Acknowledgements

We thank Professor Thierry Naas, Professor Pierre Bogaerts and Dr Romano Mattei for providing NG-Test Carba 5, KPC K-SET and the RAPIDEC® CARBA NP test, respectively.

Funding

This work was supported by internal funding.

Transparency declarations

None to declare.

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J Antimicrob Chemother 2019; **74**: 2466–2467
doi:10.1093/jac/dkz201
Advance Access publication 13 May 2019

Identification of L169P mutation in the omega loop of KPC-3 after a short course of ceftazidime/avibactam

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Sir,

We read with interest a recent article by Hemarajata and Humphries[1] entitled 'Ceftazidime/avibactam resistance associated with L169P mutation in the omega loop of KPC-2', wherein the authors report a novel mutation, conferring resistance to ceftazidime/avibactam, in the omega loop of the bla_{KPC-2} gene of a *Klebsiella pneumoniae* isolate, which emerged after 12 days of combination therapy.

Following the ECDC alert,[2] in an effort to prevent the spread of ceftazidime/avibactam-resistant, carbapenem-resistant Enterobacteriaceae (CRE), and in our role as regional reference centre, we began collecting and analysing all ceftazidime/avibactam-resistant isolates obtained from patients treated at our hospital as well as strains received from other hospitals of the Latium region. In this context, we would like to share the preliminary results of our ongoing study aimed at detecting the mechanisms responsible for ceftazidime/avibactam resistance in CRE.

Two *K. pneumoniae* strains, one susceptible (Kp-1-S) and one resistant (Kp-2-R) to ceftazidime/avibactam, were obtained from the blood cultures of a patient treated at the San Filippo Neri

Table 1. Antimicrobial susceptibility results for the clinical isolates described in this study

	MIC (mg/L), S/R								
Strain	CAZ/AVI	CAZ	FEP	CTX	ETP	IPM	MEM	TZP	GEN
Kp-1-S	2, S	>8, R	>8, R	>8, R	>8, R	>16, R	>16, R	>128, R	4, S
Kp-2-R	32, R	>8, R	>8, R	>8, R	>8, R	0.5, S	2, S	>128, R	4, S

CAZ/AVI, ceftazidime/avibactam; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; TZP, piperacillin/tazobactam; GEN, gentamicin; S, susceptible; R, resistant.

Hospital and were analysed at the 'L. Spallanzani' National Institute for Infectious Diseases in Rome. The Kp-2-R strain, which also showed decreased imipenem and meropenem MICs, was isolated after 19 days of a ceftazidime/avibactam + colistin-based treatment, which was started in order to treat bacteraemia caused by the Kp-1-S susceptible strain.

Susceptibility testing was performed using the broth microdilution method, as recommended.[2] The ST of both isolates was determined by traditional MLST of seven housekeeping genes; the bla_{KPC} alleles and porin alterations (OmpK35 and OmpK36) were investigated by Sanger sequencing.

Both isolates belonged to ST512 and harboured an insertion mutation of glycine–aspartic acid residues (GD) at amino acid positions 134–135 in the OmpK36 L3 loop, as described previously.[3] No mutations were observed in OmpK35. As shown in Table 1, the MIC of ceftazidime/avibactam increased by four 2-fold serial dilutions, i.e. from 2 to 32 mg/L. Conversely, the MICs of two out of three carbapenems were markedly decreased (Table 1). BLAST analysis revealed the presence, in the bla_{KPC-3} gene of the ceftazidime/avibactam-resistant *K. pneumoniae* isolate, of the L169P mutation, which consists of a single nucleotide substitution (T to C) resulting in a leucine-to-proline amino acid substitution at amino acid position 169.

Besides mutations in the omega loop of KPC enzymes, other mechanisms of ceftazidime/avibactam resistance have been reported, such as a decrease in permeability caused by modifications in outer membrane proteins and differences in susceptibility of KPC subtypes.[4–6] However, the mechanisms most often associated with resistance observed after ceftazidime/avibactam treatment are mutations in the bla_{KPC} gene.[7,8]

At the time of writing, the known bla_{KPC} mutations responsible for ceftazidime/avibactam resistance include A177E, D179Y, V240G, T243M and EL165-166.[9,10] The L169P mutation that Hemarajata and Humphries[1] have described in the bla_{KPC-2} gene and that was observed by us in bla_{KPC-3} is similar to D179Y (found in both bla_{KPC} variants); both mutations result in combined ceftazidime/avibactam resistance and restoration of *in vitro* activity of carbapenems.

Taken together, our results support the observation that the omega loop of KPC enzymes plays a key role in the emergence of mutations arising after antibiotic therapy; further studies aiming at understanding the mechanisms of ceftazidime/avibactam resistance should probably focus on this mutation-prone region of the bacterial genome. Our data also underscore the need for active surveillance in order to prevent the spread of any ceftazidime/avibactam-resistant strains that emerge during treatment.

Funding

This work was supported by 'Ricerca corrente' and '5X1.000' research funds from the Italian Ministry of Health.

Transparency declarations

None to declare.

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