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Letter to the Editor

Early dissemination of novel NDM-1-producing *Klebsiella pneumoniae* ST6326 to the environment

Editor: Stefania Stefani

Dear Editor,

In Latin America, the detection of carbapenemase-producing strains, especially *Klebsiella pneumoniae*, has increased during the COVID-19 pandemic. Carbapenem-resistant strains typically harbour other antimicrobial resistance genes (ARGs), leading to the extensively drug-resistant profile, which is worrying [1]. The environment plays a key role in the development and spread of antimicrobial resistance [2]. In this context, it is assumed that clinically relevant carbapenemases, even the double detection of them, may already be spreading in aquatic ecosystems; however, data remain limited. Here, we report the occurrence of novel carbapenemase-producing *K. pneumoniae* sequence type (ST) 6326 in aquatic ecosystems and the genomic analysis of a representative bla_{NDM-1}-positive strain.

Between June and July 2021, a study of carbapenem-resistant *K. pneumoniae* strains isolated from surface waters in the state of São Paulo, Brazil, was conducted. Eighty-six water samples from 58 cities were collected and filtered by membrane filters, which were placed on MacConkey agar (Kasvi, Spain) supplemented with 4 mg/L of meropenem. Subsequently, random lactose-fermenting colonies were selected and stored. Genomic DNA was extracted by PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA), and the strains were identified molecularly (Supplementary Table S1). Disk diffusion and broth microdilution methods were performed to determine the antimicrobial susceptibility. Molecular typing and detection of ARGs, virulence genes, metal tolerance genes, and plasmid replicons were performed using conventional polymerase chain reactions followed by Sanger sequencing (Supplementary Tables S2 to S6).

Four bla_{NDM-1}-producing *K. pneumoniae* strains were obtained from four aquatic ecosystems of different cities (4/86, 4.7%) (Supplementary Fig. S1). These strains were extensively drug-resistant (XDR) but susceptible to amikacin, minocycline, and tigecycline. Several ARGs, including the coexistence of bla_{NDM-1} and $bla_{CTX-M-15}$, virulence genes, metal tolerance genes, and plasmid replicons, were identified, evidencing similar genotypes. The molecular typing analysis revealed that all isolates belonged to the ST6326-KL151 (*wzi*:143) (Supplementary Table S7). This new ST was assigned in January 2023 to a clinical KPC-33-positive strain, named 186_21, isolated from human tracheal secretion in 2021 in the city of São Paulo. The ST6326 is a single locus variant (new 605 allele of *phoE*) of ST340/CG258, which is an international high-risk clone closely related to extensive drug resistance and high pathogenicity [3]. Curiously, the environmental strains were obtained from the same Brazilian state and year as the 186_21 strain. In this context, a representative environmental ST6326 strain (EW1149) was selected for the whole-genome characterization.

The whole-genome sequencing was performed using the Illumina MiSeq platform (Illumina, Inc., USA). The draft genome was *de novo* assembled by SPAdes v.3.15.2 and annotated using RAST (https://rast.nmpdr.org/rast.cgi). Molecular typing, single nucleotide polymorphism (SNP), resistome, virulome, and plasmid replicons were determined using programs available at the BIGSdb-Pasteur (https://bigsdb.pasteur.fr/) and the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Plasmid contigs were predicted by mlplasmids (https://sarredondo.shinyapps.io/mlplasmids/), and the plasmids were assembled using a multi-pronged hybrid *de novo* strategy. The plasmid sequences were manually curated by Geneious Prime® v.2023.0.4, BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and ISfinder (https://www-is.biotoul.fr/index.php).

Resistome analysis showed that the EW1149 strain presented ARGs to β-lactams (bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-9}, bla_{TEM-1A}, bla_{TEM-1B}, and bla_{SHV-11}), aminoglycosides [*aadA1, aac(3)-IIa, aac(6′)-Ib3, aph(3″)-Ib, aph(3′)-VI*, and *aph(6)-Id*], fluoroquinolones [*qnrB1, aac(6')-Ib-cr, oqxA*, and *oqxB*], tetracyclines [*tet(A)*], folate pathway antagonists (*sul2*), trimethoprim (*dfrA14*), phenicols (Δ*catB3*), and fosfomycins (*fosA*) as well as mutations in determinants of resistance for fluoroquinolones, cephalosporins, carbapenems, and colistin. Virulome analysis showed genes encoding enterobactin (*entB*), aerobactin (*iutA*), and type 3 fimbriae (*mrk* cluster). Furthermore, tolerance genes to arsenic (*arsRDABC*), copper (*pcoABCDRSE*), and silver (*silESRCBAP*) were identified. Finally, EW1149 strain harboured different plasmid replicons, including IncC3, IncFII(K), IncFIB(K), Col4401, and ColpVC. Overall, the EW1149 strain differed from the 186_21 strain mainly by the presence of different carbapenemases (Supplementary Fig. S2; Supplementary Table S8). SNP-based phylogenetic analysis showed differences of 30 SNPs between 186_21 and EW1149 genomes.

A multireplicon plasmid (~248 kb) belonged to IncFII(K7)-IncFIB(K), named pEW1149-1, that co-carried multidrug resistance and multimetal tolerance regions was detected by mapping plasmid sequences onto the 186_21 genome. On this plasmid, the $bla_{CTX-M-15}$ gene was associated with IS*Ecp1*-$bla_{CTX-M-15}$-Δ*orf477*-ΔTn*3-like*. Furthermore, an IncC3 plasmid (~136 kb), named pEW1149-2, harbouring bla_{NDM-1}, *aph(3′)-VI*, and *sul2*, was also identified. This plasmid shared high query coverage (97%) and nucleotide identity (99.99%) with bla_{NDM-1}-bearing IncC3 plasmids from *Vibrio parahaemolyticus, Citrobacter freundii*, and *Escherichia coli* of seafood and spiny eel fish from Germany and Vietnam. Contrastingly, the region harbouring the bla_{NDM-1} gene on IncC3 plasmids contained a Tn*125-like* transposon with different gene rearrangements and absence of the downstream IS*Aba125*. On the pEW1149-2, the genetic context was IS*Aba125*-bla_{NDM-1}-ble_{MBL}-*trpF-tat-cutA*-Δ*groES-groEL*, whereas on

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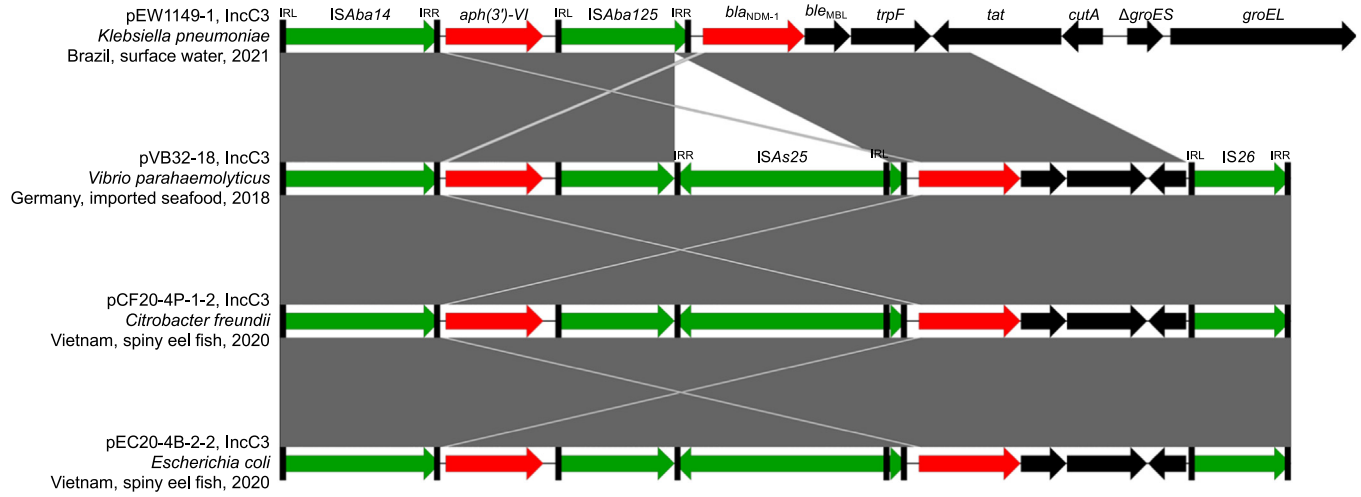

Fig. 1. Genetic context of bla_{NDM-1} in IncC3 plasmids from the environment (pEW1149-2, GenBank accession number JARVRB010000002), imported seafood (pVB32-18, GenBank accession number MN380474), and spiny eel fish (pCF20-4P-1-2, GenBank accession number AP026942; pEC20-4B-2-2, GenBank accession number AP026939). Red, green, and black arrows indicate genes related to antimicrobial resistance, insertion elements, and others, respectively. Black rectangles indicate inverted repeat (IR) sequences as follows: left (IRL) or right (IRR). The grey shading represents shared regions of homology.

the others it was ΔIS*Aba125*-IS*As25*-ΔIS*Aba125*-bla_{NDM-1}-ble_{MBL}-*trpF*-Δ*tat*-IS*26*. All plasmids contained IS*Aba14*-*aph(3′)-VI* upstream of Tn*125-like* (Fig. 1). In this regard, the two regions harboured backbones genes linked to the spread of the bla_{NDM-1} gene [4,5].

In summary, we report XDR ST6326 strains emerging from the clinical sector in Brazilian aquatic ecosystems. These findings demonstrate the early dissemination of this clone to the environment, highlight its adapting and evolution, and evidence a possible successful expansion in the One Health perspective. Therefore, continuous monitoring and genomic analysis of XDR strains are essential for a better understanding of antimicrobial resistance in the post-pandemic period.

Nucleotide sequence accession numbers

Nucleotide sequences of *K. pneumoniae* EW1149 have been deposited at GenBank under accession numbers: JARVRB010000000 (whole-genome shotgun sequencing project), JARVRB010000001 (pEW1149-1 plasmid), and JARVRB010000002 (pEW1149-2 plasmid).

Ethical Approval

Not required.

Competing interests

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.07.020.

References

[1] Romero Thomas G, Corso A, Pasterán F, Shal J, Sosa A, Pillonetto M, et al. Increased detection of carbapenemase-producing Enterobacterales bacteria in Latin America and the Caribbean during the COVID-19 pandemic. Emerg Infect Dis 2022;28 E1–8. doi:10.3201/eid2811.220415.
[2] UNEP. United Nations Environment Programme. Bracing for superbugs: strengthening environmental action in the One Health response to antimicrobial resistance. 2023. https://www.unep.org/resources/superbugs/environmental-action.
[3] Braun G, Cayô R, Matos AP, de Mello Fonseca J, Gales AC. Temporal evolution of polymyxin B-resistant *Klebsiella pneumoniae* clones recovered from blood cultures in a teaching hospital during a 7-year period. Int J Antimicrob Agents 2018;51:522–7. doi:10.1016/j.ijantimicag.2017.08.031.
[4] Bontron S, Nordmann P, Poirel L. Transposition of Tn125 encoding the NDM-1 carbapenemase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2016;60:7245–51. doi:10.1128/AAC.01755-16.
[5] Partridge SR, Iredell JR. Genetic Contexts of bla_{NDM-1}. Antimicrob Agents Chemother 2012;56:6065–7. doi:10.1128/AAC.00117-12.

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