Bacteriuria by Extended-Spectrum Beta-Lactamase-Producing Escherichia Coli and Klebsiella Pneumoniae

Isolates in a Governmental Hospital in south of Tehran, Iran

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Keywords. *Escherichia coli* infections, and *Klebsiella pneumoniae*, multiple drug resistance, beta-lactam resistance

Introduction. Antibiotic resistant mutants producing extended-spectrum beta-lactamase (ESBL) have emerged among *Escherichia coli* and *Klebsiella pneumoniae*. This study was done to determine the frequency of ESBL-producing *E coli* and *K pneumoniae* species isolated from urine samples of our patients.

Materials and Methods. A study was conducted on 164 urine isolates (124 *E coli* and 40 *K pneumoniae*) in the laboratory Loghman Hakim Hospital in Tehran, Iran, in 2007. Microbial sensitivity tests were done on Mueller-Hinton agar plates with disk diffusion method. Broad-spectrum resistance was defined as resistance to ampicillin or cephalothin; ESBL resistance, as resistance of these bacteria to one of ceftriaxone, ceftazidime, or ceftizoxime; and MDR-ESBL; as resistance to 3 of the following antibiotic groups: trimethoprim-sulfamethoxazole, aminoglycosides, fluoroquinolones, and nitrofurantoin.

Results. An ESBL resistance was detected in 52.5% of isolates with *K pneumoniae* and 45.2% of those with *E coli*. The MDR-ESBL pattern was detected in 26.8% of the isolates. These included 30.0% of the *K pneumoniae* and 25.8% of the *E coli* isolates. Broad-spectrum resistance was detected in all *K pneumoniae* isolates and 87.9% of 124 *E coli* isolates.

Conclusions. Our study showed a high rate of ESBL resistant strain of *E coli* and *K pneumoniae* and the emergency of multiple drug resistance to these bacteria in our patients in Tehran, Iran.

IJKD 2008;2:197-200
www.ijkd.org

Original Paper

INTRODUCTION

Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial drugs in the world; however, emergency of resistance to beta-lactam antibiotics in clinically important pathogens has increasingly limited their utility. Antibiotic-resistant mutants producing extended-spectrum beta-lactamase (ESBL) have emerged among gram-negative bacteria, predominantly *Escherichia coli* and *Klebsiella pneumoniae*.[1] In 1983, the first ESBL-producing organism was isolated in Germany.[2] Soon thereafter, such organisms were reported in the United States,[3,4] followed by outbreaks of infections caused by these pathogens.[5,6] In the recent years, the importance of such ESBL-mediated infections has been increasingly recognized.[7-9]

The *National Committee for Clinical Laboratory Standards* (NCCLS) recommends ESBL screening methods and confirmatory tests[10]; however, their use in microbiology laboratories has been neglected.[11] Delay in the detection and reporting of ESBL production by gram-negative bacteria is associated with prolonged hospital stay and increased morbidity, mortality, and healthcare

costs.[12,13] Antibiotic selection for treatment of serious infections due to ESBL-producing *E coli* and *K pneumoniae* (ESBL-EK) is a clinical challenge because of the complex nature of in vitro susceptibility testing and its relation to in vivo condition.[14] On the other hand, prevalence of multiple drug resistance among ESBL-EK strains is increasing.[15]

Institutional microbial sensitivity tests or local patterns of susceptibility are of the first steps that are crucial for the treatment of ESBL-producing bacteria. However, we lack enough evidence on the current epidemiology and clinical pattern of these microorganisms in our country. Accordingly, we decided to carry out a study to determine the frequency of ESBL-EK and multiple drug resistant-ESBL (MDR-ESBL) in urine samples of patients referred to our center in Tehran, Iran.

MATERIALS AND METHODS

A cross-sectional study was conducted on isolates in the laboratory of Loghman Hakim Hospital, in the south of Tehran, Iran, in 2007. In this study, only samples with significant bacterial growth were studied. Significant growth was defined as the presence of more than 10^5 colony-forming units per milliliter of *E coli* or *K pneumoniae* in the urine culture.[16] Isolated bacteria were identified by standard techniques.

All the urine cultures were done in a single laboratory and read by one investigator. A total of 164 urine isolates (124 with *E coli* and 40 with *K pneumoniae*) were included. Microbial sensitivity tests were done on the Mueller-Hinton agar plates with disk diffusion method according to the Kirby-Bauer method.[17] The following disks (Padtan Teb, Tehran, Iran) were applied onto the plates: 10-µg ampicillin, 30-µg cephalothin, 30-µg ceftriaxone, 30-µg ceftazidime, 30-µg ceftizoxime, 25-µg trimethoprim-sulfamethoxazole, 30-µg amikacin, 10-µg gentamicin, 5-µg ciprofloxacin, 10-µg norfloxacin, 30-µg nalidixic acid, and 300-µg nitrofurantoin.

Broad-spectrum resistance of *E coli* and *K pneumoniae* was defined as resistance to ampicillin or cephalothin. An ESBL-EK resistance was defined as resistance of these bacteria to one of the following drugs: ceftriaxone, ceftazidime, or ceftizoxime. An MDR-ESBL was considered as resistance to 3 of the following antibiotic groups by the ESBL-EK species: trimethoprim-sulfamethoxazole, aminoglycosides (amikacin or gentamicin), fluoroquinolones (ciprofloxacin, norfloxacin, or nalidixic acid), and nitrofurantoin. Finally, the chi square test was used with the help of the SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc, Chicago, Illinois, USA) for statistical analyses of this study. A *P* value less than .05 was considered to be significant.

RESULTS

The resistance pattern of *E coli* and *K pneumoniae* isolated form 164 urine samples are shown in the Table. Broad-spectrum resistance was detected in 149 (90.8%) of the isolates. All of the 40 *K pneumoniae* isolates and 109 of 124 *E coli* isolates (87.9%) were broad-spectrum resistant. This kind of antimicrobial

Antibiotic Resistance Pattern of Escherichia Coli and Klebsiella Pneumoniae in Loghman Hakim Hospital in Tehran, Iran*

	Microbial Sensitivity Test Results					
	Escherichia Coli			*Klebsiella Pneumoniae*		
Antibiotic	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin	14 (11.4)	0	109 (88.6)	0	0	40 (100)
Cephalothin	27 (21.8)	11 (8.9)	86 (69.3)	12 (30.0)	1 (2.5)	27 (67.5)
Ceftriaxone	54 (50.9)	2 (1.9)	50 (47.2)	14 (42.4)	0	19 (57.6)
Ceftazidime	68 (55.3)	1 (0.8)	54 (43.9)	19 (48.7)	0	20 (51.3)
Ceftizoxime	80 (64.5)	5 (4.0)	39 (31.5)	21 (52.5)	5 (12.5)	14 (35.0)
Trimethoprim-sulfamethoxazole	59 (48.4)	2 (1.6)	61 (50.0)	20 (50.0)	0	20 (50.0)
Amikacin	116 (93.6)	0	8 (6.4)	32 (80.0)	0	8 (20.0)
Gentamicin	71 (61.7)	3 (2.6)	41 (35.7)	25 (62.5)	1 (2.5)	14 (35.0)
Ciprofloxacin	77 (62.1)	1 (0.8)	46 (37.1)	27 (67.5)	1 (2.5)	12 (30.0)
Norfloxacin	74 (59.7)	1 (0.8)	49 (39.5)	28 (70.0)	0	12 (30.0)
Nalidixic acid	90 (73.2)	3 (2.4)	30 (24.4)	17 (42.5)	4 (10.0)	19 (47.5)
Nitrofurantoin	78 (62.9)	2 (1.6)	44 (35.5)	19 (47.5)	5 (12.5)	16 (40.0)

*Due to few missing data for some of the antibiotics the denominators of the percentages may vary.

resistance was significantly more common among *K pneumoniae* than *E coli* species (P = .02).

The ESBL-EK resistant isolates was observed in 77 (47.0%) of the isolates, including 21 *K pneumoniae* (52.5%) and 56 *E coli* (45.2%) isolates. There was no significant correlation between species of the isolates in the ESBL resistant pattern. Eighty percent of the *K pneumoniae* isolates (n = 32) and 93.5% of the *E coli* isolates (n = 116) were sensitive to amikacin (P = .01).

The MDR-ESBL pattern was detected in 44 isolates (26.8%). These included 12 of the *K pneumoniae* (30.0%) and 32 of the *E coli* (25.8%) isolates. There was not a significant correlation between the two kinds of isolates in the MDR-ESBL resistant pattern.

DISCUSSION

Thomson and colleagues[18] reported in 2000 that ESBL was most likely to be found in *K pneumoniae*, *K oxytoca*, and *E coli*. The highest isolation frequency of ESBL-producing *K pneumoniae* has been from the Latin America (45.4%), the western pacific (24.6%), and Europe (22.6%).[19] The frequencies of expressing ESBL-producing *E coli* phenotype was reported to be 8.5%, 7.9%, 5.3% in these areas, respectively.[19] Among 164 *E coli* and *K pneumoniae* isolates obtained in our study, 47% were ESBL resistant. We showed 51% of *K pneumoniae* and 44% of *E coli* isolates were resistant to ceftazidime. This is more than what Karlowsky and coworkers[20] reported (9.6% for *K pneumoniae*). The high rate of ceftazidime-resistant bacteria in our study is a remarkable finding; we should consider this in our empiric therapies of nosocomial infections.

In this research, the prevalence of resistance to fluoroquinolones by the ESBL-EK (more than 30%) was compatible with that reported by Burgess and colleagues.[21] Fluoroquinolones are particularly useful for the treatment of urinary tract infections, because high concentrations of the drug in the urine can be achieved.[22] A multicenter prospective study on *K pneumoniae* bloodstream infection conducted in 7 countries found that 18% of ESBL-producing isolates were resistant to ciprofloxacin.[23] Increasing resistance to fluoroquinolones has been reported up to 30%, which it is in agreement with the findings in the present study.[24]

In this study, aminoglycosides showed variable activities against *E coli* and *K pneumoniae* with about 10% amikacin resistance and 33.5% gentamicin resistance. Indeed, 80% of *K pneumoniae* and 93.6% of *E coli* isolates were sensitive to amikacin. It means sensitivity of *E coli* isolates to amikacin is much more likely than *K pneumoniae*. The risk of resistance to aminoglycosides has been reported to be increasing by 2- to 3-fold.[25] Among aminoglycosides, amikacin is likely to show the greatest percentage of susceptible strains, particularly in the United States with resistance rates of 10%.[19] It is in agreement with our findings.

In the present study, all of the *K pneumoniae* isolates and more than 85% of *E coli* isolates had broad-spectrum resistance. Messai and coworkers showed the same figures.[26] The MDR-ESBL was seen in 44 of our isolates (27%). This is in agreement with Hyle and colleagues' findings[15] that showed the annual prevalence of MDR among ESBL-EK isolates increased from 12.5% to as high as 26.9% during a 5-year study. According to Moland and associates,[27] clinical laboratories must be able to detect important beta-lactamases to ensure optimal patient care and infection control. Given the results of this study, this is just what we need.

CONCLUSIONS

Our study showed the emergence of MDR-ESBL-producing *E coli* and *K pneumoniae*. Treatment of MDR will become more complex in the coming years, because of further limitation of available drugs. Determination of resistant patterns can help us to choose the best antibiotics in such a situation.

CONFLICT OF INTEREST

None declared.

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Received May 2008
Revised July 2008
Accepted August 2008