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Emergence of KPC-31, a KPC-3 Variant Associated with Ceftazidime-Avibactam Resistance, in a ST235 Pseudomonas Aeruginosa Clinical Isolate from Argentina

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Abstract:
An increasing number of KPC-producing *P. aeruginosa* (KPC-PA) has been reported worldwide. In Argentina, KPC-2 PA has been detected since 2008, causing regional outbreaks in Patagonian and Buenos Aires districts. Ceftazidime/avibactam (CZA) has been recently used to treat several infections, including MDR/XDR KPC-PA. CZA-resistance in PA is mainly related to efflux pump, mutations in the chromosomal blaPDC or acquisition of metallo-beta-lactamases (MBL). Unlike *Enterobacterales*, KPC allelic variants that confer resistance to CZA have only been exceptionally reported in PA. ST235 is an emerging PA MDR high risk clone associated with cystic fibrosis and more virulent infections. Aim: to describe a CZA-resistant PA clinical isolate harbouring KPC-31, a D179Y variant of KPC-3, conferring resistance to CZA and exhibiting reduced carbapenemasa (CBP) activity. Bacterial identification was done with MALDI-TOF. Antimicrobial susceptibility test was performed by disk diffusion or agar dilution (CLSI/EUCAST). Colistin susceptibility was evaluated with the Drop Test. Phenotypic screening of CBP was evaluated with mCIM/eCIM and a synergism disk tests for MBL using a carbapenem and EDTA disks. Lateral flow immuno-chromatography assay (LFIA) for KPC and OXA-48like enzymes was performed. PCR multiplex against KPC, IMP, VIM, NDM, and OXA-48-like CBPs were used and Sanger sequencing to confirm gene sequences and MLST. On july 2021, PA M27432 was recovered from a tracheal aspirate from a 20 y.o. male inpatient with pneumonia, admitted in a Buenos Aires City hospital. Patient was treated with several drugs including CZA. PA M27432 was resistant to penicillins, cephalosporins, ciprofloxacin, gentamicin and amikacin. Additional testing revealed resistance to (MIC, mg/L): CZA (>256), imipenem-IMP- (8), meropenem-MER- (>32), aztreonam-ATM- (64), ceftolozane/tazobactam (>256). Combinations of MER and ATM with avibactam failed to reduce MICs, unlike IMP which achieved a significant MIC reduction (2.0 mg/L). PA M27432 remained susceptible to colistin and IMP/relebactam (MIC 1.0 mg/L). Screening of CBP production was negative by mCIM, disk synergy test and LFIA. blaKPC was positive by PCR and KPC-31 variant and ST235 was confirmed. CZA-resistant PA due to the emergence of a KPC variant in ST235 high-risk clone is of concern for public health and may represent a challenge for antimicrobial management. It is of extreme alarm that common CBP detection methods were unable to detect KPC. Thus, we advocate routine CZA susceptibility testing of PA.

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