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Evaluation of the Synergistic Effect of a Combination of Seven Antimicrobials Against a Broad-Spectrum Drug-Resistant Strain of *Acinetobacter baumannii*

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Abstract

To solve the difficulty in determining the appropriate treatment regimen for patients infected with extensively drug-resistant *Acinetobacter baumannii* (XDRAB), it is necessary to develop various strategies to increase the therapeutic effect of antimicrobial agents. The purpose of this study was to select the treatment combination showing the greatest antimicrobial effect among seven candidate antimicrobial substances. Seven strains of XDRAB were used in this study. The composition of the treatment consisted of colistin as the base and one of the seven antimicrobial substances, doripenem, minocycline, tigecycline, linezolid, fusidic acid, vancomycin, or alyteserin E4K peptide. The interaction between the drugs in each combination was evaluated by measuring the synergy rates using time-kill analysis. The synergy rates of the seven combinations tested in the time-kill assay in this study were as follows, in descending order from the combination with the highest synergy rate: colistin + minocycline (57.1%), colistin + alyteserin E4K (50.0%), colistin + tigecycline (42.9%), colistin + vancomycin (28.6%), colistin + doripenem (14.3%), colistin + fusidic acid (14.3%), and colistin + linezolid (0%). None of the combinations showed antagonism. The three combinations showing bactericidal activity and the rates of their bactericidal activity were the colistin + alyteserin E4K combination (33.3%), colistin + minocycline (14.3%), and colistin + vancomycin (14.3%). The colistin + minocycline and colistin + alyteserin E4K treatment combinations, which showed high synergy rates, can be considered promising candidates for future *in vivo* experiments evaluating combination therapies.

Keywords

Drug combinations
Synergy
Colistin
Minocycline
Antimicrobial cationic peptides

1. Introduction

To address the prevalence of multidrug-resistant *Acinetobacter baumannii* (MDRAB) and the difficulty in finding appropriate antimicrobials to treat patients infected with it, there is an urgent need to develop different strategies to increase the therapeutic effectiveness of antimicrobials and inhibit the development of resistance. Some strategies include the use of unorthodox antimicrobial combination therapies, which include not only traditionally used antimicrobials but also antimicrobials that have been underused in the treatment of patients with MDRAB infections.

Alternative antimicrobials, such as antimicrobial peptides, have also been investigated as part of new strategies to overcome antimicrobial resistance. Among the antimicrobial peptides, Alyteserin-2a (ILGKLLSTAAGLLSNL.NH_2) is a cationic α-helical peptide first isolated from the skin secretions of the midwife toad, *Alytes obstetricans* [1]. Among several alyteserin peptides, alyteserin-1c E4K peptide (hereafter abbreviated as alyteserin E4K) was reported to have potent growth inhibitory activity against multidrug-resistant *A. baumannii* strains [2].

The present study aimed to compare the combinatorial effects of different combinations in order to select a combination with superior combinatorial effects. For this purpose, the effects of different combinations of seven antimicrobials with colistin were investigated against seven weeks of extensively drug-resistant *Acinetobacter baumannii* (XDRAB), comprising commonly used agents against *A. baumannii*. That is, the colistin + doripenem combination and the colistin + minocycline combination, and the colistin + tigecycline combination, using the relatively recently developed tigecycline; Drug-drug interactions were studied for colistin + linezolid, colistin + fusidic acid, colistin + vancomycin, and colistin + alyteserin E4K, using the antimicrobial peptide alyteserin E4K, a combination of unconventional agents not previously used against Gram-negative bacteria. By comparing the antimicrobial activity of these conventional and unconventional combinations, as well as combinations containing antimicrobial peptides, we aimed to identify combinations with good synergistic effects and present them as candidates for future *in vivo* experiments.

2. Materials and methods

2.1. Subjects and specimen collection

Seven XDRAB strains that had been identified as *A. baumannii* and stored frozen were analyzed and investigated among the specimens referred for bacteriological culture at Chosun University Hospital over a five-year period from January 2008 to December 2012. The strains were grown in duplicate and used in the experiments. Species identification was performed using the VITEK 2 System (bioMérieux, Marcy-l Etoile, France) with the VITEK2 GN card (bioMérieux). $bla_{\text{OXA-51-like}}$ polymerase chain reaction (PCR) and Higgins PCR were performed on strains identified as *A. baumannii* [3,4], and if all were positive, they were identified as *A. baumannii*. Antimicrobial susceptibility testing was performed with the AST-N225 card using the VITEK2 System. The presence of XDRAB strains was determined according to the criteria proposed by Magiorakos *et al.* as follows [5]. *A. baumannii* was defined as XDRAB if it was non-susceptible to at least one agent per category in all but two of the categories of antimicrobials listed in the criteria for defining multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria for *Acinetobacter* species [5]. Strains that were non-susceptible to all tested antimicrobials, but did not complete susceptibility testing for all of the antimicrobials listed in the table, were classified as XDRAB according to Magiorakos' criteria [5]. Among the strains classified as XDRAB, bacteria with no or only one antibiotic to which they were susceptible were selected for testing.

2.2. Time-kill assay and determination of minimal inhibitory concentration (MIC) for alyteserin E4K

Concentrations of each strain in the time-kill assay were added to Mueller-Hinton (MH) broth to give a final concentration of 1×10^6 CFU/mL when mixed with antimicrobials. The antimicrobials and concentrations used were colistin 2 μg/mL [6], doripenem 8 μg/mL [7], fusidic acid 1 μg/mL [6], linezolid 8 μg/mL [8], minocycline 4 μg/mL [9], tigecycline 2 μg/mL [10], and vancomycin 32 μg/mL [11]. Alyteserin E4K was used at a concentration corresponding to 1/4× MIC for each strain. Since the MIC for alyteserin E4K in the 7 weeks of XDRAB under study ranged from 20 to 80 μg/mL, concentrations corresponding to 1/4× MIC was used for each strain (5 to 20 μg/mL). MH Broth inoculated with antimicrobials and strains was incubated for 0 and 24 hours in a 37°C shaking incubator, and the broth was then serially diluted 10-fold, and the dilutions were inoculated onto MH agar plates and the total number of colonies was counted after overnight incubation [12].

After 24 hours of incubation, a synergistic response was defined as a decrease of at least 2 $\log_{10}$ CFU/mL in the number of bacteria grown in the combination treatment compared to the number of bacteria grown in the most active single agent treatment. An indifference response was defined as an increase or decrease of less than 2 $\log_{10}$ CFU/mL, and an antagonistic response was defined as an increase of more than 2 $\log_{10}$ CFU/mL [13]. Bactericidal activity was determined when the number of bacteria decreased by 3 $\log_{10}$ CFU/mL or more after 24 hours of incubation from the initial inoculum [13].

Antimicrobials used for MIC determination and time-kill assays were from Sigma-Aldrich (St. Louis, MO, USA). Alyteserin E4K peptide (hereafter alyteserin E4K) was custom-made by AnyGen, Inc. (Gwangju, Korea) at 98% purity. The minimum inhibitory concentration (MIC) for alyteserin E4K was determined by broth microdilution assay according to Clinical and Laboratory Standards Institute guidelines [14].

3. Results

The results of the time-kill assay for the seven antimicrobial combinations are presented in **Table 1** and Supplementary Table 1 (Supplementary Table 1 in the online-only Data Supplement). No inhibition of bacterial growth was observed when the antimicrobials were used alone. When combinations of antimicrobials were used, in descending order of synergistic effectiveness, the colistin + minocycline combination had the highest synergistic effectiveness of 57.1%, followed by colistin + alyteserin E4K at 50%, colistin + tigecycline at 42.9%, colistin + vancomycin at 28.6%, colistin + doripenem and colistin + fusidic acid at both 14.3% and colistin + linezolid at 0%.

None of the seven combinations tested for antimicrobial combination effects showed antagonism between antimicrobials. No strains showed bactericidal activity against alyteserin E4K peptide and all seven antimicrobials (colistin, doripenem, tigecycline, linezolid, fusidic acid, minocycline, and vancomycin) as single agents. In terms of the bactericidal rate of the combination products, the colistin + alyteserin E4K combination was the highest at 33.3% (2/6), followed by the colistin + minocycline and colistin + vancomycin combinations at 14.3% (1/7) each, and no bactericidal activity was observed in any other combination.

4. Discussion

The combined effectiveness of the seven drug combinations tested in this study was compared to the results of previous studies that performed time-kill assays against XDRAB or MDRAB bacteria.

The colistin + minocycline combination had the highest combined efficacy rate of 57.1% in this study, but the combined efficacy rate was lower than that reported by Tan *et al.* [15], who reported a combined efficacy rate of 92.3% [16].

Table 1. Results of time-kill assay against two-drug combinations of colistin and various antibiotics or a peptide for seven extensively drug-resistant *A. baumannii* clinical isolates

Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity*
	Colistin	Doripenem	Colistin + Doripenem		Colistin + Doripenem	Colistin + Doripenem
111-27	9.2	9.2	9.9	0.7	I†	NB
111-28	9.3	9.2	9.4	0.2	I	NB
111-29	8.6	9.5	9.5	0.9	I	NB
111-31	10.1	9.8	9.2	-0.6	I	NB
64-26	9.1	9.3	3.4	-5.8	Synergy‡	NB
131-16	6.1	9.2	5.4	-0.7	I	NB
130-87	8.0	9.4	8.0	0.0	I	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Tigecycline	Colistin + Tigecycline		Colistin + Tigecycline	Colistin + Tigecycline
111-27	9.2	9.1	9.3	0.2	I	NB
111-28	9.3	9.4	8.5	-0.8	I	NB
111-29	8.6	9.9	9.0	0.4	I	NB
111-31	10.1	8.1	5.1	-3.0	Synergy	NB
64-26	9.1	9.4	6.4	-2.7	Synergy	NB
131-16	6.1	9.2	6.4	0.3	I	NB
130-87	8.0	8.5	4.5	-3.5	Synergy	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Linezolid	Colistin + Linezolid		Colistin + Linezolid	Colistin + Linezolid
111-27	9.2	8.1	9.3	1.2	I	NB
111-28	9.3	9.5	9.4	0.1	I	NB
111-29	8.6	9.4	9.0	0.4	I	NB
111-31	10.1	9.4	9.4	0.0	I	NB
64-26	9.1	9.2	9.3	0.2	I	NB
131-16	6.1	9.4	7.4	1.3	I	NB
130-87	8.0	10.0	8.3	0.3	I	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Fusidic acid	Colistin + Fusidic acid		Colistin + Fusidic acid	Colistin + Fusidic acid
111-27	9.2	9.3	3.8	-5.4	Synergy	NB
111-28	9.3	9.3	9.2	-0.1	I	NB
111-29	8.6	10.2	9.4	0.8	I	NB
111-31	10.1	9.9	9.4	-0.5	I	NB
64-26	9.1	9.3	9.4	0.3	I	NB
131-16	6.1	9.3	6.4	0.3	I	NB
130-87	8.0	9.5	8.3	0.3	I	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Minocycline	Colistin + Minocycline		Colistin + Minocycline	Colistin + Minocycline
111-27	9.2	3.3	1.8	-1.5	I	B
111-28	9.3	8.5	-1.0	-3.2	Synergy	NB
111-29	8.6	9.4	5.4	-3.2	Synergy	NB
111-31	10.1	9.4	5.8	-3.6	Synergy	NB
64-26	9.1	4.2	3.4	-0.9	I	NB
131-16	6.1	5.3	4.2	-1.2	I	NB
130-87	8.0	5.5	3.2	-2.3	Synergy	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Vancomycin	Colistin + Vancomycin		Colistin + Vancomycin	Colistin + Vancomycin
111-27	9.2	9.2	1.5	-7.7	Synergy	B
111-28	9.3	9.2	9.4	0.1	I	NB
111-29	8.6	8.2	9.1	0.9	I	NB
111-31	10.1	9.5	8.6	-0.9	I	NB
64-26	9.1	9.0	6.1	-2.9	Synergy	NB
131-16	6.1	9.2	6.4	0.3	I	NB
130-87	8.0	9.2	6.6	-1.4	I	NB
Strain	Log10 (VC) at 24 hr			Log10 (VC combination – VCMASA) at 24 hr	Interaction	Bactericidal activity
	Colistin	Alyteserin E4K	Colistin + Alyteserin E4K		Colistin + Alyteserin E4K	Colistin + Alyteserin E4K
111-27	9.1	9.1	4.2	-4.9	Synergy	NB
111-28	9.3	9.3	9.2	-0.1	I	NB
111-29	9.4	9.4	9.3	-0.1	I	NB
111-31	9.3	9.3	0.0	-9.3	Synergy	B
64-26	9.4	9.4	9.3	-0.1	I	NB
131-16	9.2	9.2	0.0	-9.2	Synergy	B
130-87	ND	ND	ND	ND	ND	ND

* ≥3 $\log_{10}$ CFU/mL reduction compared with the initial inoculums; † <2 $\log_{10}$ change in CFU/mL at 24 hours with the combination compared with the most active single agent; ‡ ≥2 $\log_{10}$ CFU/mL reduction with the combination compared with the most active single agent of 24 hours. Abbreviation: VC, viable colony count; I, Indifference; NB, non-bactericidal; B, bactericidal; ND, not done; MASA, most active single agent.

The colistin + tigecycline combination, which had the second highest efficacy rate in this study, had a 42.9% efficacy rate, which was within the range of the efficacy rates reported by others, which ranged from 4.1 to 100% [16-19].

Principe *et al.* [17] examined the interaction of colistin + tigecycline against MDRAB by first screening highly active strains using a checkerboard method, followed by a confirmatory test using a time-kill assay, and then reading only those combinations that were highly active in both assays as having a synergistic interaction. Two of 24 weeks (8.3%) showed synergy by the checkerboard screening method and one of those two weeks showed synergy by the time-kill assay, resulting in a synergy rate of 4.2% (1/24). The tigecycline/colistin drug concentrations used in this strain were 2 and 0.25 mg/L, respectively, which is lower than the tigecycline/colistin drug concentration of 2 and 2 mg/L used in this study. In addition, they recognized synergy when both tests showed synergy and did not test all 24 weeks with the time-kill technique, so these factors may have contributed to the low synergy rate in their study. Peck *et al.* [18] investigated six weeks of imipenem-resistant *A. baumannii* blood isolates in an *in vitro* time-kill assay and found that the colistin + tigecycline combination was synergistic in all six weeks (100%) when tested at a 1× MIC concentration, but in four of the six weeks (66.7%) when tested at a 0.5× MIC concentration.

Vidaillac *et al.* reported that the static time-kill analysis of colistin + vancomycin combination in 4 weeks of *A. baumannii* showed a synergistic effect in all 4 weeks (100%) at 0.5× MIC concentration and 3 weeks (75%) at 0.25× MIC concentration, but the synergistic effect of colistin + vancomycin combination in this study was significantly lower at 28.6% [20]. Since the results of Vidaillac *et al.* showed that the synergistic effectiveness rate varied depending on the drug concentration used (0.25× MIC or 0.5× MIC) [20], it is possible that some of the difference in the synergistic effectiveness rate between Vidaillac *et al.* and this study using different antimicrobial concentrations may be due to natural differences in drug concentration.

The synergistic rate of the colistin + doripenem combination in the present study was 14.3%, which is lower than the range of synergistic rates reported in other papers, which ranged from 20.8% to 100% [16,21,22]. In a 25-week time-kill assay of *A. baumannii* by Pankuch *et al.* [21], the colistin + doripenem combination was synergistic against all (100%) isolates at concentrations below the MIC. When Principe *et al.* examined the interaction of colistin + doripenem in MDRAB, 8 of 24 weeks were synergistic in the checkerboard assay and 5 of those 8 weeks were synergistic in the time-kill confirmatory assay [22]. The synergistic rate was 20.8% (5/24) because they read synergism as synergistic when both assays were positive, and only strains positive in the checkerboard assay were tested in the time-kill confirmatory assay, so the synergistic rate may be lower than in other studies. In their study, *A. baumannii* isolates sharing the same epidemiological type had different interactions with the same drug combination, which was possibly due to the heterogeneity of resistance determinants possessed by different strains [22]. This phenomenon suggests that strains of the same clone with the same genetic fingerprint and resistance pattern should be tested separately for drug combination effects [22]. If strains of the same clone tested in a study differ in their combined effectiveness against the same drug combination, it seems natural that the synergistic rates in different studies are different, and the differences are likely to be due to a combination of factors that are difficult to explain.

Phee *et al.* reported that the effect of colistin + fusidic acid combination on 11 weeks of *A. baumannii* (3 weeks MDRAB, 4 weeks XDRAB, 3 weeks PDRAB, 1 week type strain) was evaluated by disc diffusion and checkerboard method (11/11, 100%) and time-kill assay (6/6, 100%), but the synergistic effect rate of colistin + fusidic acid combination in this study was significantly lower at 14.3% [6].

Ma *et al.* reported that in a murine model of MDRAB and XDRAB injection-induced pneumonia, the colistin-linezolid combination was bactericidal and synergistic compared with colistin alone *in vivo*, suggesting the synergistic effect of colistin-linezolid combination therapy as a therapeutic alternative for severe lung infection induced by MDRAB and XDRAB [23]. In the present study, however, the colistin-linezolid combination had a synergistic rate of 0% *in vitro*.

The number of samples used in this study was only 7 weeks, so it is not clear why the synergy rates or rankings are representative of the performance of *A. baumannii* in general, but the synergy rates of the different antimicrobial combinations tested in this study tended to be lower than those reported in other studies. Several factors may have contributed to this discrepancy, including differences in strain characteristics and resistance mechanisms, regional differences, environmental influences, experimental methods and conditions, interpretation of results, and differences in antimicrobial concentrations used. The small number of strains tested in this study and the fact that only a few tests were performed on each strain made it difficult to find out the factors affecting the synergistic effectiveness of the strains or the relationship between the various factors interacting with each other, which is a limitation of this study.

Among the factors that may contribute to the differences in synergistic rates between studies, we observed that the bactericidal rate and synergistic rate varied depending on the drug combination and strain characteristics in the study by Oliva *et al.* [24]. In the time-kill test, the colistin + meropenem and colistin + tigecycline combinations showed bactericidal and synergistic activity against colistin-susceptible and colistin-low-resistant strains only, whereas the colistin + vancomycin and colistin + rifampin combinations showed bactericidal and synergistic activity against all three weeks, including colistin-high-resistant strains [24]. However, the number of strains tested in the time-kill test was only three, and whether the results of these strains are representative of the general trend requires further study. On the other hand, when drug-drug interactions were tested using a checkerboard method by combining various anti-*Acinetobacter* chemotherapeutic agents with doripenem for 22 weeks of MDRAB, 13 weeks (54.2%) showed synergism, and surprisingly, synergism was only detected in doripenem-insensitive strains [22]. Although the representativeness of the results may vary depending on the number of strains, the results of bactericidal and synergistic rates may likely vary depending on several factors, including the drug combination and strain characteristics. It is possible that the combined effects of these various factors may have led to the differences in results between the papers.

The reason for the indifference of the interaction between the two drugs in the time-kill assay, even though strain 111-27 was bactericidal, was that minocycline alone was so effective that the number of colonies did not differ by more than 100-fold compared to the combined drug treatment. This phenomenon has been observed in other meta-analysis studies. Zusman *et al.* noted that of the studies included in their meta-analysis, three studies investigating imipenem and colistin showed very high (100%) bactericidal activity with colistin monotherapy, making it impossible to demonstrate a synergistic effect [25]. When these studies were excluded, the analysis showed that the imipenem-colistin synergy rate improved to 67%. This is another example of how different strains respond differently to drug combinations, i.e. differences in response to combinations (e.g. synergy rates) are likely a reflection of the characteristics of each strain. Such differences are difficult to statistically process when studied with a small number of strains, and when calculating the percentage of potency, the potency can change significantly even if the frequency differs by only one strain, so the smaller the number of samples, the greater the difference. However, it is difficult to attach much significance to the potency obtained by testing with a small number of samples because the potency cannot

be representative of the general potency.

Among the published studies on the antimicrobial effects of antimicrobial peptides against MDR pathogens, some studies characterized various antimicrobial peptides obtained from animals, measured the MIC values of antimicrobial peptides against various drug-resistant clinical isolates, or evaluated their *in vitro* activity by observing the time-killing kinetics of single agents [2,26], but it was difficult to find studies that combined these peptides with antimicrobial agents to study their interactions. The study by Conlon *et al.* evaluated the antimicrobial activity of six cationic α-helix frog skin-derived peptides by measuring their MIC values and found that the alyteserin-1c [E4K] and B2RP [D4K] peptides were the most effective peptides against colistin-resistant strains, with MICs ranging from 4 to 16 μg/mL, was not a study that tested the effect of combining drugs [2]. To the authors' knowledge, there is no literature reporting the synergistic effectiveness of the colistin and alyteserin-1c peptide combination using time-kill analysis, and we believe this is the first study to evaluate the synergistic effect of the alyteserin E4K and colistin combination on XDRAB clinical isolates. The colistin + alyteserin E4K combination demonstrated a synergy rate of 50.0% (3/6) in XDRAB clinical isolates, the second-highest synergy rate among the seven combinations. As there are currently no other publications on this combination, it is difficult to make an objective assessment of its efficacy, and further studies are needed to evaluate its efficacy.

In conclusion, since the synergistic effect of colistin + minocycline and colistin + alyteserin E4K was high among the seven antimicrobial combinations tested in this study to identify effective antimicrobial combinations against XDRAB, we believe that colistin + minocycline and colistin + alyteserin E4K can be considered as candidate combinations for future *in vivo* studies on the effect of antimicrobial combinations. As this study was a preliminary screening study in which various combinations were tested together in a small number of samples to identify effective combinations, it is necessary to conduct follow-up studies with various combinations and methods on a larger number of strains in the future.

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Disclosure statement

The authors declare no conflict of interest.

Supplementary Materials

The online-only data supplement is available with this article at https://doi.org/10.5145/ACM.2022.25.4.3

References

[1] Conlon JM, Mechkarska M, Arafat K, et al., 2012, Analogues of the Frog Skin Peptide Alyteserin-2a with Enhanced Antimicrobial Activities against Gram-Negative Bacteria. J Pept Sci, 2012(18): 270–275.

[2] Conlon JM, Sonnevend A, Pál T, et al., 2012, Efficacy of Six Frog Skin-Derived Antimicrobial Peptides Against Colistin-Resistant Strains of the *Acinetobacter baumannii* Group. Int J Antimicrob Agents, 2012(39): 317–320.

[3] Feizabadi MM, Fathollahzadeh B, Taherikalani M, et al., 2008, Antimicrobial Susceptibility Patterns and Distribution of bla_{OXA} Genes Among *Acinetobacter* spp. Isolated from Patients at Tehran Hospitals. Jpn J Infect Dis, 2008(61): 274–278.

[4] Higgins PG, Wisplinghoff H, Krut O, et al., 2007, A PCR-Based Method to Differentiate Between *Acinetobacter baumannii* and *Acinetobacter* Genomic Species 13TU. Clin Microbiol Infect, 2007(13): 1199–1201.

[5] Magiorakos AP, Srinivasan A, Carey RB, et al., 2012, Multidrug Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: an International Expert Proposal for Interim Standard Definitions for Acquired Resistance. Clin Microbiol Infect, 2012(18): 268–281.

[6] Phee LM, Betts JW, Bharathan B, et al., 2015, Colistin and Fusidic Acid, a Novel Potent Synergistic Combination for Treatment of Multidrug-Resistant *Acinetobacter baumannii* Infections. Antimicrob Agents Chemother, 2015(59): 4544–4550.

[7] Oleksiuk LM, Nguyen MH, Press EG, et al., 2014, *In Vitro* Responses of *Acinetobacter baumannii* to Two-and Three-Drug Combinations Following Exposure to Colistin and Doripenem. Antimicrob Agents Chemother, 2014(58): 1195–1199.

[8] Mao J, Li T, Zhang N, et al., 2021, Dose Optimization of Combined Linezolid and Fosfomycin Against *Enterococcus* by Using an *In Vitro* Pharmacokinetic/Pharmacodynamic Model. Microbiol Spectr, 2021(9): e00871–21.

[9] Colton B, McConeghy KW, Schreckenberger PC, et al., 2016, I.V. Minocycline Revisited for Infections Caused by Multidrug-Resistant Organisms. Am J Health Syst Pharm, 2016(73): 279–285.

[10] Zhanel GG, Baudry PJ, Tailor F, et al., 2009, Determination of the Pharmacodynamic Activity of Clinically Achievable Tigecycline Serum Concentrations Against Clinical Isolates of *Escherichia coli* with Extended-Spectrum β-lactamases, Amp C β-lactamases and Reduced Susceptibility to Carbapenems Using an *In Vitro* Model. J Antimicrob Chemother, 2009(64): 824–828.

[11] Gordon NC, Png K, Wareham DW, 2010, Potent Synergy and Sustained Bactericidal Activity of a Vancomycin-Colistin Combination Versus Multidrug-Resistant Strains of *Acinetobacter baumannii*. Antimicrob Agents Chemother, 2010(54): 5316–5322.

[12] Kim UJ, Kim CM, Jang SJ, et al., 2020, Evaluation of Synergistic Effect of Combined Multidrug-Resistant *Acinetobacter baumannii* Treatment with Linalool and Colistin on to Expand Candidate for Therapeutic Option. Ann Clin Microbiol, 2020(23): 11–20.

[13] Park GC, Choi JA, Jang SJ, et al., 2016, *In Vitro* Interactions of Antibiotic Combinations of Colistin, Tigecycline, and Doripenem Against Extensively Drug Resistant and Multidrug-resistant *Acinetobacter baumannii*. Ann Lab Med, 2016(36): 124–130.

[14] Clinical and Laboratory Standards Institute (CLSI), 2018, Performance Standards for Antimicrobial Susceptibility Testing, CLSI document M100, Wayne, PA.

[15] Tan TY, Yong Ng LS, Tan E, et al., 2007, *In vitro* Effect of Minocycline and Colistin Combinations on Imipenem-Resistant *Acinetobacter baumannii* Clinical Isolates. J Antimicrob Chemother, 2007(60): 421–423.

[16] March GA, Bratos MA, 2015, A Meta-Analysis of *In Vitro* Antibiotic Synergy Against *Acinetobacter baumannii*. J

Microbiol Methods, 2015(119): 31–36.

[17] Principe L, D'Arezzo S, Capone A, et al., 2009, *In Vitro* Activity of Tigecycline in Combination with Various Antimicrobials Against Multidrug Resistant *Acinetobacter baumannii*. Ann Clin Microbiol Antimicrob, 2009(8): 18.

[18] Peck KR, Kim MJ, Choi JY, et al., 2012, *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): 353-360.

[19] Li J, Yang X, Chen L, et al., 2017, *In Vitro* Activity of Various Antibiotics in Combination with Tigecycline Against *Acinetobacter baumannii*: a Systematic Review and Meta-Analysis. Microb Drug Resist, 2017(23): 982–993.

[20] Vidaillac C, Benichou L, Duval RE, 2012, *In Vitro* Synergy of Colistin Combinations Against Colistin Resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* Isolates. Antimicrob Agents Chemother, 2012(56): 4856–4861.

[21] Pankuch GA, Seifert H, Appelbaum PC, 2010, Activity of Doripenem With and Without Levofloxacin, Amikacin, and Colistin Against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Diagn Microbiol Infect Dis, 2010(67): 191–197.

[22] Principe L, Capone A, Mazzarelli A, et al., 2013, *In Vitro* Activity of Doripenem in Combination with Various Antimicrobials Against Multidrug-Resistant *Acinetobacter baumannii*: Possible Options for the Treatment of Complicated Infection. Microb Drug Resist, 2013(19): 407–414.

[23] Ma XL, Guo YZ, Wu YM, et al., 2020, *In Vivo* Bactericidal Effect of Colistin Linezolid Combination in a Murine Model of MDR and XDR *Acinetobacter baumannii* Pneumonia. Sci Rep, 2020(10): 17518.

[24] Oliva A, Garzoli S, De Angelis M, et al., 2019, *In Vitro* Evaluation of Different Antimicrobial Combinations With and Without Colistin Against Carbapenem-Resistant *Acinetobacter baumannii*. Molecules, 2019(24): 886.

[25] Zusman O, Avni T, Leibovici L, et al., 2013, Systematic Review and Meta-Analysis of *In Vitro* Synergy of Polymyxins and Carbapenems. Antimicrob Agents Chemother, 2013(57): 5104–5111.

[26] Liu CB, Shan B, Bai HM, et al., 2015, Hydrophilic/Hydrophobic Characters of Antimicrobial Peptides Derived from Animals and their Effects on Multidrug Resistant Clinical Isolates. Dongwuxue Yanjiu, 2015(36): 41–47.

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