



Antimicrobial resistance pattern of *Acinetobacter*; a multicenter study, comparing European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI); evaluation of susceptibility testing methods for polymyxin

Shirin Afhami¹, Mohammad Ali Borumand², Negin Esmailpour Bazzaz¹, Hiva Saffar¹, Azar Hadadi³, Mahdi Jafary Nezhad⁴, Nahid Mirzaei Tirabadi⁵

¹Department of Infectious Diseases, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

²Department of Pathology, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran

³Department of Infectious Diseases, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

⁴Bachelor of Laboratory Sciences, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

⁵Department of Infectious Disease, School of Medicine, Antimicrobial Resistance Research Center, Immunology & Infectious Disease Research Institute, Shahid Motahari Burns Hospital, Iran University of Medical Sciences, Tehran, Iran

*Correspondence to

Nahid Mirzaei Tirabadi, Email: mirzaeitirabadi.n@iums.ac.ir

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Abstract

Introduction: *Acinetobacter* species in clinical isolates cause severe infections including meningitis, bloodstream infection, ventilator-associated pneumonia, and surgical site infections.

Objectives: In the present study, we evaluated *Acinetobacter* drug resistance using both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility test methods.

Materials and Methods: Clinical specimens of 128 patients who were admitted in three referral tertiary care teaching hospitals were enrolled in 2014. Blood and other sterile fluid samples, endotracheal secretion, ulcer, urine and other clinical specimen cultures were included, and microbial resistance of *Acinetobacter* isolates was determined and compared with disk diffusion and E-test antimicrobial susceptibility methods, using both the EUCAST and CLSI standards. Cohen's kappa coefficient was also reported.

Results: The highest percentage of resistance (96.9%) was found for meropenem and imipenem antimicrobials, and the lowest resistance (82.8%) was found for amikacin. The highest kappa agreement coefficient was for ciprofloxacin (kappa coefficient = 0.783), and the lowest kappa was for amikacin (kappa coefficient = 0.21).

Conclusion: According to the results, it is better to consider amikacin as a choice in combination with another effective antimicrobial for treatment of drug resistant *Acinetobacter*.



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Introduction

Acinetobacter, an opportunistic pathogen, has been responsible for many hospital infections. Multidrug-resistant (MDR) *Acinetobacter* isolates have been increasing worldwide over recent years and has been responsible for outbreaks of healthcare-associated infections, especially in critical care areas of hospitals (1,2). The prevalence of resistance to bacteria is 2.4% of the bloodstream infections in the hospital. These bacteria are responsible for 1.2% of surgical infections, 1.6% of urinary tract infections and 6.9% of hospital-acquired pneumonia (3,4). Treatment of *Acinetobacter* infection is often difficult

Key point

Amikacin can be chosen as an adjuvant choice in combination with other antimicrobial medications to the drug resistant *Acinetobacter*.

due to the emergence of clinical isolates of *Acinetobacter baumannii* with several classes of resistance to antimicrobial agents such as broad-spectrum beta-lactam, carbapenems, aminoglycosides and fluoroquinolones, which has been reported in various treatment centers (5-7). To reduce mortality, clinicians must be informed about the local antimicrobial susceptibility pattern of this

common nosocomial pathogen and its resistance trend for appropriate empiric and targeted treatment.

Disk diffusion is an approved method of antimicrobial susceptibility testing and can be done using most laboratories, but the poor diffusion of colistin to agar results in some problems regarding the sensitivity tests performed (8,9). Colistin disk diffusion tests have lots of errors such as false susceptibility in comparison with dilution testing methods (10,11). Interpretation of disk susceptibility testing of colistin is not achieved by the Clinical and Laboratory Standards Institute (CLSI) (8, 9, 12), and the CLSI has some recommendations to approve the susceptibility of polymyxin/colistin through disk diffusion by a minimum inhibitory concentration (MIC) method (13). Only MIC methods should be used for colistin susceptibility testing (14).

Objectives

In Iran, most laboratories are using antibiotic susceptibility testing based on the CLSI. This study aimed to determine the pattern of *Acinetobacter* antimicrobial susceptibility using the disk-diffusion method and to compare the obtained results with MIC susceptibility method based on the both CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.

Materials and Methods

Settings

This study was conducted during six months of 2015 in three tertiary care teaching hospitals of Tehran University of Medical Sciences, Iran.

Bacterial identification and antimicrobial susceptibility testing

Around 128 consecutive specimens from the microbiological samples of infected patients hospitalized in different wards of three hospitals were cultured from different sources consisted of cerebrospinal fluid, urine, blood, wound, endotracheal secret and sputum.

Initially the specimens were inoculated on blood agar and MacConkey agar medium and incubated at 37°C for 24 hours. Conventional biochemical methods such as catalase, triple sugar iron agar (TSI), indole methyl red Voges-Proskauer citrate test (IMViC), oxidase, citrate, urea urease, malonate consumption, oxidation and fermentation of sugars, motility and indole production were used to identify *A. baumannii*. The isolates were stored in brain heart infusion broth (BHI) medium containing 15% glycerol at -20°C.

All isolates were tested by the Kirby-Bauer method of disk diffusion. For this purpose, antibiotics including ciprofloxacin (5 µg), carbapenems [meropenem (10 µg), imipenem (10 µg)], amikacin (30 µg), ceftazidime (30 µg), piperacillin-tazobactam (100 µg/10 µg) (MAST, Merseyside, UK) have been used. Antibiotic susceptibility tests were performed according to the guidelines of the

CLSI (12). The antimicrobial resistance pattern was reported as sensitive (S), intermediate (I) and resistant (R). Quality control was assessed using the strains *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *E. coli* ATCC 35218 for β lactam-lactam/ β lactamase.

The E-test was performed, using both EUCAST and CLSI standards. The CLSI M100 document had polymyxin B breakpoints. A resistant breakpoint of ≥ 4 µg/mL as well as a susceptible breakpoint of ≤ 2 was considered for all isolates based on the CLSI guidelines (12). Additionally, about the same conditions were used to the EUCAST guidelines exception that susceptible breakpoint was equal and more than 2 (14).

Errors were ranked as follows; very major error, if the result of the reference method (E-test) was resistant, while that of the disk diffusion test was sensitive (false-susceptible result) and major error, if the result of the E-test was sensitive, while that of the disk diffusion test was resistant (false-resistant result). Then, the two methods of disk diffusion and E-test were compared in terms of kappa agreement percentage.

Definitions

All isolates were categorized into three types of resistance; MDR denoting multidrug resistant, XDR denoting extremely drug resistant and PDR denoting pan-drug resistant, in accordance with the literature guidelines (15-17). MDR was considered as the resistance to three or more antibiotics; fluoroquinolones (ciprofloxacin), third generation cephalosporins (ceftazidime), aminoglycosides (amikacin) and carbapenems (imipenem, meropenem), those showing resistance to all but two or less antibiotic classes were called XDR, and isolates showing resistance against all tested classes of antibiotics were categorized as PDR.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Tehran University of Medical Sciences approved this study. The institutional ethical committee at Tehran University of Medical Sciences approved all study protocols (Grant # 26175-30-03-93). Accordingly, written informed consent not taken from patients because the study was done on laboratory specimens.

Statistical analysis

Data were recorded and entered into a database. Continuous variables were compared using one-way analysis of variance, and reported as mean; categorical variables were presented as frequency. Cohen's kappa coefficient was reported as the agreement of the two applied methods. A *P* value ≤ 0.05 was considered significant. Student *t* test was used to compare the quantitative variables and chi-square test was used to compare categorical variables.

Results

Around 128 isolates were evaluated. The antimicrobial resistance pattern of 128 isolates is shown in Table 1. The results showed that the highest percentage (96.9%) of resistance was found for meropenem and imipenem antibiotics, and the lowest resistance (82.8%) was found for amikacin. In this study MDR, XDR and PDR were 63.3%, 33.6%, and 3.1%, respectively.

The frequency of *Acinetobacter* antimicrobial resistance detected by E-test method with both EUCAST and CLSI standards is shown in Table 2. The results showed that the highest percentage of resistance (96.9%) was found for meropenem and imipenem antibiotics according to both of the EUCAST and the CLSI guidelines. The lowest percentage (8.6%) of resistance (11 out of 128 isolates) was detected for colistin by both EUCAST and CLSI standards.

Table 3 shows the kappa agreement coefficients for antimicrobial resistance of 6 antibiotics. The results showed that the highest kappa agreement coefficient was between two methods of disk diffusion and E-test for ciprofloxacin (kappa coefficient = 0.78, $P < 0.001$) with both EUCAST and CLSI standards. The lowest kappa agreement coefficient was for amikacin (kappa coefficient = 0.21, $P < 0.001$).

Discussion

Acinetobacter baumannii can frequently be MDR and it is critical that appropriate susceptibility testing be performed after culture, as empirical therapy may be problematic due to resistance. Surveillance programs to monitor susceptibility can assist in delineating resistance in these isolates over time and across multiple regions globally (18-21), which may guide in choosing appropriate empirical therapy as needed and better understanding evolving resistance and can help to evaluate antimicrobial stewardship applications in hospitals (18).

In our study, the MDR rate of *Acinetobacter* was 33.6%.

Difference in multidrug resistance rates depends on the patient's setting – as MDR rate is higher in ICU and burn units – the region, and the timing of the studies, because there are increasing trends of antimicrobial resistance during recent years. *A. baumannii* MDR rates were lowest in North America (47%), ranged between 77% and 87% in Africa, Asia and Latin America, and exceeded 93% in Europe and the Middle East. Another study in Egypt showed MDR rate of 92.8% for *Acinetobacter* spp (22).

In our study, the highest resistance rate was found for meropenem and imipenem antibiotics, and the lowest resistance rate was found for amikacin and colistin. The results showed that the highest and the lowest kappa agreement coefficient was for ciprofloxacin (kappa coefficient = 0.78) and amikacin (kappa coefficient = 0.21), respectively. Lob et al reported that susceptibility to imipenem was highest in North America (64%), between 16% and 27% in Africa, Asia and Latin America, and lowest in Europe and the Middle East ($\leq 11\%$); amikacin overall was the most active of the studied agents, including against MDR isolates (of which 11%–38% were susceptible), from 20% susceptibility in Europe and Latin America to 62% in North America. Antimicrobial susceptibility profiles vary by region but resistance was high everywhere, with no drug inhibiting $>70\%$ of *A. baumannii* isolates in any region; in Europe and the Middle East the lowest susceptibility for the other studied agents, with none of them exceeding 11% susceptible (18). These figures are similar to our study and show that although historically the carbapenems were the drug of choice for the treatment of drug resistant *Acinetobacter*, currently cannot be effective drugs for the empirical treatment of infections caused by this bacterium in many centers, and monitoring of antimicrobial resistance is necessary.

In the present study, the high resistance rates of *A. baumannii* to beta-lactamase inhibitors (piperacillin/tazobactam; 94.5%), third generation of cephalosporins

Table 1. Frequency of antimicrobial sensitivity/resistance of *Acinetobacter* by disk diffusion method

Type of resistance	Antibiotic					
	Meropenem	Imipenem	Amikacin	Ceftazidime	Ciprofloxacin	Piperacillin-Tazobactam
Resistant	124 (96.9)	124 (96.9)	106 (82.8)	116 (90.6)	119 (93)	121 (94.5)
Sensitive	4 (3.1)	4 (3.1)	20 (15.6)	12 (9.4)	9 (7)	7 (5.5)
Intermediate	0 (0)	0 (0)	2 (1.6)	0 (0)	0 (0)	0 (0)

Table 2. Frequency of antimicrobial sensitivity/resistance of *Acinetobacter* by E-test method according to EUCAST and CLSI standards

Resistance Type	Antibiotic													
	Meropenem		Imipenem		Colistin		Amikacin		Ceftazidime		Ciprofloxacin		Piperacillin-Tazobactam	
	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI
Resistant	121 (94.5)	121 (94.5)	121 (94.5)	121 (94.5)	11 (8.6)	11 (8.6)	74 (57.8)	67 (52.3)	-	82 (64)	117 (91.4)	117 (91.4)	109 (85.2)	-
Sensitive	7 (5.5)	7 (5.5)	7 (5.5)	7 (5.5)	117 (91.4)	117 (91.4)	54 (42.2)	61 (47.6)	-	43 (33.6)	11 (8.6)	11 (8.6)	13 (10.2)	-
Intermediate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-	3 (2.3)	0 (0)	0 (0)	6 (4.7)	-

Table 3. Cohen's kappa coefficients for antimicrobial resistance of *Acinetobacter*

Antibiotic	Antibiotic											
	Meropenem		Ciprofloxacin		Amikacin		Ceftazidime		Imipenem		Piperacillin-Tazobactam	
	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI
Meropenem	0.716 (<i>P</i> <0.001)	0.716 (<i>P</i> <0.00)	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	-	-	0.783 (<i>P</i> <0.001)	0.783 (<i>P</i> <0.001)	-	-	-	-	-	-	-	-
Amikacin	-	-	-	-	0.26 (<i>P</i> <0.001)	0.211 (<i>P</i> <0.00)	-	-	-	-	-	-
Ceftazidime	-	-	-	-	-	-	-	0.214 (<i>P</i> =0.001)	-	-	-	-
Imipenem	-	-	-	-	-	-	-	-	-	0.716 (<i>P</i> <0.00)	-	-
Piperacillin-Tazobactam	-	-	-	-	-	-	-	-	-	-	-	0.443 (<i>P</i> <0.001)

(ceftazidime; 90.6%), and quinolone (ciprofloxacin; 93%) using disk diffusion method were consistent with other results from Jordan (23). In the study of Batarseh et al in Jordan, the resistance rates of *A. baumannii* isolates were high for ceftazidime, imipenem, piperacillin/tazobactam and quinolones, but lower for colistin.

In our study, *A. baumannii* isolates were highly resistant to all antibiotics, except for colistin, which was the most sensitive drug (117 out of 128 isolates, 91.4%) using MIC (E-test) method according to CLSI and EUCAST. There is a wide geographic variation in colistin resistance rate of *A. baumannii*. Colistin resistance was not detected in one study in Turkey (24), 1.7% in Jordan (23), 5.3% in the United States (25) and 39% (to polymyxin) in Brazil (26). Another determinant of colistin resistance is timing of the study; earlier studies show lower resistance rate and the trend of increasing antimicrobial resistance is a global concern, because colistin is a true last resort of antimicrobial treatment, but lost a considerable amount of its activity (25). Therefore, using the combination of both colistin and amikacin antimicrobial agents for the treatment of carbapenem resistant *Acinetobacter* seems prudent in our hospitals.

In the present study, the Cohen's kappa was reported for two different methods of E-test and disk diffusion, which was reported in some studies and can be the strong points of our study. Also, from practical standpoint, we evaluated available antibiotics that are prescribed in clinical settings.

No reference method has been defined against which to compare the results of colistin susceptibility testing (27). The poor performance of disk diffusion for the polymyxins (including colistin) has been attributed to poor diffusion of the polymyxins in agar (28), yielding small zones of inhibition that cannot reliably differentiate susceptible and resistant isolates. Only MIC methods can be used to test the colistin susceptibility, which is a prudent recommendation for the polymyxins as a whole (8-14). Although the E-test is a reputable method to test of the colistin susceptibility (9,29,30), variable results can occur when using different methods for colistin MIC testing and, in particular, to

use caution with the E-test, and the reliability of colistin MICs obtained by E-test is a concern, due to very major errors compared with agar dilution (11,27). Considering this limitation of E-test method, and for better evaluation of antimicrobial resistance pattern of *Acinetobacter*, designing another study and using MIC testing other than E-test is suggested. Another limitation of our study was that we evaluated the antimicrobial susceptibility test results of three referral tertiary care teaching hospitals in Tehran for *Acinetobacter*; we cannot extrapolate these data to all hospitals in Iran.

Conclusion

Trend analysis and regular evaluation of antimicrobial resistance pattern in hospitals must be as a rule and these patterns and trends can help in choosing the choice of empirical antimicrobial therapy. According to the results of this study, carbapenem resistance is high in our hospitals and empirical treatment of *Acinetobacter* with carbapenems is not effective and should be modified to combination therapy of amikacin and colistin due to low resistance of *Acinetobacter* to these antimicrobial agents. Both CLSI and EUCAST documents are helpful to test antimicrobial susceptibility of *Acinetobacter*.

Limitations of the study

This study is a kind of laboratory design conducted on specimens, however it can be considered as a basic science investigation. In this regard, we cannot manage the effect of confounders and find the causality relationship. Therefore, the results should be tested in clinical practice as clinical trials.

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Authors' contribution

SA, MAB and NEB were the principal investigators of the study. NMT, MJN, AH, NEB and SA were included in preparing the concept and design. NMT and SA revised the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft

of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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