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Case Report

In vivo emergence of cefiderocol and ceftazidime/avibactam cross-resistance in KPC-producing *Klebsiella pneumoniae* following ceftazidime/avibactam -based therapies

Stefano Amadesi^a, Alberto Amedeo^b, Matteo Rinaldi^{b,c}, Marta Palombo^a, Maddalena Giannella^{b,c}, Paolo Gaibani^{d,*}

^a Division of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

^b Clinical Infectious Diseases Unit IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

^c Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy

^d Department of Diagnostic and Public Health, Microbiology Section, Verona University, Verona, Italy

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ABSTRACT

We described the emergence of ceftazidime/avibactam and cefiderocol cross-resistance in patients with KPC-producing *Klebsiella pneumoniae* infections. All strains with ceftazidime/avibactam and cefiderocol cross-resistance showed point mutations on KPC Ω -loop. Taken together, our results indicate that prolonged exposure to ceftazidime/avibactam can confer cross-resistance to ceftazidime/avibactam and cefiderocol.

Text

Carbapenem-resistant *Enterobacteriales* (CRE) represent a significant threat to global health due to their rapid spread and limited treatment options [1]. A novel siderophore cephalosporin, Cefiderocol (CFD) has recently garnered increasing attention for its potent *in vitro* antimicrobial activity against CRE [2]. However, despite its proven clinical efficacy, the emergence of CFD resistance in different bacterial species has raised concerns [3]. Previous studies demonstrated that mutations within carbapenemase genes are associated to resistance both to CFD and ceftazidime/avibactam (CAZ/AVI), thus suggesting that similar mechanisms (e.g. D179Y mutation) are at the basis of cross-resistance to these molecules [4,5]. Of note, prolonged administration of CFD and/or other treatments, such as CAZ/AVI, have been associated with the emergence of CAZ-AVI and CFD resistance in CRE [6,7]. Furthermore, the effective role of specific mutations in CFD-resistant clinical isolates has been recently demonstrated [5]. The emergence of actual phenotypic resistance to CFD can be evaluated by investigating genetic modifications such as SNPs and indels between susceptible and resistant strains [6].

Based on these findings, we reported emergence of CFD resistance in

patients with infections caused by CRE during CAZ/AVI-based treatments.

This retrospective analysis was conducted on six selected patients, with median age of 54 years, treated between February 2017 and March 2021 at Policlinico Sant'Orsola-Malpighi in Bologna, Italy. Selected patients were diagnosed with infections caused by KPC-producing *Klebsiella pneumoniae* (KPC-Kp) and susceptible to CAZ/AVI. All patients developed resistance to CAZ/AVI and CFD following CAZ/AVI-based regimen.

Hospitalized patients were screened periodically for colonization due to CPE (carbapenemase-producing *Enterobacteriaceae*) following guidelines provided by the Emilia-Romagna Region (<https://assr.region.e.emilia-romagna.it/publicazioni/rapporti-documenti/indicazioni-pratiche-diagnosi-cpe-2017>). Briefly, rectal swabs were collected weekly during the first month from admission, and then once a month. Species identification was performed by plating rectal swabs onto a selective chromogenic medium and by evaluating selected colonies by MALDI-TOF (Bruker Daltonics, Germany) mass spectrometry. Blood samples were collected during routine diagnostic procedures and cultivated on blood agar plates. Antimicrobial susceptibility testing was performed on the MicroScan Walkaway system (Beckman Coulter,

* Corresponding author at: Department of Diagnostic and Public Health, Microbiology Section, Verona University, Verona, Italy.

E-mail address: paolo.gaibani@univr.it (P. Gaibani).

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Table 1

Antibiotic susceptibility patterns and genetic features of the 12 KPC-Kp isolates selected for this study. For each patient, a CAZ/AVI and CFD-susceptible and a resistant strain are displayed. Susceptible strains were collected before or at the beginning of therapy onset, while resistant strains were collected during or after therapy. Provided data include isolation source, isolation date, and MIC for the main antibiotics. Resistance is shown in bold.

| Patient | Days of exposure to CAZ/AVI (of which prior to RE) | Isolate name | Isolation source | MIC ($\mu\text{g/ml}$) | | | | | Carbapenemase gene (Mutation) |
|---------|--|--------------|------------------|--------------------------|----------------|---------------|-----------|---------------|-------------------------------|
| | | | | CAZ/AVI | MER | MER/VAB | IMI/REL | CFD | |
| 1 | 17 (17) | BOT-CA-S | Blood | 8 (S) | 256 (R) | 32 (R) | 2 (S) | 1 (S) | <i>bla</i> _{KPC-3} |
| | | BOT-EMO | Blood | > 256 (R) | 256 (R) | 8 (S) | 1 (S) | 16 (R) | |
| 2 | 58 (42) | CAZ26 | Rectal swab | 2 (S) | 256 (R) | 2 (S) | 1 (S) | 1 (S) | <i>bla</i> _{KPC-3} |
| | | CAZ30 | Rectal swab | > 256 (R) | 256 (R) | 0.064 (S) | 0,25 (S) | 16 (R) | |
| 3 | 9 (7) | CAZ83 | Rectal swab | 2 (S) | 8 (S) | 0.064 (S) | 0.032 (S) | 2 (S) | <i>bla</i> _{KPC-3} |
| | | CAZ85 | Blood | > 256 (R) | \leq 0.1 (S) | 0.064 (S) | 0.032 (S) | 8 (R) | |
| 4 | 32 (29) | BAT68 | Rectal swab | 4 (S) | 256 (R) | 0.5 (S) | 1 (S) | 2 (S) | <i>bla</i> _{KPC-3} |
| | | CAZ90 | Rectal swab | > 256 (R) | 256 (R) | 4 (S) | 0.25 (S) | 8 (R) | |
| 5 | 31 (28) | CAZ89 | Rectal swab | 2 (S) | 8 (S) | 0.032 (S) | 0.25 (S) | 2 (S) | <i>bla</i> _{KPC-3} |
| | | CAZ107 | Blood | > 256 (R) | 256 (R) | 0.032 (S) | 0.25 (S) | 16 (R) | |
| 6 | 19 (9) | CAZ112 | Rectal swab | 2 (S) | 256 (R) | 1 (S) | 0,5 (S) | 4 (R) | <i>bla</i> _{KPC-3} |
| | | CAZ147 | Blood | 256 (R) | 2 (S) | 2 (S) | 1 (S) | 16 (R) | |

Abbreviations: RE: resistance emergence; CAZ/AVI: ceftazidime/avibactam; MER/VAB: meropenem/vaborbactam; IMI/REL: imipenem/relebactam; CFD: cefiderocol.

California), except for CFD MIC which was evaluated separately by broth microdilution using an iron-depleted cation-adjusted Mueller Hinton Broth (CAMHB) prepared with Chelex® 100 resin, as previously explained [8]. MIC values were interpreted according to EUCAST clinical breakpoints v1.3.1 (https://www.eucast.org/clinical_breakpoints/). Whole-genome sequencing and genomic data analysis were carried out on selected susceptible strains collected before or during CAZ/AVI administration and resistant strains collected during or after therapy (depending on sample availability) using a standard workflow with Illumina platform described elsewhere [9]. A total of six patients with infections due to KPC-Kp were included in this study. Five out of six patients (83.3 %) were infected and one (patient 2) colonized. Relevant clinical data can be found in Supplementary Table 1. All patients underwent CAZ/AVI-based therapy for a median time of 25 days, and developed resistance to CAZ/AVI within a median time of 23 days of antibiotic exposure. Patients were administered the recommended dose of CAZ/AVI (2.5 g, ceftazidime 2 g/avibactam 0.5 g) every 8 hours by intravenous infusion. Following CAZ/AVI administration, the median CAZ/AVI MIC increased from 2 $\mu\text{g/ml}$ (IQR: 2-8 $\mu\text{g/ml}$) to 256 $\mu\text{g/ml}$ (IQR: 256-256 $\mu\text{g/ml}$). All patients showed an increase in CFD MICs as well, with a median value of 2 $\mu\text{g/ml}$ (IQR: 1-2 $\mu\text{g/ml}$) rising up to 16 $\mu\text{g/ml}$ (IQR: 8-16 $\mu\text{g/ml}$) (Table 1).

In order to investigate the mechanisms underlying the development of cross-resistance to CAZ/AVI and CFD during therapy, a comparative genomic analysis was conducted on longitudinal strains isolated from the same patient. Genetic features are summarized in Table 1 and detail genomic characteristics of clinical strains are shown in Table S2 in the Supplementary data. Analysis of antimicrobial resistance genes showed that no differences were observed in β -lactamase genes between samples collected from the same patient before and during therapy, except for the carbapenemase genes. In detail, all strains collected prior to therapy

harbored the KPC-3 variant, while five out of six (83.3 %) CFD and CAZ/AVI cross-resistant strains collected during therapy carried the KPC-31 variant (D179Y substitution), and one the KPC-130 variant (D179G substitution). Sequence analysis of porin-encoding genes revealed that susceptible and resistant strains collected from the same patient harbored similar porin genes. Analysis of plasmid replicons revealed that isolates collected before and during treatment had the same plasmid profile in three out of six patients. On the other hand, strains from the other three patients lost a plasmid, and one of these also acquired a plasmid during the treatment. Phylogenetic analysis showed that same patient isolates clustered together in the phylogenetic tree thus being closely related (Supplementary Figure 1).

In our study, all susceptible strains harbored KPC-3, while resistant strains carried KPC variants with substitutions within the Ω -loop, specifically KPC-31 and KPC-130. Based on these findings, we hypothesize that in our case the acquisition of KPC variants involved in CAZ/AVI resistance may have led to the development of cross-resistance to CFD.

Recent studies reported the emergence of resistant strains following prolonged antibiotic exposure. In detail, a recent retrospective study demonstrated that administration of CAZ/AVI to clinically-ill patients colonized by KPC-Kp is associated with emergence of CAZ/AVI-resistant strains [9]. In addition, another article reported that CFD-resistance can emerge in patients with CRE bloodstream infections within three weeks of CFD-based regimen [6]. Previous research demonstrated that modifications within KPC Ω -loop are associated with increased minimum inhibitory concentration (MIC) values for CAZ/AVI [10]. Moreover, KPC variants associated with CAZ/AVI-resistance have been observed to increase CFD MICs as well [4]. We demonstrated that CAZ/AVI-resistant strains exhibit higher rates of CFD resistance compared to susceptible isolates, suggesting a correlation between mechanisms leading to CAZ/AVI resistance and reduced CFD activity [7].

In our study, the acquisition of KPC variants alone explains the emergence of a CFD-resistant phenotype. However, since no population analysis profiling (PAP) was performed, a potential contribution of heteroresistance to the MIC increase for CFD should not be excluded. In addition, a recent warning from CLSI highlighted several challenges for CFD susceptibility testing in terms of results reproducibility and evaluation [11]. In conclusion, our study highlights the potential risks associated with CAZ/AVI-based therapy for KPC-Kp infections, specifically the emergence of resistance to other compounds, including CFD.

CRediT authorship contribution statement

Stefano Amadesi: Software, Methodology, Data curation. **Alberto Amedeo:** Data curation. **Matteo Rinaldi:** Data curation. **Marta Palombo:** Investigation, Data curation. **Maddalena Giannella:** Writing – review & editing, Supervision. **Paolo Gaibani:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

genome assemblies have been deposited in the NCBI database and are freely accessible. GenBank accession numbers are listed in Table 2.

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Additional information

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of IRCCS Azienda Ospedaliera Universitaria di Bologna (Project identification code 409/2019/Oss/AOUBo).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.diagmicrobio.2024.116372](https://doi.org/10.1016/j.diagmicrobio.2024.116372).

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