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Evaluation of Synergistic Effect of Combined Treatment with Linalool and Colistin on Multidrug-Resistant *Acinetobacter baumannii* to Expand Candidate for Therapeutic Option

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Abstract

Background: Acinetobacter baumannii infection is a significant health problem worldwide due to increased drug resistance. The limited antimicrobial alternatives for the treatment of severe infections by multidrug-resistant *A. baumannii* (MDRAB) make the search for other therapeutic options more urgent. Linalool, the major oil compound in *Coriandrum sativum*, was recently found to have high antibacterial activity against *A. baumannii*. The purpose of this study was to investigate the synergistic effect of linalool and colistin combinations against MDRAB and extensively drug-resistant *A. baumannii* (XDRAB). *Methods:* A total of 51 strains of *A. baumannii* clinical isolates, consisting of 10 MDRAB and 41 XDRAB were tested. We determined the minimum inhibitory concentration (MIC) of linalool for the test strains using the broth microdilution method and searched for interactions using the time-kill assay. *Results:* The time-kill assay showed that the linalool and colistin combination displayed a high rate of synergy (92.1%) (by synergy criterion 2), a low rate of indifference (7.8%), and a high rate of bactericidal activity (74.5%) in the 51 clinical isolates of *A. baumannii*. The synergy rates for the linalool and colistin combination against MDRAB and XDRAB were 96% and 92.1%, respectively. No antagonism was observed for the linalool and colistin combination. *Conclusions:* The combination of linalool and colistin showed a high synergy rate, which may be beneficial for controlling MDRAB infections. Therefore, this combination is a good candidate for *in vivo* studies to assess its efficacy in the treatment of MDRAB infections.

Keywords

Acinetobacter baumannii
Colistin
Linalool
Synergy
Time-kill assay

1. Introduction

Acinetobacter baumannii is an opportunistic gram-negative bacillus that causes a variety of infections and is associated with bacteremia, pneumonia, meningitis, and urinary tract infections [1,2]. The recent emergence of multidrug-resistant *A. baumannii* (MDRAB) has challenged clinicians in antibiotic selection [3]. The polymyxin antibiotic colistin has been underused due to its nephrotoxicity and neurotoxicity but has regained interest as one of the few antibiotics active against multidrug-resistant gram-negative bacteria, including *A. baumannii* [4,5]. However, with the increased use of colistin, resistant *A. baumannii* has emerged, posing a major threat to its usefulness [5-7]. Continuous infusion of carbapenem, bacteriophage-based therapy, and antibiotic combination therapy have been proposed as methods to restore susceptibility in MDRAB [8-10]. As the use of antibiotics can cause side effects [11], and as more and more people prefer to use ingredients that are considered safe and derived from nature [12], the combination of antibiotics with plant-derived ingredients such as essential oils has emerged as an alternative [13]. Plants produce a variety of metabolites, with primary metabolites involved in respiration and photosynthesis and associated with plant growth [14]. Secondary metabolites were formed over the past millions of years as plants fought off predators and act as chemical defenses [15]. Essential oils are a complex of volatile secondary metabolites that are extracted from flowers, leaves, roots, and seeds and have long been used as therapeutic agents due to their ability to inhibit bacterial growth [14,15]. Linalool is a monoterpene widely used in the perfume, cosmetics, and food industries, a major constituent of coriander essential oil, and has been reported to have broad-spectrum antimicrobial activity [16-18].

There have been many studies on the antimicrobial activity of antibiotics combined with essential oils or their components against various strains of bacteria [17,19]. Some studies have also been conducted on the combination of antibiotics with essential oils or plant extracts against MDRAB [20-22]. Based on the results of previous studies on combination therapy with essential oils and antibiotics, it is necessary to identify the synergistic and antagonistic effects of the active ingredients or compounds to be used in the combination due to the inter- and intra-strain variability [15-17, 23,24]. This study was conducted to evaluate the efficacy of a combination of linalool and colistin against MDRAB and extensively drug-resistant *A. baumannii* (XDRAB).

2. Materials and methods

2.1. Subjects and specimen collection

Fifty-one strains of *A. baumannii* that had been identified and stored in frozen for a total of 6 years from June 2009 to May 2015 were collected from specimens referred for bacteriological culture at Chosun University Hospital, analyzed, and investigated. The strains were used in the experiments after being grown on streaks twice. Identification was performed using the VITEK2 GN card of the VITEK2 System (bioMérieux, Marcyl, Etoile, France). Strains identified as *A. baumannii* by the VITEK2 automated analyzer were subjected to $bla_{\text{OXA-51-like}}$ PCR [25], Higgins PCR [26], and *A. baumannii* specific PCR [27], to confirm the species name, and if all were positive, they were confirmed as *A. baumannii*. Antimicrobial susceptibility testing was performed using the VITEK2 AST-N225 card of the VITEK2 System (bioMérieux). The strains used in the study were defined according to the criteria proposed by Magiorakos *et al.* in which antibiotics were divided into nine categories, MDR was defined as susceptibility to at least one antibiotic in each category in three or more categories, and XDR was defined as susceptibility to at least one antibiotic in all but two categories [28].

2.2. Antimicrobial susceptibility testing

Linalool and colistin were purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA) with the product numbers L2602 and C4461, respectively. The minimum

inhibitory concentrations (MICs) for each antimicrobial agent were determined by broth microdilution assay method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [29]. Results of susceptibility to colistin were interpreted according to CLSI breakpoint criteria [29]. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 strains were used as controls.

2.3. Time-kill assay

Time-kill assays were performed with linalool and colistin alone and with linalool and colistin combined. Each strain was added to Mueller-Hinton (MH) broth to a final concentration of 1×10^6 CFU/mL when mixed with the antibiotic. Colistin concentrations of 2 μg/mL were used, which is a clinically achievable serum concentration [30], and linalool was piloted at a concentration equivalent to 1/4 MIC. Strains were inoculated in tubes containing MH broth supplemented with antimicrobial agents, prepared to a concentration of 1×10^6 CFU/mL in a total volume of 10 mL, and incubated in a 37°C shaking incubator. After incubation at 37°C for 0 and 24 h, 10 μL of the broth was taken and serially diluted 10-fold, and the dilutions were inoculated onto MH agar plates and the total colonies were counted after overnight incubation [31].

Because different researchers tend to use different criteria, making it difficult to compare methods, two commonly used criteria were applied to determine synergism. After 24 hours of incubation, a reduction in the number of bacteria grown in the combination treatment by more than 2 $\log_{10}$ CFU/mL compared to the number of bacteria grown in the most active single agent treatment was defined as criterion 1. A reduction in the number of bacteria grown in the combination treatment by more than 2 $\log_{10}$ CFU/mL compared to the initial inoculum was defined as criterion 2 while meeting criterion 1 of synergism. Indifferent was defined as when the number of bacteria increased or decreased by less than 2 $\log_{10}$ CFU/mL, and antagonistic was defined as when the number increased by more than 2 $\log_{10}$ CFU/mL [32]. The synergy rate was calculated and compared by applying both criteria 1 and 2 for each strain. 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 [33].

3. Results

3.1. Antibiotic susceptibility of strains

The percentage of susceptibility in the homeostatic susceptibility test on the VITEK2 device for 51 strains was as follows: ampicillin-sulbactam 10.4%, aztreonam 0%, cefepime 2%, cefotaxime 0%, ceftazidime 2%, tigecycline24. 4%, ciprofloxacin 0%, gentamicin 14.2%, imipenem 4%, meropenem 4%, piperacillin 2%, piperacillin-tazobactam 2%, ticarcillin-clavulanic acid 0%, trimethoprim-sulfamethoxazole 14.8%, colistin 63.2%, minocycline 82.9%.

3.2. MIC of linalool and colistin

The 51 samples collected were identified as 10 MDRABs and 41 XDRABs. MICs of linalool ranged from 2–32 μg/mL and colistin ranged from 0.25–16 μg/mL. Linalool had an MIC_{50} of 8 μg/mL and an MIC_{90} of 16 μg/mL, while colistin had an MIC_{50} of 2 μg/mL and an MIC_{90} of 8 μg/mL.

3.3. Time-kill assay results

The time-kill assay results for all *A. baumannii* strains are presented in **Table 1**. Synergistic effects were observed in 10 out of 10 weeks (100%) of MDRAB and 39 out of 41 weeks (95.1%) of XDRAB using criterion 1. Using criterion 2, synergism was observed in 9 of 10 weeks (90%) in MDRAB and 38 of 41 weeks (92.6%) in XDRAB. No antagonism was seen (**Table 2**).

Colistin alone showed no bactericidal activity against all strains, while linalool alone showed bactericidal activity against MDRAB and XDRAB at 1

Table 1. Results of time-kill assay and bactericidal activity against two-drug combinations of linalool and colistin of 10 MDR and 41 XDR clinical isolates of *A. baumannii*

Group	Isolate	Log10(VCC of combination/ VCC of MASA) of 24 hr	Log10(VCC of 24 hr) – Log10(VCC of 0 hr)			Synergy criteria 1‡	Synergy criteria 2§	MIC	MIC	Category	Bactericidality ‖		
			LNL*	CST†	LNL*+CST†			LNL	CST	CST	LNL*	CST†	LNL*+CST†
MDR	89-92	-5.2	3.8	3.7	-1.4	S	I¶	4.0	8.0	R	N	N	N
	171-84	-6.1	0.1	2.9	-5.8	S	S	16.0	2.0	S	N	N	B
	172-91	-3.4	0.8	0.8	-2.5	S	S	16.0	2.0	S	N	N	N
	172-99	-5.1	-1.1	2.0	-6.2	S	S	4.0	1.0	S	N	N	B
	174-99	-8.1	2.9	3.1	-5.2	S	S	16.0	16.0	R	N	N	B
	180-82	-2.7	-3.4	0.1	-5.9	S	S	16.0	0.5	S	B	N	B
	181-67	-9.5	1.9	1.7	-5.3	S	S	32.0	0.5	S	N	N	B
	186-88	-5.0	-0.3	0.9	-5.2	S	S	16.0	2.0	S	N	N	B
	191-19	-8.2	3.1	4.2	-5.0	S	S	8.0	2.0	S	N	N	B
	200-92	-7.4	2.1	4.1	-5.1	S	S	8.0	2.0	S	N	N	B
XDR	69-84	-5.2	2.6	3.9	-2.5	S	S	4.0	8.0	R	N	N	N
	99-73	-6.1	0.2	0.3	-5.3	S	S	8.0	8.0	R	N	N	B
	111-27	-6.2	0.9	2.8	-5.3	S	S	32.0	2.0	S	N	N	B
	130-33	-4.1	1.8	1.9	-2.3	S	S	4.0	16.0	R	N	N	N
	139-27	-4.0	-1.3	3.1	-5.3	S	S	16.0	2.0	S	N	N	B
	148-33	-4.4	-1.4	1.3	-5.8	S	S	8.0	8.0	R	N	N	B
	150-94	-5.4	2.2	2.1	-3.2	S	S	8.0	2.0	S	N	N	B
	155-24	-2.7	1.8	3.6	-2.7	S	S	4.0	2.0	S	N	N	N
	158-46	-4.0	-1.9	-1.9	-5.9	S	S	8.0	2.0	S	N	N	B
	158-86	-7.2	2.2	3.2	-5.0	S	S	8.0	1.0	S	N	N	B
	158-87	-5.8	0.7	4.2	-5.2	S	S	8.0	8.0	R	N	N	B
	159-86	-7.0	1.0	3.2	-6.0	S	S	8.0	0.3	S	N	N	B
	160-14	-2.4	-0.2	-0.3	-2.6	S	S	4.0	16.0	R	N	N	N
	160-15	-7.0	1.7	1.7	-5.2	S	S	16.0	1.0	S	N	N	B
	160-68	-6.0	0.7	3.9	-4.9	S	S	16.0	8.0	R	N	N	B
	160-92	-6.2	0.9	3.8	-5.3	S	S	16.0	4.0	R	N	N	B
	160-93	-3.2	-3.0	1.9	-6.0	S	S	8.0	8.0	R	N	N	B
	162-83	-6.0	-0.2	2.3	-5.9	S	S	16.0	0.5	S	N	N	B
	162-88	-3.1	0.7	3.8	-2.3	S	S	4.0	8.0	R	N	N	N
	163-71	-3.6	4.4	4.3	0.8	S	I	2.0	8.0	R	N	N	N
	163-72	-1.9	-0.5	0.7	-2.5	I	I	4.0	8.0	R	N	N	N
	163-77	-7.3	2.2	2.0	-5.1	S	S	16.0	4.0	R	N	N	B
	164-35	-4.3	1.8	1.9	-2.3	S	S	4.0	2.0	S	N	N	N
	164-62	-5.8	0.6	4.0	-5.0	S	S	8.0	2.0	S	N	N	B
	165-41	-3.3	0.8	1.7	-2.4	S	S	4.0	8.0	R	N	N	N
	165-83	-8.2	2.9	2.9	-5.2	S	S	16.0	8.0	R	N	N	B
	165-83	-7.2	1.8	2.8	-5.2	S	S	16.0	8.0	R	N	N	B
	166-13	-6.4	2.0	2.9	-4.4	S	S	16.0	0.5	S	N	N	B
	166-24	-6.2	0.9	3.0	-5.3	S	S	8.0	1.0	S	N	N	B
	166-61	-8.3	3.5	4.3	-4.8	S	S	32.0	1.0	S	N	N	B
	172-89	-8.2	2.4	2.3	-6.0	S	S	8.0	2.0	S	N	N	B
	177-35	-5.2	2.9	2.9	-2.1	S	S	4.0	2.0	S	N	N	N
	189-52	-7.2	2.1	3.2	-4.9	S	S	8.0	2.0	S	N	N	B
	189-97	-7.1	1.8	1.9	-5.2	S	S	16.0	2.0	S	N	N	B
	200-19	-4.3	2.0	2.0	-2.2	S	S	8.0	2.0	S	N	N	N
	218-62	-6.0	0.8	0.6	-5.2	S	S	16.0	1.0	S	N	N	B
	219-12	-5.3	2.1	1.9	-3.2	S	S	16.0	1.0	S	N	N	B
	219-56	-6.9	1.7	3.0	-5.0	S	S	16.0	1.0	S	N	N	B
	219-67	-0.4	-4.0	4.0	-4.4	I	I	16.0	1.0	S	B	N	B
	222-23	-2.8	-3.2	1.1	-6.1	S	S	8.0	1.0	S	B	N	B
	230-54	-5.0	-1.1	-1.0	-6.1	S	S	8.0	1.0	S	N	N	B

* Linalool 1/4 MIC; † Colistin 2 μg/mL; ‡ ≥ 2 $\log_{10}$ CFU/mL reduction with the combination compared with the most active single agent of 24hr; § ≥ 2 $\log_{10}$ CFU/mL reduction with the combination compared with the most active single agent and ≥ 2 log10 CFU/mL reduction below the initial inoculum at 24 hr; ‖ ≥ 3 $\log_{10}$ CFU/mL reduction compared with the initial inoculums; ¶ < 2 $\log_{10}$ change in CFU/mL at 24 hr with the combination compared with the most active single agent. Abbreviation: MIC, minimum inhibitory concentration; LNL, linalool; CST, colistin; B, bactericidal; N, non-bactericidal; MDR, multidrug-resistant; XDR, extensively drug-resistant; S, synergy; I, indifference; VCC, viable cell count, MASA, most active single agent.

Table 2. Comparison of time-kill assay results between MDR and XDR groups against the combination of colistin and linalool

Combination of antibiotics	Interaction	MDR	XDR	Total
		No. (%)	No. (%)	No. (%)
Colistin + Linalool	Synergy (criterion 1)	10 (100.0)	39 (95.1)	49 (96.0)
	Synergy (criterion 2)	9 (90.0)	38 (92.6)	37 (92.1)
	Indifference	1 (10.0)	3 (7.3)	4 (7.8)
	Antagonism	0 (0.0)	0 (0.0)	0 (0.0)
	Bactericidal	8 (80.0)	30 (73.1)	38 (74.5)

Abbreviation: MDR, multidrug-resistant; XDR, extensively drug-resistant.

week each. In combination therapy, bactericidal activity was observed in 8 out of 10 weeks (80%) for MDRAB and 30 out of 41 weeks (73.1%) for XDRAB (**Table 2**).

4. Discussion

In this study, the combined effect of linalool and colistin was evaluated against 51 weeks of MDRAB clinical isolates and showed superior antimicrobial activity compared to single agents. In a previous study with MDRAB, a synergistic effect was observed by combining red river gum (*Eucalyptus camaldulensis*) essential oil with polymyxin B [21]. Another study combined silver plum (*Myrtus communis L.*) essential oil with polymyxin B and ciprofloxacin, respectively, and observed synergistic effects against MDRAB [20].

Treatment of *A. baumannii* with linalool showed antibacterial activity and rapid bactericidal effect, limiting biofilm formation and inhibiting the process of bacterial adhesion to surfaces [19]. Aelenei *et al.* evaluated the effect of oxacillin, erythromycin, gentamycin, ciprofloxacin, tetracycline, clindamycin, and erythromycin combined with linalool by checkerboard method [17]. *S. aureus* was studied for 3 weeks, *P. aeruginosa* for 2 weeks, *Staphylococcus epidermidis* for 1 week, and *E. coli* for 1 week, and the proportion of all antibiotic combinations with synergistic effects were examined by strain, and synergistic effects were found in 80% (12/15) of *S. aureus*, 12.5% (1/8) of *P. aeruginosa*, 42.8% (3/7) of *S. epidermidis*, and 40% (2/5) of *E. coli* [17]. Silva *et al.* performed combination therapy of linalool and imipenem and linalool and ciprofloxacin by checkerboard method against four strains including reference strain *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 25853, and clinical isolates *S. aureus* and *P. aeruginosa* [16]. The authors defined a fractional inhibitory concentration (FIC) index of 0.5 or less as "synergistic", between 0.5 and 1 as "additive", and between 1 and 4 as "unrelated". The combination of linalool and imipenem was synergistic in all four strains, while the combination of linalool and ciprofloxacin was additive in *S. aureus* and unrelated in *P. aeruginosa* [16]. The above results were evaluated on different strains and different antibiotics than in this study, so the synergistic effectiveness rate could not be directly compared. Even taking this into account, the combination of linalool and colistin showed a synergistic effect of 92% using criterion 2, making it a promising combination against MDRAB.

The mechanism by which linalool and colistin combinations work against bacteria is unclear. In general, essential oils alter the permeability of the cell wall, leading to leakage of electrolytes and loss of amino acids, ATP, and DNA, and some accumulate to disrupt the integrity of the cell wall structure and induce cell death [34]. Linalool has been reported to increase the permeability of the cell wall in both Gram-positive and Gram-negative bacteria, leading to the loss of cellular components [35]. Colistin is an amphiphilic antibiotic that interacts with lipid A (LPA)

of lipopolysaccharide (LPS) on the outer cell wall of bacteria, resulting in cell death [36]. Both linalool and colistin have antimicrobial mechanisms that act on the cell wall, and further research is needed to elucidate the mechanism of synergism.

In conclusion, the combination of linalool and colistin against *A. baumannii* was highly effective against both MDRAB and XDRAB with a high percentage of synergistic effect. There have been efforts to apply the combination of existing antibiotics to MDRAB and some previous studies have shown that the combination is effective [37], while others have shown conflicting results *in vitro* and *in vivo* [38]. This study is the first to combine the essential oil components linalool and colistin, rather than a combination of existing antibiotics, against MDRAB and found a significant synergistic effect. Currently, the combination of essential oils and antibiotics is an under-researched area, and more *in vivo* and *in vitro* studies are needed for clinical application.

Disclosure statement

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References

[1] Peleg AY, Seifert H, Paterson DL, 2008, *Acinetobacter baumannii*: Emergence of a Successful Pathogen. Clin Microbiol Rev, 21: 538–582.

[2] Munoz-Price LS, Weinstein RA, 2008, Acinetobacter Infection. N Engl J Med, 358: 1271–1281.

[3] Manchanda V, Sanchaita S, Singh N, 2010, Multidrug Resistant Acinetobacter. J Glob Infect Dis, 2: 291–304.

[4] Moffatt JH, Harper M, Harrison P, et al., 2010, Colistin Resistance in *Acinetobacter baumannii* is Mediated by Complete Loss of Lipopolysaccharide Production. Antimicrob Agents Chemother, 54: 4971–4977.

[5] Cai Y, Chai D, Wang R, et al., 2012, Colistin Resistance of Acinetobacter baumannii: Clinical Reports, Mechanisms and Antimicrobial Strategies. J Antimicrob Chemother, 67: 1607–1615.

[6] Ko KS, Suh JY, Kwon KT, et al., 2007, High Rates of Resistance to Colistin and Polymyxin B in Subgroups of *Acinetobacter baumannii* Isolates from Korea. J Antimicrob Chemother, 60: 1163–1167.

[7] Park YK, Jung SI, Park KH, et al., 2009, Independent Emergence of Colistin-Resistant *Acinetobacter* spp. Isolates from Korea. Diagn Microbiol Infect Dis, 64: 43–51.

[8] Mohammadi M, Khayat H, Sayehmiri K, et al., 2017, Synergistic Effect of Colistin and Rifampin Against Multidrug Resistant *Acinetobacter baumannii*: A Systematic Review and Meta-Analysis. Open Microbiol J, 11: 63–71.

[9] Schooley RT, Biswas B, Gill JJ, et al., 2017, Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails to Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection. Antimicrob Agents Chemother 2017;61: e00954-17.

[10] Wang D, 2009, Experience with Extended-Infusion Meropenem in the Management of Ventilator-Associated Pneumonia Due to Multidrug-Resistant *Acinetobacter baumannii*. Int J Antimicrob Agents, 33: 290–291.

[11] Rosato A, Vitali C, De Laurentis N, et al., 2007, Antibacterial Effect of Some Essential Oils Administered Alone or in Combination with Norfloxacin. Phytomedicine, 14: 727–732.

[12] Pei RS, Zhou F, Ji BP, et al., 2009, Evaluation of Combined Antibacterial Effects of Eugenol, Cinnamaldehyde, Thymol, and Carvacrol Against *E. coli* with an Improved Method. J Food Sci, 74: M379–M383.

[13] Harris R, 2002, Synergism in the Essential Oil World. Int J Aromather, 12: 179–186.

[14] Magi G, Marini E, Facinelli B, 2015, Antimicrobial Activity of Essential Oils and Carvacrol, and Synergy of Carvacrol and Erythromycin, Against Clinical, Erythromycin-Resistant Group A Streptococci. Front Microbiol, 6: 165.

[15] Bassole IH, Juliani HR, 2012, Essential Oils in Combination and Their Antimicrobial Properties. Molecules, 17: 3989–4006.

[16] Silva VA, Sousa JP, Guerra F, et al., 2015, Antibacterial Activity of the Monoterpene Linalool: Alone and in Association with Antibiotics Against Bacteria of Clinical Importance. Int J Pharmacol Phytochem Res, 7: 1022–1026.

[17] Aelenei P, Rimbu CM, Guguianu E, et al., 2019, Coriander Essential Oil and Linalool – Interactions with Antibiotics Against Gram-Positive and Gram-Negative Bacteria. Lett Appl Microbiol, 68: 156–164.

[18] Park SN, Lim YK, Freire MO, et al., 2012, Antimicrobial Effect of Linalool and Alpha-Terpineol Against Periodontopathic and Cariogenic Bacteria. Anaerobe, 18: 369–72.

[19] Alves S, Duarte A, Sousa S, et al., 2016, Study of the Major Essential Oil Compounds of *Coriandrum sativum* against *Acinetobacter baumannii* and the Effect of Linalool on Adhesion, Biofilms and Quorum Sensing. Biofouling, 32: 155–165.

[20] Aleksic V, Mimica-Dukic N, Simin N, et al., 2014, Synergistic Effect of *Myrtus communis L.* Essential Oils and Conventional Antibiotics Against Multi-Drug Resistant *Acinetobacter baumannii* Wound Isolates. Phytomedicine, 21: 1666–1674.

[21] Knezevic P, Aleksic V, Simin N, et al., 2016, Antimicrobial Activity of *Eucalyptus camaldulensis* Essential Oils and Their Interactions with Conventional Antimicrobial Agents Against Multi-Drug Resistant *Acinetobacter baumannii*. J Ethnopharmacol, 178: 125–136.

[22] Betts JW, Wareham DW, 2014, *In vitro* Activity of Curcumin in Combination with Epigallocatechin Gallate (EGCG) versus Multidrug-Resistant *Acinetobacter baumannii*. BMC Microbiol, 14: 172.

[23] Yap PSX, Yiap BC, Ping HC, et al., 2014, Essential Oils, a New Horizon in Combating Bacterial Antibiotic Resistance. Open Microbiol J, 8: 6–14.

[24] Mulyaningsih S, Sporer F, Zimmermann S, et al., 2010, Synergistic Properties of the Terpenoids Aromadendrene and 1,8-Cineole from the Essential Oil of *Eucalyptus globulus* Against Antibiotic-Susceptible and Antibiotic-Resistant Pathogens. Phytomedicine, 17: 1061–1066.

[25] 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, 61: 274–278.

[26] 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, 13: 1199–1201.

[27] Li XM, Choi JA, Choi IS, et al., 2016, Development and Evaluation of Species-Specific PCR for Detection of Nine *Acinetobacter* Species. Ann Clin Lab Sci, 46: 270–278.

[28] 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, 18: 268–281.

[29] M100. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, 2018, Wayne.

[30] 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, 36: 124–130.

[31] Kumar S, Bandyopadhyay M, Mohiruddin SK, et al., 2017, Antimicrobial Synergy Testing by Time-Kill Methods for Extensively Drug-Resistant *Acinetobacter baumannii* Isolates. J Dent Med Sci, 16: 79–84.

[32] Entenza JM, Moreillon P, 2009, Tigecycline in Combination with Other Antimicrobials: A Review of *in vitro*, Animal and Case Report Studies. Int J Antimicrob Agents, 34: 8.e1–9.

[33] 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, 19: 407–414.

[34] Wang YM, Kong LC, Liu J, et al., 2018, Synergistic Effect of Eugenol with Colistin Against Clinical Isolated Colistin-Resistant *Escherichia coli* Strains. Antimicrob Resist Infect Control, 7: 17.

[35] Zengin H, Baysal AH, 2014, Antibacterial and Antioxidant Activity of Essential Oil Terpenes Against Pathogenic and Spoilage-Forming Bacteria and Cell Structure-Activity Relationships Evaluated by SEM Microscopy. Molecules, 19: 17773–17798.

[36] Qureshi ZA, Hittle LE, O'Hara JA, et al., 2015, Colistin-Resistant *Acinetobacter baumannii*: Beyond Carbapenem Resistance. Clin Infect Dis, 60: 1295–1303.

[37] Perez F, Hujer AM, Hujer KM, et al., 2007, Global Challenge of Multidrug-Resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother, 51: 3471–3484.

[38] Dinc G, Demiraslan H, Elmali F, et al., 2015, Antimicrobial Efficacy of Doripenem and Its Combinations with Sulbactam, Amikacin, Colistin, Tigecycline in Experimental Sepsis of Carbapenem-Resistant *Acinetobacter baumannii*. New Microbiol, 38: 67–73.