5%) among the Gram negative bacilli which is similar to the findings of other workers. [18, 19, 20] Enterococcus faecalis was the commonest pathogen (71.1%) among Gram positive cocci.

The highest percentage of antibiotic resistance was observed for Tetracycline and highest sensitivity was observed with Colistin for Gram negative bacilli. The highest resistance was observed with Penicillin and highest sensitivity was observed with Vancoimycin and Linezolid for Gram posutive cocci. 71.1%Gram positive cocci were sensitive to Nitrofurantoin.

Over the last decade, the antibiotic resistance for uropathogens has increased. Though resistance to Vancomycin has been reported in Enterococci and Coagulase positive Staphylococci, [21, 22] no strain resistant to Vancomycin was observed in the present study. The increase in antibiotic resistance may be explained by inappropriate use of antibiotics. [23] Transmission of antibiotic resistance between people and or by consumption of foods from animals that already had

taken food supplemented with antibiotics.[24] The most probable reason may be for observing the high percentages of antibiotic resistance in the present study was , as our hospital is a tertiary care hospitsl in a rural set up, most of the patients were refered from other Health care set up and received one or two classes of antibiotics prior to be admitted in this hospital.

Conclusion

We hereby, conclude that, the early detection of drug resistant bacterial strains causing urinary tract infections and the biofilm forming uropathogens must be detected and thereby recurrence of UTI can be prevented. Specially, the overuse and improper use of Cephalosporins and Carbapenems will be stopped.

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