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In vitro activities of ceftazidime/avibactam alone or in combination with antibiotics against multidrug-resistant *Acinetobacter baumannii* isolates

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ABSTRACT

Objectives: Infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* (*A. baumannii*) are a growing problem because of the limited options for treatment. The number of antimicrobials that are currently being developed is still insufficient to control this global threat. Combination therapies of antibiotics with different antimicrobial mechanisms have been proposed as the best options for treating MDR *A. baumannii* infections. The objective of this study was to investigate the in-vitro effectiveness of ceftazidime/avibactam alone or in combination with antibiotics against MDR *A. baumannii* isolates using time-kill assays.

Methods: Forty clinical MDR strains were screened, and minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of ceftazidime/avibactam, colistin, levofloxacin, meropenem, tigecycline, and tobramycin were determined by microbroth dilution method. The in-vitro synergistic activities of ceftazidime/avibactam with antibiotic combinations were determined by time-kill assays at 1× MIC and 4× MIC against five MDR *A. baumannii* isolates.

Results: Based on MIC results, all isolates of *A. baumannii* were resistant to ceftazidime/avibactam, except for AB-5. All isolates were found to be resistant to meropenem and levofloxacin. At 4× MIC, all of the tested antibiotics showed bactericidal effect ($\geq$3 $\log_{10}$killing). The synergistic activities of ceftazidime/avibactam + colistin, ceftazidime/avibactam + tobramycin and ceftazidime/avibactam + tigecycline combinations at 1× MIC were observed against studied 5/5, 4/5 and 4/5 strains, respectively. Furthermore, all of the tested combinations at 4× MIC were additive at 24 h. No antagonism was observed.

Conclusions: The findings of this study suggest that a significant bactericidal effect was seen with all tested combinations. These findings present significant implications for antibiotic choice for the treatment of infections caused by MDR *A. baumannii*.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) have emerged as serious human health risks and are associated with many human diseases such as pneumonia, wound infections, meningitis, and sepsis [1]. *A. baumannii* has been emerging worldwide as an important nosocomial pathogen, particularly in intensive care units [2]. Since *A. baumannii* infections may be difficult to treat, due to the multidrug resistance (MDR) (including carbapenems), few therapeutic options like polymyxins and tigecycline have been prescribed as the agent of last resort [3,4]. However, polymyxins and tigecycline-resistant isolates of *A. baumannii* have also been reported by researchers [3,5,6]. The development of new antimicrobial agents to combat *A. baumannii* infections has been slow, with few options currently in the pipeline [7]. Thus, the use of combinations of two or more agents has been suggested as an option for combatting MDR *A. baumannii* infections [2,7]. In addition, combination therapy may also help to prevent the emergence of resistant populations.

The ceftazidime/avibactam combination was approved by the FDA in February 2015 for the treatment of complicated intra-abdominal infections in combination with metronidazole, complicated urinary tract infections, and also HAP/VAP infections caused

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by MDR pseudomonas species and MDR Enterobacteriaceae [8]. However, acinetobacter and stenotrophomonas clinical species are generally resistant to ceftazidime/avibactam [9]. Additional studies are needed to establish what the potential roles of ceftazidime/avibactam, in combination with other antibiotics, are against MDR *A. baumannii* infections.

This study investigated the synergistic and bactericidal effects of ceftazidim/avibactam alone or in combination with colistin, levofloxacin, meropenem, tigecycline, and tobramycin against MDR *A. baumannii* strains.

2. Materials and methods

2.1. Bacterial isolates

Forty nonduplicate and nosocomially acquired MDR *A. baumannii* strains isolated from blood specimens during January–June 2016 were obtained from the Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Istanbul Medipol University. All strains were identified using API 20 NE (BioM'erieux). *Pseudomonas aeruginosa* ATCC 27853 (American Type Culture Collection, Rockville, MD, USA) was used throughout the study as a reference strain to verify the accuracy of the microdilution test procedure and to ensure that minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the studied antibiotics were within the accuracy range stated by the Clinical and Laboratory Standards Institute (CLSI). Quality control for ceftazidime/avibactam was monitored with *Klebsiella pneumoniae* strain ATCC 700603, according to CLSI guidelines [10,11].

2.2. Antibiotics

All antimicrobial agents were kindly provided by their respective manufacturers. Stock solutions of ceftazidime, ceftazidime/avibactam, colistin sulfate, tobramycin, and levofloxacin were prepared according to the manufacturers' recommendations and stored frozen at −80 °C for up to 6 months. Tigecycline and meropenem solutions were prepared on the day of use.

2.3. Media

Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, MI, USA) supplemented with divalent cations to a final concentration of 25 mg/L Mg^{2+} and 50 mg/L Ca^{2+} (CSMHB) was used for all of the experiments. The broth was used within 24 h of preparation for the tigecycline [12]. Pour plates of tryptic soy agar (TSA; Difco Laboratories) were used for colony counts.

2.4. MIC and MBC determination

MICs were determined by the microbroth dilution technique, as described by the CLSI [10,11]. Serial two-fold dilutions ranging from 256–0.25 mg/L for ceftazidime, ceftazidime/avibactam, levofloxacin, meropenem, and tobramycin, and from 128 to 0.015 mg/L for tigecycline and colistin were prepared in fresh CSMHB 96-well microtiter plates. For ceftazidime-avibactam, doubling dilutions of ceftazidime were utilized in combination with a fixed 4 mg/L concentration of avibactam. The inoculum was prepared with a 4–6-h broth culture that gave a final concentration of 5×10^5 CFU/mL in the test tray. The trays were covered and placed in plastic bags to prevent evaporation, and incubated at 37 °C for 18–20 h. The MIC was defined as the lowest concentration of antibiotic giving complete inhibition of visible growth. Experiments were performed in triplicate. MBCs were determined at the conclusion of the incubation period by removing two 0.01 mL samples from each well demonstrating no visible growth and plated onto TSA. Resultant colonies were counted after an overnight incubation at 37 °C. The MBC was defined as the lowest concentration of antibiotic giving at least a 99.9% killing of the initial inoculums [13].

2.5. Determination of time-kill curves

In order to observe the dynamic profile of the bactericidal activity of ceftazidime/avibactam alone and in combination with colistin, levofloxacin, meropenem, tigecycline and tobramycin, the time-kill curve (TKC) method was performed at one and four times the MIC against AB-1, AB-2, AB-3, AB-4 and AB-5 clinical strains representing different susceptibility paterns [13]. AB-1 was susceptible to colistin and tobramycin, AB-2 was only susceptible to colistin, AB-4 was only susceptible to tobramycin, and AB-5 was susceptible to ceftazidime/avibactam and colistin. AB-3 was resistant to all studied antibiotics. This assays revealed that there are minor differences in the kinetics of bactericidal activity among the studied strains that represented different susceptibility patterns. Antibiotic-free controls were included for each strain. Inocula were quantified spectrophotometrically and added to the flasks to yield a final concentration of 1×10^6 CFU/mL. The test tubes containing CSMHB with and without (growth control) antibiotics in a final volume of 10 mL were incubated in a 37 °C calibrated shaking water bath, and viable counts were determined at 0, 2, 4, 6, and 24-h intervals after inoculation, by subculturing 0.1 mL serial dilutions onto TSA plates. All tests were performed in duplicate. Time-kill curves were constructed by plotting mean colony counts ($\log_{10}$ CFU/mL) versus time. The lower limit of detection for time-kill assays was 1 $\log_{10}$ CFU/mL. Antimicrobial carryover was controlled by the inhibition of colonial growth at the site of the initial streak according to NCCLS guidelines [13]. Bactericidal activity was defined as a $\geq$3 $\log_{10}$ CFU/mL decrease from the initial inoculum. The results were interpreted by the effect of the combination in comparison with its more active constituent. Synergy and antagonism were defined as a 2 $\log_{10}$ decrease or increase, respectively, in colony count at 24 h by the combination compared with the most active agent alone.

3. Results

3.1. Susceptibility

The in-vitro activities of the studied antibiotics against 40 MDR *A. baumannii* strains are summarized in Table 1. The MIC values of the antibiotics against the quality-control strains were within the accuracy range of the CLSI throughout the study [10,11]. Susceptibility testing demonstrated that the MIC ranges for ceftazidime/avibactam, colistin, levofloxacin, meropenem, tigecycline, and tobramycin were 8 to >256, 0.125 to >256, 2–128, 16–256, 1–32, and 0.5 to >256 mg/L, respectively. The MBC ranges for those antibiotics were 16 to >256, 0.25 to >256, 2 to >256, 32 to >256, 2–256, and 1 to >256 mg/L, respectively. Based on MIC results, all 40 MDR isolates of *A. baumannii* were resistant to ceftazidime (MIC > 256 mg/L) and ceftazidime/avibactam. Only AB-5 was susceptible to ceftazidime/avibactam (MIC = 8 mg/L). All the studied isolates were resistant to ceftazidime alone. All the studied isolates were found to be resistant to meropenem and levofloxacin (except one strain of levofloxacin), respectively. There was no major differences between bactericidal and inhibitory endpoints, the MBCs were generally two or four-fold higher than those of the MICs.

3.2. Time-kill kinetics

The results of the TKC studies showed bactericidal activity with the used antibiotics in a concentration-dependent manner, with a

Table 1
In vitro activities of antibiotics against 40 clinically obtained strains of MDR *Acinetobacter baumannii*.

Antibiotics	MIC (mg/L)			MBC (mg/L)			Susceptibility (n (%))		
	MIC range	MIC_{50}	MIC_{90}	MBC range	MBC_{50}	MBC_{90}	S	I	R
CEF	256 to >256	256	>256	>256	>256	>256	0.0	0.0	40 (100)
CEF/AVI[a]	8 to >256	64	256	16 to >256	128	>256	1 (2.5)	0.0	39 (97.5)
CS	0.125 to >256	0.5	16	0.25 to >256	2	32	29 (72.5)	0.0	11 (27.5)
LVX	2–128	16	64	2 to >256	32	256	1 (2.5)	0.0	39 (97.5)
MER	16–256	64	256	32 to >256	128	>256	0.0	0.0	40 (100)
TGC[b]	1–32	4	8	2–256	8	32	10 (25)	16 (40)	14 (35)
TOB	0.5 to >256	32	> 256	1 to >256	64	>256	4 (10)	4 (10)	32 (80)

Clinical and Laboratory Standards Institute (CLSI) breakpoints for susceptibility and resistance to: colistin are ≤2 mg/L and ≥4 mg/L, respectively; ceftazidime are ≤8 mg/L and ≥32 mg/L, respectively; levofloxacin are ≤2 mg/L and ≥8 mg/L, respectively; to meropenem are ≤4 mg/L and ≥16 mg/L, respectivelty; tobramycin are ≤4 mg/L and ≥16 mg/L, respectively.

[a] No susceptibility breakpoint for Acinetobacter has been provided by CLSI. CLSI breakpoints for susceptibility and resistance to ceftazidime alone are ≤8 mg/L and ≥32 mg/L used for comparison only.

[b] No susceptibility breakpoint for Acinetobacter has been provided by CLSI. The FDA-approved breakpoints for Enterobacteriaceae susceptibility and resistance to tigecycline are ≤2 mg/L and ≥8 mg/L, respectively.

three-log kill determined within 24 hours for the studied strains at 4 x MIC concentrations (Fig. 1b, d, f, h and j). When these antibiotics were used in combination at 1 x MIC, synergistic interactions were obtained from ceftazidime/avibactam + colistin combinations for all strains (Figure 1a, c, e, g and i). The synergistic interactions of these antimicrobial agents were also achieved with combinations of ceftazidime/avibactam + tobramycin and ceftazidime/avibactam + tigecycline used at 1× MIC for four studied strains (Fig. 1). No antagonism was observed with any combination.

4. Discussion

A rise in infections due to multidrug-resistant *A. baumannii* strains, including carbapenem-resistant isolates, has limited the treatment options [7,14]. Even extrensively drug resistant (XDR) *A. baumannii* isolates displaying resistance to all antimicrobials, including polymyxins and tigecycline, have emerged [15,16]. Thus, combination therapy has been recommended not only to combat MDR *A. baumannii* infections but also to inhibit or reduce the emergence of resistance during treatment. In the present study, the effectiveness of five antimicrobial agents alone or in combination with ceftazidime/avibactam against MDR *A. baumannii* isolates were evaluated using an in vitro time-kill analysis.

All studied MDR *A. baumannii* isolates were resistant to ceftazidime/avibactam except AB-5. Despite the limited bacterial killing of ceftazidime/avibactam against MDR *A. baumannii*, it showed bactericidal effects at high concentration at 4× MIC (except for one strain), which is clinically important considering the dose-dependent activity. Also, this study showed the synergistic and bactericidal effects of the ceftazidime/avibactam + colistin combination just at the MIC level, which was sustained for 24 h. This has very promising implications in terms of using lower doses of colistin in therapy, and thus lowering its potential toxic effects. This also holds true for the colistin-resistant isolate (AB-3 and AB-4) showing important implications for the combinations in the treatment of colistin-resistant strains. The current results, which were noted to be similar to Soudeiha et al., showed better additive activity colistin plus beta-lactam combinations like meropenem [17].

Aminoglycoside monotherapy can cause significant killing of *A. baumannii* but is followed by rapid and extensive resistance emergence in vitro and in patients [18]. The high rates of *A. baumannii* resistance highlight the urgent need for alternative treatment options such as rationally optimized combination therapies. Aminoglycoside and beta-lactam antibiotics have different mechanisms of action and resistance; there is no efflux pump that affects both of these antibiotic classes in *A. baumannii* [19]. This suggests that beta-lactams may kill aminoglycoside-resistant bacteria and vice versa (subpopulation synergy). Additionally, disruption of the outer membrane by an aminoglycoside may enhance the target site penetration of beta-lactams, since the outer membrane of *A. baumannii* is approximately 2- to 7-fold [20]. Likewise, in this study, ceftazidime/avibactam + tobramycin combinations showed good synergistic activity against four MDR *A. baumannii* strains even at the MIC level, which was sustained for 24 h.

Although levofloxacin is considered in-vitro active against *A. baumannii*, the time-kill effect of levofloxacin on *A. baumannii* is limited [21]. Safarika et al. showed that synergy between levofloxacin and imipenem was found against 58.6% of isolates of *A. baumannii* from patients with VAP [22]. However, in the current study, levofloxacin only showed additive effects in combination with ceftazidime/avibactam in both the 1× MIC and 4× MIC concentrations used.

Carbapenems, like meropenem, have previously been considered the gold standard treatment option for infections caused by *A. baumannii* [23]. However, due to the global emergence of serine- and metallo-beta-lactamase-producing strains, carbapenem susceptibility has substantially declined. Studies have suggested that combination therapy with a carbapenem agent is a seemingly superior treatment option compared with monotherapy. Lenhard et al. showed that the triple combination of polymyxin B, meropenem, and ampicillin/sulbactam sustained bactericidal activity against a polymyxin-resistant *A. baumannii* isolate in a 14-day hallow-fiber infection model [24]. The current study also showed that ceftazidime/avibactam + meropenem combinations have synergistic activity against three MDR *A. baumannii* strains even at MIC level, which was sustained for 24 h.

Studies have shown that tigecycline monotherapy has a significantly lower microbiological cure rate for treating MDR or XDR *A. baumannii* infections. This is probably due to an inadequate tigecycline plasma concentration [25]. Kengkla et al. revealed that the microbiological cure obtained with tigecycline is low in patients with bacteremia [25]. Likewise, this in-vitro study found that tigecycline monotherapy is not effective at MIC concentrations for 24 h among the studied strains. However, it did show that ceftazidime/avibactam + tigecycline combinations have good synergistic activity against four MDR *A. baumannii* strains, even at the MIC level, which was sustained for 24 h.

Moreover, single-agent studies have shown that all studied antibiotics displayed strong bactericidal effects (≥3 $\log_{10}$killing) at 4× MIC concentrations for 24 h. However, the current study could not find a difference between combinations at 4× MIC among studied strains in which combinations were additive, except one

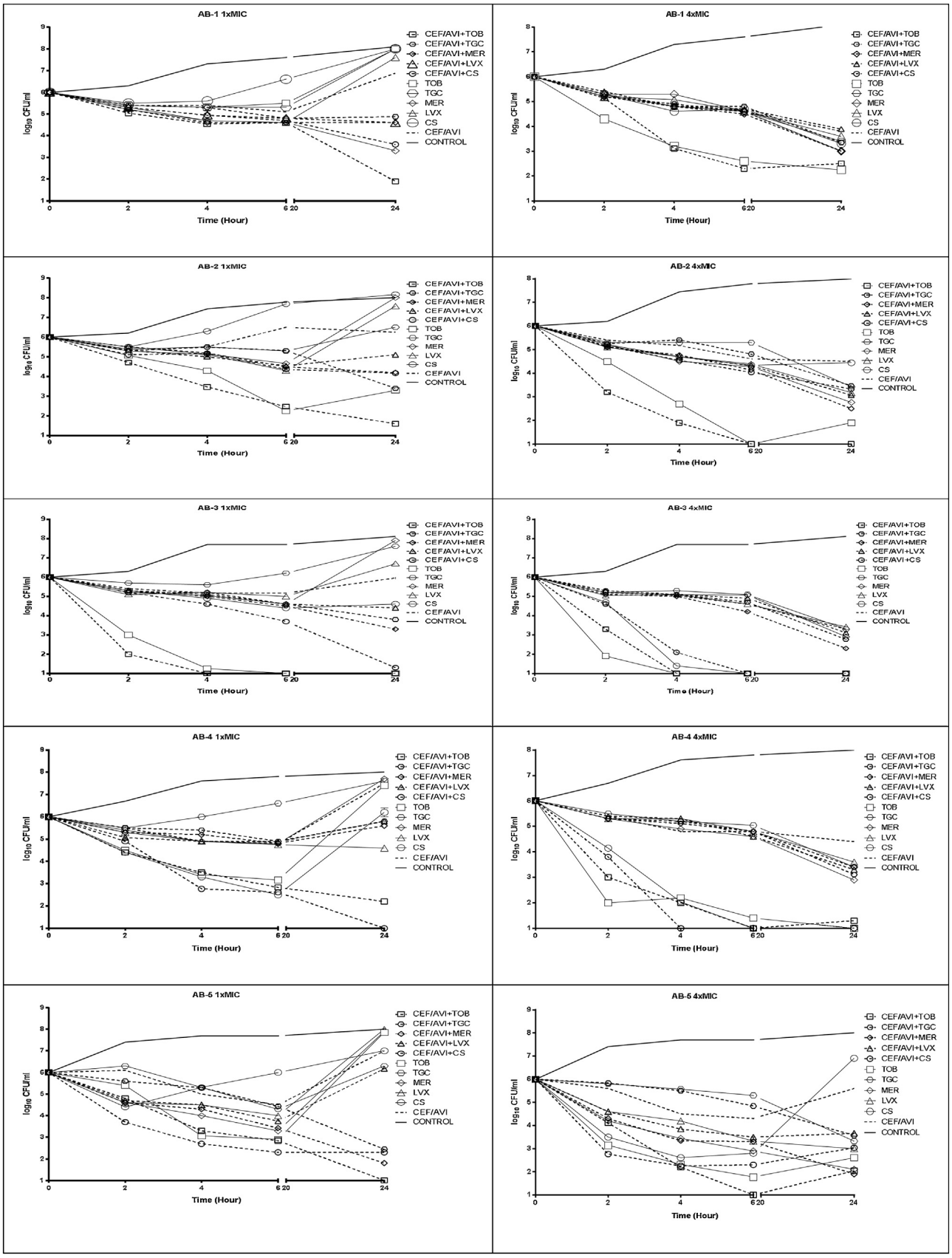

Fig. 1. Time-kill determinations for five multidrug-resistant *A. baumannii* strains after treatment with ceftazidim/avibactam (CEF/AVI) alone or in combination with colistin (CS), levofloxacin (LVX), meropenem (MER), tigecycline (TGC), and tobramycin (TOB) at at 1× MIC or 4× MIC. The *x*-axis represents the killing time, and the *y*-axis represents the logarithmic MDR *A. baumannii* survival.

strain – AB-5 – which was susceptible to both ceftazidime/avibactam and colistin. That combination showed synergism at this concentration only.

In conclusion, ceftazidime/avibactam + colistin, ceftazidime/avibactam + tigecycline and ceftazidime/avibactam + tobramycin combinations showed good in vitro activities against MDR *A. baumannii* isolates. Even though ceftazidime/avibactam has limited activity against *Acinetobacter* spp., the combinations with ceftazidime/avibactam are more warranted in order to cause synergism, thus lowering their potential toxic effects and preventing the development of resistance.

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Competing interests

None.

Ethical approval

None required.

References

[1] Shin B, Park W. Antibiotic resistance of pathogenic *Acinetobacter* species and emerging combination therapy. J Microbiol 2017;55(11):837–49.
[2] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.
[3] Arroyo LA, Mateos I, Gonzalez V, Aznar J. In vitro activities of tigecycline: minocycline and colistin-tigecycline combinations against multi- and pandrug-resistant clinical ısolates of Acinetobacter baumannii group. Antimicrob Agents Chemother 2009;53(3):1295–6.
[4] Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med 2008;358(12):1271–81.
[5] Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006;50(9):2946–50.
[6] Park YK, Jung SI, Park KH, Cheong HS, Peck KR, Song JH, et al. Independent emergence of colistin-resistant *Acinetobacter* spp. isolates from Korea. Diagn Microbiol Infect Dis 2009;64(1):43–51.
[7] Peck KR, Kim MJ, Choi JY, Kim HS, Kang CI, Cho YK, et al. In vitro time-kill studies of antimicrobial agents against blood isolates of imipenem-resistant *Acinetobacter baumannii*, including colistin- or tigecycline-resistant isolates. J Med Microbiol 2012;61(3):353–60.
[8] Duin D, Bonomo RA. Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation β-Lactam/β-lactamase inhibitor combinations. CID 2016;63(2):234–41.
[9] Sader HS, Castanheira M, Flamm RK, Farrell DJ, Jones RN. Antimicrobial activity of ceftazidime-avibactam against gram-negative organisms collected from U. S. medical centers in 2012. Antimicrob Agents Chemother 2014;58:1684–92.
[10] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A7, ed 7, Wayne, PA, USA, 2006.
[11] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twentieth informational supplement-M100-S20. Wayne, PA, USA, 2010.
[12] Jenner L, Starosta AL, Terry DS, Mikolajka A, Filonava L, Yusupow M, et al. Structural basis for potent inhibitory activity of the antibiotic tigecycline during protein synthesis. Proc Natl Acad Sci U S A 2013;110:3812–6.
[13] National Committee for Clinical Laboratory Standards. Methods for determining bactericidal activity of antimicrobial agents-approved guideline M26A. Wayne NCCLS, 1999.
[14] Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant *Acinetobacter baumannii*: beyond carbapenem resistance. Clin Infect Dis 2015;60(9):1295–303.
[15] Doi-Yhusain S, Potoski BA, McCurry KR, Peterson DL. Extensively drug-resistant *Acinetobacter baumannii*. Emerg Infect Dis 2009;15:980–2.
[16] Park YK, Choi JY, Jung SI, Park KH, Lee H, Jung DS, et al. Two distinct clones of carbapenem-resistant *Acinetobacter baumannii* isolates from Korean hospitals. Diagn Microbiol Infect Dis 2009;64:389–95.
[17] Soudehia MAH, Dahdouh EA, Azar E, Sarkis DK, Daoud Z. In vitro evaluation of the colistin-carbapenem combination in clinical isolates of *Acinetobacter baumannii* using the checkerboard, etest, and time-kill curve techniques. Front Cell Infect Microbiol 2017;7(209):1–10.
[18] Lim TP, Ledesma KR, Chang KT, Hou JG, Kwa AL, Nikolaou M, et al. Quantitative assessment of combination antimicrobial therapy against multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2008;52:2898–904.
[19] Coyne S, Courvalin P, Perichon B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. Antimicrob Agents Chemother 2011;55:947–53.
[20] Landersdorfer CB, Ly NS, Xu H, Tsuji BT, Bulitta JB. Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design. Antimicrob Agents Chemother 2013;57:2343–51.
[21] Zhanel GG, DeCorby M, Nichol KA, Wierzbowski A, Baudry PJ, Karlowsky JA, et al. Antimicrobial susceptibility of 3931 organisms isolated from intensive care units in Canada: Canadian National Intensive Care Unit Study, 2005/2006. Diagn Microbiol Infect Dis 2008;62:67–80.
[22] Safarika A, Galani I, Pistiki A, Giamarellos-Bourboulis EJ. Time-kill effect of levofloxacin on multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: synergism with imipenem and colistin. Eur J Clin Microbiol Infect Dis 2015;34:317–23.
[23] Garnacho-Montero J, Amaya-Villar R. Multi-resistant *Acinetobacter baumannii* infections: epidemiology and management. Curr Opin Infect Dis 2010;23:332–9.
[24] Lenhard JR, Thamlikitkul V, Silveira FP, Garonzik SM, Tao X, Forrest A, et al. Polymyxin-resistant, carbapenem-resistant *Acinetobacter baumannii* is eradicated by a triple combination of agents that lack individual activity. J Antimicrob Chemother 2017;72(5):1415–20.
[25] Kengkla K, Kongpakwattana K, Saokaew S, Apisarnthanarak A, Chaiyakunapruk N. Comparative efficacy and safety of treatment options for MDR and XDR *Acinetobacter baumannii* infections: a systematic review and network meta-analysis. J Antimicrobial Chemother 2018;73(1):22–32.