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# Prevalence and dissemination of *mcr-9.1*-producing non-typhoidal *Salmonella* strains from diarrhea patients throughout China during 2010–2020

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## Abstract

The emergence of mobilized colistin resistance (*mcr*) genes has raised significant concerns as they pose a public health issue. The prevalence of *mcr* genes, particularly the newly discovered *mcr-9* gene, in non-typhoidal *Salmonella* (NTS) isolates remains unclear. We characterized *mcr-9.1*-producing NTS isolates from China. Among 7,106 NTS isolates from diarrhea cases in 32 provinces during 2010–2020, 11 *mcr-9.1*-producing isolates were identified and were all not resistant to colistin. Five isolates belonged to *Salmonella* Thompson and sequence type (ST) 26, two belonged to *Salmonella* Typhimurium and ST34, two belonged to *Salmonella* Typhimurium and ST36, and two belonged to *Salmonella* 1,4,[5],12:i:- and ST34. Plasmids harboring *mcr-9.1* tended to possess the IncHI2 backbone and were ~300 kb long. All *mcr-9.1* genes shared the same flanking sequence, *rcnR-rcnA-pcoS-IS903-mcr-9.1-wbuC*. According to the NCBI data, we found that NTS serves as the primary host of *mcr-9.1*, although the prevalence of specific serotypes differed between domestic and international settings. Notably, most data came from developed countries, such as the USA. *mcr-9.1* tended to be transferred as a gene cassette or to be mobilized by a conjugational plasmid in multiple bacteria across humans, animals, and the environment. Furthermore, *mcr-9.1* frequently co-existed and was co-transferred with various genes encoding resistance to first-line drugs, reducing the effectiveness of available therapeutic options. In summary, although *mcr-9* does not mediate colistin resistance, it can silently spread with some genes encoding resistance to first-line drugs, and therefore warrants research attention.

**Keywords** Colistin resistance, *mcr* genes, *mcr-9.1*, *Salmonella*

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## Introduction

In the context of antimicrobial resistance gene (ARG) dissemination, the concept of “One Health” which underlines the interconnectivity of human, animal, and ecosystem health, becomes paramount. This concept recognizes the crucial role that multidrug-resistant (MDR) bacteria and ARGs play within this interconnected framework. Non-typhoidal *Salmonella* (NTS) are gram-negative pathogenic bacteria of the family *Enterobacteriaceae*, genus *Salmonella*, species *Salmonella enterica* and *Salmonella enterica* subsp. *enterica*. According to the surface structures expressed on the bacterial surface, including lipopolysaccharide, the flagella, and capsular polysaccharides, *Salmonella enterica* subsp. *enterica* has been classified into more than 2,600 serovars [1]. Serovars Typhi and Paratyphi, known as typhoidal serovars, can only infect humans and cause severe systemic disease. Most NTS serovars, including Enteritidis, Typhimurium, 1,4,[5],12:i:-, and Thompson, have a broad host range and cause self-limiting gastroenteritis in humans and animals [2, 3].

The MDR phenotype in NTS was initially identified in the UK in the early 1980s in isolates demonstrating resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Subsequently, reports of resistance to fluoroquinolones (*qnrB*, 24.43%, 1,736/7,106) emerged shortly after their introduction. By the mid-1980s, it had become evident that NTS possessed resistance to extended-spectrum cephalosporins, typically mediated by extended-spectrum  $\beta$ -lactamases (ESBLs) (*bla*<sub>CTX-M</sub>, 14.93%, 1,061/7,106) or AmpC-type beta-lactamases (*bla*<sub>CMY-2</sub>, 1.97%, 140/7,106) (unpublished data). In recent years, increasing numbers of reports have noted the emergence of extensively drug-resistant NTS isolates, with strains exhibiting MDR phenotypes [4]. These findings underscore the escalating global public health challenge of antimicrobial resistance.

$\beta$ -lactams (conferred by *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> gene, and so on [5]), aminoglycosides (conferred by the *aac*(6′)-*Iaa* gene), and fluoroquinolones (conferred by *qnr*, *aac*(6′)-*Ib-cr*, *qepA* [6], and *oqxAB* [7]) are frequently used to treat NTS infection in humans and animals [8]. However, the misuse and abuse of antibiotics have led to the development and spread of MDR NTS. The majority of NTS infections are associated with food-producing animals and contaminated vegetables, fruits, and other plant products [9]. The above ARGs co-exist on transferable plasmids and can be transmitted from animal-derived foods to humans [10, 11].

The first plasmid-mediated colistin resistance gene *mcr-1* was identified in late 2015 [12]. In 2019, a new *mcr* gene, *mcr-9.1*, was identified in an MDR *Salmonella* Typhimurium strain isolated in 2010 from a patient's

stool sample in the USA [13]. Unlike *mcr-1*, *mcr-9.1* seems to only reduce sensitivity to colistin, rather than conferring resistance to colistin. Therefore, it is conceivable that the *mcr-9.1* gene may circulate silently. However, the clinical use of colistin may trigger high *mcr-9.1* expression, resulting in resistance and accelerating its transmission and dissemination [14]. The *mcr-9.1* gene was widely disseminated among *Enterobacteriaceae* strains isolated from human, animal, food, and environmental samples from 21 countries spanning six continents, including but not limited to South Africa [15], the USA [16], Brazil [17], South Korea [8], Italy [18], and China [19]. The sources of isolation have expanded from patients to healthy individuals, livestock, animal-derived foods, pets, and the environment [20]. Furthermore, bacterial hosts are no longer limited to NTS, but also include *Escherichia coli*, *Enterobacter cloacae* complex [21, 22], *Morganella morganii*, *Enterobacter hormaechei* [23], *Cronobacter sakazakii* [24], and *Enterobacter kobei* [20]. These findings underscore the wide dissemination and potential risks associated with *mcr-9.1*.

We investigated the prevalence and dissemination of *mcr-9.1*-producing NTS strains in Chinese hospitals over a decade to paint a comprehensive picture of the distribution and transmission of *mcr-9.1* in China.

## Results

### Identification and characterization of *mcr-9.1*-producing isolates

Among the 7,106 NTS isolates in this study, 11 isolates carried *mcr-9.1*, five of which were isolated in 2017, three in 2018, and one in 2015, 2016, and 2019, respectively. These isolates were collected from different provinces across China, including Sichuan, Jiangsu, Anhui, Henan, Jilin, Shandong, Guangdong, Zhejiang, Fujian, and Heilongjiang. Despite the diversity in isolation years and geographic locations, all 11 strains harbored the *mcr-9.1* gene, indicating its widespread presence within NTS populations across China. To profile the resistance of the *mcr-9.1*-positive isolates in this study, Antimicrobial susceptibility test (AST) were conducted on the 11 isolates. All isolates (11/11, 100%) were resistant to ampicillin, cefotaxime, and ceftazidime, and 90.9% (10/11) were resistant to tetracycline. Several isolates were resistant to amikacin (18.2%, 2/11), ciprofloxacin (27.3%, 3/11), gentamicin (54.5%, 6/11), florfenicol (36.4%, 4/11), chloramphenicol (63.6%, 7/11) and aztreonam (18.2%, 2/11); however, all isolates were susceptible to colistin (MICs  $\leq 1$   $\mu$ g/ml) (Additional file 1). Molecular typing of these isolates showed a diverse distribution, with 5/11 belonging to *Salmonella* Thompson and sequence type (ST) 26, 2/11 being *Salmonella* Typhimurium with ST34, 2/11 being *Salmonella* Typhimurium with ST36, and

2/11 being *Salmonella* 1,4, [5],12:i:- with ST34. Further exploration into the associated ARGs revealed a range of 5 to 25 ARGs per isolate, with an average of 12.6 ARGs (Additional file 2). This suggests a significant level of antibiotic resistance within these strains.

#### Analysis of *mcr-9.1*-producing plasmids and the genetic environment of *mcr-9.1* genes

We chose long and short read sequencing illustrated that the *mcr-9.1* genes of eight isolates located in plasmids. Four out of eight were located on IncHI2A/IncHI2 plasmids, three on IncHI2A/IncHI2/IncQ1 plasmids, and one on IncHI2A/IncHI2/IncY plasmids. Notably, the latter two types of hybrid plasmids were identified as carriers of *mcr-9.1* for the first time. Genetic environment analysis revealed that all *mcr-9.1* genes in this study share a common flanking sequence, *rcnR-rcnA-pcoS-IS903-mcr-9.1-wbuC*, implying possible horizontal transfer of this resistance determinant.

To characterize *mcr-9.1*-encoding plasmids, we examined 142 *mcr-9.1*-positive plasmids, including 134 *mcr-9.1*-encoding plasmids downloaded from the RefSeq database on January 19, 2022 and the eight *mcr-9.1*-encoding plasmids obtained from hybrid assemblies in our study. Plasmid length was not related to the bacterial host species (Fig. 1A). Upon examining the relationship between the number of ARGs and incompatibility (Inc) groups, we found that the number of ARGs ranged between 7 and 17, and the number of Inc ranged between 0 and 3 (Fig. 1B). Additionally, *mcr-9.1*-encoding plasmids predominantly incorporated two Inc. The IncHI2/IncHI2A plasmid was the most widespread, accounting for 117 out of 142 instances. This information could be vital for future studies and strategies aiming at combating antibiotic resistance.

To further characterize the backbones and profiles of the 117 IncHI2/IncHI2A plasmids, a clustering tree based on Mash distance was constructed, and their bacterial hosts and lengths were analyzed. The results revealed a rich sequence diversity among the IncHI2/IncHI2A plasmids. Plasmid length varied quite significantly, from 50,622 bp to 477,340 bp, with an average of 298,141 bp. *Enterobacter* was the most common host (73 out of 117), followed by *Salmonella* (21 out of 117) (Fig. 1C). This distribution of bacterial hosts adds another layer of complexity to our understanding of these plasmids.

To outline the genetic environment of the *mcr-9.1* gene and understand the mechanism of spread, we used the flanking sequence of *mcr-9.1* in pZJ-S162 (upstream: 100,000 bp, downstream: ending) as a query sequence in the NCBI BLASTn tool. The most similar plasmid identified was p628. Using p628 as a reference sequence and the plasmids in our study as queries, we performed a

series of alignments and visualized them using BLAST Ring Image Generator (BRIG) (Fig. 2) (Version 0.95). p628 exhibited a high degree of sequence identity with pHL-19S4. Interestingly, the gene cassette *rcnR-rcnA-pcoS-IS903-mcr-9.1-wbuC* was present in all plasmids in this study [20]. A conjugation transfer test confirmed that the backbone plasmid can indeed be transferred from *Salmonella* to recipient bacteria, also, the transformants were all not resistant to colistin (MICs  $\leq 0.5$   $\mu\text{g/ml}$ ) (Additional file 3). This ability to transfer plasmids plays a crucial role in the spread of resistance genes.

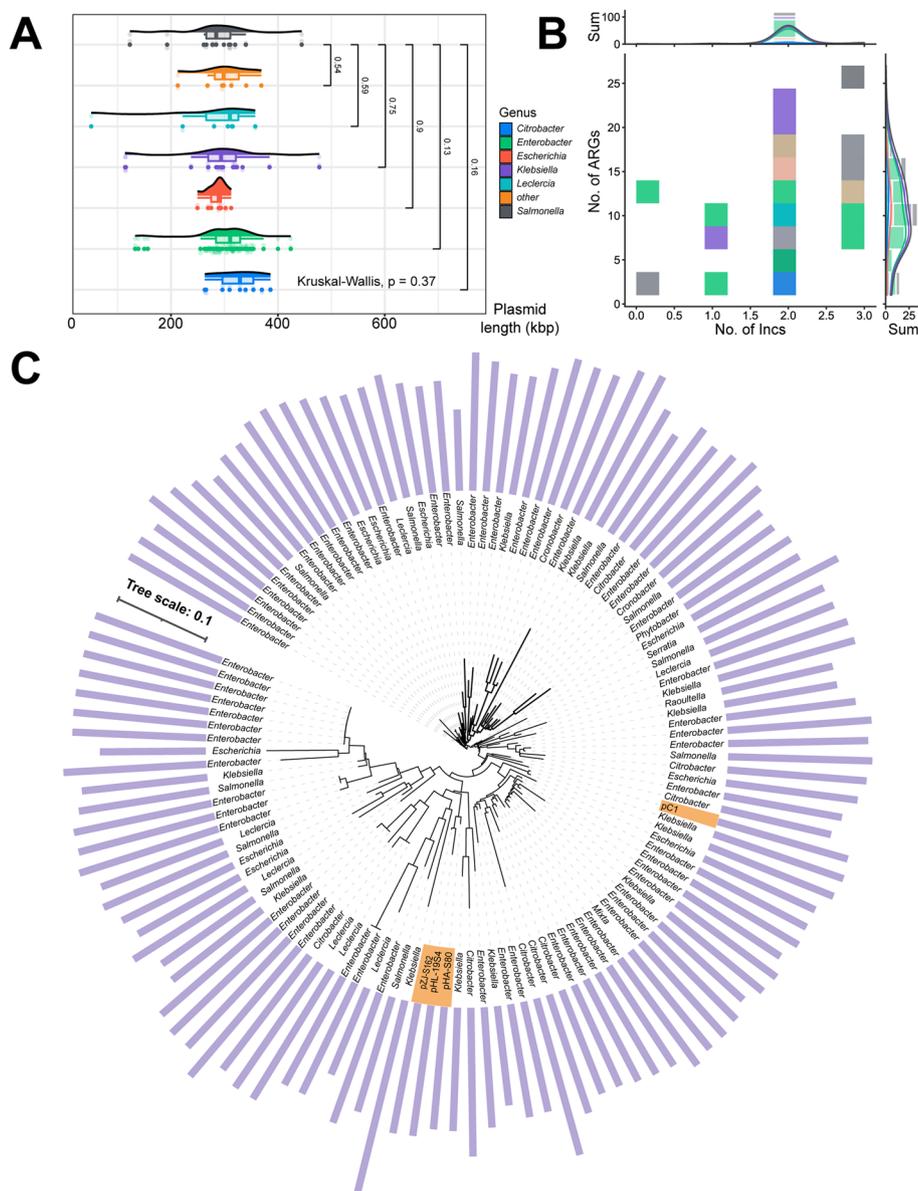
#### Co-existence and co-transfer of *mcr-9.1* with other resistance genes

The co-existence and co-transfer of *mcr-9.1* with clinically relevant resistance genes such as ESBL [11] and PMQR genes, and occasionally, *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub> [25], and *tmexCD2-toprJ2* [23], have been closely examined in previous studies. We systematically analyzed plasmids carrying *mcr-9.1* in the NCBI database. By examining the correlation between the replicon type of the plasmid and the *mcr-9.1* gene, we found that all plasmids carrying *mcr-9.1* contain the IncHI2 type plasmid backbone. It has been reported that IncHI2 plasmids serve as the transmission vector for various antibiotic-resistance genes [26], including those encoding resistance against  $\beta$ -lactams, quinolones, and aminoglycosides. Further analysis of the correlations among resistance genes revealed that *mcr-9.1* was the most strongly correlated with tetracycline resistance genes, such as *tet(D)*, followed by quinolone (*qnrA1*), sulfonamide (*sul1*), trimethoprim (*dfpA19*), macrolide (*mph(A)*), ESBLs (*bla*<sub>TEM-1B</sub>, *bla*<sub>CTX-M $\beta$</sub> , *bla*<sub>SHV-12</sub>), phenicol (*catA2*), and aminoglycoside (*aac(6')-IIc*) (Fig. 3).

#### Global prevalence and epidemiological traits of *mcr-9.1*-encoding assemblies

A total of 2,623 *mcr-9.1*-encoding assemblies were retrieved from the NCBI pathogen database as of September 18, 2022. Figure 4A shows the prevalence and distribution of these assemblies across countries. The USA accounted for most assemblies (1,382), followed by the UK with 304 assemblies, Australia with 277 assemblies, and China with 205 assemblies. Most assemblies were reported from economically developed regions.

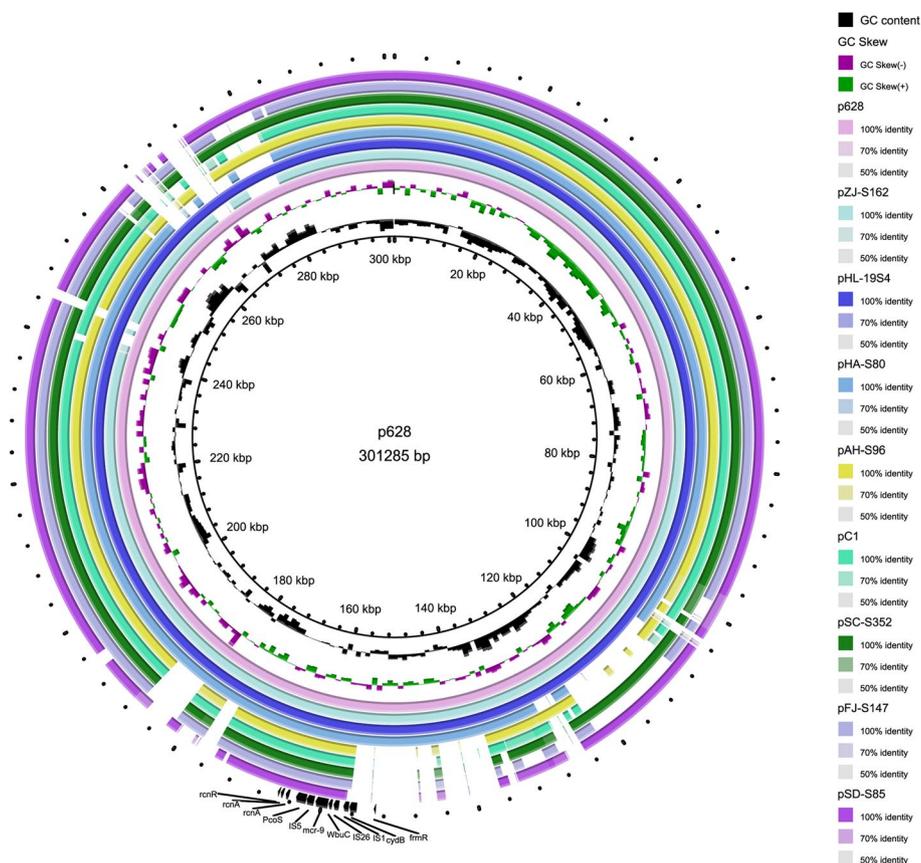
To analyze the isolation dates, geographical distribution, and bacterial hosts and origin of these hosts, a subset of 865 assemblies with unambiguous above four kinds of data was selected for further analysis. We observed that the number of assembly datasets increased over recent years, a trend likely associated with the advancements in whole-genome sequencing technology (Fig. 5). Of which, *mcr-9.1* gene was prevalent across from 29



**Fig. 1** Plasmids harboring *mcr-9.1* found in this study and the NCBI database. **A** Relationship between plasmid length and genus. **B** Relationship between the number of antimicrobial resistance genes and replicon types. **C** Clustering tree of 117 IncHI2/IncHI2A plasmids based on Mash distance

countries, the USA, the UK, and China were at the forefront of countries reporting the assemblies. *Salmonella enterica* has emerged as the primary bacterial host for *mcr-9.1* genes, followed by *Enterobacter*, *E. coli*, *Shigella*, *Citrobacter freundii*, *Cronobacter*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*. Turkeys (348/865), humans (255/865), and chickens (120/865) were identified as the principal bacterial host sources, *mcr-9.1* gene is also present in environment, swine, food, and other sources. (Fig. 4B).

To identify and analyze the dominant serotypes of *mcr-9.1*-producing NTS, the serotypes of the assemblies in the NCBI pathogen database were predicted. Serotypes Typhimurium and 1,4,[5],12:i:- (448/1,435), Saint Paul (258/1,435), and Heidelberg (191/1,435) were among the most prevalent globally. Among our isolates, we identified three predominant serotypes: Thompson (5/11), Typhimurium (4/11), and 1,4, [5],12:i:- (2/11). These serotypes are currently the most popular and dominant; hence, we speculate that these predominant NTS



**Fig. 2** Alignment of *mcr-9.1*-encoding plasmids in this study

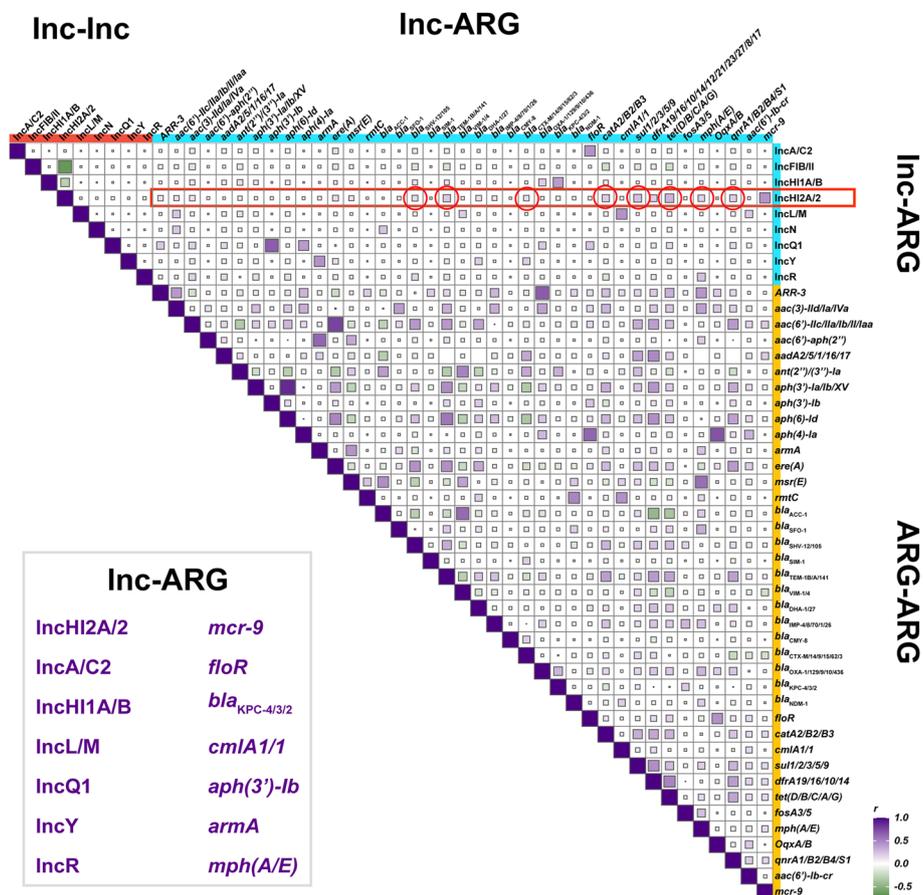
serotypes are likely to be the main bacterial hosts for *mcr-9.1* [27].

**Discussion**

The existence and spread of bacterial resistance pose a major public health threat in the twenty-first century. It makes common infections potentially more difficult to treat, leading to an increased risk of death. Additionally, developing new and effective antibiotics is expensive as well as time-consuming. The emergence of plasmid-mediated colistin resistance exacerbates this situation [28]. Colistin, which is used to treat MDR gram-negative bacterial infections, may lose its effectiveness due to the existence and spread of *mcr* genes [12]. NTS can infect food animals such as pigs and chickens, as well as humans, intercontinental spreading and proliferating among animals, humans, and the environment through foodborne transmission and contaminated water [29]. Although infection with NTS often results in self-limiting disease, such as diarrhea, in humans and the mortality rate is not high, it can cause severe disease, even death, in children or immune-compromised individuals

[30]. While all risk factors alone constitute a significant public health threat, when combined, they can undoubtedly inflict severe damage to global health.

Multiple studies have shown that *mcr-9.1* does not confer colistin resistance to bacterial strains, but rather reduces their sensitivity to colistin; however, the silent circulation of *mcr-9.1* in the environment warrants close attention [13, 31]. Genome assembly results in the NCBI database show that *mcr-9.1* is the most prevalent among *mcr* genes, even surpassing the initially discovered *mcr-1* gene. *mcr-9.1* is highly prevalent in the USA and Australia, whereas *mcr-1* is more prevalent in China [32]. Unfortunately, Chinese researchers often overlook newly discovered *mcr* variants, such as *mcr-9.1/10*, during ARGs screening. Therefore, we speculate that the prevalence of *mcr-9.1* may be underestimated, particularly in China. However, according to NCBI genome data, the prevalence of *mcr-9.1* is the highest in NTS. Thus, NTS seems to be the dominant bacterial host for *mcr-9.1*, and we should be especially vigilant about silent *mcr-9.1* transmission in NTS. More importantly, we found that the flanking sequence of *mcr-9.1*



**Fig. 3** Coexistence of antimicrobial resistance genes (ARGs) and plasmid Inc-types. Red boxes and circles indicate the coexistence of ARGs, plasmid Inc-types, or ARGs and plasmid Inc-types. The coexistence of ARGs and plasmid Inc-types is summarized in the bottom left corner

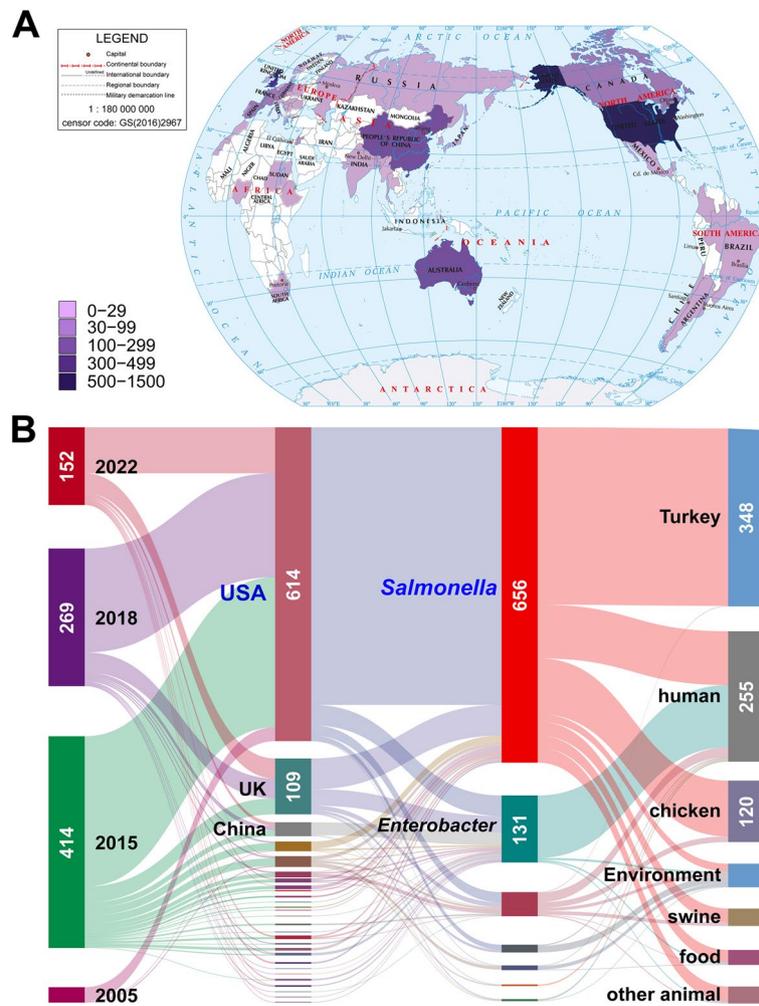
is highly conserved, consistently showing the *rcnR-rcnA-pcoS-IS903-mcr-9.1-wbuC* pattern [20].

Same as *mcr-1*, the *mcr-9.1* gene is generally located on IncHI2 plasmids, which are the most prevalent in *mcr-1*- and *mcr-9.1*-producing NTS and approximately 300 kb in length. The co-adaptive evolution of large plasmid IncHI2 and NTS improved the stability of the plasmid in bacteria [33]. These plasmids encode multidrug resistance. Although *mcr-9.1* does not induce colistin resistance in bacterial strains, it often coexists with genes that cause resistance to commonly used treatment drugs, such as ESBLs (*bla*<sub>TEM-1B</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>SHV-12</sub>) [11], macrolide (*mph(A)*) and PMQR(*qnrA1*) [25] genes [34]. Also, severe residual antibiotics in the environment, for example, tetracycline (*tet(D)*) and sulfonamide (*sul1*). The pattern of coexistence of resistance genes on plasmid IncHI2 is also the same as that of *mcr-1* genes [35].

Therefore, even without using colistin to treat NTS infections, the selective pressure from commonly used drugs may still promote the spread of plasmids carrying *mcr-9.1*, the transfer of a single plasmid amongst

bacterial strains can result in the simultaneous transfer of multiple resistance genes. This increases the risk of propagation of these resistance genes, posing a significant public health threat.

In this study, we comprehensively surveyed the prevalence and transmission of NTS strains that harbor the *mcr-9.1* gene in Chinese hospitals over the last decade. We identified 11/7,106 isolates carrying *mcr-9.1*, all of which displayed multidrug resistance but remained sensitive to colistin, the last-resort antibiotic. We found varied distributions of *Salmonella* serotypes and STs. To our knowledge, *mcr-9.1* has only been reported in serotypes Thompson, Minnesota [36], Senftenberg [37], and Typhimurium [19]. This study is the first to demonstrate the existence of *mcr-9.1* in NTS serotype 1,4,[5],12:i:-. This is the most extensive report on *mcr-9.1*-producing NTS in China to date. Previous investigations only detailed the epidemiological characteristics of one or two *mcr-9.1*-producing NTS strains [19]. Additionally, this study identified novel hybrid



**Fig. 4** Epidemic features of isolates harboring the *mcr-9.1* gene. **A** Geographical distribution of isolates harboring *mcr-9.1* in this study and the NCBI database. **B** Sankey diagram combining the isolating year, isolating country, genus of bacteria host, and sources of isolation. Numbers of isolates are indicated in white font

plasmids carrying *mcr-9.1*, suggesting a continuous expansion of *mcr-9.1* plasmids.

### Conclusion

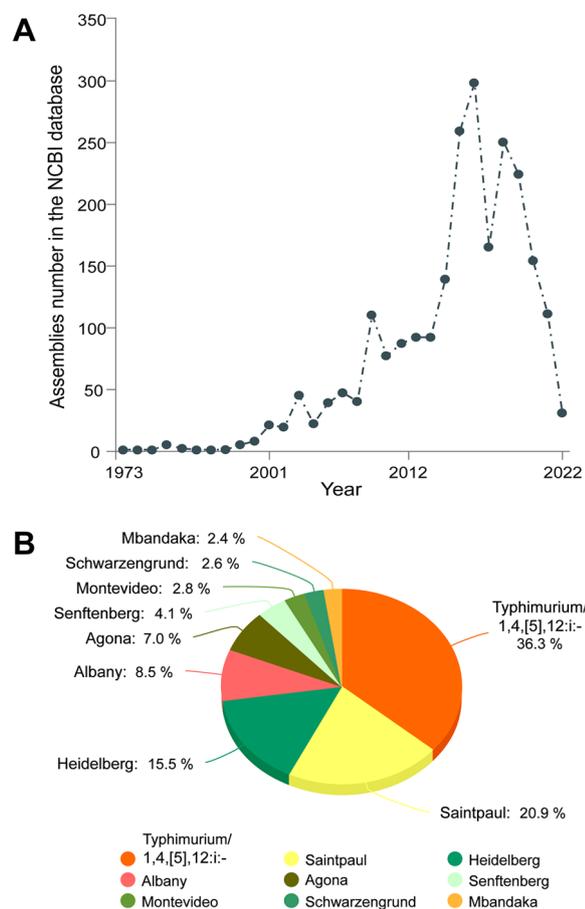
The prevalence of *mcr-9*-positive NTS isolates in China appears to be relatively low. Additionally, the level of resistance conferred by *mcr-9* is not notably high. It is noteworthy that the *mcr-9* gene can propagate through conservative flanking sequences, allowing for silent dissemination. On a global scale, the presence of *mcr-9* is primarily concentrated in specific *Salmonella* serovars, including Typhimurium, 1,4,[5],12:i:-, Saint Paul, and Heidelberg. In contrast, in China, the prevalence of *mcr-9*-positive NTS strains is mainly restricted to serovars Typhimurium, 1,4,[5],12:i:-, and Thompson. Our findings underscore the imperative for continuous surveillance and call for more research on *mcr-9.1* gene dynamics,

particularly considering its potential co-transfer under antimicrobial pressure. Future efforts should focus on multidisciplinary approaches, integrating human health, animal health, and environmental factors to effectively curtail the spread of antibiotic resistance.

### Material and methods

#### Source of NTS's data, screening of ARGs and Antimicrobial susceptibility test (AST)

As per the National Foodborne Disease Surveillance Plan of China, 32 provincial Centers for Disease Control and Prevention (CDC) laboratories are advised to submit epidemiological information and experimental data on the isolates to the China Food Safety Authority (CFSA) via the National Molecular Tracing Network for Foodborne Disease Surveillance (TraNet). In this study, 7,106 NTS isolates were sourced from diarrhea cases reported



**Fig. 5** **A** Line chart of the numbers of *mcr-9.1*-positive assemblies in the NCBI database over time (total: 865). **B** Top 10 serotypes of *mcr-9.1*-producing NTS in the NCBI database (total: 1,435)

under the active foodborne disease surveillance framework from 2010 to 2020. ARGs were screened by ABRicate (Version 1.0.1) (<https://github.com/tseemann/abricate>). ASTs and minimal inhibitory concentrations (MICs) for *mcr-9.1*-producing isolates were performed and interpreted as previously described [38]. Serotypes were predicted by SISTR\_cmd (Version 1.1.2) [39], sequence types were identified by MLST (Version 2.23.0) [40].

#### Datasets of *mcr-9.1*-producing assemblies and *mcr-9.1*-producing plasmids based on the NCBI database

To clarify the spreading of *mcr-9.1* gene across from the world, *mcr-9.1*-producing assemblies were downloaded from NCBI pathogen database, *mcr-9.1*-producing plasmids were obtained from Refseq database by BLASTn as previously described [38], on January 19, 2022.

#### Long-read sequencing and plasmid sequence analysis

To locate the *mcr-9.1* gene, eight representative isolates, having unique *mcr-9.1*-positive contigs as previously described [38], were chosen to long-read sequence. Unicycler (Version 0.4.8) was used for long-read assembling. ABRicate (Version 1.0.1) (<https://github.com/tseemann/abricate>) in PlasmidFinder database, with 90% identification threshold and 60% minimum coverage. Mashree (Version 1.2.0) were used to create a tree for plasmids based on Mash distance. BLAST Ring Image Generator (BRIG) (Version 0.95) was used to align and visualize for all plasmids in this study and a referenced plasmid obtained from NCBI.

#### Conjugation experiment

To test the transferability of *mcr-9.1* genes, conjugation experiment was performed by the donor strain *mcr-9.1*-producing isolates and the recipient strain sodium azide-resistant *E. coli* J53. Briefly, the donor and recipient strains at log phase were mixed at the donor/recipient ratio of 1/3, applied to a 0.22  $\mu$ m filter on antibiotic free Brain–Heart Infusion Agar (BHA) plates, followed by culture at 37 °C for 16 h. The putative transconjugants were selected by BHA supplemented with colistin (0.5  $\mu$ g/mL) and sodium azide (150  $\mu$ g/mL), further confirmed by Polymerase chain reaction (PCR) screening for *cdgR* genes, which were found in *E. coli* but not in NTS.

#### Abbreviations

<i>mcr</i>	Mobilized colistin resistance
NTS	Non-typhoidal <i>Salmonella</i>
ST	Sequence type
ARGs	Antimicrobial resistance genes
MDR	Multi-drug resistant
LPS	Lipopolysaccharide
ESBLs	Extended-spectrum beta-lactamases
PMQR	Plasmid-mediated quinolone resistance
<i>E. coli</i>	<i>Escherichia coli</i>
AST	Antimicrobial susceptibility test
CDC	Centers for Disease Control and Prevention
MICs	Minimal inhibitory concentrations
CFSA	China Food Safety Authority
TraNet	Tracing Network
BRIG	BLAST Ring Image Generator
PCR	Polymerase chain reaction
Inc	Incompatibility

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s44280-023-00037-5>.

**Additional file 1: Figure S1.** Antimicrobial resistance rate of *mcr-9.1*-positive isolates in this study.

**Additional file 2: Table S1.** Characterizations of 11 *mcr-9.1*-producing isolates in this study.

**Additional file 3: Table S2.** Profiles of 8 *mcr-9.1*-producing plasmids in this study.

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### Authors' contributions

Q.C. and W.L.: Methodology, investigation, data curation and visualization of these results, writing the original draft. X.Q., X.J. and X.G.: Investigation into identifying *mcr-9.1*-producing isolates, formal analysis, assisting in drafting of the manuscript. T.Y. and L.Y.: Conducted plasmid analysis. T.Y. and X.Z.: carried out conjugation assays. C.W., G.Z. and Q.Y.: These authors contributed to data curation. M.F.: contributed to the methodology. Z.S. and Y.G.: Conceptualization, supervision, funding acquisition, project administration, review and editing of the manuscript. This author provided overarching guidance throughout the project and ensured the strategic relevance and scientific rigor of the research. All authors read and approved the final manuscript.

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### Availability of data and materials

All genome assemblies of *mcr-9*-positive NTS isolates in China were registered under BioProject accession no. PRJNA1026158.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

All authors declare that they have no conflicts of interest.

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