

**Original article:****Comparison of two disk diffusion and E-test methods in determining antibiotic susceptibility of *Pseudomonas aeruginosa* strains in isolated burn infections of Ahvaz City**Changiz Ahmadizadeh<sup>1</sup>, Mehrdad Pashazadeh<sup>2\*</sup>**Abstract:**

**Background:** *Pseudomonas aeruginosa* with widely distributed in nature, for human beings is considered an opportunistic pathogen that causes infections of broad-spectrum, including administrative, respiratory, septicemia and bacteremia and sepsis in patients with the burning city of Ahvaz. **Method and Material:** A total of 95 isolates of *Pseudomonas aeruginosa* isolated from burn patients from January 2015 assemble and biochemical identification test, then they are antibiotic resistance in E. test and disk diffusion method were compared. **Findings:** From 95 different clinical isolates of *Pseudomonas aeruginosa* isolated from E. test with the highest sensitivity to the antibiotic ceftazidime, 70(68/73%) and ciprofloxacin 50 (63/52%) and gentamicin 48 (52/50%) and the antibiotic imipenem 44 (31/46%) were sensitive and disk diffusion method antibiotic ceftazidime, 67(52/70%) and the antibiotic Ciprofloxacin 51(68/53%) and safety antibiotic imipenem 49(57/51%) and gentamicin 48(52/50%) were sensitive. **Conclusion:** Statistically significant differences between E. test and disk diffusion antimicrobial susceptibility of there ( $p < 0.05$ ) and disk diffusion method can replace E. test, and also the most sensitive antibiotics, the antibiotics used The study of the isolated *Pseudomonas* is ceftazidime.

**Keywords:** *Pseudomonas aeruginosa*, disk diffusion, E. test, antibiotic sensitivity

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**Introduction**

*Pseudomonas* bacteria that put into gram-negative and aerobic organisms and can be found in water and soil, move through of two or three polar flagella. *Pseudomonas aeruginosa* which previously named *Bacillus Piosianic*, usually with the low number of fluorine construct human skin and gut<sup>2</sup>. Hospital-acquired infections are one of the most challenging problems in advanced and developing countries. After *Staphylococcus aureus* and *Escherichia coli*, *pseudomonas aeruginosa* is the third reason of hospital-acquired infections<sup>1</sup>. This gram-negative bacillus leads to infecting tissues from bones and joints to stomach and gut and also causes systemic disease by producing poisons in soft tissues of immune system deficiency patients; this condition arouses especially in HIV, cancer and severe burn. As these bacteria have low needing's to grow, it can easily survive

in environment and affect susceptible (sensitive) patients<sup>4</sup>. One of the most effective reasons for its priority as a pathogen, is its high natural resistance in comparison with most antibiotics. Therefore mortality rate caused by *pseudomonas aeruginosa* is reported by up to 50%. Deficiency in immune system and also resistance against several antibiotics are among impressive factors<sup>3</sup>. As a result of serious grounded disease, destruction of protecting membrane and skin barriers caused by using invasive medical devices and long hospitalizing, the risk of obtaining hospital infections is high especially in hospitalized patients<sup>5</sup>. Encountering with different antimicrobial factors may complicate this hospitalizing and create favorable conditions through resisting and surviving in respect to host of bacterial fluorine and or transferred strains from hospital environment. This bacteria shows more opposition against antimicrobial factors. Serious

1. Changiz Ahmadizadeh, Department of Microbiology, Science and Research Branch, Islamic Azad University, Ahar, Iran
2. Mehrdad Pashazadeh, Department of Immunology, Faculties of Medicine Bursa Uludag University, Bursa, Turkey and Immunology Division, Department of Microbiology, Health Science Institute, Bursa Uludag University, Bursa, Turkey

**Correspondence to:** Mehrdad Pashazadeh, Department of Immunology, Faculties of Medicine Bursa Uludag University, Bursa, Turkey and Immunology Division, Department of Microbiology, Health Science Institute, Bursa Uludag University, Bursa, Turkey. E-mail: mehrdad\_pashazadeh@yahoo.com

infections made by common antimicrobial-resistant anti pseudomonas are major problems<sup>8</sup>.

*Pseudomonas aeruginosa* shows very high resistance to antimicrobial drugs. These features and characteristics by using selective pressure of mutation in genes and chromosomes, leads to over expression of *ampC* gene, restraint or inactivation of *oprD*, and over expression of effluent pumps in drugs<sup>10</sup>. Moreover it can receive drug resistant factors by horizon gene transfer through coded mobile genetic elements to work with class B penmases (also called metallo-beta-lactamase, that hydrolyze all beta-lactams except aztreonam). Strains of XDRPa with extreme drug resistance against *pseudomonas aeruginosa* emerged in hospitals which become a real threat to national health<sup>9</sup>. Such infections caused by resistant microorganisms limit drug selections. Consequently these resistant strains in hospital infections are accompanied by increase in mortality and long hospitalizing<sup>11</sup>. The best way to decrease such mortalities is to prevent these infections. Antibiotic resistance is an increasingly clinical dilemma and major threat of national health. That is an international problem, however all countries are involved with this problem, but this extension in developing countries is unknown<sup>12</sup>. Considering bacteria resistance and pathogenesis against antibiotics, it is essential to find precise and effective method for evaluating sensitivity and resistance of antibiotics. Among several testing antibiotic sensitivity methods, two methods are selected, the former is disk diffusion and the latter is E-test<sup>6</sup>. The purpose of this study is to determine antibiotic resistance of *pseudomonas aeruginosa* strains in burn infections of AhvazCity to select suitable treatments, increase treatment effects, decrease considerable economic costs, and reduce hospitalization time.

### Methods

This research was done from autumn 2015 up to spring 2016, so we collected 95 isolated *pseudomonas aeruginosa* patients from Taleghani burn center of Ahvaz, which include injury, blood, urine, mucus, cornea, tracheal tube, and biopsy, and separated those using recognition and purification tests. The tests involve differential ones such as oxidase, oxidative fermentative (OF), triple sugar iron agar (TSI), and citrate. So sensitivity test is done in two ways, agar disk diffusion (Kirby-Bauer) and E-test, utilize four antibiotics including ceftazidime (30/ug), imipenem (10/ug), ciprofloxacin (5/ug), gentamicin (10/ug) to get antibiotic resistance pattern of isolated strains. We bought antibiotic disks from Padtan teb

Company and E-test strips used in this study were Italian. In this method, after providing hemogenic solutions from bacteria, first we planet all samples on muellur-hinton agar growth media with sterile swab, then place antibiotic disks on agar surface using pence, finally we put plates in incubator for 24 hours with 37<sup>0</sup>c temperature. After heating, the inhibition zone diameter resulted from antibiogram was measured according to clinical and laboratory standards institute (CLSL) instruction.

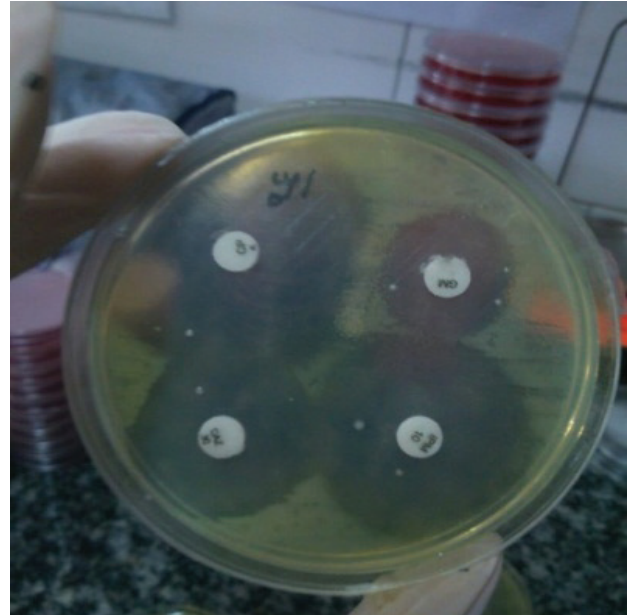


Fig1: Positive result of tested bacteria in disk diffusion method

In the second method to determine minimum inhibitory concentration (MIC) with E-test, first we sample sterile swaps from bacterial suspension of *pseudomonas aeruginosa* that is equivalent to 0/5 McFarland standard then steady spread them on special MIC determination media with E-test method. After that carefully weplace one E-test strip on media with aseptic condition then put the plate in incubator for 24 hours with 37<sup>0</sup>c temperature. When surveying the results, the number opposite to last inhibition zone diameter and beside strip was MIC with mcg/ml measurement unit.

The result of both disk diffusion test and E-test registered in data collection form. Finally all experimental results done with SPSS17 software and sensitivity or insensitivity of each strain with separate code entered in to the program. In this study p values lower than 0.05 considered meaningful (P<0.05).

### Findings

In this study 118 sample suspicious to *pseudomonas* was collected since autumn 2015 to spring 2016.

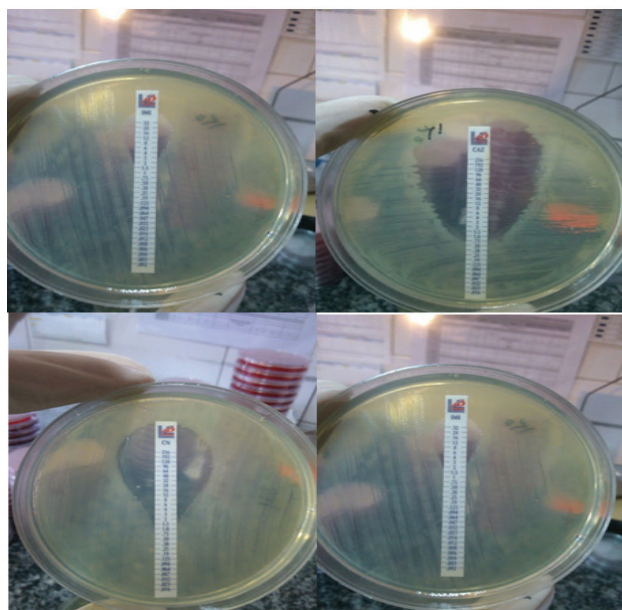


Fig.2: positive result of tested bacteria with E-test method

After doing biochemical tests, 95 pseudomonas aeruginosa sample detected and separated from different clinical ones.

Among hospitalized patients 13 samples from biopsy, 6 samples from mucus, 14 samples from injury, 13 samples from urine, 8 samples from blood, 3 samples from cornea, 7 samples from punch, and 21 samples from tracheal tube was selected. Antibiotics like ceftazidime 70 (37/68%), ciprofloxacin 50 (52/63%), gentamicin 48 (50/52%), and imipenem 44 (46/31%) were among most sensitive ones to E-test method and also antibiotics such as ceftazidime 67 (70/52%), ciprofloxacin 51 (53/68%), and imipenem 49 (51/58%) were susceptible to disk diffusion method.

Table.1. kind and number of isolations in studied samples

Kind of sample	Number of isolated stains
Biopsy	13
Mucus	6
Injury	14
Urine	23
blood	8
Cornea	3
punch	7
tracheal tube	21

Table.2. NCCLS standard indices for determination of studied antibiotics sensitivity and resistance with E-test method according to g/lit

Antibiotics	Sensitivity	Relative Sensitivity	Resistance
Ceftazidime	≤8	16	≥32
Imipenem	≤4	8	≥16
Gentamicin	≤4	8	≥16
Ciprofloxacin	≤1	2	≥4

Table.3. CLSI standard indices for determination of studied antibiotics sensitivity and resistance with disk diffusion method according to mm

Antibiotics	Sensitivity	Relative Sensitivity	Resistance
Ceftazidime	≤18	15-17	≥14
Imipenem	≤16	14-15	≥13
Gentamicin	≤15	1-14	≥12
Ciprofloxacin	≤21	16-20	≥15

Table. 4. evaluation of antibiotic susceptibility of pseudomonas aeruginosa samples with disk diffusion method

	Ceftazidime	Ceprofloxacin	Gentamicin	Imipenem
	Number (percent)	Number (percent)	Number (percent)	Number (percent)
Sensitive	(70/52)67	(53/68)51	(50/52)48	(52/57)49
Semi Sensitive	(1/05)1	(1/05)1	(2/1)2	0
Resistant	(17/89)17	(45/26)43	(47/36)45	(48/42)46

Table.5. evaluation of antibiotic susceptibility of pseudomonas aeruginosa samples with E-test method

	Ceftazidime	Ciprofloxacin	Gentamicin	Imipenem
	Number (percent)	Number (percent)	Number (percent)	Number (percent)
Sensitive	(73/68)70	(52/63)50	(50/52)48	(46/31)44
Semi Sensitive	0	0	0	0
Resistant	(21/31)25	(47/36)45	(49/47)47	(53/68)51

**Table.6. comparison of average mutual effects of measurement methods and kind of antibiotics**

	Sensitive	Semi sensitive	Resistant
(1) D.D×1	<sup>a</sup> 80/51	<sup>a</sup> 89/0	30/47
(2) D.D×2	<sup>a</sup> 86/77	<sup>a</sup> 78/1	34/20
(3) D.D×3	<sup>ab</sup> 41/50	<sup>a</sup> 72/3	41/47
(4) D.D× 4	<sup>ab</sup> 12/50	0	87/49
(5) E.test×1	<sup>ab</sup> 13/48	<sup>a</sup> 46/4	84/46
(6)E.test×2	<sup>a</sup> 58/71	<sup>a</sup> 38/2	16/25
(7) E.test×3	<sup>ab</sup> 87/49	<sup>a</sup> 0	57/49
(8) E.test× 4	<sup>b</sup> 78/33	<sup>a</sup> 54/6	90/45

Above table shows that there are sensitive meaningful differences between factors, so as number 4 antibiotic with 33/87 have least effect in E-test method and number 2 antibiotic with 78/86 have highest effect in disk diffusion method.

	Sensitive
CIP	49.96
CAZ	74.72
GN	50.14
IMI	41.95

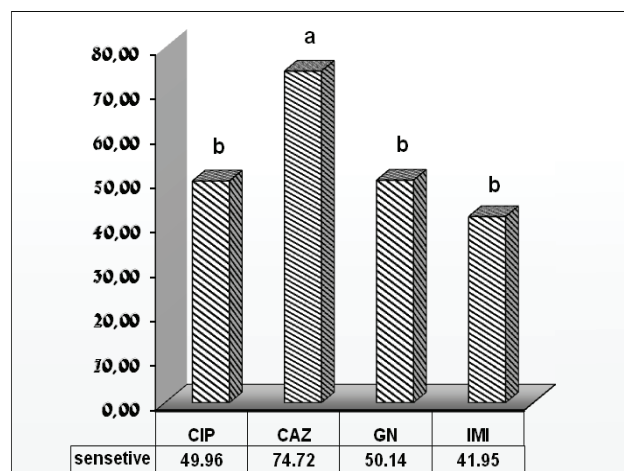


Figure 1: Comparison of Antibiotic Sensitivity

	Semisence
CIP	2.67
CAZ	2.08
GN	1.86
IMI	3.27

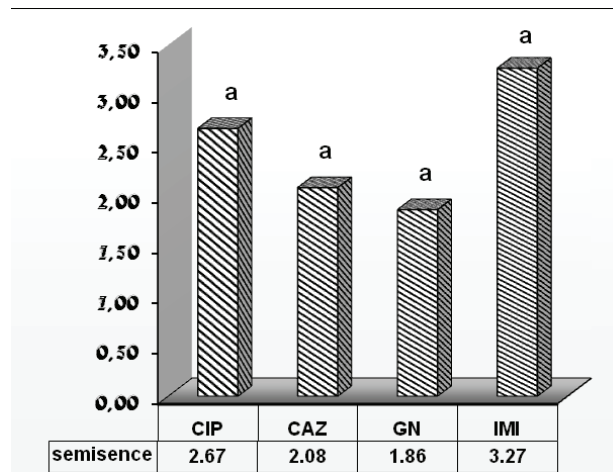


Figure 2: Comparison of Semi-sensitive antibiotics

	Resistance
CIP	2.67
CAZ	2.08
GN	1.86
IMI	3.27

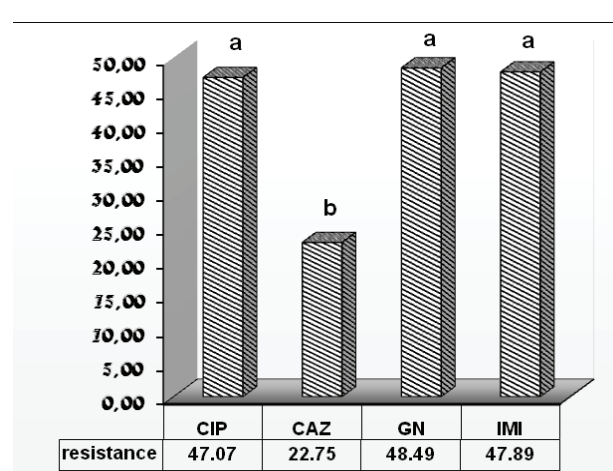


Figure 3: Comparison of Antibiotic Resistance

	1	2	3	4
E.test	48.15	71.58	49.87	33.78
D.D	51.80	77.86	50.41	50.12

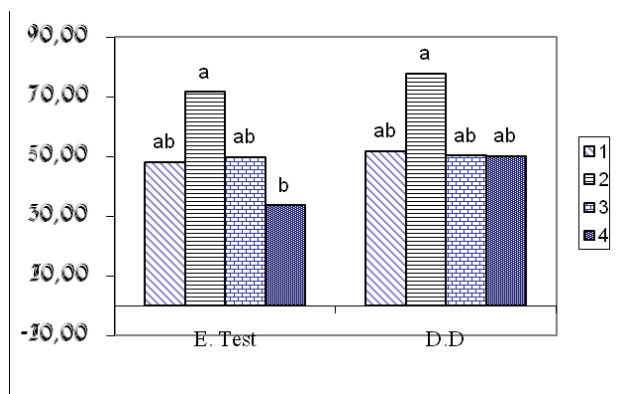


Figure 4: Comparison of methods of measurement and types of antibiotics interactions

### Discussion

Proper specification of hospital-acquired infections, type of microorganisms and their resistance pattern, and correct use of antibiotics are among important points that should be observed to reduce drug resistance<sup>7</sup>. *Pseudomonas aeruginosa* bacteria is the most important factor in hospital-acquired infections especially in immunodeficiency patients such as cancer and burn affected more one<sup>2</sup>. Treatment condition in patients with *pseudomonas aeruginosa* infection is problematic, especially when this organism inherently is resistant to several antibiotics and could obtain resistance against all antimicrobial drugs<sup>11</sup>. Mostly determination of antibiotic resistance pattern is one of the substantial elements in successful treatment of bacterial infections<sup>13</sup>. In a recent study, we survey the susceptibility of 95 isolated *pseudomonas aeruginosa* bacteria to four kinds of antibiotics like ciprofloxacin, ceftazidime, gentamicin, and imipenem with two methods of disk diffusion and E-test. Until now many studies are done to determine antibiotic susceptibility of *pseudomonas aeruginosa* bacteria separated from clinical cases which their results are different according to time and location. Algun et al. (2014) conducted a study in India and reported the lowest resistance of *pseudomonas aeruginosa* bacteria against ciprofloxacin (12.5%) that is

compatible to our study considering this bacteria's susceptibility against ciprofloxacin<sup>14</sup>. As well as Niitsuma et al. did research in 2001 in Japan and showed the resistance of *pseudomonas aeruginosa* bacteria against ceftazidime 4.6% which according to this bacteria's sensitivity to ceftazidime is in complete agreement with our findings<sup>15</sup>. Khademi et al. conducted a survey in 2013 that compared the results of two disk diffusion and E-test methods in determining antibiotic susceptibility of *Helicobacter pylori*. The recent study's findings show that using E-test in vitro can be a suitable substitution in determining antibiotic susceptibility of *Helicobacter pylori* which is in correspondence with our study in methods<sup>16</sup>. According to Pishva and his colleagues study in Esfahan that was done to compare two disk diffusion and E-test method's results about antibiotic resistance of *staphylococcus epidermis*, sensitivity and specificity was measured 95.3% and 94.7% for E-test and 86.5% and 80.9% for disk diffusion sequentially<sup>17</sup>. In this research sensitivity and specificity of E-test method is higher than disk diffusion, therefore it is not compatible with our study, because in our research the results are equal in both methods. Based on Erfani et al. research (2008) in Shariati Hospital of Tehran about comparison of both disk diffusion and E-test methods against *Escherichia coli* bacteria's sensitivity, it became clear that E-test method is more sensitive than disk diffusion which disagrees with our findings, since in recent study both methods don't have statistically meaningful differences and disk diffusion method can replace E-test<sup>18</sup>. Darvish research in Khatam Anbia Hospital of Tehran showed that isolations of *pseudomonas aeruginosa* are resistant against rifampin (50%), meropenem, gentamicin, and ceftazidime (25%), so because *pseudomonas aeruginosa* has the lowest resistance against ceftazidime, it is compatible with this research. The results of this research confirm that E-test method is more accurate than disk diffusion and can determine affective doses of antibiotic for prevention and treatment of antibiotics resistance. But it doesn't have statistically meaningful differences with disk diffusion ( $p < 0.05$ ). Therefore in vitro, applying disk diffusion method can replace E-test for determining antibiotics resistance of *pseudomonas aeruginosa* and with respect to antibiotic

susceptibility, ceftazidime sensitivity is 73/68% with E-test method and 70/52% with disk diffusion method, so among other antibiotics ceftazidime is the best option for fighting against pseudomonas aeruginosa bacteria in burn infections. According to this research findings, the comparison between two methods of E-test and disk diffusion shows that this two antibiogram methods are relatively equal, hence it is suggested that in vitro disk diffusion method

is suitable substitution for E-test, because E-test strips are expensive and using this method is not cost effective. Moreover it is necessary to conduct similar researches in other places of country to be informed about resistant strains' prevalence. Thus using this information and establishing control and evaluation systems that mostly managed by hospital-acquired infection committee, we can decrease these resistances in country.

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