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SHORT COMMUNICATION

In vitro activity of eravacycline and cefoperazone/sulbactam against extensively-drug resistant and pan-drug resistant Acinetobacter baumannii isolates from a tertiary hospital in Greece

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SUMMARY

We evaluated the in vitro activity of eravacycline and cefoperazone/sulbactam against 42 XDR and 58 PDR *Acinetobacter baumannii* isolates from blood and bronchoalveolar infections. The minimum and maximum MICs for eravacycline were 0.125 and 4 mg/L, respectively. The MIC_{50} was 2 mg/L and the MIC_{90} was 3 mg/L. The minimum and maximum MICs for cefoperazone/sulbactam were 24 and >256 mg/L, respectively. The MIC_{50} and MIC_{90} were both >256 mg/L. These novel agents were not adequate for the treatment of *A. baumannii* infections in our hospital and we recommend that microbiology laboratories perform their own evaluations before including them in clinical practice.

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Hospital-associated infections due to *Acinetobacter baumannii* resistant to multiple antibiotics include bacteraemia, pneumonia, urinary tract infections, wound infections and meningitis. Definitions vary according to resistance phenotypes; therefore, multi-drug resistant (MDR) refers to an isolate that is resistant to three or more antibiotic categories, extensively-drug-resistant (XDR) is an isolate that is susceptible to only one last-resort antibiotic, and pan-drug resistant (PDR) refers to an isolate that is resistant to all available antibiotics (Magiorakos *et al*., 2012). As expected, the emergence and spread of PDR *A. baumannii* isolates in hospital settings leads to high mortality rates and is difficult to eradicate (Karakonstantis *et al*., 2020), highlighting the urgent need for novel antibiotic agents (Jean *et al.*, 2022).

Eravacycline is a novel synthetic fluorocycline of the tetracycline class developed for intravenous and oral use with a broad spectrum of activity that includes Gram-negative and Gram-positive aerobic and anaerobic bacteria (Lee & Burton 2019). Due to modifications to the D-ring of its tetracycline core, it presents increased activity as well as resistance to tetracycline-specific resistance mechanisms of some Gram-negative species, including efflux systems and ribosomal protection proteins (Bassetti *et al*., 2019). Cefoperazone/sulbactam is a new combination of a broad-spectrum cephalosporin with a class A β-lactamase inhibitor that showed better activity against β-lactamase producing Gram-negative nosocomial pathogens than cefoperazone alone (Ku & Yu 2021). Attempting to introduce potential novel antimicrobial agents in the hospital's antibiotic armamentarium for use on difficult-to-treat *A. baumannii* infections, we assessed the *in vitro* activity of eravacycline and cefoperazone/sulbactam 2:1 against XDR and PDR *A. baumannii*, clinical isolates that clearly predominate in our hospital versus susceptible ones. To the best of our knowledge, this is the first report regarding the *in vitro* activity of eravacycline and cefoperazone/sulbactam against XDR and PDR *A. baumannii* in Greece.

The study sample included 100 XDR or PDR *A. baumannii* single isolates recovered from patients hospitalized in several departments of AHEPA University Hospital from February to June 2021; forty-three were isolated from blood and 57 from bronchoalveolar secretions. Bacterial identification and overall antimicrobial susceptibility testing were performed using the Vitek2 automated system (bioMérieux, France). Susceptibility to colistin was determined using broth microdilution (Liofilchem, Roseto degli Abruzzi, Ita-

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Table 1 - *Resistance rates (%) of the study's isolates vs tested antimicrobials (n=100).*

Antimicrobial	*Resistance rate (%)*
Imipenem	100
Meropenem	100
Ampicillin/Sulbactam	100
Amikacin	100
Gentamicin	100
Ciprofloxacin	100
Levofloxacin	100
Trimethoprim/Sulfamethoxazole	100
Colistin	58

ly). The minimum inhibitory concentration (MIC) of eravacycline and cefoperazone/sulbactam for each isolate was determined by MIC test strip (Liofilchem, Roseto degli Abruzzi, Italy) and interpreted using current EUCAST guidelines. MIC_{50} and MIC_{90} of the study sample were calculated for each antimicrobial using descriptive statistics on SPSS 21.

Among the study's isolates, 42 were XDR, being susceptible only to colistin. The remaining 58 isolates were resistant to all antibiotics (PDR) (*Table 1*). The minimum and maximum MICs for eravacycline were 0.125 and 4 mg/L, respectively. MIC_{50} for eravacycline was 2 mg/L and MIC_{90} was 3 mg/L (*Table 2*). The isolates showed minimum and maximum MICs for cefoperazone/sulbactam of 24 and >256 mg/L, respectively. The cefoperazone/sulbactam MIC_{50} and MIC_{90} were both >256 mg/L (*Table 3*).

Eravacycline and cefoperazone/sulbactam were recently proposed for the treatment of MDR *A. baumannii* infections, and studies from various parts of the world reported encouraging results. In a study from New York City, eravacycline showed enhanced *in vitro* activity with MIC_{50} 0.5 mg/L and MIC_{90} 1 mg/L (MIC range: 0.06-4 mg/L). The study included MDR and non-MDR isolates, whereas resistance to eravacycline was associated with the efflux system encoded by the adeB gene (Abdalah *et al.*, 2014). A later study from the UK evaluated the *in vitro* activity of eravacycline against carbapenem-resistant *A. baumannii*. The MIC range was 0.5-1 mg/L; however, especially for five OXA-23-producing isolates, it was 4-8 mg/L. Even though resistance to eravacycline is not related to carbapenemases, it was assumed that these isolates had upregulated endogenous efflux systems or reduced permeability (Livermore *et al.*, 2016). In a German study, eravacycline showed greater activity (MIC_{50}: 0.5 mg/L; MIC_{90}: 1 mg/L) against carbapenem-resistant *A. baumannii* than other comparators, including levofloxacin, amikacin, tobramycin and colistin (Seifert *et al.*, 2017). An international group of investigators tested the activity of eravacycline on Gram-negative bacilli isolated in clinical laboratories worldwide from 2013 to 2017. The MIC_{90} for *A. baumannii* was 1 mg/L (Morrissey *et al.*, 2019). In a recent study from China, variations among deferent *A. baumannii* phenotypes were found. Isolates susceptible to carbapenems and tigecycline were found more susceptible to eravacycline (MIC_{50}: 0.125 mg/L; MIC_{90}: 0.25 mg/L; MIC range: 0.016-0.25 mg/L) than to OXA-23 producers (MIC_{50}: 1 mg/L; MIC_{90}: 2 mg/L; MIC range: 0.5-2 mg/L) and tigecycline-resistant ones (MIC_{50}: 2 mg/L; MIC_{90}: 2 mg/L; MIC range: 2-4 mg/L) (Zhao *et al.*, 2019). Similar to previous studies, the MIC_{50} and MIC_{90} in Taiwan were 0.5 mg/L and 1 mg/L, respectively, for *A. baumannii* (Liu *et al.*, 2021). The *in vitro* activity of cefoperazone/sulbactam on *A. baumannii* isolates was evaluated in Japan and interpreted with a susceptibility rate of 97% using previously published criteria (MIC_{50}: 2 mg/L; MIC_{90}: 4 mg/L) (Yamagutchi *et al.*, 1999). In an early study from China, this antibiotic combination showed better results for imipenem-susceptible *A. baumannii* (MIC_{50}: 0.5 mg/L; MIC_{90}: 16 mg/L; MIC range: 0.03-32 mg/L) compared to imipenem-resistant isolates (MIC_{50}: 8 mg/L; MIC_{90}: 32 mg/L; MIC range: 1-32 mg/L) (Ji *et al.*, 2013). In another Chinese study, cefop-

Table 2 - *MIC distribution, MIC range, MIC_{50}, MIC_{90} and susceptibility rate of the study isolates for eravacycline (n=100).*

MIC values (mg/L)												
0.125	*0.38*	*0.5*	*0.75*	*1*	*1.5*	*2*	*3*	*4*	*MIC range (mg/L)*	*MIC_{50} (mg/L)*	*MIC_{90} (mg/L)*	*Susceptibility (%)*
1	2	2	4	10	24	33	20	4	0.125 - 4	2	3	5

Table 3 - *MIC distribution, MIC range, MIC_{50}, MIC_{90} and susceptibility rate of the study isolates for cefoperazone-sulbactam (n=100).*

MIC values (mg/L)										
24	*32*	*48*	*64*	*128*	*192*	*>256*	*MIC range (mg/L)*	*MIC_{50} (mg/L)*	*MIC_{90} (mg/L)*	*Susceptibility (%)*
1	4	3	2	1	3	86	24 - >256	>256	>256	0

erazone/sulbactam showed better results than tigecycline for the treatment of carbapenem-resistant *A. baumannii* infections (Li *et al*., 2020). More recently, 300 isolates (97.3% of which harbored bla_{OXA-23}) collected from 29 Chinese hospitals showed MIC_{50}: 64 mg/L, MIC_{90}: 128 mg/L and MIC range: 1- >256 mg/L (Wang *et al*., 2021). Two more studies from Taiwan showed very similar results with each other regarding *A. baumannii* (MIC_{50}: 32 mg/L; MIC_{90}: 64 mg/L) (Chang *et al*., 2017; Cheng Li *et al*., 2018).

Because of the recent introduction of these agents in clinical practice, EUCAST breakpoints for cefoperazone/sulbactam and eravacycline regarding *A. baumannii* are not yet available. However, despite the lack of clinical breakpoints for cefoperazone/sulbactam, the high MICs of our study clearly indicate that this compound is not active against *A. baumannii* populations in our hospital. Carbapenem resistance among *A. baumannii* is very common in our institution and is mainly mediated by OXA-23 carbapenemases. Therefore, the elevated MICs of cefoperazone/sulbactam may be correlated with carbapenem resistance and is in accord with previous findings. On the other hand, taking into consideration the EUCAST breakpoint of eravacycline for Enterobacterales (0.5 mg/L), only 5/100 isolates presented MICs ≤0.5 mg/L. These elevated resistance rates (95%) may be attributed to increased efflux activity and/or permeability issues that could not be determined precisely in the context of the present study.

Our study has some other limitations as well. It is a single-center study and its results may not reflect the overall national and international situation regarding the susceptibility profiles of *A. baumannii* to eravacycline and cefoperazone/sulbactam. Thus, it is strongly recommended that microbiology laboratories perform their own assessments regarding the susceptibility profiles of *A. baumannii* to novel antimicrobial compounds in their hospitals. Moreover, we did not use molecular epidemiology methods on our isolates since this was beyond the purpose of this work. A third limitation is that even though eravacycline is indicated for the treatment of complicated intra-abdominal infections in adults (Montravers *et al*., 2019; Heaney *et al*., 2019), the isolates included in our study were recovered from blood and bronchial secretions.

Overall, our results showed that eravacycline and cefoperazone/sulbactam cannot be the game changers for the treatment of *A. baumannii* infections in our hospital. Therefore, more research on susceptibility profiles and resistance mechanisms is needed, while at the same time, it remains mandatory to focus on the implementation and intensification of infection control measures.

Conflicts of interest

None declared. Part of this study was accepted as a poster presentation at ECCMID 2022.

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