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

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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*.

**Key point**

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

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

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

| Antibiotic         | Type of resistance |
|--------------------|--------------------|
|                    | EUCAST CLSI       | EUCAST CLSI       | EUCAST CLSI       | EUCAST CLSI       | EUCAST CLSI       | EUCAST CLSI       |
| Meropenem          | 121 (94.5)         | 121 (94.5)        | 121 (94.5)        | 11 (8.6)          | 74 (57.8)         | 67 (52.3)         |
| Imipenem           | 121 (94.5)         | 121 (94.5)        | 121 (94.5)        | 11 (8.6)          | 6 (42.2)          | 61 (47.6)         |
| Colistin           | 117 (91.4)         | 117 (91.4)        | 117 (91.4)        | 117 (91.4)        | 117 (91.4)        | 109 (85.2)        |
| Amikacin           | 74 (57.8)          | 67 (52.3)         | -                 | 62 (64)           | -                 | -                 |
| Ceftazidime        | 54 (42.2)          | 61 (47.6)         | -                 | 43 (31.6)         | -                 | -                 |
| Ciprofloxacin      | 117 (91.4)         | 117 (91.4)        | 117 (91.4)        | -                 | -                 | -                 |
| Piperacillin-Tazobactam | 119 (93)  | 9 (7)             | 0 (0)             | 3 (2.3)           | 0 (0)             | 6 (4.7)           |
methods for colistin MIC testing and, in particular, to test 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 colistin resistance, which is a prudent recommendation for the polymyxins as a whole (8-14), only MIC methods can be used to test the resistance of colistin in agar (28), yielding small zones of inhibition that cannot reliably differentiate susceptible and resistant isolates. Only MIC methods can be used to test 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 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 integrality 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 publications) have been completely observed by the authors.

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