

## Determination of Piperacillin/Tazobactam and Ticarcillin/Clavulanate Susceptibilities in *Pseudomonas aeruginosa* Isolates in Hospitalised Patients by E-test Gradient Method and Comparison of Results with Disk Diffusion Tests

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### Abstract

*Pseudomonas aeruginosa* is an important opportunistic pathogen in human infections. *P. aeruginosa* is naturally resistant to many antibiotics and mutations can result in resistance development during treatment as well. Piperacillin/tazobactam and Ticarcillin/clavulanate are  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combination with a wide spectrum of antibacterial activity. In this study was aimed to determine the susceptibility of *P. aeruginosa* isolates to Piperacillin/tazobactam and Ticarcillin/clavulanate antibiotics by Epsilometer test in the patients in intensive care units of Giresun State Hospital and to compare PIP/TZP results by Disk Diffusion method. Sensitivities of PIP/TZP were determined via Kirby Bauer disk diffusion method and MIC values of the isolates against PIP/TZP and TIC/CLA were determined by E-test. By using E-test method and in accordance with the CLSI standards, 43 (64%) isolates were found to be susceptible and 24 (36%) isolates were found to be resistant to TIC/CLA. For the PIP/TZP, 49 of the 67 isolates were susceptible, three were intermediate and 15 were resistant by using disk diffusion method. On the other hand, according to the E-test results, 63 isolates were susceptible and four isolates were resistant. When compared to eleven isolate E-test methods, the disk diffusion method was incorrectly determined to be resistant. The results of our study suggest that it would be more appropriate to use E-test method to confirm the results of the isolates which were found to be resistant against PIP/TZP by disk diffusion method.

**Keywords:** *Pseudomonas aeruginosa*; Piperacillin/tazobactam; Ticarcillin/clavulanic acid; E-test; Intensive care unit; Antibiotic resistance; Disk diffusion

### Introduction

*Pseudomonas aeruginosa* is an important opportunistic pathogen in both community and hospital-acquired infections [1]. The clinical pictures caused by *P. aeruginosa* are systemic infections, bacteraemia, wound infections, pulmonary infections, endocarditis, infections secondary to burns and trauma, and less often central nervous system infections such as meningitis. *P. aeruginosa* is naturally resistant to many antibiotics and mutations can result in resistance development during treatment as well [1,2]. This agent, which is mostly seen in intensive care, surgery and burn units of hospitals, poses a risk for immunosuppressed patients. [3]

Nosocomial infections are one of the most important causes of mortality and morbidity in hospitalized patients and can be seen as high as 54% in patients in high-risk areas such as intensive care units. Moreover, 45% of the nosocomial bacteraemia attacks occur in the patients in intensive care units [4].

$\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations obtained by adding a  $\beta$ -lactamase inhibitor to a  $\beta$ -lactam antibiotic are used in the treatment of infections caused by  $\beta$ -lactamase producing organisms and increase the antibacterial activity [5]. Both Piperacillin/tazobactam (PIP/TZP) and Ticarcillin/clavulanate (TIC/CLA) are  $\beta$ -lactam /  $\beta$ -lactamase inhibitor combination with a wide spectrum of antibacterial activity including Gram positive and negative aerobic and anaerobic bacteria.

In multicenter, randomized, double-blind clinical trials, PIP/TZP has been shown to be as effective as the corresponding comparator antibiotics. PIP/TZP has a safe and tolerable profile and remains a reliable option for empirical treatment of moderate to severe infections in hospitalized patients [6,7].

In this study, it was aimed to determine the susceptibility of *P. aeruginosa* isolates to TIC/CLA and PIP/TZP antibiotics by Epsilometer test (E-test) in the patients in intensive care units of Giresun State Hospital and to compare PIP/TZP results by Disk Diffusion (DD) method.

### Material and Methods

This study was carried out with 67 *P. aeruginosa* isolates isolated from various clinical specimens in the intensive care units of Giresun State Hospital between January 2016 and April 2016. Only a single isolate from each patient was included. Samples were plated on 5% sheep blood agar and Eosin Methylene Blue Agar (EMB, Becton Dickinson, USA). Identification of *P. aeruginosa* isolates were performed using conventional microbiological methods (Gram staining, oxidase reaction, carbohydrate oxidation, etc.) and automated bacterial identification system BD Phoenix 100 (Becton Dickinson, USA).

Sensitivities of PIP/TZP (100/10  $\mu$ g) were determined via Kirby Bauer disk diffusion method and by using antibiotic disks (Oxoid, Thermo Scientific, UK) according to the Clinical and Laboratory Standards Institute (CLSI) standards [8]. The Minimum Inhibitor Concentration (MIC) values of the isolates against PIP/TZP and

TIC/CLA were determined by E-test using E-test strips (BioMerieux, France) on Müller-Hinton agar medium. *P. aeruginosa* ATCC 27853 was used as the control bacteria.

In accordance with CLSI standards [8], the E-test reference ranges in our study were accepted to be  $\leq 16/2$  susceptible (S) and  $\geq 128/4$  resistant (R) for TIC/CLA while  $\leq 16/4$  susceptible (S) and  $\geq 128/4$  resistant (R) for PIP/TZP. Besides, disk diffusion reference ranges for PIP/TZP were accepted to be  $\leq 17$  resistant (R), 18-20 intermediate (I) and  $\geq 21$  susceptible (S).

## Results

A total of 67 *P. aeruginosa* isolates were included in the study. The isolates were obtained from 23 female and 44 male patients

hospitalized in different intensive care units. The mean age of the patients was 67 ranging from 23 to 89.

Initially, the sensitivities to PIP/TZP were determined by disk diffusion method and then MIC values of the same isolates were re-studied by E-test method for both PIP/TZP and TIC/CLA and the results obtained for PIP/TZP were compared with each other. The distribution of *P. aeruginosa* isolates by the specimens and the clinics is given in Table 1.

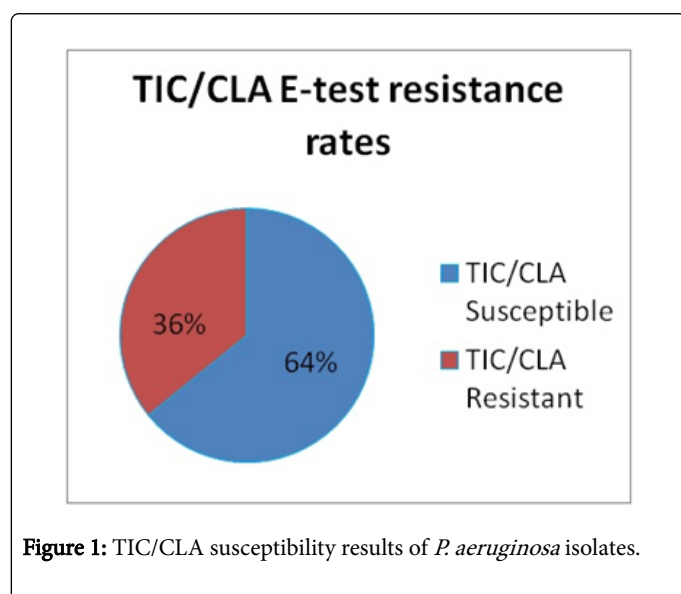
	Tracheal aspirate	Sputum	Wound	Urine	Peripheric blood	Total (%)
Internal medicine intensive care unit	11	9	2	8	1	31 (46.3)
Surgery intensive care unit	3	2	12	1	3	21 (31.3)
Reanimation unit	5	-	1	1	1	8 (11.9)
Neurology intensive care unit	1	4	-	2	-	7 (10.5)
Total (%)	20 (29.9)	15 (22.4)	15 (22.4)	12 (17.9)	5 (7.4)	67

**Table 1:** The distribution of *P. aeruginosa* isolates by the specimens and the clinics.

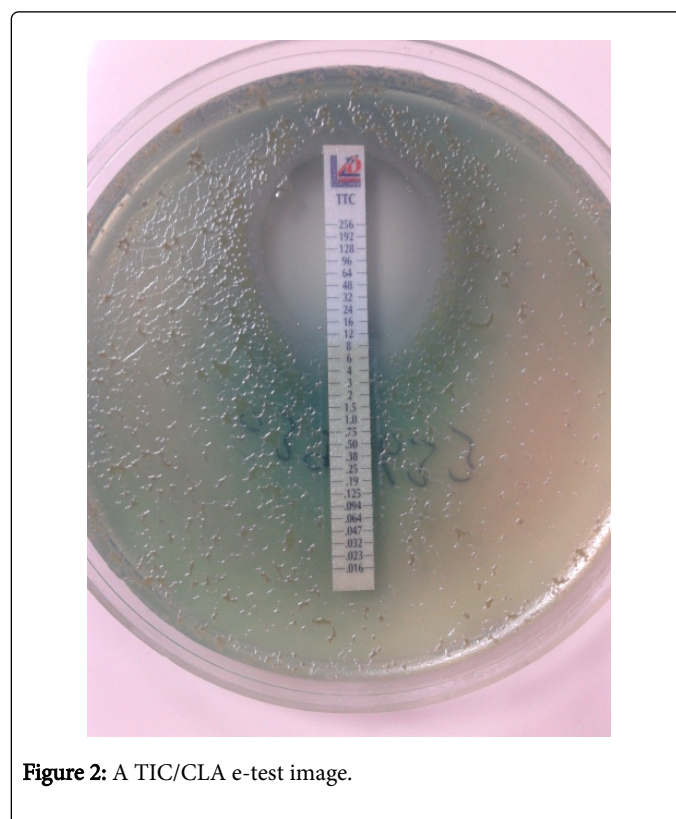
By using E-test method and in accordance with the CLSI standards, 43 (64%) isolates were found to be susceptible and 24 (36%) isolates were found to be resistant to TIC/CLA (Figures 1 and 2).

For the PIP/TZP, 49 of the 67 isolates were susceptible, 3 were intermediate and 15 were resistant by using disk diffusion method. On the other hand, according to the E-test results, 63 isolates were susceptible and 4 isolates were resistant.

The comparison of the sensitivity results of PIP/TZP by these two methods is shown in Figures 3 and 4.



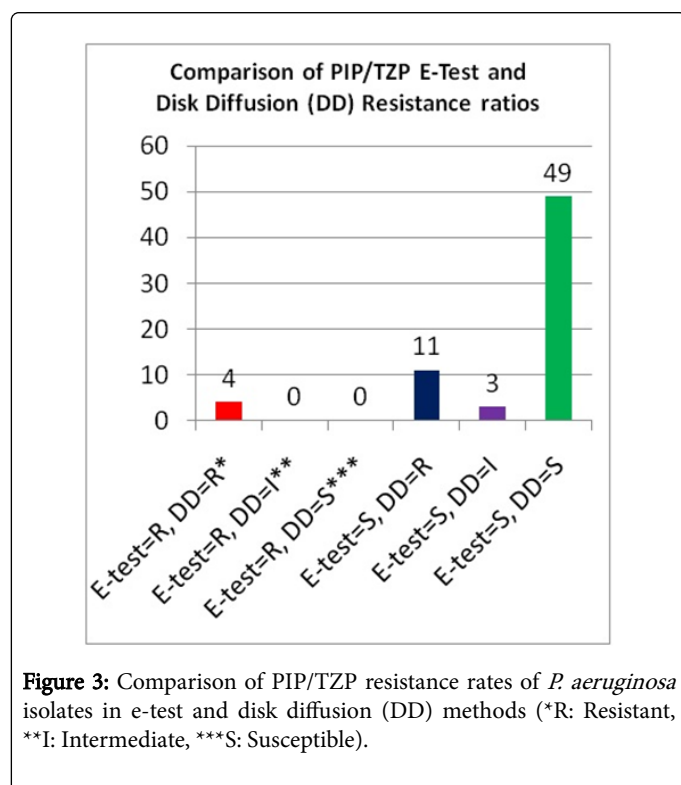
**Figure 1:** TIC/CLA susceptibility results of *P. aeruginosa* isolates.



**Figure 2:** A TIC/CLA e-test image.

## Discussion

It has been reported that *P. aeruginosa* is most frequently isolated from respiratory tract (tracheal aspirate, bronchoalveolar lavage and sputum) and blood samples. Wound and urine samples are the second most common isolates [9]. In our study, *P. aeruginosa* was isolated from tracheal aspirate, sputum, wound, urine and blood samples by the order of frequency.

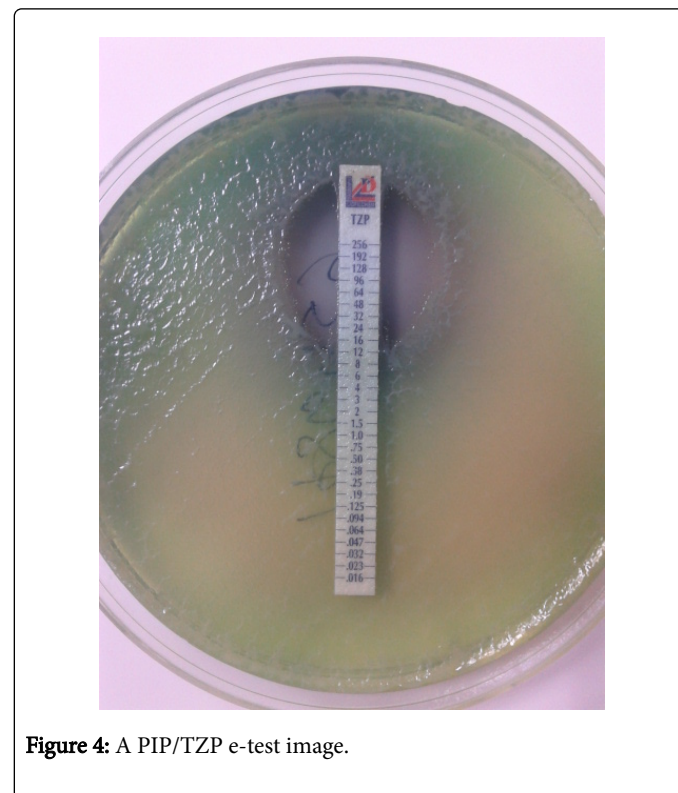


In our country, *Pseudomonas* spp. are one of the most common infectious agents among Gram negative bacteria isolated from intensive care units. Especially, the *Pseudomonas* spp. isolated from respiratory specimens may show multiple resistance profiles. In addition, a susceptible isolate can develop resistance within the treatment process. Improper use of antibacterial agents causes rapid resistance to microorganisms and treatment of infections caused by *Pseudomonas* isolates becomes more difficult every passing day. *Pseudomonas* spp. are intrinsically resistant to many antibiotics. Resistance to antibiotics in *Pseudomonas* strains is developed by different mechanisms. Hydrolysis of antibiotics with beta-lactamase enzymes and reduction of cell wall permeability to antimicrobial agents are the most important causes of resistance development [10].

Piperacillin is a beta-lactam group antibiotic and has anti-pseudomonal effect. In *P. aeruginosa* strains, resistance against the  $\beta$ -lactam antibiotics usually develops due to beta-lactamase. Tazobactam, a beta-lactamase, was added to treatment protocol considering the resistance to piperacillin [9]. Clinical trials have shown higher clinical success rates in the PIP/TZP treatment compared to various antibacterial agents, especially in intra-abdominal infections and febrile neutropenic patients [11].

Toni-Marie Gonzalzes et al. [12] indicated that if the PIP/TZP MIC value was less than or equal to 64  $\mu\text{g}/\text{mL}$ , treatment was successful in 80% of all infections caused by *P. aeruginosa* when PIP/TZP was used

alone or in combination with another antibiotic [11]. In addition, in the study of SENTRY antimicrobial surveillance program between 1997 and 2007, PIP/TZP was found to be the most effective anti-pseudomonal drug in European and Latin American countries [13]. Again in our country, Ak et al. have shown in their study that PIP/TZP and amikacin are the most effective antibiotics in *P. aeruginosa* isolates [9].



In our country, PIP/TZP resistance rates in *P. aeruginosa* isolates determined by disk diffusion method were 25% in 2005 [14], 7.8% in 2007 [15] and 8% in 2011 [10]. The resistance rates found by using E-test method were 15.4% in 2013 [16]. Additionally, the resistance rates found by fully automated identification method were 41% in 2012 [4], 51% in 2014 [2], 71% in 2015 [17] and finally 7% in 2016. Antibiotic resistance rates varied according to the years and the geographical location of the study. Furthermore the methods used were not compared with each other in any of these studies.

To our knowledge, there are just two TIC/CLA studies from Turkey. Atmaca et al. [18] determined the TIC/CLA resistance as 26 % in 1996 and Atilla et al. [19] found the resistance rates as 77.3 % in 2003 with disk diffusion method.

In a study about PIP/TZP resistance rates, it is noted that automated systems (MicroScan Walk Away, VITEK 2 and VITEK systems) generally do not accurately detect PIP/TZP resistance among clinical isolates of *P. aeruginosa* [20]. In another study investigating the *P. aeruginosa* isolates from 597 cystic fibrosis patients in the USA, it was reported that the E-test method was compatible with both mucoid and non-mucoid *P. aeruginosa* isolates when compared with the reference broth microdilution method whereas disk diffusion method was less compatible with mucoid isolates [21].

In Spain, Torres et al. were compared microdilution, Vitek 2, E-test, and disk diffusion methods for PIP/TZP resistance among 101 *P.*

*aeruginosa* isolates and they determined 77.23%, 89.11%, 88.12%, 80.20% resistance rates, respectively. The highest discordance was between the PIP/TZP results [22].

In this study, the resistance against PIP/TZP was found to be 22.4% with disk diffusion method and 6% with E-test method. It is worth reporting that 11 isolates reported to be resistant and three isolates reported to be intermediate susceptible with disk diffusion method were found to be susceptible with E-test method. It is well known that E-test method is more sensitive than disk diffusion method. Nevertheless, disk diffusion method is used as antibiotic susceptibility method in many microbiology laboratories since it is faster, cheaper and more suitable [23].

Although a previous study [24] reported that the compatibility of the E-test and Disk diffusion methods in determination of PIP/TZP sensitivity were exceptional (98%), our study revealed conflicting results.

## Conclusion and Recommendations

As a result, it should be remembered that antibiotic susceptibility may vary from one geographical region to another, from hospital to hospital, between services and even in the same unit from time to time, and hence resistance development should be monitored. The treatment should be started according to the anti-biogram result and the sensitivity test should be repeated considering the possibility of developing resistance during treatment. The results of our study suggest that it would be more appropriate to use E-test method to confirm the results of the isolates which were found to be resistant against PIP/TZP by disk diffusion method.

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