



A comparison of disk diffusion method and E-test in determining the susceptibility and resistance of *Klebsiella* and *Acinetobacter* strains to cefepime in patients with ventilator-associated pneumonia admitted to the intensive care unit

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Received 27 Nov. 2021

Accepted 20 Jan. 2022

Published online 12 Feb. 2022

Keywords: Ventilator-associated pneumonia, Intensive care unit, *Acinetobacter*, *Klebsiella*, Cefepime, Pneumonia

Abstract

Introduction: Ventilator-associated pneumonia (VAP) which is mostly caused by microorganisms such as *Acinetobacter* and *Klebsiella* is one of the causes of death among patients in the intensive care unit (ICU). **Objectives:** The aim of this study was to compare disk diffusion method and E-test in detecting the susceptibility of *Klebsiella* and *Acinetobacter* strains isolated from lung secretions of patients with VAP admitted to the ICU treated with cefepime.

Patients and Methods: In this cross-sectional descriptive study, 100 patients with VAP including 55 and 45 cases infected with *Acinetobacter* and *Klebsiella*, respectively, according to the antibiogram participated. The susceptibility and resistance of *Acinetobacter* and *Klebsiella* strains to cefepime was determined using disk diffusion method and E-test. In addition, kappa test was used to determine the sensitivity and specificity of disk diffusion method compared to E-test.

Results: The sensitivity and specificity of the disk diffusion method were 100% and 25%, respectively and its positive predictive value was 87.5% and its negative predictive value was 100%. The kappa coefficient was 0.35 ($P < 0.0001$), which indicates a significant agreement between the two tests.

Conclusion: Disk diffusion method has high sensitivity and low specificity in comparison to E-test in determining the resistance and susceptibility of *Acinetobacter* and *Klebsiella* strains to cefepime in patients with VAP admitted to the ICU.



Citation: Mahjobipoor H, Sajadi M, Honarmand A, Rahimi-Varposhti M, Yadegari L, Attarzadeh A, Mahjobipoor L, Fattahpour S. A comparison of disk diffusion method and E-test in determining the susceptibility and resistance of *Klebsiella* and *Acinetobacter* strains to cefepime in patients with ventilator-associated pneumonia admitted to the intensive care unit. *Immunopathol Persa*. 2024;10(2):e30330. DOI:10.34172/ipp.2022.30330.

Introduction

Pneumonia occurring 48 to 72 hours after endotracheal intubation is called ventilator-associated pneumonia (VAP). It is caused by a new or progressive microorganism followed by symptoms of systemic infection (fever and change in white blood cell count). The causative agent can usually be detected in the patient's sputum (1,2). VAP accounts for approximately half of all cases of hospital-acquired pneumonia (3-5). Approximately 9% to 27% of the intubated patients develop VAP with the highest risk among the patients in early days of hospitalization (6). VAP is also the second most common infection in the intensive care unit (ICU) and the most

Key point

Ventilator-associated pneumonia is a major problem in patients admitted to the ICU because antibiotic resistance exacerbates the problem. Identification of antibiotic-sensitive strains of cefepime may facilitate treatment of patients.

common in patients requiring mechanical ventilation (7, 8). Early VAP is defined as the pneumonia that develops within four days usually by antibiotic-susceptible pathogens while late VAP usually occurs more than four days after intubation and is usually due to multidrug-resistant bacteria (9). Thus, VAP increases adverse outcomes (mortality

and morbidity), hospital stay and healthcare costs in ICU patients worldwide (10). Nowadays, there is an increasing demand for new antibiotics according to the prevalence of multidrug-resistant microorganisms is increasing (11). The treatment of infections caused by gram-negative bacteria has become a huge problem because of their increasing resistance; therefore, the approach and the method conducted to evaluate their antibiotic resistance is of great importance. Two of the common gram-negative bacteria include *Klebsiella* and *Acinetobacter* (12, 13).

Currently, there are two common methods, disk diffusion method and E-test, to determine the antibiotic susceptibility of bacteria. In disk diffusion method an equal concentration of each antibiotic is placed on a paper disk and the diameter of the growth inhibition zone around this disk is measured to determine susceptibility or resistance to the antibiotic. E-test is not as common as the first method, but has a higher accuracy. In E-test different concentrations of an antibiotic from lowest to highest are placed on a paper strip and like the previous method, the diameter of the zone of inhibition around the strip is measured to detect the lowest concentration of the antibiotic inhibiting the growth of the bacteria which is called the MIC (minimum inhibitory concentration) (14, 15).

Objectives

Timely diagnosis and detection of nosocomial infections and antibiotic resistance as well as exact administration of antibiotics to reduce drug resistance is one of the most important principles that should be addressed in every hospital, especially in ICUs to reduce the lengthy hospitalization, mortality and costly healthcare for the patients. E-test is expensive and time consuming and is not easily accessible; therefore, a less expensive and accessible method such as disk diffusion method is employed instead of E-test. In this study, we aimed to determine whether the antibiotic resistance of *Acinetobacter* and *Klebsiella* isolated from the lung secretions of patients with VAP in the ICU to cefepime is due to an inherent error of the disk diffusion method (false resistance) or the bacteria is resistant to cefepime.

Patients and Methods

Study design

This descriptive cross-sectional study was performed on common gram-negative bacteria including *Acinetobacter* and *Klebsiella* of nosocomial infections of the patients in the ICU of Amin hospital located in Isfahan province during 2016-2017. Patients diagnosed with pneumonia within 48 to 72 hours after endotracheal intubation were included in the study if the gram-negative bacteria including *Klebsiella* or *Acinetobacter* was found in their culture media.

Each nosocomial infection was defined according to the US Centers for Disease Control (CDC); therefore,

blood infection or primary sepsis means positive blood culture without any other known infection by the same microorganism. Inclusion criteria were patients in ICU diagnosed with nosocomial pneumonia whose sputum secretions were positive for *Acinetobacter* and *Klebsiella* bacteria. Exclusion criteria were patients who did not provide consent and patients with less than 10 000 *Klebsiella* and *Acinetobacter* colonies in their sputum. In addition, patients who died during the study were excluded from the study. Samples included tracheal secretions (obtained by bronchoscopy or tracheal suction in patients with VAP).

Patients' sputum samples were cultured on Blood Agar and MacConkey agar medium (Merck Company, Germany) and if they were positive for gram-negative bacteria, antibiogram was studied. Then, susceptibility of *Acinetobacter* and *Klebsiella* bacteria to cefepime was determined.

The approach for detecting susceptibility was measuring minimal inhibition concentration (MIC) using E-test (AB Biodisc, Sweden). The validity of the MIC values obtained from E-test kits for each microorganism was determined by comparing with two references, Agar dilution and Broth Microdilution, according to the National Committee for Clinical Laboratory Standards (NCCLS). If the values obtained by the E-test were more than 90% consistent with the values from references, the E-test is in agreement with NCCLS reference method (essential agreement). In order to control the conditions and laboratory environment, standard and reliable raw materials and suitable environmental conditions were used and all tests were conducted and analyzed by a senior with experience in microbiology. In addition, for quality control of laboratory conditions, two reference bacterial strains with ATCC code were prepared with the help of Iran reference laboratory.

In addition to the quality control of E-test, the results were compared with special microbial growth ranges tables provided by the reference laboratory. In all cases, the results were in the acceptable range and therefore, the conditions of experiments were in the acceptable range. The samples were read after 24 hours at 37°C. The MIC was reported based on the intersection point of the growth inhibition curve with the E-test strip (MIC ≤8 and 2 means that the colony is susceptible to cefepime and >8 and 2 indicates resistance for *Acinetobacter* and *Klebsiella* respectively).

Results obtained by E-test in the laboratory were compared with numbers in NCCLS standard table.

Another method employed is disk diffusion method that is a common method for antibiogram examination in laboratories. The kits were purchased from the Mast Group Ltd (UK). In the disk diffusion method, after selecting the bacterium and preparing 0.5 McFarland standard bacterial suspension, it is transferred to Müller-Hinton agar plate and then the antibiotic discs are placed on the plate with sterile forceps. After 24 hours of incubation at 37°C, we

determined the degree of resistance or susceptibility based on the measurement of the growth inhibition zone (15-17).

Statistical analysis

Data analysis was performed using SPSS software version 18. The qualitative variables were described using frequency and 95% confidence interval and quantitative variables were provided with central tendencies and dispersion. The validity of the disk diffusion method was evaluated with the E-test result as the gold standard and the sensitivity, specificity, positive and negative predictive value, correct diagnosis and kappa coefficient of agreement were calculated and reported with 95% confidence. Independent T-test and chi-square were conducted for comparison between the two groups and $P < 0.05$ was considered as statistically significant.

Results

In this study, 100 patients (62 men and 38 women with a mean age of 46.17 ± 13.33 years) participated. Around 57% of the participants had a colony count more than 100 000/mL. Among these patients, 55 patients were positive for *Acinetobacter* and 45 for *Klebsiella*. According to E-test, 84 cases were resistant to cefepime and 16 cases were susceptible to cefepime; however based on disk diffusion method 96 cases were resistant and 4 were susceptible to cefepime. The number of colonies and E-test result was significantly different between the two strains. Therefore, in cases infected with *Acinetobacter* the number of colonies was higher and based on E-test most cases were resistant to cefepime compared to *Klebsiella* ($P < 0.05$). There were no significant differences between the cases infected based on gender, age, and disk diffusion method ($P > 0.05$; Table 1).

According to our findings, considering E-test as the gold standard, the sensitivity and specificity of the diffusion disk method are 100% and 25% respectively; while, the positive and negative predictive values are 87.5% and 100%. According to the kappa test, the kappa coefficient is 0.35 ($P < 0.0001$), which indicates a significant agreement between two tests.

Discussion

The results of our study shows that both E-test and disk diffusion method are very good with high accuracy and sensitivity in determining the resistance and susceptibility of *Acinetobacter* and *Klebsiella* detected in patients with VAP to cefepime. As mentioned in the results, the disk diffusion method is a highly sensitive test that has very similar results to the E-test which is a standard method. Other findings of this study are that the number of colonies in VAP cases caused by *Acinetobacter* is higher than *Klebsiella* and *Acinetobacter* is more resistant and less susceptible than *Klebsiella*.

Pennekamp et al studied the susceptibility of 58 strains of *Corynebacterium jeikeium* to penicillin, tetracycline, erythromycin, gentamicin, vancomycin and teicoplanin utilizing disk diffusion, agar dilution methods and E-test. They concluded that in agar dilution method 14%, 88%, 17% and 24% of strains are susceptible to penicillin, tetracycline, erythromycin and gentamicin, respectively. In addition, using disk diffusion method, the strains were susceptible to penicillin, tetracycline, erythromycin and gentamicin, from the highest to lowest, respectively. All 58 strains were susceptible to vancomycin and teicoplanin using agar dilution and disk diffusion methods. On the other hand, all of these strains were susceptible to glycopeptides employing E-test (18).

In a study comparing VITEK 2 (is a fully automated system that performs bacterial identification and antibiotic susceptibility testing), disk diffusion, E-test, broth microdilution and agar dilution methods to determine the susceptibility of *Acinetobacter baumannii* to colistin was conducted. The authors concluded that disk diffusion method is an unreliable method for determining susceptibility to colistin and VITEK 2 is an easy and reliable method for estimating the susceptibility of this bacteria to colistin. E-test and agar dilution tests are also reported as reliable methods for determining susceptibility of *Acinetobacter baumannii* to colistin (19). In another study, it was reported that disk diffusion method is an unreliable method for determining the susceptibility of Enterobacter

Table 1. Clinical information of patients

Variable		<i>Acinetobacter</i>	<i>Klebsiella</i>	Total	P value
Number		55	45	100	-
Age (year)		45.67 ± 13.28	46.77 ± 13.51	46.17 ± 13.33	0.67
Gender	Male	34	28	62	0.96
	Female	21	17	38	
Number of colonies (CFU)	>100 000	39	18	57	0.008
	100 000-50 000	12	20	32	
	<50 000	4	7	11	
Disk diffusion method	Resistant to cefepime	54	42	96	0.28
	Susceptible to cefepime	1	3	4	
E-test	Resistant to cefepime	50	34	84	0.03
	Susceptible to cefepime	5	11	16	

species to colistin (20). Rechenchoski et al studied methods for detecting the susceptibility of *Klebsiella pneumoniae* to antibiotics and concluded that the highest and lowest MICs were for tigecycline and ertapenem, respectively. In addition, the VITEK 2 system was most similar to broth microdilution as a reference (21).

Conclusion

According to the results of our study, the disk diffusion method has been very useful in determining the susceptibility and resistance of *Acinetobacter* and *Klebsiella* to cefepime and the result is similar to the E-test method. On the other hand, for patients admitted to the ICU with VAP caused by *Acinetobacter* or *Klebsiella*, the disk diffusion method seems to be effective in determining susceptibility to cefepime and methods such as E-test are no longer required. Despite the limitations of this study including its small sample size and having one center, it is the first study to compare disk diffusion method and E-test in determining the susceptibility of *Klebsiella* and *Acinetobacter* strains isolated from VAP patients' lung secretions admitted to the ICU to cefepime.

Limitations of the study

It is difficult to obtain samples from patients' lungs. Also, upon the laboratory condition, managing the effect of confounders and finding the causality relationship is complex. Thus, further studies on this subject is necessary.

Authors' contribution

Conceptualization: Hossein Mahjobipoor, Shirin Fattahpour, Ali Attarzadeh.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical issues

This research adhered to the principles outlined in the Helsinki Declaration and was approved by the Ethics Committee of Isfahan University of Medical Sciences (approval number #IR.MUI.REC.1395.3.764). Written informed consent was obtained from all participants or their parents prior to any intervention. This study was extracted from M.D., thesis of Ali Attarzadeh at this university (Thesis#395764). Moreover, ethical issues (including plagiarism, data fabrication, double publication) were completely observed by the authors.

Funding/Support

The study was financially supported by Isfahan University of Medical Sciences (Thesis #395764).

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