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ARTICLE

In vitro activity of eravacycline in combination with colistin against carbapenem-resistant *A. baumannii* isolates

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Abstract

The synergistic activity of eravacycline in combination with colistin on carbapenem-resistant *A. baumannii* (CRAB) isolates was evaluated in this study. Minimum inhibitory concentrations (MICs) of eravacycline and colistin were determined by the broth microdilution method. MICs values ranged between 1 to 4 mg and 0.5 to 256 mg l^{-1} for eravacycline and colistin, respectively. In vitro synergy between eravacycline and colistin was evaluated by using the chequerboard methodology. Synergistic activity was found in 10% of the strains, and additive effect in 30%. No antagonism was detected. Similar activity was also observed in colistin-resistant CRAB isolates. The result of this study indicates that eravacycline and colistin combination may be a potential therapeutic option for the treatment of CRAB related infections.

Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is an important nosocomial pathogen causing substantial morbidity and mortality [1, 2]. Carbapenem resistance rates in Acinetobacter strains increased and exceed 90% in some geographic regions, such as some of Eastern European Countries [3, 4]. In Asian Countries, carbapenem resistance is above 50% and increases over the years [4–7].

Current treatment options (polymyxins, tigecycline, aminoglycosides) for CRAB are limited and suffer from pharmacokinetic limitations such as high toxicity and low plasma levels [8]. In addition, increased colistin resistance is limiting current treatment options. Worldwide, the colistin resistance in *A.baumannii* isolates varies between geographic regions and ranges from 0 to 19% [4].

However, colistin resistance is underestimated due to widely used commercial succeptibility testing, and colistin resistance may be higher than established [9]. In some European Countries, the rate of colistin resistance range from 28.6 to 42.9% [10]. Therefore, to enhance clinical efficacy and to avoid toxicity, combination therapy is often used for CRAB infections [11].

Eravacycline is a novel fluorocycline that belongs to the tetracycline class of antimicrobials may be a treatment option for CRAB [12]. In vitro studies were shown that eravacycline minimum inhibitory concentrations (MICs) were found to be 2–8 fold lower than tigecycline MICs against CRAB [13–15]. The drug is also active against colistin-resistant strains [16]. Despite in vitro activity, treatment success of eravacycline in CRAB infections are unknown due to lack of clinical trials [17, 18]. Management of healthcare-associated infections (HCAI) caused by CRAB should be required combination therapy. There is no in vitro study related to the synergism of eravacycline with other antibiotics in the literature. The aim of this in vitro study is to evaluate the synergistic activity of eravacycline in combination with colistin on CRAB isolates.

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Materials and methods

Bacterial strains

Carbapenem-resistant *A. baumannii* isolates were used in the study. All strains were identified in lower respiratory tract samples in critically ill patients. Identification on species levels was determined by MALDI-TOF MS (Bruker Biotyper; Bruker Daltonics, Bremen, Germany). During testing, the isolates were cultured from frozen stocks with 5% sheep blood agar in accordance with guidelines from the Clinical and Laboratory Standards Institute [19]. All strains were incubated 35 °C before testing.

Investigation of carbapenemase resistance

MICs of meropenem was determined for all strains by the broth microdilution method [19]. MIC values of 4 µg ml^{-1} and above are taken as limit values for meropenem resistance [20]. All strains screened for carbapenemase activity by polymerase chain reaction (PCR). Eight of the most common carbapenemase genes (bla_{OXA-23}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{KPC}) were screened by an in-house multiplex PCR test [21–27]. The oligos used for the amplification of the genes as shown in Table 1.

Table 1 Oligos used for amplification

Oligos OXA, NDM, VIM, IMP, KPC	5′→3′	Amplicon size (bp)
OXA-23	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	501
OXA-48	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	733
OXA-51	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	353
OXA-58	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599
NDM	GTAGTGCTCAGTGTCGGCAT GGGCAGTCGCTTCCAACGGT	476
VIM	GTGTTTGGTCGCATATCGC CGCAGCACCAGGATAGAAG	380
IMP	GGAATAGAGTGGCTTAATTCTC CCAAACCACTACGTTATC	624
KPC	ATGTCACTGTATCGCCGTC TTTTCAGAGCCTTACTGCCC	893

OXA Oxacillinase, *NDM* New Delhi metallo-lactamase, *VIM* Verona integron-encoded metallo-β-lactamase, *IMP* Imipenem-hydrolyzing β-lactamase, *KPC* Klebsiella pneumoniae carbapenemase

Drugs

Eravacycline (Lot number: 26030) and colistin (Lot number: 16647) were provided MedChemTronica (Sweden) as laboratory-grade powders. All drugs were dissolved with dHO_2, 6.4 mg ml^{-1} stock solutions were prepared for eravacycline and colistin. All stock solutions were stored at – 20 °C throughout the study.

MIC and fractional inhibitory concentrations (FIC)

MICs of eravacycline and colistin were determined for all strains by the broth microdilution method [19]. The data obtained from broth microdilution tests were used to calculate synergy. The chequerboard microdilution panel method was used for MIC determination of the eravacycline/colistin combination.

Using 96-well U-bottom microplates, graded concentrations of antibiotics were mixed. Each antimicrobial agent was prepared to a fixed volume of 50 µl (up to a total of 100 µl volume for two antimicrobial agents), and 10 µl of bacterial suspension was added to each well. The final concentration of the test strains was a 5×10^5 CFU/ml in a total final volume of 100 µl in each well. The plates were incubated for 16–24 h at 35 °C and the presence or inhibition of microbial growth was determined visually. Eravacycline and colistin synergy were studied at least two times with the chequerboard method in all strains. The FIC index was calculated with the formula:

$$\mathrm{FIC} = \mathrm{MICAB}/\mathrm{MICA} + \mathrm{MICBA}/\mathrm{MICB}$$

The results of combination tests according to the FIC index were interpreted as follows: synergistic (FIC ≤ 0.5), additive (FIC > 0.5 and ≤1), indifferent (FIC > 1 and ≤4) and antagonistic (FIC > 4).

Results

Ten carbapenem-resistant *A. baumannii* strains were used in this study. Three of these strains were also resistant to colistin. All isolates were found to have bla_{OXA-51} and nine harbored bla_{OXA-23}. The characteristics of 10 CRAB isolates included in chequerboard experiments were shown in Table 2.

MIC values ranged between 1 to 4 mg and 0.5 to 256 mg l^{-1} for eravacycline and colistin, respectively. Chequerboard analysis showed 10% synergy, 30% additive, 60% indifference. No antagonism was observed. Both colistin and carbapenem-resistant strains showed synergistic (one strain), and additive (one strain) effect. The MIC values of eravacycline-colistin and minimum FIC values are summarized in Table 3.

Discussion

To our knowledge, this is the first study assessing the synergistic activity of eravacycline with colistin. Our study demonstrated that eravacycline had synergistic and additive activity in combination with colistin without antagonism. Our results revealed that the combination of eravacycline and colistin may be a treatment option for CRAB related infections, including colistin-resistant isolates. This preliminary in vitro results clarified that eravacycline and colistin combination should be evaluated with clinical studies.

A. baumannii is the most common nosocomial pathogen that causes bloodstream infections (BSI) or ventilator-associated pneumonia (VAP) in many medical centers, particularly in Turkey [2, 12]. Carbapenem resistance rates exceed 90% in some parts of the World [8]. Carbapenem resistance among *A. baumannii* isolates in Turkey ranges from 91 to 98% according to national health statistics [28].

Therapy of CRAB infections is generally failing and limited with colistin, mostly [15]. Because of increasing resistance rates and pharmacokinetic limitations, colistin often used in combination with other antibiotics such as meropenem, tigecycline, rifampicin. However, the optimum treatment regimen is still uncertain [1, 29–31]. There is no doubt that new therapeutic options are urgently needed for the treatment of CRAB infections.

Eravacycline is a novel fluorocycline, with a tetracycline core, that binds to the 70S ribosome submit of bacteria [15, 32]. The activity of eravacycline against Gram-negative, Gram-positive and anaerobic bacteria except *Pseudomonas aeruginosa* was demonstrated in previous studies [14, 15, 33–35]. Furthermore, eravacycline was active against multidrug-resistant bacteria, including those expressing carbapenemases [14]. It was utilized as a potent antibiotic for *A. baumannii*, including isolates associated with an acquired OXA or up-regulation of the intrinsic OXA-51-like enzyme [1, 13, 16]. The MIC 50/90 values were 0.5 and 1 mg l^{-1} and higher in tigecycline resistant strains [1, 13, 16]. The MIC values did not change with the production of OXA carbapenemases [1]. In accordance with the previous studies, eravacycline MIC values ranged from 1 to 4 mg l^{-1} for OXA-23 and OXA-51 producing CRAB strains in our study. Considering the prevalence of OXA-type carbapenemase activity in CRAB strains, eravacycline has been evaluated as a good treatment option [10, 36, 37]. However, some type of carbapenemase activity, increased expression of the efflux pumps and the presence of tigecycline resistance are associated with increasing MIC values for eravacycline [1, 8].

Table 2 Characteristics of carbapenem-resistant *A. baumannii* isolates

Isolate (AB)	Carbapenemase activity (OXA[b])	Isolation date	MIC[c] (mg l^{-1}) range MEM[d]
AB-1	OXA-23, OXA-51	2011	32
AB-2	OXA-23, OXA-51	2012	64
AB-3	OX -23, OXA-51	2012	32
AB-4	OXA-23OXA-51	2013	16
AB-5	OXA-23OXA-51	2013	32
AB-6	OXA-23OXA-51	2013	8
AB-7	OXA-51	2014	8
AB-8	OXA-23OXA-51	2014	32
AB-9	OXA-23OXA-51	2016	128
AB-10	OXA-23, OXA-51	2018	128

AB A. baumannii, *OXA* Oxacillinase, *MIC* minimum inhibitory concentration, *MEM* Meropenem

Table 3 Synergistic activity of eravacycline in combination with colistin

Isolate (AB)	MIC [b] (mg l^{-1}) range				FIC_{min}	Interpretation ADD, IND, SYN
	Alone		In combination			
	Eravacycline	Colistin	Eravacycline	Colistin		
AB-1	2	1	0.5	0.5	0.75	ADD
AB-2	1	1	1.0	0.03	1.03	IND
AB-3	2	0.5	0.03	0,5	1.01	IND
AB-4	2	1	2.0	0.03	1.03	IND
AB-5	1	1	1.0	0.06	1.06	IND
AB-6	2	32	1.0	16	1.00	ADD
AB-7	4	1	4.0	0.03	1.03	IND
AB-8	4	128	1.0	32	0.50	SYN
AB-9	2	256	2.0	4.0	1.01	IND
AB-10	4	0,5	2.0	0.03	0.56	ADD

AB A. baumannii, *MIC* minimum inhibitory concentration, FIC_{min} smallest total fractional inhibitory concentration, *ADD* additive, *IND* indifference, *SYN* synergy

Therefore, to restrict the emergence of resistance, combination therapies include eravacycline might be an option for treating CRAB infections [11]. Colistin is the most commonly used antibiotic in combination therapies in HCAI caused by CRAB [11, 38, 39]. However, colistin resistance is gradually increasing especially in CRAB strains. Colistin-resistant CRAB isolates were also included in our study. Colistin resistance was detected by broth microdilution method. However, colistin resistance mechanism could not be evaluated. This is considered to be a limitation of our study. We found synergistic and additive effect without antagonism in both colistin and carbapenem-resistant strains.

Synergistic activities of antimicrobial agents have studied by chequerboard microdilution method and simultaneous time-kill analysis. Synergy frequencies may vary depending on the method used. Compared with the time kill method, the chequerboard method reveals lower synergy frequencies [11]. In our study, the in vitro synergy between eravacycline and colistin was evaluated by using the chequerboard methodology. Therefore, we have limitations in our study. Our results should be evaluated and supported by other in vitro methods.

In conclusion, we found 10% synergy and 30% additive activity without antagonism between eravacycline and colistin in CRAB isolates. Similar activity is maintained in colistin-resistant CRAB isolates. The result of this study indicates that eravacycline and colistin combination may be a potential therapeutic option for the treatment of CRAB related infections.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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