Original Article

# High prevalence of multidrug-resistance uropathogenic *Escherichia coli* strains, Isfahan, Iran

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## Abstract

**Background and Objectives**: Urinary tract infection (UTI) is one of the most frequent infectious diseases and can occur in all age groups. *Escherichia coli* is the main cause of this infection. Multiple resistances to antimicrobial agents are increasing quickly in *E. coli* isolates and may complicate therapeutic strategies for UTI. The aim of this study was to determine the antibiotic resistance pattern and the multidrug-resistance (MDR) phenotypes in uropathogenic *E. coli* (UPEC). **Materials and Methods**: A total of 135 UPEC isolates were collected from both outpatients (91 isolates) and inpatients (44 isolates) between September, 2012 and February, 2013. In order to determine the MDR among UPEC isolates, we have tested 15 antimicrobial agents and antibiotic susceptibility was done by Kirby-Bauer disk diffusion method. **Results**: The percentage of MDR isolates (resistant to at least three drug classes such as aminoglycosides, fluoroquinolones, penicillins, cephalosporins, or carbapenems) was 68% in the inpatients and 61% in the outpatients. Antibiotic resistance to ampicillin, ceftazidim, nalidixic acid, and trimethoprim/sulfamethoxazole were higher than 50%. Amikacin, nitrofurantoin, and gentamicin showed markedly greater activity (89.1%, 85.9%, and 82.4% sensitivity, respectively) than other antimicrobial agents. Resistance to meropenem did show either in outpatients or in inpatients. **Interpretation and Conclusions:** The high prevalence of drug resistance among UTI patients calls for continuous monitoring of the incidence of drug resistance for appropriate empiric selection of antibiotic therapy. Empirical treatment of UTIs should be relied on susceptibility patterns from local studies.

**Key words:** Antimicrobial resistance, *Escherichia coli*, multidrug resistance, urinary tract infections

## INTRODUCTION

Urinary tract infections (UTIs) are the most common infections and are mostly caused by Gram-negative bacteria.[1] Almost 150 million cases of UTIs per year were reported worldwide.[2] According to an estimation, almost 40-50% of women experience UTIs once in lifetime.[3] Recurrent UTI occurs in adult women and results in high healthcare costs.[4,5] Based on the surveys, UTI is an independent risk factor for renal cell carcinoma and bladder cancer.[6,7] According to the studies, uropathogenic *Escherichia coli* (UPEC) is the most common cause of UTIs.[8-10] UPEC isolates possess multiple virulence factors that promote colonization of the bacteria and infection in the urinary tract such as fimbrial, adhesins,

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afimbrialadhesin, toxins, siderophores, and capsular polysaccharide.[10-12] The clinical experiences have shown a high rate of antibiotic resistance among uropathogens.[13-15] Excessive use of antibiotics is the most important factor in the rise of multidrug resistance (MDR) in UPEC isolates.[16] Antibiotic resistance is a serious public health emergency leading to increased mortality and morbidity.[17] According to the European Centre for Disease Prevention and Control in 2007, antibiotic resistance caused about 25,000 deaths annually which was equal to about half the number of deaths in a road accident in Europe.[18] Due to the risk of kidney damage and complications, early diagnosis and treatment of disease is important.[19,20] Since UTI cause several complex symptoms, physicians begin empirical antibiotic treatment before getting the culture results because urine culture and antibiogram results take about 4 days to be prepared.[21-23] According to reports from the USA, Japan, China, India, Saudi Arabia, Brazil, and Nepal, the prevalence of MDR *E. coli* causing UTIs is increasing.[1,24-29] The knowledge of the main bacteria usually involved in the UTIs and their antimicrobial susceptibility are necessary for appropriate empirical therapy and prevention of the emergence of antibiotic resistance. Since these data are constantly changing and may vary from hospital to hospital, each institution should determine these information and update them regularly.[30,31] The present study aimed to define the current occurrence and phenotypes of MDR *E. coli* among UTI isolates from a university medical center, Alzahra Hospital, Isfahan, Iran.

## MATERIALS AND METHODS

### Bacterial isolates

A total of 135 strains of *E. coli* causing UTIs were isolated from both outpatients (91 isolates) and inpatients (44 isolates) from Alzahra Hospital (Isfahan, Iran). The isolates were collected between September, 2012 and February, 2013. Samples were isolated from the urine. Diagnosis of *E. coli* isolates has been done according to Bailey and Scott's diagnostic microbiological methods.[32] The samples were cultured on nutrient agar, MacConkey agar, blood agar, and eosin-methylene blue agar (purchased from Himedia Company). The plates were incubated at 35°C for 24 h and the pure isolates characterized and identified according to Gram-stains and biochemical tests such as catalase, oxidative, citrate utilization, indole production, methyl red-Voges Proskauer, triple iron sugar utilization, and urea test and as described in standard bacteriological methods.[32] Quality control was tested by *E. coli* ATCC25922.

### Antibiotic susceptibility test

Bacterial susceptibility to antimicrobial agents was determined by using disk diffusion method as recommended by the Clinical and Laboratory Standards Institute guidelines.[33] The antibiotic disks used in this study were ciprofloxacin (CIP) (5 μg), norfloxacin (10 μg), ofloxacin (OFX) (5 μg), nalidixic acid (NAL) (30 μg), amikacin (30 μg), ampicillin (AMP) (10 μg), cefotaxime (CTX) (30 μg), gentamicin (GEN) (10 μg), nitrofurantoin (NOR) (300 μg), trimethoprim/sulfamethoxazole (SXT) (1.75/23.75 μg), cefoxitin (30 μg), meropenem (10 μg), cefepime (FEP) (30 μg), ceftazidime (30 μg), and cephalothin (30 μg).

*E. coli* ATCC25922 was used as a quality control strain. Then the data were entered into Whonet 5.6 (WHO, Geneva, Switzerland) software. An isolate was considered MDR if it was resistant to at least three of the antimicrobial classes such as aminoglycosides, fluoroquinolones, penicillins, cephalosporins, or carbapenems.

## RESULTS

The age distribution was determined based on the decade, and different prevalence patterns were observed in each age range as described in Table 1. In total, 68% of the participants were female, and 14% of the samples belong to children.

The resistance rates of UPEC isolates to antimicrobial agents in both outpatients and inpatients are shown in Figure 1. Antibiotic resistance to AMP, ceftazidim, NAL, and SXT were higher than 50%. The rates of resistance to AMP, SXT, ceftazidim, CTX, and FEP in outpatients were higher than in inpatients. Resistance to meropenem did show neither in outpatients nor in inpatients. Amikacin, NIT, and GEN showed markedly greater activity (89.1%, 85.9%, and 82.4% sensitivity, respectively) than other antimicrobial agents.

**Table 1: Age and gender distribution of patients diagnosed with UTI**

| | Age group | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | 1< | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 | 81-90 | 90> | Total |
| Female | 3 | 11 | 4 | 16 | 18 | 15 | 7 | 8 | 4 | 5 | 1 | 92 |
| Male | 4 | 1 | 1 | 3 | 4 | 8 | 2 | 7 | 7 | 3 | 3 | 43 |
| Total | 7 | 12 | 5 | 19 | 22 | 23 | 9 | 15 | 11 | 8 | 4 | 135 |

UTI: Urinary tract infection

Antimicrobial susceptibility test showed that the rate of MDR isolates was 68% in inpatients and 61% in outpatients. The isolates were divided into seven groups according to their resistance patterns [Table 2]. The most common MDR phenotypes are shown in Table 3. The prevalent pattern among total UPEC isolates was CIP, OFX, NOR, NAL, CTX, AMP, FEP, SXT; and it was only among outpatients.

## DISCUSSION

MDR in UPEC causing UTIs is an emerging and serious public health problem and results in treatment failure. This study provides current information about the antimicrobial resistance pattern in *E. coli* isolates from patients' urine samples in the Alzahra Hospital, Isfahan, Iran. Based on the results of the present study, there is a high resistance rate to the commonly used antibiotics in the *E. coli* isolates. We found 63% of the isolates to be resistant to three or more antibiotics. The rates of antibiotic resistance in our study were different from some studies in other countries.[18,34-37] MDR rate was higher in inpatients isolates (68%). A significant high resistance to SXT (61.2%) was found in the present study while many guidelines recommend this drug for UTIs.[38,39] In addition, CIP and OFX antibiotics recommended for UTIs, showed high resistance in data from other surveys and European countries according to Annual report of the European Antimicrobial Resistance Surveillance Network in 2012.[40-42] Excessive use of AMP in the treatment of UTIs, especially in hospitalized patients, gives a possible explanation for the existence of high resistance rate (84.2%) to this antimicrobial agent. The rates of resistance to AMP, SXT and CTX in outpatients were higher than in inpatients while the rates of resistance to fluoroquinolones and NAL in outpatients were lower than in inpatients. According to experts, resistance level >20% is used as a cut-off in guidelines on UTIs, so these antibiotics should not be recommended for the treatment of UTIs.[43,44] However, low levels of resistance to amikacin, NIT, and GEN (2.2%, 8.1%, and 16.5%, respectively) and no resistance against meropenem was observed in the present study. Based on our results, the extremely high percentage of isolates showed an MDR phenotype. Some socioeconomic and behavioral factors can contribute to antibiotic resistance such as misuse of antimicrobial agents by hospital physicians or unskilled practitioners and easy access to antibiotics without a prescription.[45] These warning resistances to the commonly used antibiotics can affect the therapeutic strategies. The data of the current study cannot be translated to the international level. The successful empirical initial treatment is based on susceptibility and resistance patterns obtaining from local data. Since these susceptibility patterns are constantly changing and may vary in different geographical regions and institutions, regular monitoring of antimicrobial agents resistance seems necessary to formulate standard treatment guidelines for empirical therapy.

**Table 2: Resistance pattern of the *Escherichia coli* strains**

| Pattern | Resistance pattern | Outpatient percentage (number of isolates) | Inpatient percentage (number of isolates) |
|---|---|---|---|
| Pattern 1 | Resistant to one drug | 17.5 (16) | 15.9 (7) |
| Pattern 2 | Resistant to two drugs | 13.1 (12) | 4.5 (2) |
| Pattern 3 | Resistant to three and four drugs | 13.1 (12) | 22.7 (10) |
| Pattern 4 | Resistant to five drugs | 4.3 (4) | 9 (4) |
| Pattern 5 | Resistant to six drugs | 13.1 (12) | 13.6 (6) |
| Pattern 6 | Resistant to more six drugs | 30.7 (28) | 22.7 (10) |
| Pattern 7 | Sensitive to all | 7.6 (7) | 11.3 (5) |

**Table 3: The most common antimicrobial resistance phenotypes showing MDR among *Escherichia coli* isolates**

| Antimicrobial resistance phenotype | Outpatient (number of isolates) | Inpatient (number of isolates) |
|---|---|---|
| CIP, OFX, NOR, NAL, CTX, AMP, CEP, SXT | 12 | 0 |
| CIP, OFX, NOR, NAL, AMP, SXT | 5 | 0 |
| CIP, OFX, NOR, AMP, FEP, CAZ | 1 | 4 |
| CTX, AMP, CEP, SXT | 4 | 0 |
| CIP, OFX, NOR, CTX, AMP, CEP, NAL, GEN | 4 | 0 |
| CTX, AMP, CEP, SXT, NAL | 3 | 0 |
| CTX, AMP, CAZ, FEP | 0 | 3 |
| AMP, SXT, NAL | 3 | 0 |
| CIP, OFX, NOR | 0 | 3 |
| CIP, OFX, NOR, CTX, AMP, FEP, CAZ | 2 | 2 |

CIP: Ciprofloxacin, NOR: Norfloxacin, OFX: Ofloxacin, NAL: Nalidixic acid, CTX: Cefotaxime, AMP: Ampicillin, CEP: Cephalothin, SXT: Trimethoprim/sulfamethoxazole, FEP: Cefepime, CAZ: Ceftazidime, GEN: Gentamicin, MDR: Multidrug resistance

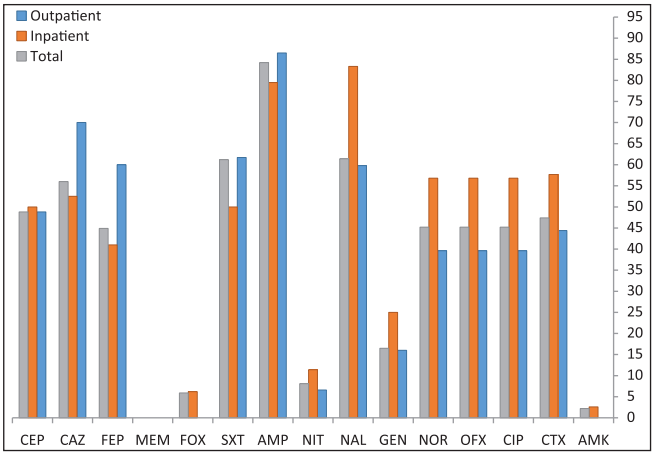


**Figure 1:** Percentages of antibiotic resistance in *Escherichia coli* isolated from outpatients and inpatients

## CONCLUSION

UTI is the most common infectious disease. As bacterial resistance to the common antimicrobial agents has increased considerably among *E. coli* causing UTIs, empirical antibiotic treatment should be reviewed periodically at a regional level.

## STUDY LIMITATIONS

One limitation of this study was the lack of extended-spectrum beta-lactamase-testing to determine the minimum inhibitory concentration of antibiotics and further analysis for a more accurate measure of antibiotic resistance.

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### Conflicts of interest

There are no conflicts of interest.

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