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SHORT COMMUNICATION

In Vitro Activity of Ceftazidime/Avibactam Alone and in Combination With Fosfomycin and Carbapenems Against KPC-producing *Klebsiella Pneumoniae*

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SUMMARY

KPC-producing *Klebsiella pneumonia* (KPC-Kp) represents a major therapeutic challenge in critically ill patients. Ceftazidime-avibactam (CAZ-AVI) is a new effective drug against KPC-Kp but, due to emerging resistant strains during monotherapy, the association with a second antibiotic has been advocated. Therefore, intravenous fosfomycin may be a possible choice for combination therapy.
The aim of this study was to evaluate the in vitro susceptibility of CAZ-AVI alone and in combination with fosfomycin and carbapenems against KPC-Kp clinical isolates by E-test method.
The combination of CAZ-AVI with carbapenems showed synergistic activity, whereas with fosfomycin showed addictive activity, suggesting that fosfomycin may be a carbapenem-sparing strategy in antimicrobial stewardship programs.

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In recent decades, KPC-producing *Klebsiella pneumoniae* (KPC-Kp) has emerged as a nosocomial pathogen causing severe infections worldwide (Pitout *et al.*, 2015). In critically ill patients, KPC-Kp infections are associated with significant morbidity and mortality attributable to limited available therapeutic options. KPC-Kp remains susceptible only to polymyxins and the treatment of severe infections is based mainly on colistin in combination with gentamycin or tigecycline with or without carbapenem (Qureshi *et al.*, 2012). Recently, ceftazidime-avibactam (CAZ-AVI) has been approved in Europe for the treatment of complicated urinary and abdominal tract infections and hospital acquired pneumonia caused by carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* (Falcone *et al.*, 2016). In particular, CAZ-AVI displays activity in vitro against class A and C beta-lactamases, including ESBLs, AmpC, KPC and also OXA-48 type enzymes (Zhanel *et al.*, 2013; Alraddadi *et al.*, 2019) In clinical trials of patients affected by complicated intra-abdominal and urinary tract infections, CAZ-AVI represents a new therapeutic option for KPC-Kp because of its efficacy comparable to that of carbapenems (Falcone *et al.*, 2016; Che *et al.*, 2019). It also appears to be a promising drug for bacteremia treatment in severely ill patients both in monotherapy and in combination therapy (Krapp *et al.*, 2017; Tumbarello *et al.*, 2019), showing similar results in mortality rate and microbiological cure (Onorato *et al.*, 2019). However, CAZ-AVI- resistant KPC-Kp strains have rapidly emerged during monotherapy, suggesting the necessity to associate a second antibiotic, although the optimal combination is still under debate (Shield *et al.* 2017). CAZ-AVI in combination with carbapenems has displayed synergistic activity against KPC-Kp and, in particular, the combination with imipenem could represent a suitable therapeutic option (Gaibani *et al.*, 2017). Recently, intravenously administered fosfomycin, a broad-spectrum antibiotic that inhibits peptidoglycan synthesis, has been used to treat infections caused by multidrug-resistant Gram-negative bacteria, including *Pseudomonas aeruginosa,* (Papp-Wallace *et al.*, 2019; Sader *et al.*, 2017) suggesting its possible use in combination with beta-lactams (Ojdana *et al.*, 2019).

In vitro activity of CAZ-AVI alone and in combination with fosfomycin and carbapenems (imipenem, meropenem, ertapenem) against KPC-Kp clinical isolates by E-test method was evaluated in this study.

Twenty non-duplicate KPC-Kp isolates were collected as part of standard patient care at Policlinico Hospital, Bari, Italy during 2018. The strains were identified as *K. pneumoniae* by Matrix Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF) assay (biòMerieux, France) and carbapenem resistance was assessed using Vitek2 system (biòMerieux, France) in accordance with the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (EUCAST, 2018). KPC production was per-

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Table 1 - *Activity of Ceftazidime-Avibactam (CAZ-AVI) alone and in combination with Meropenem (MER), Imipenem (IMP), Ertapenem (ETP) and Fosfomycin (FOS) against KPC-producing Klebsiella pneumoniae strains.*

Strains	*CAZ-AVI MIC (μg/ml)*	*MER MIC (μg/ml)*	*FICI*	*IMP MIC (μg/ml)*	*FICI*	*ETP MIC (μg/ml)*	*FICI*	*FOS MIC (μg/ml)*	*FICI*
1	1.5	48	**0.2**	4	**0.27**	>32	**0.42**	>265	**1.17**
2	0.38	24	**0.16**	4	**0.18**	>32	**0.03**	>265	**0.58**
3	4	>256	**0.18**	>32	**0.25**	>32	**0.4**	>265	**1.5**
4	1.5	96	**0.18**	6	**0.2**	>32	**0.4**	>265	**1.17**
5	1.5	32	**0.18**	3	**0.03**	>32	**0.25**	>265	**0.9**
6	1.5	3	**0.17**	1	**0.42**	3	**0.25**	32	**1**
7	1.5	48	**0.2**	3	**0.29**	>32	**0.3**	>265	**1.04**
8	0.064	8	**0.08**	8	**0.31**	3	**0.25**	16	**1**
9	1	32	**0.16**	2	**0.25**	>32	**0.4**	>265	**0.75**
10	3	96	**0.16**	>32	**0.25**	>32	**0.38**	>265	**0.71**

FICI: FIC index.

formed by phenotypic method (KPC&MBL&OXA-48 disc kit, Liofilchem, Italy). Susceptibility to CAZ-AVI was performed with E-test gradient strip (Liofilchem, Italy) and diffusion agar (sensi disk 30 μg/20 μg, Becton&Dickinson). Ten KPC-Kp strains were chosen to evaluate the activity of CAZ-AVI in combination with fosfomycin, meropenem, imipenem, and ertapenem by using a method employing two E-test strips applied at right angles to each other (Wareham *et al.*, 2006). Briefly, an inoculum equal to 0.5 McFarland turbidity standard was prepared for each strain and inoculated onto Mueller-Hinton agar. The plates were set up with the E-test strips intersecting at the MIC value of each agent tested. The nature of drug interaction was determined on the basis of calculated FIC index as follows: synergy FIC≤0.5; addictive 0.5<FIC≤1, indifferent 1<FIC< 2; antagonism FIC≥2.

All KPC-Kp strains were susceptible to CAZ-AVI using E-test (MIC_{50} 1.5 μg/ml, MIC_{90} 3 ug/ml, range 0.064-4 μg/ml) and diffusion agar (inhibition zones between 21 and 25 mm).

Table 1 shows the MICs of CAZ-AVI, meropenem, imipenem, ertapenem, fosfomycin and the FIC index of their combination with CAZ-AVI. The FIC index of CAZ-AVI in combination with each carbapenem was ≤0.5, suggesting synergistic effect against KPC-Kp isolates. In particular, carbapenems restored their susceptibility in the presence of CAZ-AVI. The FIC index of CAZ-AVI in combination with fosfomycin showed values ranging from 0.58 to 1.17, suggesting addictive activity. Both antibiotics lowered their MIC_S but fosfomycin susceptibility was not restored. The association of CAZ-AVI with a second antibiotic may be beneficial to increase the spectrum of activity or to prevent the emergence of antimicrobial resistance.

Intravenous fosfomycin as a part of combination treatment for KPC-Kp infections deserves great attention because it could be an alternative to carbapenems. In our setting, all KPC-Kp isolates were in vitro susceptible to CAZ-AVI. When CAZ-AVI was combined with carbapenems, synergistic activity was observed, in agreement with Gaibani *et al.* In addition, all KPC-Kp isolates resulted resistant to fosfomycin and its combination with CAZ-AVI suggested addictive activity as the fosfomycin MIC value lowered without restoring susceptibility.

The limitations of this study are the small number of isolates tested and the absence of clinical validation of this suggested antibiotic association; however, these data are consistent and demonstrate that the combination of CAZ-AVI and carbapenems as well as fosfomycin possesses good activity against KPC-Kp. Therefore, further in vitro studies with a larger number of isolates and a clinical trial are necessary.

In conclusion, this study shows the enhanced in vitro activity of the combination of CAZ-AVI with carbapenems and fosfomycin, compared to CAZ-AVI alone, against KPC-Kp clinical isolates. Given the limited therapeutic options for the treatment of severe KPC-Kp infections, the obtained results provide a new treatment opportunity.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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