

Microbiology

BIOFILM PRODUCTION AMONG EXTENDED SPECTRUM B-LACTAMASE PRODUCING AND NON-PRODUCING *ESCHERICHIA COLI* ISOLATES

Dhakal B¹, Lekhak B¹, Upreti MK¹, Shrestha S¹ and Tuladhar S²

¹Department of Microbiology, GoldenGate International College, Battisputali Kathmandu;

²Department of Pathology, Nobel Hospital, Sinamangal

Abstract

Resistance mechanisms like Extended Spectrum β -lactamase (ESBL) has limited the choice of antibiotics for the management of patients with bacterial disease. Further, biofilm production by bacterial pathogens make them survive under adverse conditions. The present study was undertaken over a period, of six months from July to February 2016 to determine the biofilm formation among ESBL producing and non-producing *Escherichia coli* and to assess their antibiotics susceptibility pattern.

During the study period, 1052 mid-stream urine sample was collected and processed for isolation and identification of etiologies of urinary tract infections (UTI). Antimicrobial susceptibility testing (AST) was performed by Modified Kirby Bauer Disk Diffusion method. ESBL detection was done by combined disk assay using cefotaxime and ceftazidime in alone and combination with clavulanic acid as per CLSI guideline.

A total of 270 (25.7%) *E. coli* was obtained from 1052 samples, 175 (64.8%) shows multidrug resistance, 105 were ESBL producers. Biofilm positive isolates show 73(27.0%) strong biofilm producer, 33(12.2%) moderate producer and 21 (7.8%) weak producer Congo Red Agar (CRA).

Antibiotics resistance is on increasing trend and it is necessary to take action to reduce encumbrance in the future. Advance studies in biofilm will help to prevent the virulence without any complications in therapy.

Key Words: *E. coli*, MDR, ESBL, Biofilm

Introduction

Urinary tract infection (UTI) may be defined as the pertinacious presence of micro-organism that multiplies actively within urinary tract (Chaudhary et al 2012). UTI is one of the most common bacterial infection and *E. coli* is frequently isolated bacteria in both community and hospital acquired infection (Arundathi et al 2016). UTI is common in all age groups, approximately 150 million people are affected yearly throughout the world wide (Mukharjee et al 2013). Presence of more than 10⁵ CFU/ml indicates significant bacteriuria. It is more common in females due to a close proximity of urethra with the vagina (Babu et al 2014). UTI causes many problems with millions of cases resulting the high rate of chronic diseases in developing countries every year (Kabir et al 2013).

The emergence of MDR pathogenic bacteria has become a significant global public health threat and is associated with high mortality and morbidity (Tanwar et al 2014). A common mechanism for bacterial resistance is a production of beta- lactamases that hydrolyze the drugs (Ponnusamy et al 2013). ESBLs are enzymes capable of corroborating bacterial

resistance to the penicillin, first, second, and third generation cephalosporins and Aztreonam. They are not active against the cephamycins (cefoxitin and cefotetan), and are susceptible to beta lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam (Mohanty et al 2010; Gupta et al 2013; Qin et al 2014).

Many bacteria have the capability to form biofilms when microbial cells are attached to the surface which is enclosed in a self-produced extra cellular polymeric matrix (Niveditha et al 2012). The formation of biofilms on the surface can be considered as a universal bacterial strategy for survival of the organism and to use available nutrients effectively (Poovendran et al 2013). Polymeric matrix consists polysaccharides that prevent the access of antibacterial agents like antibodies, white blood cells and antibiotics so that the bacterial cell in a biofilm can withstand host immune system and are much less susceptible to antibiotics (Poovendran et al 2013).

Materials and methods

Study site and population

The present study was conducted at Microbiology laboratory of Nobel Hospital from July 2015 to February 2016. During the study

period, 1052 mid-stream urine samples were collected from the UTI suspected patients of all ages and sex and was processed according to the standard microbiological procedures.

Isolation and identification of *E. coli*

The urine samples were processed using standard microbiological procedure. The urine samples were cultured onto the MacConkey agar (MA) and Cysteine Lysine Electrolyte Deficient Agar (CLED) plates by semi-quantitative culture technique using standard calibrated loop and incubated aerobically at 37°C for 24 hours. The isolates were identified using routine conventional methods based upon microscopic findings, colony morphology and biochemical properties.

Antimicrobial Susceptibility Testing

AST of the different isolates towards various antibiotics were performed by the Modified Kirby Bauer disk diffusion method for the commonly isolated pathogens using Mueller Hinton Agar (MHA) as recommended by CLSI. The antibiotics used were ciprofloxacin, ofloxacin, norfloxacin, gentamicin, amikacin, cotrimoxazole, ampicillin, cefixime, ceftazidime, cefotaxime, ceftriaxone, cefepime, imipenem and nitrofurantoin.

Screening and Confirmation of ESBL producers

ESBL detection was performed simultaneously with AST. Isolates were screened for possible ESBL production using ceftazidime (30µg) and cefotaxime (30µg). According to the CLSI guidelines, isolates showing ceftazidime <22mm, cefotaxime <27 mm, are the possible ESBL producing strains. The screen positive isolates i.e. suspected ESBL producers were subjected to Combined Disk Assay using ceftazidime (30µg) and ceftazidime plus clavulanic acid (30/10µg) and cefotaxime (30µg) and cefotaxime plus clavulanic acid (30/10µg). The results thus obtained were compared.

Biofilm Detection by Congo Red Agar (CRA) method

All the isolates were inoculated on CRA and on the basis of intensity of color produce isolates were classified into three categories i.e. strong producer (+++), moderate producer (++) and weak producer (+).

Quality control

Quality of each test was maintained by using standard procedures. During identification and antibiotic susceptibility testing of organism, control strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were tested primarily. For ESBL testing, *E. coli* ATCC 25922 was used as quality control.

Data analysis

All the results were entered and analyzed with Statistical Package for Social Science (SPSS) software (Version 21.0). Chi-square test was applied to test whether differences between values are significant or not. P-value < 0.05 were considered statistically significant.

Results

Out of 1052 mid-stream urine samples processed, 270 (25.7%) showed significant growth. Among them 97 (35.9%) were recovered from male patients and 173 (64.1%) from female patients.

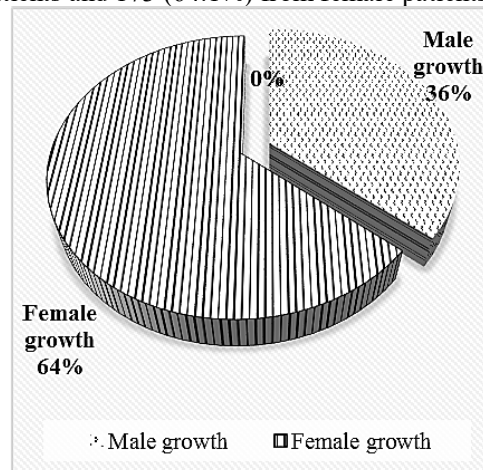


Figure 1: Culture pattern of *E. coli*

Higher incidence of UTI was observed in age groups 15-50 years (143; 53.0%) and also in inpatients than outpatients.

Table 1: Distribution of *E. coli* among age groups

Age	Male	Female	Total
>15	19(61.3%)	12(38.7%)	31(11.9%)
15-50	42(29.4%)	101(70.6%)	143(53.0%)
>50	29(30.2%)	67(69.8%)	96(35.5%)
Total	90(33.3%)	154(57.0%)	270(100%)

Among the common antibiotics used, *E. coli* was found to be more resistant to ampicillin (88.8%). Imipenem and amikacin was found to be most effective drug with susceptibility of 100% and 87.8% respectively.

Table 2. Antibiotic susceptibility pattern of *E. coli*

Antibiotics	Antimicrobial Susceptibility Pattern		
	R %	I %	S %
CIP	55.2	8.5	36.3
OF	53.0	6.7	40.4
NOR	51.1	6.3	42.6
GEN	40.0	10.4	49.6
AK	12.2	0	87.8
COT	57.4%)	5.6	37.0
AMP	88.8	20.7	9.6
CFM	77.0	10.0	13.0
CAZ	59.3	4.1	36.7
CTX	56.7	3.3	66.6
CTR	47.4	6.0	46.7
CP	48.9	6.0	45.2
IMP	0	0	100
NIT	28.1	10.7	61.1

R %: Resistance percentage, I %: Intermediate percentage, S %: Susceptible percentage

Of the total, 175 (64.8%) isolates were MDR and 95 (35.2%) were non-MDR. Among the 175 MDR isolates 93 (53.1%) were ESBL producer whereas 82 (46.9%) were ESBL non-producers. Similarly, from 95 non-MDR isolates 12(12.6%) were ESBL producer and 83 (87.4%) were ESBL non-producers. The association of MDR and ESBL production was found to be statistically significant.

Table 3. ESBL production among MDR *E. coli* isolates

	ESBL	Non-ESBL	Total	P-value*
MDR	93	82	175	
Non-MDR	12	83	95	
0.0001				
Total	105	165	270	

* Chi-square test

Out of 270 *E. coli* isolates, 27.0% were found to be strong biofilm producer, 12.2% were moderate producer and 7.8% were weak producer.

Table 5. Pattern of biofilm formation by *E. coli* isolates

Type of <i>E. coli</i>	Numbers	Percentage (%)
Strong producer	73	27.0
Moderate producer	33	12.2
Weak producer	21	7.8
Non-producer	143	53.0
Total	270	100

From 105 ESBL producing *E. coli* 42 (40.0%) were strong biofilm producer, 20 (19.1%) were moderate producer, 12 (11.1%) were weak producer and 31 (19.5%) showed no biofilm formation. There was significant association between ESBL production and biofilm formation *E. coli*. (P=0.0001).

Discussion

Out of total 1052 urine samples received for culture in the laboratory, only 270 (25.7%) specimens showed culture positive, whereas majority of sample 782 (74.3%) showed no growth. The low percentage of culture positivity among urine specimens was due to non-specific symptoms of UTI, presence of those organisms that were not able to grow on routine culture media and other suspicious factors that need for request of urine culture (Eshwarappa et al 2014). This result confirms and expands the previous finding of Khadri et al (2009) and Raghubanshi et al (2014).

Higher incidence of UTI was observed in age groups 15-50 years among which female accounting for 101 (70.5%) and male accounting for only 42 (29.4%). Higher incidence of UTI among these age groups may be due to the fact that these age groups are sexually active and highly involved in sexual activities (Khadka et al 2012, Sharma 2013).

Gram negative isolates were more prevalent than gram positive however *E. coli* was found to be predominant uropathogens accounting for 270/1052 (25.7%). *E. coli* is a common fecal commensal which can easily access into the urethra and capable of establishing infections (Elsas et al 2011). The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as health care and education programs (Amin et al 2009).

In this study, imipenem was found to be most effective drug showing (100%) susceptibility followed by amikacin (87.8%) and cefotaxime (66.7%), in a similar study carried by Kalaskar and Venkataramana (2012) imipenem was most effective oral drug against *E. coli* and other gram negative bacteria. Result obtained from this study is consistent with the finding of Asti and Sadawarte (2013), Khashef et al (2010) and Mohammadi et al (2010) who observed imipenem was the drug of choice. In the present study, most of the *E. coli* were least effective to ampicillin (88.8%), cefixime (77.0%),

cotrimoxazole (57.4%) and least resistance towards amikacin (12.2%). This result was harmony with finding of Acharya et al (2011), Chaudhary et al (2012) and Jha et al (2013). Among 270 *E. coli* isolates, 175 (64.8%) were multidrug resistant. Similar drug resistance rate was observed by Rath et al (2014), Buda (2010), Poudyal (2010). Result obtained from the present study was contrasting with result obtained by Baral et al (2012) which was only 38.2% MDR. Misuse and high levels of availability and consumption of antibiotics have led to higher incidence of resistance (Mahadevamma et al 2012). The high level of drug resistance seen among *E. coli* is mediated by β -lactamase which hydrolyses the β -lactam ring inactivating antibiotics (Forbes 2007).

In the present study, out of 175 MDR isolates 93 (53.1%) were found to be confirmed ESBL producer and from 95 non-MDR isolates 12 were ESBL producers. These results were similar to result obtained from Bartoli et al (2012) in South America. The association between MDR and ESBL production was found to be statistically significant ($P=0.0001$).

Among 270 *E. coli* isolates 127 (47%) were biofilm producers; among these 73 (27.0%) were strong producers, 33 (12.2%) were moderate producers, 21 (7.8%) were weak producers. These results agree with finding of Tajbakhsh et al (2016) in which 25.8% were strong producers, 10.1% were moderate producers and 4.7% were weak producers.

The biofilm forming ability was found to be significantly higher in ESBL positive strains than that of ESBL negative strains ($P=0.002$). the study of Subramanian et al (2012) in India also reported the higher ability of ESBL producing organisms to form biofilm in comparison to that of ESBL non-producing isolates.

Conclusion

The occurrence of UTI cases were within the reported range. ESBL production and MDR genes acquisition may worsen clinical outcomes of UTI by *E. coli*. Also, biofilm formation in vivo by ESBL producing, MDR *E. coli* might be alarming in Nepal, which emphasizes the need for judicious use of antimicrobial agent and their continuous monitoring in vitro.

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