Journal of Research in Pharmacy

Research Article

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In vitro antibacterial activity of eravacycline against multidrug-resistant *Acinetobacter baumannii* isolates

Merve ATAMAN [1, 2], Emel MATARACI-KARA [2], Berna ÖZBEK-ÇELİK [2] *

[1] Graduate School of Health Sciences, İstanbul University, Beyazıt 34126 İstanbul, Turkey.
[2] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, İstanbul University, Beyazıt 34116 İstanbul, Turkey.
* Corresponding Author. E-mail: berna.ozbek@istanbul.edu.tr (B.Ö.Ç.); Tel. +90-212-440 00 00 (13524)

Received: 17 February 2021 / Revised: 15 July 2021 / Accepted: 16 July 2021

ABSTRACT: The antibacterial effectiveness of eravacycline was compared with various antibiotics used in Acinetobacter therapy against multidrug-resistant (MDR) *Acinetobacter baumannii* strains. Antibacterial susceptibility studiea was achieved by the broth microdilution method against 52 non–duplicate, *A. baumanniii* strains to eravacycline, tobramycin, levofloxacin, cefepime, meropenem, and colistin. Eravacycline presented greater action than the comparators of the other group, cefepime, colistin, levofloxacin, and tobramycin. While the $MIC_{50/90}$ values of eravacycline were 8/16 mg/L and those for tobramycin, levofloxacin, cefepime, meropenem, and colistin were found 256/>256, 64/>256, 256/>256, 128/>256, and 0.5/256 mg/L, respectively. As a result, the present study showed that eravacycline was a potent effective antibiotic against MDR *A. baumannii*. Eravacycline could be an effective new alternative for use in particular, for the treatment of this problematic organism.

KEYWORDS: Non-fermentative; tetracycline; antibacterial agent; resistant bacteria; MIC.

1. INTRODUCTION

Multidrug-resistant (MDR) bacteria have been as a serious alert to common health. Antibiotic resistance besides its global scale endangers the effectiveness of numerous antibiotics utilized now. Also, they endanger essential therapy ways that require antibacterial remedy to be accomplished [1, 2]. One of the most severe pathogens chargeable for critical nosocomial infections is *Acinetobacter baumannii*, because of its multiple resistance mechanisms, like the decreased membrane permeability, β-lactamases production, altered target, and efflux pumps. *Acinetobacter baumannii* which clinical significance is rising especially over the last 20 years has emerged as one of the most annoying bacterial pathogens for health care worldwide. This bacterium has been known for its extraordinary capability to acquire resistance determinants and re-regulate them performing it as one of the bacterium endangering the present antibiotic therapy era. The large-scale pattern of antibacterial resistance tools that are reported for *A. baumannii* is outstanding [3]. The prompt global evolution of *A. baumannii* strains resistant to all beta-lactams, including carbapenems, illustrates the potential of this organism to answer quickly to variations in particular environmental stress. The enormous adaptive potential of *A. baumannii* and the acquisition and transfer of antibiotic resistance determinants provide to the unsuccessfulness of the largest current therapeutic procedures, including the last-line of combined. An expressive antibiotic resistance increase in *A. baumannii* mediated principally by the effect of intrinsic and/or acquired resistant mechanisms has been seeing over a decade [4, 5]. The efflux pump mechanism can not affect carbapenem antibiotics significantly; nevertheless, this mechanism implicated in resistance to various antibiotics like tetracyclines, macrolides, fluoroquinolones, aminoglycosides [6]. Colistin and tigecycline remain to be effective antimicrobial agents and they are nearly the last resort of therapeutics for MDR *A. baumannii.* [7]. Eravacycline a new totally synthetic fluorocycline antibiotic interferes with bacterial cell protein composition by binding to the 30S ribosomal subunit, therefore it is active against most Gram-negative species [8]. Eravacycline was formulated to defeat current tetracycline-specific efflux resistance and similar to the other tetracycline drugs, eravacycline has been reported to be a potent, mechanism-based protein synthesis

How to cite this article: Ataman M, Mataracı-Kara E, Özbek-Çelik B. *In vitro* antibacterial activity of eravacycline against multidrug-resistant *Acinetobacter baumannii* isolates. J Res Pharm. 2021; 25(5): 549-553.

ISSN: 2630-6344

https://dx.doi.org/10.29228/jrp.45

inhibitor agent of the bacterial ribosomes. Both the C-9 [2-(pyrrolidine-1-yl) ethanamine] and C-7 (fluorine) sites on the tetracyclic nucleus have been modified on eravacycline structures. Eravacycline varies from tigecycline structure by a couple of adjustments within a pyrrolidino acetamido group replacing the 2-tertiary-butyl glycylamido at C-9 and the D–ring structure, i.e. a fluorine atom re-placing the dimethylamine moiety at C-7 (Figure 1) [9]. This drug displays potent antibacterial activity against a large-scale of bacteria like Gram-positive, / -negative, anaerobic bacteria, MDR nosocomial pathogens, strains with gained antibiotic active efflux pumps, or bacteria with ribosomal protection [8, 9, 10]. The present investigation comparatively revealed the antibacterial activity of eravacycline against 52 multidrug-resistant *A. baumannii* isolates.

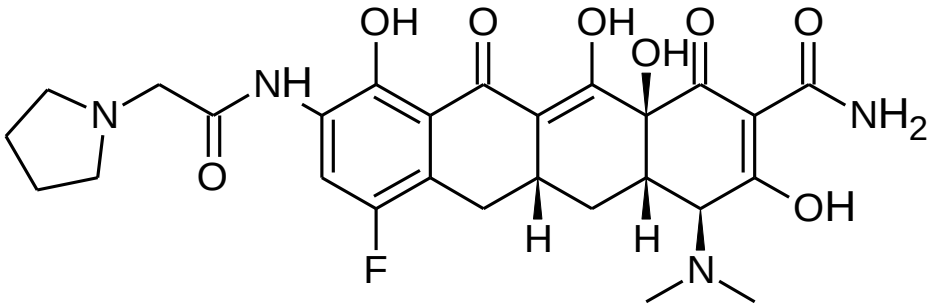

Figure 1. Chemical structure of eravacycline.

2. RESULTS

A total of 52 *Acinetobacter* strains were isolated from various clinical specimens. Tracheal aspirate was the major source of the isolates (26/52; 50%), followed by blood (19/52; 36.5%), sputum (6/52; 11.5%), and bronchial lavage (1/52; 2%) (Table S1). $MIC_{50/90}$ distribution of the tested antibiotics and their susceptibility rates are shown in Figure 2. According to gained results, all isolates exhibited MDR phenotypes and >90% of the isolates were resistant to cefepime, meropenem, tobramycin, and levofloxacin. The resistance value to colistin was considerable at 25% (≥ 4 mg/L). The $MIC_{50/90}$ values of the eravacycline were determined 8/16 mg/L. Eravacycline comparatively showed greater activity than the other antibiotics. Relatively to MIC value, eravacycline was found 2 fold active than colistin with MIC_{90} values of 8 mg/L versus 64 mg/L. Similarly, it was observed at least 4 fold more active than cefepime, meropenem, tobramycin, and levofloxacin with MIC_{90} values of 8 mg/L versus ≥ 256 mg/L. Amongst the 51 isolates which have resistance to meropenem, MICs of eravacycline ranged from 16 to >128 mg/L, and MIC_{90} values of this antibiotic were found between 16 to 256 mg/L, respectively.

3. DISCUSSION

In this study, we have presented the current progress in developing innovative strategies for fighting multidrug-resistant *A. baumannii* infections. There has been an increasing trend of antimicrobials resistance in *Acinetobacter* species isolated from different sample source. Antibiotic resistance which is emerging to seriously high levels in all parts of the world is expanding globally and endangering our ability to treat prevalent infectious diseases. Thus, there is a more prominent demand for the improvement of novel medications that exhibit antimicrobial activity against *A. baumannii*. Outbreaks prompted by MDR *A. baumannii* have been reported throughout the world with rising rates [13–14]. Moreover, resistance to carbapenems in this bacterium is a serious public health affair and the rise of carbapenem-resistance has provided clinicians with limited options. The Ambler group D β-lactamases are comprised of several oxacillinases (OXA), including plasmid-mediated OXAs with carbapenemase activity such as OXA-23, OXA-24, OXA-40, OXA-51, and OXA-58 [15]. On the other hand, the polymyxins, have appeared as significant agents in the management of *A. baumannii* infections due to extensive resistance to other antimicrobial classes. However, the treatment of colistin has already induced the rise of resistant isolates [16]. On the other hand, tigecycline which is the first glycylcycline antibiotic is one of the few new antimicrobials with activity against Gram-negative bacteria such as *Acinetobacter spp.* however, this drug was connected with some clinical adverse effects, growing antibiotic resistance, and trend to bacteremia. Notwithstanding, with this unique antibiotic, raised MICs mediated by efflux pump against *A. baumannii* have been reported [16, 17].

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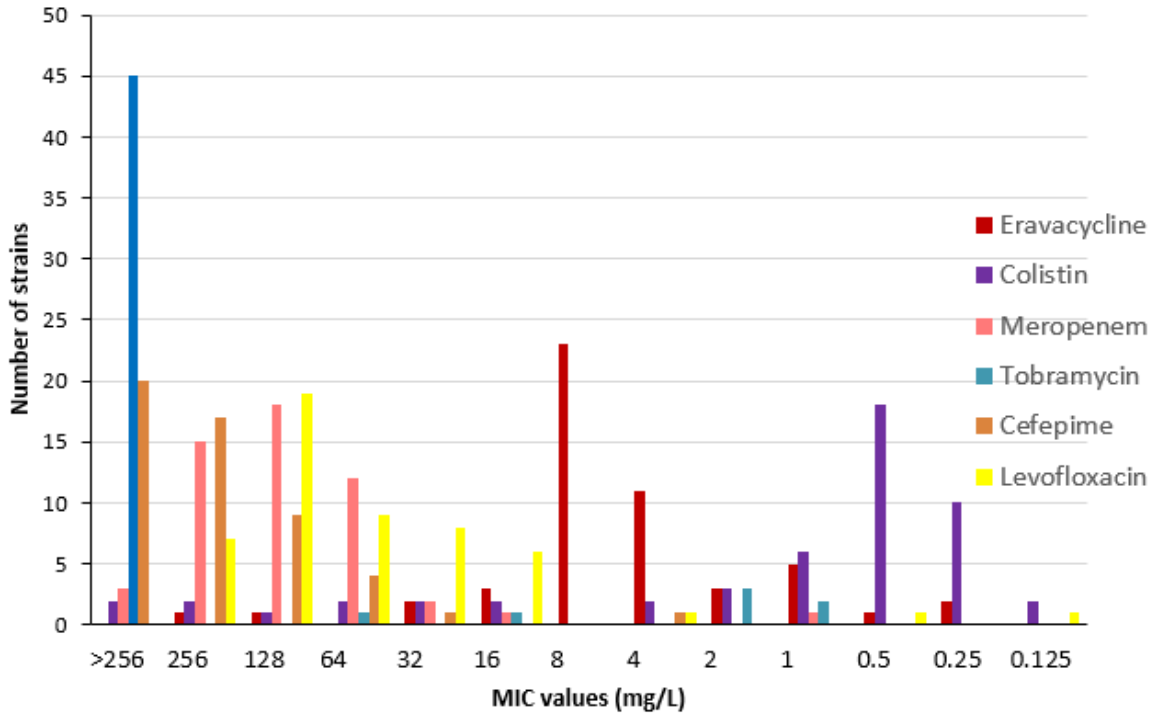

Figure 2. The distribution of MIC value of tested antibiotics.

So there is a need for new antibiotics that are not affected by the prevailing beta-lactamases besides efflux pumps. For approximately two decades, just no new antibiotics with activity against this bacterium has been presented [18, 19]. Eravacycline, a new completely synthetic fluorocycline, has been produced from the tetracyclic core structure with changes in the D ring of tetracyclic; so, it displays meaningful activity against bacteria like Gram-positive or -negative, as well as anaerobic bacteria. Additionally, it has antimicrobial activity on ESBL positive Enterobacteriaceae and bacteria with tetracycline-specific acquired resistance. In previous research with *A. baumannii* isolates expressing carbapenem-resistance, 90% of the strains displayed eravacycline MICs of ≤1 mg/L, in our study, this result was determined as 16% [10]. Another study including 158 isolates of this bacterium, of which 31% were susceptible to meropenem, eravacycline MICs were determined with $MIC_{50/90}$s of 0.5 and 1 mg/L, respectively [8]. At the present investigation, eravacycline MIC_{50} and MIC_{90} rates did not reveal any difference according to the strains which rendering different colistin resistance. According to a large study including 1097 *A. baumannii* strains, MIC_{90} value for eravacycline was determined MIC_{90} 2 µg/mL also there was no change in these respective MIC_{90} values against carbapenem-resistant or MDR strains [20]. Also, the animal researches and clinical investigations by Connors et al. [21] display that the achievable eravacycline's concentration in alveolar macrophages and epithelial lining fluid, in case of pneumonia, could be considered to evaluate the potential use of this antimicrobial in *A. baumannii* pneumonia.

4. CONCLUSION

Eravacycline seems a novel emerging option for the remedy of infectious diseases caused by this problematic bacterium. Also, this antibiotic with potent activity might be a new drug candidate in therapy for infections caused by carbapenem resistant and MDR A. *baumannii* strains.

5. MATERIALS AND METHODS

5.1. Bacterial isolates

In the research, 52 non-repeated MDR *A. baumannii* strains isolated from clinical specimens in the first six months of 2019 were used which obtained from the patients hospitalized. Multidrug resistant (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. Used bacteria through the study were classified applying the API (bioM´erieux) biochemical tests. For MIC studies, quality control strains (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) were joined to the study to ensure that determining minimum inhibitory concentration (MIC)'s of the antibiotics were within

https://dx.doi.org/10.29228/jrp.45
J Res Pharm 2021; 25(5): 549-553

the accuracy range declared by the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [11, 12].

5.2. Antibiotics

The antibiotic solvents for cefepime (Sigma-Aldrich), eravacycline (Sigma-Aldrich), meropenem (Pfizer Pharmaceuticals), tobramycin (Sigma-Aldrich), levofloxacin (Sanofi Pharmaceuticals), and colistin (Sigma-Aldrich) were prepared, according to their manufacturer recommendations. Except for meropenem, all antibiotics were saved at − 70°C for six months. Meropenem was prepared freshly on the experiment day.

5.3. Media

Mueller Hinton Broth adjusted with cations (CAMHB) was prepared daily by adding calcium (25 mg/L) and magnesium (12.5 mg/L) to sterile Mueller Hinton broth (MHB) (Oxoid, Basingstoke, UK). Bacterial colony counts were determined using Tryptic soy agar (TSA) (Oxoid) plates.

5.4. Minimum ınhibitory concentration (MIC) determinations

Fifty-two non-duplicate *A. baumannii* were examined against eravacycline, tobramycin, levofloxacin, cefepime, meropenem, and colistin. MICs for tested antibiotics were defined by the broth microdilution method explained by the CLSI. According to the method, serial two-fold dilutions, varying from 256 to 0.125 mg/L for the tested antibiotics were prepared in sterile round-bottomed 96-well microtiter plates containing sterile CAMHB. The bacterial inoculums were prepared with an approximately 5h MHB culture and a different microplate was used for each microorganism. Then, each individual bacterial inoculum was spectrophotometrically arranged with turbidity equivalent to a 0.5 McFarland and additionally diluted in CAMHB to obtain a final concentration of 5×10^5 CFU/mL in the test tray. Each test tray was stored in a plastic container to evade evaporation and then incubated at 37°C. Overnight trays were visually examined for turbidimetric growth. MIC results were evaluated according to the CLSI and EUCAST guidelines and susceptibility/resistance rates were defined according to these criteria wherever relevant [11, 12]. Each experiment was performed three times.

Acknowledgements: This study was not supported financially.

Author contributions: Concept – B.Ö.Ç., M.A.E.M.K.; Design – B.Ö.Ç., M.A..; Supervision – B.Ö.Ç.; Resources – E.M.K., M.A.; Materials – M.A., E.M.K; Data Collection and/or Processing – B.Ö.Ç., M.A.; Analysis and/or Interpretation – B.Ö.Ç., E.M.K, M.A.; Literature Search – B.Ö.Ç., M.A., E.M.K; Writing – B.Ö.Ç., M.A.; Critical Reviews – B.Ö.Ç.; E.M.K., M.A.

Conflict of interest statement: The authors declared no conflict of interest.

Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at https://dx.doi.org/10.29228/jrp.45.

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