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# Ceftazidime/avibactam-resistant meropenem-susceptible KPC-producing *Klebsiella pneumoniae*: Analysis of cases and evaluation of in vitro activity of fosfomycin-containing combinations

A. Oliva[a,*], D. Al Ismail[a], G. Arcari[b], MC. Miele[a], E. Casali[a], F. Sacco[c], L. Volpicelli[a], M. De Angelis[a], M.T. Mascellino[a], F. Cancelli[a], G. Raponi[a,c], A. Carattoli[b], M Venditti[a]

[a] *Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy*
[b] *Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy*
[c] *Microbiology and Virology Unit, University Hospital Policlinico Umberto I, Rome, Italy*

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ABSTRACT

*Objectives:* Little is known regarding outcomes and optimal therapeutic regimens of infections caused by *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) resistant to ceftazidime/avibactam (CZA) and susceptible to meropenem (MEM). Although susceptible to MEM in vitro, the possibility of developing MEM resistance overtime is a concern. We describe the clinical characteristics of patients with colonization/infection due to KPC variants with a focus on the in vitro activity of fosfomycin (FOS)-containing combinations.

*Methods:* Patients with colonization/infection due to a KPC variant were included. Fosfomycin susceptibility was performed by agar dilution method. Synergistic activity of FOS-based combinations was evaluated by gradient strip-agar diffusion method. The emergence of in vitro MEM resistance was also tested.

*Results:* Eleven patients were included: eight with infection [four with ventilator-associated pneumonia and four with bloodstream infections] and three with colonization. Previous therapy with CZA was administered to all patients (with a mean cumulative duration of 23 days). All subjects with infection received meropenem, in monotherapy (n = 4) or with amikacin (n = 2) or fosfomycin (n = 2), and achieved clinical cure. A new CZA-susceptible and MEM-resistant KPC-Kp strain was subsequently isolated in three patients (27.3%). Meropenem/vaborbactam (MVB) showed high in vitro activity, while FOS+MEM combination was synergistic in 40% of cases. In vitro resistance to MEM was observed with maintenance of CZA resistance.

*Conclusions:* Detection of KPC variants may occur within the same patient, especially if CZA has been previously administered. Although clinical success has been obtained with carbapenems, the emergence of MEM resistance is a concern. Fosfomycin plus meropenem is synergistic and may be a valuable combination option for KPC variants, while MVB may be considered in monotherapy. The detection of KPC variants in an endemic setting for KPC-Kp represents a worryingly emerging condition. The optimal therapeutic approach is still unknown and the development of meropenem resistance is of concern, which may lead to therapeutic failure in clinical practice. In these cases, the addition of fosfomycin to meropenem, or a more potent antibiotic, such as meropenem/vaborbactam, may be valuable therapeutic options.



## 1. Introduction

Since its introduction in the market, ceftazidime/avibactam (CZA) has been commonly used for the treatment of KPC (*Klebsiella pneumoniae* carbapenemase)-producing *Klebsiella pneumoniae* (KPC-Kp) infections, contributing to a reduction of mortality compared to traditional therapies [1–3]. Neverthe-

* Corresponding author. Mailing address: Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome (Italy).
*E-mail address:* alessandra.oliva@uniroma1.it (A. Oliva).

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less, its efficacy seems to be reduced in certain conditions, such as pneumonia, continuous renal replacement therapy [4], delayed source control [5], and septic thrombosis [6], where a risk of underexposure and, therefore, emergence of resistance, exists.

Indeed, KPC-Kp strains with resistance to CZA due to the production of KPC gene variants have been increasingly reported in recent years, accounting for 2% to 8% of total KPC-Kp strains [7–11]. KPCs with D179Y amino acid substitutions located in the Ω loop of KPC enzyme have been the most frequently reported KPC-2 and KPC-3 variants worldwide, conferring a particular extended-spectrum $\beta$-lactamase (ESBL)-like phenotype with susceptibility, or decreased resistance, to meropenem (MEM) [12–18].

Although this phenomenon may suggest the use of carbapenems in the treatment of CZA-resistant KPC-variants, in vitro experiments showed that the exposure of these strains to serial passages with sublethal MEM concentrations can select mutants that are also resistant to MEM [19–21], raising the question if carbapenems, either in monotherapy or in combination, represent the proper therapeutic choice in this clinical setting [7].

In this scenario, thanks to its high ability to synergize with different antimicrobials, fosfomycin (FOS) may represent a reasonable partner to carbapenems for the treatment of CZA-resistant and MEM-susceptible infections [22].

Currently, little is known regarding clinical outcomes and optimal therapeutic regimens of infections caused by KPC-Kp exhibiting resistance to CZA and susceptibility to MEM, and most evidence still comes from case reports or small studies [7,23,24]. Furthermore, limited data on FOS susceptibility and its synergistic activity against KPC variants have been described so far [25,26]. Therefore, we aimed to i) describe the clinical characteristics and outcomes of hospitalized patients with colonization or infection due to CZA-resistant MEM-susceptible KPC-Kp, ii) evaluate the in vitro activity of different antimicrobials with a focus on FOS-containing combinations, and iii) assess the in vitro emergence of MEM resistance of two representative strains harboring KPC variants in the presence of sublethal concentrations of MEM.

## 2. Materials and methods

From 2020 to 2021, patients hospitalized at Azienda Ospedaliero Universitaria Policlinico Umberto I, Rome with colonization or infection due to CZA-resistant MEM-susceptible KPC-Kp were included in the study. Demographic, clinical, and therapeutic data, as well as infection's clinical cure (defined as clinical response to treatment with resolution of symptoms/signs of the infection upon discontinuation of antimicrobials [9]) and 30-day mortality were recorded. Molecular analyses were used for typing the KPC variant. The study was approved by the local Ethical Committees (no. 0069/2022); informed consent was waived because of the retrospective nature of the research. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Institutional Review Board.

### 2.1. Microbiological analyses

Resistance to CZA and susceptibility to MEM were evaluated by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) criteria [27]. The first KPC-variant isolate from each patient was collected and further analyzed for microbiological analyses. Strain #8 was not analyzed for technical issues. Strains were recovered from blood, the lower respiratory tract, or catheter tip.

The minimum inhibitory concentration (MIC) of FOS was determined using the two-fold serial agar dilution method (AD) with supplementation of 25 mg/L of glucose-6-phosphate (G6P), as recommended in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [27]. Fosfomycin powder and G6P were supplied by Sigma-Aldrich (Sigma-Aldrich Corporation, Milan, Italy) and dissolved in saline solution.

The gradient strips (Liofilchem S.r.l., Roseto degli Abruzzi, Italy) were used for CZA and meropenem/vaborbactam (MVB) susceptibility according to the manufacturer's instructions.

Both microdilution method and gradient strips were used for MEM susceptibility. Synergistic activity of FOS-based combinations (FOS+MEM, FOS+CZA, FOS+meropenem/vaborbactam, and MVB) was evaluated by gradient strip-agar diffusion method with fixed concentrations of FOS corresponding to 0.125xMIC and 0.25xMIC [6]. Absence of bacterial growth at the tested concentrations was recorded, whereas synergism was measured by means of the Fractional Inhibitory Concentration Index (FICI). A synergistic interaction was defined as FICI $\leq$0.5.

The emergence of in vitro MEM resistance was evaluated by the daily exposure of bacteria to MEM alone at sublethal concentrations (corresponding to the first turbid tube before the MIC value after each day of incubation) for up to 14 days [19]. Every day, checks for turbidity and measurements of MEM MICs were performed. At the fourteenth day of incubation, CZA MICs were evaluated. All in vitro experiments were performed in duplicate.

### 2.2. Molecular analyses

To assess the alleles encoding for KPC variants, complete DNA sequencing of the *blaKPC* gene was obtained by amplification and Sanger sequencing. Specifically, an 892 bp amplicon was generated using the KPC FW 5'-ATGTCACTGTATCGCCGTCT-3' and KPC RV 5'-TTTTCAGAGCCTTACTGCCC-3' primer pair, with annealing at a melting temperature of 58°C.

*Klebsiella pneumoniae* strains #1, #2, #3, and #9 were subjected to whole-genome sequencing (WGS) following the procedures previously described [12]. The effective role of the KPC variants in CZA resistance, strains #1, #2, and #3 was defined, as previously described [28]. Briefly, these isolates were subjected to i) cloning into fully susceptible *Escherichia coli* DH5-alpha cells (not carrying any $\beta$-lactamase gene) of the *blaKPC* genes using the KPC_PromFW 5'-GATCCAGGTGGGTCAGTATTACT -3' and the KPCRV 5'-TTTTCAGAGCCTTACTGCCC-3' primer pair of the *blaKPC-31* and *blaKPC-70* alleles and ii) the transformation into fully susceptible *E. coli* TOP-10 cells of the pKpQIL plasmids carrying *blaKPC*.

## 3. Results and discussion

### 3.1. Clinical characteristics of patients

A total of 11 patients with CZA-resistant MEM-susceptible KPC-Kp were included (seven males and four females) with a mean age of 57 years (ranging from 40–84 years) (Table 1). Eight patients had infection (four with ventilator-associated pneumonia (VAP) and four with primary bloodstream infection [BSI]); of the remaining three patients, two had lower respiratory tract colonization and one had central venous catheter (CVC) tip culture positivity without evidence of either local infection or BSI. All patients acquired CZA-resistant MEM-susceptible KPC-Kp in the intensive care unit (ICU) after a mean duration of hospitalization in the ICU of 58 days (ranging from 24–279 days). The median time from hospital admission to CZA-resistant MEM-susceptible KPC-Kp isolation was 77 days (ranging from 24–279).

A previous therapy with CZA, either in monotherapy or in combination (three and eight patients, respectively), was administered to all patients, with a mean cumulative duration of CZA therapy of 23 days (ranging from 9–50). In detail, the majority of patients (n = 6) received one course of CZA, whereas two patients received two cycles of CZA and the remaining three patients received three courses of CZA. The reasons for a previous CZA therapy included

**Table 1**
Clinical features of hospitalized patients with ceftazidime/avibactam (CZA)-resistant and meropenem (MEM)-susceptible KPC-producer *Klebsiella pneumoniae*

| Pt (Age/sex) | Ward of CZA-R MEM-S KPC-Kp isolation | Duration of hospitalization stay before CZA-R MEM-S KPC-Kp, days | CZA-R MEM-S KPC-Kp coloniza-tion/infection | CZA therapy prior to CZA-R MEM-S KPC-Kp | Reason for prior CZA therapy | Dosage of CZA | Duration of previous CZA therapy, cumulative days (n. of CZA courses) | Therapy for CZA-R MEM-S-KPC-Kp | Dosage of MEM | Clinical cure | 30-d outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *#1* 51/M | ICU | 83 | Infection (VAP) | Yes (monotherapy) | Fever in KPC-Kp colonized pt | 2.5gr/8h | 14 (1) | MEM+AMK | 1gr/8h | Yes | Survived |
| *#2* 44/F | ICU | 62 | Colonization | Yes (monotherapy) | Fever in KPC-Kp colonized pt | 2.5gr/8h | 10 (1) | NA | NA | NA | Survived |
| *#3* 67/M | ICU | 60 | Colonization | Yes (combination) | KPC-Kp pneumonia | 2.5gr/8h | 9 (1) | NA | NA | NA | Survived |
| *#4* 66/M | ICU | 102 | Infection (VAP) | Yes (combination) | KPC-Kp BSI | 1.25gr/8h | 39 (3) | MEM+FOS | 2gr/8h | Yes | Survived |
| *#5* 45/M | ICU | 165 | Central venous catheter colonization | Yes (combination) | KPC-Kp pneumonia | 2.5gr/8h | 32 (3) | Source control | NA | NA | Survived |
| *#6* 45/F | ICU | 33 | Infection (VAP) | Yes (combination) | KPC-Kp BSI | 2.5gr/8h | 32 (2) | MEM+AMK | 2gr/8h | Yes | Survived |
| *#7* 40/M | ICU | 24 | Infection (BSI) | Yes (combination) | KPC-Kp BSI | 2.5gr/8h | 16 (1) | MEM | 2gr/8h | Yes | Survived |
| *#8* 57/M | ICU | 77 | Infection (VAP) | Yes (combination) | KPC-Kp BSI | 2.5gr/8h | 38 (3) | MEM+FOS | 2gr/8h | Yes | Survived |
| *#9* 42/F | ICU | 279 | Infection (BSI) | Yes (combination) | KPC-Kp intra-abdominal infection+BSI | 2.5gr/8h | 50 (2) | MEM | 1gr/6h | Yes | Survived |
| *#10* 59/M | ICU | 34 | Infection (BSI) | Yes (monotherapy) | Fever in KPC-Kp colonized pt | 2.5gr/8h | 10 (1) | MEM | 2gr/8h | Yes | Survived |
| #11 84/F | ICU | 47 | Infection (BSI) | Yes (combination) | KPC-Kp BSI | 2.5gr/8h | 14 (1) | MEM | 1gr/8h | Yes | Survived |

AMK, amikacin; BSI, bloodstream infection; FOS, fosfomycin; ICU, intensive care unit; NA, not applicable; VAP. ventilator-associated pneumonia.

**Table 2**
Microbiological analyses of ceftazidime/avibactam-resistant meropenem-susceptible KPC-producing *Klebsiella pneumoniae*

| Strain | Source | Type of KPC variant | *Klebsiella pneumoniae* ST | MIC FOS (AD) µg/mL | MIC MEM (Gradient strip) µg/mL | MIC MVB (Gradient strip) µg/mL | MIC CZA (Gradient strip) µg/mL | MEM+FOS Growth inhibition (µg/mL) | CZA+FOS Growth inhibition (µg/mL) | MVB+FOS Growth inhibition (µg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|
| #1 | BAL | 70 | 37 | 8 | 0.047 | 0.032 | >256 | FOS 2+ MEM 0.047 | No inhibition | FOS 2+ MVB 0.016 |
| #2 | BAL | 31 | 37 | 8 | 0.25 | 0.047 | 48 | FOS 2+ MEM 0.25 | FOS 4+ CZA 6 (Syn) | FOS 2+ MVB 0.023 |
| #3 | BAL | 31 | 37 | 64 | 0.5 | 0.047 | 48 | FOS 16+ MEM 0.125 (Syn) | No inhibition | FOS 16+ MVB 0.047 |
| #4 | Blood | 31 | NA | 256 | 0.38 | 0.023 | 16 | FOS 64+ MEM 0.125 (Syn) | No inhibition | FOS 64+ MVB 0.023 |
| #5 | Blood | 53 | NA | 32 | 2 | 0.25 | 12 | FOS 8+ MEM 2 | No inhibition | FOS 8+ MVB 0.25 |
| #6 | Blood | 31 | NA | 32 | 0.125 | 0.047 | 12 | FOS 8+ MEM 0.03 (Syn) | No inhibition | FOS 8+ MVB 0.023 |
| #7 | Blood | 31 | NA | 32 | 2 | 1 | 64 | FOS 8+ MEM 1 | No inhibition | FOS 8+ MVB 1 |
| #8* | BAL | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| #9 | Blood | 31 | 307 | 16 | 0.047 | 0.023 | 12 | FOS 4+ MEM 0.047 | No inhibition | FOS 4+ MVB 0.008 |
| #10 | Blood | 31 | NA | 16 | 2 | 0.75 | >256 | FOS 2+ MEM 1.5 | FOS 4 + CZA 64 (Syn) | FOS 2+ MVB 0.50 |
| #11 | Blood | NA | NA | 16 | 6 | 1.5 | >256 | FOS 2+ MEM 2 (Syn) | FOS 2 + CZA 12 (Syn) | FOS 4+ MVB 0.75 |

AD, agar dilution; BAL, bronchoalveolar lavage; CZA, ceftazidime/avibactam; FOS, fosfomycin; MEM, meropenem; MVB, meropenem/vaborbactam; NA, not applicable; ST, sequence type; Syn, synergism (defined as Fractional FIC Index <0.5).
*strain#8 was not tested due to a technical issue.

fever in patients with KPC-Kp colonization (n = 3), KPC-Kp pneumonia (n = 2), KPC-Kp BSI (n = 5), and complicated KPC-Kp intra-abdominal infection with subsequent BSI (n = 1).

Regarding the treatment of KPC-variant infections, all patients with infection received meropenem, in monotherapy (n = 4) or in combination with amikacin (n = 2) or fosfomycin (n = 2). The patient with CVC tip positivity was treated only with the removal of the infected catheter without antimicrobial therapy. The remaining two patients had colonization and therefore did not receive antibiotic therapy. No patient presented with septic shock at infection onset. Clinical cure and 30-day survival were observed in all patients.

Interestingly, a new CZA-susceptible and MEM-resistant KPC-Kp strain was subsequently isolated in three of 11 patients (27.3%); in detail, one patient developed a BSI after 25 days, and the other two patients developed urinary and respiratory colonization after 16 and nine days, respectively, from the KPC-variant strain.

### 3.2. Microbiological analysis: in vitro activity and synergism of fosfomycin-containing regimens

Data from in vitro analysis were carried out on 10 strains and are presented in Table 2. Strain #8 was not analyzed for technical issues. According to the current breakpoints (accessed online on 27.12.2022), eight of 10 strains were susceptible to FOS, whereas the other two strains exhibited FOS MICs of 64 and 256 µg/mL (MICs ranging from 8–256 µg/mL). Likewise, six strains exhibited full susceptibility to MEM, while the remaining strains exhibited MICs of 2 µg/mL (n = 3) and 6 µg/mL (n = 1). As expected, all the strains were susceptible to MVB (MICs ranging from 0.023–1.5 µg/mL) and resistant to CZA (MICs ranging from 12–>256 µg/mL).

All tested FOS+MEM combinations induced inhibition of bacterial growth, with full synergism observed in four of 10 strains (40%), including the two FOS-resistant strains. In these instances, FOS was inhibitory at concentrations achievable in the serum after intravenous administration [6]. On the other hand, no synergism was found regarding MVB+FOS, although absence of bacterial growth was observed in all strains (Table 2). The in vitro emergence of MEM resistance was evaluated on two representative strains (strain #2 and strain #4) harboring the KPC-31 variant and with MEM MICs of 0.125 µg/mL each at the microdilution method. As shown in Table 3, after two days of incubation, the MEM MICs had already risen to 2 and 1 µg/mL, respectively, three- and two-fold the initial MICs; full resistance to MEM was observed after six days of incubation for strain #2 and after 12 days for strain #4. At the fourteenth day of incubation with sublethal concentrations of the drug, MEM MICs were 64 (strain #2) and 32 (strain #4) µg/mL. Ceftazidime/avibactam resistance was maintained after seven and 14 days of incubation in both strains.

### 3.3. Molecular analysis

Analyzing the genes encoding for the KPC enzyme, three different alleles were observed. Specifically, strains #2, #3, #4, #6, #7, #9, and #10 encoded for the D179Y variant of KPC-3 (KPC-31), strain #1 encoded for the T268A variant of KPC-31 (KPC-70), and strain #5 encoded for a KPC-3 variant displaying a L168insLE (KPC-53).

In silico sequence type (ST) was also determined for strains subjected to WGS: strains #1, #2, and #3 belonged to ST37, and strain #9 to ST307. All the isolates had intact porins, while those belonging to ST307 also carried CTX-M-15. Furthermore, using WGS data, we investigated the differences in FOS MICs among isolates #1, #2, and #3. We found a mutation in the phosphotransferase *RcsD* gene, resulting in a Glu329Gly variant in the corresponding protein, which might be responsible for the higher FOS MIC in isolate #3 in comparison with isolates #1 and #2.

The role of two *blaKPC* carbapenemase gene alleles, *blaKPC-31* and *blaKPC-70*, was already assessed, as recently described [28]. Specifically, *blaKPC* cloning in *E. coli* DH5-alpha cells raised the CZA MIC from 0.125 mg/L to 24 mg/L in the case of *blaKPC-31* and to 48 mg/L in the case of *blaKPC-70*. The transformation of the *blaKPC*-carrying pKpQIL plasmid in *E. coli* Top10 cells raised the CZA MIC from 0.125 mg/L to 8 mg/L in both cases. MEM MICs did not increase after the transfer of the *blaKPC* genes (<0.12 mg/L).

We reported a case series including 11 patients with either colonization or infection caused by CZA-resistant MEM-susceptible KPC-Kp strains harboring, in most cases, the KPC-31 variant. The peculiarity of this study relies on the presence of microbiological data regarding the susceptibility of these strains to FOS by means of the reference gold standard agar dilution method and the evaluation of synergism of different antimicrobial combinations containing FOS.

From a clinical point of view, we found that i) the detection of a KPC variant usually follows a previous CZA-susceptible KPC-Kp within the same patient, especially if CZA has been previously ad-

**Table 3**
Evaluation of in vitro emergence of resistance to meropenem (MEM) in two strains of ceftazidime/avibactam-resistant meropenem-susceptible KPC-producing *Klebsiella pneumoniae*

| Strain | MIC MEM (BMD) µg/mL | MIC CZA (Gradient strip) µg/mL | MIC MEM Day 2 (µg/mL) | MIC MEM Day 4 (µg/mL) | MIC MEM Day 7 (µg/mL) | MIC MEM Day 14 (µg/mL) | Time to develop full MEM Resistance (days) | MIC CZA Day 7 µg/mL | MIC CZA Day 14 µg/mL |
|---|---|---|---|---|---|---|---|---|---|
| #2 | 0.125 | 48 | 2 | 8 | 32 | 64 | 6 | >256 | >256 |
| #4 | 0.125 | 16 | 1 | 4 | 4 | 32 | 12 | 16 | 16 |

MEM, meropenem; MBD, broth microdilution; CZA, ceftazidime/avibactam.

ministered and ii) that treatment with MEM, either in monotherapy or in combination, achieved clinical cure in all patients with infection.

The in vitro investigations showed that the addition of FOS at serum achievable concentrations to MEM provided growth inhibition in all the tested strains and, more importantly, resulted in full synergism in 40% of cases. Furthermore, we confirmed that in the presence of sublethal concentrations of MEM, KPC variants become also resistant to this agent.

To the best of our knowledge, no similar studies have been performed so far on this topic.

All the KPC variants were isolated in the ICU setting, with CZA antibiotic pressure observed in all patients; furthermore, five subjects had received more than one course of CZA. This observation confirms the possibility of selection for KPC variants after treatment with CZA [4–6,9,19], even though conditions traditionally associated with CZA resistance or clinical failure (pneumonia, renal replacement therapy, septic thrombosis, and delayed source control) were not present in our report [4–6,29]. This finding may suggest that additional potential conditions at risk of selecting CZA-resistant strains may exist, such as the cumulative duration of CZA therapy and the number of CZA courses in the same patient, highlighting the importance of using the new antimicrobials according to antimicrobial stewardship principles [29].

Although in our case series MEM-based regimens provided a clinical cure in all patients against KPC variants, our in vitro experiments confirmed the occurrence of early MEM resistance [19]. This phenomenon, together with the maintenance of CZA resistance over time, calls into question whether carbapenems' use in clinical practice might be associated with failure, especially in critically ill conditions [7]. Indeed, under these circumstances, MVB, which benefits from optimal PK properties and, compared to CZA, offers a higher barrier of resistance to the KPC Ω-loop binding site mutations, may represent the ideal therapeutic choice [30–32]. Even though, in our patients, the types of KPC-variant infection were all BSI or pneumonia, no patients presented with septic shock or ICS-CPE >8 at infection onset [33]. In this context, less infection severity may explain the observed clinical cure and survival rates, which appear to be far higher than the studies available so far, most of them derived from case reports or small case series.

A literature review performed by Cano et al on the use of carbapenems for the treatment of KPC variants showed that all-cause mortality was 50% and concluded that a carbapenem-based combination therapy may be a suitable option for treating patients infected with *K. pneumoniae* resistant to CAZ and susceptible to carbapenems, at least when the risk of mortality is low [23]. More recently, a systematic review of observational studies including patients with KPC-producing Enterobacterales showed that infections sustained by KPC variants were mainly treated with MEM-based combination therapy, whereas MVB was used (in combination) only in one case. The overall mortality was 37% [7].

The strength of the present report also relies on the presence of microbiological analyses investigating both the susceptibility of FOS by means of the gold standard method and the in vitro activity of FOS-containing regimens. The choice of testing FOS as a possible companion drug was mainly based on the following characteristics: i) small size, leading to a high distribution in the tissues; ii) unique mechanism of action, rendering it optimal for synergistic activity, iii) broad spectrum of action, and iii) low rate of adverse events [22].

Although available options, such as MVB or cefiderocol, may retain susceptibility towards KPC variants as single agents [34], some KPC-Kp strains with CZA resistance were also shown to be resistant, or less susceptible, to MVB [35]. In these cases, a potential synergism of MVB with FOS may be of some utility. Furthermore, a very recent survey performed by Lupia et al showed that, among respondents, the majority prefer to use MVB in combination, with the favorite partner drug being FOS [36]. On the other hand, cefiderocol is currently not considered a principal therapeutic choice in this setting [37], and CZA resistance mechanisms in KPC-producing Enterobacterales impair the in vitro activity of cefiderocol, further limiting its use [38].

Therefore, based on all these concepts, we believe that studying FOS-based synergism is crucial and responds to still unmet needs clinicians have in the treatment of infections sustained by KPC variants.

In this study, we did not test colistin (COL) because it is an old drug which has been almost completely replaced by the new beta lactams/beta-lactamase inhibitors for the treatment of KPC-Kp; therefore, we did not consider COL+FOS as a possible combination to be used in clinical practice [37]. For the same reasons, tigecycline is not commonly used for these types of infections. In our case series, most patients suffered from VAP or BSI, conditions where pharmacokinetic/pharmacodynamic (PK/PD) of the drug are suboptimal [39].

We found that, according to the breakpoints still available at the time of writing [27], most strains maintained their susceptibility to FOS. However, the most interesting findings came from the combination studies, which showed absence of bacterial growth in all the MEM+FOS combinations at achievable serum concentrations (including the strains with FOS resistance), with full synergism observed in 40% of the strains. Taken together, these findings may suggest the potential clinical use of the combination MEM+FOS for the treatment of KPC-variant infections. While the addition of MEM to FOS was shown to prevent the emergence of FOS resistance in KPC-2 producing *K. pneumoniae* strains [40], little is known regarding the possibility that FOS may prevent the development of MEM resistance in KPC variants. Further studies are therefore warranted.

The combination CZA+FOS was synergistic in three of 10 strains (30%), in line with a recent study showing 22.7% synergism without differences between strains susceptible or resistant to new beta-lactam/beta-lactamase inhibitors [41]; no synergism regarding the combination MVB+FOS was found. Although the evidence is still controversial, these in vitro results are consistent with what was recently observed by Boattini et al. in 22 KPC-Kp, including five harboring the KPC-31 variant [6,42]. However, given the paucity of similar data, additional studies investigating the potential role of MVB+FOS are warranted.

Cloning the *blaKPC* gene alleles and transforming their plasmid confirmed that the D179Y mutations have a role in CZA resistance while restoring MEM susceptibility.

In three of 11 patients (27.3%), a new CZA-susceptible MEM-resistant phenotype was observed; this result is in line with our previous report, where we highlighted the interplay between KPC-31 and KPC-3 under treatment with high dosage MEM in the same patient [43]. This finding gives additional points of discussion regarding the plasticity of KPC-Kp within the same patient and, more importantly, regarding the possibility that treatment with MEM may favor the re-appearance of the ancestral strain [43,44]. Therefore, we speculate that an alternative therapy with MVB may contribute to lowering this phenomenon.

All this considered, the question of whether to use MEM or MVB in clinical practice for the treatment of KPC-variant infections is still on the plate [7,23,24]. Indeed, carbapenems (alone or in combination with FOS) may retain a role in patients with a low risk of mortality, while MVB in monotherapy may be considered in patients with more severe infections. Unfortunately, MVB was not available at our institution during the study period and, therefore, we could not evaluate its clinical effectiveness in the setting of KPC-variant infections, which surely deserves further prospective investigations.

The present report has several limitations. First, this was a single center study; therefore, it reflects only the local epidemiology, and the observed findings cannot be generalized. Furthermore, owing to the retrospective nature of the research, only patients with less severe conditions at infection onset were included and, accordingly, our clinical results may not apply to all patients with KPC-variant infections and may be limited to patients with a low risk of mortality. Likewise, all the patients were treated before MVB was easily available at our institution; therefore, we could not evaluate the clinical effectiveness of this agent in this specific setting. Unfortunately, we could not perform microbiological synergistic studies on the three new CZA-susceptible and MEM-resistant KPC-Kp strains because the isolates were not available. The low number of patients accounts for an additional limitation; however, the emergence of a KPC variant is still a rare condition, and we believe that similar studies will contribute crucial information towards the therapeutic management of such infections.

In conclusion, the detection of KPC variants in an endemic setting for KPC-Kp represents a worryingly, although still rare, emerging condition, especially in patients previously treated with CZA. The optimal therapeutic approach is still unknown, with most of the evidence reporting treatment with carbapenems. However, the development of MEM resistance may lead to therapeutic failure in clinical practice. In these cases, the addition of fosfomycin to MEM or a more potent antibiotic, such as MVB (the latter to be considered, especially, in critically ill conditions), may represent valuable therapeutic options for KPC-variant infections.

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