

Characterization of an Extensively Drug-Resistant *Salmonella enterica* Serovar Indiana Strain Harboring Chromosomal *bla*_{NDM-9} in China

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The emergence and dissemination of New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae* are a threat to public health and a challenge for clinical therapy.^{1,2} Although NDM-producing *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*, have been increasingly reported worldwide, the *bla*_{NDM} gene has been rarely described in *Salmonella*.^{2,3} Thus far, *bla*_{NDM}, mainly *bla*_{NDM-1} and *bla*_{NDM-5}, has been reported in *Salmonella* isolated from human patients, animals, and animal-derived food products.^{3,4} Various plasmids, such as IncX3 and IncA/C, are efficient vectors for *bla*_{NDM} transmission in *Salmonella*.^{3,4} Here, we investigated the prevalence of carbapenem resistance genes in *Salmonella* in China and characterized the genetic basis for chromosome-encoding NDM-9 in a *Salmonella enterica* serovar Indiana isolate.

From July 2019 to April 2021, 445 *Salmonella* spp. isolates were obtained from food animals (33 pigs, 74 chickens, and 8 cattle) and retail meat (185 pork, 126 chicken meat, and 19 beef specimens) from 2173 samples in different geographic areas of China (including Anhui, Liaoning, Gansu, Guizhou, Henan, Hubei, Jiangsu, Guangdong, Shandong, Xinjiang provinces, and Shanghai) (Table S1). Minimum inhibitory concentrations (MICs) of meropenem were determined by the agar dilution method, and the isolates were screened for carbapenem resistance genes by PCR and Sanger sequencing.⁵ Among the isolates, one (0.24%) *S. Indiana* strain, YZ21MCS4, obtained from retail chicken meat in Yangzhou, Jiangsu province in March 2021, was resistant to meropenem (MIC=32 mg/L) and harbored *bla*_{NDM-9}. The remaining 444 isolates were susceptible to meropenem with MICs of 0.004 to 0.5 mg/L. The *S. Indiana* isolate YZ21MCS4 was further tested for susceptibility to 15 antimicrobial agents by the broth microdilution method or the agar dilution method (limited to fosfomycin). YZ21MCS4 was found to be resistant to ampicillin, cefazolin, cefotaxime, gentamicin, streptomycin, tetracycline, tigecycline, chloramphenicol, florfenicol, nalidixic acid, ciprofloxacin, fosfomycin, and sulfamethoxazole/trimethoprim, but susceptible to amikacin and colistin (Table S2). However, the *S. Indiana* isolate YZ21MCS4 failed to transfer *bla*_{NDM-9} to *E. coli* C600 via conjugation or DH5α by transformation.

The NDM-9-producing *S. Indiana* strain YZ21MCS4 was sequenced by using PacBio single-molecule real-time sequencing technology to characterize its genetic features. Sequencing data were assembled using the nonhybrid Hierarchical Genome Assembly Process version 4. The whole genome sequence was further analyzed using MLST (<https://cge.cbs.dtu.dk/services/MLST/>), ISfinder (<https://www-is.biotoul.fr/>), BLAST (<https://blast.ncbi.nlm.nih.gov/>)

[Blast.cgi](#)) and Gene Construction Kit 4.5 (Textco BioSoftware, Inc., Raleigh, NC, United States). Resistance genes and mutations were identified with ResFinder 4.1 (<https://cge.cbs.dtu.dk/services/ResFinder>) with parameter identity >90% and minimum length >60%. The *S. Indiana* isolate YZ21MCS4 belonged to ST17 and consisted of one chromosome (4,740,896 bp) and one plasmid (3374 bp). The whole genome sequences of YZ21MCS4 have been deposited in GenBank under accession no. PRJNA787409. We identified mutations within *gyrA* (S83F and D87N) and *parC* (S80R) associated with high-level ciprofloxacin resistance. All antimicrobial resistance genes, including *bla*_{NDM-9}, *bla*_{CTX-M-65}, *bla*_{OXA-1}, *fosA3*, *aph(4)-Ia*, *aac(3)-IV*, *aadA2*, *strAB*, *aac(6')-Ib-cr*, *tet(A)*, *catB3*, *floR*, *arr-3*, *sul1*, *sul2*, and *dfrA12*, were located on the chromosome of YZ21MCS4 and were clustered in two mosaic multiresistance regions (MRRs; Figure 1).

The first MRR (positions 1,000,792–1,048,079) consisted of two regions bounded by IS26. The first segment (~32 kb) contained multiple resistance genes including *aac(3)-IV/aph(4)-Ia* (aminoglycoside resistance), *sul2* (sulfonamide resistance), *floR* (florfenicol resistance), *bla*_{CTX-M-65} (extended-spectrum β -lactamase), *fosA3* (fosfomycin resistance), and *strA/B* (streptomycin resistance), and numerous mobile elements, such as IS26, ISEc59, Δ Tn5393, IS*Aba1*, ISCR2, IS1006, Δ Tn21, and ISEcp1 (Figure 1A). This segment was similar to those of multiple IncHI2 plasmids obtained from *Salmonella* isolates such as pD90-1, pSI102-1, and pC629, differed by (i) deletions of a 1122-bp fragment including one copy of IS26 (in pD90-1, pSI102-1 and pC629), (ii) the absence of a 3305-bp fragment comprising *sul2* and *strAB*, and a shorter *fosA3* resistance module (in pD90-1 and pC629), and (iii) replacement of the typical *bla*_{CTX-M-65} transposition unit (Δ ISEcp1-*bla*_{CTX-M-65}-IS903D-*iroN*- Δ mcp) and *fosA3* module (IS26-*fosA3*-*orf1*-*orf2*-*orf3*) by three hypothetical proteins (in pSI102-1) (Figure 1A). The second segment (~15 kb) was identical to the corresponding region of plasmid p0085-NDM (IncN1-IncHI2, *Salmonella enterica*, MN577015) (Figure 1A). This segment contained a core *bla*_{NDM} structure associated with an ISCR1 complex class 1 integron (Δ IS*Aba125*-*bla*_{NDM-9}-*ble*_{MBL}-*trpF*-*tat*-*cutA*-ISCR1-*sul1*-*qacE* Δ 1-*aadA2*-*gcuF*-*dfrA12*-*intI1*). This 10,219-bp structure is commonly observed among *bla*_{NDM-9}-carrying plasmids, such as pC629 (IncN1-IncHI2, *S. Indiana*, CP015725), pHNTH02-1 (IncK2, *E. coli*, MG196294), and pKPGJ-1a (IncFII_Y, *Klebsiella variicola*, CP017283) and is usually flanked by two copies of IS26 in the opposite orientation. In strain YZ21MCS4, the *bla*_{NDM} structure was flanked by one copy of IS26 and an incomplete Tn21, followed by a 4614-bp structure (Δ Tn1721-IS26- Δ Tn2-blms-*orf63*- Δ ISEncal-IS26) (Figure 1A). MRR I was flanked by two copies of IS26, but direct repeats (DRs) were not observed (Figure 1A). However, based on a detailed sequence analysis and comparison with *S. Indiana* strain SI67 (CP050783) without MRR I, we hypothesize that MRR I is inserted into the chromosome of YZ21MCS4 with the help of two copies of IS26, generating 8-bp DRs (TTATTCGG), and another copy of IS26 is also inserted into the chromosome flanked by 8-bp DRs (ATATGACT), followed by homologous recombination between it and the upstream IS26 adjacent to MRR I in opposite orientations, leading to the inversion of the 41,450-bp intervening segment (Figure 1B).

The second MRR module (positions 1,194,283–1,234,177) contained two parts and displayed high sequence identity (>99.9%) with the corresponding region of IncHI2 plasmids previously detected in *Salmonella* and *E. coli* isolates in China (Figure 1C). The first part (13,390 bp) included the [*aac(6')-Ib-cr*]*bla*_{OXA-1} [*catB3*]*arr-3*[*qacE* Δ 1]*sul1* cassette array and tetracycline resistance gene *tet(A)* variant associated with an incomplete Tn1721, which was truncated by IS26. This *tet(A)* variant differed from our previously described *tet(A)* variant associated with tigecycline in *S. Kentucky* by a single nucleotide sequence,⁶ resulting in one amino acid change (A93T). The presence of this *tet(A)* variant may explain the tigecycline resistance in *S. Indiana* YZ21MCS4 observed in this study. The second segment (~26.5 kb) corresponded to the IncHI2/ST3 plasmid backbone coding maintenance and stability functions, although a hypothetical protein was truncated by IS26 at 3' end. This MRR fragment (~39.9 kb) was probably acquired from IncHI2 plasmids and inserted into the chromosome of *S. Indiana* via an IS26-mediated mechanism. Similar mobilization was previously observed in *S. Indiana* strain SI43, possibly occurring via two separate events (Figure 1C).

Our study shows that the low prevalence of NDM-producing *Salmonella* in China is consistent with the rarity of *bla*_{NDM} detection in *Salmonella*, although the small number of tested *Salmonella* isolates is a limitation. *S. Indiana* has been increasingly reported during the past decade and has become one of the most common serovars in China, particularly in food animals and raw meat.⁷ The *bla*_{NDM} genes (*bla*_{NDM-1} and *bla*_{NDM-9}) have been previously detected

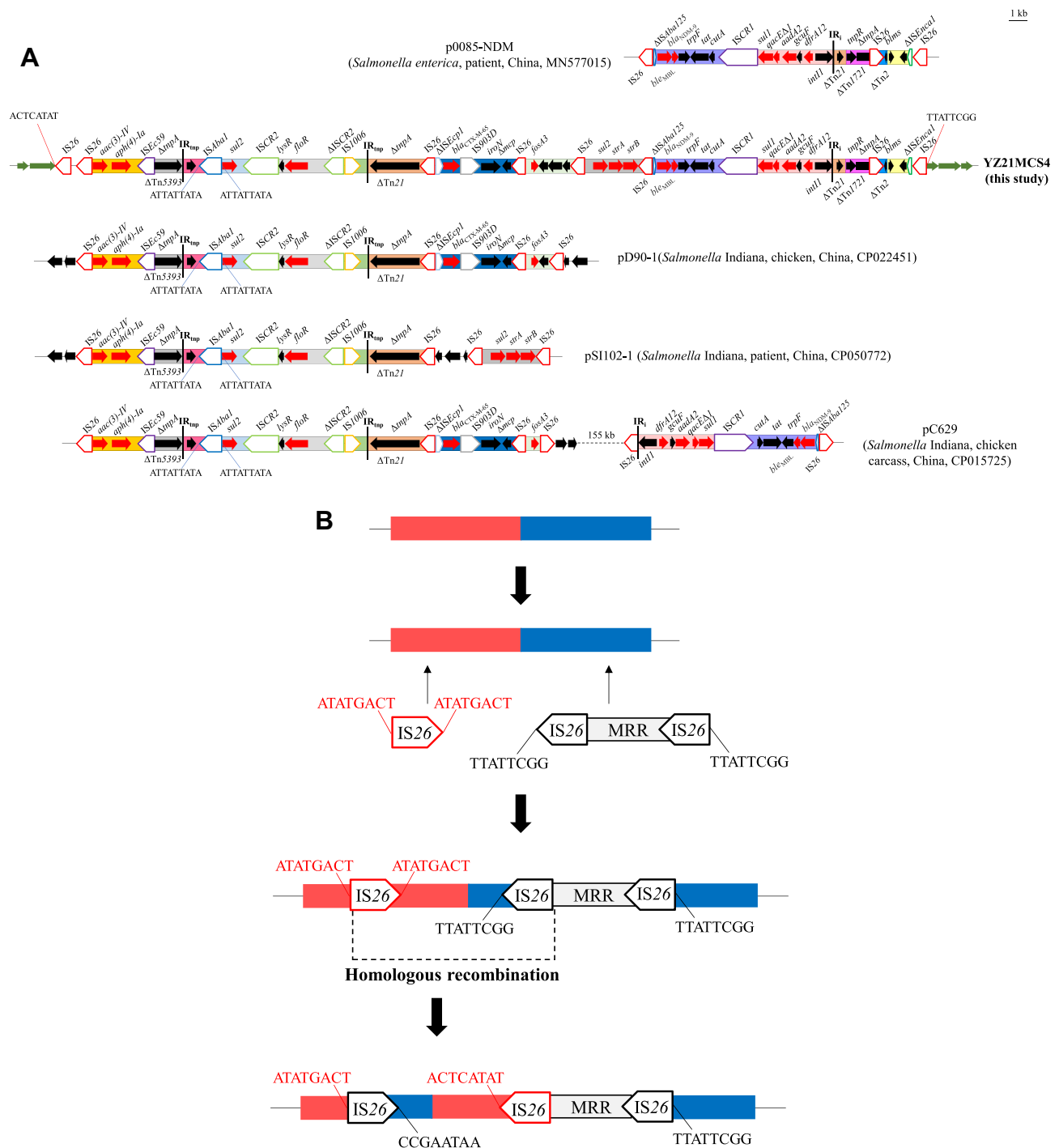


Figure 1 Continued.

in *S. Indiana* ST17 strains from chicken or chicken carcass and are associated with plasmids.^{8,9} To the best of our knowledge, this is the first report of chromosomally encoded NDM-9 in *Salmonella* species. The chromosome of *S. Indiana* strain YZ21MCS4 may capture two mosaic MRRs from IncHI2 plasmids by different mobilization events via mobile elements. The chromosomal integration of *bla*_{NDM-1} via mobile elements has been previously described in *E. coli* and *K. pneumoniae*.^{10,11} The emergence of an extensively drug-resistant *S. Indiana* strain carrying numerous chromosomally located resistance genes is alarming. This finding indicates that many clinically important genes could be

1 kb

C

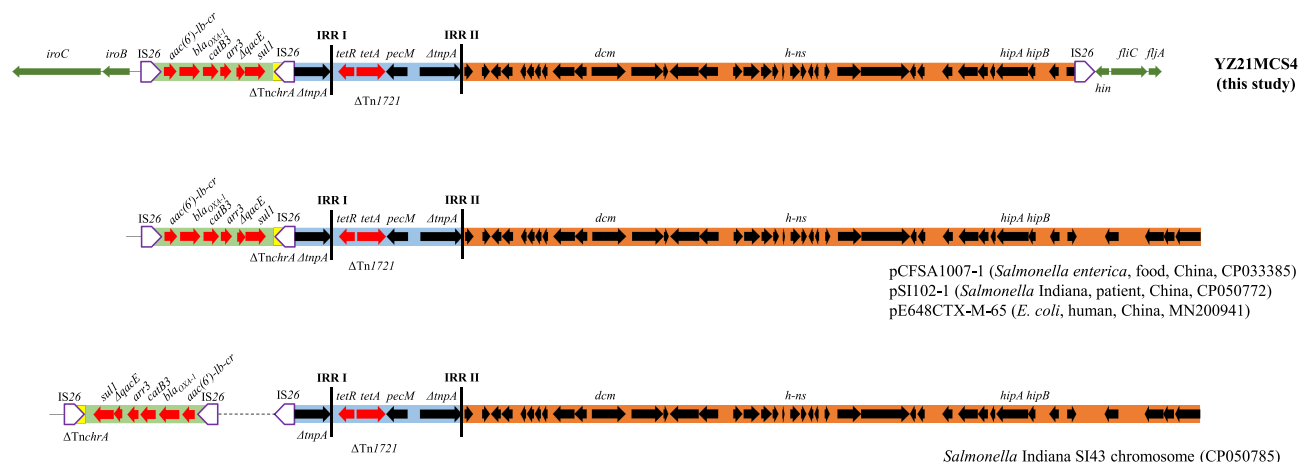


Figure 1 (A) Genetic structures of the multiresistance region I in chromosome of the isolate YZ21MCS4 in this study and comparison with other plasmids. Resistance genes are shown in red. Genes on chromosome of *Salmonella* Indiana are shown in green. Δ indicates a truncated gene or mobile element. ISs are shown as boxes labeled with their name. Tall bars represent the inverted repeats (IRs) of transposons or integron. **(B)** Proposed mechanism for the formation of multiresistance region I and its neighboring region in YZ21MCS4. It is not drawn to scale. Chromosomal DNA segments are represented by the same shape colored blue or red. Direct repeats are indicated by arrows and sequences. **(C)** Genetic structures of the multiresistance region II in YZ21MCS4 compared with similar structures. Resistance genes are shown in red. Genes on chromosome of *Salmonella* Indiana are shown in green. Δ indicates a truncated gene or mobile element. Tall bars represent the inverted repeats (IRs) of transposons.

captured and clustered in the chromosome of *S. Indiana* via mobile elements. On the other hand, wide spread of *S. Indiana* strains might facilitate the dissemination of resistance genes.

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Disclosure

The authors declare no conflicts of interest in this work.

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