Original Research Article

Detection of biofilm formation by *Escherichia coli* with its antibiogram profile

Gaurab Risal[1]*, Aayush Shrestha[1], Saroj Kunwar[2], Gajal Paudel[1], Rameshwor Dhital[1], Man Bahadur Budha[1], Rupa Nepal[1]

[1]Department of Microbiology, [2]Department of Biochemistry, Modern Technical College, Lalitpur, Nepal

*Correspondence:
Dr. Gaurab Risal,
E-mail: grvris@gmail.com

ABSTRACT

Background: In urinary tract infections, an important role is played by bacterial biofilms which are responsible for persistence infections together with the antimicrobial resistance. Higher resistance can be seen in biofilm forming uropathogens in comparison with free-floating bacteria. So, the present study was performed with a goal to find the prevalence of biofilm formation and also the antimicrobial resistant pattern of uropathogens.

Methods: A descriptive method was conducted at Modern Technical College, Sanepa, Lalitpur in samples isolated from UTI suspected patients. The overall duration of this study was approximately 3 months. Total of 50 isolated *E. coli* was tested for biofilm formation and antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines.

Results: From the 50 isolates of *E. coli*, 32 were biofilm producers (3 strong and 29 moderate) and 18 were weak/non-biofilm producers. Among the biofilm producers, cefotaxime was more resistant in 20 of the isolates followed by ceftriaxone in 16 and amoxyclav in 13, whereas amikacin was least resistant in 2 of the isolates.

Conclusions: Among the isolated *E. coli,* biofilm-forming isolates showed higher antimicrobial resistance as compared to the non-biofilm producer. Thus, uropathogen should be routinely screened for biofilm formation.

Keywords: Uropathogen, UTI, Biofilm formation, Antimicrobial resistance pattern

INTRODUCTION

Biofilm can generally be defined as a group of microbes' cell attached to each other and then these cells get to adhere to a surface with the help of self-produced matrix. The Biofilm can be formed on both living and non-living surfaces.[1,2] It is said that the formation of the biofilm begins with the attachment of free-floating microorganisms to the surface. It is also thought that the first adherence to the surface is reversible via Van der Waals force and hydrophobic effects.[3,4] Then, if not separated immediately, the adherence will become permanent. The major feature of these aggregated colonies as a biofilm is being increasingly resistant to antibiotics.[5] Gram-negative bacteria as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa* are commonly involved in biofilm formation in case of Urinary tract infections (UTIs). UTIs are considered as one of the most important causes of being diseased and health care problems affecting persons of all ages, mostly including young women, children, and the elderly. It is roughly calculated that approximately 40% of women have had a UTI at some time in their life.[6] Various methods to detect biofilms used in various researches involving biofilms are TCP, TM, and CRA.[7-9] TCP method is considered as gold standard.[7,10,11]

So, we have used this method in our study. The main motive of our study is to study the biofilm types produced by isolated *E. coli* from the urine sample and their antibiogram profile. Biofilms are currently estimated to be responsible for over 65% of nosocomial infections and 80% of all microbial infections.[12] The level of resistance is according to the stage of biofilm. Antibiotics can work most effectively in the reversible attachment step because the bacteria have not connected themselves in the matrix. Due to this, they are at risk to the action of antibiotics and immune system of the host.[13] This might be helpful in the treatment of various patients suffering from UTI.

METHODS

A descriptive research design was used to detect biofilm produced by uropathogen and its antibiogram profile. The samples were collected from Star Hospital, Sanepa height, Lalitpur. The study was carried out at Modern Technical College. The study population was urinary tract infected patients and we have limited our study in preserved *E. coli* isolates in the last three months only from October 2016 to December 2016.

Inclusion criteria

Preserved *E. coli* isolates of Urinary tract infected patients were included for study.

Exclusion criteria

Preserved samples with mixed growth were not included; uropathogens other than *E. coli* were excluded.

Laboratory procedure

The following procedure was performed in the laboratory during the study period to obtain the required result. The procedure was done according to the research of O'Toole et al.[14]

Sample selection and transportation

Among the preserved samples of uropathogens in the Star Hospital, the isolated *E. coli* bacteria were selected for the study. The selected bacteria were transferred in the preserved form to the study site, which is Modern Technical College.

Emulsification of bacteria in media for growth

One loopful of the bacterial colony from the Mac-Conkey agar was taken and emulsified in 10 ml of TSB. The emulsified colony at 37°C was incubated in an incubator for 24hrs.

Biofilm detection (by TCP method)

For biofilm detection, 10 µl of emulsified bacteria from TSB was added in 1 ml of freshly prepared TSB. 200 µl diluted sample was put in a plain microtiter plate and incubate at 37°C for 24 hrs in an incubator. Non emulsified TSB media were taken as negative control. Next day, microtiter plate was shaken and all the free cells from the microtiter were dumped out gently. Then, microtiter plate was washed 4 times by phosphate buffer to remove the unattached cells and left for 30 minutes to dry in an incubator. After that, 250 µl of 0.1% crystal violet was added tn a microtitre plate and incubated at room temperature for 10 minutes. It was washed 3 times by buffer and incubated at incubator overnight turning microtiter plate upside down. Next day, 250 µl of acetone was added in a microtiter plate to solubilize crystal violet taken by the biofilms if present. The microtiter plate was covered with aluminum foil and shaken in a shaker. 250 µl of solubilized crystal violet was transferred to a new microtiter plate. Optical Density of solution was taken at 570 nm in ELISA reader. The interpretation of biofilm production was done according to the criteria of Stepanovic et al.[15]

AST

AST was performed in the isolates by Kirby Bauer disc diffusion as recommended by CLSI, which is described as follows.[16]

For the inoculation of the MHA plates, dipping a sterile swab was dipped into the bacteria cultured in TSB. Excess inoculums were removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. The swab was passed around the edge of the agar surface. The inoculums were left to dry for a few minutes. The appropriate 6 antimicrobial-impregnated disks were placed on the surface of the agar Antimicrobial discs can be placed on the inoculated plates using a pair of sterile forceps. Discs should not be placed closer than 24 mm (center to center) on the Mueller Hinton agar plate. Each disc was gently pressed down to ensure complete contact with the agar surface and do not fall when the plate is inverted during incubation. The plates were placed in an incubator at 37°C. After overnight incubation, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm. The results should then be interpreted according to the antimicrobial susceptibility interpretation chart. The measurements were made by a ruler on the under-surface of the plate without opening the lid.

RESULTS

During the study period, a total of the 50 *E. coli* isolates was subjected for observation of biofilm. After the completion of the laboratory procedure, the result was obtained for the biofilm production by the isolates, where 3 were strong, 29 moderate and 18 being weak/non-biofilm producers. Overall, biofilm producing bacteria

are found to be more sensitive to Amikacin (49 out of 50, 98%) and bacteria are more resistant to Cefotaxime (27 out of 50, 54%).

The interpretation of biofilm production was done according to the criteria of Stepanovic et al which is shown in Table 1.[15]

Table 1: Interpretations of biofilm production.

Average OD value	Biofilm production
ODc< - ≤ 2×ODc	Non/weak
2× ODc< - ≤ 4×ODc	Moderate
>4× ODc	Strong

Optical density cut-off value (ODc) = average OD of negative control + 3 × standard deviation (SD) of negative control. The result is shown in Table 2.

Table 2: Distribution of isolates on the basis of biofilm production.

Biofilm types	No. of samples
Strong	3
Moderate	29
Weak/Non	18
Total	50

Also, after performing AST of each isolate, the antibiogram profiles of each isolate can be known and separated for strong, moderate & weak/non-biofilm producers each.

The interpretative zone diameter of used antibiotics is listed in Table 3. Antibiogram profile of strong biofilm producers is shown in Table 4.

Table 3: The interpretative zone diameter of antibiotics (in mm).

Serial No.	Antibiotics	Sensitive	Resistant	Intermediate
1	Cefepime	24	21	22-23
2	Cefotaxime	26	22	23-25
3	Ceftriaxone	23	20	21-22
4	Levofloxacin	22	19	20-21
5	Nalidixic Acid	19	13	14-18
6	Amikacin	16	13	14-15
7	Amoxyclav	19	19	14-17

Table 4: Antibiogram profiles of strong biofilm producers.

Susceptibility antibiotic	Sensitive	Intermediate	Resistant	Total
Amikacin	3			3
Amoxyclav			1	1
Cefepime	2		1	3
Ceftriaxone	2		1	3
Cefotaxime	1	1	1	3
Levofloxacin	2		1	3
Nalidixic acid	1		1	2

Table 5: Antibiogram profile of moderate biofilm producers.

Susceptibility antibiotics	Sensitive	Intermediate	Resistant	Total
Amikacin	28	1		29
Amoxyclav	4	1	12	17
Cefepime	20	1	8	29
Ceftriaxone	13	1	15	29
Cefotaxime	5	4	20	29
Levofloxacin	20		9	29
Nalidixic acid	6	5	1	12

Antibiogram profile of moderate biofilm producers is shown in Table 5.

Antibiogram profile of weak/non-biofilm producers is shown in Table 6.

According to the result in the Table 4, we can see that all 3 strong biofilm producers are sensitive to Amikacin whereas 1 of them is resistant to all other antibiotics used except for Amikacin. But, we have used Amoxyclav and Nalidixic acid in only 1 and 2 of them respectively.

Again, we can see in Table 5 that 28 out of 29 (97%) moderate biofilm producers are sensitive to Amikacin.

Also, 12 out of 17 moderate biofilm producers (71%) are resistant to Amoxyclav and 20 in 29 (69%) are resistant to cefotaxime.

Table 6: Antibiogram profile of weak/non-biofilm producers.

Susceptibility antibiotics	Sensitive	Intermediate	Resistant	Total
Amikacin	18			18
Amoxyclav	10		6	16
Cefepime	15		3	18
Ceftriaxone	14		4	18
Cefotaxime	6	6	6	18
Levofloxacin	13	3	2	18
Nalidixic acid	1		1	2

Also, in Table 6, all 18 out of 18 weak/non-biofilm producers are sensitive to Amikacin and 15 of 18 (83%) are also sensitive to Cefepime. 6 out of 16 (38%) and 6 out of 18 (33%) are sensitive to Amoxyclav and Cefotaxime respectively.

DISCUSSION

E. coli is the most frequent cause of urinary tract infections. There are several virulence factors in UPEC isolates that increase their ability to colonize and persist in the urogenital tract. An important virulence factor in various pathogenic bacteria causing human UTI is the biofilm formation according to the study conducted by Jabalameli et al.[17] Urinary tract infections are among the most common bacterial diseases worldwide which involve (infects) about 250 million people in developing countries annually.[18,19] Uropathogenic *E. coli* on its own account for 70-90% of the UTI infections and their susceptibility patterns against different antibiotics vary in different geographical regions.[19,20] Bacterial biofilms are most of the time associated with long-term persistence of bacteria in various environmental conditions. Bacteria in biofilm display dramatically increased resistance to antibiotics.[21,22]

From the given study performed by us, out of 50 isolates, 32 were biofilm producers (3 strong and 29 moderate) and 18 were none or weak biofilm producers. Among the biofilm producers, cefotaxime was most resistant in 20 of the isolates followed by ceftriaxone in 16 and amoxyclav in 13, whereas amikacin was least resistant in 2 of the isolates.

We found out that the biofilm either strong or moderate producing bacteria were found to be more resistant to the antibiotics in comparison to the weak or non-biofilm producing bacteria. But also, in some of the cases, strong biofilm producers are less resistant than some of the weak biofilm producers. So, it is not completely necessary for the biofilm producers to be resistant.

From the study carried out by Hassan et al the percentage of isolates forming strong or moderate biofilm was 64.7%

and weak/non-biofilm was 36.3% among a total of 110 isolates.[18]

ElaheTajbakhsh et al performed the study in biofilms detection by CRA method, which also focuses on the antibiotics susceptibility pattern of the isolates.[23] The resistance shown to ampicillin (87.5%) was maximum among the biofilm forming bacterial strains, which is followed by tetracycline (75%), nalidixic acid (72.5%) and co-trimoxazole (71.25%). All bacteria including both biofilm producers and non-producers were highly resistant to ampicillin, then to tetracycline followed by nalidixic acid. Against nitrofurantoin, 93.75% and 98% sensitivity was noticed for biofilm and non-biofilm producers respectively.

The results of the present and previous study demonstrated that the biofilm producing bacteria are low or high in number according to the various clinical conditions, geographical regions, and other factors. It also shows that high resistivity is shown by the biofilm producers than none producers, but it is not necessarily for each biofilm producers to be resistant to the antibiotics.

CONCLUSION

From the above study, we differentiated the biofilm producing *E. coli* into strong, moderate, weak or non-biofilm producers. Also, we can conclude that biofilm producing bacteria are more resistant to the antibiotics. Among the biofilm producers, cefotaxime was most resistant in 20 of the isolates followed by ceftriaxone in 16 and amoxyclav in 13 whereas, amikacin is more sensitive in all biofilm producing isolates, followed by cefepime in 20 of the isolates. From this study, we found out that the biofilm producing bacteria are comparatively more resistant to the antibiotics but it is not necessary to be biofilm producer to become antibiotic resistant.

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