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RESEARCH ARTICLE

EFFICACY OF MEROPENEM AND AMIKACIN COMBINATION AGAINST METALLO-BETA-LACTAMASE-PRODUCING *ACINETOBACTER* STRAINS

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ABSTRACT: Background: Multiple-drug resistance of Acinetobacter species cause difficulties in the treatment of infections. Due to decrease in success rates with monotherapy combinations that show synergistic effect in the treatment of MDR Acinetobacter infections is used. **Objectives:** The study aimed to investigate the efficacy of meropenem and amikacin combinations against metallo-beta-lactamase (MBL)-producing Acinetobacter strains isolated from clinical specimens. **Method:** The presence of MBL in strains was detected by gradient diffusion method (GDM). Fifty MBL-positive and 50 MBL-negative strains were included in the study. The activity of meropenem-amikacin combination against MBL-positive isolates was investigated by both GDM and the checkerboard method while the activity against MBL-negative isolates was investigated by the checkerboard method. **Results:** Additive or indifferent interactions between meropenem and amikacin were detected in 38 (76%) of the 50 MBL-positive strains, synergistic interactions were detected in 7(14%), and antagonistic interactions were detected in 5(10%) using GDM. Using the checkerboard method, additive or indifferent interactions between the drugs were detected in 37 (74%) and synergistic interactions in 13 (26%) of 50 MBL-positive strains while synergistic interactions were observed in 36 (72%) and additive or indifferent interactions in 14 (28%) of 50 MBL-negative strains. No antagonistic interaction was detected in the MBL-positive and MBL-negative strains using the checkerboard method. In MBL-positive strains no difference was found between the results of checkerboard and GDM. **Conclusion:** Based on our detection of 72% synergistic interactions between meropenem and amikacin on MBL-positive strains in the Gold Standard checkerboard assay, it is concluded that in vitro evidence supports meropenem and amikacin combination therapy against non–MBL-producing Acinetobacter spp. but further clinical studies are needed.

KEYWORDS: Acinetobacter, MDR, gradient diffusion method, Multiple-drug resistance

INTRODUCTION:

Acinetobacter species, especially *A. baumannii* strains that are resistant to most antibiotics, are a common problem often implicated in nosocomial infections worldwide particularly hospital outbreaks[1].

Multidrug-resistance (MDR) and pandrug-resistance in *A. baumannii* and other *Acinetobacter* spp. increasingly create difficulties in treatment of infections caused by these organisms [2]. The use of

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antibiotic combinations that show synergistic effect has become important in prevention and treatment of infections caused by MDR *Acinetobacter* strains due to reductions in the rate of success with monotherapy [3]. Although carbapenems, sulbactam, minocycline, tigecycline and colistin are the most effective antibiotics in the treatment of *Acinetobacter* species, the combination of a beta-lactam with an aminoglycoside or a fluoroquinolone is recommended for the treatment of severe infections[4,5]. Imipenem/ aminoglycosides, ceftazidime/ aminoglycosides, ceftazidime/ fluoroquinolones, imipenem/ ciprofloxacin, cefoperozone/sulbactam, sulbactam/ amikacin, colistin/ carbapenem, as well as rifampin/imipenem combinations are the most preferred combinations due to *in vitro* synergy and low resistance rates [4,5].

Beta-lactamases confer resistance in *Acinetobacter* species by hydrolysing penicillin, cephalosporins and other beta-lactam antibiotics[6]. In *A. baumannii* metallo beta lactamases (MBL) are seen as inferior to OXA-type carbapenemases but their hydrolytic activities to carbapenems are higher. Three types of MBL – imipenemase (IMP), Verona imipenemase (VIM) and Seul imipenemase (SIM) – are observed in *A. baumannii*. They mediate a high level of resistance against beta-lactams and carbapenems except monobactams which includes aztreonam[6].

The aim of this study was to investigate the efficacy of the combination of meropenem and amikacin against MBL-producing *Acinetobacter* strains isolated from clinical specimens.

METHODS :

Ethical considerations

Ethics clearance for this work was obtained from the Gaziantep University in Turkey (study approval number: 26.04.2012/194).

Bacterial isolates

Acinetobacter strains isolated from various clinical specimens (urine, abscess, wound, sputum, blood) sent to Microbiology Laboratory of Mustafa Kemal University Hospital, (Hatay, Turkey) were examined for the presence of MBL using the gradient diffusion method (GDM) [7]. Fifty MBL-producing and 50 non–MBL-producing strains were included in this *in vitro* antibiotic combination study. *Escherichia coli* ATCC 25922 was used as MBL-negative control and *Klebsiella pneumoniae* ATCC 700603 was used as MBL-positive control.

The strains determined to be resistant to at least three of these: ceftazidime, levofloxacin, gentamicin and imipenem, with Vitek 2 Automated System (bioMérieux, Marcy-l'Étoile, France) were defined as MDR.

Preparation of meropenem and amikacin stock solution

The potency of powdered meropenem trihydrate (100 mg, Sigma-Aldrich, St. Louis, Missouri, United States) and amikacin disulfate (1 g, Sigma-Aldrich, St. Louis, Missouri, United States) was calculated according to the data of analysis certifications. The liquid volume required during dilution was calculated using the following formula:

$$\text{Volume (mL)} = \frac{\text{Weight (mg)} \times \text{Potency (µg/mg)}}{\text{Concentration (µg/mL)}}$$

Detection of metallo-beta-lactamase with gradient diffusion method

The presence of MBL in strains was investigated with GDM using imipenem /imipenem-ethylenediaminetetraacetic acid (EDTA) ETEST® MBL imipenem/ imipenem-ethylenediaminetetraacetic acid strip (bioMérieux, Marcy-l'Étoile, France) in accordance with the manufacturer's instructions. Prepared bacteria suspension equivalent to 0.5 McFarland turbidity density was inoculated on Mueller-Hinton agar plate

(bioMérieux, Marcy-l'Étoile, France). The ETEST strip was carefully placed on the plate after plates were dried. According to the manufacturer's recommendations, after 16–18 h of incubation at 35 °C, minimum inhibitory concentration of imipenem (MIC_{IP})/minimum inhibitory concentration of imipenem- ethylenediaminetetraacetic acid (MIC_{IPI}) ≥ 8 was interpreted as being suggestive of MBL production.

Measurement of in vitro efficacy of meropenem and amikacin combination

Gradient diffusion method

The efficacy of meropenem and amikacin combination in 50 MBL-positive strains was investigated by GDM [8]. Prepared bacteria suspension equivalent to 0.5 McFarland turbidity density was inoculated on a Mueller-Hinton agar plate (bioMérieux, Marcy-l'Étoile, France). To determine meropenem MIC in the presence of amikacin, the amikacin E test strip (biomérieux, Marcy-l'Étoile, France) was placed on the plate, and the bottom and top of the strip were marked on plate. After 1 h of incubation at 35 °C, the amikacin E test strip was removed and the meropenem E test strip was placed in this position to coincide with the previously marked lines. The medium was then incubated for 16–24 h at 35 °C. To determine amikacin MIC in the presence of meropenem, the same procedure was repeated, but with the meropenem E test strip (bioMérieux, Marcy-l'Étoile, France) added first before the amikacin E test strip (bioMérieux, Marcy-l'Étoile, France). In each case, the MIC value was recorded as the numerical value on the E test strip corresponding to the end of the observed inhibition zone on the plate after incubation.

Specific MIC values of meropenem and amikacin, which were needed to determine fractional inhibitory concentration (FIC) values for each antibiotic, were determined using the broth microdilution method. FIC values of each antibiotic were calculated according to the following formula:

$$FIC_{Amikacin} = \frac{MIC_{Amikacin} \text{ in presence of meropenem}}{MIC_{Amikacin} \text{ (alone)}}$$

$$FIC_{Meropenem} = \frac{MIC_{Meropenem} \text{ in presence of amikacin}}{MIC_{Meropenem} \text{ (alone)}}$$

Then the value of total FIC (ΣFIC) was calculated by adding FIC values of both antibiotics. Results were evaluated according to the following criteria:

- $\Sigma FIC = FIC_{Amikacin} + FIC_{Meropenem}$
- $\Sigma FIC \leq 0.5$: synergistic interaction
- $0.5 < \Sigma FIC \leq 4$: additive or indifferent interaction
- ΣFİK > 4: antagonistic interaction

Checkerboard method

Interaction of meropenem and amikacin combination for each isolate included in this study was measured by the checkerboard method [9]. Wells of microplate not containing antibiotic were used as positive growth controls. Positive and negative control strains were also included in each plate. MICs were determined prior to performing the checkerboard test [8]. Briefly, the microdilution plates were inoculated with each bacteria to yield the appropriate density (10^5 CFU/mL) in 50 µL Mueller-Hinton broth and incubated for 24 h at 35 °C [8]. The MIC was determined as the well in the microtiter plate with the lowest drug concentration at which there was no visible growth. The MICs of meropenem, amikacin and the two in combination were determined after 24 h of incubation at 35 °C in ambient air. FICs for each isolate were calculated using GDM [8].

Determination of minimum bactericidal concentration

MIC values of both antibiotics were evaluated in row A and column 1 of the microplate. 25 µL was then transferred to sheep blood agar from wells where there was no visible growth. After incubating for 18–24 h at 35 °C, the lowest concentration of antibiotic that reduced the viability of the initial bacterial inoculum by ≥ 99.9%, was accepted as minimum bactericidal concentration for *Acinetobacter* strains.

Statistical analysis

Data were analysed using Statistical Package for Social Sciences package (SPSS for Windows, Version 16.0. [SPSS Inc., Chicago, USA]). Continuous variables were examined in terms of equality of variance and normal distribution. For the comparison between groups the Mann-Whitney U test for continuous variables and chi-square test for named variables were used. A *P*-value ≤ 0.05 was considered statistically significant.

RESULTS:

Gradient diffusion method

Ninty-four percent of all strains were *A. baumannii* and 6% were *A. lwoffii*. Ninty-four percent of MBL-positive strains were found to be resistant to meropenem and 88% resistant to imipenem. The imipenem and meropenem resistance rates in MBL-positive strains were found to be higher than in MBL-negative strains ($p < 0.05$) (**Table 1**). MIC_{50} and MIC_{90} values of meropenem, imipenem and amikacin were calculated according to MIC values determined with Vitek 2 Automated System (bioMérieux, Marcy-d'Étoile, France) (**Table 2**). 58% of the strains were determined to be MDR. 72% (36 strains) of MBL-negative and 44% (22 strains) of MBL-positive strains were found to be MDR. Multiple drug resistance status in MBL-negative strains was more than in MBL-positive strains ($p = 0.005$) (**Table 3**).

Table 1. Imipenem, meropenem and amikacin resistance rates of metallo-beta-lactamase- positive and negative strains.

Antibiotics	Susceptibility	MBL-negative *n*	Percentage	MBL-positive *n*	Percentage	Total *n*	Percentage	*P*
Imipenem	Resistant	27	54	44	88	71	71	0.001
	Intermediate	2	4	1	2	3	3	
	Susceptible	21	42	5	10	26	26	
Meropenem	Resistant	30	60	47	94	77	77	<0.001
	Intermediate	1	2	0	0	1	1	
	Susceptible	19	38	3	6	22	22	
Amikacin	Resistant	23	46	24	48	47	47	0.083
	Intermediate	4	8	11	22	15	15	
	Susceptible	23	46	15	20	38	38	

MBL, metallo-beta-lactamase.

Table 2. MIC_{50} and MIC_{90} values of amikacin, meropenem and imipenem for metallo-beta-lactamase-positive and negative strains.

Antibiotics	MBL-positive	MBL-negative
Amikacin		
MIC_{50} (µg/mL)	64	16
MIC_{90} (µg/mL)	128	64
Meropenem		
MIC_{50} (µg/mL)	16	8
MIC_{90} (µg/mL)	32	16
Imipenem		
MIC_{50} (µg/mL)	16	16
MIC_{90} (µg/mL)	16	16

MBL, metallo-beta-lactamase.

Table 3. Multidrug resistance status of metallo-beta-lactamase positive and negative strains.

MBL status	Not MDR (*n*)	%	MDR (*n*)	%	Total (*n*)	%	*P*
MBL-negative	14	28	36	72	50	100	0.005
MBL-positive	28	56	22	44	50	100	
Total	**42**	**42**	**58**	**58**	**100**	100	-

MBL, metallo-beta-lactamase; MDR, multidrug-resistant.

***In vitro* efficacy of meropenem and amikacin combination**

Results of gradient diffusion method

According to ΣFIC values calculated, based on results from the *in vitro* efficacy of meropenem-amikacin combination assay, additive or indifferent interaction between the drugs was observed against 76% (38/50) of the isolates, synergistic interaction was observed against 14% (7/50) of the isolates, while antagonistic interaction was observed against 10% (5/50) of the isolates.

Results of checkerboard method

In both MBL-positive and MBL-negative strains, the additive interaction in 51 strains (51 %) and synergistic interaction in 49 strains (49 %) was observed with the checkerboard method for the combination of meropenem and amikacin with the checkerboard method. No antagonistic interaction was found (**Table 4**).

When the results of GDM and checkerboard method were compared, additive interaction was detected in 28 (56%) of the strains and synergistic interaction was detected in 3 (6%) of the strains using both methods. Thus, there was an overlap of observed interactions (synergistic or additive) against 31 (62%) strains using the two methods. When the results of these two methods were compared, no significant difference between the methods was observed ($p > 0.05$) (**Table 5**).

In MBL-negative strains, additive interaction between meropenem and amikacin was observed against 14 strains (28%) and synergistic interaction was observed against 36 strains (72%) using the checkerboard method (**Table 6**).

Amikacin MIC ($p = 0.001$), meropenem MIC ($p < 0.001$), FIC max ($p < 0.001$), amikacin minimum bactericidal concentration ($p = 0.04$), meropenem minimum bactericidal concentration ($p < 0.001$) values determined using the microdilution method were found to be higher in MBL-positive strains than in MBL-negative strains.

Table 4. Interactions of meropenem and amikacin in the strains determined with gradient diffusion and checkerboard methods.

Methods	Interactions						Total (*n*)	%
	Antagonistic (*n*)	%	Additive (*n*)	%	Synergistic (*n*)	%		
Gradient diffusion method	5	10	38	76	7	14	50	100
Checkerboard method	0	0	51	51	49	49	100	100

Table 5. The interactions of meropenem and amikacin combination against metallo-beta-lactamase-positive strains.

Checkerboard	Gradient diffusion method						Total (*n*)	Percentage	*P*
	Antagonist	Percentage	Additive (*n*)	Percentage	Synergy (*n*)	Percentage			
Additive	5	10	28	56	4	8	37	74	> 0.05
Synergy	0	0	10	20	3	6	13	26	
Total	**5**	**10**	**38**	**76**	**7**	**14**	**50**	**100**	-

MBL, metallo-beta-lactamase.

Table 6. The interactions of meropenem and amikacin combination determined with checkerboard method in metallo-beta-lactamase-positive and negative strains.

MBL status	Results of checkerboard method				Total (*n*)	Percentage	*P*
	Additive (*n*)	Percentage	Synergy (*n*)	Percentage			
MBL-	14	28	36	72	50	100	< 0.001
MBL-	37	74	13	26	50	100	
Total	**51**	**51**	**49**	**49**	**100**	100	

MBL, metallo-beta-lactamase.

In MBL-positive strains meropenem MIC and imipenem MIC ($p < 0.001$) values detected with the Vitek 2 automated system (bioMérieux, Marcy-l'Étoile, France) were found higher than the MIC values in MBL-negative strains ($p < 0.001$).

No differences were found in minimum fractional inhibitory concentration and amikacin MIC values detected with broth microdilution method and the automated system in MBL-positive and MBL-negative strains ($p > 0.05$).

DISCUSSION:

In recent years, *Acinetobacter* species have become predominant among the bacteria that cause nosocomial infections in the intensive care units [3]. They have the potential to develop resistance to all antibiotics and thus become a difficult problem as they cause mortality in treatment[3]. Acquired resistance of *Acinetobacter* species to the beta-lactam antibiotics which are the most frequently used in the treatment of infections they cause, depends mostly on the beta lactamase enzymes[3]. MBL-producing strains possess the ability to hydrolyse all beta-lactam antibiotics, thereby becoming resistant to carbapenems, cephalosporins and cefamycin. In addition, the genes involved in the production of MBL are in the same location with aminoglycoside resistance genes. This situation restricts health care providers to use of aminoglycoside in the treatment [1].

Increasing failure in the treatment of serious infections caused by MBL-producing *Acinetobacter* strains with a single antibiotic makes it necessary to use a combination of at least two antibiotics acting by different mechanisms and showing a synergistic effect[3]. Although carbapenem, sulbactam, minocycline, tigecycline and colistin are the most effective antibiotics in the treatment of infections caused by *Acinetobacter* species, the combination of a beta-lactam with an aminoglycoside or a fluoroquinolone can be an option for successful treatment of serious infections [4,5,10].

In one study carried out in Pakistan that detected MBL among 50 carbapenem–resistant A. baumannii strains using GDM, the percentage of MBL producers was reported as 78% (39/50) 11. In another study, Gupta et al. reported the percentage of MBL as 54.1% with GDM and 41.3% with imipenem-EDTA combine disk test in 85 imipenem-resistant Acinetobacter strains in India 12.

The antibiotic combination most studied in *in vitro* assays and that is still the most preferred in clinical empirical treatment of *Acinetobacter* infections because of their synergistic activity consists of a beta-lactam and an aminoglycoside[13]. We also studied this combination in this study. In our study, these antibiotics were determined additive or indifferent in 76%, synergistic in 14% and antagonistic in 10% of 50 MBL-positive strains with GDM, the first method that we used for measuring the effectiveness of meropenem-amikacin combination. In this study, many strains were additive or indifferent because MBL production was thought to cause antibiotic resistance.

In another study carried out using GDM, Kiratisin et al. detected 44% synergy for meropenem and cefoperazone combination against *Acinetobacter baumannii*. The others showed additive or indifferent activity [13]. No antagonist interaction was reported in that study [14]. Another method used in our study to determine interaction between drugs was the checkerboard method. This method is based on microdilution and is the most commonly used and accepted standard test although it is time consuming and exhausting [13]. With the checkerboard method 74% additive or indifferent interaction, 26% synergistic interaction, and no antagonist activity was detected in 50 MBL-positive strains, while 72% synergistic interaction, 28% additive or indifferent interaction, and no antagonistic activity was detected in 50 MBL-negative strains in our study. Sopirala et al. reported 100% additive or indifferent interaction in 32 pan-drug-resistant A. *baumannii* strains with GDM and the checkerboard method using amikacin and tigecycline [9]. The synergistic, addictive or indifferent and antagonistic interaction in MBL-positive strains in this study, restricts the use of a combination of meropenem and amikacin for successful treatment of infections caused by MDR or MBL-producing *Acinetobacter* strains. On the other hand, the combination of meropenem and amikacin could be considered as an option for treatment of infections caused by MBL-negative *Acinetobacter* strains.

The MBL-producing strains are resistant to carbapenems. Many studies have investigated different antibiotic combinations and determined synergistic interactions against resistant strains. Marques et al. reported 21% synergistic interaction with the combination of sulbactam ampicillin and amikacin in *Acinetobacter* strains with the checkerboard method [14]. In a study conducted with the checkerboard method by Ozseven et al., 94.1% synergy with the meropenem and ampicillin/sulbactam combination and 88.2% with the imipenem and ampicillin/sulbactam combination were found in MDR *Acinetobacter* strains [15].

When the other studies are analysed, we thought that if the combination of meropenem and ampicillin/sulbactam or the combination of amikacin and ampicillin/sulbactam instead of the combination of meropenem and amikacin was studied, more synergistic activity would be obtained. In a study supporting this idea, Ko et al. inoculated MDR *A. baumannii* into rats [16]. After the inoculation, they administered meropenem alone [monotherapy) and then in combination with sulbactam to the rats. They observed 35% improvement with monotherapy and this increased to 87% by using the combination of meropenem and sulbactam[16]. In another microdilution-based study, combinations of polymyxin B/imipenem and meropenem/polymyxin B were studied in 34 MDR *A. baumannii* strains [16]. The researchers reported 38.2% synergistic interaction for polymyxin B and imipenem and 2.9% synergistic interaction for meropenem and polymyxin B [16].

Colistin is bactericidal but in the 1980s there was a serious reduction in its use due to nephrotoxicity and neurotoxicity [1]. Petrosillo et al. found that half of 14 patients with a diagnosis of ventilator-associated pneumonia caused by *A. baumannii* recovered when treated with a colistin and rifampicin combination in their study [17]. In another study, 32 pan-resistant *A. baumannii* strains showed 13% synergistic interaction in tigecycline/imipenem combinations with GDM and time-dependent killing method [14]. In the same study, 100% indifferent interaction was reported with an amikacin/tigecycline combination with GDM and the checkerboard method [14].

Tan et al. tested the combination of polymyxin, tigecycline and rifampin on 48 *A. baumannii* strains, 16 of which were extensively drug resistant, in their study and they reported 40% synergistic interaction with time kill method and 88.2% with the checkerboard method [18]. In another study carried out with 34 MDR *A. baumannii* strains, the researchers reported 17.6% synergistic interaction with a rifampicin/meropenem combination and 88.2% with a rifampicin/imipenem combination [15].

CONCLUSION:

In other studies, the combinations of carbapenem/sulbactam, carbapenem/tigecycline, carbapenem/colistin, carbapenem/rifampicin have been shown to have synergistic activity in resistant *Acinetobacter* strains. Many studies in which the carbapenem and the other antibiotics combinations have been tested, imipenem showed more synergistic activity than meropenem in MDR Acinetobacter strains. As the first choice in the preference stage in the treatment of infections caused by MDR Acinetobacter strains, imipenem should be more preferred than meropenem. Our results do not rationalise the use of a combination of meropenem/amikacin for satisfactory, successful treatment of infections caused by MBL producing Acinetobacter or MDR strains. Although it can be used in "non-alternative" situations based on the detection of an average of 20% synergistic interaction with the two methods, in vitro advanced studies or different combination tests are required. It is considered that the combination of meropenem/amikacin can be conveniently included among the treatment options for infections caused by non MBL-producing and/or non-MDR Acinetobacter strains

We thought that this antibiotic combination in which no antagonistic interaction was found in this study, can be the choice as a treatment option for the infections caused by non MBL producing Acinetobacter strains. But it should be supported by further studies.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

REFERENCES:

[1]. Maragakis LL, Perl TM. Acinetobacter baumannii: Epidemiology, antimicrobial resistance and treatment options. Clin Infect Dis. 2008;46:1254–1263.

[2]. Deveci O, Dal T, Tekin R, Bozkurt F, Tekin A, Dayan S. Carbapenem resistance in Acinetobacter baumannii: Where is it heading? Infez Med. 2013;21(3):211–215.

[3]. Viehman JA, Nguyen MH, Doi Y. Treatment Options for Carbapenem-Resistant and Extensively Drug-Resistant Acinetobacter Drugs. 2014 August; 74(12): 1315–1333

[4]. Vaneechoutte M, Dijkshoorn L, Nemec A, Kämpfer P, Wauters G. Acinetobacter, chryseobacterium, moraxella, and other nonfermentative gram-negative rods. In Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. Manual of clinical microbiology. Volume 1. 10th ed. Washington, DC: ASM Press; 2011, p. 714–738.

[5]. Gordon NC, Wareham DW. Multidrug-resistant Acinetobacter baumannii: Mechanisms of virulence and resistance. Int J Antimicrob Agents. 2010; 35:219–226.

[6]. Esposito S, De Simone G. Update on the main MDR pathogens: Prevalence and treatment options. Infez Med. 2017;25(4):301–310.

[7]. Walsh TR, Bolmstrom A, Qwärnstrom A, Gales A. Evaluation of a new etest for detecting metallo – Lactamases in routine clinical testing. J Clin Microbiol. 2002;40:2755–2759.

[8]. Moody J. Synergism testing: Broth microdilution checkerboard and broth macrodilution methods. In: Isenber HD, editor. Clincal microbiology procedures handbook. Volume 2. 2nd edition. Washington: ASM Press; 2004. p.5.12.1-5.12.23

[9]. Sopirala, MM, Mangino JE, Gebreyes WA, et al. Synergy testing by etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii.

Antimicrob Agents Chemother. 2010;54:4678–4683.

[10]. Dundar D, Otkun M. In-vitro efficacy of synergistic antibiotic combinations in multidrug resistant Pseudomonas aeruginosa strains. Yonsei Med J. 2010;51(1):111–116.

[11]. Kaleem F, Usman J, Hassan A, Khan A. Frequency and susceptibility pattern of metallo beta lactamase producers in a hospital in Pakistan. Infect Dev Ctries. 2010;23:810–813.

[12]. Gupta V, Sidhu S, Chander J. Metallo- β-lactamase producing non-fermentative gram-negative bacteria: An increasing clinical threat among hospitalized patients. Asian Pac J Trop Med. 2012; 5:718–721.

[13]. Kiratisin P, Apisarnthanarak A, Kaewdaeng S. Synergistic activities between carbapenems and other antimicrobial agents against Acinetobacter baumannii including multidrug-resistant and extensively drug-resistant isolates. Int J Antimicrob Agents. 2010; 36:243–246.

[14]. Marques MB, Brookings ES, Moser SA, Sonke PB, Waites KB. Comparative in vitro antimicrobial susceptibilities of nosocomial isolates of Acinetobacter baumannii and synergistic activities of nine antimicrobial combinations. Antimicrob Agents Chemother. 1997;41(5):881–885.

[15]. Ozseven AG, Cetin ES, Arıdogan BC, Ozseven L. In vitro synergistic activity of carbapenems in combination with other antimicrobial agents against multidrug-resistant Acinetobacter baumannii. Afr J Microb Res. 2012; 6:2985–2987.

[16]. Ko WC, Lee HC, Chiang SR, et al. In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant Acinetobacter baumannii strain. J Antimicrob Chemother. 2004; 53:393–395.

[17]. Petrosillo N, Chinello P, Proietti MF, et al. Combined colistin and rifampicin therapy for carbapenem-resistant Acinetobacter baumannii infections: Clinical outcome and adverse events. Eur Soc Clin Microbiol Infect Dis. 2005; 11:682–684.

[18]. Tan TY, Lim TP, Lee WH, Sasikala S, Hsu LY, Kwa AL. In vitro antibiotic synergy in extensively drug-resistant acinetobacter baumannii: The effect of testing by time-kill, checkerboard, and etest methods. Antimicrob Agents Chemother. 2011; 55:436–437.

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