

Comparison of in vitro activities of meropenem productions on *Klebsiella pneumoniae* isolated from hospitalized patients

Vergleich der In-vitro-Wirksamkeit von Meropenem-Generika verschiedener Hersteller gegen *Klebsiella pneumoniae*-Isolate hospitalisierter Patienten

Abstract

Purpose: Antimicrobial activities of meropenem products on *Klebsiella pneumoniae* isolates were determined.

Methods: 212 non-duplicated *Klebsiella pneumoniae* isolates were examined for *in vitro* meropenem susceptibility test by using the following disks, which were made from Meronem (AstraZeneca, UK), Exipenem (Exir, Iran) and Meroxan (DAANA, Iran) powders. MIC₅₀ and MIC₉₀ for meropenem antibiotics were determined.

Results: Meronem had good activities against most isolates of *Klebsiella pneumoniae*, and only a few strains had a rather high MIC. Exipenem and Meroxan showed a similar activity with Meronem.

Conclusion: Regarding the comparison of two internal generic meropenem products with the external Meronem product have shown that they are equivalents in terms of microbiological activity, as measured using the disk diffusion and MIC. In developing countries, we suggested preparing disks with antibiotic powders that can be an equivalent function in microbiological activity with standard disks. In addition, since it demonstrated significant antimicrobial activity against the *Klebsiella pneumoniae*. For use of Exipenem and Meroxan *in vivo*, it would be better to perform additional testing (activity against different species, stability etc.).

Keywords: *Klebsiella pneumoniae*, meropenem productions, agar dilution method

Zusammenfassung

Zielsetzung: Bestimmung der antimikrobiellen Wirksamkeit verschiedener Meropenem-Generika gegen *Klebsiella pneumoniae*-Isolate.

Methode: 212 nicht duplizierte *Klebsiella pneumoniae*-Isolate wurden *in vitro* auf Empfindlichkeit gegen Meropenem in Form handelsüblicher Plättchen von Meronem (AstraZeneca, UK) und Exipenem (Exir, Iran) bzw. als Meroxan-Puder (DAANA, Iran) untersucht. Bestimmt wurden jeweils die MIC₅₀ und die MIC₉₀.

Ergebnisse: Meronem war gegen die meisten Isolate von *Klebsiella pneumoniae* gut wirksam, nur einige Stämme hatten eine etwas höhere MIC. Exipenem und Meroxan erwiesen sich als vergleichbar wirksam wie Meronem.

Schlussfolgerung: Der Vergleich zweier interner Meropenem-Generika mit dem externen Standard Meronem ergab, dass alle drei Produkte sowohl im Plättchendiffusionstest als auch bezüglich der MIC in ihrer antimikrobiellen Wirksamkeit gleichwertig waren. Daher empfehlen wir für Entwicklungsländer, Plättchen mit antibiotischem Puder selbst herzustellen, da sie in ihrer antimikrobiellen Aktivität dem Standardplättchen gleichwertig sein können. Meropenem erwies sich als wirksam

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gegen die *Klebsiella pneumoniae*-Isolate. Für den Einsatz von Exipenem und Meroxan *in vivo* sollten allerdings ergänzende Tests durchgeführt werden (Wirksamkeit gegen verschiedene Species, Stabilität usw.).

Introduction

Carbapenems are the most potential β -lactam antibiotics, which developed in the 1980s, to oppose to β -lactamases resistance antibiotics. Meropenem is one of the broad-spectrum carbapenems against several clinically relevant Gram-negative aerobes and anaerobes [1], [2]. The bactericidal activity of meropenem is caused by the inhibition of cell wall synthesis through the inactivation of penicillin-binding proteins [2], [3]. Meropenem is approved by FDA for the treatment of bacterial meningitis, complicated skin and soft tissue and intra-abdominal infections. The increasing prevalence of resistance to beta-lactams [4], [5], [6] has prompted carbapenems as one of the cornerstone antibiotic classes remaining a mainstay for the treatment of patients with severe infections due to ESBL-producing Gram-negative bacteria [7], [8], [9]. The high prevalence of antibiotic resistance in *Enterobacteriaceae*, especially in *Klebsiella pneumoniae* has been achieved in Iran. Resistance often includes new agents such as carbapenems, even before the introduction in Iran [10], [11], [12]. Carbapenems are used extensively in the treatment of Gram-negative bacteria infections in teaching hospitals [13]. To know the susceptibility to meropenem of commonly isolated *Klebsiella pneumoniae* in Iran, we conducted a study to evaluate *in vitro* antimicrobial activities of meropenem products against *Klebsiella pneumoniae*, including those commonly causing nosocomial infections in Tabriz teaching hospitals in Iran.

Materials and methods

Bacterial isolates

In this study, a total of 212 isolates of *Klebsiella pneumoniae* were collected from hospitalized patients at Tabriz University Hospitals from 2012 to 2013. The obtained isolates were identified by conventional biochemical tests such as oxidase, TSI, SIM, urea, etc. In the next step, the Microgen™ GN-ID kit (Microgen Bioproducts, England) was used for final identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing including 12 antibiotics Amoxicillin-clavulanic acid (30 μ g), Cefotaxime (30 μ g), Ceftriaxone (30 μ g), Gentamicin (10 μ g), Aztreonam (30 μ g), Tetracycline (30 μ g), Ceftazidime (30 μ g), Cefepime (30 μ g), Colistin (10 μ g), Ciprofloxacin (5 μ g), imipenem (10 μ g), and meropenem (10 μ g) was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14].

Disk preparation

All isolates were examined for *in vitro* meropenem susceptibility test by using the following disks that were made from Meronem (AstraZeneca, UK), Exipenem (Exir, Iran), and Meroxan (DAANA, Iran) powders; each of these disks was compared with the other kind of meropenem products. Sterile blank diffusion disks were placed in labeled plates for meropenem products. Sterile blank disks were saturated with 20 μ l of individual stock meropenem products. After the disks were dried, these were ready to be used for disk diffusion. Commercially available antibiotic disks (meropenem; MAST, UK) were used as standards for comparison.

Agar dilution test

The susceptibilities of all *Klebsiella pneumoniae* isolates to meropenem products were determined by the agar dilution method as described by the CLSI [14]. The inoculated plates were incubated in ambient air at 35°C for 16 to 18 h. Mueller-Hinton agar was used for susceptibility testing by the agar dilution method. The minimum inhibitory concentration (MIC) of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism. Control strain, including *E. coli* ATCC 25922 was included in each set of tests.

Statistical analysis

To compare the *in vitro* activity of different meropenem products on *Klebsiella pneumoniae* isolates was determined by chi-square. SPSS, version 16 was used to perform statistical analysis, the chi-square test, when appropriate that *p* values are less than 0.05 were considered statistically significant.

Results

The isolates were collected from different infection sites of patients hospitalized in several wards. *Klebsiella pneumoniae* isolates were most frequently recovered from tracheal secretions (65%), followed by urine (16.9%), wounds (14.1%) and blood (4%). Frequency of antibiotic resistance in *Klebsiella pneumoniae* isolates is shown in Figure 1. The highest and the lowest resistance were observed in amoxicillin-clavulanic acid (61.3%), imipenem (1.8%), meropenem (0.9%) and colistin (0%) respectively. Meropenem had demonstrated significant *in vitro* antimicrobial activity against all *Klebsiella pneumoniae* isolates; 99.1% of the isolates were susceptible to meropenem. Moreover, all isolates were susceptible to colistin. Among the 212 *K. pneumoniae* isolated from Tabriz hospitalized

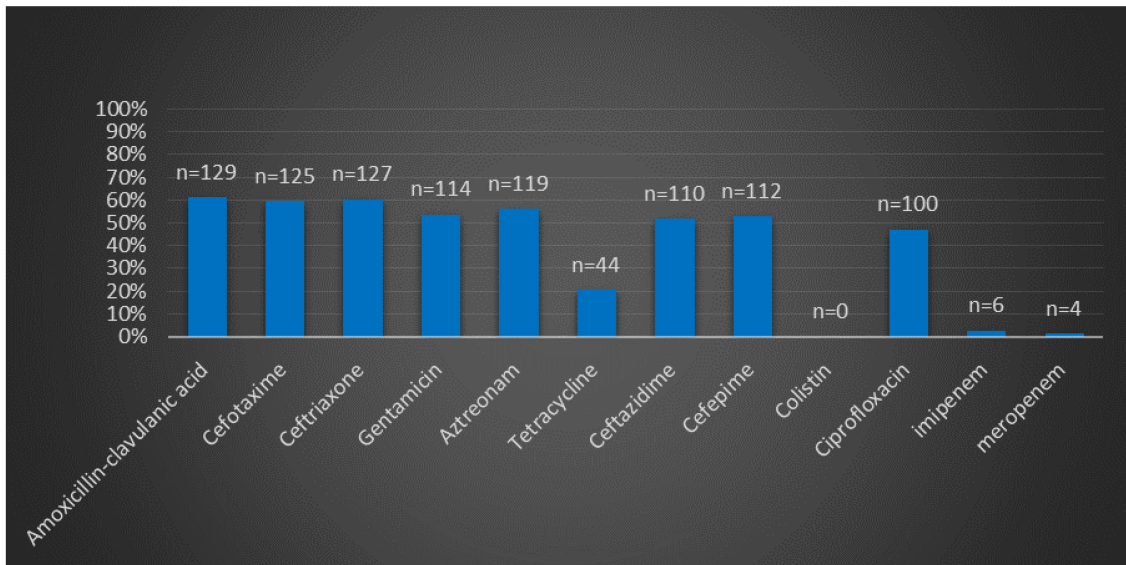


Figure 1: Antibiotic resistances pattern of *Klebsiella Pneumoniae* in Tabriz hospital, Iran

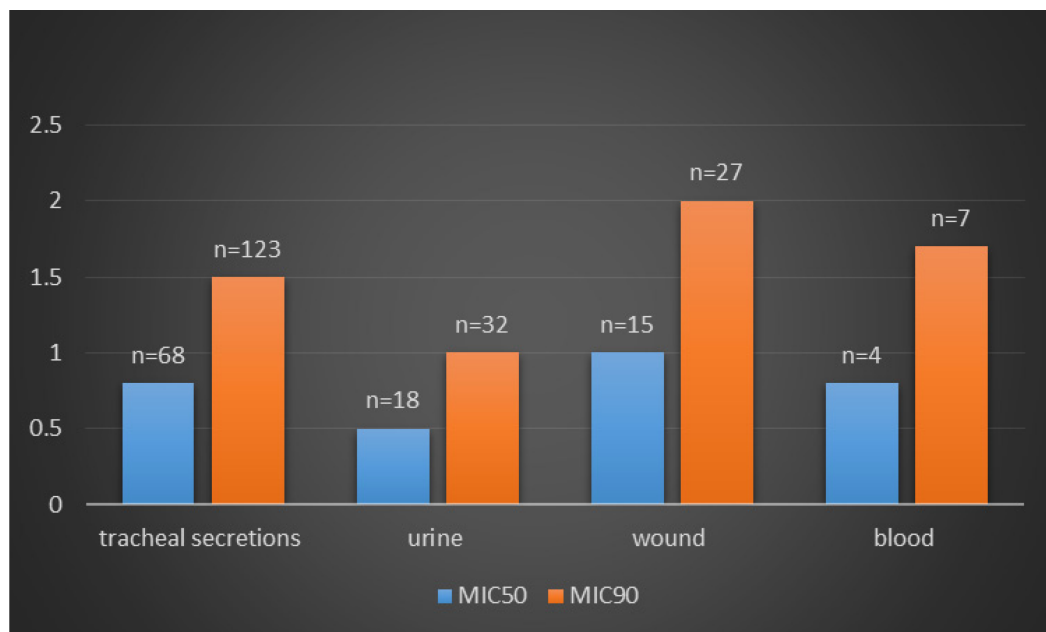


Figure 2: MIC₅₀ and MIC₉₀ of meropenem for *Klebsiella pneumoniae* isolated from different clinical sources

patients, 128 isolates showed simultaneous resistance to six antibiotics (aztreonam, cefotaxime, amoxicillin-clavulanic acid, gentamicin, ceftriaxone, and ceftazidime). Disks, which were made with three kinds of meropenem products, showed similar antibacterial activity in comparison with the standard meropenem disk (MAST, UK). In Table 1 is shown the concentration of different meropenem products (MeroneM, Exipenem and Meroxan products compare with meropenem disk (MAST, UK)) that have the similar microbiology activity. Wound infection was the main source of carbapenem-producing *K. pneumoniae*. *K. pneumoniae* isolated from wound samples was shown to produce carbapenemase at a significantly different rate ($P < 0.05$) depending on the length of stay in hospital (Figure 2). The MICs of meropenem products against all *Klebsiella pneumoniae* isolates are shown in Figure 3. MeroneM (AstraZeneca, UK) had good activities

against most isolates of *Klebsiella pneumoniae*, and only a few strains had a rather high MIC ($\geq 4 \mu\text{g/mL}$). Exipenem (Exir, Iran) and Meroxan (DAANA, Iran) showed a similar activity with MeroneM (AstraZeneca, UK).

Table 1: Comparison of concentration of meropenem products in the disks manually prepared with standard meropenem disk (MAST, UK)

Standard Disk	MeroneM (Astra Zeneca, UK)	Exipenem (Exir, Iran)	Meroxan (DAANA, Iran)
Meropenem (MAST, UK)			
10 μg	11 $\mu\text{g}/20 \mu\text{l}$	12 $\mu\text{g}/20 \mu\text{l}$	12 $\mu\text{g}/20 \mu\text{l}$

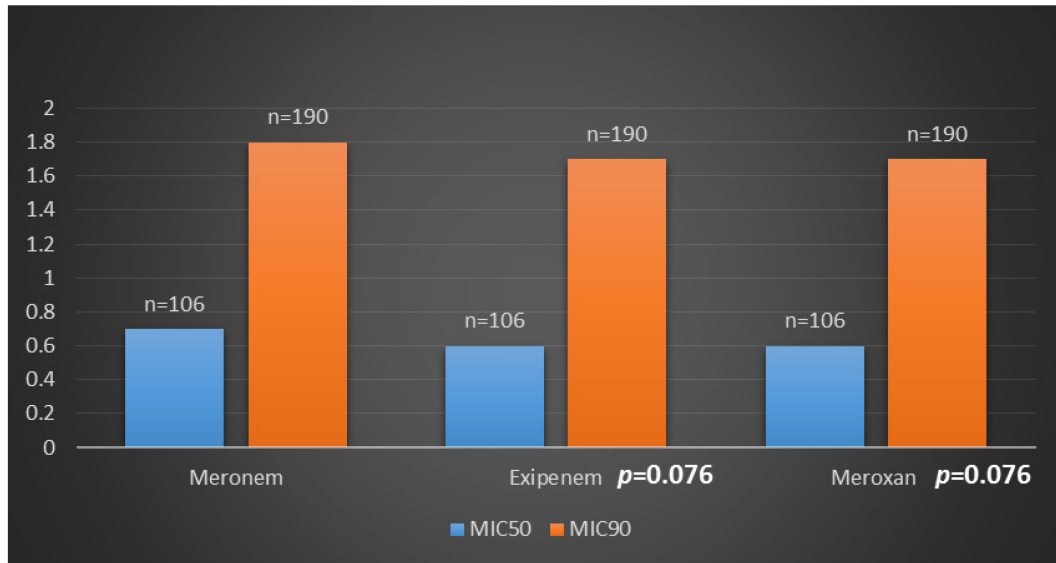


Figure 3: Comparative in vitro activity of meropenem products against *Klebsiella pneumoniae* isolates

Discussion

Bacterial resistance to antibiotic therapy is an increasing public health problem around the world [15]. Moreover, the resistance pattern of the microorganisms could be different in various populations and therefore, each of them needed to be specially planned for reduction of resistance to antibiotics especially those most commonly used for treatment [16]. Most studies demonstrated that meropenem were one of the most effective agents for the treatment of infection due to *Enterobacteriaceae*. Meropenem has been used for severe nosocomial infections, often in hospital units. Their value lies in their broad spectrum and in overcoming most resistance in Gram-negative bacilli. Exposure and use of carbapenems for the treatment of diverse infectious disease appear to be a prerequisite to the development of resistance [17]. The emergence of carbapenem resistance among clinical isolates of *Klebsiella pneumoniae* has recently raised fears that effective antimicrobial treatment options for these isolates may soon be severely limited [18]. Comparison of the results of our study and other similar studies in other countries shows that meropenem is highly active *in vitro* against all the clinical isolates of *Klebsiella pneumoniae* [19]. An excellent level of concordance between the two internal generic meropenem products company (Exipenem and Meroxan) and the Meronem (AstraZeneca, UK) has been demonstrated only for *Klebsiella pneumoniae* isolates.

The level of essential agreement by Meronem (AstraZeneca, UK) is over 90%, achieved for all *Klebsiella pneumoniae* isolates. Antimicrobial susceptibility test for all *Klebsiella pneumoniae* isolates was performed by Mast company provided disk and similar results were achieved. Our studies show that Exipenem and Meroxan are effective in the same spectrums. The disks were impregnated by various meropenem products used for disk

diffusion after drying because these disks do not have enough stability.

Antibiotics behaviors must be evaluated *in vitro* and *in vivo* to confirm their suitability for therapeutic use. Pharmaceutical equivalence or MIC values of any generic products are not useful criteria for granting therapeutic equivalence [20]. Because MIC breakpoints of meropenem products (Exipenem, Meroxan and Meronem) have not yet been obtained for Gram-negative and Gram-positive bacteria, it remains unclear whether the *in vitro* activity of antibacterial drugs is predictive of the clinical outcome. In order to do it, all generic products of meropenem should be tested *in vivo*. For *in vivo* use of Exipenem and Meroxan, it would be better to perform additional testing (activity against different species, stability etc.).

Conclusions

Regarding the comparison of two internal generic meropenem products with the external Meronem product has shown that they are equivalents in terms of microbiological activity, as measured using the disk diffusion and MIC. In developing countries, we suggested preparing disks with antibiotic powders that can be an equivalent function in microbiological activity with standard disks. In addition, in order to better validate of these generics (Exipenem and Meroxan) be equivalent to Meronem (AstraZeneca, UK), these *in vitro* findings must be further investigated (activity against different species, stability etc.) and confirmed *in vivo*.

Notes

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Competing interests

The authors declare that they have no competing interests.

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