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Original Article

Determination of the carbapenem resistance in *Escherichia coli* isolated from samples obtained from Shahrekord hospitals and determination of their minimum inhibitory concentration

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Abstract

Background and aims: Carbapenems are the final-line treatments for multidrug-resistant, gram-negative infections. The patterns of resistance to carbapenems among hospital bacterial pathogens vary widely across different hospitals in a country. Considering that *Escherichia coli* is one of the most important causes of nosocomial infections, it is essential to study its drug resistance.

Methods: In this descriptive-analytical study, a total of 80 samples of *E. coli* isolated from inpatients with urinary tract infections (UTIs) were collected in different wards (i.e., women, urology, infectious, and ICU) of Shahrekord hospitals. After the diagnosis and confirmation of bacteria by standard bacteriological methods, their sensitivity to imipenem and meropenem was investigated by the antibiogram (disk-diffusion) method. Then, the minimum inhibitory concentration (MIC) was determined by the E-test strip according to the Clinical and Laboratory Standards Institute (CLSI) standard.

Results: In this study, resistance to meropenem and imipenem by antibiogram (disc diffusion) was observed in 21 (25.26%) and 20 (25%) of the isolates, respectively. Twenty isolates had MIC ≥4 µg/mL for meropenem, 13 isolates demonstrated MIC≥4 µg/mL for imipenem, and 14 isolates had 1≤MIC<4 µg/mL and were semi-sensitive.

Conclusion: In general, *E. coli* had significant resistance to carbapenems. Therefore, rapid and accurate identification of these strains can be a major step to the treatment and control of these strains and prevention of the spread of the resistance.

Keywords: *Escherichia coli*, Carbapenems, Meropenem, Imipenem, Minimum inhibitory concentration

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Introduction

Microorganisms causing nosocomial infections lead to severe problems for patients and the health system due to antibiotic resistance (1). In some countries, the rate of nosocomial infections is high due to the lack of monitoring, the lack of infection prevention, the inappropriate use of antibiotics, and excessive overcrowding in the hospitals (2). According to Yang et al, the Enterobacteriaceae family, particularly *Escherichia coli*, is responsible for various types of infections, especially urinary tract infections (UTIs). These infections account for 30%-40% of nosocomial infections and are the most common cause of gram-negative septicemia in inpatients (4,5). The most common etiologic factor for UTI is *E. coli*, which accounts for most of these cases (6,7). Carbapenems include ertapenem, imipenem, doripenem, and meropenem (8), which are nowadays regarded as selective drugs that are used to treat serious infections in multidrug-resistant Enterobacteriaceae and produce extended-spectrum β-lactamases (9,10). In fact, they are the final-line antibiotics for the treatment of multidrug resistance (11). In addition, carbapenems are considered as selective therapeutic agents for gram-negative, penicillin- and cephalosporin-resistant bacterial infections due to their extended-spectrum and the lack of hydrolysis by β-lactamases enzymes (8). The excessive increase in antibiotic resistance among gram-negative bacteria has challenged drug therapies (12,13). In this study, the frequency of *E. coli* strains resistant to meropenem and imipenem was investigated by the disk diffusion method, followed by determining their minimum inhibitory concentrations (MICs) by using the E-test strip. Clinicians can select appropriate antibiotics to rapidly eradicate the infections caused by carbapenems by obtaining information on the prevalence and rate of

resistance to these bacteria in *E. coli* isolates from UTI samples in different regions of Iran and across the world. It also avoids a waste of time, the futile consumption of drugs, or even an increase in resistance to the mentioned bacteria.

Materials and Methods

In this study, 80 *E. coli* isolates were collected from UTI inpatients in different wards of the hospitals (e.g., urology, obstetrics and gynecology, internal medicine, and ICU) in Shahrekord during April to October, 2016. The inpatients had acquired UTI 48-72 hours after admission and their first urinary culture was negative. The study protocol was approved by Shahrekord University of Medical Sciences under the code IR.SKUMS.REC.1394.282. First, the samples were cultured on blood agar and eosin methylene blue (HiMedia Company, India) and then identified by biochemical differential tests such as triple sugar iron agar, Simon citrate, methyl red/Voges-Proskauer, indole motility, oxidase and catalase. After the identification and phenotypic confirmation of *E. coli*, the colony of pure bacteria was stored for subsequent tests. For this purpose, the pure colony was inoculated in 1.5 microtubes containing 700 µL of sterilized TSB culture medium and incubated at 37˚C overnight. After the growth, 300 µL of sterilized glycerol was added to it and stored under -70˚C in the freezer. To determine the antibiotic resistance, a suspension of pure bacteria equivalent to the 0.5 McFarland was prepared and then passaged on the surface of Mueller-Hinton agar medium and incubated at 37˚C for 18-24 hours. Antibiotic susceptibility was determined by the disk diffusion (Kirby-Bauer) method for two antibiotics, namely, meropenem (10 µg) and imipenem (10 µg) obtained from MAST Company, UK. After the incubation, the diameter of the growth inhibition zone was measured and interpreted in accordance with the CLSI Table. In addition, *E. coli* ATCC 25922 was used for the quality control of susceptibility testing. MIC was determined by using the E-test strips (Liofilchem, Italy) at a concentration ranging from 0.002 to 32 mg/L. Next, bacterial suspension was prepared from pure cultures to match the turbidity of the 0.5 McFarland and cultured on the Mueller-Hinton agar. Then, the strip was placed on the medium and incubated at 37˚C for 16-18 hours. The antibiotic release from the strip into the surrounding area formed an oval zone and the MIC extended to where it crossed the strip (14-18).

Results

The clinical samples of inpatients with UTI in different wards of Hajar and Kashani hospitals in Shahrekord were isolated, the details of which are presented in Table 1. As shown in Table 1, most isolates were obtained from the inpatients in the urology department. According to the results of the disk diffusion method, 21 and 20 isolates of *E. coli* were resistant to meropenem and imipenem, respectively, and 5 isolates had intermediate resistance (semi-sensitive).

The highest number of resistant isolates of *E. coli* to meropenem and imipenem was isolated from the urology department, and none of the isolated strains from the internal medicine department had MIC ≥4 for meropenem. The E-test strips were read from the top (high concentrations) to the bottom (low concentrations of the antibiotic) after 18 hours of incubation (Figure 1 and 2). According to the E-test results, 20 isolates were resistant to meropenem and 13 isolates to imipenem (MIC ≥4), indicating the high accuracy of the E-test method compared to the disk diffusion method for detecting resistant and semi-sensitive strains. The frequency of carbapenem-resistant and sensitive isolates is presented in Table 2.

Discussion

Opportunistic bacteria are the cause of nosocomial infections in different hospital wards and cause certain infections such as pneumonia, UTIs, and bacteremia after hospitalization. The underlying factors such as surgery, the weakness of the inpatient's immune system, and long-term hospital stay contribute to the acquisition of these infections. Further, *E. coli*, as the most common cause of UTIs, is considered as an important pathogen in the development of neonatal meningitis, respiratory infections, sepsis, fever and frequent urination, and disseminated intravascular coagulation in the inpatients in different wards of the hospital. Furthermore, antibiotic resistance is very common among the *E. coli* strains, which is due to the acquisition of multiple resistance factors. Today, bacterial resistance to various antibiotics has become a global problem and the uncontrolled administration of antibiotics for the treatment of bacterial infections has led to the selection

Table 1. The frequency distribution of *Escherichia coli* isolated from clinical ward

Clinical Ward	*E. coli* (No.)	Resistant to Meropenem (MIC≥4)	Resistant to Imipenem (MIC≥4)
Urology	33	10	7
Gynecology and obstetrics	16	4	2
Infection	14	5	1
Internal	12	0	2
Intensive care unit	5	1	1

Table 2. The frequencies of strains according to the minimum inhibitory concentrations of imipenem and meropenem

Antibiotics	R (MIC≥4)	I (4>MIC≥1)	S (MIC<1)
Meropenem	20	8	52
Imipenem	13	6	61

Note. MIC: minimum inhibitory concentration.

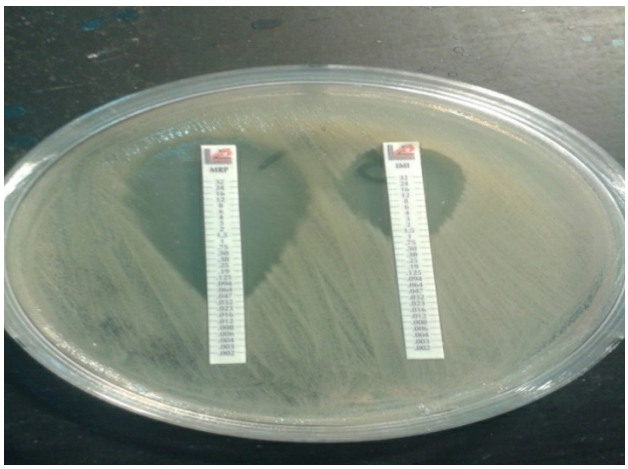

Figure 1 . The E-test strips of meropenem and imipenem for meropenem and imipenem-susceptible isolate (MIC < 1).

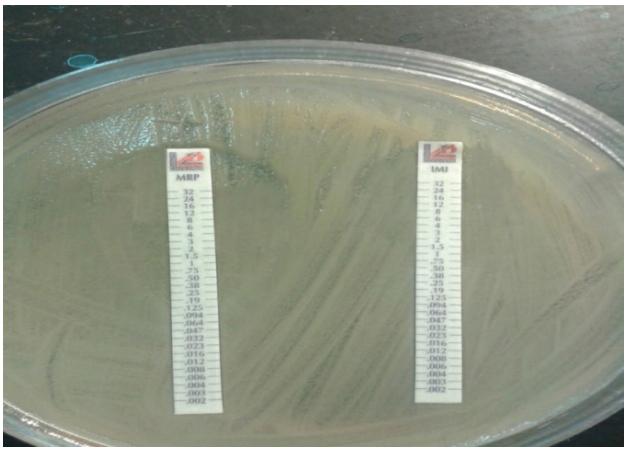

Figure 2. The E-test strips for meropenem and imipenem-resistant isolate (MIC ≥ 4)

of resistant strains (19). Antibiotic resistance patterns among nosocomial pathogenic bacteria may significantly vary from one country to another or in different regions of a country (20). Moreover, most pathogens are relatively resistant to some of the new antibiotics such as extended-spectrum cephalosporins (e.g., cefotaxime and ceftazidime). Previously, imipenem was considered the most active drug for the infections. Recently, however, evidence demonstrates the spread of imipenem-resistant strains (21,22). The wide emergence of resistance to imipenem and meropenem is a serious threat to future treatment. Additionally, antibiotic resistance and susceptibility vary from country to country due to environmental factors and the use of different antimicrobial agents (23). Previous research has also confirmed the selection of the E-test method as a precise and sensitive method for determining the susceptibility of bacteria to antibiotics (24). In a similar study conducted by Nobari et al regarding the investigation of resistance to carbapenems on 180 isolates, it was revealed that 42, 29, and 14 isolates were resistant to meropenem, ertapenem, and imipenem, respectively (25). Therefore, resistance to *E. coli* appears to be constantly changing and it is essential to determine this change in different regions. In a study by Simhon et al, the sensitivity of imipenem decreased from 98% in 1990 to 64.1% in 2000 and the ciprofloxacin sensitivity reduced from 50.5% to 13% (26). Additionally, Bora et al. investigated resistance to carbapenems in *E. coli* and *Klebsiella pneumoniae* strains by using phenotypic tests and found that 41 of 216 *E. coli* isolates and 39 of 185 *K. pneumoniae* isolates were resistant to carbapenems (27). Similarly, in their study in Al-Zahra hospital of Isfahan between the time period of March 2012 and December 2012, Moayednia et al reported that 9, 14, and 8 out of 720 *E. coli* isolates were resistant to meropenem, imipenem, and ertapenem, respectively, showing a lower resistance rate to meropenem and imipenem in the past compared to our current study (28). In a study conducted by Shahcheraghi et al in Tehran, 9 out of 244 isolates of *E. coli* were identified as meropenem-resistant, 1 isolate as imipenem-resistant, and 2 isolates as ertapenem-resistant (29). In addition, a study in a hospital in northern Palestine by Adwan et al on 79 isolates of *E. coli* showed that 30 and 35 isolates were resistant to meropenem and imipenem, respectively, which can represent an increased resistance to another region of the world (30). This is also consistent with the results of a study by Kanchanadevi and Sekaran in which 25 out of 76 *E. coli* isolates, 2 out of 9 *Klebsiella* isolates, and 42 out of 60 *Pseudomonas* isolates were resistant to imipenem according to the E-test (31). Resistance to carbapenems is very important because if the isolates from nosocomial infections are resistant to carbapenems, they will be also resistant to other antibiotics. Therefore, other antibiotics such as carbapenem-Sulbactam, colistin, or tigecycline should be used to treat these types of infections. Studying the resistance rate of *E. coli* isolates will provide adequate information for physicians to develop appropriate methods for the treatment of the infections due to this bacterium (2).

Conclusion

This study indicates a significant prevalence of resistance to the mentioned antibiotics, and this could be an alarm signal for the Healthcare.

Conflict of Interests

None.

Acknowledgments

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