Emerging ceftazidime-avibactam resistant *Klebsiella pneumoniae* producing new KPC variants in different Brazilian states

[a]Aline Valério de Lima, [a]Keila de Oliveira Lima, [a,b]Darlan Augusto da Costa Rocha, [c]Paola Cappellano, [d]Marcelle Duarte Alves, [e]Nilton Erbert Lincopan Huenuman, [f]Maria Luísa do Nascimento Moura, [g]Maura Salaroli de Oliveira, [h]Alexandre Prehn Zavascki, [a,c]Jorge Luiz Mello Sampaio

[a]Universidade de São Paulo (USP), Faculdade de Ciências Farmacêuticas – Laboratório RAMC - Resistência Antimicrobiana e Microbiologia Clínica - Avenida Professor Lineu Prestes, 580 Bloco 17 - Cidade Universitária – SP.

[b]Grupo Fleury, Departamento de pesquisa e desenvolvimento, São Paulo, SP, Brasil.

[c]Fleury Medicina e Saúde, setor de Microbiologia, São Paulo, SP, Brasil.

[d]Fleury Medicina e Saúde, setor de Microbiologia, Porto Alegre, RS, Brasil.

[e]Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

[f]Hospital Vila Nova Star, Serviço de Controle de Infecção, São Paulo, SP, Brasil.

[g]Hospital Sírio Libanês, Serviço de Controle de Infecção, São Paulo, SP, Brasil.

[h]Hospital Moinhos de Ventos, Serviço de Controle de Infecção, Porto Alegre, RS, Brasil.

The main mechanism of carbapenem resistance in *Klebsiella pneumoniae* is the expression of carbapenemases, most frequently KPC-2. In March 2019, ceftazidime-avibactam (CAZ-AVI) was approved for clinical use in Brazil. Since then, it has been widely used to treat serious infections caused by KPC-producing Gram-negative bacilli. There are reports of CAZ-AVI-resistant KPC-producing *Enterobacterales* in several countries. However, there are still no reports of this occurrence in Brazil. This study aimed to evaluate the frequency of KPC variants and clonality in CAZ-AVI resistant *K. pneumoniae*. From July/2019 to July/2021, 16 *K. pneumoniae* isolates, one per patient, resistant to CAZ-AVI, positive for bla_{KPC}, and Blue-Carba negative were detected from different sites of infection or surveillance cultures from patients admitted to private hospitals in the states of São Paulo, Rio Grande do Sul and Federal District. CAZ-AVI minimal inhibitory concentrations (MICs) were determined by broth microdilution as recommended by the BrCAST/EUCAST. Isolates were characterized by total genome sequencing on the MiSeq platform, and de novo assembly was performed using Geneious software. Resistance genes were detected using the CARD/RGI application. For the characterization of KPC variants, a local database and BioEdit software were used. The clonal profile and species identification were determined by analysis of contigs in PubMLST. No metallo-β-lactamase gene was detected in the isolates. Six isolates, with CAZ-AVI MIC ≥64 mg/L, presented KPC variants not yet described in the literature. The amino acid sequences of three of these new variants showed greater similarity, respectively 99.68%, 98.05%, and 96.86% with KPC-44, and the other three new variants showed, respectively, greater similarity with KPC- 34 (98.68%), KPC-90 (98.66%), and KPC-2 (91.28%). The other ten isolates, with CAZ-AVI MIC ≥16mg/L, had KPC variants already described in other countries, but not in Brazil: KPC-33 (n=4); KPC-61 (n=2); KPC-14 (n=1); KPC-31 (n=1); KPC-35 (n=1); and KPC-90 (n=1). Concerning multilocus sequence typing, most isolates belonged to ST11 (n=9) and were detected in SP and RS. The ST258 (n=4) was detected in SP and DF, and ST16 (n=3) was detected only in SP.

The great diversity of variants detected in two years and a half of clinical use of CAZ-AVI, the predominance of the ST11 clonal group in two states, and the KPC-33 variant depict a worrying scenario in Brazil.

Keywords: Ceftazidime-avibactam; *Klebsiella pneumoniae;* antimicrobial resistance; KPC.