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Original Article

Determination of antibiotic resistance and minimum inhibitory concentration of meropenem and imipenem growth in *Klebsiella* strains isolated from urinary tract infection in Shahrekord educational hospitals

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Abstract

Background and aims: *Klebsiella* is an opportunistic organism that is the cause of severe diseases such as pneumonia, septicemia, and urinary tract infections (UTIs). In addition, high antibiotic resistance has challenged the treatment of this bacterium. However, carbapenem antibiotics are considered as the therapeutic agents for selecting the treatment of penicillin- and cephalosporin-resistant gram-negative bacterial infections. The present study aimed to determine the resistance and minimum inhibitory concentration (MIC) of meropenem and imipenem.

Methods: A total of 80 *Klebsiella spp* isolated from UTIs were collected in various educational wards (i.e., urology, obstetrics, and gynecology, as well as the units of infectious diseases, internal medicine, and intensive care) in different hospitals of Shahrekord. The isolates were then identified by using biochemical tests. Further, disc diffusion method was employed to determine the antibiotic resistance. Furthermore, MIC was estimated by the Epsilon-test strip. Moreover, P=Q=0.50, an error of 0.05, and an accuracy of 0.11 were considered for determining the sample size (n = 80).

Results: Based on the results of disc diffusion method, 24 strains were resistant to meropenem and imipenem. Additionally, the MIC was 24 (30%) by the E-test. In addition, 24 isolates had a MIC of ≥4 µg/mL for meropenem and imipenem and thus were resistant while 18 isolates were found to have a MIC of 1≤ MIC<4 µg/mL and therefore, were considered semi-sensitive ($P<0.001$).

Conclusion: In general, *Klebsiella* strains were found to be resistant to meropenem and imipenem. Therefore, rapid and accurate identification of these strains and the selection of appropriate antibiotics can help quickly eradicate the infections caused by these bacteria. Accordingly, a waste of time, the consumption of medication, or even an increased resistance are prevented.

Keywords: *Klebsiella*, Antibiotic resistance, Meropenem, Imipenem

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Introduction

Urinary tract infection (UTI) is the second leading reported infection in patients since nearly seven million patients are annually treated by the doctors (1). The presence of over 10^5 colony forming units (CFU)/mL of a bacterium in the urine sample indicates the UTI. *Klebsiella* strains are one of the major bacterial causes of the UTI (2). *Klebsiella* is an opportunistic bacterium which is related to severe conditions such as pneumonia, septicemia, and UTIs. The importance of this bacterium, as a human pathogen, is associated with the development of hospital infections in patients with immunodeficiency and underlying diseases such as diabetes mellitus and chronic pulmonary problems (3). UTIs are the most common infections among *Klebsiella* infections. The risk of UTI acquisition is far higher than the normal rate in people with bladder infections and diabetes (4). In recent years, the emergence of *Klebsiella* strains with multidrug resistance (MDR) has been observed due to the over-the-counter and excessive consumption of the antibiotics (5). In addition, antimicrobial resistance is always a major concern for human health and affects patients in hospitals across the world (6,7). Further, carbapenem antibiotics include ertapenem, imipenem, doripenem, and meropenem. The carbapenemase enzymes belong to the molecular classes A, B, and D, which are able to inactivate carbapenem antibiotics such as meropenem and imipenem. Among the carbapenemase enzymes,

Klebsiella pneumoniae carbapenemase, which is a class A carbapenemase, is inhibited by different degrees of clavulanate and hydrolyzes penicillins and cephalosporins more than carbapenems. This enzyme is considered an extended broad-spectrum β-lactamase (ESBL) since it lacks a robust carbapenemase activity. Furthermore, class B carbapenemases (Bush group 3) are known as the metallo-beta-lactamases that hydrolyze carbapenem antibiotics except for the aztreonam and are resistant to β-lactamase enzyme inhibitors such as clavulanate. However, these lactamases are inhibited by the chelating agents such as ethylenediaminetetraacetic acid which can degrade and inhibit carbapnemase enzymes, and therefore, it is a component of the diagnostic tests for these enzymes (8, 9). Carbapenem antibiotics are the drugs of the choice for treating the infections caused by gram-negative bacilli resistant to penicillin and cephalosporins due to being a broad spectrum and lacking the hydrolysis by beta-lactams (10,11). Moreover, these antibiotics are currently regarded as the drugs of choice for treating serious infections related to MDR and ESBL-producing Enterobacteriaceae (12,13). Infections caused by the MDR gram-negative bacteria lead to higher morbidity and mortality, long-term hospital stay, increased health care costs, and limited therapeutic options (14,15). Therefore, these resistant bacteria should be identified using different methods such as antibiotic susceptibility testing, the determination of minimum inhibitory concentration (MIC), and genotype confirmatory tests including a polymerase chain reaction. Epsilon-test (E-test) is the most accurate method for determining the MIC of the antibiotics (16). If the bacterium is resistant to carbapenems, treatment with carbapenem alone cannot lead to patient recovery while it leads to the emergence of antibiotic-resistant bacteria, prolong medical treatments, or even mortality in the patients. Accordingly, the current study sought to detect meropenem- and imipenem-resistant *Klebsiella* strains by disc diffusion method in patients with UTI and to evaluate the resistance by MIC of these strains using the E-test strip.

Materials and Methods

In this descriptive-analytical study, 80 *Klebsiella* isolates (i.e., 74 *pneumonia* and 6 *oxytoca* isolates) were collected from patients with UTI in different wards (e.g., urology, obstetrics, and gynecology, in addition to infectious diseases, internal medicine, and intensive care units) of Hajar (48 isolates) and Kashani (32 isolates) Educational Hospitals during (21 April-22 October) 2016-2017. Additionally, using the statistical formula, the sample size was determined 80 by taking into account the maximum prevalence of P=Q=0.50, the error of 0.05, and an accuracy of 0.11. The inclusion criterion was *Klebsiella* isolates separated from patients with UTI 48-72 hours after hospitalization, and their primary urinary culture was negative at the time of referring to the hospital. In addition, the exclusion criterion encompassed a history of antibiotic usage. To isolate the samples, first, the information of the admitted patients including the hospitalization record and antibiotic usage within two weeks before the sampling, which was collected by using a questionnaire. The isolates were first cultured on blood Agar and eosin methylene blue (Himedia, India) and then the bacteria were identified employing biochemical differential tests such as the triple sugar iron, Simmons Citrate, methyl red, Voges–Proskauer, indole, mobility, oxidase, and catalase (Himedia, India). Following identifying and confirming the phenotypes of the *Klebsiella* bacterium (*pneumonia* and *oxytoca*), they were inoculated from the colony of the purified bacteria to 1.5 µM microtubes containing 700 µL of the sterile tryptic soy broth (Himedia, India) and incubated overnight at 37°C. After the growth of the bacteria in the medium, 300 µL of sterilized glycerol (15% concentration) was added to medium and the bacteria were stored in a freezer at -70°C to prevent the death of the bacteria in a long time. Further, the criteria proposed by the Clinical and Laboratory Standard Institute were used (17) in order to identify the *Klebsiella* strains. For this purpose, first, a suspension of the pure culture of the bacteria, equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL), was prepared and then cultivated on the Mueller-Hinton agar (Merck, Germany). Next, the culture media were incubated at 37°C for 18-24 hours. The antibiotic susceptibility of the bacteria was determined by disc diffusion method (Kirby-Bauer method) for two antibiotics, namely, meropenem and imipenem (10 µg each, MAST, England). After incubation, the diameter of the inhibition zone was measured and interpreted according to the standard antibiogram. Furthermore, the resistance of the strains to meropenem and imipenem was identified by determining the MIC using the E-test Liofilcheme (Italy) with a concentration range of 0.002-32 µg/mL. Moreover, the bacterial suspension, equal to 0.5 McFarland turbidity, was prepared from fresh and pure bacterial culture and cultivated on the Mueller-Hinton agar. Then, the strip was placed on the medium and incubated at 3 °C for 16-18 hours (18-21).

Results

The data related to the isolates from different wards of the hospital are presented in Tables 1 and 2. As shown, most samples were collected from the patients in the urology ward while the least samples were taken from the intensive care unit (ICU). A number of 34 (42.5%) and 46 (57.5%) out of 80 patients with UTI, within the five-month period of sampling, were males and females, respectively, showing a higher incidence of UTI in women. In addition, respecting the prevalence of antibiotic resistance in men and women, the number of meropenem- and imipenem-resistant samples was higher in women (n = 16) compared

Table 1. The antibiotic results for meropenem and imipenem and the number of *Klebsiella pneumoniae* isolates in different wards

Wards	*Klebsiella pneumonia* (n)	Resistant (n)	Semisensitive (n)	Sensitive (n)
Urology	22	8	4	10
Obstetrics and gynecology	14	4	2	8
Infectious diseases	20	11	1	8
Internal medicine	10	0	0	10
Intensive care unit	8	0	2	6

Table 2. The antibiotic results for both meropenem and imipenem antibiotics and the number of *Klebsiella oxytoca* isolates in different wards

Wards	*Klebsiella oxytoca* (n)	Resistant (n)	Semisensitive (n)	Sensitive (n)
Urology	2	1	0	1
Obstetrics and gynecology	1	0	1	0
Infectious diseases	2	1	0	1
Internal medicine	1	0	0	1
Intensive care unit	0	0	0	0

to men (n = 8), indicating a significantly higher prevalence of resistance to these antibiotics in women than men (Table 3). Additionally, as regards the age, the patients were within the age range of 4-72 years old. Further, most cases with UTI were 28-45 years old and the prevalence of antibiotic resistance was higher in older patients (34-45 years) compared to the younger ones. A total of 30% (i.e., 24 isolates including 22 *pneumonia* and 2 *oxytoca* isolates) were resistant to meropenem and imipenem based on the disc diffusion results (Tables 1 and 2). Furthermore, the E-test strips were read from the top to the bottom of the strip (i.e., high to low concentrations of the antibiotics) after 18 hours of incubation. The antibiotic penetration from the strip into the medium containing agar caused an oval zone. Moreover, the MIC was decided to be the point where the oval zone crossed the strip (21,22).

Table 3. The antibiogram results of meropenem and imipenem in men and women

	Totals samples	Resistant
Men	34	8
Women	46	16

Table 4. The MIC of imipenem and meropenem antibiotics in *Klebsiella pneumoniae* isolates

Antibiotic	Resistant (n) MIC≥4	Semisensitive (n) 1≤ MIC<4	Sensitive (n) MIC<1
Meropenem	22	17	35
Imipenem	22	17	35

Table 5. The MIC of imipenem and meropenem antibiotics in *Klebsiella oxytoca* isolates

Antibiotic	Resistant (n) MIC≥4	Semisensitive (n) 1≤ MIC<4	Sensitive (n) MIC<1
Meropenem	2	1	3
Imipenem	2	1	3

Based on the MICs, 24 (30%) isolates were resistant to meropenem and imipenem with a MIC of ≥4 µg/mL whereas 18 isolates had a MIC of 1≤ MIC<4 and were found to be semisensitive. Tables 4 and 5 separately present the MIC results for the *Klebsiella* species (i.e., *pneumoniae* and *oxytoca*). Only 2 out of the 6 *oxytoca* isolates were resistant to meropenem and imipenem, indicating the resistance among other *Klebsiella* species. Additionally, it demonstrates that this resistance is limited to no pneumonia.

Regarding the number of meropenem and imipenem-resistant, the majority of the isolates were separated from the patients in the infectious disease wards. None of the strains were isolated from the internal ward and intensive care unit had a MIC of ≥4 µg/mL. Based on the results of the disc diffusion method and E-test, the number of resistant isolates was equal. These isolates were completely resistant to meropenem and imipenem, and their MICs were even greater than 8 µg/mL, which confirmed the high resistance of these samples by the diffusion method. However, the number of these strains (18 isolates) was higher in semi-sensitive strains compared to the disc diffusion results (10 isolates), which was due to the higher accuracy and greater sensitivity of the E-test strip compared to the disc diffusion method regarding identifying semi-sensitive and resistant strains. The significance level for the results of the Fisher exact test was considered $P < 0.001$, and the results were found to be significant with respect to the number of strains isolated in each ward and the resistance level. Figures 1A and 1B illustrate the E-test bar for morphine and imipenem strains.

Discussion

The most common cause of UTI is *Escherichia coli*, followed by *Klebsiella* and *Staphylococcus saprophyticus* species (23). The importance of *Klebsiella*, as a human pathogenic agent, is related to the development of nosocomial infections and UTIs (24). The release of drug resistance factors in gram-negative bacteria and *Klebsiella* leads to an increase in the resistance of these microorganisms to various antibiotics. Therefore, therapeutic procedures for the patients are elongated (25,26). In the present study, 24 isolates were meropenem and imipenem resistant with MIC ≥4 while 18 isolates were found to be semi-sensitive with 1≤ MIC<4, which is in line with the results of Nobari et al in Iran, where in an investigation regarding resistance to carbapenems in 180 *Klebsiella* isolates, they observed that 42 isolates were resistant to meropenem whereas 29 and 14 isolated were resistant to ertapenem

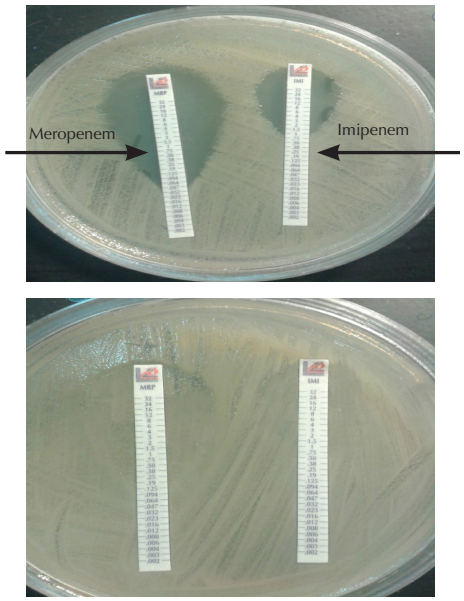

Figure 1. (A)The E-test strip of meropenem and imipenem-sensitive strain (MIC<1). (B) The E-test strip of meropenem and imipenem resistant strain.

and imipenem, respectively. In terms of the number of meropenem-resistant isolates in the total number of isolates, their results are consistent with the findings of the current study. The low number of imipenem-resistant strains compared to meropenem and ertapenem resistant ones in the study by Nobari et al may be due to several reasons such as the rate of treatment with imipenem in the study setting, the antibiotic susceptibility pattern of the studied samples, being inpatient or outpatient, and the like (27). In addition, in another study by Kanchanadevi et al in India, 25 out of 76 *E. coli* isolates, 2 out of 9 *Klebsiella* isolates, and 42 out of 60 *Pseudomonas* isolates were found to be resistant to imipenem based on the MIC results by using E-test, which is approximately consistent with the results of the current study with respect to the number of imipenem-resistant *Klebsiella* isolates per the total number of the isolates, indicating a lower prevalence of carbapenem resistance in *Klebsiella* and *E. coli* compared to *Pseudomonas* (28). Further, based on the results of previous studies, antibiotic resistance in Iran has been risen due to the excessive and over the counter use of antibiotics, as well as the acquisition of antibiotic resistance factors such as resistance genes spread through various methods including conjugation in the *Klebsiella* strains. The existence of this resistance was observed in the current study, which confirms this argument (29,30). Furthermore, in a study by Tawfik et al, 87 inpatients in the ICU were investigated, 46 of whom were colonized with different bacterial agents. All of these bacteria were examined for resistance to carbapenems, the results of which demonstrated that 20 (43.5%) *Pseudomonas aeruginosa*, 11 (23.9%) *Acinetobacter baumannii*, 3 (6.5%) *E. coli*, 2 (4.3%) *K. pneumoniae*, and 1 (2.2%) Enterobacter were resistant to carbapenems, which contradicts the results of the present study with respect to resistant *Klebsiella* isolates from the patients in the ICU. In this study, a total of 8 isolates were obtained from the patients in the ICU, none of which were resistant to meropenem and imipenem (31). Based on the findings and statistics, the prevalence of antibiotic-, meropenem-, and imipenem-resistant strains varies in different parts of the world. The results of the present study represented that *Klebsiella* strains isolated from patients with UTIs who were admitted to the wards of the hospital were significantly resistant to meropenem and imipenem, which indicates the colonization rate of *Klebsiella* strains in the hospital environment and higher sensitivity of these patients to obtain these organisms. The extensive and over-the-counter use of third-generation cephalosporins causes this resistance. If antibiotic resistance continues, antibiotics are likely to be effective in treating the infections resulted from these microorganisms. Moreover, the causes of low resistance to imipenem and meropenem compared to other antibiotics in various studies, as well as the current study, include low use of these antibiotics among the treatment teams and the lack of its use in outpatients since they are among the antibiotics used in hospitalized patients only with the physician' prescription, and the lower incidence of carbapnemase enzymes in the Enterobacteriaceae family compared to other bacteria (32-35). In a study conducted by Validi et al in Iran, of the 75 *Klebsiella* isolates obtained from the stool, blood, urine, phlegm, and wound, the least antibiotic resistance was observed with respect to meropenem and imipenem, and only one sample was resistant to these two antibiotics, which is not consistent with the results of the present study regarding the prevalence of resistance of these antibiotics. This can be due to the awareness of individuals in a region about the consequences of over-the-counter antibiotic use which makes the users predisposed to acquiring resistance, as well as the different prevalence of the genes responsible for this resistance in different regions (36). In this study, the most samples of UTI at the time of the specific sampling were from females, which indicates the higher incidence of the infection in women. This statistically corroborates with the findings of the other studies conducted in different parts of the world. The prevalence is more common in women compared to men, with almost half of the world's women experiencing this infection at least once in their lifetime (37). The proximity of the genitalia to the urethra (38), pregnancy, and sexual activity are the causes of the high prevalence of UTI in women. Additionally, many cases of UTI are treated as gastrointestinal or even respiratory infections, which increases the risk of developing pyelonephritis, urinary stones, preterm labor, and fetal death (39). In terms of age, the incidence of UTI increases with increasing age in both genders. However, the acquisition of this infection is lower in young men within the same duration, which matches the results of the current study where the number of UTI cases was lower

in the people with lower ages (40). In addition, regarding the prevalence of antibiotic resistance in men and women, in the present study, the resistance to meropenem and imipenem was higher in women compared to men and in the middle-aged and older women compared to younger ones. Further, Doosti et al in their study on 120 samples of UTI found a comparatively higher prevalence of antibiotic resistance in women than men and in people aged 27-39 years and older compared to the younger ones (41). Furthermore, Mohammadi et al observed that resistance to antibiotics and beta-lactams was higher in women compared to men among the hospitalized patients (42), which is in line with the results of the current study with respect to the prevalence of resistance in women and men.

Conclusion

In general, based on the findings of this study, *Klebsiella* is the cause of UTI and resistant to meropenem and imipenem, which is consistent with the findings of other similar studies. Accordingly, physicians' awareness of resistance to antibiotics and bacteriological agents in every region is of great importance. Therefore, controlling the increase of this resistance is needed to prevent the over-the-counter use of antibiotics. Furthermore, pharmacies should not be allowed to sell the antibiotics without a doctor's prescription. As a result, future researchers are recommended to implement further studies on genes and enzymes that contribute to resistance to carbapenems and use faster diagnostic methods such as polymerase chain reaction in subsequent studies in order to prevent the increased therapeutic costs and the spread of this resistance.

Conflict of interests

None.

Ethical considerations

The study protocol obtained the ethics code IR.SKUMS.REC.1394.282 from Shahrekord University of Medical Sciences.

Acknowledgments

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