**Enterobacter cloacae** harbouring bla<sub>KPC-2</sub> and qnrB-1 isolated from a cystic fibrosis patient: a case report

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**Abstract**

We describe the first detection of a KPC-2- and QnrB-producing *Enterobacter cloacae* from a patient with cystic fibrosis. The bla<sub>KPC-2</sub> and qnrB-1 genes were located in a 79.8-kb plasmid. The presence of bla<sub>KPC-2</sub> and qnrB-1 genes was determined by PCR and sequencing. Mobilization of plasmid containing bla<sub>KPC-2</sub> gene was assayed by conjugation.

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**Introduction**

*Enterobacteriaceae* are uncommonly isolated from respiratory specimens and appear to play a minor role in lung infection in individuals with cystic fibrosis (CF). However, increasing reports of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* in individuals with CF is a matter of concern [1,2]. This study describes the first report of a KPC-2 and QnrB-1-producing *Enterobacter cloacae* from an individual with CF.

**Case report**

The patient was a 21-year-old woman who presented weight loss and cough since birth. A diagnosis of CF was confirmed at 2 years of age, with sweat test (>90 mEq/L) and the genotype F508del/F508del. Pancreatic insufficiency was soon diagnosed and treatment was started with nutritional support, pancreatic enzymes, vitamins, physiotherapy, mucolytics and bronchodilators. *Pseudomonas aeruginosa* was first detected at the time of diagnosis and chronic colonization was assumed 5 years later (August 2003) when she started on inhaled tobramycin every other month. Although she had good treatment compliance and achieved good nutritional status (body mass index (BMI) 19–21 kg/m<sup>2</sup>), she still had frequent respiratory exacerbations that required either oral ciprofloxacin or intravenous ceftazidime and amikacin. By 15-years of age, she presented a BMI of 20.8 kg/m<sup>2</sup>; forced expiratory volume in 1 second (FEV1) 67.5%; forced vital capacity (FVC): 85.8% and sputum with imipenem-resistant *P. aeruginosa*. At this time (May 2011), *E. cloacae* was isolated and the patient was using only inhaled tobramycin. Two months after the isolation of *E. cloacae*, the patient had another exacerbation (lost weight and BMI 20.8 kg/m<sup>2</sup>; dyspnoea, more secretions) and received intravenous antibiotics for 2 weeks. During subsequent evaluations FEV1 decreased from 67.5% to 48% (rate: 4.5% each year) and FVC decreased from: 85.8% to 78% (rate: 1.95% each year) while BMI was stable (20.8 kg/m<sup>2</sup>) during the period. From this...
moment and until the latest cultures, only mucoid imipenem-resistant *P. aeruginosa* strains and methicillin-susceptible *Staphylococcus aureus* were isolated.

*Enterobacter cloacae* identification and antimicrobial susceptibility profile were investigated using the Vitek2 system (bio-Mérieux, Marcy l’Étoile, France). The strain was susceptible to amikacin, gentamicin, ciprofloxacin and colistin, with MICs of 8, ≤1, 1 and ≤0.5 mg/L, respectively, using criteria defined by the CLSI [3], with the exception of colistin, where European Committee on Antimicrobial Susceptibility Testing criteria were used. The strain was resistant to a wide variety of antimicrobials, including carbapenems (Table 1). According to phenotypic methods using EDTA and phenyl boronic acid as enzymatic blockers [4] the isolate was defined as a possible KPC producer. This was confirmed by PCR amplifying and sequencing the *bla*KPC-2 gene [5]. The presence of the quinolone-resistance gene *qnrB* was also verified by PCR [6] (Table 1). Analyses with S1 nuclease pulsed-field gel electrophoresis and Southern blot confirmed the size of the plasmid and showed the presence of both the *bla*KPC-2 and *qnrB*-1 genes in a 78.0-kb plasmid. Plasmid mobilization was successfully performed by conjugation with an *Escherichia coli* J53 strain. Selection with imipenem (1 mg/L) allowed the isolation of transconjugants harbouring the *bla*KPC-2 and *qnrB*-1 genes, with an increased MIC for meropenem and imipenem (Table 1).

**Discussion**

The presence of *Enterobacteriaceae* has seldom been described in patients with CF. However, a recent study showed the persistent isolation of *Escherichia coli* (i.e. for longer than 6 months) among German CF patients [2]. Besides that, reports of KPC-producing *K. pneumoniae* in individuals with CF supports a role for members of the *Enterobacteriaceae* as possible CF pathogens [1,2]. Our group reported the first isolation of KPC-2-producing *K. pneumoniae* from two Brazilian patients with CF patients pulmonary exacerbations. However, no KPC-producing strains grew from subsequent sputum cultures [2]. More recently, the establishment of a chronic lung infection in a patient with CF with a colistin-resistant KPC-3-producing *K. pneumoniae* was reported [1]. The isolates were persistently isolated from sputum cultures collected from the patient for at least 6 consecutive months. In contrast with previous reports, ours is the first case in which *bla*KPC-2 and *qnrB* genes were detected in *E. cloacae* from a patient with CF. This strain was recovered only once in the sputum cultures during an exacerbation episode.

Although the isolation of *Enterobacteriaceae* species may not be associated with disease severity in patients with CF, the detection of *E. cloacae* carrying a conjugative plasmid harbouring the *bla*KPC gene highlights the possibility of the spread of carbapenem resistance among different microorganisms, such as *P. aeruginosa*, in infected lungs. The presence of *qnr* genes can also be a determining factor for the selection of additional chromosome-borne mechanisms of resistance to quinolones after treatment by these antibiotics. The *qnr*-positive isolates may be a favourable background for an in vivo selection of additional chromosome-borne mechanisms of resistance to quinolones after treatment by fluoroquinolones. The *qnr* genes appear to be highly promiscuous, having the capacity to become rapidly disseminated among related and unrelated hosts. The transmissibility of the *qnr* genes makes the genomic mechanisms facilitating their movement of considerable interest and of relevance in the community and in healthcare settings. The detection of *qnrB*-1 and *bla*KPC-2 plasmid genes in *E. cloacae* isolated from a patient with CF highlights the importance of screening resistance genes in CF microbiology to aid in the infection control measures adopted for these patients.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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