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Letter to the Editor

Characterisation of a new *bla*$_{VIM-1}$-carrying IncN2 plasmid from an *Enterobacter hormaechei* subsp. *steigerwaltii*

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Sir,

The VIM-type metallo-β-lactamases represent one of the most clinically important carbapenemase families. In Enterobacterales, these enzymes are encoded by *bla* genes usually located in class 1 integrons that in turn are carried by a wide range of different incompatibility (Inc) group plasmids [1]. However, IncN2 plasmids carrying *bla*$_{VIM}$ genes have not been reported.

In May 2018, a 22-year-old German man was seen on an outpatient basis in the emergency department of Clinic Hirslanden in Zurich, Switzerland. The patient had returned from a holiday in Marrakech (Morocco), where he started suffering from a soft tissue infection of the lower leg not responsive to therapy with amoxicillin/clavulanate. A swab was taken and processed using selective agar plates (ChromID® ESBL and CARBA SMART; bioMérieux) to detect multidrug-resistant Gram-negative isolates. An *Enterobacter cloacae* complex isolate (BD-50-Eh) was identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) (Bruker). Antimicrobial susceptibility testing performed using a broth microdilution GNX2F Sensititre panel (Thermo Fisher) indicated that BD-50-Eh was resistant to different classes of antibiotics and showed slightly reduced susceptibility to carbapenems (Supplementary Table S1). BD-50-Eh was later identified as *Enterobacter hormaechei* subsp. *steigerwaltii* based on whole-genome sequencing (WGS) data and the Type (Strain) Genome Server (https://tygs.dsmz.de/).

WGS was performed using both NovaSeq 6000 (Illumina) and MinION (SQK-RBK004 library; FLO-MIN 106D R9 flow-cell; Oxford Nanopore Technologies). Adapters of Illumina and Nanopore reads were removed using Trimmomatic v.0.36 and Porechop v.0.2.4, respectively. Genome hybrid assembly was obtained with Unicycler v.0.4.8. Annotation was performed with the NCBI pipeline, and insertion sequence (IS) elements were curated with ISfinder (https://isfinder.biotoul.fr/). The final genome was analysed using tools from the Center for Genomic Epidemiology (www.genomicepidemiology.org/). Integrons were classified according to INTEGRALL (http://integrall.bio.ua.pt/). The complete genome assembly of BD-50-Eh has been deposited in GenBank (CP063224–CP063228) under BioProject PRJNA664791.

BD-50-Eh was sequence type 124 (ST124) according to the *E. cloacae* multilocus sequence typing (MLST) scheme. The strain carried four plasmids, including pBD-50-Eh_VIM-1 (65.1 kb, IncN2), pBD-50-Eh_2 [336.3 kb, IncHI2(pST1)/IncHI2A], pBD-50-Eh_3 [88.8 kb, IncFIA(HI1)/pKP1433] and pBD-50-Eh_4 [2.5 kb, Col(pHAD28)]. The following antimicrobial resistance genes (ARGs) were detected: chromosome (*bla*$_{ACT-7}$, *fosA*); plasmid pBD-50-Eh_VIM-1 [*bla*$_{VIM-1}$, *bla*$_{OXA-1}$, *aac(6')-Ib3*, *aac(6')-Ib*-cr, *aadA1*, *aph(3'')-Ib*, *aph(6)-Id*, *arr-3*, *catB3*, *dfrB1*, *mph(A)*, *qnrB19*, *sul1*] and plasmid pBD-50-Eh_2 (*bla*$_{TEM-1D}$, *aadA1*, *dfrA1*, *sul1*). Analysis of the promoter region of *bla*$_{VIM-1}$ re-

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vealed the presence of an intermediate P_1 promoter (−35 [TGGACA] and −10 [TAAACT]) and an inactive P_2 promoter (−35 [TTGTTA] and −10 [TACAGT]) (data not shown). The same P_1/P_2 promoter combination was found in *bla*$_{VIM-1}$-positive *E. cloacae* isolates susceptible to carbapenems [2]. Therefore, this finding could explain the unusual low minimum inhibitory concentrations (MICs) recorded for carbapenems in BD-50-Eh.

A search of *E. hormaechei* strains with the NCBI isolate browser (www.ncbi.nlm.nih.gov/pathogens/isolates/) resulted in 1415 genomes, of which 49 were *bla*$_{VIM}$-positive of various STs and 5 were *bla*$_{VIM}$-negative ST124 strains. A core-genome alignment of these 54 genomes and BD-50-Eh was constructed with Parsnp v.1.2. The phylogeny revealed four main clusters corresponding to the *E. hormaechei* subspecies [3] and a cluster containing the ST124 strains identified in the UK, USA, Lebanon and Nigeria (Supplementary Fig. S1). ST124 strains producing extended-spectrum β-lactamases (ESBLs) or OXA-48 carbapenemases were also reported in Chinese patients and cats in Germany, respectively, but no WGS data were available for analysis [4,5]. The 54 genomes were also investigated using PlasmidFinder. As a result, only one *bla*$_{VIM-4}$-carrying strain (SMART_1141) possessed an IncN2 replicon. However, our short-read analysis indicated that *bla*$_{VIM-4}$ was not associated with the contig containing such a replicon (data not shown).

Using BLASTn, the most similar plasmid to pBD-50-Eh_VIM-1 was the IncN2 plasmid pEC448_OXA163 (CP015078.1) from *Escherichia coli*, which shared a partial backbone, but not *bla*$_{VIM-1}$, in its genetic environment. Moreover, pBD-50-Eh_VIM-1 shared the backbone, containing the conjugation machinery (*tra* genes), with other IncN2 and IncN3 plasmids from multiple species (Fig. 1A; Supplementary Fig. S2). These data confirm that *bla*$_{VIM-1}$ and its genetic environment were exclusive to pBD-50-Eh_VIM-1. In this context, analysis of the ARG region of pBD-50-Eh_VIM-1 showed that *bla*$_{VIM-1}$ was associated with a class 1 integron flanked by two IS*26* predicted by MobileElementFinder v.1.0.3 (https://pypi.org/project/MobileElementFinder/) to produce a composite transposon (Fig. 1), which was assigned Tn*7099* (https://transposon.lstmed.ac.uk/). The same integron (In*1469*) was previously

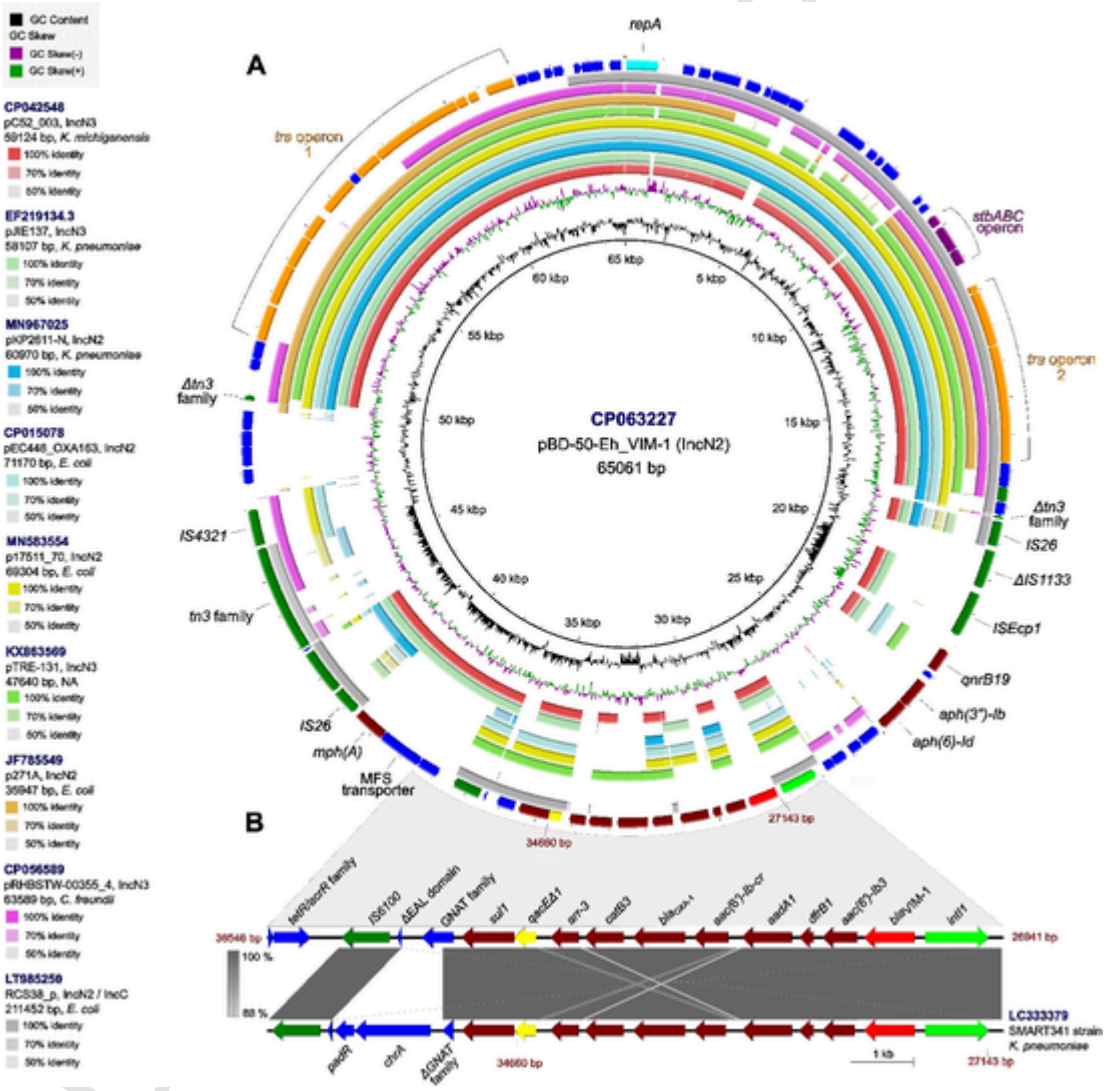

Fig. 1. Circular BLAST comparison of pBD-50-Eh_VIM-1 with other similar plasmid backbones and the integron region of *bla*$_{VIM-1}$. (A) Comparison of the sequences of nine species carrying plasmids of the IncN2 and IncN3 replicon type. Rings were constructed using BLAST Ring Image Generator (BRIG) v.0.95. Similarities with pBD-50-Eh_VIM-1 are represented by coloured rings. GenBank accession numbers, plasmid name, replicon type, plasmid size and species are indicated in the legend. Annotations above the rings correspond to the gene features of interest. Annotations for the *tra* operon, *stbABC* operon and *rep* gene are shown according to the nucleotide sequence alignment with the reference IncN2 plasmid p271A (JF785549). (B) Linear comparison of the *bla*$_{VIM-1}$ integron region against the full length (11,606 bp) of In*1469* reference sequence (LC333379). The start (27,143 bp) and end (34,660 bp) positions of the integron are shown in red. The linear BLAST comparison was generated with Easyfig v.2.2.2. BLAST similarity is represented by the grey area in between the sequence alignment. Lines represent BLAST hits.

found only in an IncR plasmid from *Klebsiella pneumoniae* strain SMART341 detected during a global survey for $bla_{IMP/VIM}$-carrying plasmids [1].

Because of the presence of mostly *E. coli* and *K. pneumoniae* and single instances in *E. cloacae* and *Citrobacter* spp. hosting IncN2 plasmids, it is likely that these genetic elements are involved in horizontal transfer. Nevertheless, conjugation experiments between BD-50-Eh and *E. coli* J53 at 37 °C and plating on MacConkey agar containing ampicillin and rifampicin (both at 100 mg/L) and a disk of imipenem (10 μg) were unsuccessful. This was possibly due to (i) the presence of the *arr-3* gene conferring rifampicin resistance that reduced the conjugation efficiency and (ii) reduced expression of bla_{VIM-1} that generated low resistance to carbapenems.

In conclusion, this is the first description of a bla_{VIM}-carrying IncN2 plasmid (pBD-50-Eh_VIM-1). This plasmid acquired bla_{VIM-1} and other ARGs inside of a transposon containing a rare integron. IncN2 plasmids are usually found in *E. coli* and *K. pneumoniae*, whereas pBD-50-Eh_VIM-1 was hosted by an *E. hormaechei* strain. This rare species is emerging as a nosocomial pathogen [3]. Surveys to study the role of *E. hormaechei* as a source for the rise and exchange of life-threatening ARGs are therefore advised.

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Conflict of interest

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2021.01.017.

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