JOURNAL OF HEALTH SCIENCES AND MEDICINE

Sağlık Bilimleri ve Tıp Dergisi

J Health Sci Med 2019; 2(2): 49-53

Research Article / Araştırma Makalesi

Determination of polymyxin B, minocycline, colistin and phosphomycin susceptibilities in *Acinetobacter baumannii* strains showing carbapenem resistant multidrug resistance phenotype

Karbapeneme dirençli, çoklu ilaç direnci fenotipi gösteren Acinetobacter baumannii suşlarında polimiksin B, minosiklin, kolistin ve fosfomisin duyarlılıklarının belirlenmesi

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ABSTRACT

Aim: In this study, the sensitivity of minocycline, polymyxin B, colistin and phosphomycin to carbapenem resistant multiresistant *Acinetobacter baumannii* (*A. baumannii*) strains (resistant to three or more antibiotic groups) isolated from patients in Ankara Training and Research Hospital It was aimed.

Material and Method: Eighty nosocomial *A. baumanii* strains with a carbapenem-resistant multidrug resistance phenotype were included in the study. Sensitivities of minocycline, polymyxin B and phosphomycin were determined by disc diffusion test and colistin susceptibility test by disk diffusion and E-test methods.

Results: Eighty (100%) polymyxin B, 75 (93,75%) minocycline sensitive and 4 (5%) medium susceptible to 80 carbapenem resistant *A. baumannii* strains were evaluated by disc diffusion method. 75 (93.5%) were sensitive to colistin, 5 (6.25%) were moderately sensitive to colistin and 2 (2.5%) were sensitive to phosphomycin. By e-test, all 80 strains (100%) were detected as susceptible to colistin. The MIC range for colistin was 0.125-1.5 µg / ml, the MIC 50 value was 025 µg / ml (the MIC range was found to be 0.5 µg / ml).

Conclusion: Polymyxin B and minocycline could be used in therapy because phosphomycin B and susceptibility rates were high in empirical treatment of carbamazepine resistant multispecific *A. baumannii* strains in our hospital. Phosphomycin could not be used in treatment because of high resistance rate.

Keywords: *Acinetobacter baumannii*, polymyxin B, minocycline, colistin, phosphomycin, susceptibilities, multidrug resistance

ÖZ

Amaç: Bu çalışmada, Ankara Eğitim ve Araştırma Hastanesi'nde yatan hastalardan izole edilen, karbapeneme dirençli, çoklu ilaca dirençli (üç ve daha fazla antibiyotik grubuna dirençli) *Acinetobacter baumannii* (*A. baumannii*) suşlarının minosiklin, polimiksin B, kolistin ve fosfomisin duyarlılıklarının belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya karbapeneme dirençli, çoklu ilaç direnci fenotipi gösteren 80 nozokomiyal kökenli *A. baumanii* suşu dahil edildi. Suşların minosiklin, polimiksin B ve fosfomisin duyarlılıkları disk difüzyon testiyle, kolistin duyarlılıkları ise disk difüzyon ve E-test yöntemleriyle belirlendi.

Bulgular: Çalışmaya alınan toplam 80 karbapeneme dirençli *A. baumannii* suşunun disk difüzyon yöntemi ile 80 (%100)'nin polimiksin B'ye, 75 (%93,75)'inin minosikline duyarlı, 4 (%5)'inin orta duyarlı, 75 (%93.5)'inin kolistine duyarlı, 5 (%6,25)'sının kolistine orta duyarlı, 2 (%2,5)'sinin ise fosfomisine duyarlı olduğu belirlendi. E-test yöntemiyle 80 suşun tamamı (%100) kolistine duyarlı olarak saptandı. Kolistin için MİK aralığı 0,125-1.5 µg/ml, MİK 50 değeri 025 µg/ml (MİK aralığı iken, MİK90 değeri 0,5 µg/ml olarak belirlendi.

Sonuç: Hastanemizde karbapeneme direçli, çoklu ilaca dirençli *A. baumannii* suşlarının ampirik tedavisinde duyarlılık oranlarının yüksek olması nedeniyle polimiksin B ve minosiklinin tedavide kullanılabileği, fosfomisinin ise direnç oranının yüksek olması nedeniyle tedavide kullanılamayacağı belirlendi.

Anahtar Kelimeler: *Acinetobacter baumannii*, polimiksin B, minosiklin, kolistin, fosfomisin, duyarlılık, çoklu ilaç direnci

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Received: 03.09.2018 **Accepted:** 09.10.2018 **Doi:** 10.32322/jhsm.456990

Cite this article as: Kınıklı S, Cesur S, Yücel M, Ataman Hatipoğlu Ç, Dinç B. Determination of polymyxin B, minocycline, colistin and phosphomycin susceptibilities in Acinetobacter baumannii strains showing carbapenem resistant multidrug resistance phenotype. J Health Sci Med 2019; 2(2): 49-53.

INTRODUCTION

Healthcare-related infections caused by *Acinetobacter baumannii (A.baumannii)* strains are an important cause of mortality and morbidity. The resistance of *A.baumannii* strains to various antibiotic strains with different mechanisms limits both treatment options and increases the costs of treatment. Carbapenem resistant multidrug-resistant *Acinetobacter baumannii* infections are a major problem in intensive care in the world and in our country (1,2).

The resistance of *A. baumannii* strains to more than three antibiotic groups are defined as multiple antibiotic resistance (3,4). There are difficulties in the treatment of *A. baumannii* infections, which have multiple antibiotic resistance, especially resistant to carbapenems. The main antibiotics that can be used in the treatment of these infections; colistin, polymyxin B, tigecycline, minocycline, phosphomycin, sulbactam and combinations of these antibiotics (5,6).

In this study, it was aimed to determine susceptibility of minocycline, polymyxin B, colistin and phosphomycin to carbapenem resistant multiple antibiotic resistant *A. baumannii* strains isolated from different clinical samples of hospitalized patients.

MATERIAL AND METHOD

A total of 80 *A. baumannii* strains isolated from different clinical specimens of patients hospitalized in Ankara Training and Research Hospital Clinical and Intensive Care Units were tested for carbamazepine resistance and multiple antibiotic resistance. Microbial cultures were incubated at 37 ° C for 24 hours after culturing of carbapenem resistant MDR-*A .baumannii* strains on Eosin-methylene blue agar (EMB) medium. Minocycline (30 µg), polymyxin B (300 IU), colistin (10 µg) and phosphomycin (200 µg) discs (Becton-Dickinson, USA) were investigated by disc diffusion method on Mueller-Hinton agar medium after the bacterial suspension was prepared at 0.5 Mc Farland turbidities colistin sensitivities were also investigated simultaneously with colistin E-test (Biomerieux, France). Sensitivities of minocycline, polymyxin B, colistin and phosphomycin were determined according to CLSI criteria (7). For the minocycline, the zone was considered sensitive if the diameter was ≥ 16 mm, moderately sensitive between 13 and 15 mm, and resistant if ≤ 9 mm (8).

For the colistin, the zone diameter was 13 mm for sensitive, 11-12 mm for moderate sensitivity, and ≤ 11 mm for resistant (7,9). Polymyxin B was sensitive if zone diameter was ≥14 mm, and resistant if ≤10 mm (7,9). For phosphomicin, the zone was considered sensitive if the diameter was ≥16 mm, moderately sensitive if 13 -15 mm, and resistant if ≤12 mm (10).

RESULTS

Distribution of clinical samples in which 80 carbapenem resistant multiresistant (MDR) strains were isolated from a total of 80 *A. baumannii* strains; 10% (n=8), 10% (n=8), 10% (n=8) and 10% (n=8) respiratory specimens were obtained in 60% (n=48) respiratory specimens (deep tracheal aspirate and bronchoalveolar lavage fluid) other samples (CSF, peritoneal fluid, urine). No colistin resistance was detected with the automated system (Vitek 2, France) in any of the 80 strains included in the study.

Antibiotic susceptibilities of a total of 80 carbapenem resistant MDR *A. baumannii* strains are shown in Table 1.

Resistance was not detected in any of the carbapenem resistant MDR 80 *A.baumannii* strains by the E-test method. The minimum inhibitory concentration (MIC) value range determined by E-test for colistin was 0.125-1.5 µg / ml. The 80 strains detected colistin-sensitive were MIC50 values of 0.25 µg / ml and MIC90 values of 0.5 µg / ml. The MIC values determined by E-test of 5 (6.25%) strains determined to be colistin-sensitive by disk diffusion method are shown in Table 2.

Table 1. Antimicrobial susceptibility of carbapenem resistant MDR ***A. baumannii*** strain (n: 80)

Antibiotics	Sensitive (S) Sayı	(%)	Intermediate (I) Sayı	(%)	Resistant (R) Sayı	(%)
Polymyxin B	80	(100)	0	(0)	0	(0)
Minocycline	75	(93.75)	4	(5)	1	(1.25)
Colistin	75	(93.75)	5	(6.25)	0	(0)
Phosphomycin	2	(2.5)	0	(0)	78	(97.5)

The most effective antibiotics to carbapenem resistant MDR *A. baumannii* strains in our study are; polymyxin B, colistin and minocycline. The rate of resistance to phosphomycin (97.5%) was found to be quite high.

Table 2. MIC values determined by E-test method in 5 (6.25%) strains determined to be medium-sensitive to colistin by disk diffusion method

Suş No	Kolistin MİK değeri (µg/ml)
1	0.19
2	0.25
3	0.25
4	0.5
5	1.5

DISCUSSION

MDR-*A. baumannii* infections are one of the most common etiologies of the most common healthcare-related infections in the world and in our country in recent years (1,2).

The treatment of MDR *A. baumannii* infections is difficult and the number of antibiotics that can be used in treatment is limited. Polymyxins consist of polymyxin B and polymyxin E (colistin). It is used in the treatment of Gram-negative bacteria (*A. baumannii, Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae etc*.), which are the most important side effects of polymyxins which are nephrotoxic and neurotoxic (11,12).

Minocycline in the treatment of MDR infections, and phosfomycin in the treatment of alternative infections (13,14). In our study, resistance to polymyxin B and colistin was not detected in 80 MDR-*A. baumannii* strains, whereas resistance to phosphomycin was 97.5% and minocycline resistance was 1.25%. According to these results, in empirical treatment of MDR-*A. baumannii* infections in our hospital, colistin, polymyxin B and minocycline could be used; but we have found that phosphomycin is not a suitable option in treatment because of its high resistance rate.

Currently, the recommended reference method for determination of colistin sensitivity in gram-negative bacteria is the bacterial microdilution method. Although there are studies reporting that the e-test method can be used, this method is not currently recommended (9,15). The fact that we did not use the bio-microdilution method in studying was a limiting factor in our study. Arroyo et al. (16), 20 of 22 (91%) *A. baumannii*. (91%) of 22 *A. baumannii* strains determined to be colistin-resistant by microbial strains were resistant by the E-test method. In our study 80 colistin resistance was not detected by the automated system (Vitek 2, France), E-test and disc diffusion method in none of the 80 ÇID-*A. baumannii* strains. Discrimination between methods was reported to be insignificant when comparing disc diffusion biotyping microdilution methods and biotyping microdilution and E-test methods for detecting resistance to polymyxin B.

Akın et al. (9) In 95 MDR *A. baumannii* strains, they investigated the resistance of colistin, polymyxin B and tigecycline by disk diffusion, E-test and biotite microdilution method. In this study, all of the strains (100%) were found to be sensitive to colistin in all three methods, 92 (96.8%) were susceptible to disk diffusion and lactate microdilution and 90 (94.7%) were sensitive to polymyxin B by E-test method. The susceptibility of *A.baumannii* strains to tigecycline is disk diffusion method, 87.4% (n=83), 82.1% (n=78) and 94.7% (n=90) respectively by e-test and microdilution method, respectively. Among the resistance rates obtained by all three methods against Polymyxin B statistically no difference was detected; While the difference between disk diffusion and biofilm microdilution methods were not significant in detecting tigecycline susceptibility, the difference between biofilm microdilution and E-test methods was found to be statistically significant.

Öksüz et al. (15) evaluated in vitro activities of colistin, polymyxin B and tigecycline in 75 MDR-*A. baumannii* strains isolated from various clinical specimens. E-test for colistin, polymyxin B and tigecycline in study was determined by disc diffusion method for other antibiotics. All of the strains were identified as MDD-*A. baumannii*, all of which were resistant to at least three antibiotic classes and 95% were found to be resistant to at least one carbapenem. All strains were found to be susceptible to colistin and polymyxin B. Of the strains, 46 (61%) were susceptible to tigecycline, 27 (36%) were moderately susceptible and two (3%) were resistant. 38% of the carbapenem-resistant strains were found to be tigecycline-resistant or moderately susceptible. MIC50 and MIC90 values for colistin, polymyxin B and tigecycline were respectively; 0.19-0.50µg / ml; 0.38-0.50µg / ml; 2-3 µg / ml. It has been reported that colistin and polymyxin B in MDR-*A.baumannii* strains have better in vitro activity than tigecycline. We did not find resistance to polymyxin B and colistin in MDR-AB strains. MIC50 and MIK90 values for colistin are respectively; 0.25 µg / ml and 0.5 µg / ml, respectively. In our study, the minocycline in the tetracycline group, such as tigecycline, was found to be resistant to only one strain. Resistance rates of polymyxin B in MDR-*A.baumannii* strains have been reported between 1% and 13% in different studies in the literature (17).

Çetin et al. (18) assessed their susceptibility to imipenem resistant 78 *A. baumannii* isolate against polymyxin B isolated from various clinical specimens by E-test. The MIC value range for 78 *A. baumannii* isolates studied was 0.064-128 µg / ml

(MIC50: 0.25 µg / ml, MIC90: 0.75 µg / ml) and 78 strains were sensitive (3%) were resistant. In our study, polymyxin B was measured by disc diffusion method, and colistin resistance was not detected by E-test and disc diffusion method.

Shail et al. (19) reported that they did not detect resistance to colistin and tigecycline by broth dilution method in 716 *A. baumannii* strain and against polymyxin B by disc diffusion method.

Phosphomycin is an antibiotic that acts by inhibiting cell wall synthesis and is frequently used in the treatment of uncomplicated urinary tract infections (20,21). Phosphomycin can be used as an alternative drug in the treatment of resistant bacterial infections, especially when used in combination with other antibiotics due to its synergistic effect (14,22). The combination of colistin and phosphomycin has been reported to have a synergistic effect, although the MIC values of phosphomicine against carbapenem resistant *A.baumannii* clinical isolates are quite high. In a study conducted in a randomized controlled trial of carbapenem resistant MDR-*A. baumannii* infections, it was reported that patients with a combination of colistin and phosphomycin had a better clinical and microbiological response than patients receiving colistin alone and had a lower mortality rate (22).

The high rate of resistance to phosphomycin in our study was remarkable (97.5%). In the literature, Falagas et al. (14) reported the rate of phosphomicine resistance in *A.baumannii* strains as 96.5%. In our study, 2 isolates (4%) were found to be resistant to colistin, 43 isolates (86%) were resistant to amikacin, while the isolates were completely resistant to amikacin (86%) in the study of colistin, phosfomycin and amikacin susceptibilities in the MDR-A. baumannii strain by liquid microdilution method, 100) were reported to be resistant to phosphomycin. MIC50 values of colistin, amikacin and phosphomycin were reported to be 0.5 mg / L,> 128 mg / L and> 512 mg / L, respectively.

The rate of 97.5% phos phomicin resistance detected in our study was similar to the literature.

STUDY LIMITATIONS

The limitations of our study were the susceptibility of strains by liquid microdilution method, which is the recommended reference method for colistin, polymyxin B and other antibiotics.

CONCLUSION

It has been determined that polymyxin B and minocycline can be used for empirical treatment because of the high susceptibility rates in the treatment of carbamazepine resistant, MDR-*A. baumannii* strains in our hospital and phosfomicine can not be used for treatment because of high resistance rate.

DECLARATION OF CONFLICTING INTERESTS

The author declared no conflicts of interest with respect to the authorship and/or publication of this article.

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