Infection and Drug Resistance

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CASE REPORT

Genomic Insights into the First Emergence of *bla*$_{NDM-5}$-Carrying Carbapenem-Resistant *Salmonella enterica* Serovar London Strain in China

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Abstract: Carbapenem-resistant *Salmonella enterica (S. enterica)* pose a significant threat to public health, causing gastroenteritis and invasive infections. We report the first emergence of a carbapenem-resistant *S. enterica* serovar London strain, A132, carrying the *bla*$_{NDM-5}$ gene in China. Whole-genome sequencing and bioinformatics analysis assigned A132 to be ST155, a multidrug-resistant clone frequently reported in China. The strain A132 exhibited resistance to multiple antibiotics, with 20 acquired antibiotic resistance genes (ARGs) identified, predominantly located on the IncFIB plasmid (pA132-1-NDM). Notably, the *bla*$_{NDM-5}$ gene was located within an IS*26* flanked-class 1 integron-IS*CR1* complex, comprising two genetic cassettes. One cassette is the class 1 integron, which may facilitate the transmission of the entire complex, while the other is the *bla*$_{NDM-5}$-containing IS*CR1*-IS*26*-flanked cassette, carrying multiple other ARGs. Genbank database search based on the *bla*$_{NDM-5}$-carrying cassette identified a similar genetic context found in transmissible IncFIA plasmids from *Escherichia coli* (p91) and *Enterobacter hormaechei* (p388) with a shared host range, suggesting the potential for cross-species transmission of *bla*$_{NDM-5}$. To our knowledge, this is the first reported case of *Salmonella* serovar London ST155 harboring *bla*$_{NDM-5}$ gene. Phylogenetic analysis indicated a close relationship between A132 and eight *S.* London ST155 strains isolated from the same province. However, A132 differed by carrying the *bla*$_{NDM-5}$ gene and four unique ARGs. Given the high transmissibility of the F-type plasmid harboring *bla*$_{NDM-5}$ and 18 other ARGs, it is imperative to implement vigilant surveillance and adopt appropriate infection control measures to mitigate the threat to public health.

Keywords: *bla*NDM-5, *Salmonella* London, ST155, carbapenem resistance, IncFIB, integron

Introduction

Salmonella is a major causative agent of gastroenteritis on a global scale.[1] Annually, non-typhoidal *Salmonella* (NTS) is responsible for an estimated 200 million to over 1 billion infections globally, resulting in 93 million cases of gastroenteritis and 155,000 fatalities.[2,3] Although NTS typically induces self-limiting gastroenteritis, invasive NTS infections frequently result in substantial mortality rates.[4] In 2017, NTS was estimated to cause 535,000 invasive infections, contributing to 77,500 deaths worldwide.[5]

The primary treatment for invasive salmonellosis previously relied on chloramphenicol, amoxicillin (ampicillin), and cotrimoxazole (trimethoprim/sulfamethoxazole). However, widespread resistance to these first-line drugs has prompted the endorsement of alternative therapeutic strategies, encompassing extended-spectrum cephalosporins (eg, ceftriaxone) and fluoroquinolones (eg, ciprofloxacin).[6] Unfortunately, the extensive use of these secondary antimicrobials has led to the development of resistance to extended-spectrum cephalosporins and fluoroquinolones in NTS strains, presenting

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a substantial hurdle in the medical management of infections.[6] Carbapenems and azithromycin are currently considered as ultimate therapeutic alternatives for treating invasive *Salmonella* infections induced by multidrug and extensively drug-resistant strains.[7]

Carbapenemase-producing *Enterobacteriaceae* pose a growing threat to public health due to their resistance to carbapenems, a crucial class of first-line antibiotics used in the treatment of multidrug-resistant (MDR) bacterial infections.[8] Although carbapenem resistance remains rare in NTS, several studies have documented the presence of carbapenemase-producing genes in *Salmonella*, including variants such as $bla_{\text{KPC-1}}$, $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-1}}$.[9,10]

In this study, we report the identification of an *S. enterica* serovar London strain carrying the $bla_{\text{NDM-5}}$ gene in China. To the best of our knowledge, this represents the first documentation of $bla_{\text{NDM-5}}$ in *S.* London. Whole-genome sequencing (WGS) was employed to elucidate the comprehensive genomic profile, coupled with extensive bioinformatics analyses, to enhance our understanding of the global dissemination of NDM.

Materials and Methods

Bacterial Isolation and Identification

The $bla_{\text{NDM-5}}$-carrying *S. enterica* serovar London strain A132 was collected once from the stool sample of a 35-year-old female patient in the First People's Hospital of Huzhou on 21st July 2023. The patient suffered from severe diarrhea, which progressed from 3 to 4 times a day before treatment to about 10 times on the day of diagnosis. The routine blood test showed that the patient's WBC (9.4 × 10^9/L), neutrophil ratio 82.5%, and high-sensitivity C-reactive protein (15.29 mg/L) were all beyond the normal range, suggesting a bacterial infection. This patient was hospitalized due to serious fever and diarrhea. The species of A132 was identified using the Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS).[11]

Antimicrobial Susceptibility Testing

The strain was cultured on LB agar plates. One hundred and forty-five microliter bacterial suspension of 0.5-McFarland turbidity was mixed with 3mL 0.45% NaCl solution. The AST-GN13 card filled with the mixture was used. The minimum inhibitory concentrations (MICs) of commonly used antibiotics, including ampicillin, amoxicillin/clavulanic acid, piperacillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, nitrofurantoin and sulfamethoxazole/trimethoprim, were determined by the Vitek2 compact system (BioMérieux, France) following the manufacturer's instructions. Antimicrobial sensitivity results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (M100-S32).[12] The *Escherichia coli* ATCC25922 was used as quality control and negative control for the antimicrobial susceptibility tests.

Whole-Genome Sequencing

Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's protocol. WGS was performed using both the PacBio Sequel II platform (Pacific Biosciences, Menlo Park, CA, USA) with the SMRT bell TM Template kit (Pacific Biosciences) and the Illumina NovaSeq platform (San Diego, CA, USA) with a TruSeq Nano library kit (Illumina) according to the manufacturer's instructions.

Bioinformatics Analysis

The long and short WGS reads were trimmed with filtlong (https://github.com/rrwick/Filtlong) and fastp, respectively.[13] Hybrid assembly was performed based on trimmed long and short reads using Unicycler v0.5.0 with default settings, resulting in a complete genome assembly.[14] WGS-based *Salmonella* serotyping was performed using SeqSero with default settings.[15] The obtained sequences were submitted to PubMLST database (https://pubmlst.org/) to determine the allele number and specific sequence type (ST). MOB-suite v3.1.4 was adopted to predict plasmid sequences from the assembly genome and identify their replicon types, mobility and host range.[16]

Gene predictions and functional annotations were performed with RAST server.[17] The presence of acquired antibiotic resistance genes (ARGs) and chromosomal resistance mutations was detected with ResFinder v4.1.[18] Virulence factors

(VFs) were screened using the VFDB database.[19] The search for insertion sequence (IS) elements and their characterization down to the family was carried out correspondingly using digIS and ISfinder.[20,21] IntegronFinder v2.0 was used to detect complete integrons.[22] *Salmonella* pathogenicity islands (SPIs) were identified with SPIFinder.[23]

Core genome single nucleotide polymorphisms (cgSNPs) were extracted with Parsnp v1.2, using the A132 complete genome as a reference.[24] Recombination sites were removed using Gubbins v3.3 with default parameters and a starting tree created by Parsnp using a GTR substitution model.[25] Pairwise SNP distances were calculated with SNP-sites.[26] The maximum likelihood phylogeny based on the concatenated data was inferred by IQ-TREE 2 with a HKY model.[27] The best topology was assessed by 1000 ultrafast bootstraps. The phylogenetic tree was visualized with the interactive Tree of Life (iTOL) web application.[28] Plasmid alignment was generated using BRIG v0.95.[29]

Results

Phenotypes and Genotypes of S. London A132

As summarized in Table 1, *S*. London A132 exhibited sensitivity solely to aztreonam, while demonstrating resistance to ampicillin, ampicillin/sulbactam, cefepime, ceftriaxone, ceftazidime, ciprofloxacin, ertapenem, imipenem, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole, so-called multidrug resistance (MDR). Additionally, an intermediate resistance pattern was observed with levofloxacin.

Based on complete genome assembly using both third- and second-generation sequencing, strain A132 was found to be composed of one chromosome (4597.8 kb) and two plasmids—IncFIB-type (111.9 kb) and IncQ1-type (6.1 kb). *In silico* typing affiliated A132 to serotype London with an antigenic profile: 3.10: l, v:1,6 (O antigen: 3, 10, H antigen Phase 1: v, H antigen Phase 2: 1.6) based on the Kauffmann-White scheme. Additionally, according to Achtman's MLST scheme, *S*. London strain A132 was classified as ST155.

Antimicrobial Resistance and Virulence-Associated Genes

ResFinder-based screening identified 20 acquired ARGs (Table 2) that conferred resistance to β-lactams ($bla_{\text{NDM-5}}$, $bla_{\text{TEM-1}}$), aminoglycoside [*aac(6')-Iaa, aac(3)-IId, aadA16, aph(6)-Id, aph(3")-Ib, aadA2*, a*ac(6')-Ib-cr*], quinolone [*aac(6')-Ib-cr, qnrB6*), sulfonamide (three copies of *sul1, sul2*), diaminopyrimidine (*dfrA12, dfrA27*), macrolide (*mphA*), tetracycline (*tetA*), chloramphenicol (*floR*) and rifamycin (*ARR-3*). Moreover, a mutation in *parC* (Trp57→Ser) was identified with the absence of *gyrA* mutation, which explained the low ciprofloxacin MICs.[30]

Using SPIFinder, A132 was found to harbor SPI-1 to SPI-5, SPI-9, and SP-12 to SP-14 on its chromosome. The VFDB led to the detection of 154 VFs (Supplementary Table S1), most of which were associated to bacterial secretion system (38% of the VFs detected, eg *hilA, invB, sopA*) and fimbrial adherence determinants (27% of the VFs detected, eg

Table 1 Antibiotic Susceptibilities of S. London A132

Antimicrobial Susceptibility	*S.* London A132		*E. coli* ATCC25922	
	MIC (mg/L)	Result	MIC (mg/L)	Result
Ampicillin	≥32	Resistant	≤2	Susceptible
Ampicillin/sulbactam	≥32	Resistant	≤4	Susceptible
Aztreonam	≤1	Susceptible	≤1	Susceptible
Cefepime	≥64	Resistant	≤0.12	Susceptible
Ceftriaxone	≥64	Resistant	≤0.25	Susceptible
Ceftazidime	≥64	Resistant	≤0.12	Susceptible
Ciprofloxacin	1	Resistant	≤0.25	Susceptible
Ertapenem	2	Resistant	≤0.12	Susceptible
Imipenem	≥16	Resistant	≤0.25	Susceptible
Levofloxacin	1	Intermediate	≤0.12	Susceptible
Piperacillin/tazobactam	≥128	Resistant	≤4	Susceptible
Trimethoprim/sulfamethoxazole	≥320	Resistant	≤20	Susceptible

Table 2 Genetic Mechanisms of Multidrug Resistance of S. London A132

Mechanism of resistance	Antibiotics	Relevant Genes
Antibiotic inactivation	Aminoglycoside	*aac(6')-Iaa, aac(3)-IId, aadA16, aph(6)-Id, aph(3")-Ib, aadA2, aac(6')-Ib-cr*
	β-lactam	bla_{TEM-1}, bla_{NDM-5}
	Quinolone	*aac(6')-Ib-cr*
	Macrolide	*mphA*
	Rifamycin	*ARR-3*
Antibiotic target replacement	Sulfonamide	*sul1, sul2*
	Diaminopyrimidine	*dfrA12, dfrA27*
Antibiotic efflux	Chloramphenicol	*floR*
	Tetracycline	*tetA*
Antibiotic target protection	Quinolone	*qnrB6*

Agf, Bcf, Fim). Several VFs related to the evasion of the immune system (ie macrophage inducible genes (*mig-14*)), magnesium uptake (*mgtB, mgtC*), stress adaptation (*sodCI*), toxin secretion (*spvB, cdtB, pltA, pltB*) and autotransporters (*ehaB*) were also encoded in the genome. All of the VFs were located on the chromosome.

Characterization of the bla_{NDM-5} Carrying Plasmid in A132

The bla_{NDM-5} gene was located on the IncFIB-type plasmid, herein designated pA132-1-NDM. Notably, pA132-1-NDM carried 95% (21/22) of the acquired ARGs. According to MOB-suite, pA132-1-NDM was predicted to be conjugative due to the presence of relaxase and mate-pair formation markers. However, the origin of the transfer (*oriT*) region was not detected. RAST annotation revealed the presence of genes associated with membrane transport (eg *virB, virC, virD, pinF*), DNA metabolism (eg *hsdM, hsdR, hsdS, rhuM*), protein folding (eg *dsbA, dsbB, dsbC, dsbD*), respiration (eg *cchl, hyp5, ccmA*) and transposable elements integrons (*int, intI1, intI2*) were also identified in pA132-1-NDM. A total of 21 insertion sequences were detected, including IS*1*, IS*3*, IS*5*, IS*6*, IS*110*, IS*1182*, IS*256*, IS*91*, IS*L3* and Tn*3* families. Moreover, pA132-1-NDM carried two complete class 1 integrons with the common *qacEΔ1-sul1* region, conferring resistance to quaternary ammonium salts and sulfonamides. These integrons integrated resistance gene cassettes, including *aac(6')-Ib-cr-aar3-dfrA27-aadA16* and *dfrA12-aadA2* gene arrays, respectively.

A megaBLAST of the pA132-1-NDM sequence retrieved five highly similar IncFIB-type plasmids from previously reported *Salmonella* strains (approximately 96% identity and 100% coverage). Information about these plasmids, which all isolated in China between 2013 and 2022, is given in Supplementary Table S2. The serotypes of these strains were London (n=4) and Thompson (n = 1), and the sources were swine (n = 2), food (n = 2) and human (n = 1). According to plasmid genome alignment, all of these plasmids were missing two regions in comparison to pA132-1-NDM (Figure 1). The two regions were 11.7 kb apart, with the first region (R1, 1.7 kb) contained only the *dfrA12* gene, while the second region (R2, 3.6 kb) comprised bla_{NDM-5} and three other genes (ble_{MBL}, *trpF*, and *dsbD*), implying two independent horizontal gene transfer events.

Comparative Analysis of the Genetic Context of bla_{NDM-5}

Detailed examination of the genetic context of bla_{NDM-5} showed that it is located within a region flanked by two IS*26* sequences, about 13 kb in length, which composed of two gene cassettes linked by an IS*CR1* (IS*91* family) element (Figure 2). One of the cassettes is the so-called class 1 integron cassette (IS*26-intI1-Ib-cr-aac(6')-arr-3-dfrA27-aadA16-qacEΔ1-sul1*) which comprised of an *intI1* gene truncated by one of the IS*26* copies, and the *aac(6')-Ib-cr-arr-3-dfrA27-aadA16-qacEΔ1-sul1* ARGs cassette. The other cassette is named IS*CR1*-IS*26*-flanked cassette (IS*CR1-dsbd-trpF-ble*$_{MBL}$-bla_{NDM-5}-IS*26*) in our study, with

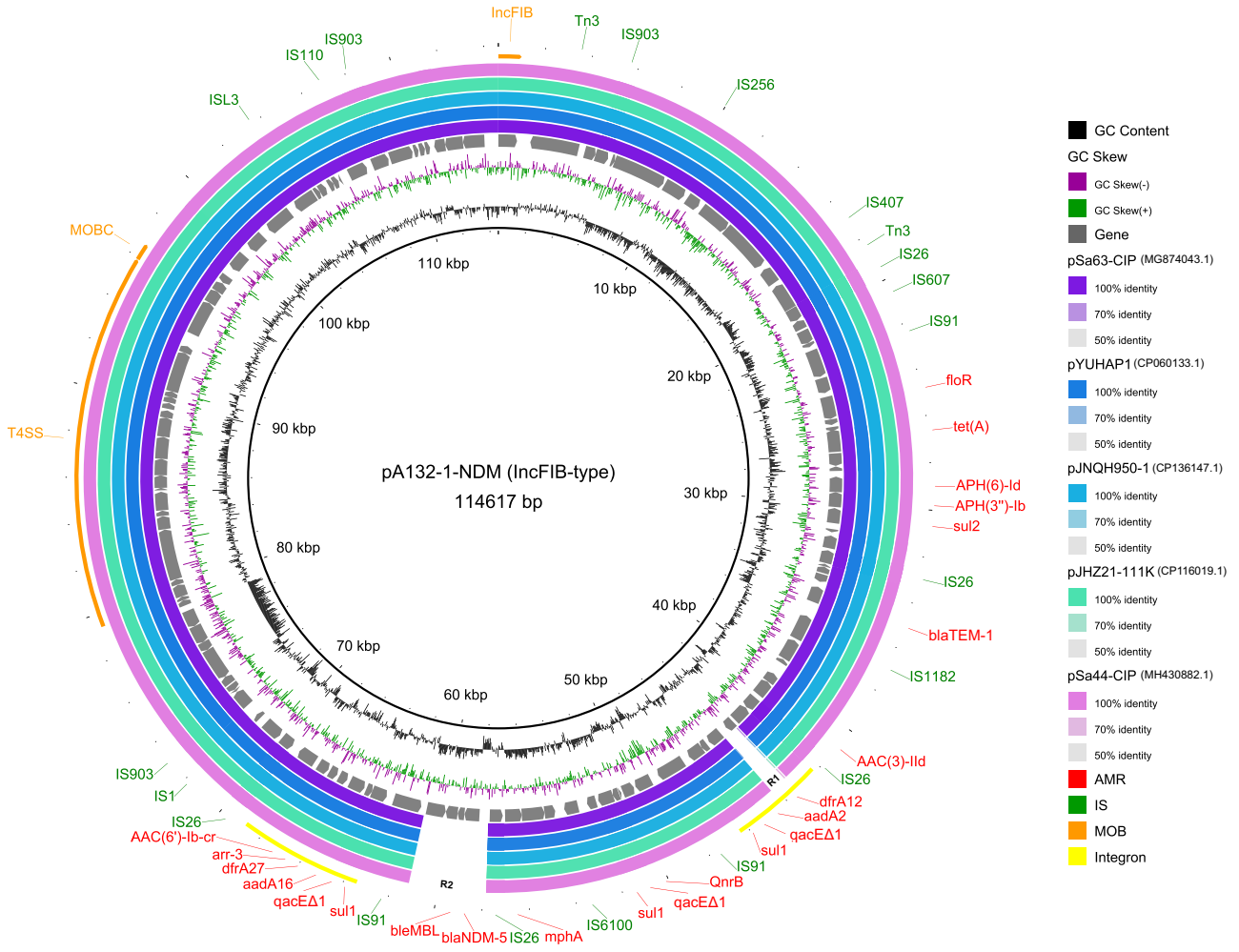

Figure 1 Genetic map of pA132-1-NDM with five other homologous IncFIB-type plasmids in *Salmonella*. Concentric rings represent the similarity between the reference sequence (pA132-1-NDM) in the inner ring and other sequences in the outer rings. The resistance and IS genes are annotated in red or green fonts, respectively. The sequence related to plasmid mobility and integron were highlighted with Orange or yellow rings, correspondingly.

genes IS*CR1, dsbd* (Dsbd superfamily protein), *trpF* (phosphor ribosyl anthranilate isomerase) and ble_{MBL} (bleomycin resistance gene) located upstream, and an IS*26* downstream. Together, the two cassettes form the so-called class 1 integron-IS*CR1* complex which is commonly found in gram-negative bacteria.[31]

To investigate the potential origin of bla_{NDM-5}-containing cassette in A132 strain, we carried out a series of NCBI BLAST search using nucleotide sequences. We found that the IS*CR1*-IS*26*-flanked cassette (IS*CR1-dsbd-trpF-*ble_{MBL}-bla_{NDM-5}-IS*26*) of pA132-1-NDM showed a perfect match with a previously reported bla_{NDM-5} harboring plasmid p91_NDM of *E. coli* (MN007141.1) with 100% identity and 100% coverage. Moreover, the BLAST search using the entire class 1 integron-IS*CR1* complex sequence in pA132-1-NDM yielded the one identified in plasmid p388 from *Enterobacter hormaechei* (CP021168.1) which exhibited a high degree of similarity (100% identity and 99% coverage). The sequence alignment of the class 1 integron-IS*CR1* complex in A132 strain with the two matched results from BLAST search (p91_NDM_*Escherichia coli* and p388_*Enterobacter hormaechei*) was shown in Figure 2. Interestingly, both p91_NDM and p388 were identified as conjugative IncFIA-type plasmids with a broad host range among *Enterobacterales*, while the pA132-1-NDM found in our study was identified as IncFIB-type plasmid.

Phylogenetic Analysis of Chinese S. London ST155 Isolates

To delineate genetic distinctions between A132 and other *S.* London ST155 strains in China, we retrieved 195 additional Chinese *S.* London ST155 strains from the EnteroBase database (up until December 2023).[32] A cgSNP analysis revealed

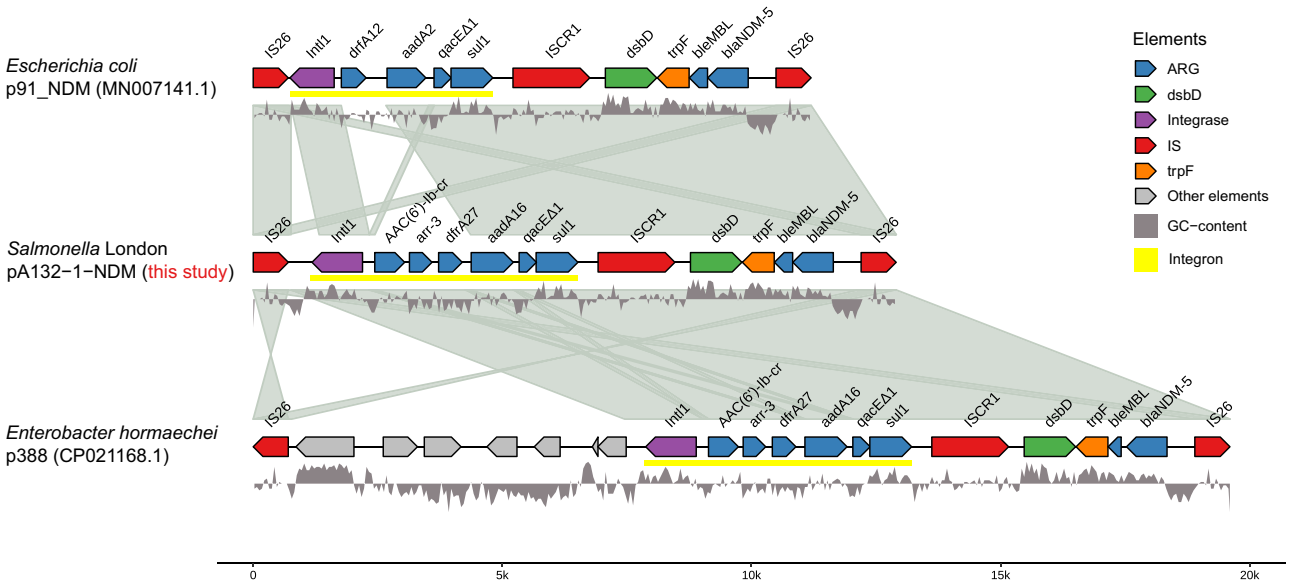

Figure 2 Schematic representation of the *bla*$_{NDM-5}$-containing class 1 integron-IS*CR1* complex found in the A132 and other two representative sequences identified in *E. coli* plasmid p91_NDM (MN007141.1) and *Ent. hormaechei* plasmid p388 (CP021168.1) from Genbank. This image was built using the R package gggenomes (https://github.com/thackl/gggenomes).

differences ranging from 14 to 169 SNPs between these strains and A132 (Supplementarys Figure S1 and Table S3). Of the 164 strains with identifiable sampling sources, the overwhelming majority originated from humans (56.7%, 93/164), swine (24.4%, 30/164) and food (14.6%, 24/164). We further narrowed down to eight closely related strains using a threshold of 40 cgSNPs (Figure 3 and Supplementary Table S3).[33] These strains were isolated from three different cities

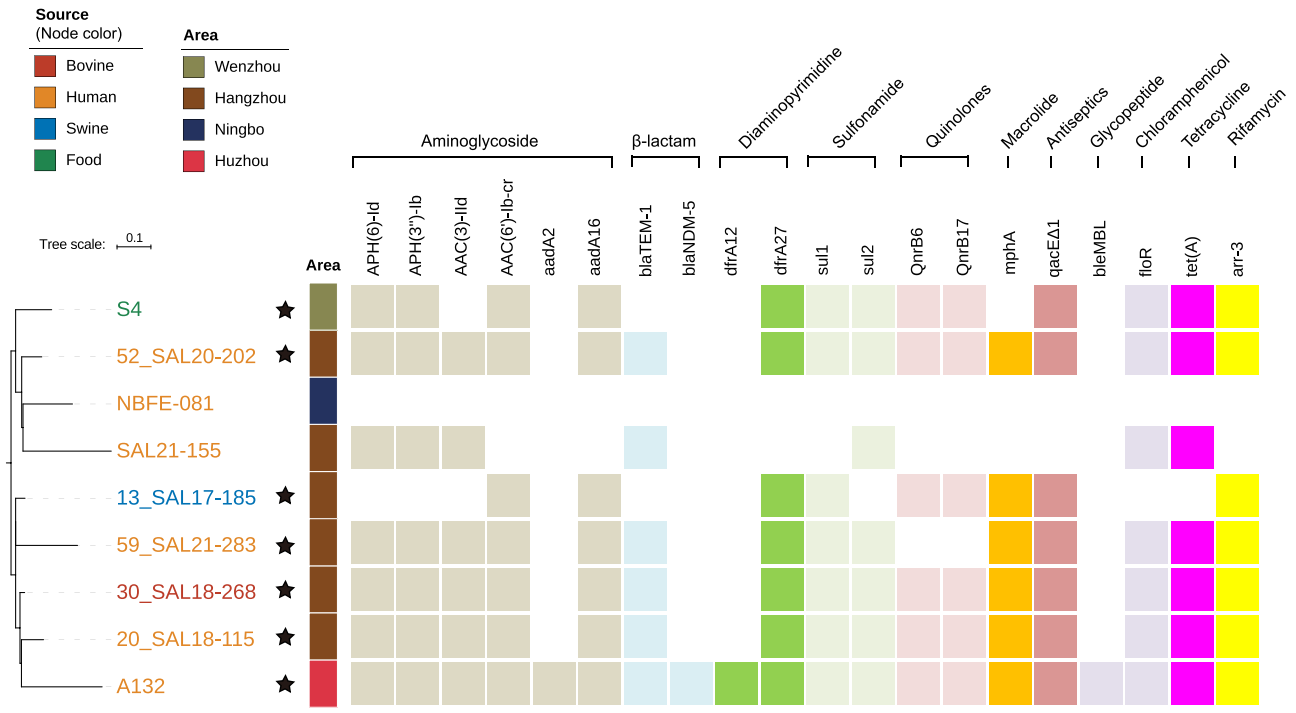

Figure 3 Phylogenetic association between A132 and its eight closely related Chinese *S.* London ST155 isolates using cgSNP strategy. Antimicrobial resistant genes were categorized into groups based on their resistance to different types of antibiotics, as shown in boxes with different colors. The absence of box indicates the absence of particular resistance gene in that strain. The source of each isolate is shown as colored terminal nodes. Different colored strips indicate the city where the strains were isolated. The black star indicates the presence of an IncFIB plasmid in that strain.

in Zhejiang Province between 2017 and 2021, including Hangzhou (n = 6), Ningbo (n = 1), and Wenzhou (n = 1). Plasmid detection through MOB-recon showed that except for NBFE-081 and 13_SAL17-185, the other six strains all carried an IncFIB-type plasmid. Apart from the strain NBFE-081 from Ningbo, the other seven strains were predicted to be MDR due to the presence of multiple common ARGs. Interestingly, compared with these closely related strains, the A132 strain in our study carried four unique ARGs, including *aadA2, dfrA12,* ble_{MBL} and bla_{NDM-5} which all located within the class 1 integron-IS*CR1* complex.

Discussion

NDM-5 carbapenemase was primarily detected in *E. coli* and remains relatively rare in other members of the *Enterobacteriaceae* family, such as NTS and *Klebsiella pneumoniae*.[34,35] However, our study reveals the emergence of a *Salmonella* strain, designated as A132, harboring the NDM-5 carbapenemase gene. A132 belongs to the serovar London, which recognized as one of the top five prevalent serovars responsible for human salmonellosis in China.[36] Furthermore, the sequence type of A132 is classified as ST155, an MDR clone that has been frequently reported in China, thus posing a significant threat to public health.[37] A132 exhibited multidrug resistance and carried a total of 20 acquired ARGs. These ARGs were in concordance with the DST phenotypes, with 95% of the ARGs located on the IncFIB plasmid (pA132-1-NDM). Previous reports in *Salmonella* have mainly identified bla_{NDM} genes on IncX3 and IncFII plasmids.[38–40] Notably, our study represents the first documentation of bla_{NDM-5} located on a transmissible IncFIB plasmid of *Salmonella*. IncFIB plasmids, often referred to as ColV plasmids, have predominantly been associated with serovars Kentucky, Typhimurium, Heidelberg and Schwarzengrund.[41–43] The results of plasmid BLAST search in Genbank database retrieved five highly homologous IncFIB plasmids in serovar London and Thompson, all isolated from China. Several studies have suggested that IncFIB plasmids likely contribute to increased colonization in the cecum of poultry, providing an explanation for their persistence in food animal populations.[42,43] These findings suggest that IncFIB plasmids may potentially contribute to the dissemination of ARGs among bacteria in various livestock species.

Based on the comparative analysis of the bla_{NDM-5} gene context in strain A132 with those in public databases using NCBI BLAST search (Figure 2), we identified the IS*CR1*-IS*26*-flanked cassette (IS*CR1-dsbd-trpF-*ble_{MBL}-bla_{NDM-5}-IS*26*) as a highly conserved and stable domain in the IncF-type plasmids of *E.coli* and *Enterobacter hormaechei*. Additionally, previous studies have reported bla_{NDM-5} gene in IncX3-type plasmids of *Enterobacteriaceae*, located in the roughly similar genetic context (IS*3000*-IS*Aba125Δ*-IS*5*-ΔIS*Aba125-*bla_{NDM-5}-ble_{MBL}-*trpF-dsbC-cutA*-IS*26*), with the bla_{NDM-5} gene flanked by IS elements and located adjacent to three other ARGs (ble_{MBL}-*trpF-dsbC*).[39,40,44] Despite the presence or absence of other gene elements in the genetic context (such as ΔIS*Aba125* and *cutA* which were not found in pA132-1-NDM), the combination of four ARGs (bla_{NDM-5}-ble_{MBL}-*trpF-dsb*) were found to be conserved across all bla_{NDM-5}-harboring IncX3- and IncF-type plasmids reported thus far. These evidences together emphasize the conservation and significance of the four ARGs as a basic unit in the transmission and expression of carbapenemase, suggesting a potential pattern for the dissemination of bla_{NDM-5} gene.[40,45]

Moreover, the location of bla_{NDM-5} gene in the vicinity of a class 1 integron seems to enhance its dissemination. The bla_{NDM-5}-containing IS*CR1*-IS*26*-flanked cassette in pA132-1-NDM was found to be linked to a complete class 1 integron via transposition of an IS*CR1* element. It was known that Class 1 integrons are widely distributed in the environmental microbial genomes and are implicated in the dissemination of many ARGs.[46] According to the model proposed by Toleman et al, the presence of IS*CR1* can mediate the fusion of class 1 integron with other IS-flanked ARGs-carrying cassettes, forming a class 1 integron-IS*CR1* complex.[31] Interestingly, the five highly similar IncFIB-type plasmids in *Salmonella* strains retrieved from the megaBLAST search all showed the same pattern of the bla_{NDM-5}-carrying cassette missing in comparison to pA132-1-NDM (Figure 1). Therefore, we suspect that the presence of bla_{NDM-5} gene in A132 may be the result of recent integration of the IS*CR1-IS26*-flanked cassette mediated by the IS*CR1* element, leading to the formation of a class 1 integron-IS*CR1* complex, as demonstrated by Toleman et al.[31] In support of our speculation, a BLAST search using the class 1 integron-IS*CR1* complex of p132-1-NDM revealed bundles of highly identical genetic structures in IncFIA plasmids of *E. coli* and *Ent. hormaechei*. These evidence collectively suggest that the bla_{NDM-5}-carrying cassette is frequently transferred as an integral complex with class 1 integron rather than as a standalone gene cassette, as also implicated in previous studies with *E. coli*.[47,48] Nevertheless, future research is necessary to validate the role of class 1 integron and IS*CR1* element in the dissemination of bla_{NDM-5} gene.

Using cgSNP-based phylogenetic analysis, we found that A132 shares a close genomic relationship with numerous Chinese *S*. London ST155 strains retrieved from EnteroBase. These prevalent Chinese *S*. London ST155 isolates have been predominantly sourced from humans, swine, and food products, suggesting that *S*. London infections in humans often originate from zoonotic transmission.[49] Therefore, it is highly probable that the patient in our study had a foodborne infection. In addition, recent studies have reported a rising prevalence of *S*. London isolates associated with foodborne human salmonellosis in Zhejiang Province.[50] The identification of eight isolates in Zhejiang Province, differing from A132 by a threshold of 40 cgSNPs, yet carrying similar plasmids and ARGs, underscores the necessity for vigilant surveillance of *S*. London pathogens.[33] Notably, A132 possesses four unique ARGs ($bla_{\text{NDM-5}}$,ble_{MBL},*trpF* and *dsbd* genes) compared to these strains. This suggests a recent acquisition of the $bla_{\text{NDM-5}}$ gene and the other four unique ARGs by A132 from the environment, possibly through horizontal gene transfer mechanisms. This acquisition further amplifies the multidrug resistance profile of *Salmonella*, presenting a formidable clinical challenge in combating antibiotic resistance.

From One Health perspective, the emergence of a $bla_{\text{NDM-5}}$-carrying *S. enterica* serovar London strain in China presents significant clinical implications.[51] Given the pivotal role of $bla_{\text{NDM-5}}$ gene in multi-drug resistance, a preliminary search on PubMed indicates a steady increase in reported cases of $bla_{\text{NDM-5}}$ over the past decades, possibly linked to the excessive usage of antibiotics. While *E. coli* accounts for the majority of reported cases, the presence of $bla_{\text{NDM-5}}$ gene in *Salmonella*, as demonstrated in our study, which is located alongside other ARGs in proximity to IS elements and housed within a highly transmissible IncFIB plasmid adds complexity to the management of infections. Therefore, sustained attention needs to be warranted, focusing on genotyping, plasmid characterization, and phylogenetic analysis to better understand the transmission dynamics of $bla_{\text{NDM-5}}$-carrying *Salmonella*, guiding public health interventions.

Conclusion

Our study documented the first isolation of an NDM-5-producing *Salmonella enterica* serovar London strain in China. The emergence of the $bla_{\text{NDM-5}}$ gene in a clinical MDR *S*. London ST155 strain represents a significant threat to public health. Through EnteroBase database search, we retrieved eight highly similar Chinese *S*. London ST155 strains from other cities in Zhejiang Province, which mostly exhibited MDR, highlighting the importance of the surveillance for *S*. London ST155. We suspect that the existence of $bla_{\text{NDM-5}}$ gene within an IS*CR1-IS26*-flanked cassette on the IncFIB plasmid is likely a result of recent horizontal gene transfer from other *Enterobacteriaceae*. The $bla_{\text{NDM-5}}$ gene carried by a transmissible IncFIB plasmid may cause an increased risk of horizontal transfer of the $bla_{\text{NDM-5}}$ gene. Moreover, the class 1 integron and IS*CR1* element in the vicinity of $bla_{\text{NDM-5}}$ gene may also benefit the mobilization and dissemination of $bla_{\text{NDM-5}}$, which highlights the necessity for implementing proactive clinical controls to mitigate the spread of $bla_{\text{NDM-5}}$ and its associated multidrug resistance.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The genome sequence of the strain has been deposited in the NCBI database under BioProject accession number PRJNA1057448.

Ethics Approval and Informed Consent

The authors certify that the patient consent form has been obtained. Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. The studies involving human participants were reviewed and approved by the Ethics Committee of the First People's Hospital of Huzhou.

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Disclosure

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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