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Short Communication

Emergence of *aph(3')-VI* and accumulation of aminoglycoside modifying enzyme genes in KPC-2-possessing *Enterobacter aerogenes* isolates from infections and colonization in patients from Recife-PE, Brazil

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Abstract

Introduction: The objective of this study was to characterize genes of aminoglycoside modifying enzymes (AMEs) in colonizing and infecting isolates of *E. aerogenes* harboring bla_{KPC} from patients at a public hospital in Recife-PE, Brazil. **Methods:** We analyzed 29 *E. aerogenes* clinical isolates resistant to aminoglycosides. AMEs genes were investigated by PCR and sequencing. **Results:** Colonizing and infecting isolates mainly presented the genetic profiles *aac(3)-IIa/aph(3')-VI* or *ant(2'')-IIa/aph(3')-VI*. This is the first report of *aph(3')-VI* in *E. aerogenes* harboring bla_{KPC} in Brazil. **Conclusions:** The results highlight the importance in establishing rigorous methods for the surveillance of resistance genes, especially in colonized patients.

Keywords: *Enterobacter aerogenes*. Aminoglycoside modifying enzymes. Antimicrobial resistance. KPC.

Enterobacter aerogenes is a gram-negative bacillus that belongs to the family Enterobacteriaceae. The bacterium is found in the environment and in the human gastrointestinal tract, and is an important opportunistic pathogen capable of colonizing and infecting hospitalized patients. *E. aerogenes* frequently causes healthcare-associated infections, such as pneumonia, meningitis, urinary tract infections, surgical site infections and sepsis[1,2].

Carbapenems were the first-line antibiotic in the treatment of infections caused by multidrug-resistant *E. aerogenes*[3]. However, resistance to carbapenems has emerged and the prevalence of carbapenem resistant isolates of *E. aerogenes* is increasing[4].

One of the major causes of carbapenem-resistance in Enterobacteriaceae is the production of *Klebsiella pneumoniae* carbapenemase (KPC). This enzyme has been frequently reported in *E. aerogenes*[5,6]. KPC-producing bacteria are often resistant to other antimicrobials, such as aminoglycosides, which may be due to the presence of the bla_{KPC} gene, genes encoding aminoglycoside modifying enzymes (AMEs), and 16S rRNA methyltransferase genes in the same plasmid[7,8].

Although studies have increasingly reported the emergence of KPC-producing gram-negative bacteria that are multidrug-resistant, little is known about the presence of bla_{KPC-2} and genes for AMEs in colonized isolates. In China, one study described the presence of *aph(3')-VI* associated with another carbapenemase, New Delhi metallo-β-lactamase (NDM)[9]. Another study based in Curitiba-PR, Brazil, reported *aac(3)-III*, *aac(6')-Ib*, and *ant(3'')-Ia* genes in four *E. aerogenes* isolates, with none detected simultaneously with the bla_{KPC-2}[10] gene. A study from Recife-PE, Brazil, described many *E. aerogenes* isolates that harbored beta-lactam resistance genes, including bla_{KPC-2}[6]. However, no AMEs genes were investigated. Therefore, the objective of this study was to characterize AMEs genes present in 29 isolates of *E. aerogenes* harboring the bla_{KPC} gene that were obtained from isolates that had colonized and infected patients at a public hospital in Recife-PE, Brazil.

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The *E. aerogenes* isolates harbored the bla_{KPC-2} gene and were resistant to one or more aminoglycosides. The isolates were from a tertiary hospital in Recife-PE, Brazil, from November 2011 to August 2012. The isolates were identified biochemically using the Bactec 9120 or Phoenix™ automated systems (BD). The presence of bla_{KPC-2} in these isolates was previously reported[6]. The minimum inhibitory concentration for antimicrobials was determined using the Phoenix™ system, according to CLSI criteria[11].

Bacterial genomic DNA was extracted using the Kit ZR Fungal/Bacterial DNA Miniprep (Zymo Research), according to the manufacturer instructions for gram-negative bacteria and quantified using a NanoDrop 2000c UV-Vis spectrophotometer. AMEs genes *aac(3)-Ia, aac(3)-IIa, aac(6')-Ib, ant(2'')-Ia*, and *aph(3')-VI* were amplified by PCR using specific primers. The PCR amplification reactions were performed in a total volume of 25 µl per tube, containing 1 µl of genomic DNA (10 ng/µl), 2.0 U of *Taq* DNA polymerase (Promega), 200 µM of deoxyribonucleotide triphosphate (dNTP) (Promega), 1.5 mM $MgCl_2$, and 1 µmol of the primers. The PCR amplifications were performed in a thermocycler (Biocycler Biosystems) for 5 minutes at 94°C for initial denaturation, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C (**Table 1**) and 1 minute at 72°C, followed by a final extension at 72°C for 5 minutes. The PCR products were subjected to electrophoresis on a 1% agarose gel. Representative isolates of positive PCR products for the tested genes were selected, purified using a commercial kit (Wizard® SV Gel and PCR Clean-Up System, Promega), and sequenced by the Sanger method to confirm the presence and variant of the genes. The nucleotide sequences were analyzed by BLAST (http://www.ncbi.nlm.nih.gov/) and Clustal W (European Bioinformatics Institute) (http://www.cbi.ac.uk/) and have been deposited in GenBank (accession numbers: KU899109, KU899110 and KU695892).

The isolates were from different patients admitted to different sectors of a public hospital in Recife-PE, Brazil. Of the 29 *E. aerogenes* clinical isolates, 15 (51.7%) were obtained from rectal swabs (surveillance culture), followed by blood (n=7), urine (n=3), tracheal secretion (n=2), catheter tips (n=1), and ascitic fluid (n=1) **(Table 1)**.

All *E. aerogenes* isolates were resistant to amoxicillin-clavulanic acid, ampicillin, cefazolin, cefotaxime, cefoxitin, ceftriaxone, ciprofloxacin, ertapenem, levofloxacin, meropenem, and piperacillin-tazobactam. Resistance to imipenem reached 93% (27/29) and 90% (26/29) for cefepime, and 82% (24/29) trimethoprim/sulfamethoxazole. In addition, 100% (29/29) of the isolates were resistant to tobramycin, 86.2% (25/29) were resistant to amikacin, and 65.5% (19/29) were resistant to gentamicin. Three aminoglycoside resistance phenotypes were identified in the isolates **(Table 1)**. Among those, the most predominant was the phenotype III, which presented resistance to all aminoglycosides tested. Phenotypes I (resistant to amikacin and tobramycin) and II (resistant to gentamicin and tobramycin) were also detected.

The most frequent aminoglycoside resistance gene was *aph(3')-VI,* which was detected in 89.6% (26/29) of the isolates and were detected in all aminoglycoside resistance phenotypes. The *aac(3)-IIa* gene was found in 51.7% (15/29) of the isolates with resistance phenotypes II and III. The *ant(2'')-Ia* gene was detected in 41% (12/29) of the samples (phenotype I and III) **(Table 1)**. The *aac(6')-Ib* and *aac(3)-Ia* genes were not found. In phenotypes I and III, resistance to amikacin could be explained by the presence of the *aph(3')-VI* gene, and the resistance to gentamicin and tobramycin by the presence of the genes *aac(3)-IIa* and *ant(2'')-Ia.*

Of the 29 isolates of *E. aerogenes*, 15 were colonization isolates and 14 were obtained from infections **(Table 1)**. Among the colonization isolates, phenotype III was the most common, presenting resistance to the three aminoglycosides tested. Phenotype I (amikacin and tobramycin) was the most observed among the infection isolates. Colonization and infection isolates presented mainly the genetic profiles *aac(3)-IIa/aph(3')-VI* or *ant(2'')-IIa/aph(3')-VI*.

Patients hospitalized for prolonged periods, mainly in the intensive care unit, are most at risk of acquiring infections by *E. aerogenes*[12,13]. In the present study, the samples analyzed were obtained from patients admitted to different sectors of a public hospital.

It is alarming that most of the genes ($bla_{KPC,}$ *aph(3')-VI, aac(3)-IIa*, and *ant(2'')-IIa*) were detected in colonization isolates obtained from cultures of surveillance samples (swab rectal). A review in 2016 showed that patients colonized by carbapenem resistant *Enterobacteriaceae* had a 16.5% increased risk of developing an infection[13]. Therefore, it is essential to identify colonized patients by isolates carrying carbapenemases and other resistance genes, for example the AMEs genes, to prevent the transmission of resistance genes and adequately treat the patients. The present results highlight the need for medical authorities to institute rigorous detection and dissemination control methods for multi-drug resistant isolates in the hospital environment, since the intestinal tract provides a favorable locus for the transfer of genes that confer resistance.

Based on the results of the susceptibility profile, several isolates of *E. aerogenes* showed resistance to the three aminoglycosides tested, and were correlated with the presence of more than one of the AMEs. Most isolates showed resistance to amikacin, which explains the high percentage of the *aph(3')-VI* gene. In China, a study showed the presence of the *aph(3')-VI* gene in isolates of *E. aerogenes* associated with NDM[10]. To our knowledge, the present study is the first report of the *aph(3')-VI* gene in *E. aerogenes* in Brazil, especially in isolates harboring the bla_{KPC} gene. This causes concern since amikacin is only recommended when resistance to gentamicin and tobramycin is observed[14]. Therefore, the presence of the *aph(3')-VI* gene and the bla_{KPC} gene in the same isolate may hamper the treatment of infections caused by these microorganisms, since aminoglycosides and carbapenems will be inactivated by the encoded enzymes by these two genes.

In this study, all isolates encoding *aac(3)-IIa* were resistant to gentamicin, suggesting that the enzyme AAC (3)-II is a determinant of resistance to gentamicin in *E. aerogenes*[14].

In Brazil, the gene *ant(2'')-Ia* has been found in other gram-negative bacteria, such as *Pseudomonas aeruginosa*[15].

TABLE 1: Origin, and phenotypic and genotypic resistance profiles to aminoglycosides in KPC-possessing *Enterobacter aerogenes* isolates from a tertiary care hospital in Recife-PE, Brazil.

Isolates	Inpatient unit	Source of isolation	Resistance phenotypes to aminoglycosides	AMEs genes
Ea2A	UCO1	Rectal swab	III	aac(3)-IIa/aph(3')-VI
Ea3A	UCO2	Rectal swab	I	aph(3')-VI
Ea4A	CARDIO	Rectal swab	III	ant(2")-IIa/aph(3')-VI
Ea5A	ICU	Rectal swab	III	aac(3)-IIa/aph(3')-VI
Ea6A	ICU	Rectal swab	III	aac(3)-IIa/aph(3')-VI
Ea8A	ICU	Rectal swab	II	aac(3)-IIa/aph(3')-VI
Ea13A	UCO1	Rectal swab	I	aph(3')-VI
Ea14A	UCO1	Rectal swab	II	aac(3)-IIa/aph(3')-VI
Ea16A	UCO1	Rectal swab	III	aph(3')-VI
Ea17A	UCO1	Rectal swab	III	aac(3)-IIa
Ea20A	UCO1	Rectal swab	III	aac(3)-IIa/aph(3')-VI
Ea23A	UCO1	Rectal swab	I	ant(2")-Ia/aph(3')-VI
Ea24A	ICU	Rectal swab	III	aac(3)-IIa/ant(2")-Ia/ aph(3')-VI
Ea29A	UCO1	Rectal swab	I	ant(2")-Ia/aph(3')-VI
Ea31A	CARDIO	Rectal swab	III	aac(3)-IIa/aph(3')-VI
Ea7A	ICU	Blood	II	aac(3)-IIa/aph(3')-VI
Ea18A	ICU	Blood	III	ant(2")-Ia/aph(3')-VI
Ea27A	UCO1	Blood	III	aac(3)-IIa/aph(3')-VI
Ea28A	ICU	Blood	I	ant(2")-Ia/aph(3')-VI
Ea32A	UCO1	Blood	I	ant(2")-Ia/aph(3')-VI
Ea33A	ICU	Blood	I	ant(2")-Ia/aph(3')-VI
Ea34A	ICU	Blood	I	ant(2")-Ia
Ea10A	CARDIO	Urine	I	aph(3')-VI
Ea11A	M. CLINIC	Urine	III	aac(3)-IIa/aph(3')-VI
Ea26A	CARDIO	Urine	III	aac(3)-IIa/ant(2")-Ia/ aph(3')-VI
Ea12A	ICU	Tip catheter	III	aac(3)-IIa/aph(3')-VI
Ea15A	ICU	Tracheal aspirates	II	aac(3)-IIa
Ea19A	ICU	Tracheal aspirates	I	ant(2")-Ia/aph(3')-VI
Ea30A	ICU	Ascitic fluid	I	ant(2")-Ia/aph(3')-VI

Ea: *E. aerogenes*. **A:** public hospital; **ICU:** intensive care unit; **UCO:** Coronary Unit; **CARDIO:** cardiology; **M. CLINIC:** medical clinic. **Phenotype I** (resistance to amikacin and tobramycin), **Phenotype II** (resistance to gentamicin and tobramycin), **Phenotype III** (resistance to amikacin, gentamicin and tobramycin).

The ANT(2")-Ia enzyme confers resistance to gentamicin and tobramycin[14]. However, in this study, this enzyme seemed to be directly related to tobramycin resistance. The *aac(6')-Ib* and *aac(3)-Ia* genes were not detected in this study, although they have already been described in *E. aerogenes* in Paraná, Brazil[11].

The simultaneous presence of resistance genes *aph(3')-VI*, *aac(3)-IIa* and *ant(2")* and bla_{KPC} in the same isolate, as demonstrated in this study, adds to the mechanisms of the dissemination of resistance to aminoglycosides and carbapenems in the hospital environment, and decreases the chances for therapeutic success of infections caused by *E. aerogenes*. Several AMEs have been already identified in different bacteria species in Brazil. However, the emergence of *aph (3 ')-VI* gene in *E. aerogenes* described here indicates the continuous dissemination of AMEs encoding genes in Brazil. These results highlight the importance in establishing rigorous methods for the surveillance of resistance genes, especially in colonizing patients.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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