

Research Article

A Comparative Analysis of Antimicrobial Susceptibility Pattern in Biofilm-Forming and Non-Biofilm Forming Uropathogens Isolated from a Tertiary Care Hospital, Lucknow

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ABSTRACT

Background: Urinary tract infections (UTIs), one of the most common bacterial diseases in the world, are often caused by uropathogens such as *Escherichia coli*. Biofilm production is critical to the persistence and recurrence of many diseases, and it also contributes to antimicrobial resistance (AMR). Understanding the relationship between resistance patterns and biofilm formation is essential for effective therapy.

Aim: To compare antimicrobial susceptibility patterns between biofilm-forming and non-biofilm-forming uropathogens and to evaluate three biofilm detection methods.

Method: Urine samples were taken from patients at a tertiary care hospital in Lucknow over the course of six months. Congo Red Agar (CRA), the Tube Method, and the Microtiter Plate (MTP) assay were used to identify uropathogenic isolates and detect biofilms. Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method in compliance with CLSI guidelines.

Result: Out of 112 isolates, 52 uropathogens yielded, which has given 60% positive result for CRA & Tube method, whereas it has only given 11% positive biofilm test for MTP method. Biofilm-positive isolates demonstrated greater resistance to aminoglycosides and tetracycline, but ESBL and MBL isolates showed maximum resistance regardless of biofilm status.

Conclusion: Biofilms are frequently formed by uropathogens, which might result in therapy failure and recurrence. Managing multidrug-resistant bacteria and recurrent UTIs requires strict antibiotic management and routine biofilm detection.

Keywords: Uropathogens, *Escherichia Coli*, Biofilm, Antimicrobial Resistance, ESBL, MBL.

INTRODUCTION

Urinary tract infections (UTIs), a significant worldwide public health concern, are among the most common bacterial infections observed in clinical practice. All age groups and genders are affected, due to anatomical and physiological considerations, women are more commonly impacted. Every year, an estimated 150 million people get UTIs, which substantially raises morbidity, medical expenses, and lost productivity. UTIs can be as simple as cystitis or as complex as kidney infections, urinary tract abnormalities, catheterisation, diabetes mellitus, immunosuppression, and recurrent infections¹. The most prevalent bacteria among them are *Escherichia coli*, which causes 70–90% of UTIs in the community and a significant portion of infections contracted in hospitals. Other uropathogens that are commonly isolated include *Klebsiella pneumoniae*, *Proteus*

mirabilis, *Pseudomonas aeruginosa*, *Enterococcus* species, *Staphylococcus saprophyticus*, *Acinetobacter* species, and *Citrobacter* species.^{2,1} The mainstay of treating UTIs is still antimicrobial therapy. However, the development of antimicrobial resistance (AMR) among uropathogens has been promoted by irrational antibiotic usage, self-medication, unfinished treatment regimens, and widespread empirical therapy. Over the past 20 years, there has been a significant rise in resistance to routinely prescribed antibiotics such as ampicillin, cotrimoxazole, fluoroquinolones, and cephalosporins. AMR is one of the biggest threats to world health, according to the World Health Organisation, and uropathogenic bacteria play a major role in this expanding problem.³ The ability of uropathogens to build biofilms is a significant role in recurrent and persistent UTIs. Biofilms

are structured communities of microbial cells enclosed within a self-produced extracellular polymeric substance (EPS) matrix and attached to either biotic or abiotic surfaces. Within the urinary tract, biofilms may develop on the uroepithelial surface, urinary catheters, stents, and other indwelling medical devices. By preventing drug penetration and protecting bacteria from host immune responses, the biofilm matrix serves as a barrier that promotes recurrent and persistent infections⁴. An estimated 150 million people globally suffer from UTIs each year, and rising antibiotic resistance and recurrence rates of uropathogens (UPs) present a significant financial burden. Global surveillance studies show that UTIs are the second most common type of infection, following respiratory tract infections.⁵ Biofilms are estimated to be the source of more than 80% of chronic and persistent microbial illnesses in humans and animals. The pathophysiology of CAUTIs is significantly influenced by biofilms. CAUTIs are linked to systemic consequences such as bacteremia and septic shock, as well as localised effects including biofilm accumulation and encrustation on the catheter and the development of cystitis and pyelonephritis.⁶ In routine urine cultures, biofilm-forming uropathogens frequently go into dormant or viable but nonculturable phases, which slows their growth. Studies show that conventional culture techniques fail to detect embedded biofilm bacteria, especially in chronic and catheter-associated UTIs, which results in inaccurate diagnosis and treatment delays⁷. Despite the fact that uropathogens have been shown to create biofilms in a number of clinical studies, there is still a lack of information comparing the antimicrobial susceptibility of isolates that form biofilms and those that do not². Since most studies have concentrated on biofilm generation or resistance patterns independently, few Indian studies have thoroughly evaluated the antimicrobial susceptibility of biofilm-forming and non-forming uropathogens.

Importance of UTI in Healthcare

UTIs are among the most common bacterial illnesses in the world, affecting over 150 million people annually, according to the WHO. According to the Centres for Disease Control and Prevention (CDC), catheter-associated urinary tract infections (CAUTIs), which make up between 30 and 40 per cent of all nosocomial infections, are among the most common healthcare-associated illnesses. The clinical and financial burden of UTIs has increased due to the growing prevalence of antimicrobial-resistant uropathogens, which raises the chance of treatment failure, lengthens hospital stays, and raises healthcare expenses^{8,9,10}. UTI is the most commonly reported infection among all HAI. Catheterisation is the major risk factor for UTI worldwide. Having the most common bacterial infection, UTI, leads to economic burden due to higher medical costs worldwide. UTI accounts 36% cases of all HAIs¹¹.

Link Between Biofilm Formation & Antibiotic Resistance

Bacteria live a sedentary lifestyle in the EPS (extracellular polymeric substance) matrix and undergo a switch in gene expression and slower metabolism. All these conditions make bacteria capable of causing increased antibiotic resistance. These bacteria are 1000 times more tolerant to antibiotics in comparison to planktonic bacteria.¹² In both human and animal healthcare, the overuse and long-term use of antibiotics have led to the rise and spread of bacteria that are resistant to many existing drugs. Biofilms make this issue worse by providing a protective environment where different microorganisms can live together and share antibiotic resistance genes with each other.¹³.

Biofilm-Forming Pathogens

Antimicrobial resistance (AMR), treatment failure, recurrent urinary tract infections (rUTIs), and catheter-associated UTIs (CAUTIs) are all caused by uropathogens' primary pathogenicity strategy, biofilm development ¹⁴.

Escherichia coli (UPEC)	Commonly found pathogen of UTI in the community and healthcare settings
Klebsiella pneumoniae	Potent biofilm former in CAUTI.
Pseudomonas aeruginosa	Forms highly organised biofilm on catheters.
Enterococcus faecalis	Persistent biofilm formation lead to recurrent UTI.

Proteus mirabilis	Catheter obstruction due to crystalline biofilm formation.
Acinetobacter baumannii	MDR-associated infection due to biofilm formation.
Candida spp.	biofilm-assisted fungal UTI.

10, 15

Mechanism of Antimicrobial Resistance in Biofilm

Limited Access to Antibiotics Via the EPS

Matrix: Tobramycin's bactericidal action in deeper biofilm layers is greatly reduced by *Pseudomonas aeruginosa* extracellular matrix. The biofilm matrix is a dynamic polymeric network that binds and delays antimicrobial drugs, shielding embedded bacterial cells from efficient drug exposure. ^{16, 17.}

Metabolic Dormancy of Persister Cells:

Specific persister cells seen in biofilms can survive at high antibiotic doses by temporarily going dormant. In addition, the biofilms' gradients of nutrients and oxygen lower metabolic activity, making antibiotics that target active processes less effective. ^{18,19.}

Overexpression Of Efflux Pump: Biofilm-associated bacteria actively export antibiotics and lower intracellular drug levels by upregulating multidrug efflux pumps., The MexAB-OprM and related components overexpression in *P. aeruginosa* biofilms greatly increases resistance to fluoroquinolones and β -lactam antibiotics. ^{20,21.}

Horizontal Gene Transfer (HGT) In Biofilms:

Antibiotic resistance genes spread more widely when dense biofilm architecture encourages plasmid-mediated gene transfer²². Compared to planktonic cells, conjugation and integron exchange are accelerated in biofilms due to their close cell contact and stable environmental circumstances. ²³

Stress Reaction and Gene Regulation

Adaptation: Biofilms' oxygen restriction and immunological pressure trigger stress-response mechanisms that improve resistance to antibiotics, resulting in biofilm formation, which contributes to the survival of chronic infections by causing adaptive gene expression changes, such as oxidative stress defences and decreased membrane permeability. ^{24,25.}

Correlation Between Biofilm & Antibiotic Resistance:

Strong biofilm development and multidrug resistance are significantly positively connected among Gram-negative clinical isolates; increased biofilm biomass was associated with higher MIC values ²⁶. Antibiotic tolerance is directly improved by biofilm formation, which also causes changes in gene expression, such as lower metabolic activity and efflux pump activation¹⁹. During biofilm maturation, adaptive resistance mechanisms are particularly activated, strengthening the mechanistic connection between the creation of structural biofilms and long-lasting antibiotic resistance²⁵.

MATERIAL & METHODS

Study Design:

Descriptive, hospital-based cross-sectional

Sample Collection:

Properly collected urine sample, by appropriate methods (midstream urine, catheterised sample, suprapubic aspirate)

Biofilm detection methods:

Detection of biofilm employs three methods, i.e., Congo red agar method, Tube method & Microtitre Plate Method.

Antimicrobial susceptibility testing:

Kirby-Bauer disk diffusion method used according to the CLSI guideline.

Sample Size Estimation:

Sample size estimation based on this formula:

$$n = z^2 p (1-p) / d^2$$

CALCULATION:

Where, sample size = n

P= 38%

Confidence level = 95%

Margin of error = 9%

Sample size = **112**

Sampling Method: convenient, non-probability purposive sampling

Methods For Biofilm Detection:

Congo Red Agar (Cra) Method:

The components of Congo Red Agar medium are 37 g/L BHI broth, 10 g/L agar base, 50 g/L sucrose, 1 L of water, and 0.8 g/L Congo Red indicator. Congo Red indicator was made as a concentrated liquid separate from other media components, autoclaved for 15 minutes at

121°C, and then put to cooled agar at 55°C. After inoculation, the isolate was cultured at 37 °C for a whole day. Colonies with a black colour demonstrated a positive outcome. Pink colonies were a sign of a poor outcome.²⁷

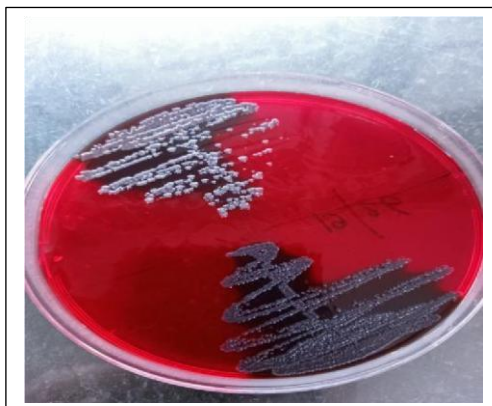
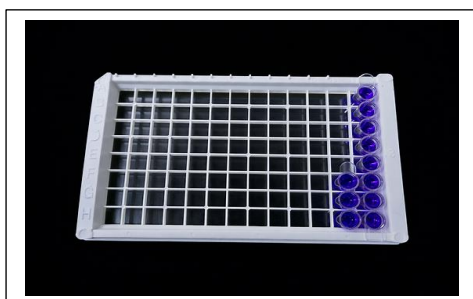


Figure 01 -Biofilm-Positive CRA, Black Colonies Showing Biofilm Formation

Microtitre Plate Method (Mtp):

Bacterial isolates from fresh agar plates were inoculated into Trypticase Soy Broth (TSB) and incubated at 37 °C for 24 h, then diluted 1:100 in fresh TSB. Aliquots (0.2 ml) were dispensed into sterile, flat-bottom 96-well polystyrene plates, with broth-only wells as negative controls, and incubated at 37 °C for 18–24 h.

Wells were emptied, washed four times with PBS (pH 7.2), and stained with 0.1% crystal violet. After rinsing and air-drying, biofilm was quantified by measuring OD at 570 nm using a micro-ELISA reader. Each assay was performed in triplicate, with mean values calculated after subtracting background OD; standard deviations were determined²⁸.



Tube

Figure 02 - Mtp Method Staining



Figure 03 - Stain Dissolving (95%)Ethanol

METHOD

A loopful of 24-h bacterial colonies was inoculated into 10 ml Tryptic Soy Broth (TSB) with 1% glucose and incubated at 37 °C. After incubation, broth was decanted and tubes rinsed with PBS (pH 7.3) to remove non-adherent cells, then air-dried and heat-fixed. Biofilm was visualised by staining with 0.1%

crystal violet, followed by washing with deionised water and drying in an inverted position. Biofilm formation was considered positive when a visible violet film appeared on the tube walls and bottom. To reduce subjectivity, three independent observers evaluated the tubes, and the assay was repeated thrice for reproducibility²⁸.

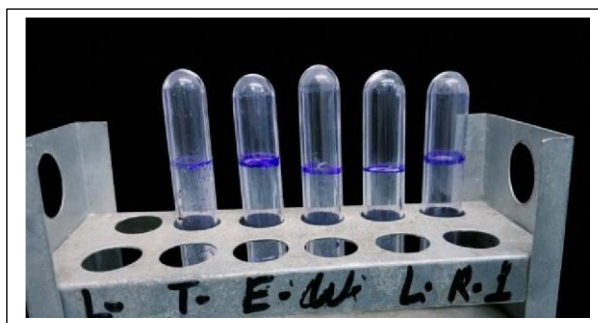


Figure 04 - Biofilm Positive Tube Method

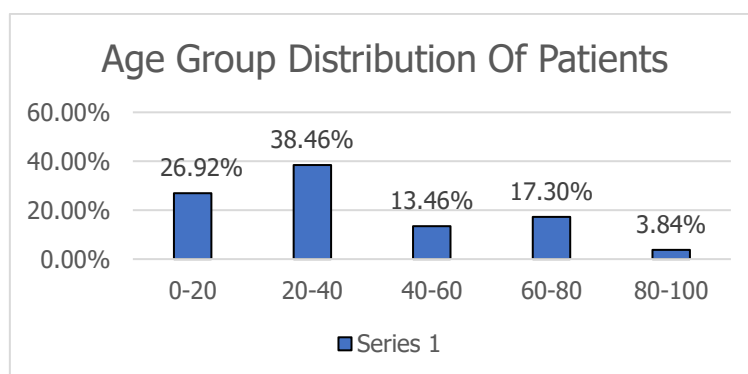
RESULT & OBSERVATION

Out of 112 samples collected from patients, clinical suspects of having UTI, 52 tested

positive for urinary pathogens. Insignificant growth was observed in 37 samples, and 23 samples tested positive for Candida species.

TOTAL	ISOLATES TESTED		
112	UROPATHOGENS	CANDIDA SPECIES	INSIGNIFICANT GROWTH
	52 (46.43%)	23 (20.53%)	37 (33.03%)

Table 01: Distribution Of Suspected UTI Isolates and Other Excluded Isolates.

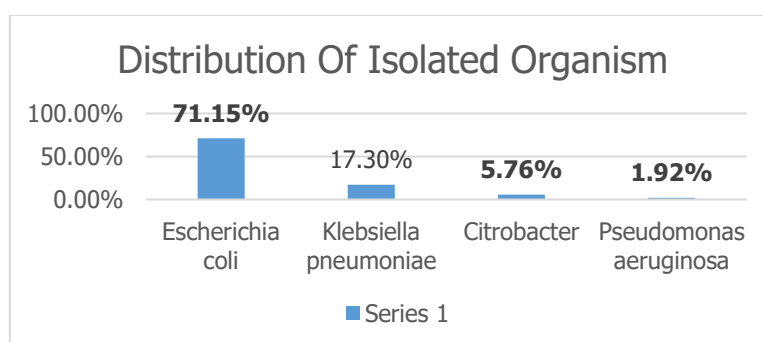


Graph 01

Interpretation (Graph 01):

Out of 52 positive tested urine isolates, the patients in the age group of 0-20 were 14, the patients in the 20- 40 age group were 21, the

patients in the 40-60 age group were 07, the patients in the 60-80 age group were 09, and the patients in the 80- 100 age group were 02.

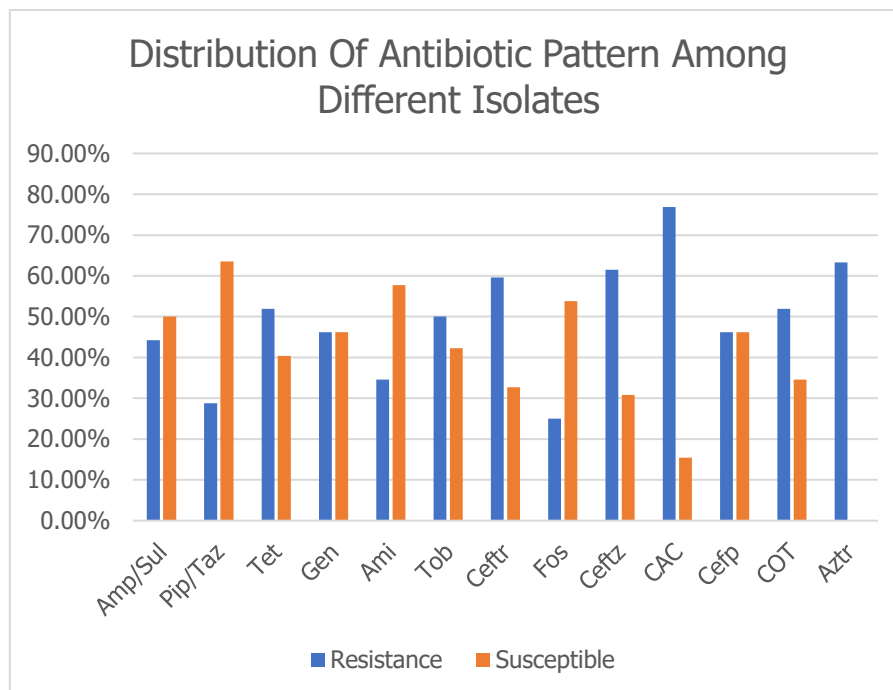


Graph 02

Interpretation (Graph 02):

Out of 52 positive urine isolates, 37 E. coli, 09 K. pneumoniae, 03 Citrobacter spp, 01 P. aeruginosa & 02 E.faecium. As the most predominant pathogen for UTI is E. coli,

due to which the maximum number of isolates are E. coli, and other pathogens are less in number.

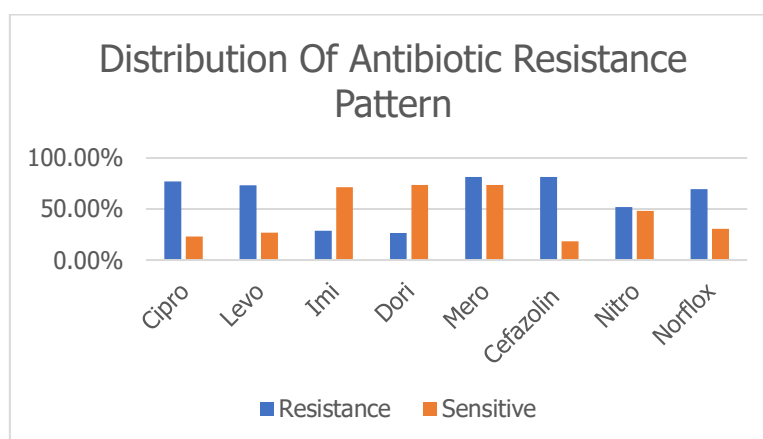


Graph 03

Interpretation (Graph 03):

The graph shows 13 antibiotics of urine culture isolates of 52 samples, in which maximum

resistance was shown by Ceftazidime clavulanic acid(76.90%), while maximum sensitivity was shown by Piperacillin tazobactam (64%)

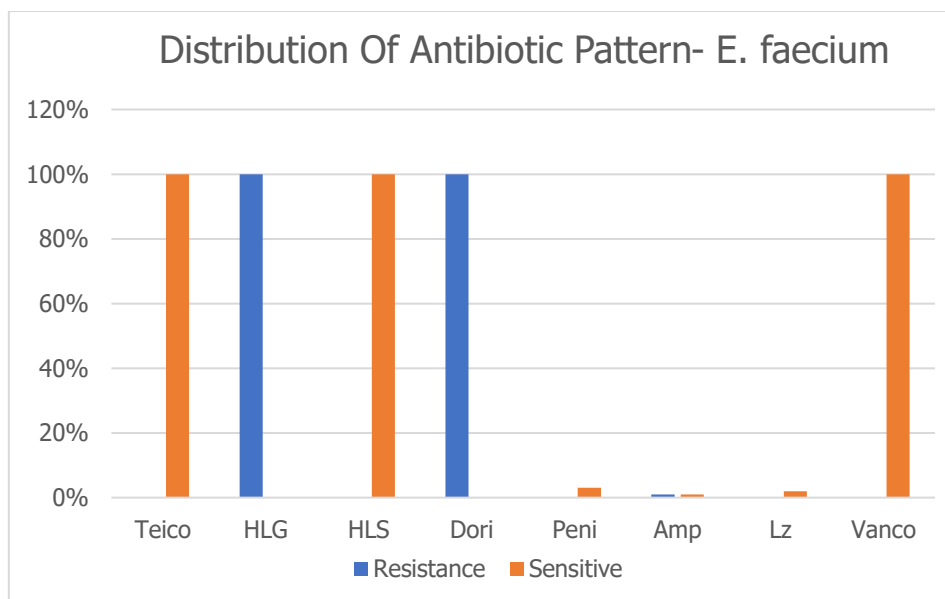


Graph 04

Interpretation (Graph04):

The above graph shows 08 antibiotics of urine culture of 52 isolates, in which Cefazolin

showing maximum number of resistance, while maximum sensitivity is shown by the carbapenem drug.

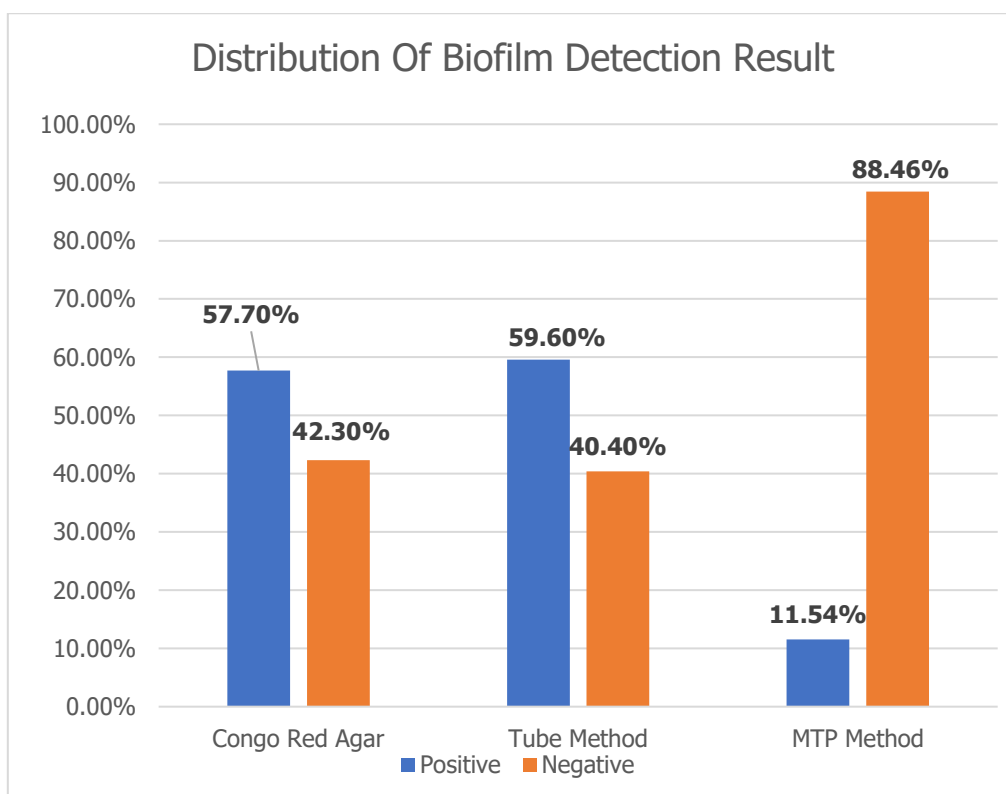


Graph 05

Interpretation (Graph 05):

The above graph shows the antibiotic panel of 02 isolates of *Enterococcus faecium* in a urine sample in a suspected UTI patient, in this 100%

resistance shown by doripenem & HLG, while Teicoplanin, HLS & Vancomycin showing 100% sensitivity



Graph 06

Interpretation (Graph 06):

Among the three methods used for biofilm detection, the Congo red agar method showed 57.70% positive biofilm of urine isolates and 42.30% negative isolates. Similarly, the Tube

method showed 59.60% of positive biofilm of urine isolates and 40.40% of negative isolates. In contrast to it, the third method, which is considered gold standard and has higher specificity & sensitivity, showed only 11.54% of

positive biofilm isolates and 88.46% of negative isolates from the urine sample

Table02: Comparison Of Biofilm Result with Antibiotic Susceptibility Pattern

Antibiotics	Biofilm Positive Resistance No.	Biofilm Negative Resistance No.
Ampicillin	12 (50.0%)	14 (50.0%)
Aminoglycoside	13 (54.2%)	12 (44.4%)
Cephalosporin	14 (58.3%)	22 (81.5%)
Fluroquinolone	19 (79.2%)	22 (81.5%)
Monobactam	12 (50.0%)	18 (66.7%)
Carbapenem	6 (25.0%)	7 (25.9%)
Urinary drugs	8 (33.3%)	17 (63.0%)
Tetracycline	13 (54.2%)	12 (44.4%)
Polymyxin -B	0 (0%)	0 (0%)
Glycopeptide	0 (0%)	0 (0%)

Interpretation (Table02):

This table illustrates the protective function of biofilm by showing that biofilm-positive isolates typically have slightly better resistance to aminoglycosides and tetracyclines. On the other hand, biofilm-negative isolates exhibit higher resistance to monobactams,

cephalosporins, and urinary medications, indicating that enzyme-mediated resistance predominates there. While carbapenem resistance is still comparatively low, fluoroquinolone resistance is consistently common in both groups

Table 03: Antibiotic Resistance Among Esbl & Mbl Isolates and Biofilm Status

Antibiotics	ESBL Only (Biofilm + / -)	MBL Only (Biofilm + / -)	ESBL & MBL (Biofilm + / -)
Ampicillin	6 / 5	3 / 2	2 / 2
Aminoglycoside	7 / 6	3 / 2	2 / 2
Cephalosporin	9 / 8	3 / 2	2 / 2
Fluoroquinolone	9 / 8	2 / 2	2 / 2
Monobactam	7 / 7	2 / 2	2 / 2
Carbapenem	5 / 5	3 / 2	2 / 2
Urinary drugs	4 / 5	2 / 2	2 / 2
Tetracycline	5 / 4	2 / 2	2 / 2
Polymyxin-B	0 / 0	0 / 0	0 / 0
Glycopeptide	0 / 0	0 / 0	0 / 0

Interpretation(Table03):

ESBL-only isolates showed high resistance across most antibiotic groups, with little difference between biofilm-positive and negative strains. MBL-only isolates were also broadly resistant, largely unaffected by biofilm status. The most critical multidrug-resistant

group was ESBL+MBL co-producers, which were consistently resistant to nearly all tested drugs. Glycopeptides and polymyxin B remained effective, as no resistance was observed. Overall, biofilm added minimal variation compared to the strong resistance

conferred by ESBL and MBL, with ESBL+MBL strains posing the greatest concern.

DISCUSSION

The Congo Red and Tube techniques revealed positive in roughly 60% of isolates, whereas the Microtiter

Plate methodology was stronger (~8%), demonstrating that biofilm production is widespread among Plate methodology was stronger 11%, demonstrating that biofilm production is widespread among uropathogens. This emphasises the diversity of detection methods and the value of employing several diversity of detection methods and the value of employing several Approaches.²⁹

Biofilm Detection

Congo Red Agar, Tube Method, and Microtiter Plate (MTP) test were the three methods used in this study to measure biofilm formation. About 58–60% of isolates were found to be biofilm positive using the Congo Red and Tube techniques, whereas only about 11% were found using the more stringent MTP method. This variation draws attention to the variations in detection systems' sensitivity and specificity. Although Congo Red and Tube techniques are quick and simple to use, their subjective interpretation may lead to an overestimation of biofilm growth. The MTP test, on the other hand, offers a quantitative evaluation and is thought to be more trustworthy, even though it might underestimate weak biofilm producers. These results highlight the significance of using a variety of techniques to get a fair and precise assessment of the incidence of biofilms in uropathogens.³⁰

Correlation Of Biofilm and Antibiotic Resistance:

Biofilm production provided an extra degree of defence, even though ESBL and MBL enzymes were the main causes of resistance. The resistance rates of biofilm-positive isolates were somewhat greater than those of biofilm-negative isolates, but the difference was not significant. This implies that biofilm improves persistence, recurrence, and survival in harsh environments, whereas enzymatic resistance mechanisms predominate. A dual mechanism of resistance is created when biofilm formation and ESBL/MBL production coexist, making infections more challenging to treat and increasing chronicity. Recurrent UTIs in clinical practice can be explained by biofilms, which serve as reservoirs for resistant bacteria that

can withstand antibiotic exposure and reappear after therapy³¹. Statistical analysis showed that this was not always the case, despite the alternative expectation that biofilm-positive isolates would show the highest resistance. Tetracycline and aminoglycoside resistance were somewhat higher in biofilm-positive isolates, but the differences were not statistically significant ($p > 0.05$). In contrast, biofilm-negative isolates showed much increased resistance to cephalosporins and urinary medications ($p < 0.05$). These results imply that although biofilm development has a role in infection persistence and recurrence, enzyme-mediated mechanisms, including the creation of ESBL and MBL, continue to be the main causes of resistance.

CONCLUSION

The most common uropathogens found were *Escherichia coli* and *Klebsiella pneumoniae*, indicating their significant involvement in UTIs. The Congo Red Agar and Tube Method identified almost 60% of isolates as biofilm producers, whereas the Microtiter Plate assay only found 11%, highlighting the necessity for various approaches to biofilm identification. Biofilm-positive bacteria are quite prevalent, which emphasises their role in treatment failure and recurrence. Antimicrobial susceptibility tests demonstrated widespread resistance in both MBL and ESBL producers, with co-producers of MBL and ESBL exhibiting resistance to almost all antibiotic classes. Glycopeptides and Polymyxin-B retained activity, suggesting their role as last-line options. Biofilm generation and ESBL/MBL production coexist, which presents a significant therapeutic challenge and raises the risk of complications and recurrence. These results highlight the significance of regular biofilm detection, stringent antimicrobial stewardship, and investigation of alternate tactics, including anti-biofilm agents and new antimicrobials. When taken as a whole, biofilm-associated multidrug resistance poses a serious clinical risk that needs immediate consideration in both hospital and community settings.

Declaration

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Conflict Of Interest Disclosure

I declare there is no known personal or financial conflicts of interest that could have influenced the work mentioned and reported in this manuscript.

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Authors Contribution

All authors contributed to the conception and design of the manuscript, Literature review, drafting and revision of the article, and approved the final version of the manuscript for publication.

Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval Statement

The study was approved by the Institutional Ethics Committee(IEC), Integral Institute of Medical Sciences and Research, Lucknow, before its commencement. The study was conducted in accordance with the ethical standards of the IEC.

IEC Ethical Approval Number-
IEC/IIMSR/2025/147

Informed Consent Statement

This study was conducted using clinical samples and patient information obtained from laboratory records after Institutional Ethics Committee approval. No direct interaction with the patient was involved.

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