



# Correlation of Multidrug Resistance and Plasmid Profile in Extended Spectrum Beta Lactamase Producing *Escherichia coli* Isolated from Patients Suspected of Urinary Tract Infection

Vivek Kumar Singh<sup>1\*</sup>, Mahesh Kumar Chaudhary<sup>2</sup> and Reshma Tuladhar<sup>3</sup>

<sup>1</sup>Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal.

<sup>2</sup>Department of Microbiology, KIST MCTH, Imadole, Lalitpur, Nepal.

<sup>3</sup>Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal.

## Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/BJMMR/2016/21451

### Editor(s):

(1) Shashank Kumar, Department of Biochemistry, University of Allahabad, Allahabad, India.

### Reviewers:

(1) Charbell Miguel Haddad Kury, Municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil.

(2) Ilham Zahir, University Sidi Mohamed Ben Abdellah, Morocco.

(3) M. Luisa Jordao, National Institute of Health Dr Ricardo Jorge, Portugal.

(4) Teresita Sainz-Espuñes, Universidad Autónoma Metropolitana-Xochimilco, Mexico.

Complete Peer review History: <http://sciencedomain.org/review-history/11764>

Original Research Article

Received 18<sup>th</sup> August 2015  
Accepted 14<sup>th</sup> September 2015  
Published 9<sup>th</sup> October 2015

## ABSTRACT

**Aims:** To determine the prevalence of extended spectrum beta lactamase producing *Escherichia coli* (ESBL *E. coli*) strains among the total isolates and study the association between the antibiotic resistance and plasmid profiles of the isolates.

**Methodology:** A total of 1258 urine samples were collected. Identification of Bacterial isolates was done using standard biochemical tests, antibiotic susceptibility pattern was determined using the Kirby - Bauer's disk diffusion method and confirmation of the ESBL *E. coli* was done following CLSI guidelines. Isolation of Plasmid DNA of ESBL positive strains was done by alkaline lysis method.

**Results:** Out of 303 isolates, 198 were *E. coli*. The isolates were tested for antibiotic sensitivity, MDR, ESBL and plasmid profiles. 59.09% of the *E. coli* isolates exhibited multi-drug resistance. 41(58.57%) out of 76 ESBL *E. coli* isolates possessed plasmids. Few isolates possessed single

\*Corresponding author: Email: [viveksingh1424@gmail.com](mailto:viveksingh1424@gmail.com);

plasmid while other had multiple plasmids with different size ranged from 1 kb to 10 kb.

**Conclusions:** High prevalence of ESBL *E. coli* was found with good association between the antibiotic resistance and plasmid profiles of the isolates.

*Keywords: ESBL; MDR; plasmid; susceptibility; urinary tract infection.*

## 1. INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent infectious diseases, with a substantial financial burden on society. UTIs account for more than 100,000 hospital admissions annually, most often for pyelonephritis [1]. Microorganisms can reach the urinary tract by haematogenous or lymphatic spread, but there is abundant clinical and experimental evidence to show that the ascent of microorganisms from the urethra is the most common pathway that leads to a UTI, especially organisms of enteric origin (e.g. *Escherichia coli* and other *Enterobacteriaceae*).

Beta Lactam antibiotics are commonly used to treat bacterial infections. The groups of antibiotics in this category include penicillins, cephalosporins, carbapenems & monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of,  $\beta$ -Lactamases mediated bacterial resistance, which subsequently led to the development of Extended Spectrum Beta Lactamase (ESBL) producing bacteria. ESBLs are enzymes that mediate resistance to extended spectrum e.g., third generation cephalosporins as well as monobactams such as aztreonam [2]. These enzymes catalyze the hydrolysis of the,  $\beta$ -lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs have been reported worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa* [3]. However, these are most common in *Klebsiella pneumoniae* & *E. coli* [4]. ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community [5].

The first plasmid mediated,  $\beta$ -lactamase in Gram-negative bacteria, TEM-1, was described in the early 1960s [6]. Afterwards it was detected from *Klebsiella* in Europe 1980, in Germany 1983, and in France 1985 [7]. Genetic control of

beta-lactamase production resides either on plasmids or on the chromosome, while expression is either constitutive or inducible [8].

The development of extended spectrum cephalosporins in the early 1980s was regarded as a major addition to our therapeutic armamentarium in the fight against beta-lactamase mediated bacterial resistance. The emergence of *Escherichia coli* and *Klebsiella pneumoniae* resistant to ceftazidime & other cephalosporins seriously compromised the efficacy of these life saving antibiotics [7]. Drug resistance is mostly plasmid mediated and the situation is worsening due to the evolution of drug resistance plasmid genes.

There is a large reservoir of resistant genes, in bacterial genomes and in extra-chromosomal pieces of DNA (plasmids) that encode different mechanisms of drug resistance [9]. The transmission of antibiotic resistance, often to several drugs simultaneously, from one bacterium to another is attributed to plasmids. Understanding antibiotic resistance patterns and molecular characterization of plasmids is epidemiologically useful [10]. Although conventional antimicrobial susceptibility testing methods are useful methods for detecting resistance profiles and for selecting potentially useful therapeutic agents, they are insensitive tools for tracing the spread of individual strains within a hospital or region. Molecular methods like plasmid profiling provide powerful tools to track bacterial strains and contribute to the evaluation of nosocomial infection outbreaks, recurrent infection and clonal dissemination of specific pathogens [11]. They are also used as a means of providing additional information, to detect and evaluate the mode of dissemination of MDR pathogens [12]. Plasmid analysis has also proved a useful method for differentiating bacterial isolates [13,14]. The number and size of the plasmids present is used as the basis for strain identification. This strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections and community acquired infections [15] caused by a variety of species of Gram negative rods.

The ESBLs producing organisms are important causes of nosocomial infections. Due to the increase of different resistance mechanisms the treatment options are narrowing against Gram-negative bacteria.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

This study was a cross sectional study.

### 2.2 Place and Duration of Study

Central Department of Microbiology, Tribhuvan University and Department of Microbiology, KIST Medical College Teaching Hospital from 2013 to 2014.

### 2.3 Specimen Collection

The patient was given a sterile, dry, wide-necked leak-proof Hi-Media container of 100ml capacity for collection of 10-20 ml of clean-catch mid-stream urine (CC-MSU). The patient was given instructions for the collection of CC-MSU.

### 2.4 Culture of Specimen

The urine sample was cultured onto the MacConkey agar and Blood agar medium by the semi-quantitative culture technique using a standard loop.

### 2.5 Identification of the Isolates

Identification of significant isolates was done by using microbiological techniques which involves morphological appearance of the colonies, staining reactions and biochemical properties [16,17,18].

### 2.6 Antibiotic Susceptibility Testing

The antibiotic sensitivity testing was performed according to the recommended Kirby-Bauer sensitivity testing method [2].

### 2.7 Quality Control for Test

Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. During identification of organism, for each test ATCC

positives and negatives controls was taken simultaneously. *E. coli* ATCC 25922 was used as negative control for quality control of ESBL tests. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4mm and the pH at 7.2-7.4. Similarly antibiotic discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures. The quality of each test was maintained by using the products of Merck.

### 2.8 Screening Test for ESBLs

Isolates were screened for ESBL production by using disc diffusion of cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CTR) and Aztreonam (AT) placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations.

### 2.9 Confirmatory Test for ESBLs

Phenotypic confirmatory test for ESBL producers was done by combination disc method for all the ESBL producing isolates as per CLSI 2013 guidelines.

In this test a disc of ceftazidime (30 µg), cefotaxime (30 µg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10 µg) were used for each isolates. Both the discs were placed 25 mm apart, center to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37°C. Increase in 5 mm or more zone diameter for the antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

Interpretation: When there is an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime with Clavulanic acid (30/10 µg) versus the inhibition zone diameter around Ceftazidime disk (30 µg) alone, it confirms ESBL production.

### 2.10 Plasmid Profiling

Plasmid DNA was isolated from ESBL isolates according to Sambrook & Russel [19]. *E. coli* isolates were subcultured in Luria-Bertani broth at 37°C and 3 ml of overnight culture was subjected to plasmid DNA extraction by centrifugation at 5000 rpm for 5 min. After

washing in Tris- ethylene diamine tetraacetic acid (EDTA) buffer, the pellet was added to the freshly prepared mixture of NaOH and SDS, to which Potassium acetate was added. The microfuge tube was inversely mixed and centrifuged at 13,000 rpm for 10 min. The supernatant was transferred into a new microfuge tube with adding Phenol: Chloroform and centrifuged at 13,000 rpm for 10 min. The upper aqueous phase was collected in a clean microfuge tube to which Chloroform was added and centrifuged at 13,000 rpm for 10 min. The supernatant was transferred into a new microfuge tube and cold 95% ethanol was added to precipitate bacterial DNA. The pellet was washed with 70% ethanol and dissolved in 60  $\mu$ L of TE buffer.

0.8% agarose gel was prepared by melting 0.8 gm agarose in 100 ml of diluted TAE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) using a microwave oven. The melted agarose was allowed to cool to about 50°C and 0.5  $\mu$ g/ml ethidium bromide was mixed, shaken and was poured into gel tray, then a comb was placed. After solidification of the gel, the comb was removed. The gel was placed in a horizontal electrophoresis apparatus filled with TAE buffer.

Around 80 -150 micrograms per ml of isolated Plasmid DNA was mixed with 1  $\mu$ l of 6X gel loading buffer. The mixture was slowly loaded into the well using disposable micropipette tips. Marker of 1 Kbp molecular weight was loaded in one well to determine the size of the Plasmid DNA. Electrophoresis of the plasmid DNA was carried out at 100 volts for 2 hours.

The amplified products of the study samples were visualized by using UV trans-illuminator. The gel was photographed by a digital camera and transferred data to computer for further documentation [20-22].

### 2.11 Ethical Clearance

Institutional Ethical Committee clearance (ethical committee of KIST Medical College Teaching Hospital, Nepal) was obtained before starting this study.

### 2.12 Statistical Analysis

Statistical package for social sciences (SPSS) version 16 was used to analyze the data.

## 3. RESULTS

A total of 303 uropathogens were recovered from urine samples of inpatients and outpatients of the hospital, with their provisional diagnosis written on their requisition form received from various wards like Medicine, Surgery, Obstetrics and Gynecology, Dermatology, Nephrology, Pediatrics, Orthopedics and Intensive care units. Out of 1258 samples processed for culture, 303 (24.08%) samples showed significant bacteriuria (i.e.  $>10^5$  cfu/ml), while 955 (75.91%) samples showed insignificant growth (i.e.  $\leq 10^5$  cfu/ml), growth of contaminants or no growth. Sixteen different species of bacteria were isolated among which *E. coli* (198 out of 303 isolates) was predominant. 117 out of 198 *E. coli* were found to be MDR strains.

By the screening test ESBL production in *E. coli* was noticed in 98 (49.49%) isolates. Of the 98 ESBL screen positive isolates, 76 isolates were found to be ESBL producers by Combination disc method. Most of the bacteria showed ESBL positive by both combination (Ceftazidime/ Ceftazidime: clavulanic acid, and Cefotaxime/ Cefotaxime: clavulanic acid). *E. coli* showed maximum ESBLs production in ceftazidime/ ceftazidime: clavulanic acid (CAZ/CAZC) combination.

All the antibiotics used were more resistant in ESBL producers. Among them ciprofloxacin, ampicillin and nalidixic acid were more resistant, on the other hand amikacin (98.43%) and imipenem (100%) were more sensitive.

### 3.1 Plasmid Profiling

Seventy six isolates of ESBL *E. coli* isolated from urine samples of UTI suspected patients were screened for plasmids. The overall frequency of *E. coli* isolates containing plasmids was 53.9% (41 isolates where 1-7 plasmids were detected), while thirty five strains (46.1%) lacked any plasmid. Some strains contained plasmids of  $>10$  kb, but mainly the isolated plasmids ranged from 1 kb to 10 kb. The plasmid distributions of the isolates and antimicrobial resistance pattern are shown in Table 1 containing single plasmid, Table 2 containing two plasmids, Table 3 containing three plasmids, Table 4 containing four plasmids, Table 5 containing five plasmids, Table 6 containing six plasmids and Table 7 containing seven plasmids respectively. Up to 7 plasmids occurred in the isolates with sizes ranging from 1.0 kb to 10.0 kb. The abbreviations

used in the tables for the antibiotics are ampicillin (Amp), amoxicillin/clavulanic acid (Amc), ciprofloxacin (Cip), cotrimoxazole (Cot), gentamycin (Gen), nalidixic acid (NA), nitrofurantoin (Nit), norfloxacin (Nx) and ofloxacin (Of).

**Table 1. Plasmid profiles and antimicrobial resistance of 11 isolates of *E. coli* harbouring 1 plasmid**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	10	Amp, Cot, Amc
2	2	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
3	1	Amp, Gen, Nit, NA, Cot, Amc,
4	6	Amp, Nit, NA, Cot, Amc,
5	1	Amp, NA, Nx, Cip, Cot, Amc, Of
6	1	Amp, NA, Nx, Cip, Cot, Amc, Of
7	10	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
8	10	Amp, NA, Nx, Cip, Cot, Amc, Of
9	10	Amp, Gen, Amc
10	2	Amp, NA, Nx, Cip, Cot, Amc, Of
11	10	Amp, NA, Nx, Cip, Cot, Amc, Of

**Table 2. Plasmid profiles and antimicrobial resistance of 10 isolates of *E. coli* harbouring 2 plasmids**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	1,10	Amp, Nit, NA, Nx, Cip, Cot, Amc, Of
2	3,4	Amp, Gen, NA, Nx, Cip, Cot, Amc, Of
3	3,4	Amp, NA, Amc
4	4,6	Amp, Nit, NA, Cot, Amc
5	2,10	Amp, Nit, NA, Nx, Cip, Cot, Amc, Of
6	2,10	Amp, Gen, NA, Nx, Cip, Cot, Amc, Of
7	1,2	Amp, Nit, Gen, NA, Nx, Cip, Cot, Amc, Of
8	3,4	Amp, Nit, NA, Nx, Cip, Cot, Amc, Of
9	1,2	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
10	1,10	Amp, NA, Nx, Cip, Cot, Amc, Of

**Table 3. Plasmid profiles and antimicrobial resistance of 8 isolates of *E. coli* harbouring 3 plasmids**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	2,4,6	Amp, NA, Nx, Cip, Cot, Amc, Of
2	1,2,10	Amp, Nit, NA, Nx, Cip, , Amc, Of
3	2,3,7	Amp, Gen , NA, Nx, Cip, Cot, Amc, Of
4	2,4,6	Amp, Nit, NA, Nx, Cip, Cot, Amc, Of
5	5,7,10	A mp, NA, Amc
6	7,8,10	Amp, NA, Nx, Cip, Amc, Of
7	1,2,10	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
8	1,2,10	Amp, Gen, NA, Nx, Cip, Cot, Amc, Of

**Table 4. Plasmid profiles and antimicrobial resistance of 3 isolates of *E. coli* harbouring 4 plasmids**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	2,4,6, >10	Amp, NA, Nx, Cip, Cot, Amc, Of
2	1,3,4,10	Amp, NA, Nx, Cip, Cot, Amc, Of
3	1,2,3,4	Amp, NA, Nx, Cip, Amc, Of

**Table 5. Plasmid profiles and antimicrobial resistance of 3 isolates of *E. coli* harbouring 5 plasmids**

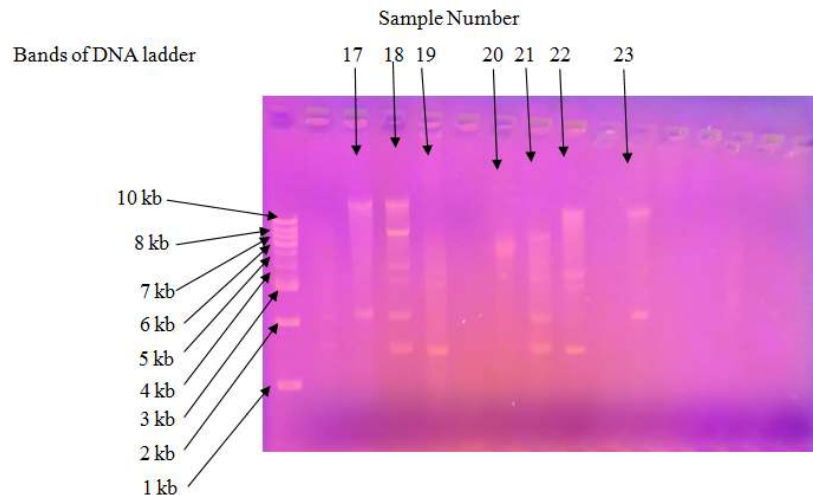
S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
3	3,4,5,6,7	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
5	3,4,5,6,7	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
21	1,2,3,4,7	Amp, Gen, NA, Nx, Cip, Cot, Amc, Of

**Table 6. Plasmid profiles and antimicrobial resistance of 4 isolates of *E. coli* harbouring 6 plasmids**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	1,2,3,4,7,10	Amp, NA, Nx, Cip, Cot, Amc, Of
2	1,2,3,4,8,10	Amp, Gen, Nit, NA, Nx, Cip, Cot, Amc, Of
3	1,2,3,4,10,>10	Amp, NA, Nx, Cip, Cot, Amc, Of
4	1,2,6,7,8,10	Amp, Gen, NA, Nx, Cip, Amc, Of

**Table 7. Plasmid profiles and antimicrobial resistance of 2 isolates of *E. coli* harbouring 7 plasmids**

S. no.	Plasmid sizes (kb)	Drugs bacteria resistant to
1	1,2,3,4,7,10,>10	Amp, NA, Nx, Cip, Cot, Amc, Of
2	1,2,5,6,7,8,10	Amp, Gen, NA, Nx, Cip, Cot, Amc, Of

**Photograph 1. Plasmid profiles of ESBL *E. coli***

#### 4. DISCUSSION

The prevalence of UTI in the population was 24.08%. This is higher than the 20.2% rate recorded by Getachew [23], and 21.8% rate recorded by Mahmood [24]. It is lower than prevalence rate of 60% significant bacteriuria recorded by Kolawole [25] and 80% by Durgesh et al. [26]. The high prevalence may be due to genuine population susceptibility because factors like sexual intercourse, pregnancy, low socio-economic status, are common among Nepali male and female.

Resistance rates to commonly used antimicrobial agents have increased over the years among common uropathogens and this resistance rates vary from country to country. Among the 198 *E. coli* isolates, 117 (59.1%) isolates were MDR-strains.

Of the 198 *E. coli* isolates, 98 (49.5%) were resistant to ceftriaxone and all were subjected for phenotype confirmatory test for ESBL, combination disc method. Total 76 isolates were confirmed as ESBL producers.

In the study, resistance to third generation cephalosporins was found to coexist with resistance to antibiotics like ampicillin, nalidixic acid, ciprofloxacin, ofloxacin, amoxicillin/clavulanic acid as also reported by several studies indicating multidrug resistant pattern. Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids [27-32].

Above 90% ESBL positive isolates were found resistant to ampicillin, quinolones, fluoroquinolones and amoxicillin/clavulanic acid. 100% sensitivity to imipenem and 98.43% sensitivity to amikacin advocates the usage of amikacin and carbapenem antibiotics as the therapeutic alternative to beta-lactam antibiotics as indicated in many studies [32,33]. Nitrofurantoin was considered the most effective of the drugs that can be orally administered; which represent an alternative for oral empiric therapy of uncomplicated UTI. Nitrofurantoin in this study demonstrates excellent activity against UPEC isolates i.e. 68%; both in complicated and uncomplicated UTIs and also in community acquired and hospital acquired infections. The study showed good co-resistance between nitrofurantoin, fluoroquinolones and ESBL. Moreover, the production of ESBLs has been associated with decreased susceptibility to nitrofurantoin [34-36]. As in UPEC infection, rising rates of resistance to antimicrobial drugs limits the choice of drugs that can be used to treat infections with these potent pathogens.

Aztreonam was found 100% resistant in this study, which correlates with the study done by Sasirekha et al. [37]. Most of the ESBL producing organisms were found to be coresistance to fluoroquinolones, aminoglycosides and cotrimoxazole, which correlates with the study done by Denholm [38] and Jabeen [39]. This was due to the genes encoding these  $\beta$ -lactamases were often located on large plasmids that also encode genes for resistance to others antibiotics, including aminoglycosides, tetracycline, sulfonamides, trimethoprim and chloramphenicol [7]. We found such associated resistance with fluoroquinolones 84.4%.

In this study we used two combinations with clavulanic acid (CAZ/CAZC and CTX/CTXC) and found that *E. coli* showed maximum ESBLs production in CAZ/CAZC combination, which correlates with other studies [40,41].

By definition, plasmids do not carry genes essential for the growth of host cells under non-stressed conditions but most plasmids confer positively selectable phenotypes including antimicrobial resistance genes among others [42]. Plasmid profiling is also an important tool for epidemiological typing and has got diagnostic value as well. In view of these results, the studies on *E. coli*, focusing on the changes on molecular level, could provide valuable insights for its management. Thirty five strains (46.1%) do not contained plasmid whereas in 41 strains (53.9%), 1-7 plasmids were detected. Three strains contained plasmids of >10 kb, but mainly the isolated plasmids ranged from 1 kb to 10 kb. In this study, plasmids of molecular weight 2 kb was most common among isolates. Clinical isolates of *E. coli* are known to harbour plasmids of different molecular size ranging from 2-3 kb to 6.5 kb and maximum 26 kb. Danbara [43] reported the plasmid size between 3.9 kb and 50kb in *E. coli* strains isolated from patients suffering from traveler's diarrhea. All the plasmid containing strains were resistant to ampicillin (100%). Majority of the isolates (92.7%) with plasmid showed multidrug resistance.

## 5. CONCLUSIONS

High prevalence of extended spectrum beta lactamase producing *Escherichia coli* (ESBL *E. coli*) strains was found among the total isolates. The study showed good association between the antibiotic resistance and plasmid profiles of the isolates as the plasmid containing strains showed high resistance towards different antibiotics.

## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Foxman B. Epidemiology of urinary tract infections: Incidence, morbidity and economic costs. Am J Med. 2002;113(1):5-13.

2. Wayne PA. CLSI, Performance standards for antimicrobial susceptibility testing, twenty-third informational supplement, CLSI Document M100-S23. Clinical and Laboratory Standards Institute; 2013.
3. Friedman C, Callery S, Jeanes A, Piaskowski P and Scott L. Best Infection Control Practices for Patients with Extended Spectrum Beta Lactamase Enterobacteriaceae. International Infection Control Council; 2005.
4. Agrawal P, Ghosh A, Kumar S, Basu B and Kapil K. Prevalence of extended spectrum, lactamases among *E. coli* and *K. pneumoniae* isolates in tertiary care hospital. *Ind J Patho Microbiol.* 2008;51:139-142.
5. Pitout JDD, Gregson DB, Church DL, Elsayed S and Laupland KB. Community-wide outbreaks of clonally related CTX-M-14 Beta-Lactamase-Producing *Escherichia coli* Strains in the Calgary Health Region. *J Clin Microbiol.* 2005;43:2844-2849.
6. Datta N, Kontomichalou P. Penicillinase synthesis controlled by infectious r factors in *Enterobacteriaceae*. *Nature.* 1965; 208:239-241.
7. Perez F, Endimiani A, Hujer KM and Bonomo RA. The continuing challenge of ESBLs. *Cur Opin in Pharma.* 2007;7:459-469.
8. Chiang CS and Liaw GJ. Presence of  $\beta$ -lactamase gene TEM-1 DNA Sequence in Commercial Taq DNA Polymerase. *J of cli Microbiol.* 2005;43:530-531.
9. Soulsby L. Resistance to antimicrobials in humans and animals. *Br J Med.* 2005;331:1219-1220.
10. Hassan SH. Sensitivity of Salmonella and Shigella to antibiotics and chemotherapeutic agents in Sudan. *J Trop Med Hyg.* 1985;88:243-248.
11. Sader HS, Pfaller MA, Hollis RJ. The use of molecular techniques in the epidemiology and control of hospital infectious. *Clin Lab Med.* 1995;15:407-431.
12. Pfaller MA, Ehrhardt AF, Jones RN. Frequency of pathogen occurrence and antimicrobial susceptibility among community-acquired respiratory tract infections in the respiratory surveillance program study: Microbiology from the medical office practice environment. *Am J Med.* 2001;111:4-12.
13. Waschmut IK, Griffin PM and Wells JG. *Escherichia coli* O157:H7, a cause of hemorrhagic colitis and hemolytic uremic syndrome. *Acta Paediatr Jpn.* 1991;33:603-612.
14. Dorn CR, Silapanuntakul R, Angrick EJ. Plasmid analysis and epidemiology of Salmonella Enteritidis infection in three commercial layer flocks. *Avian Dis.* 1992;36:844-851.
15. Fornasini M, Reeves RR and Murray BE. Trimethoprim resistant *Escherichia coli* in households of children attending day care centers. *J Infect Dis.* 1992;166:326-330.
16. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 11th edition, Mosby, Inc. USA; 2002.
17. Cheesbrough M. Medical laboratory manual for tropical countries microbiology, 1st ELBS ed. Cambridge: University Press; 1984.
18. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney practical medical microbiology. 14<sup>th</sup> Edition, Churchill Livingstone, New York, USA; 1999.
19. Sambrook J, Russell DW. Molecular cloning. A Laboratory Manual, 3rd edition, CSHL press; 2001.
20. Sharma J, Sharma M, Roy P. Detection of TEM and SHV genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian J Med.* 2010;132:332-336.
21. Lal P, Kapil A, Das BK, Sood S. Occurrence of TEM and SHV gene in extended spectrum beta lactamases (ESBLs) producing *Klebsiella* spp. isolated from a tertiary care hospital. *Ind J Medica.* 2007;125:173-178.
22. Pagani L, Amico ED, Migliavacca R, DiAndrea MM, Giacobone E, Amicosante G, Romero E, Rossolini GM. Multiple CTX-M-Type Extended-Spectrum,  $\beta$ -Lactamases in Nosocomial Isolates of *Enterobacteriaceae* from a Hospital in Northern Italy. *J of Clinical Microbiol.* 2003;41:4264-4269.
23. Getachew T. Bacterial pathogens implicated in causing urinary tract infection (UTI) and their antimicrobial susceptibility pattern in Ethiopia Revista CENIC. Centro Nacional de Investigaciones Cientificas Cuba. 2010;41:1-6.
24. Mahmood MA. Prevalence and antimicrobial susceptibility of pathogens in urinary tract infections. *J of Al-Nahrain University.* 2011;14(4):146-152.
25. Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade



- KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital. *Intl J of Med and Med Sciences*. 2009; 1:163-167.
26. Wasnik DD, Tumane PM. Prevalence and antibacterial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. *Asian J of Biomed and Pharma Sciences*. 2012;2(15):1-3.
27. Levy SB. Microbial resistance to antibiotics an evolving and persistent problem. *Lancet*. 1982;2:83–88.
28. Levy SB. The challenge of antibiotic resistance. *Sci. Am*. 1998;278:46–53.
29. Watanabe T. Infective heredity of multidrug resistance in bacteria. *Bacteriol Rev*. 1963;27:87–115.
30. Barber M. Infection by penicillin resistant Staphylococci. *Lancet*. 1948;2:641–644.
31. Crofton J and Mitchison DA. Streptomycin resistance in pulmonary tuberculosis. *Br. Med. J*. 1948;2:1009–1015.
32. Bradford PA. Extended-spectrum  $\beta$ -lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. *Clin Microbiol Rev*. 2001;14:933-951.
33. Al-Agamy MH, Ashour ME, Wiegand I. First description of CTXM  $\beta$ -lactamase-producing clinical *Escherichia coli* isolates from Egypt. *Int. J. Antimicrob. Agent*. 2006;27:545-548.
34. Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother*. 2009;53:2227–2238.
35. Laupland KB, Church DL, Vidakovich J, Mucenski M, Pitout JD. Community-onset extended-spectrum  $\beta$ -lactamase (ESBL) producing *Escherichia coli*: Importance of international travel. *J Infect*. 2008;57:441–448.
36. Rodriguez-Bano J, Navarro MD. Extended-spectrum  $\beta$ -lactamases in ambulatory care: A clinical perspective. *Clin Microbiol Infect*. 2008;14:104–110.
37. Sasirekha B, Manasa R, Ramya P, Sneha R. Frequency and antimicrobial sensitivity pattern of extended spectrum  $\beta$ -Lactamases Producing *E. coli* and *Klebsiella pneumoniae* Isolated In A Tertiary Care Hospital. *Al Ameen J Med Science*. 2010;3:265-271.
38. Denholm JT, Huysmans M, Spelman D. Community acquisition of ESBL-producing *Escherichia coli*: A growing concern. *Med J Australia*. 2009;190:45-46.
39. Jabeen K, Zafar A, Hasan R. Frequency and sensitivity pattern of extended spectrum beta lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. *J Pakistan Med Associat*. 2005;55:436-439.
40. Rahman MM, Haque JA, Hossain MA, Sultana R, Islam F, AHM Islam S. Prevalence of extended spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka Bangladesh. *Intl J of Antimicrob Agents*. 2004;24:508-510.
41. Thomson KS, Sanders CC and Washington JA. High-level resistance to cefotaxime and ceftazidime in *Klebsiella pneumoniae* isolates from C level and, Ohio. *Antimicrob Agents Chemother*. 1991;35:1001-1003.
42. Carattoli A. Plasmids in Gram negatives: Molecular typing of resistance plasmids. *Int. J. Med. Microbiol*. 2011;301:654–658.
43. Danbara H, Kamase IY, Shinahawa M, Arita H, Makino A, Yashikawa M. *Microbial Path*. 1987;3:269-278.

© 2016 Singh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:  
The peer review history for this paper can be accessed here:  
<http://sciedomain.org/review-history/11764>