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# PREVALENCE AND CHARACTERIZATION OF MULTI-DRUG RESISTANT *ESCHERICHIA COLI* FROM URINE SAMPLES

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**Abstract**

Isolation, identification and characterization of *Escherichia coli* were carried out in terms of biochemical, serological, antibiogram, plasmid profile and culture condition of urine samples. Out of 50 urine samples, 36 were positive for *E. coli* that were confirmed by biochemical (e.g. oxidase, kligler's iron agar, indole, methyl red-voges proskauer and citrate utilization) tests and 4-methyl-umbelliferyl-β-D-glucoronide (MUG) test. Twenty seven strains gave positive result with different antisera whereas nine strains were untypable (UT), respectively. Thirty six strains were also tested by antibiogram against ten different antibiotics. Most *E. coli* strains were resistant to bacitracin, ampicillin, novobiocin, kanamycin and streptomycin. Eighty three per cent strains were sensitive to ciprofloxacin and gentamycin while 11 and 12% showed resistance to ciprofloxacin and gentamycin, respectively. By plasmid profile analysis of the 36 strains seven different plasmid patterns were observed. Comparison of the plasmid profiles with the antibiogram results indicated the presence of resistant (R) plasmid. Thirty four isolates of *E. coli* contained a common 25 kb plasmid that may possibly be responsible for drug resistance in this study. The results suggested that the prevalence of multi-drug resistant and new serotype of *E. coli* may be increasing rapidly which is alarming for treatment of urinary tract infection in Bangladesh.

## Introduction

Urinary tract infections (UTIs) having *Escherichia coli* as an etiologic agent are common with an estimated annual global incidence of at least 250 million cases. Preventive measures are therefore important as detection and treatment are costly for both patients and healthcare systems.(1) In women and children, non-complicated UTIs account for the highest number of infection observed. UTI may occur in the lower urinary tract or may involve both lower and upper urinary tract which is characterized by a syndrome involving dysuria, frequency, urgency and occasional suprapubic tenderness.(2) UTIs are often treated with broad-spectrum antibiotics. Fluoroquinolone

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are preferred as initial agents for empiric therapy of UTI in an area where resistance is likely to be a great concern. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens.[3,4] The extensive and misuse of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which in recent years, has become a major public health problem world-wide.[5]

There are fundamentally different genetic mechanisms underlying for intrinsic and acquired resistance. The intrinsic resistance of an organism is a stable property encoded in the chromosome and shared by the members of the species. Acquired resistance may develop either from changes or mutation to genomic DNA or by acquisition of resistance genes through mobile genetic elements (plasmid, bacteriaphage, transposons etc.) by gene transfer mechanisms such as conjugation, transformation, transduction and site specific integration. Antimicrobial resistance genes emerge either by being mobilized from obscure strains or by evolving from obscure ancestral genes. Resistant genes may be situated on chromosomes, plasmids, integrons or on transposons.[6]

The resistance pattern of etiologic agents of UTI has not been studied extensively in our country. The etiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years both in community and hospital nosocomial infections.[7,8] The incidence of drug resistance is increasing at an alarming rate and causes serious problems in the treatment of infectious diseases against multidrug resistant (MDR) bacteria. Therefore, the aim of this study was to isolate, identify multidrug resistant (MDR) *E. coli* causing UTI by different biochemical, serological and molecular characteristics. Present also different from some of the previous works[9,10] that have been done in Bangladesh with respect to sampling site, strain variation, antibiotics used and other molecular and serological approaches. Therefore, this study was important particularly for clinician in order to facilitate the treatment and management of UTI infected patients. Moreover, such data would also help the drug development authorities to formulate guideline for antibiotic use or develop new antibiotics for UTI treatment.

**Materials and Methods**

A total of 50 urine samples were collected from two diagnostic centers of Dhaka City, Bangladesh. One hundred microlitters of each urine sample were plated on MacConkey agar plates (Difco, USA). Bright pink lactose fermenting colonies were selected as presumptive isolates of *E. coli*. Presumptive *E. coli* colonies were grown again on eosine methylene blue (EMB) agar plates to observe for green metallic sheen production as confirmatory test. Presumptive and confirmatory colonies were further identified by cultural, microscopic and biochemical tests. Morphological characteristics (size, shape, surface texture, edge, elevation, colour, opacity etc.) were studied after 24 h incubation at

37°C on MacConkey and EMB agar plates. Biochemical tests were performed according to the methods described in the Bergey`s Manual of Systematic Bacteriology.[11]

Fluorogenic procedure with the substrate MUG has become common for the identification of *E. coli* isolated from both human and environmental samples. *E. coli* are able to produce glucoronidase enzyme that cleaves the MUG substrate, providing a fluorescent end product methylumbelliferons that is detectable under a long-wave UV light. For satisfactory results, incubation temperatures were kept between 225 and 44.5°C.[12] In this test, agar plates containing MUG were incubated at 37 and 42°C.

Thirty six isolates were serotyped by slide agglutination test[13] using commercially available 11 polyvalent antisera each containing a mixture of different monovalent antisera (Table 1). All of the polyvalent antisera were purchased from Denka Seiken Co. Ltd., Japan.

**Table 1. Polyvalent antisera used for serotyping of *E. coli*.**

| Polyvalent antisera | Mixture of monovalent antisera |
|---|---|
| Polyvalent 1 | O1, O26, O86a, O111, O119, O127a, O128 |
| Polyvalent 2 | O44, O55, O125, O126, O146, O166 |
| Polyvalent 3 | O18, O114, O142, O151, O57, O158 |
| Polyvalent 4 | O6, O27, O78, O148, O159, O168 |
| Polyvalent 5 | O20, O25, O63, O153, O167 |
| Polyvalent 6 | O8, O15, O115, O169 |
| Polyvalent 7 | O28c, O112ac, O124, O136, O144 |
| Polyvalent 8 | O29, O143, O152, O164 |
| Polyvalent II | O26, O55, O119, O126 |
| Polyvalent III | O86, O114, O124, O127, O128 |
| Polyvalent IV | O44, O112, O125, O1 |

Antimicrobial susceptibility was determined on Muller-Hinton agar plates following the Bauer-Kirby method.[14] The following antibiotics were used at the concentration indicated in this study: ampicillin (10 μg/ml), nalidixic acid (30 μg/ml), ciprofloxacin (5 μg/ml), erythromycin (15 μg/ml), streptomycin (10 μg/ml), chloramphenicol (30 μg/ml), bacitracin (10 μg/ml), gentamycin (10 μg/ml), kanamycin (5 μg/ml) and novobiocin (30 μg/ml). All antibiotic disks were commercially available and purchased from Oxoid, *In Vitro* Diagnosticum, USA.

Plasmid DNA was prepared by SDS/alkali lysis method[15] and electophoresed using 0.8% agarose in TBE buffer at 100 V for 30 minutes, in a horizontal electrophoresis system. Gels were stained with 0.1 μg/ml of ethidium bromide for 20 minutes. The gels were viewed on an ultraviolet transilluminator and photographed by using a Polaroid camera. DNA ladder (1 kb) was used as marker to measure the size of the isolated plasmids.

**Results and Discussion**

Of the 50 urine samples processed only 36(72%) samples showed positive results for *E. coli*, 10% samples were found to be positive for *Klebsiella* sp. The high incidence of *E. coli* suggested that it's the major etiologic agents of UTI as compared to *Klebsilla* sp., *Pseudomans aeroginosa*, *Acinetobacter baumanii* etc.[16]

The MUG assay is based on the enzyme activity of β-glucoronidase (GUS) which cleaves the substrate 4-methylumbelliferyl β-D-glucoronide, to release 4-methy-umbelliferron (MU). When exposed to long-wave UV light (365 nm) light 4-methyl-umbelliferone exhibit a bluish fluorescence that is easily visualized in the medium or around the colonies. Several report suggested that over 95% *E. coli* produces GUS, including anaerobic, non gas producing strains. Enterohemorrhagic *E. coli* (EHEC) of serotype O157 : H7 has been shown to be GUS negative strain.[17,18] In this study, it was found that 84% *E. coli* were GUS positive and 14% were GUS negative strain, respectively at 37 and 42°C. These data concluded that MUG test is a very suitable test for identification and confirmation of GUS producing bacteria in clinical samples.

In the present study, 11 types of polyvalent antisera were used each of which are mixtures of enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), entero-toxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAEC) monovalent antiserum designed on the basis of their O antigen (Table 1). Out of thirty six *E. coli* strains, twenty seven showed positive results to the different polyvalent antisera. Nine *E. coli* strains that could not be serotyped (Table 2) may belong to other serogroups (either H antigen or new serogroup). According to our result, 75% of the isolated *E. coli* strains were found to carry O antigens which may be highly virulent and responsible for urinary tract infection, diarrhoea etc.[19]

**Table 2. Serotype profile of *E. coli* isolates.**

| Strain | Positive with polyvalent antisera |
|---|---|
| AK 1, 2, 8, 14, 16, 30 | 1 |
| AK 3, 12, 15, 19 | 2 |
| AK 22 | 3 |
| AK 11 | 4 |
| AK 19, 33 | 5 |
| AK 32 | 6 |
| AK 4, 17 | 7 |
| AK 20, 23 | 8 |
| AK 6, 26, 34, 36 | II |
| AK 28 | III |
| AK 5, 27, 31 | IV |
| AK 7, 9, 10, 13, 21, 24, 25, 29, 35 | Untypable |

The drug resistance pattern of thirty six isolates of *E. coli* against ten different antibiotics is presented in Fig. 1. Thirty per cent *E. coli* were completely resistant to ampicillin, novobiocin, streptomycin, bacitracin, kanamycin and nalidixic acid, respectively. These *E. coli* were referred to as multi-drug resistant (MDR) strains and are a major public health concern. Eighty three per cent of isolates were sensitive to ciprofloxacin and gentamycin, respectively. Among the 36 isolates, 20 per cent and 25 per cent were found to be resistant to chloramphenicol and to erythromycin, respectively. These data proved to be an alarming indication that the drug resistant *E. coli* strains are increasing day by day.[19]

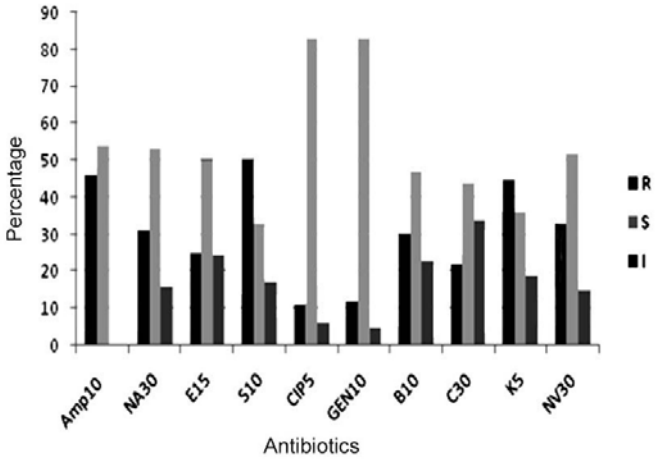


Fig. 1. Antibiotic sensitivity pattern of *E. coli* to different drugs. R - resistant, S - sensitive and I - intermediate, Amp = ampicillin, NA – nalidixic acid, E – erythromycin, S – strptomycin, GEN – gentamycin, B – bacitracin, C = chloramphenicol, KS = kanamycin sulfate, NV – novobiocin. The numerical number indicated the concentration of drugs used in this study.

To determine whether the drug resistance properties are plasmid or chromosome-mediated the plasmid profile of 36 *E. coli* isolates were studied. Of these isolates, 34 strains were found to carry plasmids which were also found to be multi-drug resistant strains. This study revealed seven different patterns of plasmids among the 34 isolates (Fig. 2A-B, Table 3). Eighteen isolates contained only a 25 kb plasmid while the other 16 isolates harbored more than one plasmid. All thirty four strains that carried the 25 kb plasmid were also found to be MDR; this is an indication that the plasmid may be responsible for resistant to major drugs.[20] But further proof for this is necessary which could be done by curing the respective plasmids. Two isolates AK-35 and AK-36 did not reveal any plasmid but still showed resistance to several drugs e.g. ampicillin, erythromycin and kanamycin. For these two isolates drug resistance properties may be related to chromosome-mediated mechanisms e.g. drug efflux pumps, β- lactamases enzyme etc.[21]

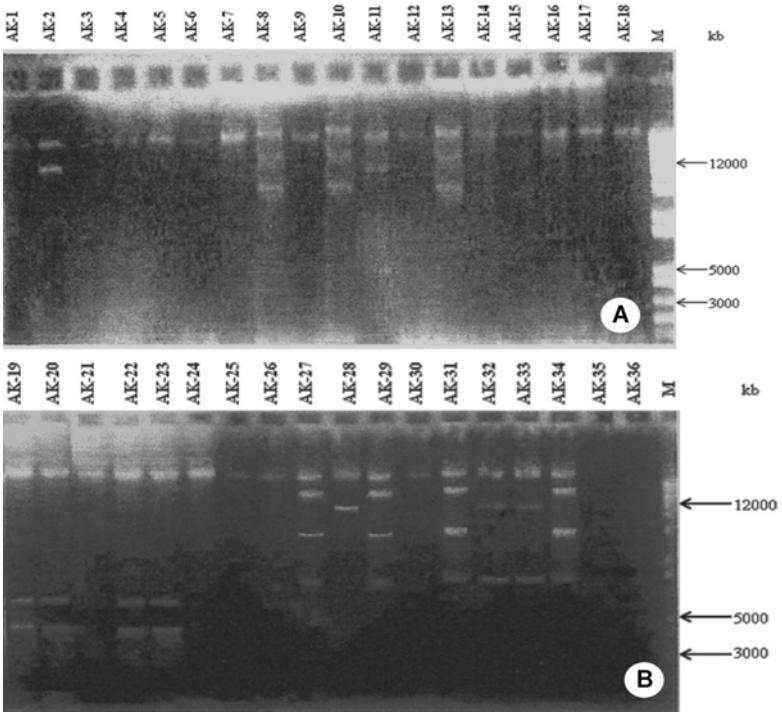


Fig. 2A-B). Plasmid profiles of *E. coli* strains isolated from different clinical samples.

**Table 3. Plasmid profile of the *E. coli* isolates.**

| *E. coli* strains | Plasmid size (kb) |
|---|---|
| AK - 1, 3, 4, 5, 6, 7, 9, 12, 14, 15, 16, 17, 18, 21, 24, 25, 26, 30 | 25 |
| AK - 2, 28, 11 | 25, 12 |
| AK - 8, 10, 13 | 25, 14, 10 |
| AK - 19, 20, 22, 23 | 25, 5, 3.5 |
| AK - 27, 29, 31, 34 | 25, 14, 10, 7 |
| AK - 32, 33 | 25, 11, 7 |
| AK - 35, 36 | No plasmid |

This study suggests that it is very alarming to observe the increase in the drug resistant and sero-untypable strain of *E. coli* that may be involved as the causative agents of urinary tract infection, a common problem in Bangladesh. These problems may have arisen due to misuse of antibiotics or prolonged use of single antibiotics. Therefore, immediate precautions should be taken by the government to stop the selling of

antibiotics over counter without prescription, increase the public health concern and ensure the proper use of antibiotics by doctors and patients, respectively.

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