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IncA/C Plasmid Carrying bla_{NDM-1}, bla_{CMY-16}, and *fosA3* in a *Salmonella enterica* Serovar Corvallis Strain Isolated from a Migratory Wild Bird in Germany

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A *Salmonella enterica* serovar Corvallis strain was isolated from a wild bird in Germany. This strain carried the IncA/C_2 pRH-1238 plasmid. Complete sequencing of the plasmid was performed, identifying the bla_{NDM-1}, bla_{CMY-16}, *fosA3*, *sul1*, *sul2*, *strA*, *strB*, *aac(6′)-Ib*, *aadA5*, *aphA6*, *tetA*(A), *mphA*, *floR*, *dfrA7*, and *merA* genes, which confer clinically relevant resistance to most of the antimicrobial classes, including β-lactams with carbapenems, fosfomycin, aminoglycosides, co-trimoxazole, tetracyclines, and macrolides. The strain likely originated from the Asiatic region and was transferred to Germany through the *Milvus migrans* migratory route.

The New Delhi metallo-β-lactamase (NDM) is one of the most widespread carbapenemases. NDM-producing strains have been identified worldwide in *Enterobacteriaceae* but also in *Acinetobacter* spp. and *Pseudomonas aeruginosa* from clinical cases and colonized patients (1). However, one of the most intriguing aspects of the wide diffusion of NDM is the emergence of positive carriers in livestock and in companion and wild animals, denouncing contamination by wildlife and the environment (2, 3). The bla_{NDM-1} gene has been identified in different genetic environments but is always associated with remnant portions of the Tn*125* transposon (4). The bla_{NDM-1}-carrying genetic determinants have been localized on a variety of rare and frequent plasmid types (5). Here, the complete sequence of the bla_{NDM-1}-carrying IncA/C plasmid (pRH-1238), identified in a *Salmonella enterica* serovar Corvallis strain isolated from a wild bird (*Milvus migrans*) in Germany (6), was determined.

The NDM-carrying plasmid was transferred by conjugation from the *S.* Corvallis 12-1738 isolate to the sodium azide-resistant *Escherichia coli* K-12 strain J53, selecting transconjugants on cefoxitin (10 μg/ml) plus azide (100 μg/ml; MICs determined for donor and transconjugant strains are reported in Table S1 in the supplemental material). The transferred NDM-positive plasmid was classified as IncA/C by a PCR-based replicon typing method (7). The complete DNA sequence of the pRH-1238 plasmid was obtained using a 454-FLX genome sequencer (Roche Diagnostics, Monza, Milan, Italy) on a library obtained using plasmid DNA purified by the Invitrogen PureLink HiPure plasmid filter midiprep kit (Invitrogen, Milan, Italy), according to the manufacturer's protocol. Assembly of DNA sequences was done using the GS-FLX gsAssembler software, followed by PCR-based gap closure of contigs. Homology and phylogenetic trees were obtained by aligning the IncA/C DNA plasmid sequences downloaded from GenBank, using the DNAman phylogenetic analysis software (Lynnon BioSoft, Vaudreuil, Quebec, Canada) for quick alignment. Unrooted phylogenetic trees were generated by the maximum-likelihood method.

The pRH-1238 plasmid (GenBank accession no. KR091911) is 187,683 bp in size, with a G+C content of 51.7%, and it contains 173 predicted coding sequences (CDSs). pRH-1238 belongs to type 1 A/C_2, as defined by the presence of the entire *rhs* gene in the ARI-A region (type 1 A/C_2 reference plasmid pR148). pRH-1238 shows the two typical resistance islands, designated ARI-A and ARI-B, and the A/C_2 replicon (8). Homology and phylogenetic analysis performed on the entire plasmid DNA sequence revealed high relatedness and 82% nucleotide identity with the plasmid pMR0211 (GenBank accession no. JN687470) identified in a *Providencia stuartii* strain isolated from a patient in Afghanistan (9). These two plasmids share an origin, which is different from the other NDM-IncA/C_2 plasmids identified in the United States, Canada, Kenya, Australia, and Oman (Fig. 1 and 2). The best homology between the two plasmids is observed in the replicon, conjugation (Tra1 and Tra2 transfer regions), and ARI-B regions; both pRH-1238 and pMR0211 carried the RepAciN and NDM regions, localized in the ARI-A region (Fig. 2).

pRH-1238 carried the IS*Ecp1*-bla_{CMY-16} element located in the Tra1 region, while the bla_{NDM-1} gene was located within ARI-A. This resistance island contains a complete Tn*21* transposon carrying the entire mer_{21} locus (Fig. 2). Most of the previously fully sequenced IncA/C plasmids (pMR0211, pKP1-NDM-1, pNDM-US, pNDM102337, pNDM10469, pNDM-KN and pNDM10505) carried truncated Tn*21* transposons with deleted mer_{21} loci. The IR_{tnp} and IR_{mer} inverted repeats of the Tn*21* transposon were interrupted by IS*4321L* and IS*4321R* elements in the opposite ori-

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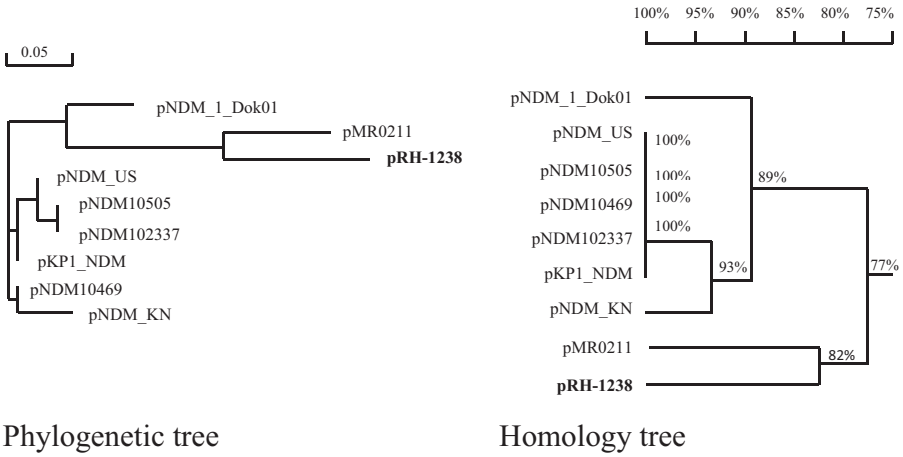

FIG 1 Phylogenetic and homology trees of IncA/C plasmids. The phylogenetic and homology analyses were performed by aligning sequences of the following NDM-1-IncA/C plasmids (GenBank accession numbers): pNDM-1_Dok01 (AP012208), pMR0211 (JN687470), pKP1-NDM-1 (KF992018), pNDM-US (CP006661), pNDM102337 (JF714412), pNDM10469 (JN861072), pNDM-KN (JN157804), and pNDM10505 (JF503991). The phylogenetic (left) and homology (right) trees were obtained using the DNAman software (Lynnon BioSoft, Vaudreuil, Quebec, Canada) for quick alignment. Unrooted phylogenetic trees were generated by the maximum-likelihood method. The bar corresponds to the scale of sequence divergence.

entation (Fig. 2). These insertion sequences show target site specificity and have been found to interrupt the terminal ends of members of the Tn*21*-like subgroup transposons (10). The bla_{NDM-1} gene environment shows the IS*CR1* and the Tn*402 tniA* transposase genes in a complex class 1 integron carrying the *aac(6′)-Ib* gene cassette (Fig. 2). Immediately upstream of the bla_{NDM-1} gene, the partial sequence of the remnant Tn*125* with the IS*Aba125* element, the *aphA6* gene, and a partial IS*Aba14* were identified, as previously described in pMR0211. Downstream of the bla_{NDM-1} gene, the ble_{MBL} gene, encoding bleomycin resis-

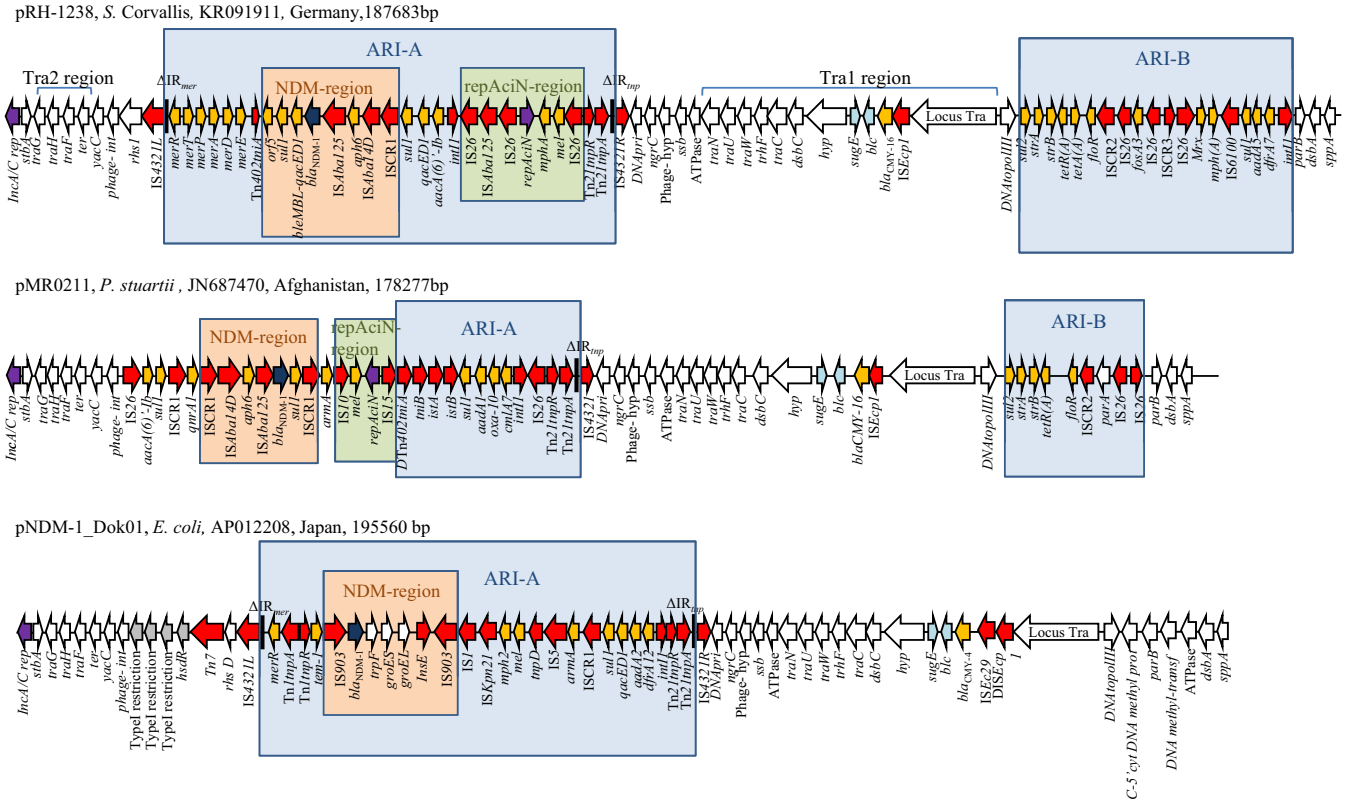

FIG 2 Major structural features of pRH-1238 plasmid compared with pMR0211 and pNDM-1_Dok01 plasmids identified in *P. stuartii* and *E. coli*, respectively. Orange arrows indicate antibiotic resistance genes, and red arrows indicate transposon-related genes (*tnpA* and *tnpR*) or insertion sequences. The bla_{NDM-1} gene is indicated by blue arrows. The *repAciN* gene is indicated by violet arrows. The white arrows indicate plasmid scaffold regions in common among IncA/C plasmids. The black bars indicate inverted repeat (IR) sites.

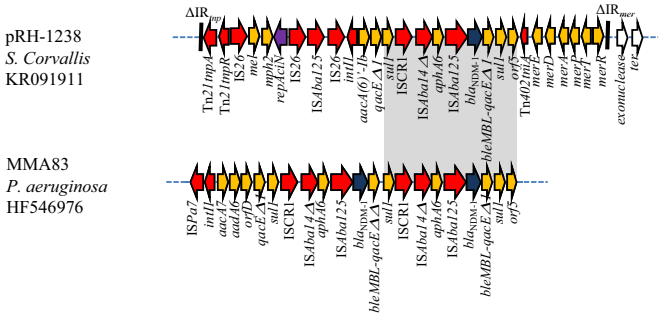

FIG 3 Comparison of the bla_{NDM-1} gene environments between pRH-1238 plasmid and the genetic context of the genome of the *P. aeruginosa* MMA83. Orange arrows indicate antibiotic resistance genes, and red arrows indicate transposon-related genes (*tnpA* and *tnpR*) or insertion sequences. The bla_{NDM-1} gene is indicated by blue arrows. The *repAciN* gene is indicated by the violet arrow. The white arrows indicate plasmid scaffold regions that are in common among IncA/C plasmids. The black bars indicate IR sites.

tance, fused with *qacEΔ1*, and the *sul1* and *orf5* genes were found. A similar bla_{NDM-1} genetic environment was previously identified in the chromosome of *P. aeruginosa* MMA83 strain (a comparison among the two structures is shown in Fig. 3) (11).

pRH-1238 contains the macrolide resistance determinant *mphA-mel-repAciN* flanked by two IS*26* elements integrated in the Tn*21* transposon of the ARI-A region (Fig. 2). The *mphA-mel-repAciN* module was previously described in IncA/C plasmids pVC1447 from *Vibrio cholerae* (GenBank accession no. KM083064), pPG010208 from *E. coli* (GenBank accession no. HQ023861), and pTR2 from *Klebsiella pneumoniae* (GenBank accession no. KJ187752), but it was located in the ARI-B region.

The ARI-B region of the pRH-1238 plasmid contains the *sul2* (sulfonamide resistance), *strA* and *strB* (streptomycin resistance), *tetR* and *tetA*(A) (tetracycline resistance), *floR* (chloramphenicol/florfenicol resistance), and *fosA3* (fosfomycin resistance) genes and a class 1 integron carrying the *dfrA7* (trimethoprim resistance) and *aadA5* (streptomycin resistance) gene cassettes (Fig. 2).

The *fosA3-orf1-orf2-orf3* element, conferring resistance to fosfomycin, was identified on this plasmid, flanked by two IS*26* elements, as previously described (12). FosA3 is rarely detected in Europe, while it is emerging among *E. coli* and in *K. pneumoniae* CTX-M-producing strains from animals and patients in China, Japan, and South Korea. It has been suggested that the increasing prevalence of *fosA3* is due to dissemination of the IncI1 and IncN plasmids rather than clonal expansion of specific strains (13, 14). Recent reports from China described *fosA3* on IncFII plasmids in *S. enterica* serovars Derby and Enteritidis from chickens purchased at the market in Hong Kong (15) and the copresence of both bla_{NDM-1} and *fosA3* genes in a clinical *E. coli* isolate (16). Furthermore, the colocalization of the bla_{NDM-1} and *fosA3* genes on IncA/C plasmids was demonstrated in *E. coli* and *Citrobacter freundii* clinical isolates in a report denouncing the high incidence and endemic spread of NDM-1-positive *Enterobacteriaceae* in the Henan Province of China (17).

pRH-1238 is the first completely sequenced bla_{NDM-1}-*fosA3*-IncA/C plasmid and is of particular interest because it was identified in *Salmonella* from a wild animal in Germany (6). The report of this broad-host-range plasmid, which confers multidrug resistance in *Salmonella* identified in wildlife in Europe, is worrisome. This strain has been found thanks to the German *Salmonella* routine monitoring system, but it is possible that plasmids of this type are highly represented in other commensal bacteria that are not under surveillance. The structure of the plasmid, the presence of the *fosA3* gene that is still so rare in Europe but frequent in China, and the differences observed with the other previously fully sequenced NDM-1-IncA/C_2 plasmids identified in bacteria from Western countries are all clues supporting a possible origin of this plasmid in the Asiatic region. This strain probably traveled in the gut of the wild bird *M. migrans*, which is a migratory diurnal raptor, and its route of migration includes Europe and northern Asia during the summer and wintering in sub-Saharan Africa and southern Asia (18). The report in Germany of this *M. migrans* bird carrying the *S.* Corvallis strain with the NDM-1-*fosA3*-IncA/C plasmid suggests that new routes of environmental diffusion of multidrug-resistant bugs from Eastern to Western countries are possible, for instance, according to wild bird migratory paths.

Nucleotide sequence accession number. The complete sequence of the pRH-1238 plasmid was deposited in GenBank under accession no. KR091911.

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