Reliability of MIC Gradient Strips (E-test) in Detection of Colistin Resistant \textit{Acinetobacter baumannii} Caused an Outbreak in a Teaching Hospital in Tehran

\textbf{Aims} \textit{Acinetobacter baumannii} strains have become an important treat in nosocomial infection control. The reliable detection of these strains plays a critical role in treatment procure. The aim of this study was to evaluate the three different methods in detection of colistin resistant \textit{A. baumannii} strains.

\textbf{Materials & Methods} Eighty-three \textit{A. baumannii} strains were isolated from hospitalized patients of a teaching hospital in Tehran during 1 year (2016-2017). All isolates were genetically confirmed by Polymerase Chain Reaction (PCR). The resistance to colistin was determined with disc diffusion, E-test, and micro broth dilution method.

\textbf{Findings} According to the results of micro broth dilution as a gold standard, 43\% of the isolates were resistant to colistin, while this percentage was 23\% and 44\% through E-test and disc diffusion methods, respectively. The positive and negative predictive value (PPV and NPV) of this method was 43\% and 57\%, respectively. The sensitivity and NPV index of E-test for the detection of colistin resistant strains was 76\% and 68\%.

\textbf{Conclusion} Detection of colistin MIC by E-test strips has been commonly used in clinical laboratories to recognize the colistin susceptible strains. The NPV and sensitivity of E-test method demonstrated that this method has inefficacy to accurate determination of colistin susceptible strains. Thus, using standard protocol micro broth dilution with qualified materials should be stabilized and replaced instead of disc diffusion or even using E-test in clinical laboratories.

\textbf{Keywords} \textit{Acinetobacter baumannii}; Colistin Resistant; E-test; Micro broth dilution
Introduction

Acinetobacter baumannii is the most common pathogen in clinical samples among other species in this genus. A. baumannii can cause variety infections, including respiratory tract, wound infection, meningitis, and bacteremia [1]. A. baumannii has been detected as a 5th causing agent in ventilator-associated pneumonia (VAP) and 13th in central line-associated bloodstream infection [2]; it has also been recognized as one of the 6 top dangerous pathogens causing nosocomial infection outbreaks according to the Infectious Diseases Society of America (IDSA) [3]. The ability of A. baumannii to tolerate the harsh environments make it as an endemic pathogen in health care units, which can survive on inanimate surfaces for months [4]. A. baumannii intrinsically is resistant to several classes of antibiotics and has a great tendency in acquisition of resistance factors. Invasive procedures, wrong antibiotic diets, and immunocompromised hosts in the hospitals have been leaded to prevalent the multi-drug resistant A. baumannii (MDR) strains among hospitalized patients in the recent decade.

According to the results of different studies, there are many risk factors in acquisition of infection caused by MDR A. baumannii, including, environmental contamination, colonized healthcare stuffs, surgery, previous exposure to antibiotics specially carbapenems, or cephalosporins, using instruments like catheters or ventilators [5-9]. The potent treatments for MDR A. baumannii infection are extremely limited since many strains have become resistant to all available antibiotics [10].

Almost the only remaining antibiotic for the treatment of MDR A. baumannii is colistin, which is a cationic bactericidal polypeptide for Gram negative bacteria. The mechanism of colistin is related to the electrostatic interaction with lipid A part of lipopolysaccharide (LPS) in outer membrane of Gram negative bacteria and destabilization of cytoplasmic membrane [11]. This antibiotic also is a potent substitution for cure in patients infected with resistant Pseudomonas aeruginosa. The resistant strains have been emerged via the wide and excessive clinical usage of this antibiotic, [12, 13]. The first report of colistin-resistant A. baumannii was from Czech Republic in 1999 and after a while, this resistance increased year by year in all over the world [13, 14]. It has been demonstrated that modification in lipid A by adding some cationic residues or loosing of Lipid A are the mainly colistin resistance mechanisms, which are lead to decrease the negative charge of LPS in outer membrane of bacterial cells. The current detection of resistance among clinical isolates play a critical role in efficient antibiotic prescribing; thus, the infection specially the nosocomial can be controlled in a better manner. Since clinical diagnostic of antimicrobial resistance especially against colistin is a basic and critical step in treatment of A. baumannii infection, the validation of diagnostic method should be considered. Most of the clinical laboratories perform Epsilometer test (E-test) as a reliable method for detection of colistin resistance according to the Infectious Diseases Society of America (IDSA) [3]. This method is really time-benefit, but the point is that is it completely reliable for Micro broth substitution in detection of colistin resistant strains?

With regard to the mentioned points, the aim of this study was to evaluate the three different clinical methods for detection of colistin resistance among clinical A. baumannii isolates and to investigate the false positive and negative results of these methods.

Materials and Methods

Eighty-three A. baumannii strains were isolated from hospitalized patients during 1 year (2016-2017). Clinical samples were different and contained urine, blood, sputum, and cerebrospinal fluid (CSF). The patients were hospitalized in different units, but mostly from intensive care units (ICUs) including neurosurgical ICU, internal ICU, surgical ICU.

Identification of isolates: All the primary identified A. baumannii strains were transferred to Antimicrobial Resistance Research Center laboratory and were subjected to conventional biochemical tests including, Gram staining, oxidase, simon citrate, triple sugar iron agar, oxidative/fermentative glucose, and growth on 42°C and gelatinase. In all biochemical tests A. baumannii ATCC 19606 was used as a positive control.

Genetic confirmation of A. baumannii isolates: Although the accurate identification of genus and species of clinical isolates is a principle step in determination of antimicrobial resistance, mostly the isolates have wrongly been identified by clinical laboratories. In this study, all the 83 A. baumannii were confirmed by PCR, using specific primers (oxa51-F: TAATGCTTTGATCGGCCTTG; oxa51-R: TGGATTGCACTTTCATCATCTTGG) amplified bla-oxa51 genes, which are definite for A. baumannii strains [15]. The whole genome of bacteria was extracted with boiling method and used as a DNA template. The PCR reaction was performed in 25 µl mixture composed of 12 µl commercial master mix (including dNTPs, superTaq DNA polymerase, dNTPs, and Taq-buffer), 0.5 µl of each primer with 10 pmol concentration, 5 µl DNA template, and sterile distilled water up to 25 µl. The PCR program was set as follow, initial denaturation at 95°C for 5min, 35 cycles repeat of denaturation at 95°C for 30s, annealing at 52°C for 30s, and extension at 72°C for 45s, followed by final extension at 72°C for 10min. The genomic DNA of A. baumannii ATCC 19606 and E. coli ATCC25922 were used as a positive and negative, respectively.

Determination of Colistin resistant A. baumannii, using 3 standard methods: According
to the Clinical and Laboratory Standard Institute (CLSI) guideline, 3 different methods have been validated for the detection of resistance to antibiotics in bacterial strains. Recently, it has been demonstrated that disc diffusion is not reliable for the detection of colistin resistant strains and has been omitted from CLSI 2107, while E-test strips is still valid. In the present study, disc diffusion, using E-test strips and micro broth dilution methods, were performed and compared to each other. For determination of colistin resistant strains, the micro broth dilution, which determines the Minimum inhibitory concentration (MIC) of antibiotic, has been reported as a gold standard method. The overnight culture of A. baumannii strains on BHI agar were suspended in sterile normal saline to the turbidity equal to 0.5 McFarland. The colistin MIC for all isolates was determined, using E-test strips (bioMérieux, Inc., La Balme les Grottes, France) ranging from 0.01 to 128 μg/ml according to the manufacturer instruction. The disc diffusion and micro broth dilution were performed according to the CLSI 2017 guideline. Briefly, the concentration of colistin from 0.5 μg.ml⁻¹ to 128 μg.ml⁻¹ was poured in 96-well microplates by serial dilution for micro broth dilution method and the MIC of colistin was determined for all strains in duplicate.

**Positive and negative predictive value (PPV and NPV, respectively), specificity and sensitivity of different methods:** The PPV, NPV, sensitivity, and specificity indices for diagnostic methods were evaluated, using the following formula:

$$\begin{align*}
PPV &= \frac{\text{true positive}}{\text{true positive} + \text{false positive}} \times 100 \\
NPV &= \frac{\text{true negative}}{\text{true negative} + \text{false negative}} \times 100 \\
Sensitivity &= \frac{\text{true positive}}{\text{true positive} + \text{false negative}} \times 100 \\
Specificity &= \frac{\text{true negative}}{\text{true negative} + \text{false positive}} \times 100
\end{align*}$$

The true negative and positive were determined according to the results of micro broth dilution method as a gold standard [16].

**Findings**

86 out of 90 biochemical identified A. baumannii strain were genetically confirmed and included into the study. The PCR product of oxa-51 gene among confirmed isolates has been shown in Figure 1. According to the results of micro broth dilution, 37 out of 86 (43%) isolates were resistant to colistin; this number was 20 (23%) with E-test and 44 (51%) with disc diffusion method. The PPV, NPV indices, sensitivity, and specificity of E-test and disc diffusion comparing with the micro broth dilution method is mentioned in Table 1.

**Discussion**

The mortality of hospitalized patients causing by A. baumannii infections has been significantly increased in the recent decade [4, 17]. Up to the early 1970s, A. baumannii was susceptible to the wide range of antibiotics, while during 4 decades, most of reliable treatment for A. baumannii infections were took away because of the emergence of resistant strains especially carbapenems-resistant strains [18-20]. Since colistin is a last line treatment for MDR A. baumannii strains, resistance profile against this antibiotic is critically considerable [21, 22]. Thus, accurate detection of A. baumannii and colistin resistance profile of local strains are the important keys to reduce the antimicrobial resistance and surveillance the nosocomial infection caused by A. baumannii.

In the present study, resistance to colistin among isolate was strongly higher than the previous reports in Iran. In a study conducted by Vakili et al., the colistin resistance rate was reported 11.6% among A. baumannii isolated from medical and surgical ICUs [23]. There are some other results in contrast, which are reported 20% and 15% of colistin resistance among clinical A. baumannii isolates in Tehran and Shiraz during 2011 to 2012 [24], while according to the systematic review done by Moradi et al. during 13 years from 2011 to 2013, no significant increase in the rate of resistance to colistin has been reported [25]. The disparities among results of studies might be due to using unreliable methods.

![Figure 1](image-url)
Although the disc diffusion has been invalidated by CLSI guideline to evaluate the colistin resistance, it has still been used in clinical laboratories. The disc diffusion method determined the same percentage of colistin resistance in comparison with micro broth dilution (p≥0.05), but the main point is that the results were not reliable since NPV and PPV of the former method were too low. The E-test method has been validated as an alternative method instead of micro broth dilution; as we can see in this study, using E-test has an acceptable specificity and also has 80% ability to recognize the true resistant samples, but has deficiency in sensitivity and recognizing the susceptible samples.

The results of the present study are in agreement with other studies, which revealed E-test is not a reliable method in diagnosis of colistin resistance. Chew et al. performed a study on the evaluation of some commercial susceptibility testing methods, including E-test with two which demonstrated that E-test has 12% major error rate in comparison with micro broth dilution. The errors rates were higher in lower MICs, which might be associated to poor micro broth dilution. In some other studies, it has been revealed that E-test underestimated the MIC value of colistin in comparison with micro broth dilution, resulting in increasing the number of false susceptible strains [27, 28].

Conclusion

Determination of colistin resistant A. baumannii strains is critical in national administration of colistin for carbapenem resistant A. baumannii. Although user friendly commercial micro dilution methods such as E-test have been developed and approved to time saving in treatment procedure, they are not completely reliable and trustful in comparison with micro broth dilution method. Thus, using standard protocol with qualified materials should be stabilized and replaced instead of disc diffusion or even using E-test in clinical laboratories.

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References


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