ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 02 | February - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIBIOTIC RESISTANCE PATTERN OF BIOFILM FORMING UROPATHOGENS ISOLATED FROM CATHETERIZED PATIENTS IN TERTIARY CARE TEACHING HOSPITAL FROM MAHARASHTRA – A PILOT STUDY

Microbiology

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ABSTRACT

Background: CAUTI account for more than 40% of various hospital acquired infections. Many uropathogens have been reported to be resistant against commonly available antibiotics. In addition, if isolate is a biofilm producer, antibiotic effectiveness is reduced or fully prevented. Therefore, treating CAUTI in patients with biofilm producing MDR microorganisms has been a challenging task to clinicians. So, this study was done with the aim to know the prevalence of biofilm formation and antibiotic resistance pattern among gram negative bacilli in urinary isolates from catheterized patients. **Material and Methods:** This prospective observational study was done in Microbiology laboratory, RCSMGMC and CPR Hospital, Kolhapur, Maharashtra. Urinary samples from catheterized patients, submitted to laboratory, were processed as per standard guidelines. Gram negative bacillary isolates from those samples were included in the study. The isolates were tested for biofilm formation by Congo red agar method. Antimicrobial susceptibility tests were performed by Kirby-Bauer disc diffusion method as per CLSI guidelines. **Results:** A total of 50, Gram negative bacilli were tested for biofilm formation as well as antimicrobial susceptibility testing. It has been found that 70% of isolates produced biofilm. The most common biofilm producing isolate was Escherichia coli (36%) followed by Klebsiella pneumoniae(10%). On analysis of antimicrobial susceptibility pattern, the biofilm producing isolates showed 83%, 86%, 86%, 77%, and 63% resistance to co-amoxyclav, ceftazidime, ciprofloxacin, norfloxacin and cotrimoxazole respectively as compared to non-biofilm producers that showed 50%, 40%, 50%, 40% and 40% resistance to the respective antibiotics. Most of the biofilm producing strains showed multi-drug resistance phenotype. **Conclusion:** E. coli was the most frequent isolate, of which 36% were biofilm producers.The antibiotic susceptibility pattern in the present study showed fluoroquinolones were the least active drugs againsturopathogens. The uropathogens showed highest sensitivity to nitrofurans and aminoglycosides. Significant correlation between biofilm production and multidrug resistance was observed in our study.

KEYWORDS

INTRODUCTION

Catheter associated urinary tract infection (CAUTI) is one of the most common health care associated infections (HAI) as well as secondary bloodstream infections. These infections are most likely caused by bacteria entering the body during catheter insertion, as a result of prolonged or unnecessary use of urinary catheters [1]. Biofilm formation is a process where microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that leads to alteration in phenotype of organisms with respect to growth rate and gene transcription [2].

Biofilms have major medical significance as they decrease susceptibility to antimicrobial agents & decrease the rate of cell multiplication of organisms and leads to development of chronic infections[2]. Risk factors for CAUTI include age, female gender, diabetes, and prolonged catheterization time [3].The duration of catheterization is the most important factor in the development of bacteriuria, with a risk of 3–7% daily [4]. Uropathogenic Escherichia coli (UPEC) is the most common pathogen for CAUTI [5]. So this study was carried out to know the prevalence of biofilm formation and antibiotic resistance pattern among gram negative bacilli in urinary isolates from catheterized patients.

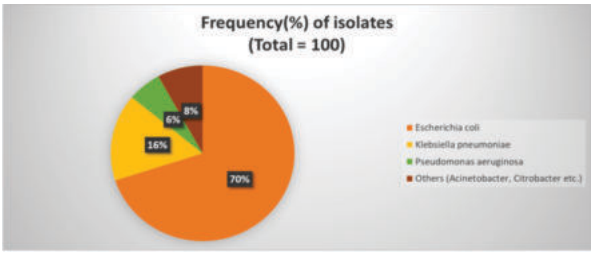
MATERIAL & METHODS

A prospective observational study was done in Microbiology laboratory, RCSMGMC, and CPR hospital, Kolhapur, Maharashtra. Urinary samples from catheterized patients, submitted to laboratory, were processed as per standard guidelines[6].

All samples were processed for aerobic bacterial culture by inoculating on blood agar, Mac-Conkey agar and Congo red agar by semi quantitative method. The growth on these media were observed and analyzed after incubating for 24 hours at 37◦C.The isolates grown on blood agar and/or Mac-Conkey agar were identified based on the colony morphology, Gram's staining and standard biochemical tests.Gram negative bacillary isolates from those samples were included in the study. Biofilm detection was done by using Congo red agar(CRA) method[7].The black colonies with dry metallic consistency were considered as positive test. Non-biofilm producers usually remained pink. Antimicrobial susceptibility tests were performed by Kirby-Bauer disc diffusion method on Muller-Hinton agar as per CLSI guidelines[6]

RESULTS & DISCUSSION

A total of 100 Gram negative bacilli were tested for biofilm formation as well as antimicrobial susceptibility testing.

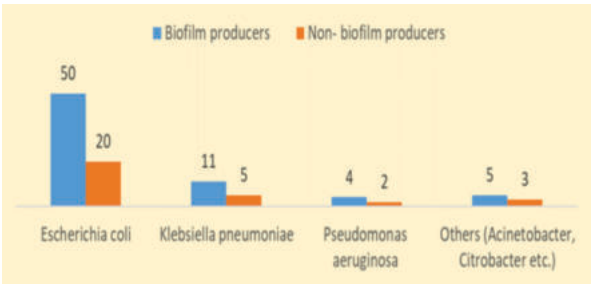


Percentage distribution of Gram negative bacilli from catheterized urine samples

From the above pie chart, it has been found that, out of 100 isolates, E. coli is the most frequent isolate which is around 70 (70%), followed by Klebsiella pneumoniaewhich is around 16 (16%). Pseudomonas aeruginosa and other gram negative bacilli (like Acinetobacter spp, Citrobacter spp. etc.) have lesser frequency which is around 6 (6%) and 8 (8%) respectively, from catheterized urine samples.

Screening of 100 urinary isolates for biofilm formation

Bacteria	Biofilm producers (%)	Non- biofilm producers(%)	Total
Escherichia coli	50 (71%)	20 (29%)	70
Klebsiella pneumoniae	11 (69%)	5 (31%)	16
Pseudomonas aeruginosa	4 (67%)	2 (33%)	6
Others (Acinetobacter, Citrobacter etc.)	5 (50%)	3 (50%)	8
Total	**70**	**30**	**100**



Graph of 100 urinary isolates as per biofilm formation

In this study 70% of strains were in vitro positive for biofilm production and 30% were negative for biofilm production.

As per each isolated bacteria, it has been found that, out of 70 isolates of E. coli, 50 (71%) were biofilm producers and 20 (29%) were non-biofilm producers. Similarly, for Klebsiella pneumoniae, out of 16 total isolates, 11 (69%) were biofilm producers and 5 (31%) were non-biofilm producers. Out of 6 Pseudomonas aeruginosa, 4 (67%) produced biofilm and 2 (33%) did not produce biofilm. Among other gram negative bacilli which include Acinetobacter spp, Citrobacter spp. etc. out of 8 isolates, 5 (50%) were biofilm producers and 3 (50%) were non-biofilm producers.

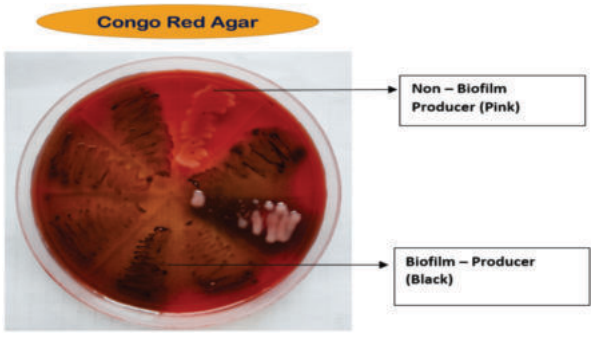
it was also observed that, 50% E. coli isolates were biofilm producers followed by Klebsiella pneumoniae (11%), Pseudomonas aeruginosa (4%) and other gram negative isolates (5%). So, E. coli is the most common biofilm forming pathogen in our study.

Antibiotic resistance pattern of biofilm and non-biofilm producers

Antibiotics	Resistance		
	Biofilm producers (70%)	Biofilm non-producers (30%)	Resistance of all isolates (n = 100)
Co-amoxyclav	58 (83%)	15 (50%)	73 (73%)
Ceftazidime	60 (86%)	12 (40%)	72 (72%)
Ciprofloxacin	60 (86%)	15 (50%)	75 (75%)
Norfloxacin	54 (77%)	12 (40%)	66 (66%)
Co-trimaxazole	44 (63%)	12 (40%)	56 (56%)
Gentamicin	41 (58%)	12 (40%)	53 (53%)
Nitrofurantoin	14 (20%)	8 (6%)	22 (22%)

On analysis of antimicrobial susceptibility pattern, the biofilm producing isolates showed 83%, 86%, 86%, 77%, 63% and 58% resistance to co- amoxyclav, ceftazidime, ciprofloxacin, norfloxacin, cotrimoxazole and gentamicin respectively as compared to non-biofilm producers that showed 50%, 40%, 50%, 40%, 40% and 40% resistance to the respective antibiotics.

The biofilm producers showed more susceptibility for Nitrofurantoin as compared to resistance i.e. 20% resistance by biofilm producers and 6% by non-biofilm producers.



Almost all the biofilm producing isolates displayed relatively more percentage of resistance to tested antimicrobials than biofilm non producers.

CONCLUSION

E. coli is the most frequent isolate, of which 71% are biofilm producers. The antibiotic susceptibility pattern in the present study showed fluoroquinolones are the least active drug against uropathogens. The uropathogens showed highest sensitivity to nitrofuran. There is a significant correlation between biofilm production and resistance to multiple antibiotics.Antimicrobial resistance is an issue of global concern, and biofilm producing isolates show higher antimicrobial resistance as compared to biofilm non-producers.

As biofilm production was detected in 70%of our isolates, it is necessary to follow standard guidelines of catheter care bundle to reduce the incidence of CAUTI mainly in terms of reducing the device utilization ratio and average catheter days per patient. Implementing care bundle and auditing the adherence to each element should be included as a part of routine HIC practices with a view to prevent device associated nosocomial infections in patients.

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