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Emergence During The COVID-19 Pandemic Of *Klebsiella Pneumoniae* ST307 Co-producing KPC-3 And NDM-1 Carbapenemases

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Abstract:
In Argentina, *bla*$_{KPC-2}$ and *bla*$_{NDM-1}$ are the most dominant circulating allelic variants. Dual carbapenemase producers emerged in our country during the COVID-19 pandemic, being *Klebsiella pneumoniae* (KPN) co-producing KPC and NDM of primary concern. In 2017 Argentina reported the first KPN with *bla*$_{KPC-3}$ in a ST307 isolate, which is an emerging high-risk clone, globally associated with antimicrobial resistance determinants such as CTX-M-15, KPC-2/3, OXA-48/181, NDM-1 and VIM-1. Here, we describe for the first time the emergence of two KPN ST307 isolates co-producing KPC-3 and NDM-1.**Methods** KPN M25910 (rectal swab) and M27284 (blood), were submitted in Nov. 2020 and Jun. 2021, respectively, to the National Reference Laboratory from two different hospitals and cities. Identification was performed by MALDI-TOF MS. Antimicrobial susceptibility test was performed by disk diffusion and/or microdilution/strip (CLSI/EUCAST).Enzymatic carbapenemase activity was determined by mCIM/eCIM (CLSI). Aztreonam (AZT)/avibactam (AVI) MIC was evaluated placing an AZT strip on Mueller-Hinton agar plate with 4ug/ml AVI. KPC and metallo-β-lactamase (MBL) co-production was evaluated by disk synergy tests between ceftazidime/AVI (CZA)/EDTA and AZT/boronic acid (BOR). PCR and Sanger sequencing were used for MLST and to confirm resistance genes.**Results** Both KPN were resistant to carbapenems, AZT, amikacin, gentamicin, ciprofloxacin, colistin, CZA (MIC >256 µg/ml) and susceptible to minocycline, tigecycline, fosfomycin and AZT/AVI (MIC 0.19 µg/ml).mCIM/eCIM displayed no inhibition zones, indicating the presence of a serine carbapenemase. Double-disk synergy test was positive for AZT/BOR as well as for

CZA/EDTA, suggesting the co-production of Class A and B carbapenemases, respectively. PCR was positive for bla_{KPC}, bla_{NDM} and *amp*C bla_{CMY}. *mcr-1* was negative for both KPN. Amplicons sequencing confirmed bla_{KPC-3}, bla_{NDM-1} and ST307 in both isolates.**Conclusions** To our best, these are the first isolates of KPN ST307 dual producer of KPC-3 and NDM-1 in Argentina. Phenotypic detection of carbapenemase combinations is complex and challenging. Therefore, the disk synergy CZA/EDTA and AZT/BOR could be an appropriate screening of KPC and MBL combinations in low/middle resource labs when molecular test or lateral flow are not available. The finding of KPN ST307 carrying bla_{KPC-3} plus bla_{NDM-1} highlights the relevance of epidemiological surveillance to understand the dynamics and dissemination of these mechanisms during the COVID-19 pandemic.

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