Case Report

Recurrent urinary tract infection due to co-infection with extended spectrum β -lactamase-producer uropathogenic *Escherichia coli* and enteroaggregative *E. coli*

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Introduction: Urinary tract infections (UTIs) are the most common bacterial infections in women of all ages. The emergence of resistance to newer classes of antibiotics, including third generation cephalosporins, has limited the drug choices for treatment of UTI. We report here a recurrent UTI due to co-infection with multiple-antibiotic-resistant (MAR) *Escherichia coli* pathotypes.

Case presentation: A 73-year-old woman with diabetes mellitus type 2 presented with fever, nausea, vomiting, burning sensation, painful and frequent urination. She was diagnosed with recurrent UTI (RUTI) due to co-infection with pansensitive enteroaggregative *E. coli* (EAEC) and MAR extended spectrum β -lactamase (ESBL)-producing uropathogenic *E. coli* (UPEC) and treated with azithromycin and levofloxacin (each for 10 days). Unfortunately, she did not respond. Plasmid profile analysis showed that the MAR *E. coli* strain harboured multiple plasmids including ~38 and ~80 MDa plasmids. PCR for detection of β -lactamase genes showed that the isolate was negative for blaTEM, blaSHV, blaOXA and blaCTX-M. To the best of our knowledge, this is the first report of co-infection with an EAEC and MAR ESBL-producer UPEC in Bangladesh.

Conclusion: We recommend that all pathotypes of *E. coli* as well as the other more common uropathogens should be considered in the diagnosis of RUTI and multiple antibiotics should be prescribed only in severe conditions.

Keywords: Bangladesh; EAEC; ESBL; multiple antibiotic resistance; recurrent urinary tract infection.

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Introduction

Urinary tract infections (UTIs) are frequent bacterial infections worldwide and the most commonly encountered bacterial infection, particularly in women. It is estimated that ~150 million cases of UTI occur worldwide, as many as 50 % of women and 12 % of men experience at least one symptomatic UTI during their lives and ~25 % of affected women have recurrent UTI (RUTI) (Foxman, 2010). Non-pathogenic *E. coli* are widely distributed in nature and an important part of the normal flora in the human gastrointestinal tract. *E. coli* causing UTI are classified as uropathogenic *E. coli* (UPEC); these strains account for 75–90 % of uncomplicated UTIs but are also an important aetiological agent of bacteraemia, sepsis and meningitis (Russo & Johnson, 2000; Yamamoto, 2007).

There are 14 serogroups of UPEC that are most frequently found in UTIs and 75 % of UTIs are caused by serogroups O4, O6, O14, O22, O75 and O83 (Li *et al.*, 2010). O2, O4, O6 and O75 are the most common serogroups causing UTIs worldwide (George & Manges, 2010). Outbreaks due to particular UPEC strains have been reported from different parts of the world (George & Manges, 2010), indicating that these strains are capable of causing infections in the elderly and the immunosuppressed, including people with diabetes, HIV and chronic heart disease (Foxman, 2010). UPECs have distinct virulence factors, including adhesins, toxins, cytotoxins, siderophores and surface components, that enable them to colonize and invade the urinary tract (Foxman, 2010). Colonization can lead to development of a quiescent intracellular reservoir in the bladder. Activation of this quiescent intracellular reservoir results in RUTIs (Anderson *et al.*, 2003).

The increasing incidence of UTIs due to extended spectrum β -lactamase (ESBL)-producing *E. coli* is an emerging threat and of great concern throughout the

Abbreviations: EAEC, enteroaggregative *Escherichia coli*; ESBL, extended spectrum β -lactamase; MAR, multiple-antibiotic-resistant; UTI, urinary tract infection; RUTI, recurrent UTI; UPEC, uropathogenic *E. coli*.

globe (Foxman, 2010). In addition, the ESBL-producing strains are also acquiring resistance to other, non- β -lactam antibiotics, which limits the empiric choice of treatment (Falagas *et al.*, 2010; Foxman, 2010). However, limited data are available on UTIs caused by co-infection with ESBL-producing UPEC and enteroaggregative *Escherichia coli* (EAEC) in older people with diabetes in Bangladesh.

It is important that all health-care professionals should be aware of co-infection with multiple-antibiotic-resistant (MAR) UPEC and EAEC owing to their potential for life-threatening complications. Here, we present a case report of an elderly woman with RUTI due to mixed infection with EAEC and MAR ESBL-producing *E. coli*.

Case presentation

A 73-year-old woman with diabetes mellitus type 2 and hypertension who came to Bangladesh on vacation with her family presented with fever, headache, fatigue, body ache, nausea, vomiting, abdominal pain, morning rise of temperature, shivering, polyuria, dysuria, pain in the pelvic area

and back, and cloudy urine. Her past medical history was significant for recurrent UTIs for 15 years. She had a history of taking the following medications for the last 8 years: astatine (20 mg) (serastatin), lisinopril (5 mg), oxycodone–acetaminophen, calcium and gabapentin (300 mg). She also had insulin (8 units per day) for the last 3 years.

At the time of admission her blood pressure was 180/80 mmHg, her pulse rate was 80 beats min^{-1} , her respiration rate was 32 breaths min^{-1} , and her body temperature was 36.7 °C. Her abdomen was slightly tender but not distended. Microbiological tests of blood and urine are shown in Table 1. As the patient had a history of allergy to sulfonamides and trimethoprim, the antibiotic azithromycin (400 mg twice a day) was initially started after a urine sample had been taken for bacteriological analysis. Urine culture revealed lactose-fermenting colonies on MacConkey agar and biochemical tests showed properties of *E. coli*. Two types of colony, typical of EAEC with *aaiC* and *aatA* genes and of UPEC, were further confirmed using API 20E.

Antimicrobial sensitivity tests showed that the EAEC was pansensitive while the MAR UPEC strain was resistant to

Table 1. Biochemical analysis of blood sample and routine microscopic analysis of urine sample of the patient

Test	Result	Reference value for female	Unit
Blood sample			
Haemoglobin	13	11.5–47.0	g dl^{-1}
ESR (Western method)	14	≤ 15	mm in 1st hour
Red blood cell count	4.39	3.5–5.5	10^{12} l^{-1}
Total leucocyte count	6.01	4.0–11.0	10^9 l^{-1}
Differential leucocyte count			
Neutrophil	57.0	40–75	%
Band	0	00–04	%
Lymphocyte	31.8	20–45	%
Monocyte	5.7	02–10	%
Eosinophil	5.2	01–06	%
Basophil	0.3	00–01	%
Platelet	228	150.0–450.0	10^9 l^{-1}
Sodium	138.2	135–146	mmol l^{-1}
Potassium	4.1	3.5–5.3	mmol l^{-1}
Chloride	107.5	97–106	mmol l^{-1}
Glucose [F]	5.1	4.2–6.1	mmol l^{-1}
Glucose [2ABF]	4.3	4.2–7.8	mmol l^{-1}
Hba1c	7.33	4–6.2	%
Serum creatinine	54.9	44–97	$\mu\text{mol l}^{-1}$
Total cholesterol	5.6	3.6–6.3	mmol l^{-1}
HDL cholesterol	1.3	0.90–16.8	mmol l^{-1}
LDL cholesterol	3.9	1.7–4.5	mmol l^{-1}
Triglyceride	0.9	0.4–1.5	mmol l^{-1}
Total cholesterol/HDL ratio	4.26	<4.4	
LDL/HDL ratio	2.95	<2.9	
Urine sample			
Red blood cell cell count	1–2		hpf $^{-1}$
Pus cell cell count	Numerous		hpf $^{-1}$
Epithelial cell cell count	3–5		hpf $^{-1}$

Abbreviations: HDL, high-density lipoprotein; hpf, high power field; LDL, low-density lipoprotein.

ampicillin, amoxicillin–clavulanate, cotrimoxazole, ciprofloxacin, ceftriaxone, cefixime and norfloxacin but susceptible to amikacin, azithromycin, gentamicin, mecillinam, imipenem, meropenem and nitrofurantoin. After 10 days of azithromycin treatment, a culture of urine was positive for only the MAR UPEC strain. At that point, levofloxacin (500 mg per day) was started and continued for 10 days. Unfortunately, the patient also did not respond to levofloxacin. Several days after this, she flew home with her family. In a telephone call 1 month later, she conveyed that she had not recovered from the illness. She confirmed that she continued taking cranberry juice as medication and had started to use vaginal Vagifem (oestradiol) tablets to prevent vaginal dryness.

Discussion

E. coli is a very diverse species of bacteria found naturally in the intestinal tract of all humans and animals. Diarrheagenic *E. coli* are classified into six major pathotypes, each with distinct phenotypic and genetic make-up (Rasko *et al.*, 2011). The diarrheagenic pathotypes of *E. coli* are also diverse in terms of disease potential and age and host specificity. *E. coli* can cause a range of human diseases, including UTI, neonatal meningitis and sepsis. UTIs are the most common form of extra-intestinal infections with *E. coli*. *E. coli* is the most common aetiology of UTIs and is responsible for 80–90 % of community-acquired UTIs. UPEC are different from the normal flora of *E. coli* in terms of better adaptability to living within the urinary tract and evading the host's immune response (Foxman, 2010). UPEC are very heterogeneous in nature. UPEC strains harbour several pathogenicity islands, which may encode adhesins, toxins, iron uptake systems, secretion mechanisms and capsules in order to enable them to develop successful UTI (Foxman, 2010). UPEC has multiple molecular pathways (for example, biofilm formation and urothelial cell invasion) that may contribute to pathogenesis. In addition, EAEC is the most recently identified diarrheagenic *E. coli* and is increasingly recognized as an emerging enteric pathogen that can cause infection in a wide range of age groups (Harrington *et al.*, 2009; Huang *et al.*, 2004). The annual incidence of EAEC infections ranges from 0.1 to 12 cases per 100 000 population. EAEC-mediated extra-intestinal disease was first reported by Erik and colleagues in 2012 (Boll, 2012). EAEC has virulence factors that can cause symptomatic UTI (Herzog *et al.*, 2013; Olesen *et al.*, 2012). In Bangladesh, the true incidence of EAEC infections is unknown. UTI due to UPEC is likely to be under-diagnosed due to improper laboratory diagnostic methods and lack of awareness of the epidemiologic significance. UPEC can spread efficiently from person to person via intimate activity or faecal–oral transmission (Foxman, 2010). Transmission might differ in different environmental settings (Huppertz *et al.*, 1997).

The first line of drugs to treat UTI includes ampicillin, cotrimoxazole and ciprofloxacin. However, emergence of resistance to these antimicrobial agents has complicated the situation and limits the empiric choice of treatment. The reported patient was allergic to cotrimoxazole; hence the treating physician initially prescribed her a macrolide antibiotic before doing culture and sensitivity tests. Prescribing antibiotics prior to getting sensitivity test results is a common phenomenon in low-resource countries like Bangladesh. This contributes to the emergence of multiple antibiotic resistance among enteric pathogens (Larsson *et al.*, 2000). A major concern regarding ESBL-producing *E. coli* is its high rate of co-resistance to non- β -lactam antibiotics, particularly quinolones, cotrimoxazole and aminoglycosides (Azap *et al.*, 2010). In this study, the EAEC strain was uniformly sensitive to all tested antimicrobial agents whereas the UPEC showed resistance to ampicillin, ciprofloxacin, ceftriaxone and cefixime. The patient also did not adequately respond to fluoroquinolone (levofloxacin) therapy. When tested for antimicrobial drug susceptibility using the disc diffusion method with commercially available discs, two different types of *E. coli* isolate were recovered. The first type of isolate, a MAR ESBL-producing *E. coli* strain, was resistant to β -lactams, including ampicillin, ceftriaxone (MIC $>64 \mu\text{g ml}^{-1}$) and cefixime. The other type of isolate was a pansensitive EAEC. ESBL production was confirmed using a double disc diffusion test as described elsewhere (Ahmed *et al.*, 2012). The isolates were also resistant to amoxicillin–clavulanate, cotrimoxazole, ciprofloxacin and norfloxacin, but susceptible to amikacin, azithromycin, gentamicin, mecillinam, imipenem, meropenem and nitrofurantoin. Although the patient's physician first prescribed azithromycin for 10 days and later levofloxacin for another 10 days, unfortunately the patient did not recover by the time she returned home. Alternative antibiotic choices for treatment of patients with ESBL-producing bacterial infection are limited. Carbapenems remain the most effective drugs of choice, but these antibiotics need to be administered by an intravenous or intramuscular route (DeBusscher *et al.*, 2009). PCR specific for β -lactamase resistance genes of the ceftriaxone-resistant isolate showed that this strain was negative for *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA} and *bla*_{CTX-M} genes. This finding indicates that ESBL production in the MAR UPEC strain might be due to another class of genes.

Plasmid profile analysis of cefixime-resistant *E. coli* and all sensitive EAEC strains showed that both strains harboured an ~ 80 MDa plasmid which is common in *E. coli* (Talukdar *et al.*, 2013). In addition, the multidrug-resistant UPEC strain also harboured plasmids 38, 3.4 and 2.5 MDa in size (Figure 1). These plasmids contribute to resistance to different classes of antibiotics (Talukdar *et al.*, 2013). The MAR *E. coli* strain persisted in the reported patient despite appropriate treatment with antimicrobial drugs. Thus, because of the long duration of carriage of this highly resistant strain, potential for dissemination to others is high. The infrequency of earlier reports on

Fig. 1. Agarose gel electrophoresis of plasmid DNA of *E. coli*. Lanes: A, *E. coli* R-1 and Sa; B, *Shigella flexneri* 1c; C, MAR ESBL-producer *E. coli*; D, EAEC; E, *E. coli* PDK-9; F, *E. coli* V-517.

community-acquired ESBL-mediated infection in the United States does not suggest a high prevalence of these pathogens in the community. The wider distribution of ESBL-producer bacteraemia in patients visiting emergency departments has been reported by other investigators (Lee *et al.*, 2012). Although regularly culturing urine samples for microbiological analysis is difficult, without continuous surveillance to detect and control ESBL-producer pathogens, the frequency of these pathogens can only be expected to increase.

The current lack of global routine surveillance systems for detecting EAEC and ESBL-producer UPEC, likely rendering the burden of these organisms under-reported, makes it difficult to address the extent to which these aetiological agents are causing extra-intestinal infection. To the best of our knowledge, our study is the first report of direct detection of co-infection of MAR ESBL-producer UPEC

and EAEC due to RUTI in a diabetic patient in Bangladesh. Due to the high prescription of antibiotics for humans and livestock animals, antibiotic resistance has been increased in UPEC strains, and we recommend using antibiotics only in severe conditions and applying multi-antibiotic prescriptions.

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