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Klebsiella pneumoniae co-producing KPC and RmtG, finally targeting Switzerland

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ABSTRACT

A carbapenem- and pan-aminoglycoside-resistant *Klebsiella pneumoniae* strain was isolated from a Brazilian patient hospitalized in a Swiss hospital. The clinical isolate carried genes encoding the KPC-2 carbapenemase and the RmtG 16S rRNA methyltransferase. This is the first report of a carbapenem-resistant *K. pneumoniae* producing RmtG in Europe.

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Klebsiella pneumoniae is a leading cause of hospital-associated bacterial infections. The acquisition of a multidrug resistance profile has been associated to the predominance of some specific sequence types (STs) in clinical settings. Among the main threats, strains producing carbapenemases are considered as the most worrying ones. In this regard, carbapenem-resistant *K. pneumoniae* strains of clonal complex 258 (CC258 that includes ST11, ST258, ST340 and ST437) that produce KPC-type carbapenemases have disseminated worldwide and have been often responsible for nosocomial outbreaks (Woodford et al., 2011). Worryingly, the co-occurrence of 16S rRNA methyltransferases, such as ArmA, RmtB, RmtD, and RmtG enzymes, that confer high-level and broad-spectrum resistance towards aminoglycosides has been increasingly reported among KPC-producing *K. pneumoniae* in recent years (Cerdeira et al., 2016; Cheng et al., 2016; Quiles et al., 2015). Once KPC and a 16S rRNA methyltransferase are co-produced, it almost leads to pandrug resistance. We report here the emergence of a *K. pneumoniae* of clonal complex 258 co-producing KPC-2 and RmtG in Switzerland.

K. pneumoniae strain KP05–2017 was isolated from a rectal swab upon admission in May 2017 in a Swiss hospital. It was recovered from a 73-year-old patient who had been hospitalized in Brazil the month before for treatment of a hemorrhagic shock caused by perforation of a gastric ulcer.

Disk diffusion assay (Sanofi-diagnostic Pasteur, France) was performed to test the antimicrobial susceptibility while E-test (bioMérieux, La Balme-les-Grottes, France), and broth dilution techniques were used to assess minimal inhibitory concentrations (MIC). Antimicrobial susceptibility was interpreted according to the CLSI guidelines. Isolate

KP05–2017 was resistant to ceftriaxone (MIC of 6 mg/L), but remained susceptible to ceftazidime and cefepime (MIC of 3 and 8 mg/L, respectively). It exhibited resistance to imipenem, ertapenem and meropenem with MICs < 32 mg/L. Carbapenemase activity was assessed using the Carba NP test (Nordmann et al., 2012). Since the isolate was resistant to amikacin, gentamicin, tobramycin and netilmicin, the occurrence of a 16S rRNA methyltransferase was tested and confirmed by using the Rapid Aminoglycoside NP test that is based on a rapid culture of bacteria in the presence of defined concentrations of gentamicin and amikacin (Nordmann et al., 2017). Isolate KP05–2017 was also resistant to sulfonamide, nalidixic acid, ciprofloxacin, chloramphenicol, tetracycline, trimethoprim-sulphamethoxazole and the combination of ceftolozane-tazobactam (MIC 24 mg/L). It remained susceptible only to fosfomycin and exhibited MIC values of colistin, the ceftazidime-avibactam association and tigecycline at 2 mg/L, 1.5 mg/L, and 0.38 mg/L, respectively.

Multiplex PCRs performed to detect Ambler class A, B and D genes and 16S rRNA methyltransferase genes (Bercot et al., 2011; Poirel et al., 2011) followed by sequencing revealed the presence of the *bla*_{KPC-2} and *rmtG* genes. Analysis of the plasmid content obtained with the Kieser method (Kieser, 1984) identified three plasmids of ca. 10, 45 and 150 kb in size. Molecular profiling showed that KP05–2017 belongs to the sequence type 11. Mating-out assays were performed to establish the transferability of the *bla*_{KPC-2} and *rmtG* genes using the azide-resistant *Escherichia coli* J53 as recipient strain. Transconjugants were selected on LB agar plates containing sodium azide (100 mg/L), amikacin (50 mg/L) and gentamicin (50 mg/L), indicating the transferability of the ca. 45-kb plasmid carrying the *rmtG* gene. However, no transconjugant was obtained when selecting on imipenem (1 mg/L).

Genomic DNA was subjected to whole genome sequencing using an Illumina MiniSeq platform (Illumine, San Diego, CA, USA), which

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generated 4,564,240 reads with an average size of 144.3 bp. De novo assembly of the reads was performed using CLC Genomics Workbench version 7.5.1 (Qiagen, France). The final size of the draft genome was of 5,779,331 bp, with an average CG content of 56.8%.

The analysis of the resistome using ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>) identified genes conferring resistance to β -lactams (*bla*_{TEM-1C} and *bla*_{KPC-2}), aminoglycosides (*aadA2*, *aadA5*, *strA* and *rmtG*), macrolides, lincosamides and streptogramin B [*erm(B)* and *mph(A)*], phenicols (*cmlA1*), sulphonamides (*sul1* and *sul2*), trimethoprim (*dfrA14*, *dfrA15* and *drfA17*) and tetracycline [*tet(D)*]. Plasmid finder (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) revealed the presence of the IncFIB(*Mar*) and IncB/O replicons, with the latter one located in the 48 kb plasmid carrying the *rmtG* gene. PHASTER (<http://phaster.ca/>) unveiled the presence of four intact, four incomplete and three putative prophages.

The successful and worldwide dissemination of KPC-2 producing *K. pneumoniae* strains belonging to CC258, which includes sequence type 11, is now well established. The emergence of clones co-producing 16S rRNA methyltransferases further complicates the treatment of infections due to these pathogens, with extremely limited therapeutic options left. The occurrence in Switzerland of a *K. pneumoniae* clone co-producing KPC-2 and RmtG, with the latter enzyme being so far reported in America and India (Bueno et al., 2013; Filgona et al., 2015; Hu et al., 2014; Poirel et al., 2014), underlines how importation of multidrug-resistant clones may occur despite the high standards of antibiotic stewardship programs and hygiene control in effect in the Swiss healthcare institutions.

Of note, and despite elevated MICs, isolate KP05–2017 remained susceptible to ceftazidime and cefepime, suggesting a low-level of expression of the *bla*_{KPC-2} gene. Notwithstanding, isolate KP05–2017 could grow on CHROMagar mSuperCarba chromID® (CHROMagar, Paris, France), CARBA SMART and CHROMID® ESBL media (bioMérieux, La Balme-les-Grottes, France) (data not shown), indicating that such isolate, despite exhibiting low MICs of some broad-spectrum cephalosporins, may be well detected with those screening media when used for screening of carriers.

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Accession number. The draft genome sequence of the *K. pneumoniae* KP05–2017 has been deposited to the GenBank under the accession number NSLD00000000.

Transparency Declarations

None to declare.

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