Enterobacter cloacae harbouring bla_{KPC-2} 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_{KPC-2} and *qnrB-1* genes were located in a 79.8-kb plasmid. The presence of bla_{KPC-2} and *qnrB-1* genes was determined by PCR and sequencing. Mobilization of plasmid containing bla_{KPC2} gene was assayed by conjugation.

Keywords: Antimicrobial resistance, bla_{KPC-2}, Cystic fibrosis, *Enterobacteriaceae*, *qnrB-1*
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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^2), 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^2; 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^2; 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^2) during the period. From this

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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 (bioMérieux, Marcy l'Etoile, 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).

TABLE 1. Phenotypic and molecular characterization of a QnrB-1 and KPC-2 *Enterobacter cloacae* strain 12615 isolated from a brazilian CF patient and its transconjugant

	E. cloacae 12615		TC-12615-bla_{KPC}, *qnrB*		J53 *Escherichia coli* (recipient)	
Specimen	Sputum		–		–	
Resistance genes[a]	bla_{KPC-2}, *qnrB-1*		bla_{KPC}, *qnrB*		NA	
MIC (mg/L)[b] [category]						
Amikacin	8	[S]	≤2	[S]	≤2	[S]
Cefepime	2	[I]	≤1	[S]	≤1	[S]
Cefoxitin	≥64	[R]	≤4	[S]	≤4	[S]
Ceftazidime	16	[R]	≤1	[S]	≤1	[S]
Ceftriaxone	≥64	[R]	≤1	[S]	≤1	[S]
Cefuroxime	≥64	[R]	≥64	[R]	4	[S]
Ciprofloxacin	1	[S]	≤0.25	[S]	≤0.25	[S]
Colistin	≤0.5	[S]	≤0.5	[S]	≤0.125	[S]
Gentamycin	≤1	[S]	≤1	[S]	≤1	[S]
Imipenem	≥16	[R]	8	[R]	0.25	[S]
Meropenem	≥16	[R]	4	[R]	≤0.25	[S]
Tigecycline	2	[I]	≤0,25	[S]	≤0.25	[S]
Piperacillin/tazobactam	≥128	[R]	≥128	[R]	≤4	[S]

Abbreviations: NA, not applicable; I, intermediate; R, resistant; S, susceptible.
[a]Resistance genes for clinical sample were detected by PCR and sequencing. For transconjugant strain, standard PCR was used.
[b]Antimicrobial susceptibility tests were interpreted according to CLSI criteria, except for tigecycline and polymyxins, which were interpreted according to European Committee on Antimicrobial Susceptibility Testing guidelines.

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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