

Basic Sciences in Medicine

PATTERN OF RESISTANCE TO BETA-LACTAMS IN *E. COLI* ISOLATED FROM URINARY TRACT INFECTIONS IN IRAN

**S. M. HOSSEINI-MAZINANI, H. JAFAR-NEJAD,
AND S. GHANDILI**

*From the National Research Center for Genetic Engineering and Biotechnology,
Tehran, I.R. Iran.*

ABSTRACT

A total of 385 urine specimens from suspected urinary tract infections were subjected to bacteriological analysis and susceptibility testing at a major university hospital laboratory in Tehran.

A battery of eight beta-lactam antibiotics, commonly prescribed in Iran, and three third-generation cephalosporins (ceftazidime, ceftriaxone and ceftizoxime) that are usually reserved for limited use in the hospital setting were tested against *E. coli* urine isolates, using Kirby-Bauer disk diffusion method.

E. coli was the most common isolate from both the inpatient (37.8%) and outpatient (30.5%) populations. The susceptibility testing revealed a high degree of resistance to oxacillin, ampicillin, amoxicillin, carbenicillin and cephadrine. Furthermore, a marked difference in the sensitivity/resistance ratio between the inpatient and outpatient populations was observed.

The *E. coli* isolates exhibited the highest rate of resistance to oxacillin, ranging from 49.4% in the outpatients to 81.6% in the inpatients. The frequency of the isolate resistance to first-generation cephalosporins was less widespread. Despite the absence of resistance to third-generation cephalosporins among the outpatient isolates, a significant resistance to ceftazidime (9.6%) and ceftriaxone (8.8%) was observed among the outpatient bacterial isolates. The susceptibility testing of the *E. coli* isolates to a combination of amoxicillin and clavulanic acid revealed 6.9% resistance among outpatient and 12.5% among inpatient isolates.

These results are particularly significant in that this study was performed immediately before the introduction of Co-amoxyclav to the Iranian drug market and can be served as a basis for monitoring the spread of resistance to this drug in Iran. *MJIRI, Vol. 16, No. 4, 209-212, 2003.*

Keywords: antibiotic resistance, clavulanic acid, third-generation cephalosporins, urinary tract infection.

INTRODUCTION

Beta-lactams are the most frequently prescribed class of antibiotics worldwide. Consequently, resistance to

Correspondence: S. M. Hosseini-Mazinani, National Research Center for Genetic Engineering and Biotechnology, P.O.Box: 14155-6343, Tehran, Iran.
Email: hosseini@nrcgeb.ac.ir, Tel: (98)-21-641-9826, Fax: (98)-21-641-9834.

Resistance to Beta-Lactams in UTI Isolates of *E. coli*

these agents has become a major problem especially in the past two decades after the introduction of newer extended spectrum cephalosporins; monobactams, carbapenems, and beta-lactamase inhibitor/beta-lactam combination.¹⁻⁴ This increase of resistance is clearly observable among gram-negative bacilli. This study was performed on 385 urine samples being submitted to the laboratory of a major university hospital.

MATERIAL AND METHODS

Bacteria

A total of 385 bacterial isolates from urinary samples of inpatients (n = 254) and outpatients (n = 131) with urinary tract infection were studied at the bacteriology laboratory of Vali-e-Asr hospital, Tehran, Iran during a seventy-day period in autumn 1996. The isolates were identified according to conventional criteria. The *E. coli* isolates were selected and subjected to a standard battery of identification tests at a reference laboratory.

These included eight commonly prescribed beta-lactam antibiotics, and three third-generation cephalosporins that are usually reserved for use in the hospital setting. In addition, the *E. coli* isolates were tested for resistance to the combination of amoxycillin with a beta-lactamase inhibitor, clavulanic acid, which had not been introduced to Iran's drug market at that time.

Antimicrobial susceptibility tests

The *E. coli* isolates were subcultured to LB (Luria Bertani) agar and incubated overnight at 37°C. A few similar colonies from the overnight culture were transferred into approximately 4-5 mL LB broth medium. The turbidity of suspension was adjusted to that of a 0.5 McFarland barium sulfate standard against a white background with contrasting black lines. Agar disc diffusion tests were carried out according to the method of Bauer et al. under strict adherence to NCCLS criteria^{5,6} where applicable. Antibiotic discs were purchased from Difco Laboratories (Detroit, USA) and Padtan Teb (Tehran, Iran). The following discs were used: amoxycillin (25 µg),

ampicillin (10 µg), ceftizoxime (30 µg), cephalothin (30 µg), cephalexin (30 µg), cefazoline (30 µg), ceftazidime (30 µg), carbenicillin (100 µg), oxacillin (1 µg), cephradine (30 µg) and ceftriaxone (30 µg). The *E. coli* isolates were also tested against a combination of amoxycillin and clavulanic acid. Zone sizes for the antimicrobials tested were determined according to the recommendations of the manufacturers.

RESULTS

Table I shows the frequency of bacterial species isolated from 385 urine specimens. The bacterial isolates included *Escherichia coli* (35.3%), *Klebsiella* species (11.2%), *Pseudomonas* species (10.9%), *Proteus* species (6.8%), *Enterobacter* species (3.6%), enterococci (3.1%), *Staphylococcus aureus* (3.1%), and other species (25.9%). *E. coli* was the most common isolate from both the inpatient (37.8%) and outpatient (30.5%) populations.

Table II shows the pattern of resistance of the *E. coli* isolates to eight beta-lactam antibiotics commonly prescribed in Iran. The susceptibility testing revealed a high degree of resistance to oxacillin, ampicillin, amoxycillin, carbenicillin and cephradine. Furthermore, a marked difference in the sensitivity/resistance ratio between the inpatient and outpatient populations was observed. The *E. coli* isolates exhibited the highest rate of resistance to oxacillin, ranging from 49.4% in the outpatient to 81.6% in the inpatient populations. Resistance to the first-generation cephalosporins was less widespread. For cefazolin, it ranged from 25.3% (among the outpatients) to 41.9% (among the inpatients), and for cephalothin, from 18.4% to 29.4% in the corresponding populations.

The results of susceptibility testing to three third-generation cephalosporins (ceftazidime, ceftriaxone and ceftizoxime) that are usually reserved for use in the hospital setting are shown in Table III. Despite the absence of resistance among the outpatient isolates, a significant resistance to the cephalosporins was observed among the inpatient bacterial isolates.

In contrast to the fact that at that time no beta-

Table I. Bacterial species isolated from patients with urinary tract infection.

Bacterial species	Inpatient isolates	Outpatient isolates	Total
	No (%)	No (%)	No (%)
<i>E. coli</i>	96 (37.8)	40 (30.5)	136 (35.3)
<i>Pseudomonas</i> spp.	40 (15.7)	2 (1.5)	42 (10.9)
<i>Klebsiella</i> spp.	36 (14.2)	7 (5.3)	43 (11.2)
<i>Proteus</i> spp.	17 (6.7)	9 (6.9)	26 (6.8)
<i>Enterobacter</i> spp.	10 (3.9)	4 (3)	4 (3.6)
Enterococci	1 (0.4)	11 (8.4)	12 (3.1)
<i>S. aureus</i>	6 (2.4)	6 (4.6)	12 (3.1)
Other	48 (18.9)	52 (39.7)	100 (25.9)
Total	254	131	385

Table II. Resistance of *E. coli* isolates to beta-lactam antibiotics commonly prescribed in Iran.

Antibiotics	Inpatient isolates	Outpatient isolates
	No (%)	No (%)
Oxacillin	111 (81.6)	43 (49.4)
Ampicillin	108 (79.4)	35 (40.2)
Amoxycillin	110 (80.9)	43(49.4)
Carbenicillin	97 (71.3)	32 (36.8)
Cephadrine	108 (79.4)	43 (49.4)
Cephalexin	52 (38.2)	18 (20.7)
Cephalothin	40 (29.4)	16 (18.4)
Cefazolin	57 (41.9)	22 (25.3)

Table III. Resistance of inpatient *E. coli* isolates to third-generation cephalosporins.

Antibiotics	Inpatient isolates
	No (%)
Ceftazidime	13 (9.6)
Ceftriaxone	12 (8.8)
Ceftizoxime	12 (8.8)

lactamase inhibitor/beta-lactam combinations was introduced to Iran's drug market, 17(12.5%) and 6(6.9%) isolates were found to be resistant to co-amoxyclav from the inpatients and outpatients, respectively.

DISCUSSION

As shown in the study, *E. coli* was the most common bacterial species isolated from UTI cases either inpatients or outpatients. This is in accordance with previously published data in Iran.⁷ The significant difference between the inpatient and outpatient percentages of *Pseudomonas* species and *Klebsiella* species emphasizes the importance of hospital-acquired UTI in Iran.

Our results show an increase in the resistance of *E. coli* isolates of urinary tract infections to cephalothin (21.1%), as compared to 7% of a previous study conducted in 1981.⁸ This increase may be due to the indiscriminate use of cephalosporins in the country.

The pattern of resistance to third-generation cephalosporins is both disappointing and promising. In comparison to a report on *E. coli* isolated from burn patients⁹ in Mashhad, Iran, which showed no single case of ceftazidime-resistance in *E. coli* isolates, we have found an 8-9% resistance rate to this group of antibiotics. Meanwhile, since all resistant strains were isolated from inpatient UTIs, appropriate prescription of third-generation cephalosporins may prevent this phenotype from distribution in the society.

Finally, as the samples were collected prior to the introduction of any beta-lactam/beta-lactamase inhibitor combination to the Iranian drug market, the extent of resistance observed to co-amoxyclav can serve as a baseline for monitoring the spread of this phenotype in this country.

ACKNOWLEDGEMENT

We are very grateful to Dr. Manoochehr Dezfulian for his critical review of the manuscript and to Dr. Bahram Fathollah-Zadeh, for his assistance in gathering the samples.

REFERENCES

1. Shannon K, King A, Phillips I: Prevalence of resistance to beta-lactam antibiotics in *Escherichia coli* isolated from blood from 1969-1991. *J Antimicrob Chemother* 30: 661-72, 1992.
2. Vatopoulos AC, Philippon A, Tzouveleki LS, et al: Prevalence of a transferable SHV-5 type beta-lactamase in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Greece. *J Antimicrob Chemother* 26: 635-48, 1990.
3. Ehrhardt AF, Sanders CC: Beta-lactam resistance amongst *Enterobacter* species. *J Antimicrob Chemother* 32 (suppl B): 1-11, 1993.
4. Piddock LJ, Walters RN, Jin YF, et al: Prevalence and mechanism of resistance to 'third-generation' cephalospor-

Resistance to Beta-Lactams in UTI Isolates of *E. coli*

- ins in clinically relevant isolates of *Enterobacteriaceae* from 43 hospitals in the UK, 1990-1991. *J Antimicrob Chemother* 39: 177-187, 1997.
- 5 . Bauer AW, Kirby WM, Sherris JC, et al: Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493-6, 1999.
 - 6 . National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial disk susceptibility tests. 5th ed. NCCLS document M2-A5. Villanova/Pa: NCCLS, 1993.
 - 7 . Saremi A, Abtahi F: Etiologic agents and drug sensitivity in urinary tract infections: a survey of 786 urine cultures. *Med J Islam Rep Iran* 7: 13-6, 1993.
 - 8 . Dezfulian M, Naghashfar Z: Transmissible drug resistance in certain enteric bacilli isolated in Tehran. *Chemotherapy* 27: 334-9, 1981.
 - 9 . Ghanaat J: Evolution of ceftazidime antimicrobial activity in infections caused by burns. *Med J Islam Rep Iran* 6: 59-61, 1992.