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Letter to the Editor

Enterobacter roggenkampii producing KPC-3 collected from a hospital sink drain in Portugal during the COVID-19 pandemic

Editor: Professor A Tsakris

Sir,

Enterobacter roggenkampii is a recently described species belonging to the *Enterobacter cloacae* complex, a group increasingly associated with multidrug resistance and nosocomial infections [1]. The emergence of carbapenem resistance has become a public health concern worldwide, with the environment playing a key role in the spread of these genes, including among *Enterobacter* spp. [2]. In this study, we report an environmental KPC-3-producing *E. roggenkampii* strain recovered during a *Klebsiella pneumoniae* outbreak in a hospital centre in northern Portugal during the COVID-19 pandemic.

Before the COVID-19 pandemic, infection prevention and control measures were routinely used to reduce the spread of antimicrobial resistance, but the emergence of the COVID-19 pandemic has caused major disruptions in health care systems that threatened the effectiveness of these strategies. Despite further improvements in hand and environmental hygiene, mainly implemented in COVID-19 wards, resource constraints affected infection prevention efforts in non-COVID-19 wards, further increasing the risk of contamination and spread of infections [3]. In August 2020, during the COVID-19 pandemic and as a result of additional searches in the hospital environment due to an outbreak caused by *K. pneumoniae* strains, a KPC-3-producing *E. roggenkampii* strain unrelated to the outbreak was recovered from an operating room sink drain.

Antimicrobial susceptibility testing by disk diffusion test was performed for this strain, which showed resistance to amoxicillin-clavulanic acid, cefoxitin, cefotaxime, ceftazidime, gentamicin, ciprofloxacin, aztreonam, imipenem, meropenem, doripenem, ertapenem, and to ceftazidime/avibactam, but was susceptible to tigecycline.

Additionally, whole-genome sequencing was performed on an Illumina HiSeq NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) using paired-end reads (2 × 151 bp). Raw data quality was assessed using FASTQC v.0.11.9. Trimming and *de novo* assembly were performed using CLC Genomics Workbench 12.0.3 (QIAGEN). Antimicrobial resistance was performed by CGE (https://www.genomicepidemiology.org/), and multilocus sequence typing (MLST) performed by both CGE and BIGSdb (https://pubmlst.org). Virulence factors were analysed using Virulence Finder (https://cge.food.dtu.dk/services/VirulenceFinder/) and Virulence Factor Database (http://www.mgc.ac.cn/VFs/), while plasmid replicon identification was performed using PlasmidFinder (https://cge.food.dtu.dk/services/PlasmidFinder/).

The environmental *E. roggenkampii* strain co-produced the bla_{KPC-3}, bla_{MIR-3}, and *fosA2* genes and belonged to clone ST501. This strain carried several virulence genes, including adhesion associated genes (*mrkC, fimACDFHIZ, cfaB, csgCDFG, hofBC, bcfDFG, stgABD*), antiphagocytosis related gene (*wbfY*), iron uptake genes (*iucABCD, iutA, entABCEFS, fes, fepABCDG, shuV, chuAS)*, regulatory genes (*rcsAB*), secretion system related genes (*clpV|tssH, vasE|tssK, vgrG|tssI, vipA|tssB, vipB|tssC, clpV, epsE, flgBCDEFGHIJKLM, flhABCD, fliACDEFGHIJLMNPQRZ, clpV1, yst1O, exeDF, iagB),* toxin genes (*ast*), autotransporter genes (*cdiAB, ebaH),* endotoxin (*htrB*), invasion (*cheBRWYZ, motA*), magnesium uptake (*mgtC*), motility-related genes (*motB*) and efflux pump genes (*acrAB*). Additionally, this strain carried the plasmid replicons IncFIA(HI1), IncHI2, IncHI2A, IncN. Furthermore, among the antimicrobial resistance genes found, only the bla_{KPC-3} gene was encoded in plasmids, which was designated as pKPC-3_FMUL402.

Compared with the NCBI database, this *de novo* assembled pKPC-3_FMUL402 plasmid showed high similarity to several plasmids from *Escherichia coli* and *K. pneumoniae*, indicating that the plasmid carrying the bla_{KPC-3} gene could spread to other Enterobacterales. The alignment of the *de novo* assembled pKPC-3_FMUL402 plasmid was performed using the pWI1_KPC-3 (54,518 bp; GenBank accession number LT838197.1) plasmid obtained from a KPC-3-producing *E. coli* strain as the backbone (Fig. 1a). The bla_{KPC-3} gene was located within the Tn*4401* isoform d transposon and was flanked upstream by *tnpR, tnpA, istA*, and *istB* and flanked downstream by IS*Kpn6* (Fig. 1b).

Previous studies have shown that bla_{KPC-3} is predominantly produced by *K. pneumoniae* in hospital settings in Portugal, and that this gene is associated with the Tn*4401*d transposon [4]. Of note, the Tn*4401*d transposon has also been shown to carry a novel worldwide variant of the bla_{KPC-3} gene with great clinical importance [5]. Furthermore, the high homology of the pKPC-3_FMUL402 plasmid with plasmids carried by *K. pneumoniae* strains in the NCBI database, coupled with the fact that the bla_{KPC-3} found in this strain is also harboured within a Tn*4401*d transposon, suggests that this environmental strain may contribute to the spread of bla_{KPC-3} to other Enterobacterales, especially *K. pneumoniae* strains responsible for infections in clinical settings. The pKPC-3_FMUL402 plasmid also harboured several key mobilization genes, such as the *traABCDEFGIJKLMNO* operon, further suggesting its ability to spread across bacterial strains.

In addition, reports of carbapenemase-producing *E. roggenkampii* strains are scarce, with most strains producing mainly metallo-β-lactamases [2]. In fact, to our knowledge, no reports of *E. roggenkampii* carrying KPC-3 have been previously found in the literature to date, which is worth highlighting.

Herein, we report the first worldwide KPC-3-producing *E. roggenkampii* strain recovered from an operating room sink drain during the COVID-19 pandemic. This study highlights the impor-

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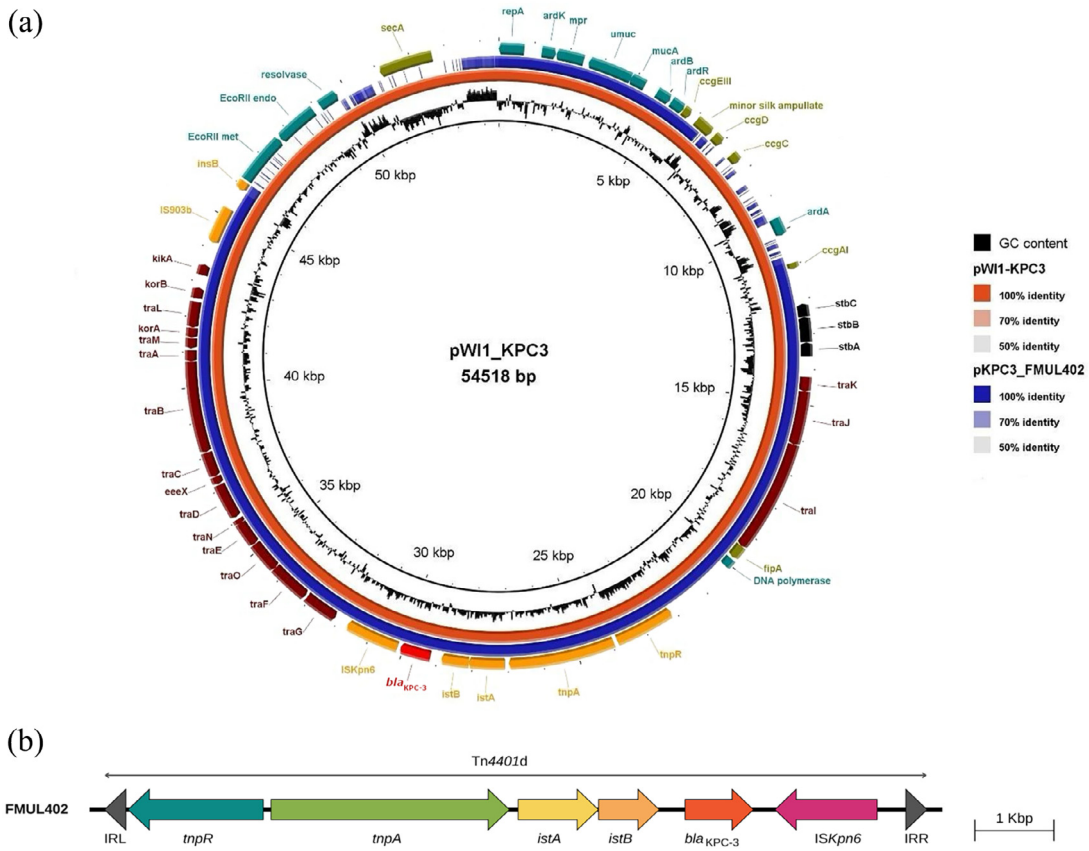

Fig. 1. (a) Plasmid alignment between de novo assembled pKPC3_FMUL402 found on a KPC-3-producing environmental *Enterobacter roggenkampii* strain (in blue) and plasmid pWI_KPC3 (GenBank acc. LT838197.1), used as backbone plasmid reference (in orange). Genes are represented by coloured blocks: Red – carbapenemase gene; Orange – transposons, insertion sequences (IS), and transposase genes; Maroon – conjugation-association genes; Teal – DNA replication, regulation, and restriction systems; Black – genes associated with partition and stability systems; Olive – other genes; (b) genetic background surrounding bla_{KPC-3}.

tance of additional infection prevention and control measures to eliminate genetic reservoirs of antibiotic-resistant strains in hospital settings.

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Ethical approval

The study was approved by Centro Hospitalar de Entre o Douro e Vouga Ethics Committee (Nr. CA-330/2020-0t_MP/AC). The study proposal was analysed and dismissed from evaluation by the Ethics Committee of the Lisbon Academic Medical Centre of the Faculty of Medicine, University of Lisbon, Portugal (Nr. 248/21).

Sequence information

The Whole Genome Shotgun project has been deposited at GenBank under the accession number PRJNA1246589.

Declaration of competing interests

C.C. received research grants administered by the university and honoraria for serving on the speakers' bureaus of Pfizer and MSD, which are not related to the present study. All other authors declare no competing interests.

References

[1] Ji Y, Wang P, Xu T, Zhou Y, Chen R, Zhu H, et al. Development of a one-step multiplex PCR assay for differential detection of four species (*Enterobacter cloacae, Enterobacter hormaechei, Enterobacter roggenkampii*, and *Enterobacter kobei*) belonging to *Enterobacter cloacae* complex with clinical significance. Front Cell Infect Microbiol 2021;11:677089. doi:10.3389/fcimb.2021.677089.

[2] Mateos M, Hernández-García M, Del Campo R, Martínez-García L, Gijón D, Morosini MI, et al. Emergence and persistence over time of carbapenemase-producing *Enterobacter* isolates in a Spanish University hospital in Madrid, Spain (2005-2018). Microb Drug Resist 2021;27:895–903. doi:10.1089/mdr.2020.0265.

[3] Stevens MP, Doll M, Pryor R, Godbout E, Cooper K, Bearman G. Impact of COVID-19 on traditional healthcare-associated infection prevention efforts. Infect Control Hosp Epidemiol 2020;41:1249. doi:10.1017/ice.2020.141.

[4] Rodrigues C, Bavlovic J, Machado E, Amorim J, Peixe L, Novais Â. KPC-3-producing *Klebsiella pneumoniae* in Portugal linked to previously circulating non-CG258 lineages and uncommon genetic platforms (Tn*4401*d-IncFIA and Tn*4401*d-IncN). Front Microbiol 2016;7:1000. doi:10.3389/fmicb.2016.01000.

[5] Mendes G, Santos ML, Ramalho JF, Bruschy-Fonseca A, Lito L, Mendes-Pedro D, et al. Genomic characterisation of a novel KPC-98-producing clinical *Klebsiella pneumoniae* strain conferring resistance to ceftazidime/avibactam. Int J Antimicrob Agents 2023;62:107013. doi:10.1016/j.ijantimicag.2023.107013.

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