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Characterization of a carbapenem-resistant *Escherichia coli* from dairy cattle harbouring bla_{NDM-1} in an IncC plasmid

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NDM-producing Enterobacteriaceae have increased worldwide in human infections, but are still rare in food-producing animals.[1] The only NDM type described in cattle so far was NDM-5,[2–4] always carried by IncX plasmids. To the best of our knowledge, this study represents the first description of a bla_{NDM-1} gene in *Escherichia coli* isolated from cattle, carried in an IncC plasmid.

In July 2020, *E. coli* isolates were recovered on ChromID® Carba Smart plates (bioMérieux) from rectal faeces collected from dairy calves in the Basque Country (Spain) within a longitudinal study conducted to monitor antimicrobial resistance (AMR) in commensal *E. coli*. MICs determined by broth microdilution (Thermo Scientific™ Sensititre™ AST plates EUVSEC1 and EUVSEC2) and interpreted using epidemiological cut-off values as developed by EUCAST (http://www.eucast.org) showed microbiological resistance to all β-lactams tested, including temocillin and carbapenems, and sulfamethoxazole and trimethoprim (Table S1, available as Supplementary data at *JAC* Online). Genomic DNA from *E. coli* strain EC1110 was extracted using a NZY Microbial gDNA Isolation Kit (NZYTech) and Illumina WGS was performed as previously described[5] and further complemented by Oxford Nanopore–ONT sequencing (MinION Mk1C, SQK-LSK109 library and R9.4.1 flow-cell). Base-calling of ONT reads on Guppy (HAC mode) was followed by adapter removal with Porechop[6] and discharge of shorter reads (<1000 bp) by Filtlong retaining only the best 1000 Mbp. A hybrid assembly (Illumina-ONT) was generated with Unicycler[7] and analysed as described elsewhere.[5] Details on bioinformatic tools are available in Table S2.

The Illumina-ONT assembly produced two contigs, i.e. the chromosome (4 816 563 bp) and an IncC plasmid (145 165 bp) that contained the bla_{NDM-1} gene and nine other AMR genes (GenBank JADWPF000000000, BioProject PRJNA680938 and BioSample SAMN16926619) (Figure 1). *E. coli* EC1110 belonged to serogroup O74: H23 and was assigned to a novel MLST type (ST-11626; cgST-151275). It carried a fimH60 fimbrial adhesion allele and harboured virulence-associated genes related to adhesion (CFA/I and Type 1 fimbriae), iron uptake (*ent* and *fep*) and invasion of brain endothelial cells (*ompA, ibeB* and *ibeC*). The IncC plasmid, named pEC1110_NDM-1, was assigned to pST-3 and included genes for the initiation of replication (*repA*), conjugative transfer (*tra*) and plasmid partitioning (*stb* and *par*) (Figure 1a). AMR genes were located in accessory modules of AMR islands, ARI-A and ARI-B. The bla_{NDM-1} gene was in ARI-A flanked upstream by IS*Aba125* and downstream by the bleomycin resistance gene ble_{MBL}, followed by a truncated Δbla_{DHA-1} gene (Figure 1b). Other AMR genes in ARI-A were the sulphonamide-resistance *sul1* gene (two copies), the trimethoprim-resistance *dfrA12* gene, genes that confer resistance to amikacin [*aph(3')-VI*] and streptomycin (*aadA2*), the *qacEΔ1* gene (quaternary ammonium compound resistance) and a mercury-resistance operon (*merDACPTR*). The sulphonamide-resistance gene *sul2* was present in ARI-B, downstream of IS*91* (Figure 1b).

A BLAST search showed that pEC1110_NDM-1 was related to IncC bla_{NDM-1}-harbouring plasmids in *Providencia stuartii* (pMR0211; 94% coverage, 99.9% identity), *Salmonella enterica* subsp. *enterica* serovar Corvallis (pSE12-01738–2; 92% coverage, 99.9% identity) and *E. coli* (pM214_AC2; 88% coverage, 99.9% identity). Moreover, it was also similar to IncC plasmids in Enterobacteriaceae that do not carry bla_{NDM} genes, isolated from food-producing animals, like *S. enterica* subsp. *enterica* serovar Newport p34530-1 isolated from cattle in the USA (92% coverage, 99.9% identity) and *E. coli* pEC3-1/2a from a chicken in China (91% coverage, 99.9% identity), or from human cases, like pRMH760 from *Klebsiella pneumoniae* (89% coverage, 99.8% identity), the first IncC plasmid described in detail (Figure S1).

The genetic environment of bla_{NDM-1} in pEC1110_NDM-1 had features present in other bla_{NDM-1}-harbouring strains. The IS*Aba125*-bla_{NDM-1}-ble_{MBL}-*trpF*-Δbla_{DHA-1}-*ampR* region was conserved in the partial sequence of the plasmid of *E. coli* DVR22 (JF922606; 4036 bp), the first description of an NDM-1 carbapenemase-producing *E. coli* in Spain.[8] Homology with the PGI1-*Pm*PEL genomic island of *Proteus mirabilis* was nearly 100% in the segment that extended from the IS*CR1* upstream of bla_{NDM-1} to the *ampR* gene (Figure S2). Insertion of bla_{NDM-1} from a circular molecule mediated by IS*CR1* was proposed and IS*CR1* was also associated with acquisition of the bla_{DHA}-*ampR* gene region as part of a class 1 integron.[9] On the other hand, the gene synteny of ARI-A in pEC1110_NDM-1 was highly conserved compared with the same region in p34530-1 (Figure S2). Despite lacking the 9639 kb fragment from the IS*CR1* element to the *ampR* gene where the

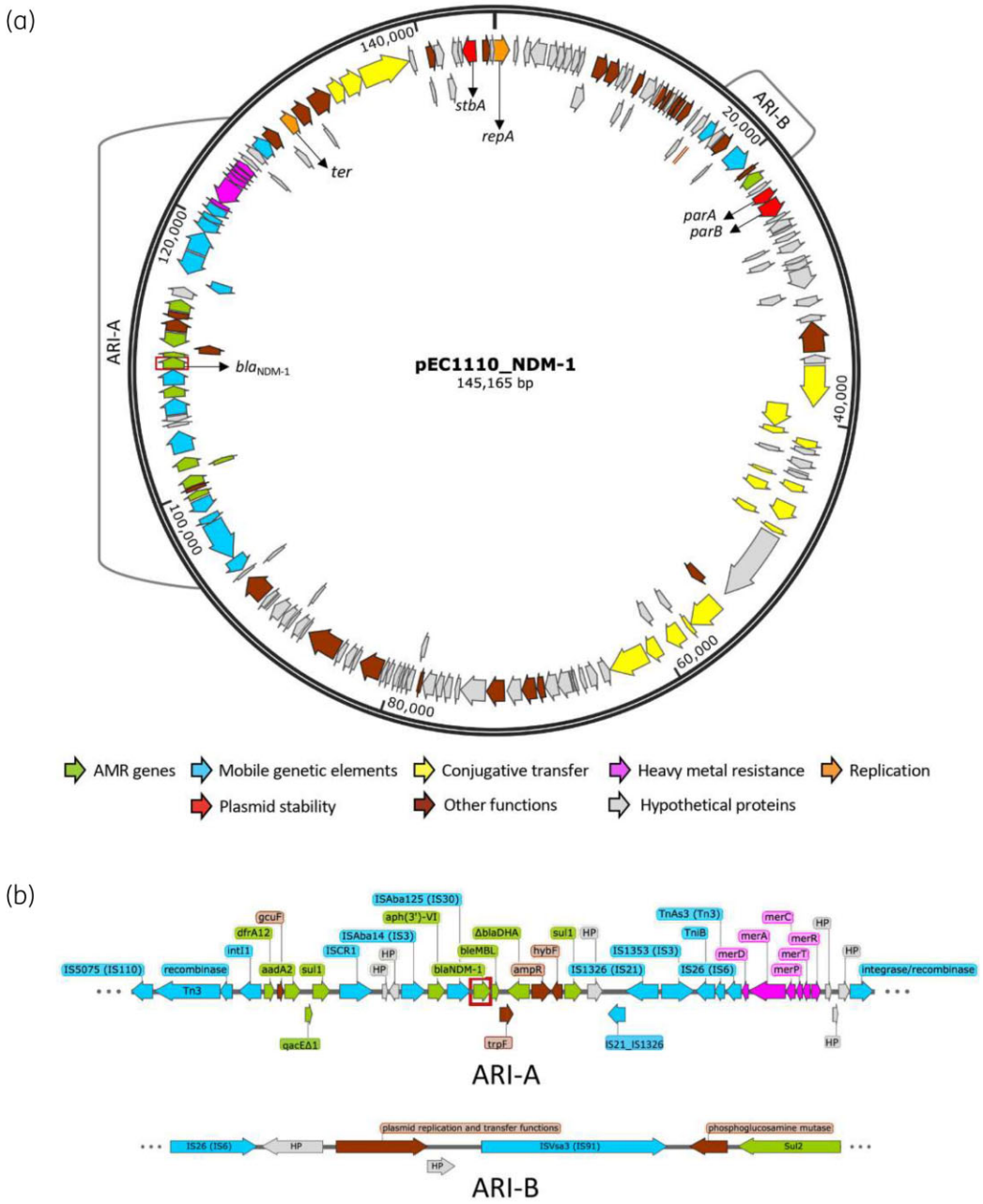

Figure 1. (a) Circular representation of the pEC1110_NDM-1 IncC plasmid from *E. coli* strain EC1110 isolated from dairy cattle. (b) Schematic representation of the genetic context of the bla_{NDM-1} gene in the ARI-A region of the pEC1110_NDM-1 plasmid and the *sul2* gene in the ARI-B region. The position of the bla_{NDM-1} gene is highlighted by a red square. Coding sequences, represented by arrows indicating the translational direction, are named above and coloured according to the key. IS designations are followed by the family name in brackets. Annotations were graphically depicted using SnapGene (v.5.2.4) (http://www.snapgene.com/).

bla_{NDM-1} gene is located in pEC1110_NDM-1, both plasmids shared a *sul1*-type class 1 integron structure (intI1-*dfrA12-gcuF-aadA2-qacEΔ1-sul1*), suggesting a common origin. Whereas only *sul2* was present in pEC1110_NDM-1 ARI-B, other IncC plasmids usually contain several additional AMR genes, such as *floR, strA, strB* and *tet*(A).[10] Different-sized deletions reported in ARI-B are considered potentially useful evolutionary and epidemiological markers.[10] pEC1110_NDM-1 ARI-B showed an IS*26*-mediated deletion of 12 451 bp upstream of *parA* and *parB* genes that removed part of the plasmid backbone. The presence of several different mobile genetic elements suggests that a series of recombination events was likely at the origin of the different resistance-gene arrays identified on pEC1110_NDM-1.

In conclusion, to the best of our knowledge, this is the first description of a bla_{NDM-1}-harbouring plasmid in an MDR *E. coli* isolated from cattle. This IncC plasmid also carried genes for aminoglycoside,

sulphonamide and trimethoprim resistance. The occurrence of NDM-1 plasmid-mediated carbapenem resistance in *E. coli* in cattle is worrisome since it might pose a risk for resistance spread in food-producing animals. However, this was the only detection after monitoring the herd for over 2 years, suggesting a sporadic event.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 and Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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