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# Journal Pre-proof


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# *In vitro* activity of ceftazidime/avibactam against carbapenem-non-susceptible *Klebsiella penumoniae* isolates collected during the first wave of SARS-CoV-2 pandemic: a Southern Italy, multi-center, surveillance study.

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## Highlights

- During the first pandemic period CAZ/AVI resistance remained infrequent in southern Italy
- The presence of KPC-31 in a ST101 isolate was described for the first time
- The spread of the high-risk epidemic ST147 NDM-1 producing clone was reported

## 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 unfavorable pharmacological profile (2).

Ceftazidime/avibactam is the first commercialized beta-lactam/beta-lactamase inhibitor combination active against some CPE and, due also to 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-lactamases (MBLs); amino acids changes in the carbapenemase enzyme (especially D179Y substitution in KPC-type); 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 non-susceptible isolates.

## 2. Methods

For a 6 months period (Jan-Jun 2020), 4 Microbiology Laboratories distributed across Southern Italy collected and stored all consecutive non-replicate 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, ceftazidime/avibactam, ceftolozane/tazobactam, ciprofloxacin, colistin, ertapenem, gentamicin, levofloxacin, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole (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$^{TM}$ 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://pathogen.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, ceftolozane-tazobactam, piperacillin-tazobactam, meropenem, ertapenem 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, comparing 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 due to 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 rRNAmethylase-encoding *armA* gene (4).

The VIM-Kp strain ($bla_{\text{VIM-1}}$) belonged to ST45, and the NDM-Kp strain ($bla_{\text{NDM-1}}$) to ST147. The NDM-Kp isolate described in this work belongs to one of the two main sub-populations of ST147, characterized by the capsular locus KL64 (wzi64) and the locus O2v1, and grouped into the clade called ST147KL64 clade 1 which was associated with a large outbreak, in Tuscany (Italy), began in 2018-2019 (5) and never declared over .

## 4. Conclusions

During the first wave of SARS-CoV-2 pandemic, ceftazidime-avibactam resistance rate among CR-Kp clinical isolates 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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**Conflict of interest**

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Competing interests: None

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| Isolate | ST | Acquired resistance genes | Plasmids | Virulence traits | Capsular type |
|---|---|---|---|---|---|
| Kp_LE1 | ST45 | *aac(6')-Ib4*, *aadA1*, *aphA15*, *bla*$_{VIM-1}$, *qnrS1*, *catB2*, *sul1*, *dfrA14* | IncFIB(K) | Yersiniabactin: *ybt1*; ICEKp4 YbST: 78-1LV | *wzi149* K locus: KL62, K62; O locus: O1/O2v1, Type O2a |
| Kp_FG1 | ST147 | *aac(6')-Ib'*, *aac(6')-Ib-cr*, *aadA*, *aph(3')-VI*, *armA*, *bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{OXA-9}$, *bla*$_{TEM-1D}$, *dfrA5*, *sul1*, *sul2*, *arr-3*, *catB3*, *mph(A)*, *mph(E)*, *qnrS1* | IncFIB(pQil), IncR | Yersiniabactin: *ybt9*; ICEKp3 YbST: 184-3LV Aerobactin: *iuc1* AbST: 63-1LV | *wzi64* K locus: KL64; O locus: O2v1 |
| Kp_FG2 | ST101 | *armA*, *bla*$_{KPC-31}$ | IncR, ColRNAI | Yersiniabactin: *ybt9*; ICEKp3 YbST: 183 | *wzi137* K locus: KL17, capsula K17; O locus: O1/O2v1, Type O1 |

**Table 1.** Genetic features of ceftazidime/avibactam resistant *K. pneumoniae* isolates.