British Microbiology Research Journal
15(6): 1-11, 2016, Article no.BMRJ.27393
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Multidrug Resistance Phenotype and Plasmid Profiling of *Escherichia coli* Isolates Causing Urinary Tract Infections in North East Part of Bangladesh

Md. Fazle Rabbee[1], Musammat Kulsuma Begum[1], Md. Jahidul Islam[1], Parveen Afroz Chowdhury[2], Osul Ahmed Chowdhury[3], Fatema Tuj Zohora[1], Pijush Sutradhar[1], Kamrul Islam[1] and Abul Kalam Azad[1*]

[1]Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh.
[2]Department of Dermatology and Venereal Diseases, Sylhet Womens Medical College Hospital, Mirboxtolla, Sylhet, Bangladesh.
[3]Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AKA and PAC conceived the project. Author AKA designed the work, supervised all procedures and wrote the manuscript. Authors MFR, PAC and OAC were involved in sample collection and processing. Authors MFR, MKB and MJI equally contributed by carrying out the work and analyzed results with the close supervision of authors AKA and KI. Authors FTZ and PS did some experiments with the supervision of author AKA. Authors MFR, MKB and PAC participated in writing the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/27393
Editor(s):
(1) Vijay Kumar Eedunuri, Greehey Children's Cancer Research Institute, UT Health Sciences Center, San Antonio,Texas, USA.
Reviewers:
(1) Oshodi, Adebola John, University of Ilorin, Ilorin, Nigeria.
(2) Ronald Bartzatt, University of Nebraska, USA.
Complete Peer review History: http://sciencedomain.org/review-history/15398

Original Research Article

Received 31st May 2016
Accepted 27th June 2016
Published 14th July 2016

ABSTRACT

Aim: Urinary tract infections (UTIs) caused by *Escherichia coli* have become a significant worldwide public health concern and the situation is now worsening by the ability of bacteria to develop resistance to antimicrobial agents. The main aim of our study is to determine the probable link of antibiotic resistance patterns with plasmid profile of *E. coli* isolates causing Urinary tract infections (UTIs).

**Corresponding author: E-mail: dakazad-btc@sust.edu;*

Study Design: The design of this study was to i) identify bacteria causing UTIs, ii) investigate antimicrobial susceptibility, iii) analyze plasmid profiling and iv) find possible link between of antimicrobial susceptibility and plasmid profile.
Place and Duration of Study: Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh, from January 2011 to January 2015.
Methods: Urine samples were collected from 94 patients suspected with UTI. Bacterial isolates from the infected urine samples were identified based on morphological, cultural and biochemical characteristics. Antibiogram of bacterial isolates was performed by standard disc diffusion method. Plasmid isolation of all *E. coli* isolates was done by mini alkalysis method. Plasmid profiling was visualized following agarose gel electrophoresis.
Results: Fifty patients of them were infected with UTI. Among the 50 UTI positive patients, 29 were infected with *E. coli.* All the *E. coli* isolates were resistant to amoxicillin and nalidixic acid. Overall resistant phenotype of *E. coli* isolates to cefixime, ciprofloxacin, ceftriaxone and azithromycin was 68.96%, 65.51%, 55.17% and 47.5% respectively. However, imipenem and gentamicin were found very effective as 96.55% and 82.75% of *E. coli* isolates were sensitive to these drugs, respectively. Approximately 90% of the *E. coli* isolates were resistant to three or more antibiotics and were defined as multidrug resistant (MDR). Plasmid profiling showed that multiple plasmids of different sizes between 24.5 and 0.5 kb were present in most of the MDR *E. coli* isolates. However, no plasmid was found in several MDR *E. coli* isolates.
Conclusion: Our data revealed that multidrug resistance pattern of *E. coli* isolates causing UTI was very alarming in Bangladesh and might be plasmid-mediated in most cases and also chromosomal DNA mediated in some cases.

Keywords: Antibiogram; MDR; plasmid profiling; UTI.

ABBREVIATIONS

AMX, Amoxicillin; ATCC, American Type Culture Collection; AZM, Azithromycin; CFM, Cefixime; CIP, Ciprofloxacin; CTR, Ceftriaxone; EMB, Eosin-methylene blue; GN, Gentamicin; MDR, Multi-Drug Resistant; NIT, Nitrofurantoin; OD, Optical density; SXT, Sulphamethoxazole; UTI, Urinary tract infection.

1. INTRODUCTION

Urinary tract infection (UTI) is an infection that affects part of the urinary tract. Clinically, UTI is the most frequently diagnosed bladder (cystitis) and kidney (pyelonephritis) disorders [1]. The term cystitis has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness. Pyelonephritis, an infection of the kidneys with potential for bacteremia, clinically presents with flank pain, fever, nausea, vomiting and malaise [2]. Acute pyelonephritis is a severe acute systemic infection caused by uropathogenic *E. coli* clones with virulence genes clustered on "pathogenicity islands" [3]. UTIs reach up to 150 million cases annually worldwide [4]. It is the most common in woman and accounts for significant morbidity and mortality, and health care costs [5-6].

The pathogens causing UTIs are almost always predictable, with *Escherichia coli* the primary etiologic agent among both outpatients and inpatients [7,8]. This organism is responsible for up to 90% of all community-acquired and almost 50% of nosocomial UTIs and it has clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections [4,9]. Many other groups of bacteria have also been implicated as causative agents of UTIs such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus spp. and Streptococcus spp* [10,11].

The therapeutics used in the treatment of UTIs, involves a short course of antibacterial drugs such as amoxicillin, cephalexin, clarithromycin, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin, norfloxacin, tetracyclines, sulphamethoxazole etc [12-17]. But problems arise when bacterial species acquired resistance against antibacterial drugs in course of infection. Bacteria can acquire antibiotic resistance in the presence of antibiotics through mutations, transfer of plasmids from resistant bacteria through conjugation, stimulation of the stress response system, horizontal gene transfer, transposition or by up taking extracellular DNA from milieu [18-20]. There are a number of reasons why bacterial resistance should be a concern for physicians. First, resistant bacteria

are becoming common in healthcare institutions [21]. Secondly, bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients [22].

Drug-resistant bacterial infections are on the rise all over the world. Bacteria are adapting to the commonly used antibacterial drugs and resistant strains are developing through a process of natural selection. The problem is exacerbated by several factors, including abuse, underuse or misuse of antimicrobials, poor patient compliance, and poor quality of available drugs. In Bangladesh, misuse of antibiotics is increasing day by day due to availability of pharmacies and increased prescribing rate of antibiotics by physicians for pleasing the patients [23]. There are many evidence of increasing antibiotic resistance in Bangladesh resulting from misuse of antibiotics [24,25] which leads to further increased cost of treatment, adverse drug effects and antibiotic resistant bacteria [26]. Consequently, antibiotic resistance or multidrug resistance pattern of *E. coli* causing UTIs in Bangladesh may vary from that in other countries. From clinical urine samples, we herein isolated and identified *E. coli* isolates and analyzed their antibiogram to study the pattern of antibiotic resistance or multidrug resistance of these isolates causing the UTI. We further performed their plasmid profiling to discuss the correlation between antibiotic resistance and the plasmids present in the isolates.

2. MATERIALS AND METHODS

2.1 Sample Collection

Midstream urine samples were collected in sterile containers from 94 patients suspected with UTI by physicians of three reputed medical college hospitals and medical services at North East region of Bangladesh. Patients were of different ages of both sexes. At each time of collection, precaution was taken to prevent cross-contamination of the sample. Patients were advised by the health care personnel to wash their hands first and then the penis or vulva and surrounding area four times, with front-to-back strokes, using a new soapy sponge each time. Each patient was also demonstrated how the midstream urine sample would be collected. Consent was obtained from each patient that he/she had no hostility to use the urine samples for research purpose. After collection, the urine samples in an insulated foam box with ice were transported immediately to the laboratory. Microbiological examinations were done as soon as they arrived at the laboratory.

2.2 Isolation and Identification of *E. coli* Isolates from Urine Samples

Urine specimens collected were cultured on duplicate plates of nutrient agar (OXOID Limited, Basingstoke, Hampshire, England) (1% peptone, 0.5% yeast extract, 0.5% NaCl and 1.5% agar; pH 7.0), eosin methylene blue (EMB) agar (OXOID Limited, Basingstoke, Hampshire, England) (1% peptone, 1% lactose, 0.2% K_2HPO_4, 0.04% Eosin Y, 0.0065% methylene blue, and 1.5% agar; pH 7.2) and MacConkey agar (OXOID Limited, Basingstoke, Hampshire, England) (1.7% peptone, 0.3% proteose peptone, 1% lactose, 0.5% NaCl, 0.15% bile salts, $0.1x10^{-3}$% crystal violet, 0.003% neutral red, and 1.5% agar; pH 7.2) at 37ºC for 18-24 hour. Bacterial isolates that produced green metallic sheen with black center colonies on EMB agar and red/pink colonies on MacConkey agar plates were presumptively assumed as the cultural characteristics of *E. coli*. These typical colonies were further sub-cultured on EMB agar to obtain pure culture of green metallic sheen with black center colonies. The pure culture of presumptive *E. coli* were sub-cultured on nutrient agar and then in nutrient broth to preserve in -80ºC for further studies. *Enterobacter aerogenes* is normally found on grains and plants but may be found in human and animal feces [27]. *E. coli* and *E. aerogenes* have close similarity in their morphological and cultural characteristics. In order to differentiate *E. coli* from *E. aerogenes* and other Gram-negative bacteria that cause UTIs, the presumptive *E. coli* isolates were subjected to further identification using Gram-staining and some biochemical tests. In brief, some of the biochemical tests used for identification of the *E. coli* were IMViC (Indole, Methyl Red,Voges-Proskauer and Citrate), triple sugar iron (TSI) and lactose fermentation tests. These biochemical tests were performed as described by Cappuccino and Sherman [28] and Bergey's Manual of Systematic Bacteriology [29].

2.3 Analyses of Antimicrobial Susceptibility of *E. coli* Isolates

Susceptibility of *E. coli* isolates to antimicrobial agents was determined by the modified Kirby-Bauer [30] disk diffusion method on Mullar-Hinton agar (OXOID Limited, Basingstoke, Hampshire, England) as recommended by Clinical and Laboratory Standard Institute

Antimicrobial Susceptibility Testing [31]. *E. coli* ATCC25922 was run in parallel to compare the antibiotic susceptibility of *E. coli* isolates. Diluted fresh broth cultures of *E. coli* isolates of exponential growth phase with the same OD_{600} were homogenously spread on Mullar-Hinton agar plates. It was verified by viable growth analysis that each cell suspension had similar viable cell count. The liquid part of the cell suspension on the plate was allowed to be absorbed onto the media and the antibiotic disks (antibiotics which are usually prescribed in Bangladesh for the UTI patients) were then aseptically placed on the media. The disks were pressed gently with sterile needle so that there was good contact between the antibiotic discs and the agar surface. The antimicrobial disks (OXOID Limited, Basingstoke, Hampshire, England) used in this study were amoxicillin (AMX), azithromycin (AZM), cefixime (CFM), ceftriaxone (CTR), ciprofloxacin (CIP), gentamicin (GN), imipenem (IPM), nitrofurantion (F), nalidixic acid (NA) and sulphamethoxazole (SXT). The plates were incubated at 37ºC overnight. After incubation, the plates were observed and the diameters of the zone of complete inhibition were measured in mm. The zone of inhibition was analyzed to determine the pattern of antibiogram in accordance to the manufacture's instruction.

2.4 Extraction and Purification of Plasmids

Plasmids were extracted by alkaline lysis method [32] from 16 hour cultured cells of *E. coli* isolates and *E. coli* ATCC25922. All the plasmids were then subjected to electrophoresis onto 0.7% agarose gel using standard protocol [33,34]. The gels stained with ethidium bromide were visualized and recorded on an Ultraviolet Transilluminator (UVP, High Performance transilluminater; USA and canon camera, PowerShort A3200 IS). The number and molecular sizes of the plasmid DNA were determined on the basis of mobility through agarose gel compared to that of the standard DNA ladders (Genei Pvt. Ltd., Bangalore, India).

3. RESULTS

3.1 Isolation and Identification of *E. coli* Isolates Causing UTI

Cultural analysis of the 94 urine samples used in this study revealed that 50 samples (approximately 50%) were infected with significant number of bacteria. All the samples that showed growth on nutrient agar revealed the growth on EMB and MacConkey agar plates. This result indicated that all 50 UTI patients might be infected with Gram-negative bacteria since EMB and MacConkey agar usually inhibits the growth of Gram-positive bacteria. Among the 50 UTI positive patients, 19 (38.0%) were male and 31 (62.0%) were female, indicating that females are more infected than males. About 46 and 33% of the UTI positive patients were in age between 61-80 and 41-60 years, respectively. Rest of the UTI positive patients were at the age of below 41 years. This result indicated that the emergence of UTI raised with the increase in age of the patient. When these bacterial isolates were subjected to identification based on cultural characteristics on EMB and MacConkey agar plates and biochemical tests, *E. coli* were identified in 29 UTI positive patients (58%). All *E. coli* isolates produced small green metallic sheen with black center colonies on EMB agar and red or pink colonies on MacConkey agar plates (Fig. 1). On EMB agar, although *E. coli* usually produces green metallic sheen colonies, *E. aerogenes* may rarely shows metallic sheen [27]. However, *E. aerogenes* generally produces large pinkish mucoid colonies and no other bacteria causing UTI produce green metallic sheen colonies. On MacConkey agar, *Klebsiella* and *E. aerogenes* produce large mucoid pinkish colonies, and *Pseudomonas* and *Proteus* that may also cause UTIs produce colorless colonies. Bacterial isolates that were presumptively identified as *E. coli* based on cultural characteristics on EMB and MacConkey agar were motile and Gram-negative, and were further identified based on some biochemical characteristics (Table 1). All these isolates could produce indole from tryptophane in indole test and sufficient acid from glucose in methyl red test. In contrast, these isolates were unable to produce acetylmethylcarbinol in a glucose peptone medium in Voges-Proskauer test and to utilize citrate as sole carbon source in citrate test. Table 1 further showed that all the presumptive *E. coli* isolates were positive for catalase and triple sugars iodine tests but negative for oxidase test. All of these biochemical tests supported the notion that the 29 presumtive isolates were *E. coli*.

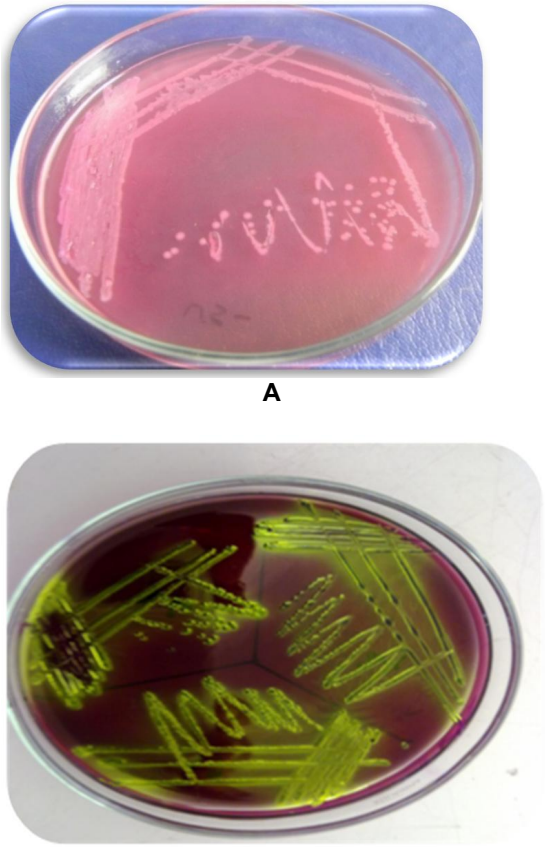
3.2 Antibiogram of *E. coli* Isolates

To compare the antibiogram of *E. coli* isolates with that of *E. coli* ATCC25922, disks of ten antimicrobials which are usually prescribed for UTI patients in Bangladesh were used. The zone of inhibition produced by these antimicrobial

disks against *E. coli* ATCC25922 and *E. coli* isolates (Fig. 2) were compared with the standard clear zone diameters of these disks against enterobacteriaceae (Table 2) [31]. However, the *E. coli* ATCC25922 strain was sensitive to all the ten antimicrobial disks. In contrast, 55.17%, 65.51%, 68.96%, 55.55% and 47.5% *E. coli* isolates were resistant to CTR, CIP, CFM, SXT and AZM respectively. In addition, all *E. coli* isolates were resistant to NA. Analysis of antimicrobial resistance profiles showed that all of the *E. coli* isolates were resistant to more than one antibiotics and more than 90% isolates were MDR (Table 4). MDR was defined as resistance to three or more of the antimicrobial agents tested. The majority of MDR *E. coli* isolates were resistant to five or more antimicrobial agents. Antibiogram analysis showed that almost 97 and 83% of *E. coli* isolates were sensitive to IPM and GN, respectively. This result indicated that among the ten antimicrobial agents used in this study, only IPM and GN were the most successful drugs.

3.3 Plasmid Profiling of *E. coli* Isolates

Electrophoresis of extracted plasmid onto agarose gel demonstrated that 26 of 29 *E. coli* isolates (~90%) contained plasmid DNA (Fig. 3 and Table 4). Most of the isolates showed 1 to 8 plasmid bands with sizes ranging from 1.5 to 24.5 kb. The most common plasmid of 24.5 kb was observed at least in 24 isolates. At least 10 *E coli* isolates harbored only the 24.5 kb plasmid. Eight plasmid DNA bands were observed in isolates 5, 15 and 29, and seven and five bands were observed only in isolates 4 and 25, respectively. Table 4 further showed that four plasmid DNA bands were found in four isolates; three bands in four isolates; and two bands in three isolates. However, no plasmid band was observed in isolates 18, 23 and 27.



A

B

Fig. 1. Cultural characteristics of *E. coli* isolates on MacConkey agar (A) and EMB agar (B). A typical observation of a repeated experiment (n=4) is shown

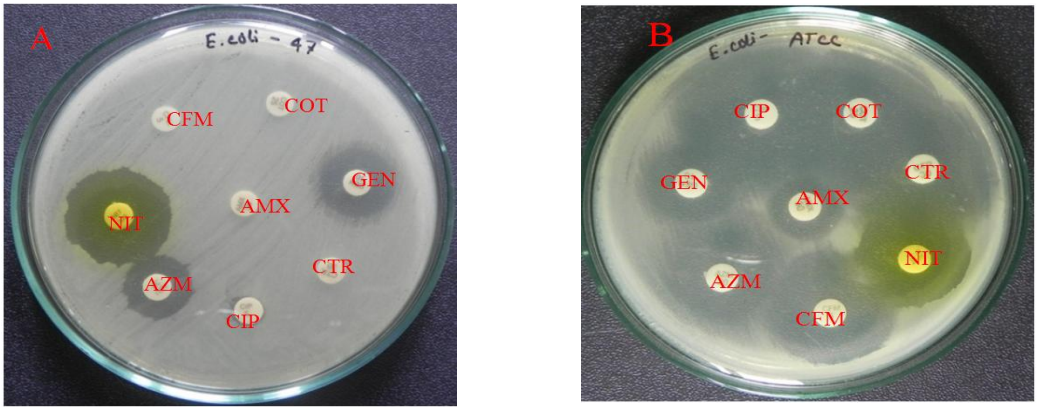


Fig. 2. Antibiotic susceptibility pattern of a *E. coli* isolate (A) and *E. coli* ATCC 25922 (B). A typical antibiogram of three independent experiments is shown

Table 1. Biochemical tests that were used for identification of *E. coli* isolates causing UTI

Isolates	M	G	IT	MR	VP	C	TSI	Ca	O
U-1	+	-	+	+	-	-	+	+	-
U-2	+	-	+	+	-	-	+	+	-
U-3	+	-	+	+	-	-	+	+	-
U-4	+	-	+	+	-	-	+	+	-
U-5	+	-	+	+	-	-	+	+	-
U-6	+	-	+	+	-	-	+	+	-
U-7	+	-	+	+	-	-	+	+	-
U-8	+	-	+	+	-	-	+	+	-
U-9	+	-	+	+	-	-	+	+	-
U-10	+	-	+	+	-	-	+	+	-
U-11	+	-	+	+	-	-	+	+	-
U-12	+	-	+	+	-	-	+	+	-
U-13	+	-	+	+	-	-	+	+	-
U-14	+	-	+	+	-	-	+	+	+
U-15	+	-	+	+	-	-	+	+	-
U-16	+	-	+	+	-	-	+	-	-
U-17	+	-	+	+	-	-	+	+	-
U-18	+	-	+	+	-	-	+	+	-
U-19	+	-	+	+	-	-	+	+	-
U-20	+	-	+	+	-	-	+	+	-
U-21	+	-	+	+	-	-	+	+	-
U-22	+	-	+	+	-	-	+	+	-
U-23	+	-	+	+	-	-	+	+	-
U-24	+	-	+	+	-	-	+	+	-
U-25	+	-	+	+	-	-	+	+	-
U-26	+	-	+	+	-	-	+	+	-
U-27	+	-	+	+	-	-	+	+	-
U-28	+	-	+	+	-	-	+	+	-
U-29	+	-	+	+	-	-	+	+	-

Here, M, Motility; G, Gram test; MR, Methyl red test; VP, Voge's-Proskauer; TSI, Triple sugar iron; IT, Indole test; C, Citrate test; Ca, Catalase test; O, Oxidase test. + and - indicate the positive and negative result of the test. E. coli isolates were numbered serially according to sample codes

Table 2. Standard diameters for clear zone of inhibition for *Enterobacteriacea* to determine resistant, intermediate sensitive and sensitive profiling [31]

Name of the antibiotics	Zone diameter (mm)		
	Resistant	Intermediate	Sensitive
AMX	≤ 13	14-17	≥18
IPM	≤13	14-15	≥16
AZM	≤13	14-17	≥18
CIP	≤15	16-20	≥21
NA	≤13	14-18	≥19
CTR	≤13	14-20	≥21
GN	≤12	13-14	≥15
F	≤14	15-16	≥17
SXT	≤10	11-15	≥16
CFM	≤15	16-18	≥19

3.4 Relation of Plasmid Profiling with Antibiotic Resistance Pattern of *E. coli* Isolates

Analysis of antibigrams of 29 *E. coli* isolates from patients of UTIs has demonstrated that the rate of resistance to AMX and NA is the highest among the antimicrobial agents used in this study. The 24.5 kb plasmid might be responsible for AMX resistance since all *E. coli* isolates harbored this plasmid and showed resistance to AMX. However, this plasmid might also be responsible for resistance to NA and other antibiotics because at least 10 of 29 isolates having only this plasmid DNA band were MDR (Table 4). Isolates showing resistant to CIP contained different sizes of plasmid DNA but a

plasmid ranging from 2.0 kb to 3.0 kb was found frequently. Isolates resistant to CFM and CTR had plasmids approximately ranging from 2.0 kb to 15.0 kb. On the other hand, three isolates in which no plasmid was observed were MDR.

4. DISCUSSION

Bacterial resistance to antibiotics is an increasing-problem worldwide [35]. This study was carried out to reveal the relationship between antibiotic resistance and plasmid profiles of *E. coli* isolates from different UTI patients. Identification of the causative organism and its susceptibility to antimicrobial agents is important, so that proper drug can be chosen to treat the patient in early stages of UTI [36].

Antibiotics used in the treatment of UTIs play an important role. However, antibiotic resistance which has been reported earlier [37,38] is increasing day by day due to excessive use and mal-administration of the antibiotics. We found very high frequency of resistance to antimicrobial agents normally used to treat UTI patients. It clearly indicated very limited possibilities of using these drugs in the treatment of UTI patients. Resistance of *E. coli* isolates to AMX and NA was so high (100%) that these should not be recommended for empirical treatment of UTI. Indeed, doctors in Bangladesh do not prescribe AMX currently for patients with UTIs. Third generation cephalosporins are very effective therapy and frequently used in the treatment of UTI patients. However, expression levels of extended-spectram β-lactamases are reducing the clinical utility of this class of antibiotics [39]. We herein observed considerable rate of resistance to CFM and CTR (Table 3), which are the therapeutics of UTIs. This might be because of possible acquision of genes for third generation cephalosporins [35]. *E. coli* isolates from UTI patients were found not only resistant to AMX and third generation cephalosporins but also resistant to CIP (Table 3). This might be due to emergence and spread of genes encoding CTX-M -type β-lactamases (class A) and AmpC-type β-lactamases (Class C) enzymes in these isolates [40-41]. However, resistance pattern of *E. coli* to IPM was only about 4%. This antimicrobial agent is highly stable against β-lactamase and has an unusual property of causing a post antibiotic effect on gram-negative bacteria [42].

Approximately 90% of *E. coli* isolates from UTI patients of the present study were MDR. In 2000, only 7.1% MDR *E. coli* were reported in patients of the USA [37], whereas in 2011, 77% MDR *E. coli* were reported in patients in Iran [43] indicating rapid and threatening emergence of antibiotic resistance in *E. coli* causing the UTI. However, the emergence of MDR *E. coli* causing UTI may vary depending on geographical locations, legislation for selling and practicing of antibiotics and also the quality of them. About 82% of MDR *E. coli* isolates harbored multiple plasmids of different sizes ranging from 24.5 to 3 kb. In these isolates, there might be a possible correlation between antibiotic resistance and number of plasmids (Table 4). These MDR *E. coli* isolates might be potential causes of infection due to potential reservoir of resistance genes that could be transferred between strains of the same or between different bacterial species of UTI pathogens including *Pseudomonas, Klebsiella* etc. by horizontal evolution [44]. However, the number of plasmids did not always correlated to the multidrug resistance (Table 4). The possible reason might

Table 3. Antibiotics susceptibility pattern of *E. coli* isolates from urine samples of UTI patients

Antibiotics	Concentr-ation (µg)	Sensitivity pattern of isolated *E. coli* isolates			Sensitivity pattern of *E. coli* ATCC strain		
		%R	%I	%S	%R	%I	%S
AMX	10	100	0.00	0.00	-	-	100
IPM	10	3.44	0.0	96.55	-		100
AZM	15	47.5	27.5	25.0	-	-	100
CIP	05	65.51	10.34	24.14	-		100
CFM	05	68.96	13.80	17.24	-	-	100
CTR	30	55.17	6.89	37.93	-	-	100
GN	10	10.34	6.89	82.75	-	-	100
NA	30	100	0.0	0.0	-	-	100
F	300	60	30	10	-	-	100
SXT	10	55.55	-	44.45	-	-	100

Here, R, Resistance; I, Intermediate Sensitive and S, Sensitive

Table 4. The correlation between antibiotic resistance and copy number of plasmid

E. coli isolates	Name of resistant antibiotics	No. of bands	Band sizes (kb)
01	CTR, CIP, AMX, CFM, AZM, NA, F	2	24.5, 2
02	AMX, NA, F	1	24.5
03	AMX,NA, F, CFM	1	24.5
04	CTR, CIP, NA, AMX, CFM, F	7	24.5, 7, 6, 5, 4, 2.8, 1.8
05	CTR, CIP, AMX, CFM, NA, F	8	24.5, 9, 7, 6.5, 5.2, 5.5, 2.8 , 2.2
06	CTR, CIP, AMX, CFM, AZM, NA, F	4	20, 3.8, 3.2, 2.8
07	AMX, CFM, NA	1	24.5
08	CTR, CIP, CFM, NA, AMX	4	15, 5, 3, 2.8
09	CTR, CIP, AMX, NA, CFM, GN, AZM	1	24.5
10	AMX, NA, CFM	1	24.5
11	AMX, NA	2	24.5, 1.6
12	CIP, AMX, NA	2	24.5 , 8.5
13	CTR, CIP, AMX,NA, CFM	4	24.5, 7, 2, 1.7
14	AMX,NA, F	1	24.5
15	CTR, CIP, NA, AMX, CFM ,SXT	8	24.5 , 9 ,8, 6.4,5.1, 2.8 ,1.8
16	CTR, CIP, AMX, NA, CFM ,SXT, F	1	24.5
17	CIP, AMX, CFM, NA, F	3	24.5, 10, 2
18	AMX, NA, F	0	No band found
19	CTR, CIP, AMX, CFM, NA	3	24.5, 2.8, 1.7
20	CIP, AMX, NA	3	24.5, 5.1, 2.8
21	CTR, CIP, AMX, CFM, NA	1	24.5
22	AMX, NA	4	24.5,9.1 , 6, 4
23	CTR, CIP, AMX, CFM, NA, F	0	No band found
24	CIP, AMX, NA, F	3	24.5, 9 , 7
25	CTR, CIP, AMX, CFM,GN,NA	5	24.5, 5, 4 ,2.8, 2
26	CTR, AMX, CFM, GN, NA	1	24.5
27	CTR, AMX, CFM, NA, F	0	No band found
28	IPM,CTR,AMX,CFM,GN,CIP, NA	1	24.5
29	AMX, NA	8	24.5, 15, 9, 6.6, 5.5, 4.5,3.5, 2.8

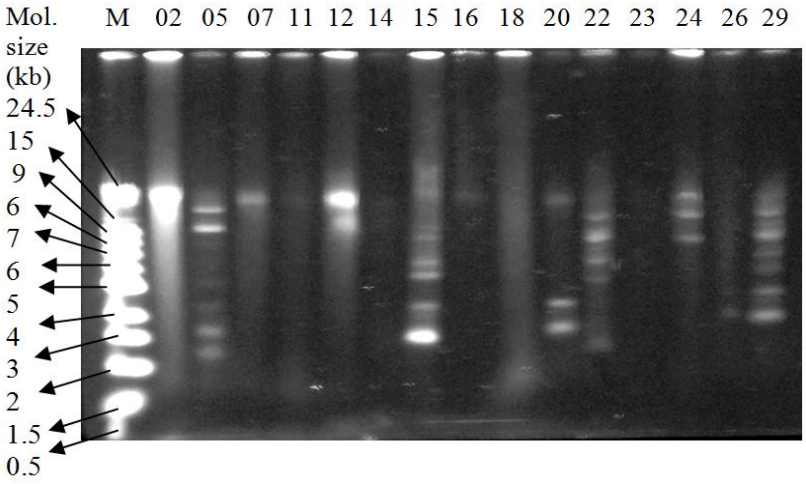


Fig. 3. A representative electrogram on agarose gel of plasmid obtained from *E. coli* isolates

The number above the agarose gel indicates the isolate number. M indicate DNA ladder with known molecular size of DNA fragment. The molecular size (kb) of the DNA ladder is indicated by arrows

be because of the presence of more than one antibiotic resistant genes in one plasmid band or one gene product might be responsible for resistance against three or more than three antibiotics. However, it is necessary to identify which plasmid is responsible for resistance

against a particular type of antibiotics or which enzymes are expressed by the isolates for antibiotic resistance property or multidrug resistance property. Currently we are performing research to find out the specific gene(s) in each plasmid to elucidate the emergence of antibiotic resistance or multidrug resistance property in *E. coli* isolates causing UTIs in Bangladesh. Again, few strains were MDR without having any plasmid. This result supports the notion that drug resistance or multidrug resistance to antimicrobials in some cases might also be chromosomal DNA-mediated.

5. CONCLUSION

Plasmid profile analysis with antibiogram is very important for differentiating MDR *E. coli* isolates. The antimicrobial susceptibility and resistance profile of all isolates in this study revealed that IPM and GN possess the higher efficacy while AMX and NA possess lower efficacy against *E. coli* isolates. Plasmid profiling of all isolates showed that the number of plasmids apparently had no relationship with multidrug resistance in all isolates as indicated by the observation that some plasmidless strains were MDR. As antibiotic resistance pattern in *E. coli* isolates are continuously evolving, surveillance studies are essential for safe and effective empiric therapy as well as to avoid treatment failure in the patients of UTI.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ejrnaes K, Sandvang D, Lundgren B, Ferry S, Holm S, Monsen T, et al. Pulsed-field gel electrophoresis typing of *Escherichia coli* strains from samples collected before and after pivmecillinam or placebo treatment of uncomplicated community-acquired urinary tract infection in women. J Clin Microbiol. 2006;44(5):1776-81.
2. Rasko DA, Phillips JA, Li X, Mobley HL. Identification of DNA sequences from a second pathogenicity island of uropathogenic *Escherichia coli* CFT073: Probes specific for uropathogenic populations. J Infect Dis. 2001;184(8):1041-9.
3. Welch R, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, et al. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. Proc Natl Acad Sci. 2002;99(26):17020-17024.
4. Gupta K, Sahm DF, Mayfield D, Stamm WE. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: A nationwide analysis. Clin Infect Dis. 2001;33(1):89-94.
5. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrob Agents Chemother. 2002;46(8):2540-5.
6. Stamm WE, Norrby SR. Urinary tract infections: Disease panorama and challenges. J Infect Dis. 2001; 183(Supplement 1):S1-S4.
7. Kariuki S, Revathi G, Corkill J, Kiiru J, Mwituria J, Mirza N, et al. *Escherichia coli* from community acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. J Infect Dev Ctries. 2007;1(03):257-262.
8. Taneja N, Chatterjee SS, Singh M, Singh S, Sharma M. Pediatric urinary tract infections in a tertiary care center from north India. 2010;131:101-105.
9. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis. 1999;5(1):18-27.
10. Bhowmick BK, Rashid H. Prevalence and antibiotic susceptibility of *Escherichia coli* isolated from urinary tract infection (UTI) in Bangladesh. Pak J Biol Sci. 2004;7(5): 717-720.
11. Zhanel GG, Karlowsky JA, Harding GK, Carrie A, Mazzulli T, Low DE, et al. A Canadian national surveillance study of urinary tract isolates from outpatients: Comparison of the activities of trimethoprim-sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. Antimicrob Agents Chemother. 2000;44(4):1089-1092.
12. Aronson JK. Meyler's side effect of drugs used in anesthesia. 15th ed. Oxford: Elsevier Science and Technology; 2008.
13. Colgan R, Williams M. Diagnosis and treatment of acute uncomplicated cystitis. Am Fam Physician. 2011;84(7):771-776.

14. Gaines K. Phenazopyridine hydrochloride: the use and abuse of an old standby for UTI. Urol Nurs. 2004;24(3):207.
15. Glass SK, Pearl DL, McEwen SA, Finley R. A province-level risk factor analysis of fluoroquinolone consumption patterns in Canada (2000–06). J Antimicrob Chemother. 2010;65(9):2019-2027.
16. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103-e120.
17. Zalmanovici Trestioreanu A, Green H, Paul M, Yaphe J, Leibovici L, et al. Antimicrobial agents for treating uncomplicated urinary tract infection in women. Cochrane Database Syst Rev. 2010;10.
18. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Med. 2006;119(6):S20-S28.
19. Hawkey P. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62(Suppl 1):i1-i9.
20. Barlow M. What antimicrobial resistance has taught us about horizontal gene transfer. Horizontal Gene Transfer: Springer. 2009;397-411.
21. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: A three-year analysis. Clin Infect Dis. 1999;29(2):239-44.
22. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: Risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother. 2005;49(2):760-6.
23. Fagbule D, Kalu A. Case management by community health workers of children with acute respiratory infections: Implications for national ARI control programme. Am J Trop Med Hyg. 1995;98(4):241-6.
24. Dore MP, Sepulveda AR, Mura I, Realdi G, Osato MS, Graham DY. Explanation for variability of omeprazole amoxicillin therapy? Tolerance of *H-pylori* to amoxicillin. Gastroenterology. 1997;112(4): A105- A105.
25. Nahar S, Mukhopadhyay AK, Khan R, Ahmad MM, Datta S, Chattopadhyay S, et al. Antimicrobial susceptibility of *Helicobacter pylori* strains isolated in Bangladesh. J Clin Microbiol. 2004;42(10): 4856-8.
26. Abdulhak AAB, Al Tannir MA, Almansor MA, Almohaya MS, Onazi AS, Marei MA, et al. Non prescribed sale of antibiotics in Riyadh, Saudi Arabia: A cross sectional study. BMC Public Health. 2011;11(1):538.
27. Chan ECS. Microbiology. 5th ed. New Delhi: Tata McGraw-Hill Pub; 2007.
28. Cappuccino J, Sherman N. Microbiology: A laboratory manual, 6th ed. Benjamin/ Cummings, New York; 2001
29. Whitman WB, Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Ludwig W, et al. editors. Bergey's manual® of systematic bacteriology. 2nd ed. New York City, New York, USA: Springer; 2009.
30. Bauer AW, Kirby WM, Sherris JC,Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
31. Ferraro MJ. Performance standards for antimicrobial susceptibility testing. NCCLS; 2001.
32. Sambrook J, Russell DW. Molecular cloning: a laboratory manual (3-volume set), 3rd ed. Cold Spring Harbor, New York; 2001.
33. Azad AK, Sawa Y, Ishikawa T, Shibata H. Heterologous expression of tulip petal plasma membrane aquaporins in Pichia pastoris for water channel analysis. Appl Environ Microbiol. 2009;75(9):2792-2797.
34. Azad AK, Yoshikawa N, Ishikawa T, Sawa Y, Shibata H. Substitution of a single amino acid residue in the aromatic/arginine selectivity filter alters the transport profiles of tonoplast aquaporin homologs. Biochim Biophys Acta Biomembr. 2012;1818(1):1-11.
35. Chehregani A, Azimishad F, Alizade H. Study on antibacterial effect of some *Allium* species from Hamedan-Iran. Int J Agr Biol. 2007;9(6):873-876.
36. Khan M, Shah S. Experience with gram negative bacilli isolated from 400 cases of urinary tract infection (UTI) in Abbottabad. J Ayub Med Coll Abbottabad. 1981;12(4):21-23.
37. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA. Multidrug-resistant urinary tract isolates of *Escherichia coli*: Prevalence and patient

demographics in the United States in 2000. Antimicrob Agents Chemother. 2001;45(5): 1402-1406.
38. Olowe O, Okanlawon B, Olowe R, Olayemi A. Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, south western Nigeria. Afr J Microbiol Res. 2008;2(1):8-11.
39. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14(4):933-51.
40. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev. 2009;22(1):161-82.
41. Cao X, Cavaco LM, Lv Y, Li Y, Zheng B, Wang P, et al. Molecular characterization and antimicrobial susceptibility testing of *Escherichia coli* isolates from patients with urinary tract infections in 20 Chinese hospitals. J Clin Microbiol. 2011;49(7): 2496-501.
42. Neu HC. Resistance of *Pseudomonas aeruginosa* to imipenem. Infect Control Hosp Epidemiol. 1992;13(1):7-9.
43. Farshad S, Anvarinejad M, Tavana AM, Ranjbar R, Japoni A, Zadegan RM, et al. Molecular epidemiology of *Escherichia coli* strains isolated from children with community acquired urinary tract infections. Afr J Microbiol Res. 2011;5(26):4476-83.
44. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006;119(6):S3-S10.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15398