Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal

Sanjeev Neupane¹, Narayan Dutt Pant²*, Saroj Khatiwada³, Raina Chaudhary⁴ and Megha Raj Banjara¹

Abstract

Background: Escherichia coli is the most predominant causative agent of urinary tract infection (UTI). Recently, increase in drug resistance among the uropathogenic bacteria has caused great problem in treatment of UTI. The main objective of this research is to determine the correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli.

Methods: The urine samples collected from the patients suspected of urinary tract infections (visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal between July to December 2013) were cultured in cystine lactose electrolyte deficient (CLED) agar by using semi quantitative culture technique. Extended spectrum beta lactamase (ESBL) production was detected by combined disc diffusion technique and biofilm formation was detected by Congo red agar method. Chi-square test was applied and p-value < 0.05 was considered statistically significant.

Results: Out of 1480 urine samples, E. Coli was isolated from 208 (14.1%) samples. Of total 69 (33.2%) ESBL producing uropathogenic strains of E. coli, 20 (29%) were strong biofilm producers, 22 (31.9%) were moderate biofilm producers, 11 (15.9%) were weak biofilm producers and 16 (23.2%) were biofilm non producers. Whereas among 139 ESBL non producing E. coli, 22 (15.8%) were strong biofilm producers, 20 (14.4%) were moderate biofilm producers, 13 (9.4%) were weak biofilm producers and 84 (60.4%) were biofilm non producers. Among total 108 biofilm producing E. coli, maximum resistance was observed toward cephalaxin followed by amoxicillin and highest susceptibility was seen toward amikacin.

(Continued on next page)
Conclusion: The ability of biofilm formation was found to be significantly higher in ESBL producing strains of *E. coli* than that in ESBL non producing strains (*p* < 0.05). There was higher resistance rate to antimicrobial agents among biofilm producing strains of *E. coli* than that in biofilm non producing strains. According to our antimicrobial susceptibility pattern for *E. coli*, to start preliminary treatment for UTI in Nepal, we recommend to use amikacin or nitrofurantoin. Further, for the treatment of the UTI, the antibiotics should be selected on the basis of the urine culture and sensitivity report.

**Keywords:** *Escherichia coli*, Urinary tract infection, Extended spectrum beta lactamase, Biofilm, Nepal

**Background**

Urinary tract infection (UTI) is one of the most common bacterial infections acquired both in the community and hospital settings, affecting all age groups [1, 2]. Worldwide, around 150 million cases of UTI are diagnosed each year [3] and *Escherichia coli* is identified as the most common cause of UTI, accounting for 80 to 85% of the cases [4–6].

Recently the haphazard uses of antibiotics have resulted in the worldwide spread of antibiotic resistance among the bacteria causing a major problem [7]. The emergence and worldwide rapid increase in prevalence of extended spectrum beta-lactamase (ESBL) producing bacteria that are multidrug resistant, pose treatment problem resulting in high morbidity, high mortality, and increased health care costs [8]. Biofilm production is a mechanism exhibited by several microbes to survive in unfavorable conditions. The bacterial biofilm is a structured community of bacterial cells enclosed in polymeric matrix and adherent to a surface [9]. Biofilm producing uropathogenic bacteria may be responsible for many recurrent UTIs [10]. The bacteria enclosed in the biofilm are highly resistant to antibiotic treatment [9].

In this study we are investigating the incidence of the ESBL producing *E. coli* in causing UTI. Further we are determining the correlation between biofilm formation and drug resistance with commonly used antibiotics (for treatment of UTI) along with ESBL production in *E. coli* isolated from the urine samples of the patients suspected of urinary tract infections. This is the first this type of study conducted in Nepal.

**Methods**

A cross sectional study was conducted among the patients suspected of urinary tract infections (having symptoms like burning micturition, frequent or intense urge to urinate, back pain or lower abdominal pain, fever or chills etc.) visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal since May to October 2013.

Total 1480 mid stream urine samples collected from the patients (out patients and in patients) suspected of urinary tract infections were cultured by the semi-quantitative culture technique [11]. The patients having laboratory or radiological evidence of other infections as the cause of the symptoms, patients with urinary catheterization and those who already have received antibiotics were excluded from our study. The bacterial isolates from the urine samples were identified by using microbiological techniques as described in the *Bergey’s* manual which include morphological appearance of the colonies, staining reactions and biochemical properties. The antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion technique as recommended by clinical and laboratory standards institute (CLSI) [12].

**Detection of ESBL producers**

Among the uropathogens isolated from suspected cases of UTI, only the strains of *E. coli* were subjected for detection of ESBL production. The phenotypic confirmation of the ESBL producing strains was done by combined disk method (Fig. 1) [12].

**Detection of biofilm producers**

Detection of biofilm production was done for both ESBL positive and ESBL negative strains of *E. coli* by Congo red agar method (CRA) (Fig. 2) [13].

On the basis of the intensity of color change of CRA medium after inoculation of the organisms, which is directly proportional to the amount of biofilm produced...
by the organisms, the biofilm producing organisms were
classified into three categories as strong biofilm pro-
ducers, moderate biofilm producers and weak biofilm
producers [14, 15].

Data analysis
SPSS version 16.0 statistical software package was used
for statistical analysis. Chi-square test was applied.
P-value < 0.05 was considered statistically significant.

Results
Out of 1480 mid stream urine samples 278, (18.8 %)
samples showed significant growth (≥ 10^5 cfu/ml). E.
coli was isolated from 208 (74.82 %) samples. Out of
208 E. coli isolates 69 (33.2 %) were found to be ESBL
producing organisms and 139 (66.8 %) were ESBL non
producers. Among 208 E. coli isolates 42 (20.19 %) were found
to be strong biofilm producers, 42 (20.19 %) were
moderate biofilm producers, 24 (11.538 %) were weak
biofilm producers and 100 (48.076 %) were biofilm
non producers.

Antibiotic resistance pattern of E. coli among biofilm
producers and biofilm non producers
The antibiotic resistance among biofilm producing E. coli
was found significantly higher than that of biofilm non
producing E. coli (p < 0.05). The correlation between
biofilm production and antibiotic resistance was found
statistically significant (p < 0.05) in most of the antibiotics
ciprofloxacin, ofloxacin, norfloxacin, amikacin, gentami-
cin, cotrimoxazole, cephalaxin, cefixime, cefazidime, cefo-
taxime, ceftriaxone and cepfepime) but the correlation was
not found to be significant in case of amoxicillin and
nitrofurantoin (Table 1).

Antibiotic susceptibility pattern of the uropathogenic E.
coli isolated
Of total 208 E. coli isolates, the highest numbers of
the strains were susceptible to amikacin followed by
nitrofurantoin, gentamicin, ceftriaxone and cefepime.
Similarly least numbers of the strains were susceptible
to amoxicillin (Table 1).

Association of ESBL production and biofilm formation
among E. coli isolates
Out of 69 ESBL producing uropathogenic strains of E. coli,
20 (29 %) were strong biofilm producers, 22 (31.9 %) were
moderate biofilm producers, 11 (15.9 %) were weak
biofilm producers and 16 (23.2 %) were biofilm non
producers. Whereas among 139 ESBL non producing E. coli,
22 (15.8 %) were strong biofilm producers, 20 (14.4 %)
were moderate biofilm producers, 13 (9.4 %) were weak
biofilm producers and 84 (60.4 %) were biofilm non
producers. The ability of biofilm formation was found to
be significantly higher in ESBL producing strains of E. coli
than that in ESBL non producing strains (p < 0.05).

Discussion
Among 208 E. coli isolates, 108 (51.92 %) were biofilm
producers. This finding agrees with the findings of differ-
ent authors from different parts of the world [16, 17].
Biofilm protects bacteria from host defense mechanisms,
along with the antibiotics [18].
In this study, the antibiotic resistance of biofilm produ-
cing E. coli was found significantly higher than that of
biofilm non producing E. coli (p < 0.05). Among biofilm
producing E. coli, higher antibiotic resistance was observed
in strong and moderate biofilm producers. The association
between biofilm production and antibiotic resistance was
found to be statistically significant (p < 0.05) except in case
of amoxicillin and nitrofurantoin. Microorganisms growing
in a biofilm are intrinsically resistant to many antibiotics
increasing the antibiotic resistance up to 1000 folds and
high antimicrobial concentrations are required to inactivate
organisms growing in a biofilm [19, 20]. This may be
because of the insufficient concentration of the antibiotics
reaching some areas of the biofilms and metabolic inactive-
ness (along with the presence of active antibiotic degrad-
ation mechanisms contributing to halt the accumulation of
the drugs up to an effective concentration) of the bacteria
located at the base of the biofilms [9].
The biofilm forming ability was found to be significantly
higher in ESBL positive strains of uropathogenic E. coli
than that of ESBL negative strains (p < 0.05). The study by
Subramanian et al. in India also reported the higher ability
of the ESBL producing organisms to form biofilm in
comparison to that of ESBL non-producing isolates. It has
been postulated that during occurrence of the large
numbers of the chromosomal gene rearrangements upon
acquisition of the ESBL plasmids the bacteria express several virulence genes [21].

ESBLs are enzymes that are responsible for resistance of bacteria toward third generation cephalosporins and monobactams [22]. Most of the plasmids responsible for ESBL production carry genes encoding resistance to other drugs also [23]. Due to frequent presence of cross-resistance to several other classes of antibiotics (like aminoglycosides and fluoroquinolones), in ESBL-producing organisms, the treatment of the infections by these bacteria are often present as the therapeutic challenges [22]. Further higher ability of the ESBL producing organisms to form biofilm makes the treatment even more difficult, increasing the mortality and severity of the infections [21]. Macro- lides (erythromycin, clarithromycin, and azithromycin) are known to have antibiofilm activity against biofilm producing organisms by inhibiting a key component of the biofilm, alginate. And several studies have recommended, the combined therapy (being macrolides one of the first antibiotics chosen) as the treatment of choice in infections caused by biofilm producing organisms [9].

Increasing irrational and haphazard use of antibiotics, sales of substandard antibiotics and transmission of drug resistant bacteria among people may be responsible for the rise in antibiotic resistance among the bacteria [24]. Antimicrobial resistance has become a serious global public health issue. Infections caused by drug resistant bacteria are responsible for increased morbidity and mortality [25]. The selection of the antibiotics for treatment of the bacterial infections should be based on culture and sensitivity reports.

Conclusion
The ability of biofilm formation was found higher among ESBL producing strains of E. coli. There was higher resistance rate among biofilm producing E. coli isolates to almost all the antimicrobial agents except a few. According to our antimicrobial susceptibility pattern for E. coli, to start preliminary treatment for UTI in Nepal, we recommend to use amikacin or nitrofurantoin. Further, for the treatment of the UTI, the antibiotics should be selected on the basis of the urine culture and sensitivity report.

Limitations of the study
Due to lack of easy availability of the advanced laboratory in Nepal and due to lack of the fund we could not confirm the ESBL producing and biofilm producing organisms by using molecular technology.

Abbreviations
ATCC: American type culture collection; CLED: Cystine lactose electrolyte deficient agar; CLSI: Clinical and laboratory standards institute; CRA: Congo red agar; ESBL: Extended spectrum beta lactamase; MHA: Mueller Hinton agar; SPSS: Statistical package for the social sciences; UTI: Urinary tract infection.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
SN designed the study, collected and processed the samples and analysed the data, NDP designed the study, analysed the data and prepared the manuscript, SK analysed the data, RC and MRB monitored the study. All authors read and approved the final manuscript.

Table 1 Antibiotic resistance pattern of E. coli among biofilm producers and non producers along with the antibiotic susceptibility pattern of all the E. coli isolates

| Antibiotics used | Resistance pattern of biofilm producers and biofilm non producers | Total Resistant | Total susceptible |
|------------------|---------------------------------------------------------------|----------------|------------------|
|                   | Strong producers N = 42                                      |                |                  |
|                   | Moderate producers N = 42                                     |                |                  |
|                   | Weak producers N = 24                                         |                |                  |
|                   | Non producers N = 100                                         |                |                  |
| Ciprofloxacin     | 32 (76.2)                                                    | 45 (45.0)      | 126 (60.6)       |
|                   | 32 (76.2)                                                    |                | 82 (39.4)        |
| Ofloxacin         | 30 (71.4)                                                    | 39 (39.0)      | 113 (54.3)       |
|                   | 30 (71.4)                                                    |                | 95 (45.7)        |
| Norfloxacin       | 33 (78.6)                                                    | 45 (45.5)      | 126 (60.6)       |
|                   | 32 (76.2)                                                    |                | 82 (39.4)        |
| Gentamicin        | 24 (57.1)                                                    | 26 (26.0)      | 82 (39.4)        |
|                   | 26 (61.9)                                                    |                | 126 (60.6)       |
| Amikacin          | 7 (16.7)                                                     | 5 (5.0)        | 26 (12.5)        |
|                   | 11 (26.2)                                                    |                | 182 (87.5)       |
| Cotrimoxazole     | 27 (64.3)                                                    | 17 (70.8)      | 42 (42.0)        |
|                   | 29 (69.0)                                                    |                | 93 (44.7)        |
| Amoxicillin       | 40 (95.2)                                                    | 22 (91.7)      | 186 (89.4)       |
|                   | 37 (88.1)                                                    |                | 22 (10.6)        |
| Cephalexin        | 41 (97.6)                                                    | 22 (91.7)      | 175 (84.1)       |
|                   | 38 (90.5)                                                    |                | 33 (15.9)        |
| Cefixime          | 37 (88.1)                                                    | 20 (83.3)      | 157 (75.5)       |
|                   | 41 (97.6)                                                    |                | 51 (24.5)        |
| Cefazidime        | 31 (73.8)                                                    | 15 (62.7)      | 122 (58.7)       |
|                   | 36 (85.7)                                                    |                | 86 (41.3)        |
| Cefotaxime        | 34 (81.1)                                                    | 14 (58.3)      | 117 (56.2)       |
|                   | 34 (81.0)                                                    |                | 91 (43.8)        |
| Ceftriazone       | 29 (69.0)                                                    | 15 (62.5)      | 100 (48.1)       |
|                   | 28 (66.7)                                                    |                | 108 (51.9)       |
| Cefepime          | 31 (73.8)                                                    | 12 (50.0)      | 103 (49.5)       |
|                   | 27 (64.3)                                                    |                | 105 (50.5)       |
| Nitrofurantoin    | 12 (28.6)                                                    | 6 (26.0)       | 57 (27.4)        |
|                   | 11 (26.2)                                                    |                | 151 (72.6)       |
Acknowledgements
The authors would like to thank all who contributed directly or indirectly in
conduction of this research.

Author details
1 Central Department of Microbiology, Tribhuvan University, Kirtipur,
Kathmandu, Nepal. 2 Department of Microbiology, Grande International
Hospital, Dhapasi, Kathmandu, Nepal. 3 Department of biochemistry, CIST
College, Kathmandu, Nepal. 4 Shree Birendra Hospital, Chhauni, Kathmandu,
Nepal.

Received: 17 December 2015 Accepted: 9 February 2016
Published online: 15 February 2016

References
1. Ramesh N, Sumathi CS, Balasubramanian V, Palaniappan KR, Kannan VR.
Urinary tract infection and antimicrobial susceptibility pattern of
extended spectrum beta lactamase producing clinical isolates. Adv Biol Res. 2008;2:78–82.
2. Dromigny JA, Nabeth P, Perrier Gros Claude JD. Distribution and
susceptibility of bacterial urinary tract infection in Dakar, Senegal.
Int J Antimicrob Agents. 2002;20:339–47.
3. Gupta K. Increasing antimicrobial resistance and the management of
uncomplicated community acquired urinary tract infections. Int J Antimicrob. 2001;135:41–50.
4. Nicolle LE. Uncomplicated urinary tract infection in adults including
uncomplicated pyelonephritis. Urol Clin North Am. 2008;35:1–12.
5. Bhatta CP, Shrestha B, Khadka S, Swar S, Shah B, Pun K. Etiology of
urinary tract infection and drug resistance cases of uropathogens.
J Kath Med coll. 2012;2:114–20.
6. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA.
Multidrug-resistant urinary tract isolates of E. coli: prevalence and
patient demographics in the United States. J Antimicrob Chemother. 2001;45:1402–6.
7. Goldstein FW. Antibiotic susceptibility of bacterial strains isolated from
patients with community-acquired urinary tract infections in France.
Multicentre Study Group. Eur J Clin Microbiol Infect Dis. 2000;19:112–7.
8. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy
associated with extended-spectrum –lactamase production in
Enterobacteriaceae bacteremia: a systemic review and meta-analysis.
J Antimicrob Chemother. 2007;60:913–20.
9. Soto SM. Importance of biofilms in urinary tract infections: new therapeutic
approaches. Adv Biol. 2014;2014(2014):543974.
10. Rajaveer M, Muller-Premru M, Zakotnik B, Bertok DZ. Virulence factors and
biofilm production among Escherichia coli strains causing bacteremia of
urinary tract origin. J Med Microbio. 2008;57:1329–34.
11. Cheesbrough M. District laboratory practice in tropical countries, part II.
2nd ed. New York: Cambridge university press; 2006. p. 112–3.
12. Clinical Laboratory Standards Institute. (CLSI) CLSI document M100S-S22.
Performance standards for antimicrobial susceptibility testing: Twenty
second informational supplement ed. Wayne: CLSI, 2012.
13. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of
biofilm formation among the clinical isolates of staphylococci: an evaluation of
three different screening methods. Indian J Med Microbio. 2006;24:25–9.
14. Poovendran P, Vidyha N, Murugan S. Antimicrobial susceptibility pattern of
ESBL and non-ESBL producing uropathogenic Escherichia coli (UPEC) and
their correlation with biofilm formation. Int J Microbiol Res. 2013;4:56–63.
15. Chriistenso G, Simpson WA, Bisno AL, Beachey EH. Adherence of slime
producing strains of Staphylococcus epidermidis to smooth surface. Infect
Immun. 1982;37:318–26.
16. Anandkumar H, Sahan G, Vinodkumar CS, Rao A, Srinivasa H.
Detection of Cell Surface Hydrophobicity and Biofilm formation
among ESBL and non-ESBL producing uropathogenic Escherichia coli.
J Med Educ. Res. 2012;2:12–20.
17. Nair BT, Bhat KG, Shantaram M. In vitro biofilm production and virulence
factors of uropathogenic Escherichia coli. Int J Pharm Bio Sci. 2013;4:951–6.
18. Hanna A, Berg M, Stout V, Razatos A. Role of capsular colanic acid in adhesion
of uropathogenic Escherichia coli. Appl Environ Microbiol. 2003;69:4474–81.
19. Thien-fah UK, George PA. Mechanism of biofilm resistance to antimicrobial
agents. Trends Microbiol. 2001;9:34–9.
20. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms.
Lancet. 2001;358:135–8.
21. Subramanian P, Umadevi S, Kumar S, Stephen S. Determination of
relation between biofilm and extended spectrum β-lactamases
producers of Enterobacteriaceae. Scho Res J. 2012;2:2–6.
22. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of
plasmid-mediated AmpC-b lactamases in Enterobacteriaceae lacking
chromosomal AmpC-b lactamases. J Clin Microbiol. 2005;43:3110–3.
23. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases a clinical
update. Clin Microbiol Rev. 2005;18:657–86.
24. Gautam R, Chapaqain ML, Acharya A, Rayamajhi N, Shrestha S, Ansari
S, et al. Antimicrobial susceptibility patterns of Escherichia coli from various
clinical sources. JCMC. 2013;114–7.
25. Awasthi TR, Pant ND, Dahal PR. Prevalence of Multidrug Resistant Bacteria
in Causing Community Acquired Urinary Tract Infection Among the Patients
Attending Outpatient Department of Seti Zonal Hospital, Dhangadi, Nepal.
Nepal J Biotechnol. 2015;3(1):55–9.