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LETTER

Prophage mediate the emergence of NDM-13-positive monophasic *Salmonella* Indiana by imprecise excision

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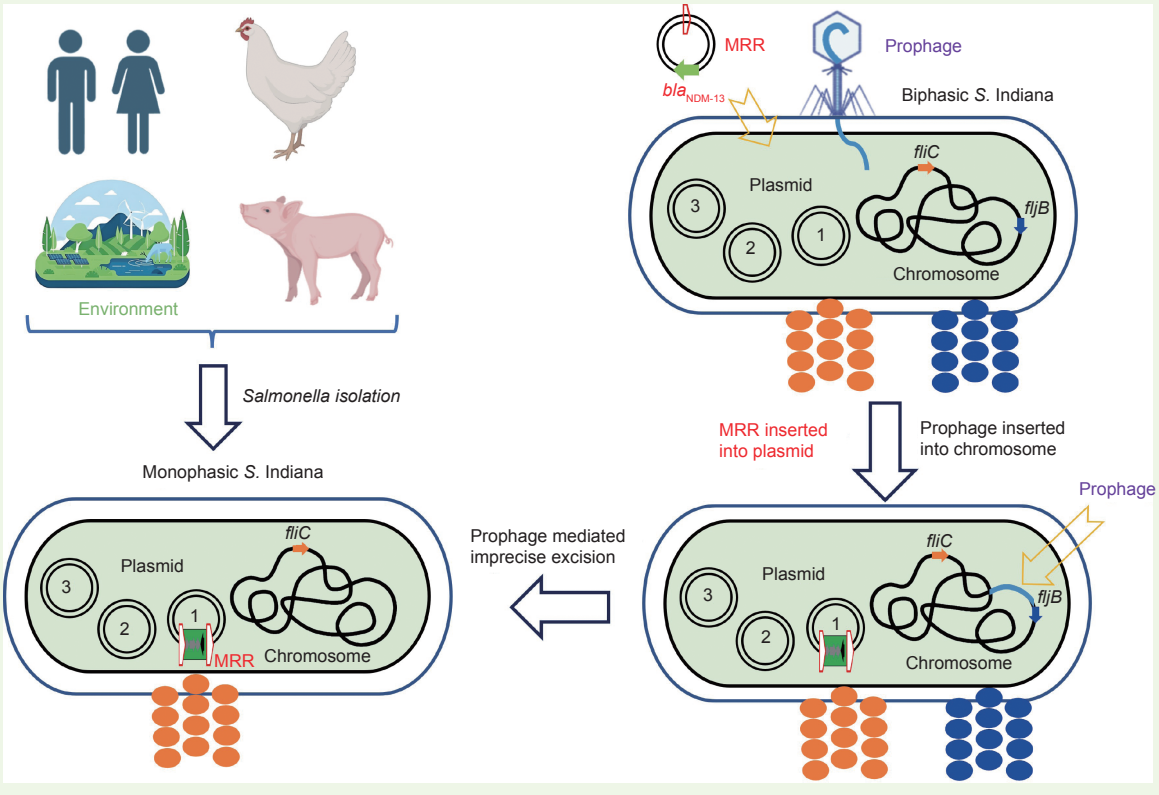
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Highlights

- A monophasic *Salmonella* Indiana variant was isolated from chicken meat in China.
- The variant arose *via* prophage-mediated imprecise excision replacing the *fljAB* operon and adjacent genes with ΔΦSM.
- An IncHI2 plasmid carrying two MRRs including the carbapenem resistant gene bla_{NDM-13} was reported in *Salmonella* for the first time.

Graphical abstract



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Salmonella, a prominent foodborne pathogen, presents a serious threat to both humans and animals, causing severe diseases like typhoid, paratyphoid, and various gastrointestinal conditions, including diarrhea, vomiting, and gastroenteritis (Mead *et al.* 1999; Majowicz *et al.* 2010; Liu *et al.* 2025). Until now, more than 2,600 serovars of *Salmonella* enterica have been identified (Lu *et al.* 2025), among which *S*. Indiana is highly prevalent in poultry, particularly in chicken and turkey (EFSA and ECDC 2020). Since its first isolation from an infant in the United States in 1955, reports of *S*. Indiana infections have been steadily increasing worldwide, with most cases linked to poultry consumption and characterized by gastroenteritis and diarrhea (Bai *et al.* 2015; Chao *et al.* 2017; Du *et al.* 2022). Traditional slide agglutination tests for serotyping have limited ability to distinguish monophasic variants, such as *S*. Typhimurium and monophasic *S*. Typhimurium. Whereas whole-genome sequencing combined with MLST provides a rapid and accurate approach, significantly enhancing epidemiological surveillance and outbreak investigations. Meanwhile, the rise of antimicrobial resistance has intensified public health concerns. Carbapenems, regarded as last-resort antibiotics against multidrug-resistant Gram-negative bacteria, are increasingly compromised by the global dissemination of carbapenemase genes such as NDM. In this study, we examined the prevalence of carbapenem-resistant *Salmonella* in food animals and conducted a comprehensive genomic analysis of NDM-positive *Salmonella* strains.

From April 2021 to December 2022, a total of 179 *Salmonella* spp. strains were isolated from various sources, including food animals (2 pigs and 39 chickens), retail meat (29 pork, 41 chicken meat and 8 chicken carcass), human (44 patients) and the environment (16 chicken slaughterhouse). These strains were collected from 1,748 samples, which included pig feces, chicken feces, chicken intestinal contents, sheep feces, pork, chicken meat, mutton, healthy volunteers, patients and the environment swabs in Anhui Province, Jiangsu Province, Xinjiang Uygur Autonomous Region, and Beijing City of China (Appendix A). Antimicrobial susceptibility testing was performed against 14 antimicrobial agents, including ampicillin, cefazolin, cefotaxime, meropenem, gentamycin, amikacin, streptomycin, tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin, colistin, fosfomycin, and sulfamethoxazole/trimethoprim, using the broth microdilution method or agar dilution method (limited to fosfomycin) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI M100-2022 2022). The presence of carbapenemase genes (bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA-48}) was detected by PCR and sequencing (Poirel *et al.* 2011). Among these isolates, one (0.56%) isolate YZ22MCS8 obtained from retail chicken meat in Yangzhou, Jiangsu Province, China, in March 2022, was confirmed to be carbapenem-resistant and carried the bla_{NDM-13}. None of the other tested carbapenemase genes was detected, consistent with previous reports of the low prevalence of bla_{NDM} in *Salmonella* (Wang *et al.* 2020; Woh *et al.* 2021). Phenotypically, YZ22MCS8 was resistant to meropenem (MIC=8 mg L^{-1}) and exhibited additional resistance to ampicillin, cefazolin, cefotaxime, streptomycin, nalidixic acid, ciprofloxacin, and sulfamethoxazole/trimethoprim (Table 1). Serovar identification using the classic slide agglutination test revealed that the YZ22MCS8 strain exhibited the antigenic formulae of 4,12:z:-. Notably, the phase-2 flagellar antigen was undetectable, suggesting that this strain may be a monophasic variant of serovars, such as *S*. Indiana, *S*. Kiambu, *S*. Koenigstuhl, *S*. Loubomo, *S*. Neftenbach, *S*. Perston and *S*. Shubra.

To further clarify the serovar identity, whole-genome sequencing (WGS) combined with the *Salmonella In Silico* Typing Resource (SISTR) was performed to predict the serovar by analyzing antigen genes and cgMLST gene alleles. The SISTR analysis indicated that YZ22MCS8 possessed the antigenic formulae of 4,12:z:- and was classified as *S*. Indiana based on comparison with 330 core genome MLST alleles. To validate this classification, 20 representative strains available from 5 related serovars were retrieved from public databases for phylogenetic analysis using 3,002 cgMLST alleles. The resulting phylogeny further supported this classification, revealing that YZ22MCS8 clustered closely with *S*. Indiana strains, particularly with strain SAL_HC4751AA (Fig. 1-A). Both strains shared the same core genome sequence type (cgST), cgST229604, indicating a high degree of genetic relatedness. Genome-based *Salmonella* serotyping has been confirmed as an effective approach for predicting *Salmonella* serotypes and identifying novel *Salmonella* variants (Banerji *et al.* 2020). Finally, YZ22MCS8 was identified as a monophasic variant of *S*. Indiana based on genome-based methods.

Homology analysis revealed that, compared to the reference strain *S*. Indiana YZ20MCS6 (GenBank accession number CP098829), YZ22MCS8 had lost the *fljAB* operon fragment and seven surrounding genes. These deleted regions were replaced by an 8.9 kb truncated prophage (ΔΦSM), which is derived from the *S*. Mbandaka prophage (ΦSM). Further comparative genome analysis indicated that the generation mechanism of monophasic *S*. Indiana involved the horizontal acquisition of ΦSM from *S*. Mbandaka. During an imprecise excision event of ΦSM, the *fljAB* operon and its surrounding genes were lost, leaving a ΔΦSM behind (Fig. 1-B). Similar prophage-mediated imprecise excision events have been reported in the emergence of monophasic *S*. Virchow and the acquisition of lysogenic conversion genes from an ancestral host (Wang *et al.* 2021; Boyd and Brüssow 2022). In contrast, the generation of monophasic *S*. Typhimurium typically involves IS*26*/IS*1R*-mediated excision, which also leads to the loss of the *fljAB* operon (Wang *et al.* 2023a).

To further characterize the multidrug-resistant (MDR) isolate, the genome sequences were analyzed for multi-locus sequence typing (MLST), antimicrobial resistance genes, and chromosomal mutations using MLST and ResFinder (https://genomicepidemiology.org/services/). MLST analysis revealed that YZ22MCS8 belonged to sequence type ST17. Its genome consisted of one chromosome (4,721,124 bp) and 3 plasmids, designated pYUYZMCS8-1 to pYUYZMCS8-3 (Table 1). The genomic sequences have been deposited into the European Nucleotide Archive database under the accession number PRJEB88601. Analysis of antimicrobial

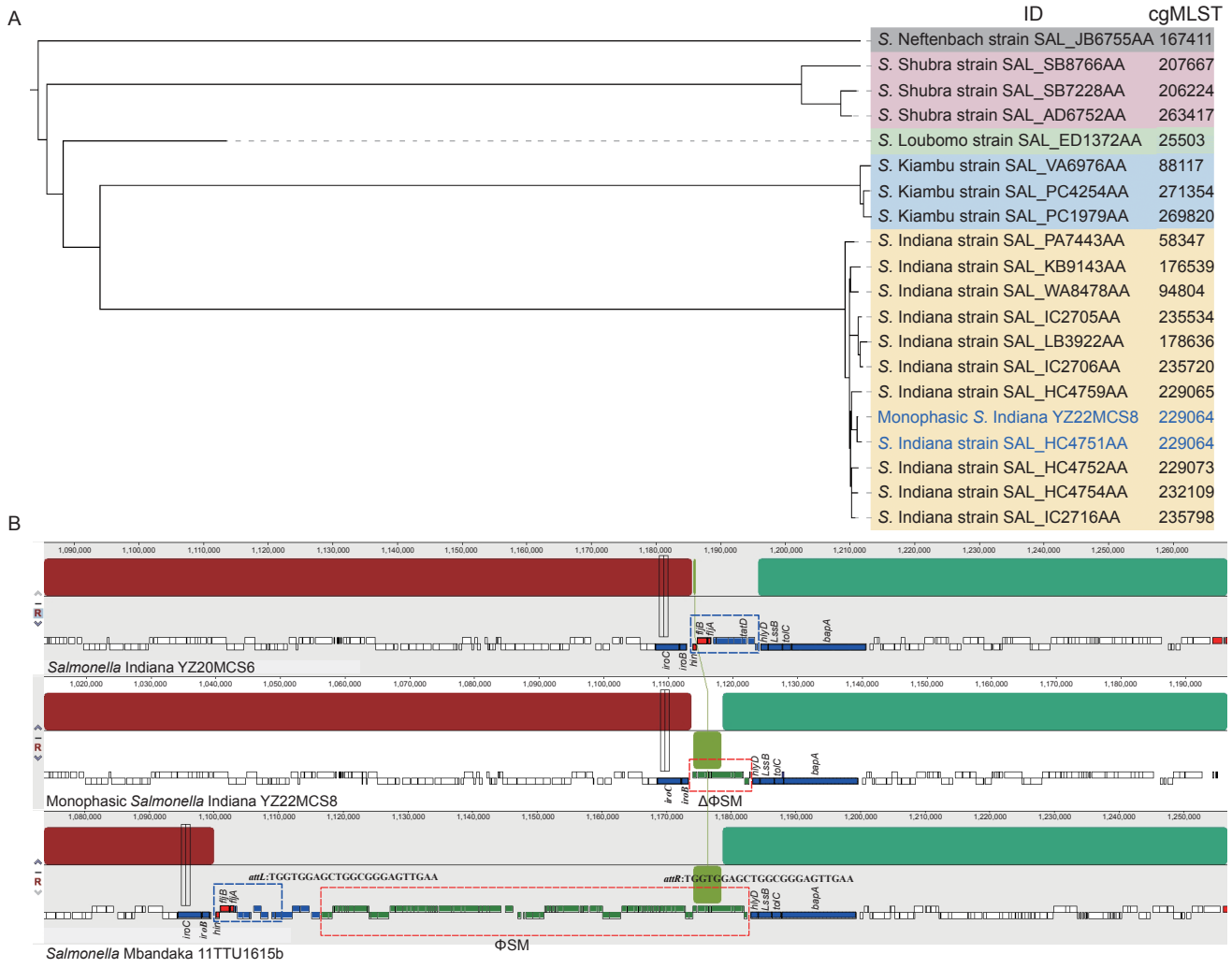


Fig. 1 Phylogenetic and comparative genomic identification of a monophasic *Salmonella* Indiana. A, phylogenetic relationship of the 20 *Salmonella* isolates based on 3,002 cgMLST alleles. A neighbor-joining tree is constructed based on the core genome MLST alleles of 20 *Salmonella* strains. The strain YZ22MCS8 isolated in this study is labelled in blue color. The cgSTs are displayed on the right side of the picture. B, comparative genome analysis of the *fljAB* operon in *S*. Mbandaka, *S*. Indiana and its monophasic variant. MAUVE software was used to compare the sequence of the *fljAB* operon and its neighboring regions in monophasic *S*. Indiana YZ22MCS8 with the corresponding sequences in *S*. Indiana YZ20MCS6 and *S*. Mbandaka 11TTU1615b. The red rectangle represents the prophage ΦSM and truncated prophage ΔΦSM in the genome, whereas the blue rectangle represents the *fljAB* operon and surrounding genes absent in monophasic *S*. Indiana YZ22MCS8.

resistance genes revealed that all identified genes, including *bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *bla*$_{NDM-13}$, *aph(3′)-IIa*, *aadA22*, *strA/B*, *sul2*, *mph*(A), and *erm*(B), were co-located on the largest plasmid pYUYZMCS8-1. Mutations within *gyrA* (S83F and D87N) and *parC* (S80R) were identified to be responsible for resistance to nalidixic acid and ciprofloxacin. The genotype was consistent with their antimicrobial patterns. Plasmid pYUYZMCS8-1 had a size of 225,517 bp, and was classified as IncHI2/ST3 plasmid. It was similar in organization to other IncHI2 plasmids, including regions for replication, multi-resistance, conjugal transfer, maintenance, and stability. However, compared to plasmid p8C59-NDM, the 45.4-kb segment corresponded to partial conjugative transfer region, including *traJGIH* and *trhRYFHG* was absent in pYUYZMCS8-1 due to IS*26* mediated transposition (Appendix B-a). It may explain the failure of this *bla*$_{NDM-13}$-carrying plasmid pYUYZMCS8-1 to *Escherichia coli* C600 *via* conjugation. In contrast, the plasmid pYUYZMCS8-1 could be successfully introduced into *S*. Typhimurium by electroporation.

Interestingly, in addition to the typical multi-resistance region (MRR) containing *bla*$_{TEM-1B}$, *bla*$_{CTX-M-65}$, *strA*, *strB* and *sul2*, an additional 51.3 kb MRR was identified inserted into the *dam* gene, containing 5 antimicrobial resistance genes: *aph(3′)-IIa*, *aadA22*, *bla*$_{NDM-13}$, *mph*(A), and *erm*(B) (Appendix B-b). Comparative genomic analysis revealed that both ends of this MRR contain IS*26* insertion sequences in the same orientation, indicating that the region was inserted into the *dam* gene *via* IS*26*-mediated composite transposon transposition, resulting in truncation of the *dam* gene. Further BLASTn analysis showed that the *bla*$_{NDM-13}$ gene was located on a gene background containing IS*26*-Δ*ble-aph(3′)-IIa*-

Table 1 Characteristics of *bla*$_{NDM}$-carrying *Salmonella enterica* serovar Indiana YZ22MCS8 in this study

Strain	YZ22MCS8
Source	Chicken meat
MLST	ST17
Mutations	*gyrA* (S83F, D87N), *parC* (S80R)
Resistance genes	*bla*$_{TEM-1B}$, *bla*$_{CTX-M-65}$, *bla*$_{NDM-13}$, *aph(3´)-Ia*, *aadA22*, *strA*/*B*, *sul2*, *mph*(A), *erm*(B)
Plasmids (size)	pYUYZMCS8-1 (225,517 bp)
	pYUYZMCS8-2 (4,018 bp)
	pYUYZMCS8-3 (3,373 bp)
Minimum inhibitory concentration (mg L^{-1})	
Ampicillin	>128
Cefazolin	>128
Cefotaxime	>128
Meropenem	8
Gentamycin	0.5
Amikacin	1
Streptomycin	>256
Tetracycline	2
Chloramphenicol	4
Nalidixic acid	>256
Ciprofloxacin	8
Colistin	0.5
Fosfomycin	2
Sulfamethoxazole/ Trimethoprim	>64

ΔIS*50-tat-trpF-ble*$_{MBL}$-*bla*$_{NDM-13}$-ΔIS*Aba125*-IS*1294*. This identical gene background was previously identified in the IncFII plasmid pYZLc23-1_NDM-13_96k (GenBank accession number CP123269), which originated from *E. coli* isolated from chickens. In contrast, a distinct genetic background for the *bla*$_{NDM}$ gene was observed in other IncHI2 plasmids from *Salmonella*, such as pHN2IYZ1903 and pSA17155 (Appendix B-c). IncHI2 plasmids are widely prevalent among dominant *Salmonella* serovars (e.g., *S*. Indiana, *S*. Typhimurium and *S*. 4,[5],12:i:-) and play a crucial role in the dissemination of antimicrobial resistance genes, including *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-55}$, and *qnrS1* (Du *et al*. 2022; Wang *et al*. 2023b). Previous studies have demonstrated that IncX3, IncF, and IncA/C plasmids are key drivers of the global spread of the *bla*$_{NDM}$ gene within Enterobacteriaceae (Wu *et al*. 2019). However, recent research showed that the IncHI2/ST3 plasmid has surpassed IncX3 as the primary plasmid vector for *bla*$_{NDM-5}$ transmission in some farms (He *et al*. 2023). Therefore, the presence of the *bla*$_{NDM-13}$ resistance gene on IncHI2 plasmids poses a potential risk for the further dissemination of carbapenem resistance.

In this study, we report a novel monophasic *S*. Indiana isolated from retail chicken meat in China. The variant was generated through prophage-mediated imprecise excision, which led to the deletion of the *fljAB* operon and surrounding sequences, resulting in the generation of monophasic *S*. Indiana. Although a low prevalence of *bla*$_{NDM}$ was observed among *Salmonella* isolates from various sources, the presence of the pandemic IncHI2 plasmid poses a significant risk for the rapid and global dissemination of *bla*$_{NDM}$ and other resistance genes in *Salmonella* and other Enterobacteriaceae species. The *bla*$_{NDM-13}$ resistance gene is reported in *Salmonella* for the first time in our study. Continued surveillance of these critical resistance genes and monophasic variants in both human and environmental samples is warranted.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.10.021

References

Bai L, Lan R, Zhang X, Cui S, Xu J, Guo Y, Li F, Zhang D. 2015. Prevalence of *Salmonella* isolates from chicken and pig slaughterhouses and emergence of ciprofloxacin and cefotaxime co-resistant *S. enterica* serovar Indiana in Henan, China. *PLoS ONE*, **10**, e0144532.

Banerji S, Simon S, Tille A, Fruth A, Flieger A. 2020. Genome-based *Salmonella* serotyping as the new gold standard. *Scientific Reports*, **10**, 4333.

Boyd E F, Brüssow H. 2002. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends in Microbiology*, **10**, 521–529.

Chao G, Wang C, Wu T, Zhang X, Chen J, Qi X, Cao Y, Wu Y, Jiao X. 2017. Molecular epidemiology and antibiotic resistance phenotypes and genotypes of salmonellae from food supply chains in China. *Food Control*, **77**, 32–40.

CLSI M100-2022. 2022. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. Clinical and Laboratory Standards Institute, Wayne.

Du P, Liu X, Liu Y, Li R, Lu X, Cui S, Wu Y, Fanning S, Bai L. 2022. Dynamics of antimicrobial resistance and genomic epidemiology of multidrug-resistant *Salmonella enterica* serovar Indiana ST17 from 2006 to 2017 in China. *mSystems*, **7**, e0025322.

EFSA and ECDC (European Food Safety Authority & European Centre for Disease Prevention and Control). 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. *EFSA Journal*, **18**, e06007.

He W, Gao M, Lv L, Wang J, Cai Z, Bai Y, Gao X, Gao G, Pu W, Jiao Y, Wan M, Song Q, Chen S, Liu J H. 2023. Persistence and molecular epidemiology of *bla*$_{NDM}$-positive Gram-negative bacteria in three broiler farms: A longitudinal study (2015–2021). *Journal of Hazardous Materials*, **446**, 130725.

Liu G, Lü X, Tian Q, Zhang W, Yi F, Zhang Y, Yu S. 2025. Deletion of *Salmonella* pathogenicity islands SPI-1, 2 and 3 induces substantial morphological and metabolic alternation and protective immune potential. *Journal of Integrative Agriculture*,

24, 272–289.

Lu J, Wu H, Wu S, Wang S, Fan H, Ruan H, Qiao J, Caiyin Q, Wen M. 2025. *Salmonella*: Infection mechanism and control strategies. *Microbiological Research*, **292**, 128013.

Majowicz S E, Musto J, Scallan E, Angulo F J, Kirk M, O'Brien S J, Jones T F, Fazil A, Hoekstra R M, ICEDBIS (International Collaboration on Enteric Disease 'Burden of Illness' Studies). 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*, **50**, 882–889.

Mead P S, Slutsker L, Dietz V, McCaig L F, Bresee J S, Shapiro C, Griffin P M, Tauxe R V. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases*, **5**, 607–625.

Poirel L, Walsh T R, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*, **70**, 119–123.

Wang Z, Gu D, Hong Y, Hu Y, Gu J, Tang Y, Zhou X, Zhang Y, Jiao X, Li Q. 2023a. Microevolution of *Salmonella* 4,[5],12:i:-derived from *Salmonella enterica* serovar Typhimurium through complicated transpositions. *Cell Reports*, **42**, 113227.

Wang Z, He J, Li Q, Tang Y, Wang J, Pan Z, Chen X, Jiao X. 2020. First detection of NDM-5-positive *Salmonella enterica* serovar Typhimurium isolated from retail pork in China. *Microbial Drug Resistance*, **26**, 434–437.

Wang Z, Jiang Z, Xu H, Jiao X, Li Q. 2023b. Prevalence and molecular characterization of *mcr-1*-positive foodborne ST34-*Salmonella* isolates in China. *Microbiological Research*, **274**, 127441.

Wang Z, Xu H, Chu C, Tang Y, Li Q, Jiao X. 2021. Genomic identification of multidrug-resistant *Salmonella* Virchow monophasic variant causing human septic arthritis. *Pathogens*, **10**, 536.

Woh P Y, Yeung M P S, Goggins W B 3rd, Lo N, Wong K T, Chow V, Chau K Y, Fung K, Chen Z, Ip M. 2021. Genomic epidemiology of multidrug-resistant nontyphoidal *Salmonella* in young children hospitalized for gastroenteritis. *Microbiology Spectrum*, **9**, e0024821.

Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. 2019. NDM metallo-β-lactamases and their bacterial producers in health care settings. *Clinical Microbiology Reviews*, **32**, e00115–18.

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