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

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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 blaNDM-1, all these isolates carried blaCTX-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 blaNDM-1 and qnrB4 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 blaNDM-1, blaCTX-M-15 and qnrB4 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 blaNDM-1 in a 94,795bp contig flanked by a truncated ISAba125 at the right boundary and by a bleomycin-resistance gene (bleMBL) 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 \textit{qnr}B4 gene was found in a 16,569bp contig in which were also observed IS\textit{CR1}, genes encoding permeases (\textit{sap}A-B-C), phage shock proteins (\textit{psp}A-B-C-D) and AmpC \textit{bla}\textit{DHA-1} (GeneBank accession number KF646592). This same region has been reported in different plasmids of other bacterial species (8).

\textit{bla}_{\text{CTX-M-15}} was integrated in the chromosome associated with an upstream \textit{ISEcp1} element in a 277,989bp contig (part of it is in GeneBank accession number KF727590). This transposition unit was inserted into the \textit{flhC} gene that encodes a flagellar transcriptional activator protein (9). In fact, these isolates showed no motility.

This study showed the detection of a multiresistant \textit{E. hormaechei} clone carrying relevant resistance genes \textit{(bla}\textit{NDM-1}, \textit{bla}_{\text{CTX-M-15}} and \textit{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.

\textbf{Acknowledgments}

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References


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.
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