

Letter



Genomic Epidemiology of Foodborne *bla*_{NDM-9} Gene-carrying Extensively Drug-resistant (XDR) *Salmonella enterica* Serovar Indiana ST17

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Antimicrobial resistance (AMR) has become a critical global public health challenge in the 21st century. Since the initial isolation of a *bla*_{NDM-1}-carrying and carbapenem-resistant *Klebsiella pneumoniae* from an Indian hospital in 2009^[1], the escalating prevalence of New Delhi metallo- β -lactamase (NDM)-encoding genes (*bla*_{NDM}) has transformed carbapenem resistance into a worldwide phenomenon, transcending national and regional boundaries^[2]. Up to 90 distinct NDM variants have been reported globally according to the NCBI GenBank Pathogens database. Plasmid-mediated horizontal gene transfer (HGT), which occurs both within and across bacterial species, has significantly accelerated the global dissemination of *bla*_{NDM}-related genes and the associated resistance^[3]. Carbapenem-resistant pathogens were responsible for 50,000–100,000 deaths globally in 2019^[4]. Although NDM-1 has been relatively well characterized^[5], the epidemiological profiles of other NDM variants require continued surveillance and in-depth investigation. The novel NDM-9 variant (GenBank accession no. KC999080) was first identified in 2013 from a clinically significant isolate of *Klebsiella pneumoniae* ST107 strain PPH1303 with a high level of resistance to carbapenems recovered from the urine culture of a pediatric patient in Beijing, China, who had acute lymphocytic leukemia and had undergone allogeneic stem cell transplantation^[6].

To characterize the genomic epidemiology of foodborne carbapenem-resistant *Salmonella* harboring *bla*_{NDM} genes across China and to

elucidate the genetic environments and potential transmission mechanisms of these genes, we investigated the prevalence of *bla*_{NDM} genes using PCR^[7] among 2,877 foodborne *Salmonella* strains isolated from diverse food sources. This work was carried out across 31 provinces of the Chinese mainland in 2022, and included a positive strain, 22S130, which was isolated from retail chicken thigh sample in Hebei Province. It was sequenced using the QNome-3841hex (Qitan Tech, Chengdu, China) and Illumina Novaseq PE150 platform (Novogene, Beijing, China). Assembly of the Qitan genome was performed using flye v2.9, followed by error correction with minimap2 v2.29-r1283 and racon v1.5.0. Consed v29.0 was employed to generate the single, complete and closed chromosome/plasmid. Additionally, Serovar was predicted with SeqSero2. MLST v2.09, PlasmidFinder v2.1, and ResFinder v4.7.2 available at Center for Genomic Epidemiology (CGE) were utilized for multilocus sequence typing, plasmid replicon, and AMR genotypes identification, respectively. Antimicrobial susceptibility testing (AST) was determined by micro-broth dilution using Biofosun[®] Gram-negative panel (Fosun Diagnostics, Changsha, China). Furthermore, Clinical and Laboratory Standards Institute guidelines (CLSI M100-S34 and M31-A3) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, v2025) were consulted for the resistance interpretation. To further analyze the genetic environment and perform plasmid structural comparative studies, Easyfig v2.2.2 and BLAST Ring Image Generator (BRIG, v0.95) were used,

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respectively.

In this study, 22S130, the positive strain isolated from the retail chicken thigh sample in Hebei Province, was identified as *S. enterica* serovar Indiana of the MLST type ST17. Among the selected 86 *bla*_{NDM} gene positive *Salmonella* isolates from NCBI GenBank database, 20.93% (18/86) were identified as serovar Indiana ST17, followed by Typhimurium (18.60%, 16/86, 12 ST34, and 4 ST36), monophasic Typhimurium ST34 (15.12%, 13/86), Senftenberg ST14 (12.79%, 11/86), Kentucky ST198 (9.30%, 8/86), and other 13 serovars (Table 1). These data revealed that foodborne *Salmonella* carrying the *bla*_{NDM} gene exhibited considerable serovar diversity, with *S. Indiana* ST17 constituting a significant proportion. While *S. Indiana* ST17 dominated the serovar diversity, the co-circulation of 16 additional serovars signifies a broad reservoir for *bla*_{NDM} transmission, highlighting emergent threats from the Typhimurium (including its monophasic variant) and Senftenberg lineages.

We observed that 22S130 exhibited severe multidrug resistance (MDR) against 18 antimicrobial agents belonging to 11 distinct classes. Excluding susceptibility to tigecycline and intermediate resistance to colistin, the strain demonstrated resistance to all the remaining 16 tested antimicrobial agents across 10 classes, including carbapenems (imipenem), the ultimate drug for clinically treating severe infections caused by MDR Enterobacteriaceae. Following the international expert consensus criteria for drug resistance classification^[8], *Salmonella* isolate 22S130 qualified as an extensively drug resistant (XDR) isolate.

Genomic analysis revealed multiple diverse resistance determinants of this isolate (Table 2). Seven β -lactamase genes, including *bla*_{CTX-M-55}, three variants of *bla*_{TEM}, *bla*_{OXA-1}, and *bla*_{NDM-9}, which collectively confer resistance to various β -lactams. With the exception of the chromosomally-encoded *bla*_{CTX-M-55}, the remaining six β -lactamase genes were localized within plasmid p22S130. The chromosomally located *aac(6')-laa* gene, along with the plasmid-borne *aac(6')-lb-cr*, *aadA2*, *aph(4)-la*, *rmtB*, and *aac(3)-IV* genes, collectively mediate aminoglycoside resistance. The *mph(A)*, *tet(A)*, *fosA3*, and *ARR-3* genes located on the plasmid conferred resistance to azithromycin, tetracycline, fosfomycin, and rifampicin, respectively. Folate pathway inhibitor resistance involved genes such as *sul1*, *sul2*, and *dfrA12* while resistance to phenicol was conferred by *catB3* and *floR*. In addition to these antimicrobial resistance genes (ARGs), we identified chromosomal mutations contributing to quinolone resistance in *gyrA* (p.S83F, p.D87N) and *parC* (p.T57S, p.S80R). These mutations significantly enhanced the potential for quinolone resistance when combined with the plasmid-borne *aac(6')-lb-cr* gene. Moreover, we observed the biocide tolerance gene *qacE* on plasmid p22S130. The convergence of XDR phenotype, carbapenemase (*bla*_{NDM-9}), and enhanced fluoroquinolone resistance in *S. Indiana* ST17, highlights its role as a priority AMR vector.

The genetic environment related to *bla*_{NDM-9} in p22S130 is shown in Figure 1. Through the comparative analysis involving our *bla*_{NDM-9} fragment and ten homologous sequences from Enterobacteriaceae (including *Salmonella*,

Table 1. serovars of *bla*_{NDM}-carrying *Salmonella* isolates retrieved from NCBI

Serovar	Number	Percentage	MLST types
Indiana	18	20.93%	ST17
Typhimurium	16	18.60%	ST34 (n = 12), ST36 (n = 4)
I 4,[5],12:i:-	13	15.12%	ST34
Senftenberg	11	12.79%	ST14
Kentucky	8	9.30%	ST198
Enteritidis	4	4.65%	ST11
Mbandaka	3	3.49%	ST413
Havana	2	2.33%	ST578
Typhi	2	2.33%	ST1
Others ^a	9	10.47%	-

Note. ^aOthers included the following serovars of *Salmonella*: Corvallis, Geraldton, Goldcoast or Brikama, I -;e,h:1,5, I 4:-, London, Molade or Wippra Rissen, Stanley.

Escherichia coli, and *Klebsiella pneumoniae*; 9 plasmid-derived and 1 chromosomal), we identified a novel and highly conserved composite transposon structure harboring bla_{NDM-9}, which was "IS26-bla_{NDM-9}-ble_{MBL}-trpF-dsbD-cutA-ISCR1." This conservative structure was manifested in: (i) all the three bacterial species examined, (ii) diverse plasmid incompatibility types, and (iii) geographical origins and sources of isolation. This structural conservation suggests that the identified genetic environment may be prevalent across diverse Enterobacteriaceae, potentially functioning as a stable transmission vehicle for disseminating bla_{NDM-9}. Two key features flanked the identified bla_{NDM-9} composite transposon in most of these 11 sequences: (1) a mercury

resistance operon (*merEDACPTR*) appearing upstream and (2) an MDR transposon (*ISCR1-sul1-aadA2-dfrA12-int11-IS26*) appearing downstream, implying potential co-selection under mercury exposure, a phenomenon documented in wastewater systems^[9]. Notably, several sequences, including that of 22S130, lacked the upstream *mer* cluster, the region of which was replaced by diverse insertion sequences (ISs) (e.g., IS103, IS150, IS15, and IS26), suggesting potential complex recombination events mediated by these elements. This may reflect niche-specific adaptations, wherein antibiotic pressure supersedes mercury selection. While the functional implications of these flanking structures on the stability and dissemination of the

Table 2. Antimicrobial susceptibility of *Salmonella* Indiana 22S130 to a panel of antimicrobial agents and aquired antimicrobial resistance-encoding genes identified in the bacterial genome with online retrieval in Resfinder database

Antimicrobial class	Antimicrobial Agent (abbreviation)	MIC (mg/L)	R/I/S ^a	Resistance genes or point mutation	
				Chromosome	Plasmid
β-Lactam combination agents	Ampicillin/sulbactam (SAM)	> 64/32	R		
Penicillins	Ampicillin (AMP)	> 64	R		bla _{TEM-1B}
	Cefotaxime (CTX)	> 16	R		bla _{TEM-214}
Cephalosporins	Ceftazidime (CAZ)	> 64	R	bla _{CTX-M-55}	bla _{TEM-206}
	Cefazolin()	> 16	R		bla _{TEM-141}
	Cefoxitin (FOX)	> 64	R		bla _{OXA-1} bla _{NDM-9}
Carbapenems	Imipenem (IMP)	4	R		
Macrolides	Azithromycin (AZM)	64	R		mph(A)
Aminoglycosides	Gentamicin (GEN)	> 32	R	aac(6')-Iaa,	aac(6')-Ib-cr, aadA2, aph(4)-Ia, rmtB, aac(3)-IV
	Amikacin (AK)	> 128	R		
Tetracyclines	Tetracycline (TET)	> 32	R		tet(A)
	Tigecycline (TGC) ^b	≤ 0.25	S		
(Fluoro)Quinolones	Nalidixic acid (NAL)	> 64	R		aac(6')-Ib-cr ^f
	Ciprofloxacin (CIP)	> 4	R		
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole (SXT)	> 4/76	R		sul1, sul2, dfrA12
Phenicol	Chloramphenicol (CHL)	> 64	R		catB3, floR
	Florfenicol (FFC) ^{c,d}	> 32	R		
Polymyxins	Polymyxin E (Colistin, CT) ^d	1	I		
Fosfomycins	Fosfomycin (FOS) ^e	-	-		fosA3
	Rifampicin ^e	-	-		ARR-3

Note. ^aR, resistant; I, intermediate; S, susceptible, R/I/S according to the CLSI guidelines M100-S34, 2024; ^bR/I/S according to EUCAST clinical breakpoints, 2025; ^cR/I/S according to the CLSI guidelines M31-A3, 2008; ^dUsed as a feed additive in animal production; ^eNo antimicrobial resistance phenotype data; ^faac(6')-Ib-cr also mediate resistance to fluoroquinolones such as ciprofloxacin, but MIC of ciprofloxacin does not always increase above ECOFF.

*bla*_{NDM-9} transposon remain unclear and require further experimental validation to elucidate their mechanistic roles, they may facilitate co-selection under heavy metal or antibiotic pressure.

A comparison between the genetic environment flanking the *bla*_{NDM-9} gene in *Salmonella* 22S130 (this study) and that in *K. pneumoniae* PPH1303 (initial *bla*_{NDM-9} discovery)^[6] revealed that the *bla*_{NDM-9} structure in both the strains contained an identical minimal mobile element (including *bla*_{NDM-9}, *ble*_{MBL}, and *trpF*) and shared conserved downstream *dsbD* and *cutA* genes. However, the genetic context adjacent to *bla*_{NDM-9} in our strain lacked the GroES and GroEL heat shock chaperonin-encoding genes and carried distinct ISs on the right flank. This suggests that the right-terminal genetic environment of *bla*_{NDM-9} in these two strains likely diverged because of an additional ISs insertion event.

Notably, the extracted fragments carrying *bla*_{NDM-9} exhibited exceptionally dense and diverse MGEs, including transposons (Tn3-like and IS26-flanked), integrons (*int11*), and ISs (*ISCR1* and *IS26*) among others, suggesting that the current configuration of these sequences may have undergone complex MGE-mediated homologous recombination events during their evolutionary history.

Concurrently, we also observed that all ten *bla*_{NDM-9}-carrying plasmids in this study exhibited

hybrid replicon types, with distinct differences in hybrid configurations existing across bacterial species: the three *Escherichia coli*-derived plasmids were of the IncB/O/K/Z_2 types, whereas the four *Salmonella*-derived plasmids exhibited the IncHI2/IncHI2A/IncN/RepA_1_pKPC-CAV1321 profiles, and the two *Klebsiella pneumoniae*-derived plasmids belonged to the IncQ1/repFIB/repHI5B types. Hybrid plasmid replicons have been reported to mediate various critical resistance mechanisms. Examples include IncFIB(K)/IncHI1B/IncX3 hybrid plasmids mediating NDM-IMP carbapenemase expression^[10]; IncFIA/FIB/FIC(FII) hybrid plasmids facilitating the co-transfer of *bla*_{NDM-5} and *fosA3*^[11]; and IncC-IncX3 hybrid plasmids co-carrying *bla*_{NDM-4}, *tet(X)*, and *tmexCD3-toprJ3* that confer resistance to both carbapenems and tigecycline^[12]. We speculate that the plasmid hybridization in this study may be associated with the diverse MGEs mentioned earlier, and with multiple homologous recombination events that catalyzed the fusion of distinct plasmid backbones. This hybrid configuration likely confers integration of varied replication initiation systems to maintain plasmid stability, expand the host range to facilitate cross-species transmission, enhance dissemination of host bacterial clones, and further facilitate accelerated global dissemination of the *bla*_{NDM-9} gene and carbapenem resistance. Three *bla*_{NDM-9}-carrying *Salmonella* plasmids sharing high

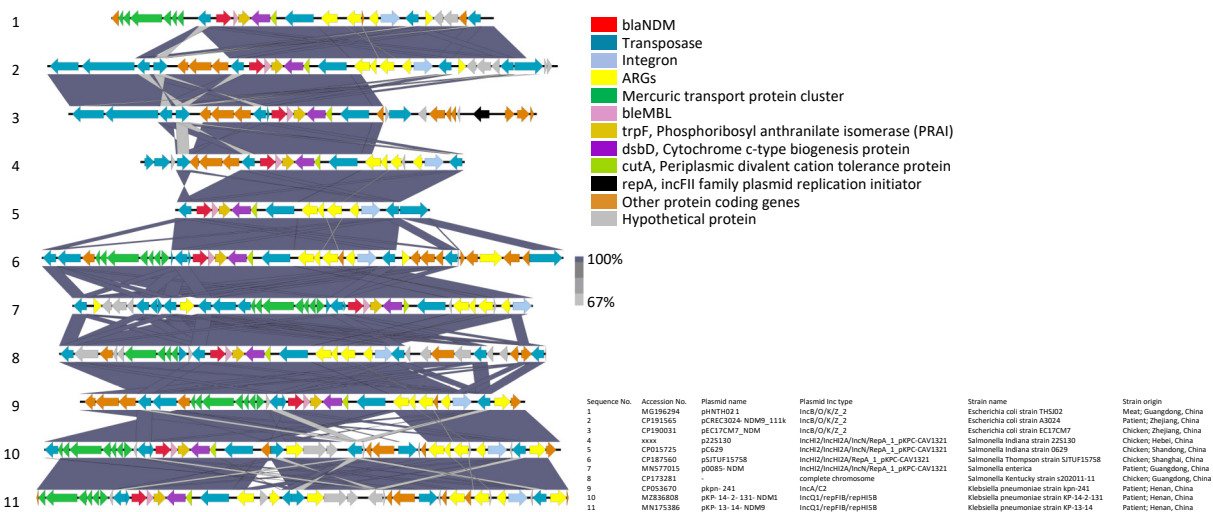


Figure 1. Genetic environments related to *bla*_{NDM-9} gene in bacterial plasmids. The figure was generated by Easyfig (v2.2.2). Confirmed and putative open reading frames (ORFs) are indicated by block arrows and their orientations with different colours, and arrow size is proportional to the predicted ORF length. *bla*_{NDM-9} gene is indicated by a red arrow, while genes encoding mobile elements (Insertion sequence, IS) are indicated by dark blue arrows. Regions of homology between the plasmids ranging from 67% to 100% are indicated by the graded shaded regions between sequences.

similarity with p22S130 were selected for comparative plasmid structural analysis between pSJUF15758 (from *S. Thompson*, CP187560), p0085-NDM (from *Salmonella* spp., MN577015.1), and pC629 (from *S. Indiana*, CP015725, previously isolated from a whole chicken sample in Qingdao, China in our laboratory). These plasmids demonstrated nearly identical backbone structures (Supplementary Figure S1), with the bla_{NDM-9} gene consistently located in the distal region and exhibiting high sequence conservation in the bla_{NDM-9} genetic environment structure (Figure 1). Compared with p22S130, all three plasmids lacked a 130-165 kb segment encoding serine/threonine-protein kinase toxin HipA, DNA-binding protein H-NS, chromosome partition protein Smc, NA-cytosine methyltransferase, Tn3 family transposase TnAs1, tetracycline resistance protein TetC, and multiple putative proteins. This structural variation suggests p22S130 may have acquired enhanced tetracycline resistance through transposon activity, consistent with our strain's minimum inhibitory concentration (MIC) > 32 µg/mL.

Additionally, three *Salmonella* strains with a chromosomally integrated region identical to the ~17-kb bla_{NDM-9}-containing region of p22S130 were identified: *S. Indiana* YZ21MCS4 (CP089313; chicken, Yangzhou, 2021), *S. Indiana* QT6365 (CP103966; diarrheal patient, Wenzhou, 2018), and *S. Kentucky* s202011_11 (CP173281; chicken, Guangzhou, 2020). Given that reports of the bla_{NDM-9} gene in *Salmonella* have thus far been predominantly confined to the serovar Indiana ST17 lineage (whether plasmid-borne or chromosomally integrated), we hypothesized that this lineage possesses competitive advantages over other *Salmonella* lineages, likely attributable to its abundance of MGEs flanking bla_{NDM-9} and its enhanced feasibility for forming hybrid plasmids. These genomic features not only facilitate inter-strain gene transfer but also enable vertical transmission of bla_{NDM} via chromosomal integration events.

In this study, we conducted a genomic epidemiological analysis of an XDR *Salmonella* Indiana ST17 strain, which was isolated from retail chicken carrying the bla_{NDM-9} gene. In addition to harboring multiple resistance genes and exhibiting carbapenem resistance, this strain possessed a novel and highly conserved composite transposon structure harboring bla_{NDM-9}. The unique genetic environment of this structure, combined with the plasmid's hybrid replicon types, likely collectively mediated the rapid bla_{NDM-9} dissemination. Our

results demonstrate that *Salmonella* Indiana ST17 is emerging as a primary epidemic clone and reservoir for diverse bla_{NDM} variants, and exhibits significant potential for disseminating carbapenem resistance via HGT, a process in which MGEs critically accelerate transmission dynamics. Given that ARGs have been categorized as emerging contaminants affecting public health and environmental safety, with the bla_{NDM} gene being the pivotal and widespread one across multiple stages of the food chain, implementing AMR surveillance is imperative. The One Health framework, recognized as a vital tool for addressing the interconnections among humans, animals, and the environment^[13], provides an essential strategy for elucidating carbapenem resistance mechanisms, tracking transmission pathways, and conducting comprehensive surveillance.

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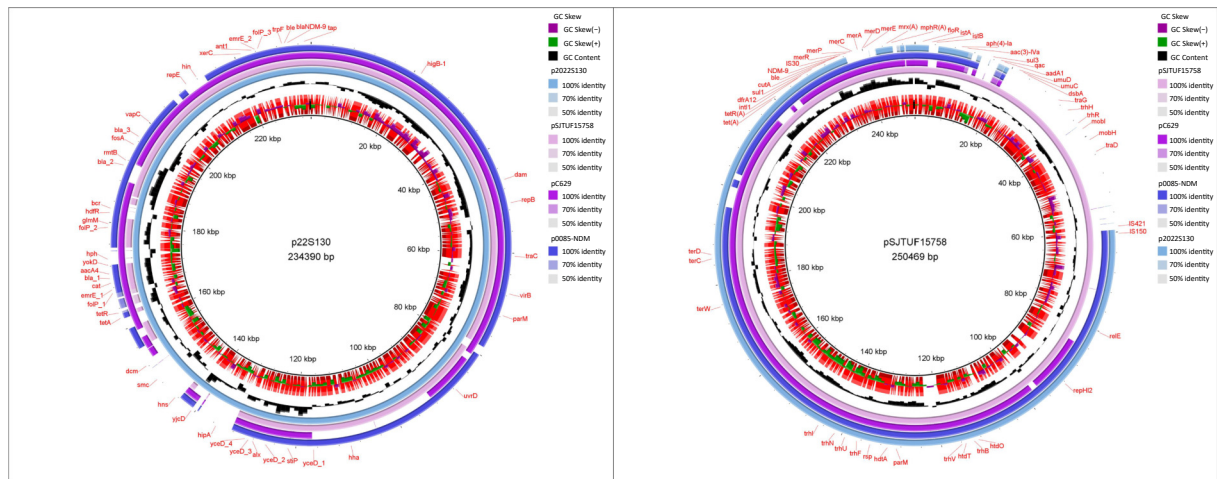
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REFERENCES

1. Thomas CA, Cheng ZS, Bethel CR, et al. The directed evolution of NDM-1. *Antimicrob Agents Chemother*, 2023; 67, e0071423.
2. Fu B, Xu J, Yin DD, et al. Transmission of bla_{NDM} in Enterobacteriaceae among animals, food and human. *Emerg Microbes Infect*, 2024; 13, 2337678.
3. Hu JC, Han M, Yan RY, et al. Mobile genetic elements contributing to horizontal gene transfer of bla_{NDM} among *Escherichia coli* in the community setting. *Microb Pathog*, 2024; 196, 106996.
4. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*, 2022; 399, 629–55.

5. Yao SJ, Yu JQ, Zhang TY, et al. Comprehensive analysis of distribution characteristics and horizontal gene transfer elements of *bla*_{NDM-1}-carrying bacteria. *Sci Total Environ*, 2024; 946, 173907.
6. Wang XJ, Li HN, Zhao CJ, et al. Novel NDM-9 metallo- β -lactamase identified from a ST107 *Klebsiella pneumoniae* strain isolated in China. *Int J Antimicrob Agents*, 2014; 44, 90–1.
7. Du PC, Zhang P, Wang J, et al. Molecular characterization of two novel NDM-1-producing atypical enteroaggregative *Escherichia coli* isolates from patients. *Plasmid*, 2021; 115, 102568.
8. Rafailidis PI, Kofteridis D. Proposed amendments regarding the definitions of multidrug-resistant and extensively drug-resistant bacteria. *Expert Rev Anti Infect Ther*, 2022; 20, 139–46.
9. He DH, Li J, Yu WH, et al. Deciphering the removal of antibiotics and the antibiotic resistome from typical hospital wastewater treatment systems. *Sci Total Environ*, 2024; 926, 171806.
10. Jia XM, Jia PY, Zhu Y, et al. Coexistence of *bla*_{NDM-1} and *bla*_{IMP-4} in one novel hybrid plasmid confers transferable carbapenem resistance in an ST20-K28 *Klebsiella pneumoniae*. *Front Microbiol*, 2022; 13, 891807.
11. Zhao WB, Li WJ, Du XD, et al. Hybrid IncFIA/FIB/FIC(FII) plasmid co-carrying *bla*_{NDM-5} and *fosA3* from an *Escherichia coli* ST117 strain of retail chicken. *Int J Food Microbiol*, 2022; 382, 109914.
12. Hirabayashi A, Dao TD, Takemura T, et al. A transferable IncC-IncX3 hybrid plasmid cocarrying *bla*_{NDM-4}, *tet(X)*, and *tmexCD3-toprJ3* confers resistance to carbapenem and tigecycline. *mSphere*, 2021; 6, e0059221.
13. Sun YF, Liu DL, Wu YF, et al. Annual summary of global infectious diseases in 2024. *Zoonoses*, 2025; 5, 995.



Supplementary Figure S1. Representation of plasmid p22S130. Coding genes were shown in the circle, based on BRIG (BLAST Ring Image Generator) tool. (A) Plasmid p22S130 was used as the reference genome sequence itself. Individual rings range from 1 (inner ring) to 4 (outer ring): ring 1, backbone; ring 2, GC skew of p22S130; ring 3, GC content of p22S130; ring 4, p22S130 conservation plot; ring 5, pSJTUF15758 conservation plot; ring 6, pC629 conservation plot; ring 7, p0085-NDM conservation plot. (B) Plasmid pSJTUF15758 was used as the reference genome sequence itself. Individual rings range from 1 (inner ring) to 4 (outer ring): ring 1, backbone; ring 2, GC skew of pSJTUF15758; ring 3, GC content of pSJTUF15758; ring 4, pSJTUF15758 conservation plot; ring 5, pC629 conservation plot; ring 6, p0085-NDM conservation plot; ring 7, p22S130 conservation plot.