The emergence of multidrug-resistant Enterobacteriaceae strains producing carbapenemases, such as NDM-1, has become a major public health issue due to a high dissemination capacity and limited treatment options. Here we describe the draft genome of three NDM-1-producing isolates: Providencia rettgeri (CCBH11880), Enterobacter hormaechei subsp. oharae (CCBH10892) and Klebsiella pneumoniae (CCBH13327), isolated in Brazil. Besides blaNDM, resistance genes to aminoglycosides [aadA1, aadA2, aac(6’)-Ib-cr] and quinolones (qnrA1, qnrB4) were observed which contributed to the multidrug resistance profile. The element ISAba125 was found associated to the blaNDM gene in all strains.

Key words: NDM-1 - Brazil - Enterobacteriaceae

The emergence of carbapenemase-producing Enterobacteriaceae has become a major public health issue worldwide due to a high dissemination capacity and limited treatment options (Nordman et al. 2011). NDM is a metallo-beta-lactamase first reported in 2009 (Yong et al. 2009) and, now, it has already been detected in several countries worldwide. In Brazil, this carbapenemase was first described in a Providencia rettgeri isolate from the city of Porto Alegre, state of Rio Grande do Sul (RS) (South Region of Brazil), in 2013 (Carvalho-Assef et al. 2013). Then, the detection of six clonally related NDM-producing Enterobacter hormaechei subsp. oharae (CCBH10892) and Klebsiella pneumoniae (CCBH13327), isolated in Brazil. Besides blaNDM, resistance genes to aminoglycosides [aadA1, aadA2, aac(6’)-Ib-cr] and quinolones (qnrA1, qnrB4) were observed which contributed to the multidrug resistance profile. The element ISAba125 was found associated to the blaNDM gene in all strains.

Initialy all of the strains were tested against different antimicrobial drugs and showed multidrug resistance profiles. Genomic DNA of all strains was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Germany). Whole genome shotgun libraries from each strain were prepared with the Nextera XT DNA Sample Prep kit (Illumina Inc, USA), according to the manufacturer’s instructions, and sequenced on an Illumina MiSeq system with the MiSeq Reagent v.2 500 cycles kit. Sequence reads were then trimmed and filtered using a Phred score >20. The software Geneious v.6.1.7 ( Biomatters Ltd, New Zealand) was used to perform de novo assembling. Rapid Annotation using System Technology v.2.0 server was used for genome annotation. Acquired resistance genes were analysed using the ResFinder platform (genomicepidemiology.org). The detailed features of all isolates can be found on Table. For the P. rettgeri isolate (CCBH11880) we obtained 656,560 paired end reads of 250 base pairs (bp), which were assembled into 80 contigs. The G+C content for this strain was 41%, considered common for this species. The estimated genome size, comprising all contigs, was 4,999,177 bp. Overall, 4,670 protein coding sequences were found and 89 RNAs were annotated (79 tRNA and 10 rRNA). Acquired resistance genes were searched using the ResFinder platform and different resistance genes were observed such as: aadA1 (GenBank JSEQ01000006.1; 199,767-200,555 bp), strA (GenBank JSEQ01000017.1; 91,398-92,201 bp), strB (GenBank JSEQ01000017.1; 92,201-93,037 bp), aadB (GenBank JSEQ01000041.1; 326-859 bp), aac(6’)-Ib (GenBank JSEQ01000017.1; 2,882-3,400 bp), qnrD (GenBank JSEQ01000028.1; 1,671-2,315 bp), blaOXA-10 (GenBank JSEQ01000017.1; 2,014-2,814 bp), blaNDM-1 (GenBank JSEQ01000024.1; 412-1,224 bp), ereA (GenBank JSEQ01000025.1; 295-1,521 bp), msr(E) (GenBank JSEQ01000023.1; 37,090-38,565 bp), mph(E) (GenBank JSEQ01000023.1; 36,150-37,034 bp), floR (GenBank JSEQ01000017.1; 96,173-97,386 bp), catA1 (GenBank JSEQ01000031.1; 870-1,529 bp), sul2 (GenBank JSEQ01000017.1; 3,823-4,749 bp).

Initially all of the strains were tested against different antimicrobial drugs and showed multidrug resistance profiles. Genomic DNA of all strains was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Germany). Whole genome shotgun libraries from each strain were prepared with the Nextera XT DNA Sample Prep kit (Illumina Inc, USA), according to the manufacturer’s instructions, and sequenced on an Illumina MiSeq system with the MiSeq Reagent v.2 500 cycles kit. Sequence reads were then trimmed and filtered using a Phred score >20. The software Geneious v.6.1.7 (Biomatters Ltd, New Zealand) was used to perform de novo assembling. Rapid Annotation using System Technology v.2.0 server was used for genome annotation. Acquired resistance genes were analysed using the ResFinder platform (genomicepidemiology.org). The detailed features of all isolates can be found on Table. For the P. rettgeri isolate (CCBH11880) we obtained 656,560 paired end reads of 250 base pairs (bp), which were assembled into 80 contigs. The G+C content for this strain was 41%, considered common for this species. The estimated genome size, comprising all contigs, was 4,999,177 bp. Overall, 4,670 protein coding sequences were found and 89 RNAs were annotated (79 tRNA and 10 rRNA). Acquired resistance genes were searched using the ResFinder platform and different resistance genes were observed such as: aadA1 (GenBank JSEQ01000006.1; 199,767-200,555 bp), strA (GenBank JSEQ01000017.1; 91,398-92,201 bp), strB (GenBank JSEQ01000017.1; 92,201-93,037 bp), aadB (GenBank JSEQ01000041.1; 326-859 bp), aac(6’)-Ib (GenBank JSEQ01000017.1; 2,882-3,400 bp), qnrD (GenBank JSEQ01000028.1; 1,671-2,315 bp), blaOXA-10 (GenBank JSEQ01000017.1; 2,014-2,814 bp), blaNDM-1 (GenBank JSEQ01000024.1; 412-1,224 bp), ereA (GenBank JSEQ01000025.1; 295-1,521 bp), msr(E) (GenBank JSEQ01000023.1; 37,090-38,565 bp), mph(E) (GenBank JSEQ01000023.1; 36,150-37,034 bp), floR (GenBank JSEQ01000017.1; 96,173-97,386 bp), catA1 (GenBank JSEQ01000031.1; 870-1,529 bp), sul2 (GenBank JSEQ01000017.1; 3,823-4,749 bp).
Genetic information about three NDM-1-producing isolates from Brazil

| Isolate feature | Providencia rettgeri (CCBH1880) | Enterobacter hormaechei (CCBH10892) | Klebsiella pneumoniae (CCBH13327) |
|-----------------|---------------------------------|-------------------------------------|---------------------------------|
| NCBI accession  | JSEQ01000017.1; 90,522-91,337 bp | JSBO01000014.1; 552-1,343 bp         | JSER010000014.1; 552-1,343 bp   |
| BioProject      | PRJNA264579                     | PRJNA264581                         | PRJNA264954                     |
| Isolation source| Surgical wound                  | Rectal swab                         | Rectal swab                     |
| City/state of origin | Porto Alegre/RS                        | Porto Alegre/RS                     | Rio de Janeiro/RJ               |
| GC content (%)  | 41                               | 54.5                                | 56.6                            |
| Paired end reads (n) | 656,560                          | 2,283,589                           | 1,748,579                       |
| Genome coverage  | 4,999,177                        | 5,373,562                           | 6,023,847                       |
| Estimated genome size (bp) | 4,670                             | 5,134                               | 5,722                           |
| Contigs (n)      | 80                               | 58                                  | 106                             |
| N50             | 282,487                          | 277,989                             | 133,213                         |
| Coding sequences (n) | 89                                | 102                                 | 99                              |
| RNAs (n)        | 79                               | 89                                  | 86                              |
| rRNA (n)        | 10                               | 13                                  | 13                              |
| Resistance genes | aadA1, strA, strB, aabB, aac(6’)-Ib, qnrD, bla<sub>SHV-99</sub>, ere(A), msr(E), mph(E), floR, catA1, sul1, sul2, tet(A), dfrA1, dfrA8 | aadA2, aph(3’)-Ia, strA, strB, bla<sub>SHV-99</sub>, ere(A), sul1, tet(A), tet(D) | aadA2, aac(3)-Ia, bla<sub>SHV-99</sub>, qoxA, qoxB, qnrA1, catA1, sul1, tet(D), dfrA8 |

bp: base pair; NCBI: National Center for Biotechnology Information; RJ: state of Rio de Janeiro; RS: state of Rio Grande do Sul.

Genome sequencing of K. pneumoniae isolate (CCBH11880) generated 2,283,589 paired end reads of 250 bp, yielding 58 contigs after assembly (Geneious v.6.1.7) with 106 contigs was achieved (GenBank JSER010000014.1; 552-1,343 bp, aac(3)-Ia (GenBank JSER010000058.1; 14,738-15,598 bp), bla<sub>SHV-99</sub>, msr(E), mph(E), floR, catA1, sul1, sul2, tet(A), dfrA1, dfrA8 (GenBank JSER010000033.1; 334-843 bp).

The announcement of the whole-genome sequence of three NDM-1-producing Enterobacteriaceae strains also provides basis for other studies, which will certainly increase our understanding of the role of this species in the drug resistance scenario.
Nucleotide sequence accessions - These Whole Genome Shotgun project have been deposited in DDBJ/ENA/GenBank under the accessions JSEQ00000000, JSBO00000000 and JSER00000000 for \textit{P. rettgerii} (CCBH1880), \textit{E. hormaechei} (CCBH10892) and \textit{K. pneumoniae} (CCBH13327), respectively. The versions described in this paper are the first version (JSEQ00000000, JSBO00000000 and JSER00000000).

REFERENCES

Carvalho-Assef AP, Pereira PS, Albano RM, Berião GC, Chagas TPG, Timm LN, da Silva RCF, Falci DR, Asensi MD 2013. Isolation of NDM-producing 	extit{Providencia rettgeri} in Brazil. \textit{J Antimicrob Chemother} 68: 2956-2957.

Carvalho-Assef AP, Pereira PS, Albano RM, Berião GC, Tavares CP, Chagas TP, Marques EA, Timm LN, da Silva RC, Falci DR, Asensi MD 2014. Detection of NDM-1, CTX-M-15 and \textit{qnrB4} producing \textit{Enterobacter hormaechei} isolates in Brazil. \textit{Antimicrob Agents Chemother} 58: 2475-2476.

Nordmann P, Naas T, Poirel L 2011. Global spread of carbapenemase-producing Enterobacteriaceae. \textit{Emerg Infect Dis} 17: 1791-1798.

Pereira PS, Borghi M, Albano RM, Berião GC, Lopes JCO, Asensi MD, Carvalho-Assef APD 2014. First description of NDM-1-producing \textit{Klebsiella pneumoniae} in Brazil. 24th European Congress of Clinical Microbiology and Infectious Diseases, P1196, Poster Session V, Worldwide spread of carbapenem resistance. Available from: escmid.org/escmid_library/online_lecture_library/?search=1&current_page=1&search_term=polyana+pereira&timeperiod%5B%5D=ly.

Pillonetto M, Arend L, Vespero EC, Pelisson M, Chagas TP, Carvalho-Assef AP, Asensi MD 2014. The first report of NDM-1-producing \textit{Acinetobacter baumannii} ST 25 in Brazil. \textit{Antimicrob Agents Chemother} 58: 7592-7594.

Rozales FP, Ribeiro VB, Magagnin CM, Pagano M, Lutz L, Falci DR, Machado A, Barth AL., Zavascki AP 2014. Emergence of NDM-1-producing Enterobacteriaceae in Porto Alegre, Brazil. \textit{Int J Infect Dis} 25: 79-81.

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR 2009. Characterization of a new metallo-β-lactamase gene, \textit{bla\textsubscript{NDM-1}}, and a novel erythromycin esterase gene carried on a unique genetic structure in \textit{Klebsiella pneumoniae} sequence type 14 from India. \textit{Antimicrob Agents Chemother} 53: 5046-5054.