# Comparative Analysis of Three Methods for Determination of Imipenem Resistance in *Pseudomonas aeruginosa*

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**ABSTRACT**

***Background*:** These days, the antibiotic resistance of *Pseudomonas aeruginosa* isolates to imipenem has significantly increased. Therefore the study of resistance to imipenem in this organism to imipenem in determining the appropriate treatment is crucial and necessary. The goal of this study is to compare three phenotypic methods of E-test, disk diffusion and micro broth dilution in the study of resistance to imipenem in clinical isolates of *P. aeruginosa*.

***Methods*:** Within a 6-month interval, 120 clinical specimens were collected and evaluated. All isolates were identified as *P. aeruginosa* by standard biochemical tests and amplification of 16S rRNA gene. Three phenotypic methods of E-test, disk diffusion, and micro broth dilution were used to determine imipenem resistance in *P. aeruginosa* isolates.

***Results*:** Of the 96 *P. aeruginosa* isolates studied for their resistance to imipenem by the use of E-test, disk diffusion and micro broth dilution methods, 38.5% of the strains in micro broth dilution method and 33.3% in the two methods of E-test and disk diffusion were resistant to imipenem. The rate of sensitivity and specificity of disk diffusion and E-test methods were 100%, 90.1%, and they were 100% and 83.1% for micro broth dilution, respectively.

***Conclusion*:** With regard to the results obtained from the comparison of the three methods 100% agreement were observed among the antimicrobial susceptibility results obtained by the E-test and disk diffusion methods ($P \geq 0.05$). Therefore, the use of disk diffusion method can be an appropriate replacement for E-test method with regard to its being easy and cost-effective.

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## Introduction

The antibiotic resistance of Gram-negative rod bacteria especially in enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter baumannii* has become a growing problem around the world. To a large extent, the resistant bacteria make treatment problematic (1). The introduction of carbapenems into the world of medicine has been a great advancement in the treatment of diseases resulting from the β-lactam resistant bacteria. Due to the wide spectrum of their activity and their resistance against hydrolysis by most β-lactamase, carbapenems are selective medicines for the treatment of infections resulting from Gram-negative bacilli resistant to penicillin or cephalosporin (2). Among the carbapenems we can refer to imipenem, meropenem, andertapenem with a wide spectrum compared with other antibiotics of β-lactam (3). The resistance of *P. aeruginosa* strains to carbapenems is increasing in many countries (4). Many studies have been performed in different countries regarding resistance to antibiotics with very different results in terms of time and place. With regard to the point that nowadays different phenotypic methods such as E-test, disk diffusion, and micro broth dilution have been used for the determination of antibiotic sensitivity of strains in laboratories and different environmental factors are effective in the growth of bacteria and the results of the tests, therefore the use of a precise, sensitive, and reliable method whose results are less affected by different environmental factors seems necessary. With regard to the growth and outbreak of infections resulting from *P. aeruginosa* among the patients on the one hand, and their increasing resistance to antibiotics on the other hand, the determination of the resistance pattern of the strains to antibiotics plays an important role in the selection and adoption of suitable treatment. The goal of this study is to compare the three phenotypes methods of E-test, micro broth dilution and disk diffusion in determining resistance to imipenem among the *P. aeruginosa* strains.

The present research is a cross-sectional and descriptive study. Sampling was done from April to August 2014 and 120 different clinical samples were collected from the inpatients in different wards of Fatemeh Alzahra hospital in Najafabad; Alzahra and Imam Kazem hospitals in Isfahan. A total of 120 clinical isolates 48 isolates belong to urine and 72 isolates belong to burn wounds. The standard tests including Gram staining and culture in Mac Conkey agar, blood agar, cetrimide agar, Muller-Hinton agar, OF test and oxidase test were performed to identify of *P. aeruginosa* isolates. To determine the molecular identity of the strains, the 16S rRNA gene was amplified by the use of Colony-PCR method. The primer sequences, F: GGGGGATCTTCGGACCTCA and R: TCCTTA GAGTGCCCACCCG were used (5). PCR conditions were as follows: The initial denaturation at 94 degrees for 5 minutes; 25 cycles for denaturation at 94 °C for 5 minutes, annealing at 53 °C for 40 seconds and extension at 72 °C for 50 seconds; the final extension at 72 °C for 6 minutes were done (5). Then, the PCR products were separated in 1% agarose gel containing DNA Green Viewer by electrophoresis.

The antibiotic resistance pattern of P. aeruginosa strains to imipenem was studied by Kriby-Bauer standard method. *P. aeruginosa* ATCC 27853 was used as the quality control strain. Briefly, the 24-hour culture of bacterial suspension with half McFarland turbidity was prepared and inoculated on the surface of Mueller-Hinton agar medium in a sweeping manner. An imipenem antibiotic disc (10 µg, Mast) was placed in the center of the plate with sterile forceps. Then, the plates were incubated for 24 hours at 37 °C centigrade and the inhibition zone was measured. The rate of the microorganism sensitivity to antibiotic was determined on the basis of the Clinical and Laboratory Standards Institute (CLSI 2013) standard (6).

The MIC values of imipenem were determined by micro broth dilution method. For this purpose, the bacterial suspension with a turbidity of 0.5 McFarland was prepared in a Muller-Hinton broth medium from the 24-hour culture of the strains.

The stock solution of imipenem (Sigma-Aldrich) was prepared at a concentration of 10 mg/ml and two-fold serially diluted in water, after being mixed with bacterial suspension, a final concentration 4 to 512 µg/ml was obtained. Next, 50 µl of each dilution were added in a 96-well microplate and then 50 µl of the bacterial suspension inoculated to it. 100 µl of bacterial suspension and 100 µl of Muller-Hinton Broth were inoculated in separate wells for positive and negative controls, respectively. The microplate was incubated for 20 to 24 hours at 37 °C centigrade. The absorption rates of the wells were read before and after incubation by ELISA Microplate Reader; then they were compared to determine the value of MIC. The antibiotic resistance of the strains to imipenem was determined according to the suggestions of CLSI.

The strips (LiofilChem) impregnated with different concentrations of imipenem were used in this method to determine MIC. First, the bacterial suspension with a concentration of 0.5 McFarland was prepared and inoculated in Muller Hinton Agar medium through spread method. After a few minutes, the strip was placed on the medium and the plate incubated for 24 hours. The last point of the crescent-shape inhibition zone cutting the strip was considered as MIC (7).

Statistical analysis was performed by the use of SPSS software version 16 and by the use of McNemar test, the sensitivity and specificity of the three tests were determined.

Of the 120 clinical samples, 96 *P. aeruginosa* isolates were identified on the basis of biochemical tests and colony-PCR reaction was showed that the 16S rRNA gene was present in all the strains.

**Table 1.** Frequency of resistant *P. aeruginosa* strains to imipenem.

| Micro broth dilution | Disk diffusion | E-test |
|---|---|---|
| 38.5% | 33.3% | 33.3% |

MICs ≥ 16 µg/ml for imipenem were the breakpoint to designate resistant isolates for micro broth dilution and E-test methods according to the CLSI guidelines. The frequencies of imipenem resistance evaluated by the three methods is shown in Table 1.

McNemar test showed that there was no significantly difference between the results of E-test and disk diffusion (P value =1), but there was a meaningful difference between the results of micro broth dilution test, E-test and Disk diffusion (P value = 0.03). The rate of the sensitivity and specificity of E-test were 100% and 90.1%, respectively (Table 2).

**Table 2.** Comparison of sensitivity and specificity of the used methods.

| method | sensitivity | specificity |
|---|---|---|
| E-test | 100% | 90/1% |
| Micro Broth Dilution | 100% | 83/1% |
| Disk diffusion | 100% | 90/1% |

According to our results the two methods of E-test and disk diffusion had a similar result. This finding is consistent with the studies conducted by Akhi *et al.* on comparison of E test and disk diffusion test for antibiotic resistance testing of *Bacteroides fragilis* (8). Also in a study conducted by Hadadi *et al.* the resistance of four micro-organisms including: *P. aeruginosa*, *Acinetobacter*, *E. coli*, and *Klebsiella pneumoniae* to imipenem, cefepime, ceftazidime, and ciprofloxacin antibiotics was studied by two methods of Disk diffusion and E-test and the rate of this sensitivity for *P. aeruginosa* isolates to the above antibiotics by E-test method was 69.6%, 30.4%, 26.1%, and 34.8%, respectively and by the Disk diffusion method it was reported to be 78.3%, 17.4%, 15.2%, and 37%, respectively. In all the organisms under study there was a meaningful consistency between the two methods of Disk diffusion and E-test to all the antibiotics studied (9). However, this result is not consistent with the other studies in which tested other

antibiotics and bacteria (7). The resistance of isolates to imipenem was not comparable to that found in other studies and countries. Fazeli *et al*. Isolated 66 *P. aeruginosa* strains from intensive care unit (ICU) in Isfahan and studied the resistance of the strains to imipenem by disk diffusion methods. The antibiotic resistance of the isolates to imipenem was 66.6% (6) of the total 96 clinical strains, 33.3% of the strains showed resistance to imipenem antibiotic in the present study. Many studies have been done with different results in term of time and place of study regarding the drug resistance of *P. aeruginosa* separated from clinical samples. With regard to the results of the present study, the high levels of antibiotic resistance were observed among the *P. aeruginosa* separated from Isfahan hospitals. The rate of resistance to this antibiotic is 33.3% in Isfahan which is higher than that in most parts of the world. The rate of resistance to imipenem by micro-broth dilution has been reported to be 13.4%, 12%, and 14% in Russia, Canada, and Spain, respectively. Of the 96 clinical strains in the present study, 38.5% showed resistance to imipenem antibiotic by micro-broth dilution test. Resistance to this antibiotic is more than that in most parts of the world. The reason for this fact is probably the use of excessive amount of this antibiotic in therapeutic regimes (10).

## Conclusion

In spite of the fact that E-test is a faster, more precise, and more sensitive method for the study of resistance to imipenem, the use of disk diffusion method considering its being easy and cost-effective can be a suitable replacement for E-test method.

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## Conflict of interests

No conflict of interests is declared.

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