BMC Microbiology

RESEARCH Open Access

Investigation of the in vitro antimicrobial activity of eravacycline alone and in combination with various antibiotics against MDR *Acinetobacter baumanni* strains

Merve Ataman[1,2] and Berna Özbek Çelik[3*]

Abstract

Background *Acinetobacter baumannii* is an obligately aerobic, non-motile, non-fermenting, gram-negative, opportunistic pathogen. The fact that this pathogen, which is the leading cause of nosocomial infections, is naturally resistant to many antibiotics and quickly acquires new resistance mechanisms gradually limits the antibiotic options that can be used in treatment. So, our study aims to investigate the in vitro antibacterial effects of eravacycline, a new tetracycline-class antibiotic, and compare this antibiotic with the antibiotics used in the clinic to treat the infection caused by *A. baumannii.* Also, eravacycline was tested in combination with meropenem or colistin against *A. baumannii* strains, which are resistant to colistin and meropenem. The antibiotic susceptibility of strains was determined by the microbroth dilution method. In addition, the agar dilution method determined the mutant inhibition concentration (MPC) values of the studied antibiotics. To investigate the effects of the antibiotics mentioned in our study on biofilm formation, the biofilm-forming abilities of the strains were evaluated by the crystal violet staining method. The bactericidal and synergistic effects of the studied antibiotics alone or in combination were determined by the time-dependent killing curve (TKC) method.

Results The present antibacterial susceptibility experiments showed that 98% of the strains were multi-drug resistant (MDR). Our results in mutant inhibition studies showed that eravacycline is an antibiotic with the potential to prevent the emergence of resistant mutants with its low MPC value. When the effects of antibiotics on biofilm formation were investigated in our thesis study, it was determined that 95% of our strains formed biofilm. In biofilm inhibition experiments, it was observed that eravacycline at minimum inhibitory concentration (MIC) inhibited biofilm formation by 84% alone, 86% combined with colistin, and 85% combined with meropenem. Our combination experiments showed that 1×MIC eravacycline-meropenem and 4×MIC eravacycline-colistin combinations were synergistic against *A. baumannii* strains. In addition, the combination of 4×MIC eravacycline-meropenem also showed bactericidal activity at the 24th hour. No antagonist effects were detected in our combination studies.

Conclusion Present results reveal essential pharmacodynamic data on eravacycline, a new antibiotic for treating *A. baumannii* infections, which poses a global threat.

*Correspondence:
Berna Özbek Çelik
bernaozbek@hotmail.com

Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Clinical trial number Not applicable.

Keywords Eravacycline, Antibiotic combination, *Acinetobacter baumannii*, Mutant prevent concentration, Biofilm Inhibition

Background

A. baumannii, a gram-negative, nonmotile, catalase-positive, oxidase-negative pathogen that thrives well in the presence of oxygen, is prevalent in hospital intensive care and burn units [1, 2]. *A. baumannii* is a significant nosocomial pathogen that can lead to severe infections, including ventilator-associated pneumonia, catheter-associated bacteremia, urinary tract infections, soft tissue infections, septicemia, meningitis, and endocarditis [3].

The high frequency of *A. baumannii* in nosocomial infections is due to this bacterium's environmental resistance, which includes its capacity to thrive in environments with limited nutrients and dryness, its resistance to disinfectants, and its extended survival on both biotic and abiotic surfaces. In addition, *A. baumannii* poses challenges in treatment due to its virulence factors, which enable it to avoid the innate immune system [4, 5]. Furthermore, these bacteria frequently have intrinsic and acquired antibiotic-resistance mechanisms that cause MDR variants to appear [6, 7]. *A. baumannii* exhibits multiple drug-resistant mechanisms, with the most prevalent modification of penicillin-binding proteins and the chromosomal efflux pump system [8]. The enormous adaptive potential of *A. baumannii* and the acquisition and transfer of antibiotic resistance determinants contribute to the failure of the most used therapeutic approaches today [9].

Research indicates that MDR *A. baumannii* has demonstrated resistance to multiple antibiotic classes, including β-lactams, cephalosporins, and carbapenems. Recently, the percentage of MDR has increased from 23 to 63%, which is four times higher than the rate reported in other MDR Gram-negative bacteria [10].

According to the 2019 Antibiotic Resistance Threats Report by the Centers for Disease Control and Prevention, *A. baumannii* has emerged as a significant threat in healthcare [11, 12]. Currently, *A. baumannii* exhibits resistance to several primary antibiotics, and its infections are commonly related to MDR [13]. The increased usage of first-line beta-lactam antibiotics, such as cephalosporins, has resulted in the rapid emergence of beta-lactam-resistant *A. baumannii* strains. Thus, the carbapenem antibiotics, which are the most resistant to the beta-lactamases except for the carbapenamases, have become the preferred antibiotics for MDR *A. baumannii* infections. Nevertheless, the spread of carbapenem-resistant strains hampers the efficacy of carbapenems and leads to treatment insufficiency. Colistin, previously disregarded because of its systemic toxicity, has been reintroduced by guidelines as a potential treatment for MDR *A. baumannii* infections. Currently, it is reported that there are resistant *A. baumannii* strains, and colistin resistance rates are rapidly increasing for both monotherapy and combination treatment protocols [14, 15]. There is an urgent demand for new medicines to treat infections caused by MDR *A. baumannii* effectively.

A new tetracycline compound, eravacycline, is a new type of antibiotic called a fully synthetic fluorocycline. It has a similar structure to tigecycline and, like other tetracyclines, inhibits bacterial protein synthesis by binding to the 30 S ribosomal subunit [16]. It is different from tigecycline in the D-ring structure by two changes: a fluorine atom replaces the dimethylamine moiety at C-7, and a pyrrolidinoacetamido group replaces the 2-tertiary-butyl glycylamido at C-9. Eravacycline has exhibited antimicrobial efficacy against Gram-positive, Gram-negative, and anaerobic bacteria, including drug-resistant *Enterobacteriaceae* and *A. baumannii*. On the other hand, it is least influenced by acquired tetracycline efflux determinants and ribosome protection. These properties of eravacycline make it a promising candidate for treating infections caused by multidrug-resistant pathogens [17, 18].

Materials and methods

Bacterial isolates

From January 1, 2021, to December 31, 2022, 100 non-duplicated *A. baumannii* isolates were collected from intensive care patients at two hospitals. All isolates were identified using the VITEK 2 compact system. The *Pseudomonas aeruginosa* standard strain ATCC 27,853 was used to confirm that MIC values were within the accuracy range declared by the Clinical and Laboratory Standards Institute (CLSI).

Antibiotics

Eravacycline dihydrochloride (Tetraphase Pharmaceuticals), meropenem trihydrate (Astra Zeneca Pharmaceuticals), colistin sulfate (Sigma-Aldrich), cefepime (Sigma-Aldrich), tobramycin (Sigma-Aldrich), and levofloxacin (Sanofi Pharmaceuticals) were used in experiments. According to their manufacturer recommendations, stock solutions of tested antibiotics were prepared in water for injection at 5.120 mg/L. Stock solutions of eravacycline, colistin, cefepime, tobramycin, and levofloxacin were frozen at -80 ° C and used within six

months. Meropenem solutions were prepared on the day of use.

Media

Cation-adjusted Mueller–Hinton Broth (CAMHB) was prepared daily by adding 25.0 mg of calcium per litre to liquid and 12.5 mg of magnesium per litre to Mueller–Hinton broth (Oxoid Ltd.). This medium was used for MICs and TKC studies. Tryptic soy agar (Difco Ltd.) was used for colony counts.

Determination of MIC

MICs were determined by the microbroth dilution technique described in the CLSI guidelines [19]. To perform the test series, test tubes were prepared with a broth medium to which different concentrations of the antimicrobial agent were added [20]. Serial twofold dilutions ranging from 256 to 0.125 mg/L for the tested antibiotics were prepared in fresh CAMHB 96-well microtiter plates. The bacterial culture was prepared according to CLSI M07 11 ed, standards [20]. To create a broth culture, the sterile liquid growth medium CAMHB was inoculated with bacteria and placed in an incubator at the appropriate temperature. The inoculum was prepared with a 4- to 6-hour broth culture. Each bacterial strain was spectrophotometrically adjusted to OD600 = 0.12–0.13, corresponding to approximately 1×10^8 colony-forming units per millilitre (CFU/ml) and further diluted in CAMHB to obtain a final concentration of 5×10^5 CFU/ml in the test tray. The trays were placed in plastic bags to avoid evaporation, incubated at 37 °C for 18–20 h, and visually inspected for growth. CLSI interpretative criteria for the susceptibility of *P. aeruginosa* ATCC 27,853 were used.

Determination of the MPC

The MPC for eravacycline, meropenem, colistin, tobramycin, levofloxacin, and cefepime was determined for the *A. baumannii* ATCC 19,606 standard strain. An initial inoculum of approximately 10^{10} CFU/mL was prepared from overnight cultures grown in CAMHB for 24 h of incubation with shaking at 37 °C. In this assay, 10^{10} CFU of bacteria were applied to TSA plates containing antibiotics, each differing by two-fold dilutions (eravacycline concentration 2 to 128 mg/L, meropenem 4 to 256 mg/L, colistin 4 to 256 mg/L, tobramycin 8 to 512 mg/L, levofloxacin 2 to 1280 mg/L, cefepime 16 to 1024 mg/L). Plates were incubated for 48 h at 37 °C. The MPC was recorded as the lowest antibiotic concentration at which no colonies grew on an agar plate [21].

Detection of biofilm formation by the microtiter plate assay

In the study, the biofilm formation ability of the 100 *A. baumannii* on polystyrene plates was performed using the microtiter plate assay. In brief, the turbidity of each isolate grown overnight in 5 ml Tryptic Soy Broth (TSB) supplemented with 1% glucose was adjusted to 0.5 McFarland and diluted in fresh TSB-glucose, yielding a final concentration of approximately 1 × 107 CFU/ 200 µl. An aliquot of 200 µl of this suspension was added to the wells of a 96-well tissue culture microtiter plate (Greiner). Sterile TSB-glucose was used as a negative control, while *A. baumannii* strain was used as a positive control. Microplates were incubated at 24 h at 37 °C. After incubation, the waste media was aspirated gently. The plates were washed with sterile phosphate-buffered saline three times to remove planktonic cells in microplate wells and then air-dried and fixed with 200 µL of 99% methanol for 15 min. The wells were decanted to dry in the air and stained with 200 µL of 0.1% crystal violet solution (in water) for 5 min. The excess stain was gently rinsed off with tap water, and the plates were air-dried. The stain was re-solubilized by adding 200 µL of 95% ethanol and shaking the plate on an orbital shaker for 30 min. The optical density (OD) was measured at OD595 nm using a spectrophotometer. The results were categorized as non-biofilm, weak, moderate and strong biofilm producers based on the OD values [22]. The findings were analyzed based on the cut-off point between the optical density averages of the negative control (ODc) and the tested strains (OD). The strains were categorized as non-biofilm producers (OD ≤ ODc), weak (ODc < OD ≤ 2×ODc), moderate (2×ODc < OD ≤ 4×ODc) and strong biofilm producers (4×ODc < OD) [23].

Biofilm attachment assays

An overnight culture of strong biofilm-producing *A. baumannii* strain was diluted 1/50 to obtain 1×10^7 CFU/200 µl in TSB supplement with 1% glucose and added to each well of a 96-well tissue culture microtiter plate with 1/10 the MICs of eravacycline, meropenem and colistin and their combinations. The plates were incubated for 1, 2, and 4 h at 37 °C. Six wells were used for each alone antibiotic or antibiotic combination. The positive control was the *A. baumannii* strain in TSB supplemented with 1% glucose without antibiotics. After incubation, wells were washed with PBS solution and measured at OD 595 nm to measure bacterial cell density [24].

Inhibition of biofilm formation

Strong biofilm-producing *A. baumannii* strain 1×10^5 CFU/ 200 µl in TSB supplement with 1% glucose was incubated at 37 °C, 24 h, with antibiotics (eravacycline, meropenem and colistin) or their combinations at 1 MIC, 1/10 the MIC, and 1/100 the MIC in 96-well tissue culture microtiter plates. Six wells were used for each antibiotic or combination. The positive control was the *A. baumannii* strain in TSB supplemented with glucose

Table 1 In vitro activities of tested antibiotics against 100 *A. baumannii* strains

Antibiotics	MIC (µg/ml)					Serum concentration (µg/mL)
	MIC range	%50	%90	$MIC_{50/90}$	%R	
TOB	0.25->256	64–128	256 - >256	1/4	%68 (68)	4–10 [26]
LVX	< 0.125-256	8–16	64–128	1/8	%95 (95)	6.4 [27]
CPM	4->256	128–256	256 - >256	1/2	%98 (98)	39.1 [28]
MEM	1->256	64–128	128–256	1/2	%99 (99)	26 [29]
CST	< 0.125->256	0.25–0.5	32	1/128	%24 (24)	2 [30]
ERV	0.25-32	2–4	4–8	1/2	*	1–10 [31]

Note: *According to the CLSI 2022 guideline, eravacycline susceptibility data for tested bacteria has not been established. TOB, tobramycin; LVX, levofloxacin; CPM, cefepime; MEM, meropenem; CST, colistin; ERV, eravacycline. R, resistance

without antibiotics. After incubation, wells were washed with PBS solution and measured at OD 595 nm to measure bacterial cell density.

Determination of TKC

TKC assays were performed to observe the dynamics of the bactericidal activity of eravacycline, meropenem and colistin alone on five isolates of *A. baumannii* at 1×MIC and 4×MIC. TKC assays were also performed to evaluate the concentration-dependent bactericidal and synergistic activity of eravacycline when combined with colistin and meropenem at 1×MIC and 4×MIC, following the CLSI [25]. The broth culture from TKC assays was sampled for colony counts at 0, 2, 4, 6, and 24 h. In this method, 20 CFU/ml was the lower detection limit. Bactericidal activity was defined as a log 10 CFU/ml decrease ≥ 3 in the original inoculum within 24 h. synergy and antagonism were defined as a log 10 CFU/ml decrease or increase > 2, respectively, with CFU/ml at 24 h for the antibiotic combination compared with the most active antimicrobial agent alone. The additive effect was described as a log 10 CFU/ml decrease in colony count < 2 at 24 h by the combination compared with the most active single antimicrobial alone.

Statistical analysis

The study's statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Two-way ANOVA tests followed by Tukey's multiple comparison tests were used to determine the statistical significance among the control and antimicrobial groups. P-value < 0.0001 indicated statistical significance.

Results

Susceptibility

Table 1 shows the MICs of tobramycin, levofloxacin, cefepime, meropenem, colistin and eravacycline against the 100 strains of *A. baumannii.*

The results show that 98% of the isolates tested exhibited the MDR phenotype, defined as an isolate that is not susceptible to at least one agent in at least three antimicrobial classes. The MIC ranges of tobramycin, levofloxacin, cefepime, meropenem, colistin and eravacycline were found to be 0,25->256 mg/L, < 0,125–256 mg/L, 4->256 mg/L, 1->256 mg/L, < 0,125->256 mg/L and 0,25–32 mg/L, respectively. According to the CLSI breakpoint, the results revealed that 31%, 3%, 1%, 1%, and 76% of tested strains were susceptible to tobramycin, levofloxacin, cefepime, meropenem, and colistin, respectively. Eravacycline could not be included because the CLSI guidelines do not recommend the MIC susceptibility breakpoints for eravacycline against *A. baumannii.* The lowest MIC50 results were 0.25–0.5 obtained with colistin. Colistin was the second most effective antibiotic, with a MIC90 value of 32 mg/L. The lowest MIC90 results were 4–8, obtained with eravacycline. Eravacycline showed greater activity than the other antibiotics (Table 1).

Table 2 Results of MPC values of *A. baumannii* ATCC 19,606 strain

Antibiotics	MIC (µg/ml)	MPC (µg/ml)	MPC / MIC
TOB	8	128	16
LVX	0.5	1	2
CPM	32	1024	32
MEM	2	8	4
CST	2	> 256	> 256 / 2
ERV	2	4	2

TOB, tobramycin; LVX, levofloxacin; CPM, cefepime; MEM, meropenem; CST, colistin; ERV, eravacycline

Determination of MPC

The MPC experiments with the *A. baumannii* ATCC 19,606 strain determined the potential of tobramycin, levofloxacin, cefepime, meropenem, colistin, and eravacycline to inhibit the emergence of mutant strains. The MIC and MPC results for tested antibiotics are shown in Table 2. The MPC results obtained against the strain for tobramycin, levofloxacin, cefepime, meropenem, colistin, and eravacycline were 16, 2, 32, 4, > 128, and they were twofold higher than their MIC value, respectively.

The MPC values for tobramycin, cefepime, and colistin were above the breakpoint value for each antibiotic. For levofloxacin, MPC against *A. baumannii* ATCC 19,606

were below the breakpoint value. No breakpoints for eravacycline have been generated yet; eravacycline, similar to levofloxacin, showed an MPC effect at a concentration twice its MIC value.

Biofilm formation

The biofilm-forming abilities of 100 MDR *A. baumannii* strains were tested. In the biofilm-positive *A. baumannii* strains, 32% of isolates were strong producers, while 34% and 29% were moderate and weak, respectively. The biofilm-forming abilities of 100 MDR *A. baumannii* strains were tested. Of the 95 biofilm-positive *A. baumannii* strains, 32% of isolates were strong producers, while 34% and 29% were moderate and weak, respectively. Five strains were not biofilm producers (Table 3).

Table 3 The biofilm-forming capabilities of 100 MDR *A. baumannii* strains [23]

Microorganisms	No biofilm (0)	Weak biofilm (+)	Moderate biofilm (+ +)	Strong biofilm (+ + +)
A. baumannii ($n=100$)	5% (5)	29% (29)	34% (34)	32% (32)

Biofilm attachment assay

When incubated the 1/10 × MIC of antibiotics with strong biofilm-forming strains of *A. baumannii* for 1, 2–4 h at 37 °C to determine the adherence to the wells, all tested antibiotics inhibited biofilm attachment processes. Inhibition rates of adhesion showed a time- and concentration-dependent effect. Eravacycline was found to be more efficient after four hours of incubation. The most potent agent for inhibition of adhesion was eravacycline (up to 30%). This result was statistically significant ($P<0.0001$) against the control. However, no significant antibiofilm inhibition effect was observed in the other antibiotics tested compared to the control (Fig. 1).

Inhibition of biofilm formation

The rates of biofilm formation inhibition were dependent on concentration, and the highest inhibition rates were seen at 1×MICs for all agents, as expected. Eravacycline showed significant inhibitory activity against biofilm formation of *A. baumannii* strains at 24 h. Eravacycline was the most efficient agent for inhibiting biofilm formation (84%). The concentration-dependent increase in antibiofilm activity was seen in eravacycline, and 80% antibiofilm activity was detected for meropenem. While it was observed that the eravacycline-colistin combination inhibited biofilm formation by 86% at 1×MIC, this rate was similarly 85% at the eravacycline-meropenem concentration. Significant biofilm inhibition was observed for all studied antibiotics at 1×MIC values (Fig. 2).

Results of TKC

According to the susceptibility test results, time-kill studies were performed with four antibiotics (meropenem, colistin, and eravacycline) against clinical 4 *A. baumannii* isolates and *A. baumannii* ATCC 19,606. Each chosen strain has represented a different antibiotic susceptibility pattern. The results of the TKC are given in Fig. 3.

The data obtained from the TKC experiments revealed that meropenem, colistin, and eravacycline alone did not display any bactericidal effect (at least 3log killing) against tested strains within 24 h, either at 1×MIC or 4×MIC.

In the present study, to explore the antimicrobial activities of antibiotics in combination, we combined eravacycline, a new synthetic, halogenated tetracycline antibiotic, with meropenem and colistin, which had already been included in the *A. baumannii* treatment protocols.

The synergistic activity of the eravacycline-colistin combination at 4×MIC was demonstrated for one of

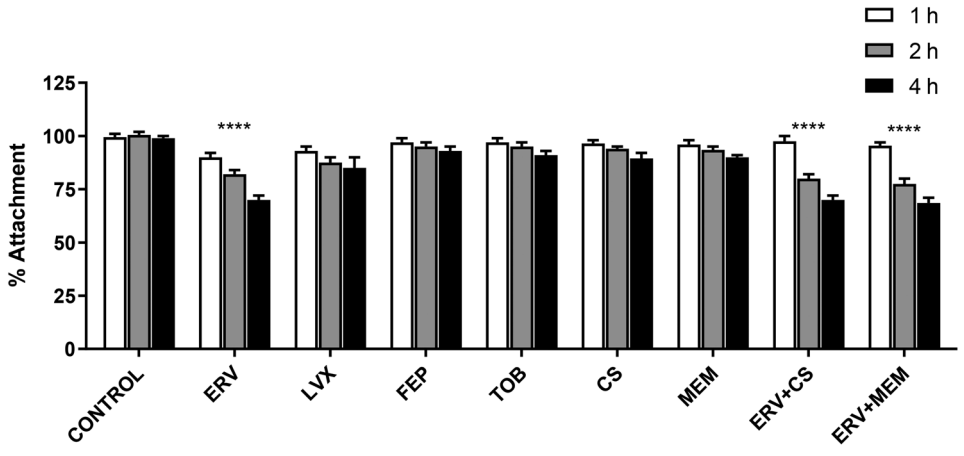

Fig. 1 Inhibition of surface attachment of *A. baumannii* ATCC 19,606 strain in wells containing antibiotics

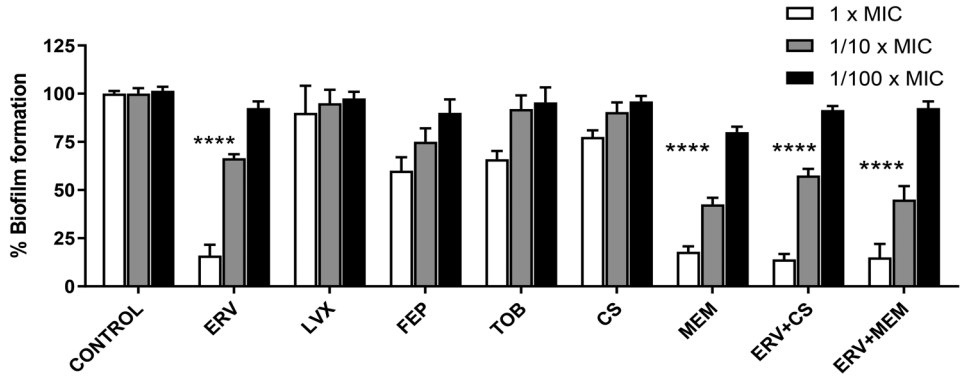

Fig. 2 Inhibition of the formation of biofilm by the *A. baumannii* ATCC 19,606 strain by antibiotics

five strains (AB-29); however, the same combination at 1×MIC had an additive effect in all studied strains. On the other hand, when the eravacycline-meropenem combination was used at 1×MIC, it showed a synergistic effect against one of four strains (AB-35); this combination at 4×MIC had an additive effect against all tested strains (Table 4).

Discussion

A. baumannii has become an extremely dangerous pathogen responsible for causing life-threatening infections in communities and hospitals. In the past, traditional broad-spectrum antibiotics were successful in treating infections caused by *A. baumannii*. However, only a limited selection of antibiotics can efficiently combat this pathogen. One of the most significant challenges posed by this pathogen is the crisis of antibiotic resistance worldwide [32].

Because of the lack of effective antimicrobials, the clinical management of infections caused by MDR *A. baumannii* has become more complicated and innovative infection management methods have become necessary.

The present study provides data to promote the development of new approaches to combat MDR *A. baumannii* infections. The present study has investigated the in vitro effects of various antibiotics alone or combined with eravacycline, a new, fully synthetic fluorocycline, against five strains of *A. baumannii*. MDR is the characterisation of resistance to at least one antimicrobial agent across three or more categories [33]. According to the current study results, 98 out of 100 randomly collected isolates were classified as MDR, indicating that the resistance rate in *A. baumannii* is a crucial problem.

Similarly, Liu et al. investigated the resistance rates of 128 MDR *A. baumannii* strains in 2018–2020 in China. A 100% resistance rate was detected against ten antibiotics, including tobramycin, levofloxacin, cefepime, and meropenem [34].

On the other hand, according to the SENTRY Antimicrobial Surveillance Program results, colistin resistance in *A. baumannii* was around 6.1% in Europe from 1993 to 2003, while by 2013–2016, the rate had increased dramatically to 10.4%. Turkey is also one of the countries with the highest rates of antibiotic resistance [35, 36]. In the present study, *A. baumannii* isolates showed colistin resistance with a rate of 24%. Also, a study conducted in Korea utilized broth microdilution testing to determine the prevalence of colistin resistance, and it displayed a resistance rate of 30.6%, which was higher than the present study [37].

Although carbapenems are the gold standard medicines for treating *A. baumannii* infections, meropenem resistance was very high, as seen in the present data, and it had the lowest susceptibility percentage among the antibiotics tested in the investigation. Unfortunately, these results make this antibiotic unsuitable for empirical treatment for treating *A. baumannii* infections. The present investigation also observed a 24% colistin resistance rate; this antibiotic was the most sensitive against tested *A. baumannii* strains. Studies conducted both internationally and domestically corroborate the results. Colistin is still used as a last resort to treat infections caused by MDR *A. baumannii* and *P. aeruginosa* [38, 39].

There are no established breakpoints for eravacycline against *A. baumannii*; however, the MIC90 values of eravacycline against *A. baumannii* were the lowest among the tested antibiotics. According to the results, the MIC90 of eravacycline against *A. baumannii* was eight µg/ml. Eravacycline showed significant in vitro efficacy against MDR *A. baumannii* isolates. It displayed the lowest MIC50 value (4 µg/ml) among the antibiotics tested, compared to colistin (0.5 µg/ml). When compared, the eravacycline MIC 50/90 results were under the serum concentration of eravacycline. This data means that eravacycline might be an alternative option for treating MDR *A. baumannii* infections.

In a study conducted in Turkey, Ozger et al. discovered that in 10 carbapenem-resistant *A. baumannii* (CRAB) strains, MIC values for eravacycline and colistin ranged from 1 to 4 µg/ml and 0.5 to 256 µg/ml, respectively [40].

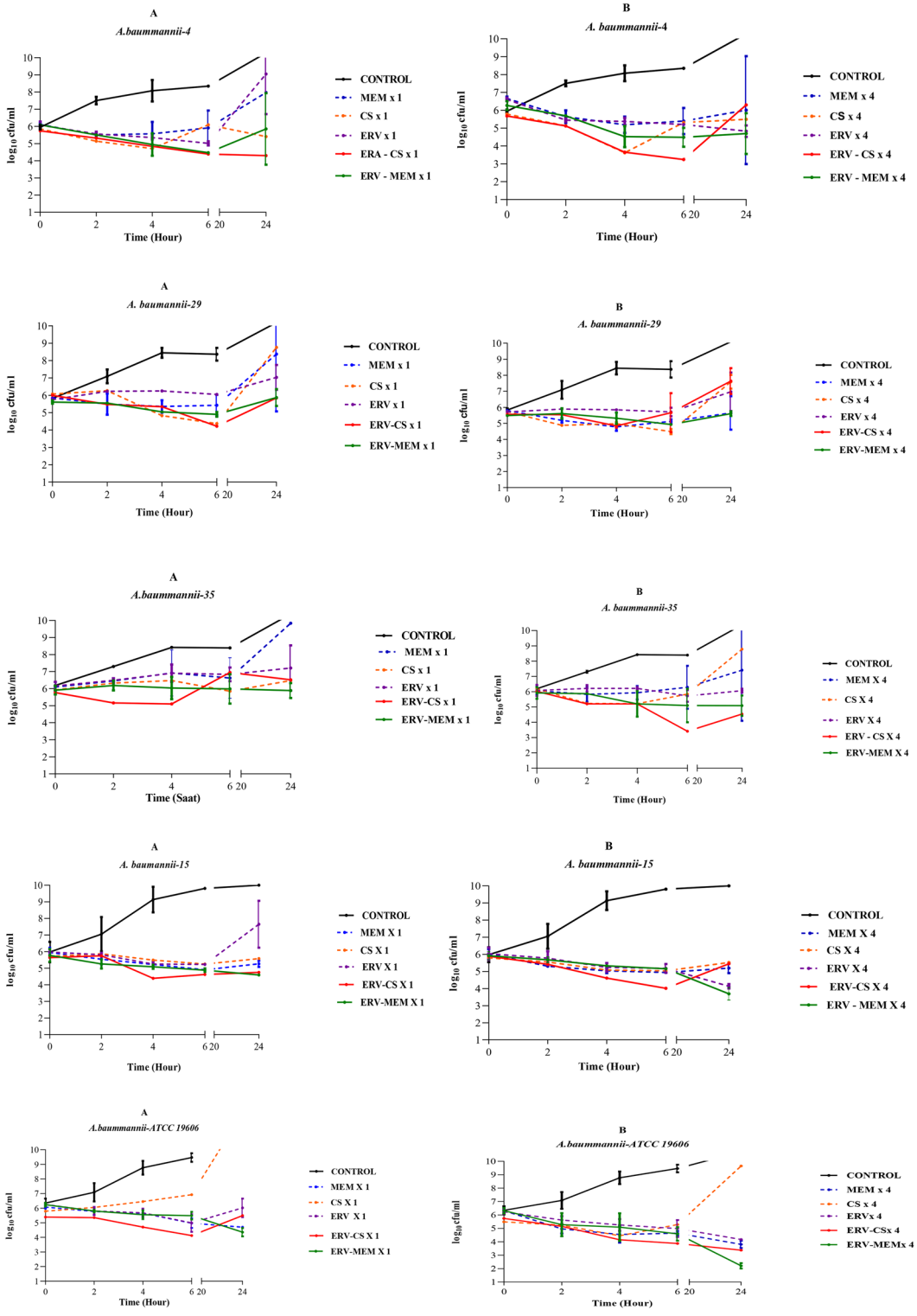

Fig. 3 TKC analysis for meropenem, colistin, and eravacycline alone and eravacycline-colistin and -meropenem combination against 5 *A. baumannii* strains. The mean value of antimicrobial effects against five bacteria at 1×MIC (A) and 4×MIC (B). ERV, eravacycline; MEM, meropenem; CS, colistin

Table 4 Bactericidal and synergistic effects of tested antibiotic combinations against five *A. baumannii* strains

Strain	Antibiotics			Combination effects							
				Erv-Mem				Erv -Cst			
				1xMIC		4xMIC		1xMIC		4xMIC	
	Erv	Mem	Cst	S/A	Bct	S/A	Bct	S/A	Bct	S/A	Bct
AB- 4	2	8	8	A	-	A	-	A	-	A	-
AB-29	4	16	4	A	-	A	-	A	-	S	-
AB-35	2	16	4	S	-	A	-	A	-	A	-
AB-15	0,5	1	4	A	-	A	+	A	-	A	-
AB-ATCC 19,606	2	2	2	A	-	A	-	A	-	A	-

Note: S, Synergy: A, Additive; Bct, Bactericidal effect

According to the EUCAST guideline, the ranges of eravacycline MIC values observed in only 30 *A. baumannii* strains are as follows: 9, 2/29, and 4/29 for 0.25, 0.5, 1, 2, and 4 µg/ml.

(https://mic.eucast.org/search). In our study, the MIC distributions for 0.25, 0.5, 1, 2, 4, 8, 16, and 32 µg/ml were 3/100, 4/100, 9/100, 22/100, 29/100, 28/100, 3/100, and 2/100, respectively. The clinical strains used in these two experiments differed, which might explain the differences in MIC distribution.

Li et al. conducted a study in China in 2022 to investigate the susceptibility values of eravacycline, imipenem, ceftazidime, cefoperazone-sulbactam, ciprofloxacin, amikacin, and polymyxin B against MDR *A. baumannii* strains. The MIC90 values of these antibiotics were found to be 1, 128, >512, 128, 512, >512, and 1 µg/ml, respectively. The study showed that eravacycline and polymyxin B had the lowest MIC90 (1 µg/ml) value [41].

The results of the present investigation revealed that eravacycline exhibited a promising antibacterial action, as evidenced by its lower MIC values compared to other antibiotics tested.

These results encourage the hope of using eravacycline as an alternative to antibiotic therapy, as it shows fewer adverse effects than colistin. They also suggest that eravacycline may be a new treatment for MDR *A. baumannii* infections, which have reached alarming levels.

The MPC was described as a novel in vitro measurement of antimicrobial susceptibility and the frequency of a microorganism to mutate and develop resistance to a given antibiotic [42].

In their study, Cai et al. discovered that the MPC range of colistin against 30 MDR *A. baumannii* strains was approximately 32 to >128 µg/ml. Moreover, the MPC90 was higher than 128 µg/ml. The MPC/MIC values for 24 (80%) of the 30 strains in the study were ≥128 µg/ml [43]. These results are consistent with our study's colistin MPC/MIC results.

A lower MPC/MIC ratio indicates a greater ability to prevent the emergence of mutant strains [44]. The MPC was described as a novel in vitro measurement of antimicrobial susceptibility and the frequency of a microorganism to mutate and develop resistance to a given antibiotic. In the present study, eravacycline had the lowest MPC/MIC value of all the antibiotics examined with levofloxacin; MPC was two-fold higher than MIC (MPC/MIC = 2).

Eravacycline with a low MPC value can inhibit the development of drug-resistant mutants. So, the MPC can be beneficial in developing therapeutic antibiotic regimens, especially for the long-term therapy of immunodeficient individuals.

There is a notable rise in biofilm infections related to medical equipment such as prosthetic joints, cardiac pacemakers, catheters, and shunts [45]. So, studies on the antibiofilm activity of an antimicrobial agent are of great importance, and these studies mainly concentrate on the early phases of biofilm development. The microorganism's antibiotic resistance is more significant when it reaches the mature biofilm stage. For this reason, it is essential for the antibacterial effect to prevent the bacteria's irreversible adhesion to a surface in the early stage of biofilm production. The present study determined that 95% of the tested isolates formed biofilms, and this significant biofilm production ability makes them particularly problematic in nosocomial infections.

In the present study, when the inhibition of biofilm formation against microbial biofilms was tested, it was observed that 1×MIC eravacycline solution inhibited biofilm formation by 84%. The antibiofilm activity of meropenem and colistin alone was found to be 80% and 22.5%, respectively, under the same test conditions. On the other hand, the eravacycline-colistin combination at 1×MIC inhibited biofilm formation by 86%, while the eravacycline-meropenem combination similarly inhibited it at 85%. These results suggest that the antibiofilm effect of eravacycline is adequate to prevent the development of biofilm-associated infections.

Antibiotic combinations synergising in vitro in immunosuppressed individuals have been linked to promising clinical outcomes [46]. On the other hand, combination regimens can provide broader coverage than monotherapy for possible pathogens, increase the likelihood of bactericidal antibiotic concentrations at the infection

site, reduce the probability of heteroresistant evolution, and provide synergy [47–49]. For this reason, especially in immunosuppressive patients, they offer a potential solution to the problems of *A. baumannii* infections, which are resistant to carbapenems and other antibiotic choices.

In the present study, it was investigated meropenem and colistin, the antibiotics of choice for treating *A. baumannii*, in combination with eravacycline for their bactericidal and synergistic effects at 1×MIC and 4×MIC for 24 h. TKC assay results showed that eravacycline produces bactericidal activity when combined with meropenem, which are commonly used to treat Acinetobacter infections. This bactericidal effect was determined against the strain *A. baumannii*-15, which is susceptible to meropenem and resistant to colistin. This effect was present even when eravacycline alone and colistin alone were not bactericidal at 24 h.

When the experimental results were evaluated for synergy, the 1×MIC eravacycline-meropenem combination showed a synergistic effect against *A. baumannii*-35 at 24 h, while it had an additive effect against the other four strains (Fig. 1). In addition, the fact that *A. baumannii*-35, a strain with synergistic effects with a combination of 1×MIC, was resistant to carbapenems (meropenem MIC 16 µg/ml) highlights the clinical implications of the synergistic effect that we identified in our research. Furthermore, it was detected that the additive effects against five strains used with 4×MIC antibiotic combinations of eravacycline-meropenem (Fig. 1).

A synergistic effect was observed in one (*A. baumannii*-29) of the five strains we used with the 4×MIC eravacycline-colistin combination and an additive effect against four strains (Fig. 1). The synergistic effect of the 4×MIC eravacycline-colistin combination on the *A. baumannii*-29 strain showing colistin resistance (4 µg/ml) shows the clinical importance of the obtained synergistic effect. At the end of 24 h, additive effects against all tested strains with 1×MIC antibiotic combinations of eravacycline-colistin were detected.

The current in vitro experiment results provide the first evidence by the TKC method that the synergistic activity of eravacycline combinations in strains resistant to meropenem and colistin indicates that these combinations are candidates for evaluation in vivo clinical investigations. Combination therapies with agents with different mechanisms of action will slow down resistance development, which has become a significant clinical problem, especially in colistin-resistant *A. baumannii* strains.

According to a study by Li et al., using the checkerboard method to investigate the synergistic effect of eravacycline against carbapenem-resistant gram-negative strains [41], the eravacycline-polymyxin B combination was found to be the most effective combination against Escherichia coli and Klebsiella pneumoniae with 30% synergism. Also, Deolankar et al. conducted a study to evaluate the effectiveness of eravacycline in combination with amikacin, meropenem, ceftazidime, levofloxacin, ampicillin-sulbactam, and trimethoprim-sulfamethoxazole against an MDR *A. baumannii*. That strain also had a low MIC against eravacycline. As a result of the study, additive and synergistic combinations were observed only for eravacycline and amikacin [50].

Another study by Ozger et al. investigated the activity of eravacycline in combination with colistin on ten CRAB isolates using the checkerboard synergy test; no antagonism was observed between eravacycline and colistin, and 10% synergistic and 30% additive effect was observed [51]. This study's findings substantiate our research and demonstrate that combining colistin and eravacycline can be a therapeutic alternative for treating CRAB and MDR *A. baumannii*-related infections.

Conclusion

In conclusion, eravacycline, a recently developed antibiotic derived from tetracycline, might be an option for treating *A. baumannii* infections. This is due to its low MIC and MPC, its enhanced bactericidal activity when combined with meropenem and colistin, and its ability to prevent the formation of biofilms. Additionally, the gained data have shown synergy at 1×MIC and 4×MIC, implying that these results might present promising alternatives for treating severe *A. baumannii* infections. Our findings, which include critical eravacycline pharmacodynamic parameters, will guide future clinical research.

The present study has some limitations. The number of bacterial isolates included was limited, and they may not represent all *A. baumannii* isolates. In this work, we only performed time-kill tests to assess the effectiveness of antibiotic combinations in vitro. Using the 4×MIC of colistin presented a challenge in achieving adequate concentration levels to treat the infection.

Acknowledgements
None.

Author contributions
Conceptualization: B.O.C, M.A.; Supervision: B.O.C.; Resources: M.A.; Materials: B.O.C., M.A.; Data Collection and/or Processing: M.A.; Analysis and/or Interpretation: B.O.C., M.A.; Literature Search: B.O.C., M.A.; Writing: M.A., B.O.C.; Critical Reviews – B.O.C. All authors reviewed and confirmed the manuscript.

Funding
The present work was supported by the Research Fund of Istanbul University. BAP. Project No. TDK-2019-34283.

Data availability
Data is provided within the manuscript.

Declarations

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee Istanbul, Turkey, under the ethical code 2019/454.

Informed consent

All enrolled subjects provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Pharmaceutical Microbiology, Istanbul University Institute of Graduate Studies in Health Sciences, Beyazıt, Istanbul 34116, Turkey
[2]Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul Aydın University, Istanbul 34295, Turkey
[3]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, Beyazit, Istanbul 34116, Turkey

Received: 12 November 2024 / Accepted: 20 March 2025
Published online: 26 March 2025

References

1. Alp E, Coruh A, Gunay GK, Yontar Y, Doganay M. Risk factors for nosocomial infection and mortality in burn patients: 10 years of experience at a university hospital. J Burn Care Res. 2012;33(3):379–85.
2. Sieniawski K, Kaczka K, Rucińska M, Gagis L, Pomorski L. A*cinetobacter baumannii* nosocomial infections. Pol Przegl Chir. 2013;85(9):483–90.
3. Havenga B, Reyneke B, Waso-Reyneke M, Ndlovu T, Khan S, Khan W. Biological control of *Acinetobacter baumannii*: in vitro and in vivo activity, limitations, and combination therapies. Microorganisms. 2022;10(5):1052.
4. Monem S, Furmanek-Blaszk B, Łupkowska A, Kuczyńska-Wiśnik D, Stojowska-Swędrzyńska K, Laskowska E. Mechanisms protecting *Acinetobacter baumannii* against multiple stresses triggered by the host immune response, antibiotics and Outside-Host environment. Int J Mol Sci. 2020;21(15):5498.
5. Jeffreys S, Chambers JP, Yu JJ, Hung CY, Forsthuber T, Arulanandam BP. Insights into *Acinetobacter baumannii* protective immunity. Front Immunol. 2022;13:1070424.
6. Lee CR, Lee JH, Park M, et al. Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol. 2017;7:55.
7. Li P, Zhang S, Wang J, et al. Uncovering the secretion systems of *Acinetobacter baumannii*: structures and functions in pathogenicity and antibiotic resistance. Antibiot (Basel). 2023;12(2):195.
8. Marchand I, Damier-Piolle L, Courvalin P, Lambert T. Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the aders two-component system. Antimicrob Agents Chemother. 2004;48(9):3298–304.
9. Chen C, Ke S, Li C, Chang C. The comparison of genotyping, antibiogram, and antimicrobial resistance genes between carbapenem-susceptible and -resistant *Acinetobacter baumannii*. Comp Immunol Microbiol Infect Dis. 2014;37(5–6):339–46.
10. Giammanco A, Calà C, Fasciana T, Dowzicky MJ. Global assessment of the activity of Tigecycline against multidrug-resistant gram-negative pathogens between 2004 and 2014 as part of the Tigecycline evaluation and surveillance trial. mSphere. 2017;2(1):e00310–16.
11. Centers for Disease Control and Prevention. Acinetobacter in healthcare settings. Centers Disease Control Prev Consulté Le. 2019;30:05–22.
12. Towner KJ. Acinetobacter: an old friend, but a new enemy. J Hosp Infect. 2009;73(4):355–63.
13. Ayoub Moubareck C, Hammoudi Halat D. Insights into *Acinetobacter baumannii*: A review of Microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. Antibiot (Basel). 2020;9(3):119.
14. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens. 2021;10(3):373.
15. Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN. Multidrug-resistant *Acinetobacter baumannii* as an emerging concern in hospitals. Mol Biol Rep. 2021;48(10):6987–98.
16. Zhanel GG, Cheung D, Adam H, et al. Review of Eravacycline, a novel fluorocycline antibacterial agent. Drugs. 2016;76(5):567–88.
17. Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, Quale J. Activity of Eravacycline against *Enterobacteriaceae* and *Acinetobacter baumannii*, including multidrug-resistant isolates, from new York City. Antimicrob Agents Chemother. 2015;59(3):1802–5.
18. Livermore DM, Mushtaq S, Warner M, Woodford N. In vitro activity of Eravacycline against Carbapenem-Resistant *Enterobacteriaceae* and *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2016;60(6):3840–4.
19. CLSI, Clinical & Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. M100. 2022. https://clsi.org/standards/products/microbiology/documents/m100/. Accessed 1 Mar 2023.
20. CLSI, Clinical & Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. M07. 2018. https://clsi.org/standards/products/microbiology/documents/m07/. Accessed 1 May 2023.
21. Drlica K. The mutant selection window and antimicrobial resistance. J Antimicrob Chemother. 2003;52(1):11–7.
22. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of Staphylococcal biofilm formation. J Microbiol Methods. 2000;40(2):175–9.
23. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Res Int. 2016;2016:2475067.
24. Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun. 2008;76(9):4176–82.
25. CLSI. Clinical & Laboratory Standards Institute. Methods for determining bactericidal activity of antimicrobial agents. Approved Guideline M26-A CLSI, 1999.
26. Demirkan K. Terapötik Ilaç Monitorizasyonu. J Crit Intensive Care. 2007;7(3):365–9.
27. Günal E, Erdem H, Kinolonlar. İç Hastalıkları Dergisi. 2014;21:69–85.
28. Pais GM, Chang J, Barreto EF, et al. Clinical pharmacokinetics and pharmacodynamics of cefepime. Clin Pharmacokinet. 2022;61(7):929–53.
29. Sepúlveda RA, Downey P, Soto D, et al. Plasma and renal cortex meropenem concentrations in patients undergoing percutaneous renal biopsy. Biomed Res Int. 2019;2019:1368397.
30. Garnacho-Montero J, Timsit JF. Managing *Acinetobacter baumannii* infections. Curr Opin Infect Dis. 2019;32(1):69–76.
31. Scott LJ, Eravacycline. A review in complicated intra-abdominal infections. Drugs. 2019;79(3):315–24.
32. Bergogne-Bérézin E, Towner KJ. *Acinetobacter spp.* as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 1996;9(2):148–65.
33. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis. 2010;2(3):291–304.
34. Liu H, Hu D, Wang D, Wu H, Pan Y, Chen X et al. In vitro analysis of synergistic combination of polymyxin B with 12 other antibiotics against MDR *Acinetobacter baumannii* isolated from a Chinese tertiary hospital. J Antibiot (Tokyo). 2022;20–6.
35. Gales AC, Seifert H, Gur D, Castanheira M, Jones RN, Sader HS. Antimicrobial susceptibility of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and *Stenotrophomonas maltophilia* clinical isolates: results from the SENTRY antimicrobial surveillance program (1997–2016). Open Forum Infect Dis. 2019;6(Suppl 1):S34–46.
36. Ozekinci T, Habip Z, Onder N, Koçoglu ME. Antibiotic resistance of *Acinetobacter baumannii* strains isolated in 2015–2018 years. Van Med J. 2020;27(3):340–4.
37. Ko KS, Suh JY, Kwon KT, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother. 2007;60(5):1163–7.
38. Çağlan E, Nigiz Ş, Sancak B, Gür D. Resistance and heteroresistance to colistin among clinical isolates of *Acinetobacter baumannii*. Acta Microbiol Immunol Hung. 2020;67(2):107–11.
39. Mumcuoglu İM, Caglar H, Erdem D, Aypak A, Gun P, Kursun S, et al. Evaluation of the secondary bacterial infections of respiratory tract in Covid 19 patients. J Infect Dev Ctries. 2022;16(7):1131–7.

40. Ozger HS, Cuhadar T, Yildiz SS, et al. In vitro activity of Eravacycline in combination with colistin against carbapenem-resistant *A. baumannii* isolates. J Antibiot (Tokyo). 2019;72(8):600–4.
41. Li Y, Cui L, Xue F, Wang Q, Zheng B. Synergism of Eravacycline combined with other antimicrobial agents against carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii*. J Glob Antimicrob Resist. 2022;30:56–9.
42. Blondeau JM. New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. Vet Dermatol. 2009;20(5–6):383–96.
43. Cai Y, Li R, Liang B, Bai N, Liu Y, Wang R. In vitro antimicrobial activity and mutant prevention concentration of colistin against *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2010;54(9):3998–9.
44. Zhao X. Clarification of MPC and the mutant selection window concept. J Antimicrob Chemother. 2003;52(4):731–3.
45. Mishra A, Aggarwal A, Khan F. Medical device-associated ınfections caused by biofilm-forming microbial pathogens and controlling strategies. Antibiot (Basel). 2024;13(7):623.
46. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria.
47. Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F, et al. 2015 ESC guidelines for the management of infective endocarditis. Eur Heart J. 2015;36:3075–123.
48. Drusano GL, Neely M, Van Guilder M, Schumitzky A, Brown D, Fikes S et al. Analysis of combination drug therapy to develop regimens with shortened duration of treatment for tuberculosis. PLoS ONE. 2014;9(7).
49. Xu X, Xu L, Yuan G, Wang Y, Qu Y, Zhou M. Synergistic combination of two antimicrobial agents closing each other's mutant selection windows to prevent antimicrobial resistance. Sci Rep. 2018;8(1):1–7.
50. Deolankar MS, Carr RA, Fliorent R, Roh S, Fraimow H, Carabetta VJ. Evaluating the efficacy of Eravacycline and Omadacycline against extensively Drug-Resistant *Acinetobacter baumannii* patient isolates. Antibiot (Basel). 2022;11(10):1298.
51. Ozger HS, Cuhadar T, Yildiz SS, Gulmez ZD, Tunccan OG, Kalkanci A, et al. In vitro activity of Eravacycline in combination with colistin against OXA-type carbapenemase producing *Klebsiella pneumoniae* isolates. Gazi Med J. 2021;32:276–80.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.