RESEARCH PAPER

Analysis Of Antibiotic Sensitivity Profile Of Biofilm-Forming Uropathogenic Escherichia Coli

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Abstract

Biofilms are group of microorganisms which are embedded within a self produced matrix of extracellular polymeric substance which adhere to each other. They are found to be involved in a wide range of infections in the body like urinary tract infections. Biofilms are considered to be highly resistant to antimicrobial agents. E.coli is the most common organism causing both community as well as hospital acquired uti leading to serious health issues. The aim of the study was to analyse the antibiotic sensitivity profile of biofilm forming uropathogenic e.coli. Out of 160 urinary isolates 109(68%) showed strong biofilm production. Among them 68 (42.5%) were strong biofilm producer, 41(25.62%) were moderate biofilm producer and 51 (31.87%) were weak/non biofilm producer. A significant association ($p<0.05$) was seen between biofilm formation and resistance towards antimicrobial drugs ampicillin (86%), cefuroxime (81.58%), ciprofloxacin (72%) and ceftriaxone (71.48%). They were sensitive to higher antibiotics like imipenem, piperacillin tazobactam, nitrofurantoin, and amikacin. The study emphasized the necessity for developing alternative therapeutic approaches to overcome multi drug resistance arising from biofilm formation of upec.

Keywords: Uti, E.coli, Biofilm, Antibiogram, Mdr, Upec.

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INTRODUCTION

One of the most prevalent infections and major contributor to morbidity in the human population is still urinary tract infection.Upto 40-50% of nosocomial infections are thought to be caused by UTI's, which affect an estimated 150 million people worldwide each year.[1]One of the main causes of disease and in certain situations, a cause of death such as pyelonephritis and urosepsis are urinary tract infections (UTI). *E. coli* is a gram negative, facultative anaerobic, versatile bacterium mostly found in the lower intestine of humans and endothermic animals. They colonize in the host and in the environment. Escherichia coli is comparatively more common (80%) in community-acquired infections and 50% in hospital-acquired infections among the etiological agents causing the illness[2,3]

A biofilm is often described as a collection of microbial cells that are joined to one another and are able to stick to a surface thanks to a matrix that they have created on their own. Both living and non-living surfaces can develop biofilm. The adhesion of free-floating bacteria to the surface is thought to be the first step in the creation of the biofilm.[4,5]Additionally, it is believed that hydrophobic effects and Van der Waals force might reverse the initial adherence to the surface. The adhesion will then become irreversible if it is not separated right away.[6,7]

About 40% of women are estimated to have experienced a UTI at some point in their lives.[8] TCP, TM, and CRA are some of the biofilm detection techniques employed in different biofilm-related studies.[9,10,11]

Biofilm formation is an important virulence factor in many nosocomial infections so that in 65% of the nosocomial infections biofilm are formed.[12] Biofilm is the term used to describe UPEC's capacity to form micro-colonies in the uroepithelium. Because of its capacity for antibiotic resistanceincluding increased virulencethis phenomena is well-known[13]

Even with our wide range of highly effective antimicrobial medications, recurring UTIs are currently a major health issue for many women.The

bacterial virulence factors displayed by uropathogenic *E. coli* (UPEC), which allow the bacteria to colonize and aid the organism overcome human defenses and infiltrate the urinary system, may be the cause of recurrent and relapse UTIs[14]

Antimicrobial drugs cannot penetrate biofilm due to its high polysaccharide content.Chronic infections may occur as a result of the biofilm's slow pace of cell multiplication and the limited penetration of antimicrobial medications.In addition to causing recurring infections, biofilm-forming bacteria show increased resistance to antimicrobial medications used to treat UTIs[15] .Recent years have shown an alarming rise in antibiotic resistance to UTIs, which suggests a major risk to human health.[16]

The spread of antibiotic-resistant microorganisms in diverse environments has clearly grown to be a significant global concern.[17]A significant clinical worry in the case of biofilm-related infections is the biofilm-forming isolates' resistance to antibiotic therapy.[18,19] Bacteria are shielded from antibiotics, immune cells and host proteins by biofilm[20]

MATERIAL AND METHOD

Study design

A cross sectional study was done in Santosh medical collegein the department of microbiology. The study population was confined to urinary tract infected patients and atotal of 160 urinary isolates were taken between January 2025 to july 2025

Processing of sample

Urine samples were inoculated by a wire loop to deliver 0.001ml of specimen on Cysteine lactose electrolyte deficient agar (CLED Agar), which was then incubated for 18–24 hours at 37^{O}C in an aerobic environment. Significant bacteriuria was defined as a pure growth of the gram-negative isolate on a colony count of at least 10^5 colony forming units. The strains were isolated and identified based on their morphology in Gram's staining, culture Characteristics, and biochemical tests. After another 24 hours of incubation, plates showing no growth were classified as negative cultures.

Congo red agar method

Congo red indicator and brain heart infusion (BHI) agar were used to make CRA medium (Hi-media India Pvt. Ltd.). First, a concentrated aqueous solution of Congo red stain was made and autoclaved for 15 minutes at 121ºC. After that, it was added to the BHI agar that had been autoclaved with sucrose at 55ºC. Test organisms were added to CRA plates, which were then aerobically incubated for 24 hours at 37ºC.

Red colonies were classified as biofilm non producers (BFNPs)and black colonies with a dry, crystalline consistency were classified as biofilm producers(BFPs).

Three repetitions of the exam were conducted.[21]

Antibiotic sensitivity testing

The Kirby-Bauer disc diffusion method was used to test for antibiotic sensitivity on Mueller-Hinton agar as per CLSI[22]. AST was carried out in the isolates using Kirby Bauer disc diffusion, which is explained as follows. A sterile swab was dipped into the bacteria grown in Trypic soy broth (TSB) in order to inoculate the MHA plates.

By firmly pressing and rotating the swab against the tube's side above the liquid's level, extra inoculums were eliminated. The swab was vigorously pressed and rotated against the tube's side above the liquid level in order to remove any excess inoculums. Three times, the medium's surface was streaked with the swab and each time the plate was rotated at a 60˚ angle. The swab was run along the agar surface's edge. A few minutes were given for the inoculums to dry.

The agar's surface was covered with the proper six antimicrobial-impregnated disks. Using a pair of sterile forceps, antimicrobial discs can be applied to the inoculated plates. On the Mueller Hinton agar plate, discs shouldn't be positioned closer than 24 mm. To guarantee full contact with the agar surface and prevent it from falling when the plate is inverted during incubation, each disc was carefully pressed down. The plates were kept at 37˚C in an incubator. Each zone's diameter was measured and noted in millimeters following an overnight incubation period. The antimicrobial susceptibility interpretation chart should then be used to interpret the data. Without lifting the lid, a ruler was used to measure the plate's underside.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS 28. A chi-square ($\chi 2$) test was performed and a value of $p<0.05$ was statistically significant

RESULTS

During the study period, a total of the 160 *E. coli* isolates were subjected for biofilm observation.After the completion of the laboratory procedure, the result was obtained for the biofilm production by the isolates, where 68 were strong, 41 moderate and 51 being weak/non biofilm producers.Biofilm forming E. coli developed significantly higher degree of resistance towards antimicrobial drugs Ampicillin (86%), Cefuroxime(81.58%), Ciprofloxacin(72%)

and Ceftriaxone(71.48%).They were sensitive to higher antibiotics like Imipenem, Piperacillin-tazobactam, Nitrofurantoin, and Amikacin.

Table 1 : Analysis of biofilm production

s.no	Biofilm production	No.of isolates
1.	Strong	68
2.	Moderate	41
3.	Weak/ non biofilm	51
4.	total	160

Table 2: Antibiotic sensitivity pattern of biofilm producing and non biofilm producing UPEC

S.No	Antibiotic	Biofilm Resistant n (%)	Biofilm Sensitive n (%)	Non-Biofilm Resistant n (%)	Non-Biofilm Sensitive n (%)	p-value
1	Amoxyclav	107 (98.17 %)	2 (1.83 %)	44 (86.27 %)	7 (13.73 %)	0.004
2	Norfloxacin	103 (94.50 %)	6 (5.50 %)	41 (80.39 %)	10 (19.61 %)	0.048
3	Gentamicin	103 (94.50 %)	6 (5.50 %)	20 (39.22 %)	31 (60.78 %)	o.oo1
4	Nalidixic acid	101 (92.66 %)	8 (7.34 %)	38 (74.51 %)	13 (25.49 %)	0.021
5	Ampicillin	100 (91.74 %)	9 (8.26 %)	44 (86.27 %)	7 (13.73 %)	0.62
6	Ofloxacin	98 (89.91 %)	11 (10.09 %)	41 (80.39 %)	10 (19.61 %)	0.19
7	Ceftazidime + Clavulanic acid	85 (77.98 %)	24 (22.02 %)	39 (76.47 %)	12 (23.53 %)	0.88
8	Cefoperazone + Sulbactam	82 (75.23 %)	27 (24.77 %)	33 (64.71 %)	18 (35.29 %)	0.36
9	Co-trimoxazol	81 (74.31	28 (25.69	20 (39.22	31 (60.78	o.oo1
10	Imipenem	13 (11.93 %)	96 (88.07 %)	2 (3.92 %)	49 (96.08 %)	0.15
11	Nitrofurantoin	27 (24.77 %)	82 (75.23 %)	8 (15.69 %)	43 (84.31 %)	0.34
12	Piperacillin + Sulbactam	46 (42.20 %)	63 (57.80 %)	20 (39.22 %)	31 (60.78 %)	0.72

ASSOCIATION BETWEEN ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION

In comparison with non-biofilm producers, biofilm-producing isolates showed stronger resistance to antibiotics.Amoxyclav ,Norfloxacin, Gentamicin, Nalidixic acid, Co-trimoxazole are highly significant as $p < 0.05$. Gentamicin and Co-trimoxazole show the strongest association with biofilm production ($p < 0.001$). **):**

Ampicillin, Ofloxacin, Ceftazidime+Clavulanic acid, Cefoperazone+Sulbactam, Imipenem, Nitrofurantoin, Piperacillin+Sulbactam show no significant difference as $p > 0.05$.

DISCUSSION

Urinary tract infections are most commonly caused by *E. coli.* The ability of UPEC isolates to colonize and endure in the urogenital tract is enhanced by a number of virulence factors. According to the study, biofilm development is a significant virulence factor in many pathogenic bacteria that cause UTIs in humans [23]. From the present study performed by us , out of 160 isolates 109 were biofilm producers (both strong and moderate).

According to the Congo red method, 59.4% of the strains in the investigation conducted by Sevanan et al. produced biofilm [24] and according to the Hassan et al. study 64.7% of isolates formed a strong or moderate biofilm [25]

A study by Rajput et al. shows that the proportion of biofilm formation using the congo red agar technique is 20.09 percent, which is in contrast to the findings mentioned above. Additionally, Najiri et al.'s investigation found that the rate of biofilm formation was 27% [27]

Comparatively , UPEC that generate biofilms are more resistant to antibiotics than those that do not. Since microorganisms growing in a biofilm are naturally resistant to many antibiotics, which can enhance antibiotic resistance up to 1000 times, high concentrations of antimicrobials are required to render them inactive [28]

This might be the result of the antibiotics' insufficient concentration reaching certain areas of the biofilm and the metabolic inactivity of the bacteria at the biofilms' bases [29,30]

Ampicillin (86%), Cefuroxime (81.58%), Ciprofloxacin (72%), and Ceftriaxone (71.48%) exhibited the highest levels of resistance in the current study .

Ponnusamy P et al.[31] conducted a similar study in which they found a strong correlation between biofilm production and resistance to many antibioticsRisal G. et al.'s study also produced findings that were comparable[32]

However, a study by Sevanan et al.[24] revealed that biofilm producers had the least amount of resistance to amoxyclav and cephalexin

CONCLUSION

We have found a strong correlation between multidrug resistance and biofilm formation.We think that the treatment of UTI therapy may benefit from the identification of biofilm formation.Most of the isolates are biofilm producers and are resistant to ampicillin, cefuroxime, ciprofloxacin and ceftriaxone.Therefore, choosing the right antibiotic treatment for a UTI will be aided by understanding how E. coli forms biofilms and their pattern of drug sensitivity.Additionally, it aids in preventing the spread of drug-resistant strains.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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