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In vitro activity of ceftazidime/avibactam against carbapenem-nonsusceptible *Klebsiella pneumoniae* isolates collected during the first wave of the SARS-CoV-2 pandemic: a Southern Italy, multicenter, surveillance study

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**ABSTRACT**
Objectives: Carbenapenemase-producing Enterobacterales (CPE) represent a serious threat for human health being frequently resistant to most of available antibiotics classes. Recently, ceftazidime/avibactam (CAZ/AVI) has been approved for treatment of infections by Gram-negative bacteria, including class A CPE (including KPC-producing *K. pneumoniae*). Following CAZ/AVI commercialization, resistance to this combination has been reported. The aim of this study was to evaluate the prevalence of CAZ/AVI resistance among carbapenem-resistant *K. pneumoniae* (CR-Kp) isolates recovered from bloodstream infections (BSI) and hospital-acquired pneumonia (HAP), representative of the contemporary southern Italy epidemiology, during the first pandemic wave of SARS-CoV-2.

Methods: From Jan’20-Jun’20, 4 Laboratories, collected all consecutive, non-replicated CR-Kp from BSIs and HAPs. All isolates were subjected to i) MALDI-ToF identification; ii) antimicrobial susceptibility testing by microdilution method. CAZ/AVI resistant (CAZ/AVI-R) isolates were screened for presence of most common carbapenemase genes and subjected to whole genome sequencing for characterization.

Results: A total of 89 isolates were collected. The majority of strains retained susceptibility to colistin, gentamicin and amikacin. Three strains (3/89, 3.4%) were CAZ/AVI-R (MIC range 16/4-64/4 mg/L). Among CAZ/AVI-R, one was KPC-type producer (an ST101) while the remaining where NDM-type and VIM-type producers and belonged to ST147, and ST45, respectively.

Conclusion: During the pandemic period, in southern Italy, CAZ/AVI resistance remained infrequent but high-risk *Klebsiella pneumoniae* epidemic clones, producing the KPC-31 variant and class B carbapenemases were reported from some of the included centers.

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1. Introduction

Among resistance mechanism/pathogen combinations, carbapenemase-producing Enterobacterales (CPE) are particularly problematic [1]. In fact, therapeutic options for infections caused by CPE are extremely limited, and the usefulness of
antibiotics with possible residual activity is compromised by the associated toxicity and/or the unfavourable pharmacological profile [2].

Ceftazidime/avibactam is the first commercialized beta-lactam/beta-lactamase inhibitor combination active against some CPE, and, also because of its safety and efficacy features, it has been increasingly used for treatment of infections caused by KPC-type carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) [2]. Despite the recent introduction and its proven effectiveness against Gram-negative bacteria, some countries are already reporting resistance against CAZ/AVI [3]. Resistance to CAZ/AVI can be linked to the production of metallo-beta-lactamas (MBLs); amino acids changes in the carbapenemase enzyme (especially D179Y substitution in KPC-type); and changes in cell permeability and expression of efflux pumps [3].

The COVID-19 pandemic effect on antimicrobial resistance is incompletely understood, but monitoring the activity of key antimicrobial agents is of paramount importance during this period. It can be speculated that the perturbations on the healthcare systems caused by pandemic waves could have modified the pre-existing antimicrobial resistance burden.

The objectives of our study were: (i) to obtain a collection of clinical isolates of carbapenem-resistant *K. pneumoniae* (CR-Kp) from bloodstream infections (BSI) and hospital-acquired pneumonia (HAP, including ventilator-associated pneumonia [VAP]) representative of the southern Italy epidemiology, during the first wave of SARS-CoV-2 pandemic; (ii) to monitor the in vitro activity of CAZ/AVI and comparator drugs within this collection; (iii) to characterize the CAZ/AVI resistance mechanism in nonsusceptible isolates.

### 2. Methods

For a 6-month period (January to June 2020), four microbiology laboratories distributed across southern Italy collected and stored all consecutive nonrePLICATE CR-Kp from BSIs (blood culture) and HAP/VAP (broncho-alveolar lavage). All isolates were subjected to identification at the species level by MALDI-TOF technology (Bruker, Germany). Antimicrobial susceptibility testing was performed by reference microdilution method according to CLSI standards with the following antibiotics: amikacin, cefepime, ceftazidime, ceftriaxone/avibactam, cefepime/tazobactam, cefoxitin, cefotaxime, gentamicin, levofloxacin, meropenem, piperacillin/tazobactam, and trimethoprim/sulphamethoxazole (interpretation according to EUCAST breakpoints v. 12.0; [https://www.eucast.org/clinical_breakpoints/]). Ceftazidime/avibactam-resistant isolates (CAZ/AVI-R) were subjected to next-generation sequencing on MiSeq Illumina platform (Illumina, Inc., San Diego, CA, USA) or MinION™ sequencer (Oxford Nanopore Technologies plc, Oxford Science Park, UK). This Whole Genome Shotgun project was deposited at DDBJ/ENA/GenBank under the BioProject PRJNA853728. Analysis of genetic features of sequenced isolates was performed using the web application Pathogenwatch ([https://pathgen.watch](https://pathgen.watch)).

### 3. Results

Overall, during the study period a total of 89 CR-Kp isolates were collected by participating laboratories. Of these, 39 (44%) were from BSIs, while 50 (56%) were from HAP/VAP episodes. The most active drug was ceftazidime/avibactam followed by colistin, gentamicin, and amikacin, with resistance rates of 3.4%, 13%, 25%, and 36%, respectively. Trimethoprim/sulfamethoxazole retained activity in one-third of cases. All isolates were resistant to third- and fourth-generation cephalosporins, cefotaxime/tazobactam, piperacillin/tazobactam, meropenem, etaperpen, and fluoroquinolones. The three CAZ/AVI-R isolates (MIC range 16–64 mg/L) carried different carbapenemases (KPC type, VIM type, and NDM type, respectively) and were clonally unrelated. A summary of the genetic features of ceftazidime/avibactam-resistant *K. pneumoniae* strains is shown in Table 1.

The KPC-Kp strain belonged to ST101 and encoded for KPC-31. To the best of our knowledge, our paper describes, for the first time, the presence of KPC-31 in a ST101 isolate. KPC-31 is a single amino acid variant of KPC-3 (D179Y) that had been shown to confer a ceftazidime/avibactam-resistant phenotype associated with susceptibility or decreased resistance to carbapenems, compared to KPC-3 [3]. In this study, the strain KPC-31 was susceptible to meropenem with a MIC of 2 mg/L. ST101 and derivative STs are emerging and can be considered high-risk clones because of the antibiotic resistance profile and the presence of virulence determinants [4]. In fact, ST101 isolates (including that described in this work) are mainly associated with the KL17 (wzi-137) and ybt loci and carry an extended set of resistance genes, including the 16S rRNA methylase-encoding *armA* gene [4].

The VIM-Kp strain (*bla*VIM-1) belonged to ST45, and the NDM-Kp strain (*blaNDM-1*) to ST147. The NDM-Kp isolate described in this work belongs to one of the two main subpopulations of ST147, characterized by the capsular locus KL64 (wzi64) and the locus O2v1 and grouped into the clade called ST47KL64 clade 1, which was associated with a large outbreak in Tuscany, Italy), beginning in 2018–2019 [5] and never declared over.

### 4. Conclusions

During the first wave of the SARS-CoV-2 pandemic, the ceftazidime-avibactam resistance rate among CR-Kp clinical iso-
lates from BSIs and HAP/VAP in southern Italy remained extremely low (3 isolates out of 89; 3.4%). CAZ/AVI-R isolates belonged mostly to well-known high-risk clones (ST101 and ST147) encoding for the KPC-31 and NDM-1 carbapenemase, respectively. The spread of these high-risk clones should be carefully monitored performing adequate molecular surveillance studies.

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**Competing interests**

None declared.

**Ethical approval**

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