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

Authors
Boustanshenas M.[1] *MSc*,
Defaee S.[2] *MD*,
Majidpour A.[3] *PhD*,
Afshar M.[1] *MSc*,
Mohammadi Azad Z.[1] *MSc*,
Ferasati M.[2] *MD*,
Namdari Moghadam N.[2]
MSc, Jamshidi-Makiani M.*[3]
PhD*

[1]Antimicrobial Resistance Research Center, Institute of Immunology & Infectious Disease, Iran University of Medical Sciences, Tehran Iran
[2]Infectious Disease Department, Medicine Faculty, Iran University of Medical Sciences, Tehran, Iran
[3]"Antimicrobial Resistance Research Center, Institute of Immunology & Infectious Disease" and "Infectious Diseases & Tropical Medicine Department, Medicine Faculty", Iran University of Medical Sciences, Tehran, Iran

*Correspondence
Address: Floor 3, NO.30, Rasoul-e-AKaram Hospital, Niayesh Street, Tehran, Iran. Postal Code: 1445613131
Phone: +98 (21) 64352397
Fax: +98 (21) 66554063
mahinjamshidi42@gmail.com.

A B S T R A C T

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

Materials & Methods Eighty-three *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.

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%.

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.

Keywords *Acinetobacter baumannii*; Colistin Resistant; E-test; Micro broth dilution

C I T A T I O N   L I N K S

[1] Epidemiology and impact of imipenem resistance in Acinetobacter ... [2] Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention ... [3] Bad bugs, no drugs: No ESKAPE! an update from the Infectious ... [4] The epidemiology and control of Acinetobacter ... [5] An outbreak due to multiresistant Acinetobacter baumannii in a burn unit: Risk factors for acquisition and ... [6] Risk-factors for the acquisition of ... [7] Risk factors for acquisition of imipenem-resistant Acinetobacter ... [8] Multidrug resistant Acinetobacter baumannii: Risk factors for appearance of imipenem resistant strains on patients formerly with susceptible ... [9] Risk factors for healthcare-associated extensively drug-resistant ... [10] Comparison of outbreak and nonoutbreak ... [11] Towards antibacterial strategies: Studies ... [12] Colistin, mechanisms and prevalence of ... [13] Colistin resistance of Acinetobacter ... [14] Characteristics of Acinetobacter ... [15] Usefulness of phenotypic and genotypic ... [16] Measures of diagnostic accuracy ... [17] Emergence of resistance to carbapenems ... [18] Multidrug resistance among Acinetobacter ... [19] Acinetobacter spp. as nosocomial ... [20] Acinetobacter baumannii: Evolution of antimicrobial ... [21] Pharmacokinetics of colistin methanesulfonate ... [22] Colistin heteroresistance in Acinetobacter ... [23] Detection of colistin sensitivity in clinical isolates of Acinetobacter ... [24] Emergence of rifampicin, tigecycline, and colistin-resistant Acinetobacter baumannii in Iran, spreading of MDR strains of novel International ... [25] Antibiotic resistance of Acinetobacter baumannii in Iran: A systemic review of the published ... [26] Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive enterobacteriaceae: Comparison of sensititre, microscan, vitek 2, and etest with broth ... [27] Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and ... [28] Evaluation of colistin stability in agar and comparison of four methods for MIC ...

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 tolerant 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. baummanii* 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 *(oxa*51-F: TAATGCTTTGATCGGCCTTG; *oxa*51-R: TGGATTGCACTTTCATCATCTTGG) amplified *bla-oxa*51 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. baummanii* ATCC 19606 and *E. coli* ATCC25922 were used as a positive and negative, respectively.

Determination of Colistin resistant *A. baummanii*, 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 1024 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:

$$PPV = [true\ positive/true\ positive + false\ positive] \times 100$$

$$NPV: [true\ negative/true\ negative + false\ negative] \times 100$$

$$Sensitivity: [true\ positive/true\ positive + false\ negative] \times 100$$

$$Specificity: [true\ negative/true\ negative + false\ positive] \times 100$$

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.
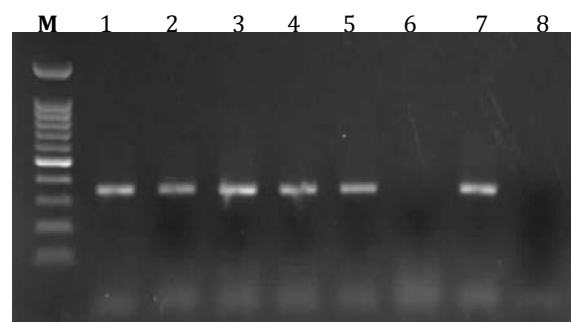
Figure 1) Amplification of oxa-51 gene for confirmation of *A. baumannii* isolates. M: 1kb DNA size marker; lane 1-6: different primary identified *A. baumannii*; lane 7: *A. baumannii* ATCC19606 as positive control; lane 8: *E. coli* ATCC as a negative control

Table 1) Statistical analysis of different methods for detection of colistin resistance (Values based on %)

Method	Resistance to colistin	PPV	NPV	Specificity	Sensitivity
Micro broth dilution	43	-	-	-	-
E-test	23	80	68	91	76
Disc Diffusion	44	43	57	48	51

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. baumanni* infection 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.

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 ($p \geq 0.05$), 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 diffusion of colistin in lower concentration [26]. 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 rational 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.

Authors' Contribution: Boustanshenas M. (First author), Methodologist/ Original researcher/ Statistical analyst (35%); Defaee S. (Second author), Original researcher (10%); Majidpour A. (Third author), Discussion author (5%); Afshar M. (Fourth author), Methodologist (5%); Mohammadi Azad Z. (Fifth author), Methodologist (5%); Ferasati M. (Sixth author), Discussion author (5%); Namdari Moghadam N. (Seventh author), Statistical analyst (10%); Jamshidi-Makiani M. (Eighth author), Original researcher (25%).

References

1- Lautenbach E, Synnestvedt M, Weiner MG, Bilker WB, Vo L, Schein J, et al. Epidemiology and impact of imipenem resistance in Acinetobacter baumannii. Infect Control Hosp Epidemiol. 2009; 30(12):1186-92.
2- Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol. 2013;34(1):1-14.
3- Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: No ESKAPE! an update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1-12.
4- Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006;42(5):692-9.
5- Simor AE, Lee M, Vearncombe M, Jones-Paul L, Barry C, Gomez M, et al. An outbreak due to multiresistant Acinetobacter baumannii in a burn unit: Risk factors for acquisition and management. Infect Control Hosp Epidemiol. 2002;23(5):261-7.
6- Cisneros JM, Rodríguez-Baño J, Fernández-Cuenca F, Ribera A, Vila J, Pascual A, et al. Risk-factors for the acquisition of imipenem-resistant Acinetobacter baumannii in Spain: A nationwide study. Clin Microbiol Infect. 2005;11(11):874-9.
7- Lee SO, Kim NJ, Choi SH, Hyong Kim T, Chung JW, Woo JH, et al. Risk factors for acquisition of imipenem-resistant Acinetobacter baumannii: A case-control study. Antimicrob Agents Chemother. 2004;48(1):224-8.
8- Ye JJ, Huang CT, Shie SS, Huang PY, Su LH, Chiu CH, et al. Multidrug resistant Acinetobacter baumannii: Risk factors for appearance of imipenem resistant strains on patients formerly with susceptible strains. PLoS One. 2010;5(4):e9947.
9- Chan MC, Chiu SK, Hsueh PR, Wang NC, Wang CC, Fang CT. Risk factors for healthcare-associated extensively drug-resistant Acinetobacter baumannii infections: A case-control study. PLoS One. 2014;9(1):e85973.
10- Dijkshoorn L, Aucken H, Gerner-Smidt P, Janssen P, Kaufmann ME, Garaizar J, et al. Comparison of outbreak and nonoutbreak Acinetobacter baumannii strains by genotypic and phenotypic methods. J Clin Microbiol. 1996;34(6):1519-25.
11- Wiese A, Gutsmann T, Seydel U. Towards antibacterial strategies: Studies on the mechanisms of interaction between antibacterial peptides and model membranes. J Endotoxin Res. 2003;9(2):67-84.
12- Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin. 2015;31(4):707-21.
13- Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: Clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother. 2012;67(7):1607-15.
14- Hejnar P, Kolár M, Hájek V. Characteristics of Acinetobacter strains (phenotype classification, antibiotic susceptibility and production of beta-lactamases) isolated from haemocultures from patients at the Teaching Hospital in Olomouc. Acta Univ Palacki Olomuc Fac Med.

1999;142:73-7.

15- Szejbach A, Mikucka A, Bogiel T, Gospodarek E. Usefulness of phenotypic and genotypic methods for metallo-beta-lactamases detection in carbapenem-resistant Acinetobacter baumannii strains. Med Sci Monit Basic Res. 2013;19:32-6.

16- Šimundić AM. Measures of diagnostic accuracy: Basic definitions. EJIFCC. 2009;19(4):203-11.

17- Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: Clinical impact and therapeutic options. Int J Antimicrob Agents. 2012;39(2):105-14.

18- Queenan AM, Pillar CM, Deane J, Sahm DF, Lynch AS, Flamm RK, et al. Multidrug resistance among Acinetobacter spp. in the USA and activity profile of key agents: Results from CAPITAL Surveillance 2010. Diagn Microbiol Infect Dis. 2012;73(3):267-70.

19- Bergogne-Bérézin E, Towner KJ. Acinetobacter spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 1996;9(2):148-65.

20- Doi Y, Murray GL, Peleg AY. Acinetobacter baumannii: Evolution of antimicrobial resistance-treatment options. Semin Respir Crit Care Med. 2015;36(1):85-98.

21- Li J, Rayner CR, Nation RL, Deans R, Boots R, Widdecombe N, et al. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. Antimicrob Agents Chemother. 2005;49(11):4814-5.

22- Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in Acinetobacter and its association with previous colistin therapy. Antimicrob Agents Chemother. 2008;52(1):351-2.

23- Vakili B, Fazeli H, Shoaei P, Yaran M, Ataei B, Khorvash F, et al. Detection of colistin sensitivity in clinical isolates of Acinetobacter baumannii in Iran. J Res Med Sci. 2014;19 Suppl 1:S67-70.

24- Bahador A, Taheri M, Pourakbari B, Hashemizadeh Z, Rostami H, Mansoori N, et al. Emergence of rifampicin, tigecycline, and colistin-resistant Acinetobacter baumannii in Iran, spreading of MDR strains of novel International Clone variants. Microb Drug Resist. 2013;19(5):397-406.

25- Moradi J, Hashemi FB, Bahador A. Antibiotic resistance of Acinetobacter baumannii in Iran: A systemic review of the published literature. Osong Public Health Res Perspect. 2015;6(2):79-86.

26- Chew KL, La MV, Lin RTP, Teo JWP. Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive enterobacteriaceae: Comparison of sensititre, microscan, vitek 2, and etest with broth microdilution. J Clin Microbiol. 2017;55(9):2609-16.

27- Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. Clin Microbiol Infect. 2018;24(8):865-70.

28- Turlej-Rogacka A, Xavier BB, Janssens L, Lammens C, Zarkotou O, Pournaras S, et al. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. Eur J Clin Microbiol Infect Dis. 2018;37(2):345-53.