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In vitro* activity of cefiderocol in combination with new β-lactam/β-lactamase inhibitor combinations (βL-βLICs) against multidrug resistant KPC-producing *Klebsiella pneumoniae

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New β-lactam/β-lactamase inhibitor combinations (βL-βLICs) have been recently developed for the treatment of difficult-to-treat (DTR) infections caused by multi-drug-resistant (MDR) microorganisms.[1] Although the new βL-βLICs represent a promising effective option for treatment of DTR infections due to carbapenemase-producing Enterobacterales (CPE), the emergence of strains resistant to these molecules poses further considerations in their clinical use [1]. Recently, a novel siderophore cephalosporin, cefiderocol (CFD), has been developed for treatment of challenging DTR infections due to MDR pathogens. Although CFD exerts potent *in vitro* activity against most MDR Gram-negative microorganisms, recent studies reported the increase of CFD-resistant strains thus limiting its clinical impact [2,3]. In this context, combination antimicrobial treatments have been proposed to overcome the emergence of DTR pathogens [4].

Aim of this study was to evaluate the in vitro activity of CFD in association with ceftazidime/avibactam (CAZ-AVI), meropenem/vaborbactam (MER/VAB), and/or

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imipenem/relebactam (IMI-REL) and fosfomycin (FOS) against a collection of MDR KPC-producing *K. pneumoniae* (KPC-Kp) clinical isolates.

Phenotypic and genotypic characteristics of KPC-Kp strains included in this study are shown in Table 1 and 2. Strains were grouped accordingly to the antimicrobial susceptibility patterns to new βL-βLICs and CFD (Group 1; CAZ-AVI, MER/VAB, IMI-REL and CFD -resistant; Group 2; CAZ-AVI and CFD -resistant; Group 3; CFD -resistant; Group 4; susceptible to new βL-βLICs and CFD). *In vitro* synergy testing was evaluated by calculating the FIC index (FICI <=0.5, synergy; 0.5< FICI <=4, indifferent; FICI >4, antagonist) as previously described [5]. Overall, in vitro synergistic activity was observed for 28 out of 80 (35%) combinations tested against CFD-resistant KPC-Kp and for 13 out of 40 (32.5%) against CFD-susceptible strains (Figure 1). In particular, CFD in combination with CAZ-AVI, MER-VAB, IMI-REL or FOS showed a synergy rate respectively of 45%, 35%, 45% and 15% against CFD-resistant strains and 60%, 40%, 30% and 0% against CFD-susceptible KPC-Kp. Importantly, CFD reduced the MICs of CAZ-AVI, MER-VAB, IMI-REL and FOS respectively of 4, 12, 3.2 and 3.5 –folds against CFD-resistant strains and of 3.4, 15.6, 2.65 and 2 –fold against CFD-susceptible KPC-Kp (Figure 2).

Examination of the synergy results based on phenotypic traits showed that CFD in combination with CAZ-AVI exhibited high synergistic activity (FICI <=0.5) against both KPC-Kp -susceptible to CFD and βL-βLICs or KPC-Kp -resistant to CFD and novel βL-βLICs (Figure 3). Also, CFD in combination with MER-VAB showed higher synergistic values against KPC-Kp resistant to CFD and at least one βL-βLICs. Interestingly, CFD in combination with IMI-REL exhibited higher FICI than other combinations against KPC-Kp susceptible to βL-βLICs and re-sistant to CFD. Interestingly, CFD restored the IMI-REL activity against 8/9 (88.8%) Kp strains co-producing mutated KPC and OXA-181 that are resistant to CFD and all βL-βLICs.

In conclusion, our findings demonstrated that CFD in combination with novel βL-βLICs showed synergistic activity [FICI <0.5] against all KPC-Kp isolates, including CFD –susceptible and –

resistant strains. Although CFD exhibited synergistic activity differentially depending on the phenotypic traits of KPC-Kp strains, here we demonstrated that CFD reduced the MICs for IMI-REL below the resistance breakpoints against 29 out of 30 (96.6%) KPC-Kp strains. Further study are necessary to evaluate the clinical impact of this combination and establish the efficacy of this regimen in the treatment of infections due to KPC-Kp producers.

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Figure 1. Fractional inhibitory concentration index (FICI) of cefiderocol in combination with (**a**) ceftazidime/avibactam (CAZ-AVI), (**b**) meropenem/vaborbactam (MER-VAB), (**c**) imipenem/relebactam (IMI-REL), (**d**) fosfomycin (FOS) against cefiderocol -resistant (CFD-R) and -susceptible (CFD-S) KPC-producing *K. pneumoniae* strains. Dotted lines represent the cut-off for synergism.

Figure 2. MICs distribution of CFD in combination with CAZ-AVI against CFD -susceptible (**A**) and -resistant (**B**) strains, with MEM-VAB (**C**: CFD -susceptible **D**: CFD-resistant), with IMI-REL (**E**: CFD -susceptible **F**: CFD-resistant), with FOS (**G**: CFD -susceptible **H**: CFD-resistant. Dotted lines represent the clinical breakpoints.

Figure 3. FICIs distribution of CFD in combination with CAZ-AVI (A), MER-VAB (B), IMI-REL (C) and FOS (D) against KPC-producing *K. pneumoniae* strains grouped on the basis of resistance patterns to CFD and BL-BLICs. Dotted lines represent the cut-off for synergism.