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EVALUATION OF CELL SURFACE HYDROPHOBICITY AND BIOFILM PRODUCTION IN CATHETER-ASSOCIATED URINARY TRACT INFECTION (CAUTI) PATHOGENS AND THEIR ASSOCIATION WITH DRUG-RESISTANCE

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ABSTRACT:
Background: Catheter-associated urinary tract infections (CAUTIs) constitute for nearly 40% of hospital-acquired infections globally. Urinary catheterization facilitates microbial adhesion and biofilm formation, a key survival mechanism that enhances microbial tolerance to antimicrobial agents and environmental stresses. The biofilm production not only shields microorganisms from antimicrobial agents but also facilitates the transfer of antimicrobial resistance. Cell Surface Hydrophobicity (CSH) is an important virulence factor, also enhances microbial adherence, promoting colonization and persistent infection.
Aims & Objectives: The study was conducted to detect organisms responsible for CAUTI, determine their antimicrobial susceptibility patterns, detect the ability to produce Biofilm and Cell Surface Hydrophobicity (CSH) and assess the correlation with drug resistance.
Materials and Methods: A cross-sectional laboratory-based study was conducted over 5 months on 50 samples. Isolates were identified based on colony morphology, Gram staining characteristics, and standard biochemical tests, and tested for antimicrobial resistance. Biofilm production was assessed using two methods: Tissue Culture Plate method & Congo Red Agar method. The CSH was evaluated using an inverted microscope and the association between the two and drug resistance was assessed.
Results: Among 50 isolates from CAUTI, Klebsiella pneumoniae (32%) was the most frequently isolated pathogen, followed by Escherichia coli (26%), Proteus mirabilis (20%), Pseudomonas aeruginosa (18%), and Candida albicans (4%). Biofilm production was seen in 42 (84%) isolates, while 26 (52%) exhibited CSH. Increased antimicrobial resistance was observed and multidrug resistance (MDR) was seen in 43 (86%) isolates. Fisher's exact test s howed statistically significant association between the virulence factors and antimicrobial resistance as biofilm and CSH producing organisms had significantly higher resistance to Ampicillin (100% vs. 62.5%; p=0.003) and Amoxiclav (78.5% vs. 37.5%; p=0.030).
Conclusion: Biofilm formation and CSH are highly prevalent among CAUTI uropathogens and are significantly associated with increased antimicrobial resistance and multidrug resistance. These factors contribute to bacterial adhesion, persistence, and treatment failure, emphasizing the importance for routine detection, proper catheter care practices and judicious antibiotic use.

KEYWORDS: Catheter-associated Urinary Tract Infection (CAUTI), Cell Surface Hydrophobicity, Biofilm Production, Uropathogens, Virulence factors

INTRODUCTION

Urinary tract infections (UTIs) represent a substantial portion (30-40%) of all healthcare-associated infections, constituting a significant burden on individual patients and public health systems1. Among these, catheter-associated urinary tract infections (CAUTIs) emerge as the most prevalent nosocomial infections, occurring subsequent to urinary catheter insertion2. The impact of CAUTIs on patient morbidity and mortality is significant, underscoring the urgent need for effective management strategies3.

A wide range of uropathogens such as E. coli, Proteus, Enterococcus, Pseudomonas, Enterobacter, Serratia, and Candida spp are responsible for colonization of indwelling urinary catheters 3,4,5.

Biofilms production represents complex communities of microorganisms encased within an extracellular polymeric matrix, adhering to either biotic or abiotic surfaces5. This matrix not only shields microorganisms from antimicrobial agents but also facilitates the transfer of antimicrobial resistance genes, thereby conferring greater resistance to commonly used antibiotics among biofilm producing bacteria. The restricted diffusion of antimicrobials within biofilm matrices and the close proximity of microorganisms promote the exchange of plasmids and other genetic elements, contributing to the emergence of multidrug resistant (MDR) strains6,7. Bacterial adhesins can be characterized according to their hydrophobicity, with pathogenic strains of E. coli generally exhibiting greater surface hydrophobicity

than non-pathogenic strains. Microbial surface hydrophobicity is an important factor influencing bacterial adhesion to a wide range of biotic and abiotic surfaces7,11.
MDR pathogens significantly impact healthcare delivery by limiting therapeutic options, ultimately resulting in poor patient outcomes, including high case fatality rates8.
Hence the study was performed to evaluate Cell Surface Hydrophobicity and Biofilm Production in Catheter-associated Urinary Tract Infection (CAUTI) Pathogens and their association with drug-resistance.

MATERIALS AND METHODS

This prospective Hospital-based, cross-sectional observational study was conducted in the Department of Microbiology in collaboration with the Departments of Medicine, Surgery, and Intensive Care Units of Navodaya Medical College Hospital Research Centre, Raichur, Karnataka
Patients with indwelling urinary catheters admitted to various wards and intensive care units who developed catheter-associated urinary tract infections were selected. The samples were collected after getting informed consent. A total of 50 significant CAUTI isolates were included in the study. Urine specimens were collected aseptically from the sampling port of indwelling urinary catheters using sterile syringes and transported immediately to the microbiology laboratory for Isolation, identification and antibiotic susceptibility pattern.

Isolation and Identification of Organisms

Urine samples were cultured using the semi-quantitative standard loop technique on Blood agar and MacConkey agar. Plates were incubated aerobically at 37°C for 18–24 hours. Organisms were identified based on colony morphology, Gram staining characteristics, and standard biochemical tests9.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to CLSI guidelines. Antibiotics tested included Ampicillin, Amoxiclav, Ciprofloxacin, Cefuroxime, Cotrimoxazole, Amikacin, Levofloxacin, Nitrofurantoin, Piperacillin-Tazobactam, and Meropenem10.

Biofilm Production Assay

Standardization of procedures for screening biofilm production was carried out by two methods:

1. Tissue Culture Plate (TCP) Method
2. Congo Red Agar (CRA) Method

Tissue Culture Plate (Tcp) Method

Isolates were inoculated in Tryptic Soy Broth (TSB) and incubated for 18 hours at 37oC and diluted in 1:100 with fresh medium. Individual wells of sterile polystyrene in a 96 wells plate were filled with 200 µl aliquots of diluted culture and incubated at 37oC for 18 hours. After incubation the wells were washed four times with PBS (pH 7.2) to remove free floating 'planktonic' bacteria. Biofilms formed by adherent sessile organisms in plate were fixed with 200 µl 99% methanol in each well and stained with 0.1% (w/v) crystal violet for five minutes. Excess stain was rinsed off by placing the plate under running tap water. The plates were air dried and dye attached to well was dissolved in 200 µl of 33% glacial acetic acid. Optical Density (OD) of each well was determined by using ELISA Reader (Biotek ELX 800-MS) at 490 nm (OD490 nm). These OD values were considered as an index of bacteria adhering to surface and forming biofilms.
The experiment was performed in triplicate and the data was then averaged. To compensate for background absorbance, OD readings from sterile medium, fixative and dye were averaged and subtracted from all test values. The mean OD value obtained from media control well was deducted from all the test OD values.

Classification of bacterial adherence by TCP method:

Mean OD value	Adherence	Biofilm formation
<0.120	None	None/Weak
0.120-0.240	Moderate	Moderate
>0.240	Strong	High

Congo Red Agar (Cra) Method

This method of screening biofilm formation requires the use of specially prepared solid medium. The medium was composed of Brain Heart Infusion Broth with 5% sucrose and Congo red as indicator.
Composition:
i. Brain Heart Infusion Broth - 37 gms/lt
ii. Sucrose - 50 gms/lt
iii. Agar - 15 gms/lt
iv. Congo red indicator - 0.8 gms/lt
Congo red satin was prepared as a concentrated aqueous solution and autoclaved (121oC for 15 minutes) separately from the other medium constituents, and was added when the agar had cooled to 55oC. Plates of the medium were inoculated and incubated aerobically at 37oC for 24 hours.
Positive result was indicated by black colonies with dry crystalline consistency. A darkening of colonies with the absence of a dry crystalline colonial morphology indicated an intermediate result and weak/none slime producers usually remained pink with occasional darkening at the center of the colony11.

Detection Of Cell Surface Hydrophobicity (Csh)

Bacteria were tested for their hydrophobic property by using different molar concentrations of ammonium sulphate. Those which aggregated with salt particles and formed clumps were considered hydrophobic. A strain of E.coli which was hemolytic, MRHA positive and consistently positive for cell surface hydrophobicity was used as positive control. The strain negative for MRHA, non-lytic, and consistently negative for cell surface hydrophobicity was used as negative control.

The E.coli strains grown on MA plates were inoculated into 1 ml of PBS pH 6.8 and turbidity were matched with Mc Farland tubes 6 and 7 to get a colony count of 5x109 CFU/ml. Different molar concentrations of ammonium sulphate namely 0.4M, 0.1M, 1.0M, 1.4M and 2.0M concentrations were prepared as under. Molecular weight of ammonium sulphate-132.0 g. 1.0 molar-1.32 gms, 1.4 molar-1.85 gms, 2.0 molar-2.64 gms, 0.4 molar-0.538 gms, 0.1 molar-0.132 gms of ammonium sulphate was made up to 10ml with distilled water.

40 µl of 0.2 M PBS pH6.8 was taken in first column of VDRL slide and 40 µl of 1M, 1.4 M, and 2M concentration of ammonium sulphate were taken in each well of other columns of VDRL slide. 40 µl of E.coli suspension was added to each of these wells. The clumps formed in different molar concentrations of ammonium sulphate was observed under inverted microscope- binocular (0.32) at 20x magnification and scored positive on 1 to 4 scales (1+ to 4+). Strains were considered hydrophobic if they aggregated in concentrations of ≤1.4 M11.

Figure 1: Detection of Biofilm formation

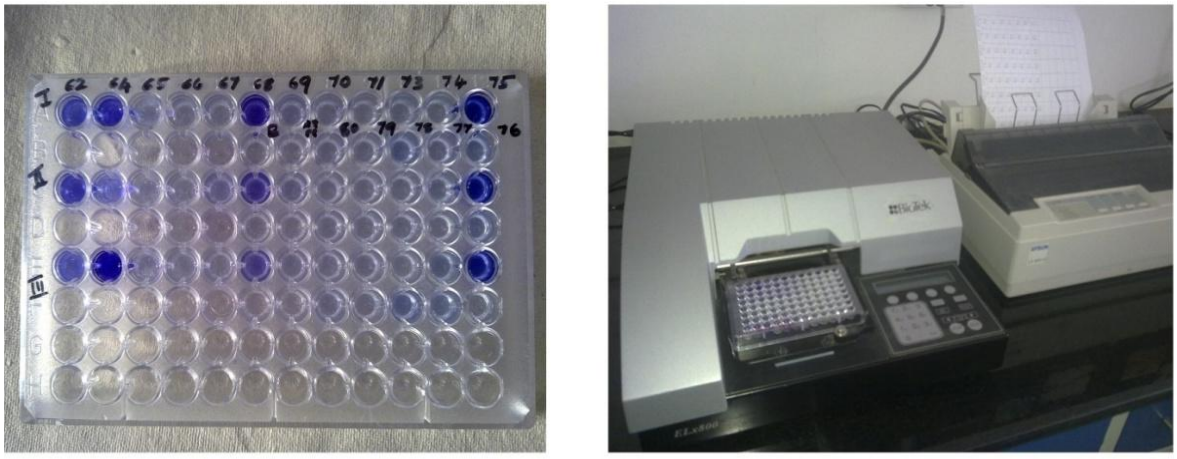


Plate-1: Biofilm production by Tissue Culture Plate (TCP) method

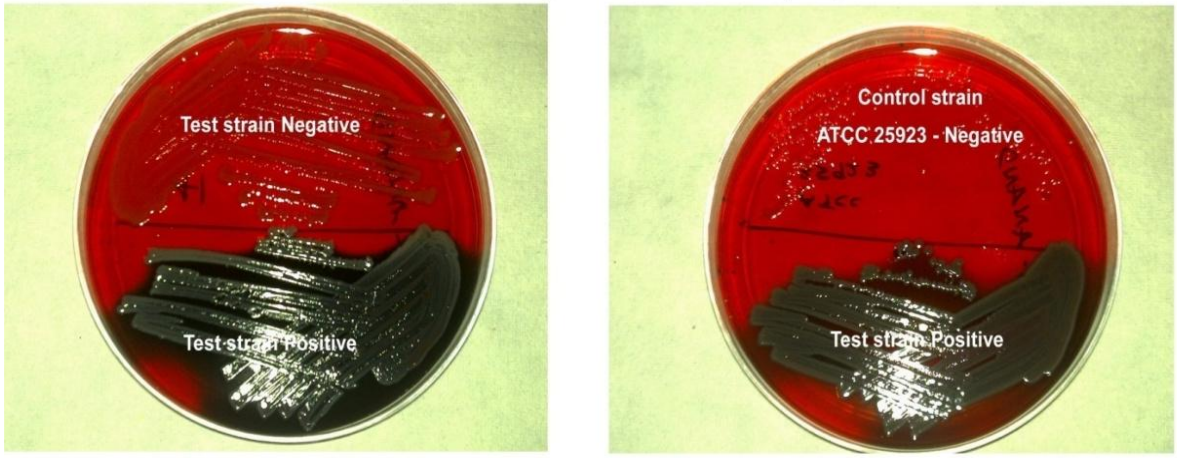
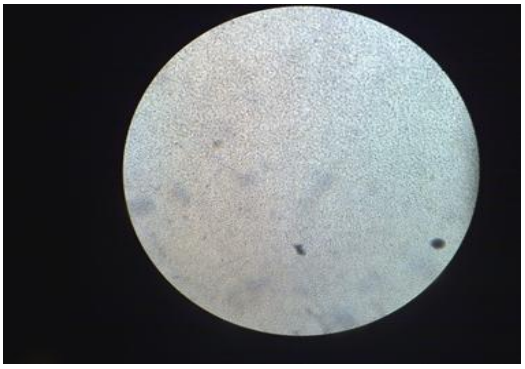


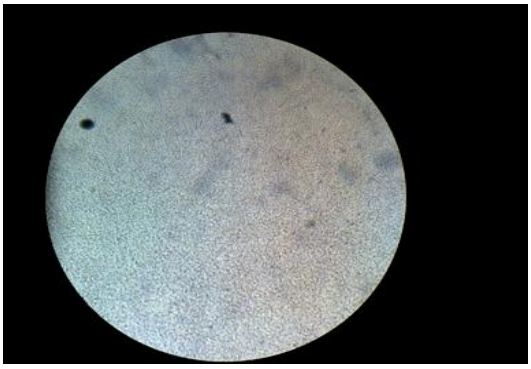
Plate-2: Biofilm production by Congo red Agar (CRA) method

Figure 2: Detection of Cell surface hydrophobicity

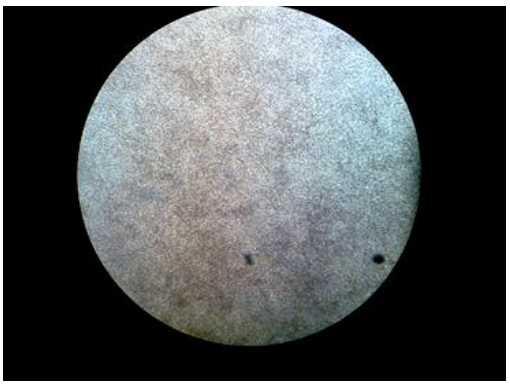
Observation of clumps under inverted microscope



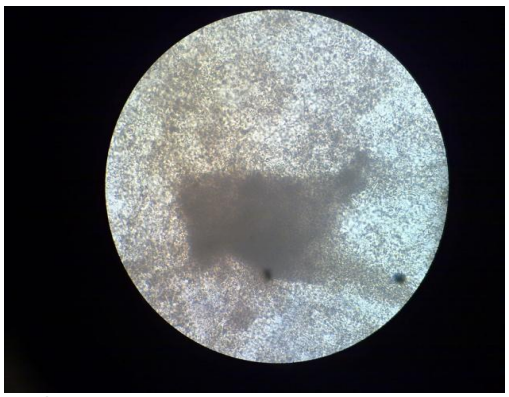
Salt aggregation at 0.4M$(NH_4)_2SO_4$ concentration



Salt aggregation at 1.0M$(NH_4)_2SO_4$ concentration



Salt aggregation at 1.4M $(NH_4)_2SO_4$ concentration



Salt aggregation at 2.0M $(NH_4)_2SO_4$concentration

Statistical Analysis

Data were entered and analyzed using SPSS software version [Specify]. Categorical variables were expressed as frequencies and percentages. Chi-square test or Fisher's exact test was used to assess associations between biofilm production, cell surface hydrophobicity, and antimicrobial resistance. A p-value <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee. Informed consent was obtained from all participants before enrollment.

RESULTS:

Table 1: Organisms isolated from Catheter-associated urinary tract infections (CAUTIs)

Sl. No	Organisms isolated in significant number	No. of Organisms	%
1	Klebsiella pneumoniae	16	32
2	Escherichia coli	13	26
3	Proteus mirabilis	10	20
4	Pseudomonas aeruginosa	09	18
5	Candida albicans	02	04

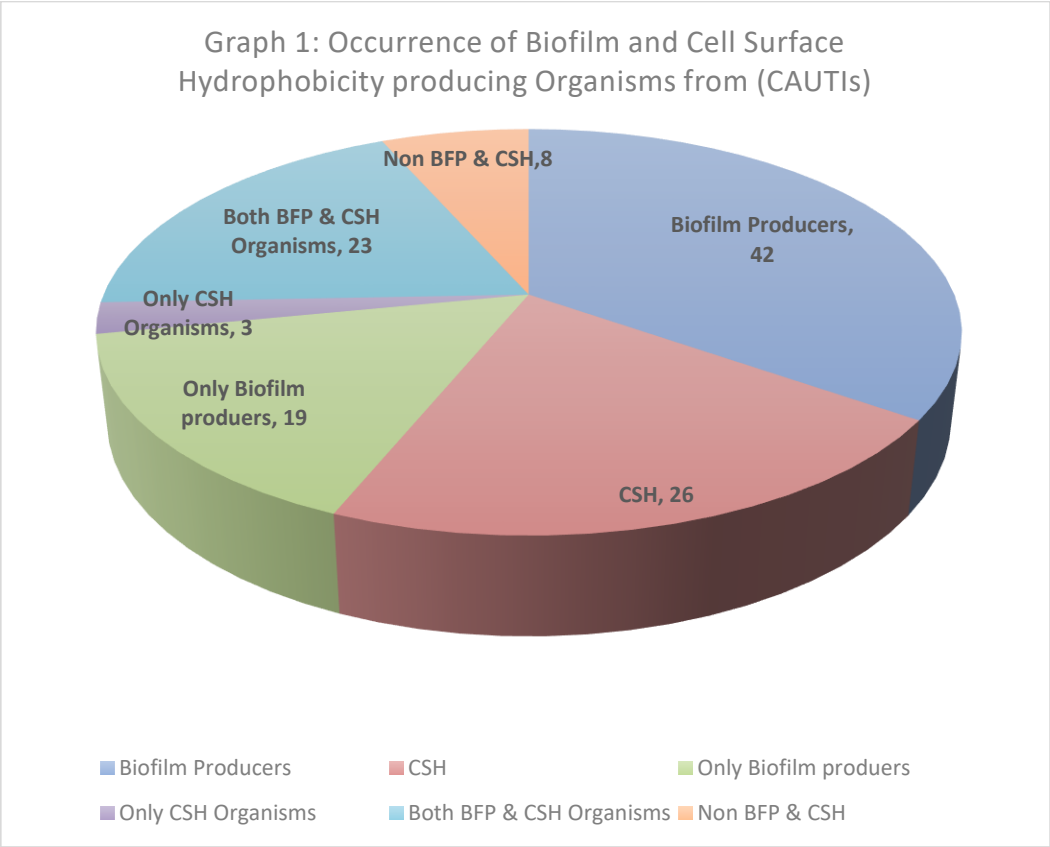


Table 3: Antibiotic resistance pattern among biofilm & CSH producers and non-biofilm & CSH producers uropathogens of CAUTI

Drugs	Resistance among all isolates (n=50)	Biofilm & CSH Producers (42)	Non- Biofilm & CSH Producers (8)	p-value	S/NS
Ampicillin	47 (94%)	42 (100%)	5 (62.5%)	0.003	S
Amoxiclav	36 (72%)	33 (78.5%)	3 (37.5%)	0.030	S
Ciprofloxacin	32 (64%)	28 (66.7%)	4 (50%)	0.436	NS
Cefuroxime	6 (12%)	5 (11.9%)	1 (12.5%)	1.000	NS
Meropenem	0	0	0	NA	NS
Cotrimoxazole	28 (56%)	24 (57.1)	4 (50%)	0.429	NS
Amikacin	21 (42)	18 (42.8%)	3 (37.5%)	1.000	NS
Levofloxacin	25 (50%)	22 (52.3%)	3 (37.5%)	0.702	NS
Nitrofurantoin	16 (32%)	14 (33.3%)	2 (25%)	0.416	NS
Piperacillin+Tazobactam	26 (52%)	23 (54.8%)	3 (37.5%)	0.126	NS

Fisher's exact test showed a statistically significant association between biofilm/CSH production and resistance to Ampicillin ($p = 0.003$) and Amoxiclav ($p = 0.030$). Resistance to Ciprofloxacin, Cefuroxime, Cotrimoxazole, Amikacin,

Levofloxacin, Nitrofurantoin, and Piperacillin–Tazobactam did not differ significantly between biofilm/CSH producers and non-producers ($p > 0.05$). Meropenem resistance was not observed in either group
Note: Because the non-biofilm group has only 8 isolates, Fisher's exact test is preferred over the Chi-square test for publication-quality analysis.

Table 4: Multidrug resistance among biofilm-producing and CSH-producing uropathogens of CAUTI.

Variables	Multidrug Resistance		Total	Chi - Square	df	p-value
	Yes (n= 43)	No (n=07)				
Biofilm & CSH Producers	38	04	42	4.368	1	0.0366
Non- Biofilm & CSH Producers	05	03	08			

The association between Biofilm & CSH production and multidrug resistance is statistically significant at the 5% level ($p < 0.05$).
There was a significant association between Biofilm & CSH production and multidrug resistance among CAUTI uropathogens ($\chi^2 = 4.37$, df = 1, $p = 0.037$).
Among the 50 microbial isolates recovered from patients with catheter-associated urinary tract infections (CAUTIs), Klebsiella pneumoniae was the most predominant pathogen, accounting for 16 (32%) isolates. This was followed by Escherichia coli, which constituted 13 (26%) isolates. Proteus mirabilis and Pseudomonas aeruginosa were isolated in 10 (20%) and 9 (18%) cases, respectively. Candida albicans was the least frequently isolated organism, representing only 2 (4%) of the total isolates. These findings indicate that Gram-negative bacilli were the major etiological agents of CAUTIs in the study population as shown in Table-1.
Out of the 50 CAUTI isolates studied, 42 (84%) were identified as biofilm producers, while 26 (52%) exhibited cell surface hydrophobicity (CSH). Among the isolates, 23 (46%) demonstrated both biofilm-forming ability and CSH, indicating a substantial overlap between these virulence characteristics. Nineteen (38%) isolates were exclusively biofilm producers, whereas only 3 (6%) isolates exhibited CSH alone. Eight (16%) isolates were negative for both biofilm production and CSH. These findings suggest that biofilm formation is a common virulence trait among CAUTI pathogens and is frequently associated with cell surface hydrophobicity.
The pie chart visually demonstrates the distribution of biofilm-producing and CSH-positive organisms among CAUTI isolates. Biofilm producers constituted the largest proportion (42 isolates), followed by CSH-positive organisms (26 isolates). A considerable number of isolates (23) possessed both biofilm-forming ability and CSH, highlighting the close association between these virulence factors. In contrast, only a small proportion of isolates exhibited CSH alone (3 isolates), while 8 isolates lacked both characteristics. The graphical representation emphasizes the predominance of biofilm formation among CAUTI pathogens and its frequent coexistence with cell surface hydrophobicity as mentioned in Graph-1.
Analysis of antimicrobial resistance revealed high levels of resistance among CAUTI isolates, particularly among biofilm and CSH-producing organisms. Resistance to Ampicillin was observed in 47 (94%) isolates overall and was significantly higher among biofilm & CSH producers [42/42 (100%)] compared with non-producers [5/8 (62.5%)] ($p = 0.003$). Similarly, resistance to Amoxiclav was significantly greater among biofilm & CSH producers [33/42 (78.5%)] than among non-producers [3/8 (37.5%)] ($p = 0.030$). No statistically significant differences were observed for Ciprofloxacin, Cefuroxime, Cotrimoxazole, Amikacin, Levofloxacin, Nitrofurantoin, or Piperacillin–Tazobactam ($p > 0.05$). Notably, no resistance to Meropenem was detected in either group. These findings suggest that biofilm formation and CSH are significantly associated with increased resistance to Ampicillin and Amoxiclav among CAUTI pathogens as shown in Table-3.
Among the 50 CAUTI isolates, multidrug resistance (MDR) was observed in 43 (86%) isolates. Of the 42 biofilm and CSH-producing organisms, 38 (90.5%) exhibited MDR, whereas only 5 (62.5%) of the 8 non-biofilm and non-CSH-producing isolates were multidrug resistant. Statistical analysis demonstrated a significant association between biofilm/CSH production and multidrug resistance ($\chi^2 = 4.368$, df = 1, $p = 0.0366$). These findings indicate that isolates possessing biofilm-forming ability and cell surface hydrophobicity are more likely to exhibit multidrug resistance, highlighting the clinical importance of these virulence factors in CAUTI pathogens as depicted in Table-4.

DISCUSSION

Catheter-associated urinary tract infection (CAUTI) remains one of the most common healthcare-associated infections worldwide, primarily due to prolonged catheterization and the ability of microorganisms to form biofilms on catheter surfaces. Biofilm formation enhances bacterial persistence, promotes antimicrobial resistance, and contributes to recurrent infections. In the present study, Klebsiella pneumoniae (32%) was the most frequently isolated pathogen, followed by Escherichia coli (26%), Proteus mirabilis (20%), Pseudomonas aeruginosa (18%), and Candida albicans (4%). These findings are consistent with previous reports that identified Klebsiella spp., E. coli, Proteus spp., and Pseudomonas spp. as the predominant uropathogens responsible for CAUTIs 12,15. Ramadan et al. reported that

Klebsiella pneumoniae exhibited the highest biofilm-forming potential among CAUTI isolates, highlighting its significant role in catheter-associated infections 12. Similarly, recent reviews have emphasized that E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa are among the principal pathogens implicated in biofilm-mediated CAUTIs 15.

Biofilm production was observed in 84% of isolates in the present study, indicating that biofilm formation is a major virulence factor among CAUTI pathogens. This prevalence is comparable to the 82.85% prevalence of biofilm-dependent CAUTIs reported by Ramadan et al. 12 and is higher than the 58.46% biofilm positivity reported by Paul et al. 13. Differences in biofilm prevalence across studies may be attributed to variations in study populations, catheter duration, bacterial species distribution, and methods employed for biofilm detection. The high prevalence observed in the present study supports the concept that catheter surfaces provide an ideal environment for bacterial attachment, microcolony formation, and maturation of biofilms, thereby facilitating persistent infection14.

Cell surface hydrophobicity (CSH) is recognized as an important determinant of bacterial adherence and colonization. In the current study, 52% of isolates exhibited CSH, and nearly half (46%) demonstrated both biofilm production and CSH. This finding suggests a strong relationship between hydrophobicity and biofilm formation. Nigudgi et al. demonstrated significantly higher rates of both CSH and biofilm production among virulent ESBL-producing uropathogenic E. coli, indicating that these factors contribute synergistically to bacterial pathogenicity and colonization 11. Hydrophobic bacterial surfaces facilitate stronger interactions with catheter materials and host tissues, thereby promoting biofilm establishment and persistence11,12.

Antimicrobial resistance among biofilm-producing organisms is a growing concern in clinical practice. In the present study, biofilm- and CSH-producing isolates demonstrated significantly higher resistance to Ampicillin (100% vs. 62.5%; p=0.003) and Amoxiclav (78.5% vs. 37.5%; p=0.030) compared with non-producers. Similar observations were reported by Paul et al., who found that biofilm-producing uropathogens exhibited markedly higher resistance to Ampicillin and Amoxiclav than non-biofilm producers12. Ramadan et al. also reported increased resistance among biofilm-forming CAUTI pathogens, emphasizing the protective role of the biofilm matrix against antimicrobial penetration12. The extracellular polymeric substance surrounding biofilm communities restricts antibiotic diffusion, alters bacterial metabolic activity, and facilitates horizontal transfer of resistance genes, thereby contributing to treatment failure and chronic infection 14.

Interestingly, resistance to Ciprofloxacin, Cefuroxime, Cotrimoxazole, Amikacin, Levofloxacin, Nitrofurantoin, and Piperacillin-Tazobactam did not differ significantly between biofilm producers and non-producers in our study. Similar variability has been reported in previous investigations. Gajdács et al. observed that the relationship between biofilm formation and antimicrobial resistance is complex and may vary depending on bacterial species, resistance mechanisms, and environmental factors. Therefore, while biofilm formation undoubtedly contributes to antimicrobial tolerance, its association with resistance to individual antibiotics may not always be uniform 14.

Multidrug resistance (MDR) was detected in 86% of isolates in the present study. Furthermore, MDR was significantly associated with biofilm and CSH production ($\chi^2 = 4.368$, $p = 0.0366$). Among biofilm- and CSH-producing isolates, 90.5% were MDR compared with 62.5% of non-producers. These findings closely parallel those reported by Paul et al., who observed MDR in 80.64% of biofilm producers compared with only 38.88% of non-producers 10. The association between biofilm formation and MDR may be explained by the enhanced ability of biofilm communities to exchange resistance genes through plasmids and other mobile genetic elements, in addition to the reduced susceptibility conferred by the biofilm matrix itself 13,15.

The present study highlights the critical role of biofilm formation and cell surface hydrophobicity in the pathogenesis of CAUTIs and their contribution to antimicrobial resistance. The significant association between biofilm production and multidrug resistance emphasizes the need for routine screening of these virulence factors in clinical microbiology laboratories. Early detection of biofilm-producing uropathogens may guide appropriate antimicrobial therapy and infection-control measures. Additionally, minimizing unnecessary catheterization, reducing catheter dwell time, and developing anti-biofilm catheter materials may help reduce the burden of CAUTIs caused by multidrug-resistant organisms15.

Overall, the findings of this study reinforce the growing body of evidence that biofilm formation and cell surface hydrophobicity are important virulence determinants that contribute significantly to antimicrobial resistance and multidrug resistance among CAUTI pathogens.

Conclusion

The present study demonstrated that biofilm formation and cell surface hydrophobicity are highly prevalent among uropathogens causing catheter-associated urinary tract infections (CAUTIs). Klebsiella pneumoniae was the predominant isolate, followed by Escherichia coli, Proteus mirabilis, and Pseudomonas aeruginosa. A substantial proportion of isolates exhibited biofilm-forming ability and cell surface hydrophobicity, indicating their important role as virulence determinants in the pathogenesis of CAUTIs.

Biofilm- and CSH-producing isolates showed significantly higher resistance to commonly used antibiotics, particularly Ampicillin and Amoxiclav, compared with non-producers. Furthermore, a significant association was observed between biofilm/CSH production and multidrug resistance, highlighting the contribution of these virulence factors to the emergence and persistence of antimicrobial-resistant infections.

The findings emphasize that biofilm formation and cell surface hydrophobicity enhance bacterial adherence, colonization, persistence, and resistance to antimicrobial therapy, thereby increasing the risk of treatment failure and recurrent infections. Routine detection of these virulence factors, along with antimicrobial susceptibility testing, may aid in the early identification of high-risk pathogens and facilitate appropriate therapeutic interventions.

Effective infection-control measures, strict catheter care practices, minimization of catheterization duration, and judicious use of antibiotics are essential to reduce the burden of CAUTIs caused by multidrug-resistant biofilm-forming organisms. Further studies involving larger sample sizes and molecular characterization of virulence and resistance determinants are recommended to better understand the mechanisms underlying biofilm-associated antimicrobial resistance.

Acknowledgement: We sincerely acknowledge the contribution of Dr. Veereshappa, Statistician and Laboratory technologists of the Dept. of Microbiology, Navodaya Medical College Hospital & Research Centre, Raichur.
Source Of Funding: The project was funded by Rajiv Gandhi University of Health Sciences,
Bangalore.
Conflict of Interest: The authors declare no conflict of interest.

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