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Research Article

Identification and Antibiotics Combination Testing against Multiple-Drug Resistance *Acinetobacter baumannii* isolates

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ABSTRACT

Infection caused by *Acinetobacter* species is one of the significant agents of hospital-acquired infections worldwide, which can rapidly extend antibiotic resistance. This study evaluated the synergistic effects of combinations of different medications on the multi-drug resistance (MDR) *Acinetobacter baumannii.* In total of 70 *Acinetobacter baumannii* strains were collected from clinical specimens of patients hospitalized in ICU (Intensive Care Unit). For evaluating all isolated MDR strains of *Acinetobacter baumannii*, the minimum inhibitory concentration (MIC) of selected antibiotics, fractional inhibitory concentration index (FICI), and multiple combination bactericidal testing (MCBT) were used. The result showed the maximum resistance for imipenem (100%), meropenem (100%), cefotaxime (98.6%), and ceftazidime (98.6%), and minimum resistance for tetracycline (65.7%) and gentamicin (68.6%). The MCBT test for meropenem-gentamicin-tetracycline antibiotics demonstrated 65.6% and 6.3% of MDR *Acinetobacter baumannii* strains killed at high and low concentrations of antibiotics, respectively (P=0.04). Besides, 34.4% and 93.8% of MDR *Acinetobacter baumannii* strains lived at high and low concentrations of meropenem-gentamicin-tetracycline antibiotics, respectively, which were statistically significant (P=0.04). There was a significant relationship between the MIC of gentamicin-meropenem in the combination (Checkerboard) (P=0.0001). Antibiotic interaction effects frequencies were 6.3%, 53.1%, and 40.6% for synergy, incremental, and indifference, respectively. In this study, a significant difference was shown between the FICIs of meropenem (0.68±0.27) and gentamicin (0.003±0.003) (P=0.0001). Also, there was a significant correlation between the FICI factor and the antibiotic's antimicrobial interaction by MCBT (r=0.95, P=0.0001). It seems that the combination of meropenem-gentamicin-tetracycline antibiotics had additive antibacterial effects and could be used to suppress MDR *Acinetobacter baumannii* isolated.

1. Introduction

Multi-drug resistance (MDR) is a common form of clinical resistance. MDR bacteria have developed resistance to one or more of the antibiotics used to trea them. Multi-drug

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resistance in an organism may develop when antibiotics are not used properly (WHO, 2022). The MDR has now become a global health concern that has threatened the impact of antibiotic therapy and has also challenged the efforts to produce new antibacterial agents (WHO, 2022; Nwobodo et al., 2022). The critical group of MDR gram negative bacteria includes *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae*, which cause severe infections like pneumonia and blood stream infections in hospital-admitted patients (Parmanik et al., 2022). *A. baumannii* is a natural flora of the skin and can be located in the oral cavity, throat, and tonsils. *Acinetobacter baumannii* is a Gram-negative, opportunistic pathogen, causing severe infections difficult to treat. The *A. baumannii* infection rate has increased year by year in human medicine and it is also considered as a major cause of nosocomial infections worldwide. (Nocera et al., 2021). *A. baumannii* species can be inherently resistant to some antibiotics through the transfer of antibiotic resistance genes by mobile genetic elements include Integrons, plasmids or transposons (Wieczorek et al., 2008). In a study in 2019 all *Acinetobacter* spp. were multidrug-resistant (MDR) due to considerable resistance to fluoroquinolones (95%), cephalosporins (93% - 98%), penicillins (97%), carbapenems (94% - 95%), and beta-lactamase inhibitors (87% - 100%) and the resistance rate of *Acinetobacter* spp. against antibiotics varied from 0.9% for colistin to 100% for piperacillin-tazobactam (Sedaghat et al., 2019). In severe infections with MDR Gram-negative bacteria, using combination antibiotic therapy is essential for critically ill patients (Tamma et al., 2012). Carbapenem is a beta-lactam antibiotic used in the last antibiotics, especially to control severe MDR infections (Amudhan et al., 2011; Coyne et al., 2011; Terzi et al., 2015). Currently the major threat of antibiotic-resistant bacteria is from MDR Gram-negative organisms, particularly those which have developed resistance to carbapenem. Along with carbapenem- resistant *Acinetobacter baumannii* (CRAB) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), carbapenem-resistant *Enterobacteriaceae* (CRE) are among the top tier of the WHO list of antibiotic-resistant "priority pathogens" that pose the greatest threat to human health (Willyard., 2017). Due to unpredictable MDR patterns of clinical strains of *A. baumannii*, it is imperative to know the institutional prevalent susceptibility profiles. Today combination antibiotic therapy is used in critically ill patients due to widespread emergence of multidrug resistance organisms (Wang et al., 2022).Therefore, this study was directed to isolate the *A. baumannii* species from different clinical samples to define these isolates' sensitivity to antibiotics and aimed to assess the antimicrobial resistance and synergistic effects of combinations of other medications on MDR *A. baumannii* isolates.

2. Materials and Methods

2.1. Sampling and patients

In this study, a total of 70 clinical specimens of *A. baumannii* were isolated from hospitalized patients in Milad Hospital, Tehran, Iran for three months (from September 2018 to December 2018). Entry criteria was patients that were hospitalized in intensive care unit and basis the results of clinical cultures performed during their index hospitalization.

This study was approved by the Ethical Committee of the Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.

2.2. Isolation and identification of Acinetobacterbaumannii

A. baumannii was isolated from injuries and sore of patients in intensive care units (ICU) and confirmed based on differential diagnostic tests such as catalase, oxidase, urease, choline, and lysine, as well as the culture in different media including, blood agar, MacConkey agar, BHI, Methylred-Vegesproskaure broth (MR-VP), Sulfide, Indole, Motility (SIM), oxidative-fermentative (OF), triple sugar iron agar (TSI), and Simmons' citrate agar (Wang el al., 2012). All *A. baumannii* isolates were molecular identified based on polymerase chain reaction of *16srRNA* and carbapenemase genes, *blaOXA-21 and blaoxa 23.*

2.3. Determination of Antibiotic susceptibility profile

The 0.5 McFarland Standards were used to standardize the approximate number of bacteria in a suspension**,** which is equivalent to 1.5×10^8

bacterial cells per mm^3. The optical density (OD) of 0.5 McFarland standard (1 mc, 625 nm) was between 0.05 and 0.1. Müller Hinton medium autoclaved, divided into 8 cm plates, placed at 37 ° C for 30 hours. Susceptibility testing for all isolates was determined by the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI 2019) (Melvin .,2019). The discs of antibiotics include cefotaxime, piperacillin, tetracycline, amikacin, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, ceftazidime, imipenem, meropenem, and piperacillin-tazobactam with distinct concentrations were prepared from Padtanteb Company (Iran) and used.

For the Kirby-Bauer disk diffusion susceptibility test, firstly, the *A. baumannii* samples were cultured on a blood agar medium and kept at 37 ° C for 24 hours to prepare fresh microbial culture. The bacterial colony was then dissolved in physiological saline and placed in an incubator for 1 to 3 hours to equal the turbidity of 0.5 McFarland standard. Then *A. baumannii* was cultured in Müller Hinton agar medium linearly by using sterile forceps. The antibiotic discs were placed on the plate's surface and placed in an incubator (37° C for 16-17 hours). Finally, the growth inhibition zone diameter was measured using an accurate ruler and recorded according to the CLSI (2019) standard for reporting the sensitivity, semi-sensitivity, and resistance to antibiotics. After the Kirby-Bauer disk diffusion susceptibility test, the antibiotic resistance and susceptibility were assessed in all studied strains (Melvin., 2019). The MDR strains were selected when the strains were resistant to three or more different antibiotic classes.

2.4. Determination of minimum inhibitory concentration (MIC)

The MIC was used to determine the lowest concentration of an antimicrobial required to inhibit the visible growth of *A. baumannii* after incubation. The MIC of meropenem, gentamycin, and tetracycline was determined by the broth micro-dilution method based on CLSI (2019) standard in 96-well micro-plates. According to the manufacturers' instructions, Meropenem, gentamicin, and tetracycline powder were dissolved in sterilized deionized water. Then, different concentrations of antibiotics (256, 128, 64, 32, 16 and 8 µg/ml) were prepared and 100 µl of the prepared dilutions were added to each well of the 96-well micro-plate. The exact concentration of the bacterial suspension (10^6 CFU/ml) was set to 0.5 McFarland and diluted in the ratio of 1/100. Then, 100 µl of the microbial suspension was added to each well, and the plates were incubated at 35 ℃ for 18-24 h. The lowest antibiotic concentration showed no growth and was determined by ELIZA reader (Biotek, USA) in 620 nm, as MIC.

2.5. Determination of fractional inhibitory concentration (FIC)

The FIC was evaluated to determine the synergistic effect of two or more antibiotics intended to be used in combination. The FIC is determined for each antibiotic by dividing the MIC of each antibiotic when used in combination with the MIC of each antibiotic when used alone. Following the fractional inhibitory concentration index (FICI) was calculated from the total of antibiotic FICs, and the results were assessed based on the Checkerboard method according to the European Committee for the Evaluation of Antimicrobial Susceptibility (EUCAST) guidelines (Polsfuss., 2014). Accordingly, if the FICI is less than or equal to 0.5, the interaction is synergy. If greater than 0.5 to one was incremental, and more than one or less than two was inferior or similar to or greater than two indifferences.

FIC index =FIC of antibiotic A + FIC of antibiotic B

2.6. Determination of multiple combination bactericidal testing (MCBT)

According to the protocol, the synergistic effect of the three antibiotics mentioned by MCBT was also studied based on the Seidel bacteria test. In this method, the bactericidal properties of antibiotics were investigated, two upper concentrations (the concentration that the strain was known to be resistant to), and a lower concentration (the concentration that the strain was known to be sensitive to) were used according the CLSI, 2019 standard table (Melvin., 2019). 30 µl of the meropenem, gentamycin and tetracycline antibiotics (10 µl from each antibiotic) were prepared in low (2, 4,

4 µg/ml, respectively) and high (8, 16, 16 µg/ml, respectively) concentrations, After adding 70 µl of bacterial suspension (106 CFU/ml) in each well-containing antibiotics, they were incubated (24 hours) and evaluated.. Then 100 µl of the wells that did not show any growth were added to plates containing Müller-Hinton agar and incubated for another 24 hours after surface culture. After this period, dishes that had no change were reported as MCBT positive.

2.7. Statistical analysis

Statistical package for social sciences (SPSS) software (Version 19, SPSS Inc., IBM) was used for all analyses. The one-sample t-test, independent t-test, and Spearman were used to compare the variables in groups, between groups, and evaluate the correlation, respectively. In all statistical analyses, $p<0.05$ was considered statistically significant.

3. Results

In this study, the 70 clinical specimens of *A. baumannii* were identified and confirmed by catalase, oxidase, ureas, choline, and lysine, and culture in the different media. The frequency of 16srRNA, *blaoxa*21, *blaoxa*23, and *blaoxa*58 genes were investigated. All isolates were positive for *blaoxa*21, 90% for *blaoxa*23, and not for blaoxa58 gene. So based on 16srRNA and *blaoxa*21 results, the identity of *Acinetobacter baumannii* isolates was confirmed. Then, samples were preceded for an antibiotic susceptibility test by Kirby Bauer disc diffusion.

3.1. The Kirby-Bauer disk diffusion susceptibility profiles

As shown in Table 1, the percentage of resistance and the concentration of antibiotics used have been reported. Maximum resistance was respectively recorded for imipenem (100%), meropenem (100%), ceftazidime (98.6%), cefotaxime (98.6%), ciprofloxacin (95.7%), piperacillin (94.3%), trimethoprim-sulfamethoxazole (90%), as well as minimum resistance was observed for amikacin (77.1%), piperacillin-tazobactam (75.7%), gentamicin (68.6%) and tetracycline (65.7%).

3.2. MDR Acinetobacterbaumannii isolates

After disc diffusion susceptibility, bacterial strains resistant to three or more different classes of antibiotics were selected MDR strains. It showed that 98.57% (69 isolates) of *A. baumannii* was resistant to three or more antibiotic classes and selected as MDR strain. A total of 32 isolates were phenotypically resistant to three or more antibiotics including100% resistance to meropenem, gentamicin, and tetracycline, identified as MDR strains, and selected for the next steps.

3.3. MIC of MDR Acinetobacter baumannii isolates

Following 32 isolates of MDR *A. baumannii* strains were assessed for the bacterial growth inhibitory concentrations of meropenem, gentamicin, and tetracycline antibiotics according to the CLSI 2019 standard by broth microdilution method (Table 2). The results confirmed the resistance to the meropenem, gentamicin, and tetracycline antibiotics obtained from the Kirby-Bauer disk diffusion susceptibility test. As shown in Table 2, the lowest and highest bacterial growth inhibitory concentrations of meropenem antibiotics were 8 µg/ml (3.1%) and 16 µg/ml (28.1%) respectively. The highest inhibitory concentration of gentamicin was 512 µg/ml (90.6%), while concentrations 64, 128, and 256 µg/ml had the same inhibitory frequency (3.1%). The lowest and highest inhibitory concentrations of tetracycline were 256 µg/ml (9.4%) and 32 µg/ml (25%), respectively, for MDR strains. Also, a significant relation was observed between MIC of meropenem (69.75±64.84 µg/ml), gentamicin (478.00±110.23 µg/ml), and tetracycline (77.50±71.31 µg/ml) in pure condition and combination of three antibiotics in MDR A. *baumannii* strains (P=0.0001) which reported in Table 3.

3.4. Fractional inhibitory concentrations (FIC) results

The FIC was calculated using the Checkerboard method to evaluate the antimicrobial interaction between the meropenem and gentamicin antibiotics. As shown in Table 4, a significant relation was demonstrated between the MIC of gentamicin and meropenem in the combination form by the Checkerboard method (P=0.0001). Although the

effects of the interaction of both appropriate antibiotics, gentamicin, and meropenem, on the MDR *A. baumannii* strains were investigated. Antibiotic interaction effects, frequencies for synergy, incremental, and indifference have been shown in Figure 1. A significant difference was demonstrated between the FICIs of meropenem (0.68±0.27) and gentamicin (0.003±0.003) (P=0.0001) (Table 4). There was a significant correlation between the FICI factor and the antibiotics antimicrobial interaction by MCBT in meropenem and gentamicin (r=0.95, P=0.0001).

3.5. Combined and synergistic effects of gentamycin, meropenem, and tetracycline antibiotics with MCBT method

In addition to the previous Checkerboard method which investigated the effect of two antibiotics on bacteria, another technique called the Seidel bacteria test was used to evaluate up to three antibiotics. The MCBT test for meropenem-gentamicin-tetracycline antibiotics demonstrates that respectively 65.6% and 6.3% of MDR *A. baumannii* strains were killed at high and low concentrations (P=0.04). Besides, respectively 34.4 and 93.8 % of MDR *A. baumannii* strains lived at high and low concentrations, statistically significant (P=0.04) (Table 4).

Table 1. Results of determination of antibiotic susceptibility by disc-diffusion testing of *Acinetobacter baumannii* isolates

Antibiotics		Disk Content(µg)	Interpretation of zone diameters					
			Sensitive (%)	**Semi-sensitive (%)**	**Resistant (%)**	**Sensitive (mm)**	**Semi-sensitive (mm)**	**Resistant (mm)**
Piperacillin-Tazobactam	**PI.TZ**	100	8.6	15.7	75.7	≥21	18_20	≤17
Meropenem	**MEN**	10	0	0	100	≥18	15_17	≤14
Imipenem	**IPM**	10	0	0	100	≥22	19_21	≤18≥
Ceftazidime	**CAZ**	30	1.4	0	98.6	>18	15_17	≤14
Trimethoprim-Sulphamethoxazole	**SXT**	1.25/23.75	7.1	2.9	90.0	≥16	11_15	≤10
Gentamicin	**GM**	10	25.7	5.7	68.6	≥15	13_14	≤12
Ciprofloxacin	**CP**	5	4.3	0	95.7	≥21	16_20	≤15
Amikacin	**AN**	30	8.6	14.3	77.1	≥17	15_16	≤14
Tetracycline	**TE**	30	15.7	18.6	65.7	≥15	12_14	≤11
Piperacillin	**PIP**	100	2.9	2.9	94.3	≥21	18_20	≤17
Cefotaxime	**CTX**	30	0	1.4	98.6	≥23	15_22	≤14

Table 2. Evaluation of the bacterial growth inhibitory concentrations of meropenem, gentamicin, and tetracycline.

Antibiotics	**Antibiotic Concentrations (µg/ml)**	**512**	**256**	**128**	**64**	**32**	**16**	**8**
Meropenem	**Bacterial growth inhibition (%)**	-	6.3	21.9	25.0	15.6	28.1	3.1
Gentamicin		90.6	3.1	3.1	3.1	-	-	-
Tetracycline		-	9.4	21.9	21.9	25.0	21.9	-

Table 3.MIC and FIC of antibiotics

MIC	Mean	P-value	FIC	Mean	In group (p-value)	Between FICI and FIC (p-value)
Meropenem	69.75±64.841	0.0001	**Meropenem**	0.68±0.27	0.0001	0.0001
Tetracycline	77.50±71.336	0.0001	**Gentamicin**	0.003±0.001	0.0001	0.0001
Gentamicin	478.00±110.235	0.0001	**FICI**	0.68±0.27	0.0001	-

p-value<0.05 was reported as statistically significant.

Table 4. The results of MIC, FIC, FICI and the antimicrobial interaction of MEN and GMon *A.baumannii*

Strain No.	MIC MEN (µg/ml)	MIC GM (µg/ml)	MIC Combined MEN (µg/ml)	MIC Combined GM (µg/ml)	MIC MEN	MIC MEN	MIC MEN	interaction
1	**16**	**512**	**16**	**1**	**1**	**0.001953**	**1.001953**	**indifferent**
2	32	512	32	1	1	0.001953	1.001953	indifferent
3	32	512	16	1	0.5	0.001953	0.501953	additive
4	32	512	16	1	0.5	0.001953	0.501953	additive
5	128	512	128	1	1	0.001953	1.001953	indifferent
6	128	512	128	1	1	0.001953	1.001953	indifferent
7	128	512	128	1	1	0.001953	1.001953	indifferent
8	16	512	8	1	0.5	0.001953	0.501953	additive
9	32	512	32	1	1	0.001953	1.001953	indifferent
10	128	512	128	1	1	0.001953	1.001953	indifferent
11	256	512	128	1	0.5	0.001953	0.501953	additive
12	16	512	8	1	0.5	0.001953	0.501953	additive
13	16	512	8	1	0.5	0.001953	0.501953	additive
14	16	128	8	1	0.5	0.007813	0.507813	additive
15	16	512	8	1	0.5	0.001953	0.501953	additive
16	256	512	128	1	0.5	0.001953	0.501953	additive
17	128	512	128	1	1	0.015625	1.001953	indifferent
18	8	256	8	1	1	0.003906	1.001953	indifferent
19	128	512	64	1	0.5	0.001953	0.501953	additive
20	64	512	16	1	0.25	0.001953	0.251953	synergistic
21	64	64	32	1	0.5	0.015625	0.515625	additive
22	64	512	32	1	0.5	0.001953	0.501953	additive
23	128	512	16	1	0.125	0.001953	0.126953	synergistic
24	64	512	32	1	0.5	0.001953	0.501953	additive
25	64	512	16	1	0.5	0.001953	0.501953	additive
26	64	512	16	1	1	0.001953	1.001953	indifferent
27	64	512	128	1	1	0.001953	1.001953	indifferent
28	16	512	128	1	1	0.001953	1.001953	indifferent
29	32	512	128	1	0.5	0.001953	0.501953	additive
30	64	512	8	1	1	0.003906	1.001953	indifferent
31	16	512	32	1	0.5	0.001953	0.501953	additive
32	16	512	128	1	0.5	0.001953	0.501953	additive

Table 5. Multiple Combination Bactericidal Test (MCBT) of meropenem, gentamycin, and tetracycline on the *Acinetobacter baumannii* MDR strains

Antibiotics	Concentrations(µg/ml)	MCBT	Frequency (%)	
Meropenem	2	**Low concentration**	Positive (lived)	93.8
Gentamycin	4			
Tetracycline	4		Negative (killed)	6.3
Meropenem	8	**High concentration**	Positive (killed)	65.6
Gentamycin	16			
Tetracycline	16		Negative (lived)	34.4

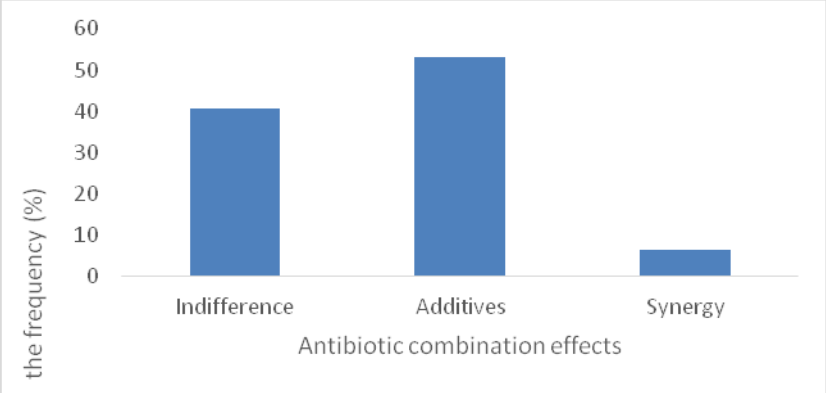

Figure 1. The antibiotic combination effects frequency

4. Discussion

In this study, antibiotic resistance patterns of *A. baumannii* strains were investigated against different antibiotic classes. The maximum antibiotic resistance was in imipenem (100%), meropenem (100%), ceftazidime (98.6%), cefotaxime (98.6%), ciprofloxacin (95.7%), and trimethoprim-sulfamethoxazole (90%). According to a new study in Iran pooled prevalence of resistance to antibiotics was as follows: imipenem 85.9%, meropenem 88%, ceftriaxone 93.5%, ciprofloxacin 92.2%, gentamicin 81.8%, ceftazidime 92.2%, cefotaxime 93% ,cefepime 91.9%, , amikacin 74.4% .(Keisha et al., 2023). In a study by Teo in 2015, the antibiotic resistance was imipenem (100%), meropenem (100%), and doripenem (98%) (Teo., 2015). In a study by Sharma et al in 2021, Meropenem resistant Acinetobacter were 100% towards cefotaxime, ampicillin and ceftriaxone. All meropenem resistant Acinetobacter were multidrug-resistant, 77 (82.8%) isolates were extensively drug-resistant that was excluding one pan drug-resistant (Sharma et al., 2021). This study showed that 98.57% of isolated *A. baumannii* were resistant to three or more antibiotics. In 2024 Gharaibeh et al reported that more than 90% of *A. baumannii* isolates were resistant to monobactam, carbapenem, cephalosporins, Fluoroquinolones, penicillin, and β-lactam agents and were MDR isolates (Gharaibeh et al., 2024). In a study on 75 strains of *A. baumannii* isolated from military and civilian warfare in Iraq and Afghanistan, it was reported that among the 15% of strains that were tested for nine antibiotics, 89% of isolates were resistant to at least three antibiotic classes (Hujer et al., 2006). It was shown that the multiple antibiotic resistance of *A. baumannii* was increasing and 70% of this *Acinetobacter* were MDR strain (Bayuga et al., 2002; Joshi et al., 2003).

Investigators have shown that aminoglycosides have synergic effects on Gram-negative bacteria such as *P. aeruginosa* and *A. baumannii* (Wang et al., 2022; KONG et al., 2010). Studies suggested disruption of the outer membrane by aminoglycoside may enhance the target site penetration of β-lactams (Obara et al.,

1991; Sato et al ., 1991). In the current study, the lowest and highest bacterial growth inhibitory concentration of meropenem was respectively 8 µg/ml (3.1%) and 16 µg/ml (28.1%); tetracycline, 256 µg/ml (9.4%), and 32 µg/ml (25%) and also the highest inhibitory concentration of gentamicin was 512 µg/ml (90.6%). The lowest were 64, 128, and 256 µg/ml (3.15%). The isolation of *A. baumannii* as a hospital pathogen and its resistance to various antibiotics causes mortality and serious problems (Kurutepe et al., 2016; Pachón-Ibáñez et al., 2004). Increasing antibiotic-resistant strains cause a reduction in treatment options in patients with acute infection (Yavuz et al., 2006; Kurutepe et al., 2016). It is recommended to avoid resistance and failure in treating multidrug resistance strains, antibiotic compounds should be used (Marques et al.,1997). According to our findings, the synergistic, incremental, and indifference frequency of meropenem and gentamicin interaction on the MDR *A. baumannii* strains were respectively 6.3, 53.1, and 40.6%. In high and low concentrations of meropenem, gentamicin, and tetracycline antibiotics, the MCBT test was respectively positive in 65.6 and 93.8 and negative in 34.4% and 6.3%. Previously the MIC of meropenem, colistin, and sulbactam antibiotics respectively was reported as 256, 2, and 256 µg/ml (An et al., 2016). Also, the synergistic effects of meropenem-colistin and meropenem-sulbactam antibacterial were investigated through the Checkerboard method and respectively were 52% and 16% (30). In another study, the synergistic effect of meropenem-ampicillin sulbactam (94%), meropenem-sulbactam (70%), rifampin-colistin (80%), and colistin-imipenem (100%) combinations were reported by the Checkerboard method (Pongpech et al., 2011). In another study, only 8.3% synergy was reported for the combination of tigecycline-imipenem (Principe et al.,2009). Also, the synergistic effects of imipenem-ampicillin-sulbactam, ampicillin-rifampin, meropenem-cefoperazone-sulbactam, and meropenem-polymyxin B combinations were evaluated by the Checkerboard method and concluded that the meropenem-ampicillin sulbactam have the highest synergistic effect (94.1%) on *Acinetobacter* (Arıdoğan., 2012). Some studies had performed the combination of imipenem-rifampin (Saballs et al., 2006) and carbapenem-polymyxin (Pongpech et al., 2011; van Belkumet al., 2014), by the Checkerboard method to treat CRAB cases. It was also shown that the meropenem-colistin combination could increase the synergistic and incremental effects to 92% and 8% in *Acinetobacter baumannii* MDR strains (Pongpech et al., 2011; van Belkumet al., 2014). There is a controversial report about the side effects of carbapenem combinations with other antibiotics (Rattanaumpawan et al., 2011). In 2015, the effect of polymyxin B combination with imipenem, meropenem, doripenem, rifampin, and tigecycline was investigated by MCBT. It showed the combination of imipenem, meropenem, and rifampin with polymyxin B had an antimicrobial effect on all strains of *Acinetobacter baumannii* (Teo et al., 2015). In the present study, the combination of gentamicin, meropenem, and tetracycline antibiotics showed a high effect on MDR strains and the maximum dose of antibacterial activity of drugs was 65.5%. This method has been used in other studies (Teo et al., 2015; Haja et al., 2012). Today, most of the pathogens affecting hospital infections are resistant to antibiotics and other antimicrobial agents because of the unnecessary use of antibiotics. So, it is difficult, costly, and sometimes impossible to treat these types of resistant infections. During the past three decades, the bacteria have changed from a suspected pathogenic organism to an important infectious agent in hospitals. Therefore, to prevent microbial resistance catastrophe, it is important and necessary to use antibiotics, discontinue the resistant antibiotic after sensitivity report, and give an alternative the sensitive drug.

Conclusion

This study's specific focus was assessing the antimicrobial resistance and synergistic effects of different medication combinations on MDR *A. baumannii* isolates. According to this study, it seems that the combination of meropenem, gentamicin, and tetracycline antibiotics had additive antibacterial effects on CRAB and could be used to suppress isolated MDR *A. baumannii*. However, further studies such as *in vitro* bactericidal activity evaluations with a larger number of bacteria, *in vivo* activity, and

clinical efficacy are needed to confirm this finding.

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Competing interests

All authors declare no financial or commercial competing interest.

Consent for publication

Applicable

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran Ethical Committee of IR.IAU.VARAMIN.REC. 1397.004. Ethical code.

Refereces

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