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Research Article

Relationship between Biofilm Formation and Antibiotic Susceptibility Pattern in Uropathogenic Escherichia coli

Rejina Shrestha[1*], Anisha Shrestha[1], Binod Khadka[1], Rosy K.C.[1], Manju Shree Shakya(Hada)[1], Anil Kumar Sah[2]

[1]Department of Microbiology, Tri-Chandra Multiple Campus, Ghantaghar Kathmandu, Nepal
[2]Annapurna Research Centre, Maitighar, Kathmandu, Nepal

Abstract

Escherichia coli are one of the most common isolates from the urine sample. The ease of treatment of the UPEC mediated UTI is hindered by many factors developed within the pathogen-biofilm being one of the factor resulting in the resistance of pathogen against the prevalent antibiotics and emergence of MDR cases. Biofilm formation by *E.coli* is a pathogenic mechanism in which the organism covers itself by exopolysaccharide coat and the organism becomes resistant to antibiotics which are used to tackle the pathogen. The study was done to understand the relationship between biofilm formation and antibiotic susceptibility pattern in Uropathogenic *E. coli.* For this study, a total of 350 urine sample was analyzed and 48 UPEC isolates were isolated from suspected urinary tract infected patients at Annapurna Neuro Hospital, Kathmandu from April 2017 to September 2017. The isolates were characterized by biochemical tests and were subjected to AST which was done by modified Kirby- Bauer disk diffusion method. In-vitro biofilm production by these isolates was determined by Congo red agar method. The most effective antibiotic was found to be Imipenem (100%), followed by Nitrofurantoin (87.5%) and Amikacin (83.3%). Biofilm production was found in 60.4% of isolates. These isolates forming biofilm were found to be highly resistant to antibiotics. Biofilm production makes the organism to be more resistant to antibiotics and virulent as compared to non-biofilm producers.

Keywords: UPEC; Antibiotic Resistance; Biofilm

Introduction

Urinary tract infections (UTIs) are one of the major public health concerns in developed and developing countries and represent one of the most common nosocomial infections. Uropathogens causing urinary tract infection is one of the major health problems (Zaki and Elewa, 2015). The prime etiological agent causing UTI is *E. coli* (80%) (Säemann, 2008). *E. coli* strains that cause urinary tract infections are termed as UPEC (Mobley *et al.,* 2009). UPEC strains are accountable to cause acute infections and recurrent infections that do not respond to common antimicrobial treatments. The main intention behind treatment of an uncomplicated UTI is to resolve the symptoms and sterilization of the urine (Chaulin, 2005). Resistance against antibiotics complicates the treatment of UTI and is also often allied with a higher patient morbidity rate, greater expenses of re-evaluation and re-treatment, greater hospitalization rates and greater usage of broader-spectrum antibiotics (Hooton *et al*., 2004). Proper understanding of the mechanisms by which uropathogenic micro-organisms manifest resistance towards antimicrobials (both intrinsic and acquired resistances) is obligatory to augment treatment approaches for UTI.

By forming a biofilm, pathogen develops a mechanism that obstructs the eradication of organisms. Biofilm production facilitates and enhances survival of an organism against various antibiotic therapies which results in chronic and persistent infections and leads to the complications in the treatment (Chakraborty *et al.,* 2011). Biofilms are the micro-bacterial communities of the causative organisms

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[1]*Corresponding author
Rejina Shrestha,
Department of Microbiology, Tri-Chandra Multiple Campus, Ghantaghar Kathmandu, Nepal
Email: regshrestha3@gmail.com

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tending to colonize the bladder mucous membrane. Favoring the long-term continuing persistence of microbes, these microcolonies are impervious to several antibiotics, and lead to the advancement of multidrug-resistant strains of microorganisms responsible for setbacks in untreatable UTI (Mittal *et al.*, 2015).

The main purpose of study was to find out whether the UPEC isolated from the urine sample at Annapurna Neuro Hospital were biofilm producers or not. Biofilm production is a pathogenic mechanism in bacteria increasing the persistence of organism in the host. The increasing antibiotic resistance in UPEC samples is a major problem in the hospital and the knowledge of the relationship between biofilm production and AST pattern will help to take required steps to solve the problem.

Methods

Isolation of Pathogenic Escherichia coli

350 urine samples from the UTI suspected patients at Annapurna Neuro Hospital were cultured on Mac Conkey Agar and were subjected to incubation at 37°C for 24 hours. Pink lactose fermenting colonies were then subjected to identification by gram stain, catalase and oxidase test, biochemical tests which included Indole test, Methyl Red-Voges Proskauer, Citrate test, Urease test and Triple Sugar Iron Test.

Antibiotic Susceptibility Testing

The antibiotic sensitivity testing of the isolates towards various antibiotic discs was performed by modified Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI, 2014) using Muller Hinton agar. The antibiotics tested were Amikacin (AK, 30µg), Ampicillin (AMP, 10µg), Ceftriaxone (CTR, 30µg), Co-Trimoxazole (COT, 25µg), Imipenem (IPM, 10µg), Nalidixic Acid (NA, 30µg), Nitrofurantoin (NIT, 300µg), Norfloxacin (NX, 10µg) and Ofloxacin (OF, 5µg). The broth culture of the test organism comparable to 0.5 McFarland was uniformly carpeted on the surface of the MHA plates and antibiotics discs were placed over the lawn culture. The MHA was inoculated at 37°C for 18 hours (or overnight) and then zone of inhibition around antibiotic discs was noted and reported as "Resistant" or "Sensitive" according to CLSI guidelines (CLSI, 2014). MDR isolates were defined as those which showed resistance to ≥3 of the following 6 classes of drugs carbapenems (imipenem), flouroquinolones (ofloxacin), aminoglycosides (amikacin), Nitrofurans (Nitrofurantoin), Cotrimoxazole (a mixture of sulphamethaxazole and trimethoprim) and 4-quinolone (Nalidixic acid and norfloxacin). Control strain of *E. coli* (ATCC) was used in parallel as part of quality control when using new batches of media or antibiotics (Cheesebrough, 2000).

Biofilm Formation Assay

Biofilm detection of the identified uropathogenic *E. coli* was performed using Congo red agar method. Congo red agar method is a process of phenotypic characterization of biofilm production using Congo red agar plates. In this process, Congo red agar plates were prepared using 37gm/l of Brain Heart Infusion broth, sucrose 50gm/l, agar 10gm/l and congo red dye 0.8gm/l. Aqueous solution of Congo red dye was prepared and autoclaved separately and mixed with brain heart infusion agar with sucrose at 55°C (Freeman et al., 1989). The category of biofilm production was differentiated on the basis of color of colony on Congo Red Agar. Strong biofilm producers formed deep black colonies on CRA and non-biofilm producers formed pinkish to white colonies on CRA.

Results and Discussion

Antibiotic Susceptibility Pattern of Uropathogenic E. coli

Among the antibiotics used, Imipenem (100%), Nitrofurantoin (87.5%) were most effective against Uropathogenic *E. coli* followed by Amikacin (83.3%). Uropathogenic *E. coli* isolates showed maximum resistance against Ampicillin (79.2%) followed by Nalidixic acid (70.8%) (Table 1). Fig.1 shows Growth of E. coli on Mac Conkey Agar and Fig.2 shows Antibiotic Susceptibility Test of UPEC on Muller Hinton Agar. A similar study showed that *E. coli* isolated from urine was highly sensitive to Amaikacin (87%) (Das et al., 2006) and to nitrofurantoin (85%) (Kibret and Abera, 2014) and completely (100%) sensitive to imipenem (Ponnusamy et al., 2012).

Imipenem is a carbapenem class of antibiotic and is resistant to most β-lactamases so is sensitive to all UPEC and is the most effective antibiotic for treatment of UPEC. The consistent and high-level susceptibility of *E. coli* to nitrofurantoin may be influenced by nitrofurantoin's narrow spectrum of activity, limited indication (treatment of acute cystitis), narrow tissue distribution (low or undetectable serum concentrations) and limited contact with bacteria outside the urinary tract (Hooper, 2000).

Fig 1: Growth of *E. coli* on Mac Conkey Agar

Table 1: Antibiotic Susceptibility pattern of uropathogenic *E. coli*

Organism	Antibiotic used	Susceptible		Resistant	
		No	%	No	%
***E. coli* (n=48)**	Amikacin	**40**	**83.3**	8	16.7
	Ampicillin	10	20.8	**38**	**79.2**
	Cotrimoxazole	19	39.6	29	60.4
	Ceftriazone	28	58.3	20	41.7
	Nalidixic acid	14	29.2	**34**	**70.8**
	Nitrofurantoin	**42**	**87.5**	6	12.5
	Norfloxacin	20	41.7	28	58.3
	Ofloxacin	27	56.3	21	43.7
	Imipenem	**48**	**100**	0	0

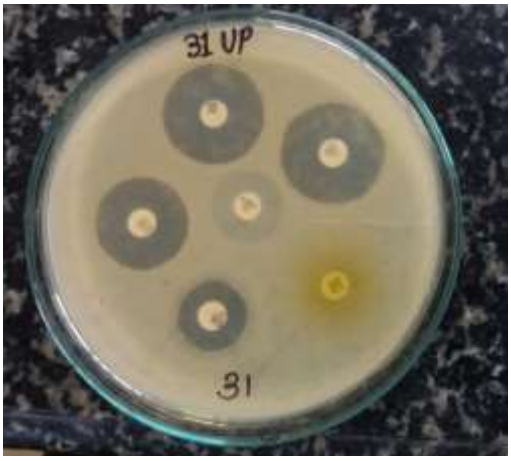

Fig 2: Antibiotic Susceptibility Test of UPEC on Muller Hinton Agar

Biofilm Production in Uropathogenic Escherichia coli

Among the 48 isolated uropathogenic *Escherichia coli*, 29 (60.4%) were biofilm producer while 19 (39.6%) were non-biofilm producers. In a similar study performed by (Ponnusamy et al., 2012) among total 100 isolated UPEC, 60% of the isolates were biofilm producer in which 23% isolates were strong biofilm producer, 37% were moderate biofilm producer and remaining 40% were non-biofilm producer.

Fig 3: Biofilm production by UPEC on Congo Red Agar (A. and B. Biofilm producers; C., D., and E. Non biofilm producers)

Relationship between Biofilm Production and AST

Both biofilm producer and biofilm non-producer strains were 100% sensitive towards Imipenem. 86.2% of biofilm producer were sensitive towards Nitrofurantoin while 89.5% of biofilm non-producer were sensitive to this drug. In case of Amikacin drug, 82.8% of biofilm producer were sensitive towards it, while 84.2% of biofilm non-producer were sensitive towards it.

Similarly both biofilm producer and non-producer showed maximum resistance against Ampicillin. 86.2% of biofilm producer showed resistance against Ampicillin while only 68.4% of biofilm non-producer showed resistance against Ampicillin. This result is in accord with study performed by (Tajbakhsh et al., 2016) in which biofilm producers showed maximum resistance to Ampicillin (87.5%) whereas the resistivity of non-biofilm producer to ampicillin was 80%. In addition, for nitrofurantoin resistivity to the drug for biofilm producers was 6.25% whereas for non-biofilm producer was 2%. In a similar study, both biofilm producer and non-producer were highly resistance to Ampicillin followed by Nalidixic acid. Similar study by (Mittal S et al., 2015) also tabulated higher resistivity pattern among the biofilm producers than the non-producers. The above data indicates that there is increase in resistivity among the biofilm producers which relates that the chances of increase in resistivity due the biofilm production. This result is supported by statement "Bacteria in biofilm display dramatically increased resistance to antibiotics" given by (Graham and Galloway, 2001).

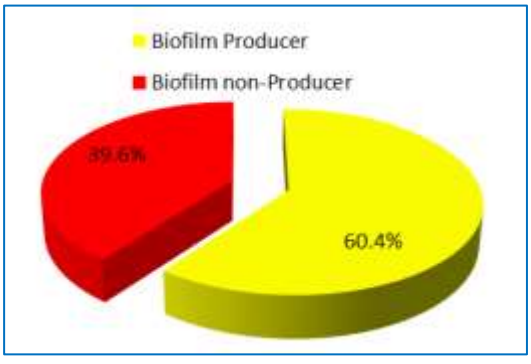

Fig. 4: Biofilm production in UPEC

Table 2: Relationship between biofilm production and AST

Antibiotics	Biofilm Producer (n=29)		Non-biofilm producer (n=19)	
	Sensitive (S)	Resistance (R)	Sensitive (S)	Resistance (R)
Amikacin	24 (82.8%)	5 (17.2%)	16 (84.2%)	3 (15.8%)
Ampicillin	4 (13.8%)	25 (86.2%)	6 (31.6%)	13 (68.4%)
Cotrimoxazole	11 (37.9%)	18 (62.1%)	8 (42.1%)	11 (57.9%)
Ceftriazone	16 (55.2%)	13 (44.8%)	12 (63.2%)	7 (36.8%)
Nalidixic acid	7 (24.1%)	22 (75.9%)	7 (36.8%)	12 (63.2%)
Nitrofurantoin	25 (86.2%)	4 (15.8%)	17 (89.5%)	2 (10.5%)
Norfloxacin	11 (37.9%)	18 (62.1%)	9 (47.4%)	10 (52.6%)
Ofloxacin	15 (51.7%)	14 (48.3%)	12 (63.2%)	7 (36.8%)
Imipenem	29 (100%)	0 (0%)	19 (100%)	0 (0%)

Ethical Approval

Ethical approval was taken from Nepal Health Research Council (NHRC).

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