Laboratory Evaluation of Iranian Commercially Provided Antibiotic Disks With Conventional E-Test Method for Susceptibility Testing in Three Most Isolated Multi-drug Resistant Organisms

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Citation

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Abstract

Antimicrobial susceptibility testing is one of the most important tasks of clinical microbiology laboratories. This study was performed to compare accuracy of susceptibility testing carried out by disk diffusion method and E-test minimal inhibitory concentration (MIC) for detection antimicrobial resistance among three prevalent gram-negative organism isolated in an Iranian hospital . These organisms included Pseudomonas aeroginosa, Acenitobacter bumannii, and Klebsiella pneumoniaew which isolated from different Baquiatalah hospital wards .Disk diffusion method were carried out initially in Baqyatalah hospital microbiology laboratory. All isolates were sent to Iranian reference microbiology laboratory for detection of MIC by E-test method. Of 100 isolates, 52 isolates were Acinetobacter bumannii, 32 Pseudomonas aeroginosa and 16 Klebsiella pneumonia. All isolated strains were resistant to ciprofloxacin, imipenem, and amikacin by disk diffusion method. However there were significant differences compared with E-test method. Many strains were susceptible to above mentioned antibiotics by E-test method .Our study revealed that there was a controversy results between disk diffusion method and MIC determination technique by E-test. It may be due to lack of suitable internal quality control as well as poor quality of commercially provided disks in our country.

INTRODUCTION

Antimicrobial susceptibility testing is one of the most important tasks of clinical microbiology laboratories for efficient guidance to physicians on therapeutic options. Susceptibility testing is also an important first step in providing surveillance data for use in local and national aggregate databases ([1,2,3]). Susceptibility testing is performed daily in diagnostic laboratories by standard methods. There are many different methods for susceptibility testing. However disk diffusion method has been extensively used for this objective.

Quality assurance must be applied for antimicrobial susceptibility testing using internal quality control protocols for monitoring of precision and accuracy of the methods ([4,5,6]). Additional external quality control assessment is necessary in quality assurance of identification and susceptibility testing methods. In Iran, external quality control carry out by Iranian Health Reference Laboratories which one of its duty is to evaluate all Iranian commercially provided reagents and materials such as antibiotics susceptibility disks.

The incidence of nosocomial infections caused by Gram-negative pathogens is increasing ([7]), and infections caused by organisms such as Klebsiella pneumoniae Acinetobacter baumannii, and Pseudomonas aeruginosa are more commonly resistant to traditional antimicrobial agents, including aminoglycosides, fluoroquinolones and broad-spectrum cephalosporins ([8]). The most important mechanism of resistance to beta-lactam antibiotics among Gram-negative bacilli involves the production of beta-lactamases. Extended-spectrum beta-lactamases are often associated with multi-drug resistance phenotypes, which can pose a significant therapeutic challenge ([9]). Disk diffusion method is a common method for susceptibility testing carrying out routinely in clinical microbiology laboratories in our country.

The aim of this study was to compare the results of disk

diffusion susceptibility testing method with MIC determination using standard E-test on isolated multi-drug resistant strains of gram negative organisms.

MATERIALS AND METHODS

Specimens: This study was a descriptive cross sectional study from April 2006 since March 2007. All three most prevalent gram negative organisms isolated from specimens collected from Baquiatalah Hospital wards entered to the study. These organisms were Pseudomonas aeroginosa, Acenitobacter bumannii, and Klebsiella pneumoniae.

100 isolates were collected from all hospitalized patients during the study period. These organisms were isolated from blood, urine, cerebral spinal fluid (CSF), Bronchoalveolar lavage (BAL), surgical wound and bed sore. All patients who got the infection 48 hours after hospitalization were considered having nosocomial infection.

All isolated organisms were identified by using standard bacteriological protocols ($_{10}$), susceptibility testing were performed by disk diffusion method as recommended by clinical laboratory standard institute ($_{11}$). MIC for above mentioned antibiotics carried out by E-test MIC method (AB Biodisk Solona, Sweden) as recommended by manufacture .

Isolated organisms: Hundred received organisms were from six different hospital wards included bronchoalveolar lavage, wound, urine, and blood specimens. Of 100 isolates 52 strain were Acinetobacter bumannii, 32 strain Pseudomonas aeroginosa and 16 strain Klebsiella pneumonia. The number of different collected specimens and isolated organisms are shown in Table 1.

Applied standard organisms: Following organisms were used as quality control strains. Organisms for quality control of disk diffusion method were E.coli (ATCC 25922), S. aureus (ATCC 25923), and P. aeruginosa (ATCC 27853). Standard organisms for the checking the quality of the Mueller-Hinton medium were the above mentioned organisms plus E.faecalis (ATCC 29212). At last, Standard organisms for quality control of the E test were e E.coli (ATCC 25922), S.aureus (ATCC 29213), and P. aeruginosa (ATCC 27853)

Analytical method: Data were entered into a database using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL) 95% Confidential Interval (CI) were applied for description quality variables, frequency and relative-frequency. Chi-square test were used for comparison the quality variables based on others numerical variables. T-test was applied to compare quantity variables between two groups. A two–tailed p-value <0.05 was considered statistically significant.

Susceptibility testing and MIC determination by E-test: Briefly bacterial suspension was prepared with standard procedure and inoculated on Mueller-Hinton medium. Applied antibiotic disks were provided from Iranian commercially Co. (Padtan-Teb) (imipenem 10 µg, amikacin 30 µg, and ciprofloxacin 5 µg). The results were interpreted by standard tables as recommended by CLSI ($_{11}$). E-test strips were provided from AB Bio-disk Co. (Sweden) and performed according to the instructions recommended by manufacture.

RESULTS

All These isolated organisms were resistant to ciprofloxacin imipenem, and amikacin by routine disk diffusion susceptibility testing method.

E-test MIC: MIC pattern of these isolated organisms were quite different in comparison with routine disk susceptibility testing results. Some of these organisms were sensitive by E-test MIC method while all showed resistance by disk diffusion (Table 2).

Real sensitivity rate of various collected isolates: Among these three applied antibiotics, imipenem showed the highest MIC different results when compared with susceptibility testing that were due to all collected isolates (P value <0.05). Amikacin revealed less difference than imipenem but had higher rate than ciprofloxacin especially for wound specimens isolates (Table 3). Disk diffusion susceptibility testing had the best result in ciprofloxacin when compared with its E-test MIC results for all collected specimens.

Figure 1

Table 1: Number of different specimens in studied organisms

Isolated organisms	Wound	BAL	Urine	Blood	Others	Total
Acinetobacter Bumani	8	32	1	2	9	52
Pseudomonas aeroginosa	13	15	1	1	2	32
Klebsiella pneumoniae	8	0	0	4	4	16
Total	29	47	2	7	15	100

Figure 2

Table 2: MIC results determined by E test for isolated organisms for Ciprofloxacin, Imipenem, and Amikacin

	Ciprofloxacin (No.)			Imipenem (No.)			Amikacin (No.)		
	S	I	R	S	I	R	S	I	R
Acinetobacter bumnanni	2	0	50	29	2	21	10	3	39
Pseudomonas aeroginosa	4	1	27	18	0	14	10	7	15
Klebsiella pneumoniae	1	0	15	14	1	1	5	2	9

S: sensitive, I: intermediate, R: resistant

Figure 3

Table 3: Frequency rate of observed sensitivity in E-test MIC of wound, brunch and blood

	Wound (n=29)	BAL (n=47)	Blood (n=7)	Others (n=15)	Total
Imipenem	23	20	7	9	63
Amikacin	16	4	1	4	25
Ciprofloxacin	2	2	0	2	6

BAL: Bronchoalveolar lavage

DISCUSSION

Nosocomial infections are the main cause of mortality and morbidity in hospitalized patients, because of their underlying disease conditions. Micro-organisms isolated from these patients may be multi –drug resistant and it can be difficult to detect emerging antimicrobial patterns and to ensure the efficiency of reporting strategies for antimicrobial resistance. Failure of the test to predict antimicrobial resistance can result in increase morbidity or mortality. A wide variety of antimicrobial susceptibility systems are available to clinical microbiology laboratory. They must be reliable and priciest because their results will guide antimicrobial therapy ([8,9]).

In our country disk diffusion method is used as routine susceptibility testing method because of its low cost and easily technical performance. However our study revealed that the results of disk diffusion method are not enough confident and there was high rate of false resistant results meaning very major error. E-test results showed real resistant rate to imipenem was 36% in all isolates, while it was 100% in disk diffusion methods. Real resistant rate were 63% and 92% for amikacin and ciprofloxacin respectively while in disk diffusion method all isolates were resistant. It means disk diffusion method is not enough reliable method for susceptibility testing Our previous studies also showed poor quality of commercially provided antibiotic disks in our country([12]) However there are other multiple factors that may affect the performance of susceptibility tests and standardized methods are more likely to be reproducible than un-standardized methods. Quality assurance is the overall process by which the quality of the test results can be guaranteed. A major part of these processes is the internal quality control testing, which is routinely undertaken to monitor precision accuracy the test procedure, the performance the reagent use in the test and the performance of persons carried out the test. However there are additional aspects that contribute to quality assurance, including participation in external quality assessment schemes, internal quality assessment and the validation the process, in which atypical or contradictory results can be detected. Education is an important part of the quality assurance processes as an understanding the techniques, together with their limitation and pitfalls, contributes significantly to the recognition, resolution and avoidance of errors ([13,14]).

CONCLUSION

Our study revealed that there was a controversy results between disk diffusion method and MIC determination technique. It may be due to lack of suitable internal quality control as well as poor quality of Iranian commercially provided antibiotic disks.

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