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Detection of NDM-1, CTX-M-15 and qnrB4-producing *Enterobacter hormaechei* isolates in Brazil

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Running Title: NDM-1-producing *E. hormaechei* in Brazil

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Abstract: Not applicable

Text:

After the first description of NDM-1-carbapenemase in a *Providencia rettgeri* isolate in Brazil in February 2013 (1), the public hospital where this isolate was recovered began an active surveillance in search of asymptomatic carriers and at the hospital environment. Furthermore, a retrospective study of carbapenem-resistant isolates stored at that hospital since 2012 was performed. Six NDM-1-producing *Enterobacter hormaechei* subps. *oharae* isolates, identified by the Vitek2 system and *hsp60*-genotyping, were recovered and characterized by phenotypic assays and molecular techniques, such as PCR and DNA sequencing (2,3). One isolate (CCBH10892) was recovered from a rectal swab of a patient at the Intensive Care Unit (ICU) in September 2012 (a time period prior to the isolation of NDM-positive *P. rettgeri*). The others were isolated from March to May 2013 from rectal swabs of patients (n=4) and a sink (n=1) located in the same ICU.

PFGE of *Xba*-I-digested DNA (4) showed that all isolates belonged to the same clone, considered to be multidrug-resistant as they were susceptible only to amikacin (MIC range to 8-16mg/L) and polymyxin B (MIC ≤1mg/L) by E-test method (Figure 1). Besides $bla_{\text{NDM-1}}$, all these isolates carried $bla_{\text{CTX-M-15}}$, *qnrB4* and *aac(6)-Ib* genes, detected by PCR and sequencing. Plasmid analysis by restriction digests with S1 nuclease and southern blotting (5) showed that the $bla_{\text{NDM-1}}$ and *qnr*B4 genes were located on the same plasmids, ranging in size from 420 to 490kb (Figure 1).

To obtain a comprehensive in-depth view of the genetic structure surrounding the $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$ and *qnr*B4 genes, the genomic sequence of the CCBH10892 isolate was determined on an Illumina MiSeq system. A total of 1,149,470 reads (5,373,710bp) were assembled with Geneious assembler (Biomatters) to generate 56 contigs. We found $bla_{\text{NDM-1}}$ in a 94,795bp contig flanked by a truncated IS*Aba*125 at the right boundary and by a bleomycin-resistance gene (ble_{MBL}) at the left (GeneBank accession number KF727591). This region shared 99% identity with the NDM region present in a plasmid carried by a *K. pneumoniae* isolate from Taiwan (6). In this contig, some

conjugation and plasmid transfer genes and a replication protein gene belonging to the IncF group were also observed. However, this replicon could not be detected by the PBRT scheme (7).

The *qnr*B4 gene was found in a 16,569bp contig in which were also observed IS*CR1*, genes encoding permeases (*sap*A-B-C), phage shock proteins (*psp*A-B-C-D) and AmpC $bla_{\text{DHA-1}}$ (GeneBank accession number KF646592). This same region has been reported in different plasmids of other bacterial species (8).

$bla_{\text{CTX-M-15}}$ was integrated in the chromosome associated with an upstream IS*Ecp1* element in a 277,989bp contig (part of it is in GeneBank accession number KF727590). This transposition unit was inserted into the *flh*C gene that encodes a flagellar transcriptional activator protein (9). In fact, these isolates showed no motility.

This study showed the detection of a multiresistant *E. hormaechei* clone carrying relevant resistance genes ($bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$ and *qnr*B4) in asymptomatic carriers and at the hospital environment, alerting that our current views on the extent of the spread of NDM in Brazil may well be underestimated.

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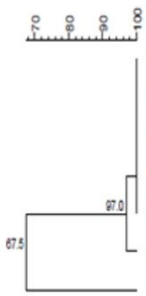
Figure 1: Characteristics of 6 NDM-producing *E. hormaechei* subsp. *oharae* isolates obtained in a hospital in Rio Grande do Sul, Brazil.

*CCBH14397 – *E. hormaechei* isolate epidemiologically unrelated to NDM-producing isolates

Results of antimicrobial susceptibility were interpreted according to CLSI breakpoints, except for tygecicline and polymixin in which the EUCAST breakpoints were used.



Number	Date	Site	Ward	ERT	MER	IPM	CTX	CAZ	AMI	GEN	CIP	TIG	POL	blaNDM-1 plasmid
CCBH10892	Sep/12	rectal swab	ICU	32 (R)	24 (R)	32 (R)	256 (R)	256 (R)	8 (S)	256 (R)	32 (R)	2 (R)	0.5 (S)	490kb
CCBH12047	Mar/13	rectal swab	ICU	12 (R)	32 (R)	32 (R)	256 (R)	256 (R)	8 (S)	256 (R)	12 (R)	1.5 (R)	1.0 (S)	490Kb
CCBH12102	Mar/13	rectal swab	ICU	32 (R)	32 (R)	24 (R)	256 (R)	256 (R)	8 (S)	256 (R)	24 (R)	2 (R)	0.5 (S)	490kb
CCBH12180	Apr/13	sink	ICU	32 (R)	32 (R)	32 (R)	256 (R)	256 (R)	16 (S)	256 (R)	32 (R)	2 (R)	0.5 (S)	490kb
CCBH12503	May/13	rectal swab	ICU	32 (R)	32 (R)	32 (R)	256 (R)	256 (R)	12 (S)	6 (I)	32 (R)	2 (R)	0.5 (S)	460kb
CCBH12049	Mar/13	rectal swab	ICU	32 (R)	32 (R)	32 (R)	256 (R)	256 (R)	8 (S)	256 (R)	32 (R)	16 (R)	0.5 (S)	420kb
CCBH14397 *	Oct/13													