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. Although NDM-producing Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, have been increasingly reported worldwide, the \textit{bla}_{\text{NDM}} gene has been rarely described in \textit{Salmonella}. Thus far, \textit{bla}_{\text{NDM}}, mainly \textit{bla}_{\text{NDM-1}} and \textit{bla}_{\text{NDM-5}}, has been reported in \textit{Salmonella} isolated from human patients, animals, and animal-derived food products. Various plasmids, such as IncX3 and IncA/C, are efficient vectors for \textit{bla}_{\text{NDM}} transmission in \textit{Salmonella}. Here, we investigated the prevalence of carbapenem resistance genes in \textit{Salmonella} in China and characterized the genetic basis for chromosome-encoding NDM-9 in a \textit{Salmonella enterica} serovar Indiana isolate.

From July 2019 to April 2021, 445 \textit{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. 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 \textit{bla}_{\text{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 \textit{bla}_{\text{NDM-9}} to \textit{E. coli} C600 via conjugation or DH5\textalpha{} 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/
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. Indian 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<sub>NDM-9</sub>, bla<sub>CTX-M-65</sub>, bla<sub>OXA-1</sub>, fosA3, aph(4)-Ia, aac(3)-IV, aadA2, strAB, aac(6')-Ib-cr, tet, 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 (floR-fenicol resistance), bla<sub>CTX-M-65</sub> (extended-spectrum β-lactamase), fosA3 (fosfomycin resistance), and strAB (streptomycin resistance), and numerous mobile elements, such as IS26, ISEc59, ΔTn5393, ISAbal, ISCR2, IS1006, ΔTn21, 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<sub>CTX-M-65</sub> transposition unit (ΔISEcp1-bla<sub>CTX-M-65</sub>-IS903D-iroN-Dmcp) 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<sub>NDM</sub> structure associated with an IScri1 complex class 1 integron (ΔISaba125-bla<sub>NDM-9</sub>-ble<sub>MBL</sub>-trpF-tat-cut4-ISCRI-sul1-qacEΔ1-aadA2-gcuF-dfrA12-intI1). This 10,219-bp structure is commonly observed among bla<sub>NDM-9</sub>-carrying plasmids, such as pC629 (IncN1-IncHI2, S. Indiana, CP015725), pHNTH02-1 (IncK2, E. coli, MG196294), and pKPGJ-1a (IncFII<sub>V</sub>, Klebsiella variicola, CP017283) and is usually flanked by two copies of IS26 in the opposite orientation. In strain YZ21MCS4, the bla<sub>NDM</sub> structure was flanked by one copy of IS26 and an incomplete Tn21, followed by a 4614-bp structure (ΔTn1-721-1S26-ΔTn2-blms-orf3-ΔISEna1-IS26) (Figure 1A). MRR I was flanked by two copies of IS26, but direct repeats (DRs) were not observed (Figure 1A).

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<sub>OXA-1</sub>|catB3|arr-3|qacEΔ1|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, 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<sub>NDM</sub> 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. The bla<sub>NDM</sub> genes (bla<sub>NDM-1</sub> and bla<sub>NDM-9</sub>) have been previously detected.
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
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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