Genetic characterization of multidrug-resistant *Salmonella typhimurium* carrying *mcr-1* in China

Running title: Genetic characterization of *S. Typhimurium*

Haoran Zhang[1,2†], Yong Huang[1†], Beibei Liang[2†], Jing Xie[2], Ying Xiang[2], Xinying Du[2], Chaojie Yang[2], Hongbo Liu[2], HONGBo Liu[2], Hui Wang[2], Mingjuan Yang[2], Ligui Wang[2], Xiaofeng Hu[2], Lianqun Jin[2], Zhengquan Yuan[2*], Shaofu Qiu[2*], and Hongbin Song[2*]

[1] Academy of Military Medical Sciences, Beijing, China; address: 20 Fengtai Eastern Avenue, Beijing, 100071, China.

[2] Center for Disease Control and Prevention of PLA, Beijing, China; address: 20 Fengtai Eastern Avenue, Beijing, 100071, China.

[*]**These authors contributed equally to this work.**

*** Corresponding author**

Mailing address: Center for Disease Control and Prevention of PLA, Beijing, China; Beijing, China; address: 20 Fengtai Eastern Avenue, Beijing, 100071, China;

Tel: (86)-10-66948475

Fax: (86)-10-66948475

E-mail: qiushf0613@hotmail.com Or hongbinsong@263.net Or yzhengquan66@163.com.

Abstract

Background: With the rapid emergence of plasmid mediated polymyxin resistance gene *mcr-1*, multidrug-resistant *Salmonella* caused great troubles in clinical treatment and attracted extensive attention. Here we report multidrug-resistant *Salmonella* strains harboring *mcr-1* in China, which are resistant to both polymyxin, traditional antibiotics and even clinically wide-used antibiotics.

Methods: We screened 1454 strains of *Salmonella* collected in our laboratory from 2006 to 2018 from 3 provinces or regions for *mcr-1* by PCR. Antimicrobial susceptibility testing was determined. Plasmid conjugation assays were carried out to analysis the transferability of polymyxin resistance. Genetic polymorphism analysis of *Salmonella* was performed using the PFGE, and the plasmid profiles were characterized by S1-PFGE and southern blotting. The plasmids harboring *mcr-1* were sequenced and compared.

Results: Eleven *S. Typhimurium* isolates harboring *mcr-1* with polymyxin resistance (MICs 4μg/ml) were identified from intestinal infections and foods in China. All *S. Typhimurium* isolates were multidrug-resistant to traditional antibiotics and even clinically wide-used antibiotics. Three types of plasmids harboring *mcr-1* were recovered (*IncHI2, IncX4 and IncI2*). Compared with the reference plasmid, IncX4 and IncI2 plasmids had extremely similar typical backbone, and contain only *mcr-1* resistance gene. However, IncHI2 were the most diverse type of plasmid due to containing a large MDR region, including $bla_{\text{CTX-M}}$, *oqxB*, *sul*, *aph*, *aadA* and bla_{TEM}. IncHI2 plasmids were observed to contain only one or no insertion sequence ISApl1 around *mcr-1*, without forming a circular intermediate.

Conclusion: With the horizontal transfer of different types of plasmids, *mcr-1* is widely spread worldwide. These prevalent plasmids are responsible for resistance to polymyxin, traditional antibiotics and even clinically wide-used antibiotics resulting from transmission of *mcr-1* and other resistance

genes. Our studying emphasizes the necessity to jointly monitor its international epidemic and preemptive further upgrade.

Keywords: Plasmid; Polymyxin; *S. Typhimurium*; Drug resistance

Introduction

The emergence and evolution of multi-drug-resistant (MDR, resistance to three or more classes of antimicrobials) bacteria, including gram-negative bacteria, pose a growing threat to public health [1, 2]. *Salmonella* is one of the common pathogens causing bacterial intestinal infections and diarrhea in both developed and developing countries. In recent years, powerful fluoroquinolones and cephalosporins with a broad antibacterial spectrum have been developed as the preferred drugs in the clinical treatment of *Salmonella* infection [3]. In addition, azithromycin has been recommended for the clinical treatment of *Salmonella* infection due to its superior curative effect on intestinal bacteria in 2017. But with the increase in the MDR rate of *Salmonella* isolate, the drug sensitivity of fluoroquinolones, cephalosporins and azithromycin is also decreased, and the MDR *Salmonella* causing great troubles in clinical treatment have been reported in countries across six continents [4-6]. Polymyxin, as one of the colistin antibiotics, is currently the last line of defense the severe infection with MDR-Gram-negative bacteria [7]. A plasmid-mediated horizontally transferable colistin resistance gene, *mcr-1*, was first found in China in 2015, which plays an important role in the transmission of colistin resistant strains [8]. The colistin resistance gene *mcr-1*, was first detected from *E. coli* harboring a conjugative IncI2 plasmid in China. It encodes a phosphoethanolamine transferase which is modification of the lipid A. The propagation of polymyxin resistance gene *mcr-1* is closely related to these types of plasmids, which include IncHI1, IncHI2, IncI2, IncX4, IncF, IncFI, IncFII and IncP[3, 9]. It may provide the insight about potential of multiple drug resistance genes transfer between *Salmonella* species in

the intestine. Here, our study was aimed to characterize the *Salmonella enterica serovar Typhimurium* (*S. Typhimurium*) harboring *mcr-1* plasmid isolated from patients, pork and pork products.

MATERIALS AND METHONDS

Bacterial Isolation, Serotyping, mcr-1 Gene Screening

We conducted a retrospective *mcr-1* study on 1454 strains of *Salmonella* collected in our laboratory from 2006 to 2018 from 3 provinces or regions (Shanghai 1046, Guangdong 209，Guangxi 199). These isolates were recovered from the stool samples of patients and market were strictly identified according to the national microbiological examination standard and stored in the glycerol tube at -80℃. *Salmonella* strains were identified through the use of biochemical test kit (API20E system Merrier; bioMérieux Vitek, Marcy-L'Etoile, France) according to the manufacturer's instructions. According to the Kauffmann-White scheme, *Salmonella* strains were serotyped on slides by a microtiter agglutination test for O and H antigens, strictly following the manufacturer's instructions (SSI, Copenhagen, Denmark). We screened all historical *Salmonella* strains for *mcr-1* by PCR using the published primers according Liu study [8].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was determined using broth microdilution in Sensititre Gram Negative AST Plates for *Salmonella* strains (Thermo Fisher Scientific, Inc., West Sussex, United Kingdom) including 14 different antimicrobials: ceftriaxone (CRO), tetracycline (TET), ceftiofur (XNL), cefoxitin (FOX), gentamicin (GEN), ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT), sulfisoxazole (FIS), nalidixic acid (NAL), streptomycin (STR), azithromycin (AZI), and amoxicillin/clavulanic acid 2:1 ratio (AUG2). The susceptibility to colistin is to use the dye WST (Dojindo Molecular Technologies, Inc., Japan) by a microbial viability

assay kit. A reference strain of *Escherichia coli* ATCC 25922 strain was performed in the test as quality control [10].

Plasmid conjugation assays

Plasmid conjugation assays were determined using a standard *E. coli j53* as the recipient, and the *mcr-1* positive *Salmonella* strains as donors. The donor bacteria cultured overnight were mixed with the recipient bacteria in a ratio of 1:3, and were harvested, re-suspended in 80μL. The mixture was incubated for mating at 37℃ for 12-18h in 5ml LB liquid broth. Then a Muller-Hinton agar (BD Biosciences, San Jose, CA) plate containing 100 mg/L sodium azide and 2 mg/L polymyxin B was to a selective medium for *E. coli j53* transconjugants. Putative transconjugants were confirmed by antimicrobial susceptibility testing and detection of *mcr-1* with PCR.

Pulsed-Field Gel Electrophoresis (PFGE), S1-PFGE and Southern blotting

Genomic polymorphism analysis of *Salmonella* strains was performed using the pulsed field gel electrophoresis (PFGE) after a slight modification of the pulseNet standardized PFGE protocol for *Salmonella* [11]. These isolates were digested with *XbaI* (Takara, Dalian, China) at 37℃, and the *salmonella* enterica var. Braendrup H9812 strain was used as the reference. Electrophoresis running on a CHEF MAPPER variable angle system (Bio-Rad, California, America) with the parameters set at 2.16s-63.8s for 19h were performed following a previously methods [12]. The plasmid profiles were characterized by S1-PFGE. The endonuclease S1 nuclease (Takara, Dalian, China) was used to digest at 37℃, and electrophoresis running set at 0.22s-26.29s for 15h. Following this, the southern blotting with digoxigenin-labelled *mcr-1* probe sequences by using the published primers [8] was performed to step of membrane transfer, molecular hybridization, and probe detection following a previously

reported method [13]. The images were captured by a Gel Doc 2000 system (Bio-Rad), and were imported into the BioNumerics software (v6.0) database for processing and further analysis.

Whole Genome Sequencing and Bioinformatic Analysis

The *mcr-1* positive *Salmonella* strains preserved in our laboratory were sequenced based on the second-generation genome sequencing technique. The DNA was extracted from the overnight cultured strains according to the instructions of the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The mate-pair library was constructed by nucleic acid protein analyzer Qsep100 to obtain DNA fragments (not less than 500bp, not more than 800bp) and sequenced by MiSeq platform sequencer. Sequence reads were assembled into draft continuous sequences (contigs) by Newbler [14] and NxTrim [15], and then splice it through GapFiller, Cytoscape. Complete plasmid genomes were annotated using the online annotation server RAST. Identification of insertion sequence (IS), plasmid replicons and resistance genes is performed by ISfinder (https://www-is.biotoul.fr/search.php), PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and ResFinder [16], respectively. Multiple plasmids compared by Mauve, Brig and CLC Genomics Workbench. The circle figure of multiple plasmids for comparison were drawn by DNAploter, Circos et al [17].

RESULT

Characterization of MDR salmonella harboring mcr-1

A total of 1454 *Salmonella* strains were preserved in our laboratory from different samples collected from Shanghai (1046), Guangdong (209) and Guangxi (199), we found 11 strains of *S. Typhimurium* harboring *mcr-1* gene. Among them, eight *mcr-1* positive *S. Typhimurium* strains were identified from fecal samples of patients, and three *mcr-1* positive *S. Typhimurium* strains were from pork and pork products. All eleven *mcr-1* positive *S. Typhimurium* strains showed resistance to polymyxin (MICs

4μg/ml), and were resistance to multiple antimicrobial agents, including gentamicin, ampicillin, sulfamethoxazole, streptomycin, tetracycline and chloramphenicol. Moreover, six of eight strains isolated from patients were resistant to ceftriaxone and cefotaxime. Among three strains isolated from pork and pork products, one was resistant to ceftriaxone and cefotaxime, and another one was insensitive to azithromycin (Table 1).

PFGE, plasmid profiling and southern blotting

Eleven *mcr-1* positive *S. Typhimurium* and four *mcr-1* negative *Salmonella* were analyzed by PFGE. Based on the PFGE results of fifteen isolates of *Salmonella* strains, four clusters were formed among the strains, which were divided according to about 85% homology. In Shanghai, the similarity of five *mcr-1* positive *Salmonella* strains isolated from pork and humans in 2015 was above 95% with the *mcr-1* negative *Salmonella* isolated from humans in 2012. The *mcr-1* negative *Salmonella* strains isolated from pork in 2014 had high homology with *mcr-1* positive *Salmonella* isolated from human in 2015 and lower similarity with *mcr-1* negative *Salmonella* isolated from pork in Guangdong. Further analysis of plasmid characteristics, S1-PFGE and southern blotting showed that all isolates carried different plasmids, of which two strains carried two plasmids, others carried one plasmid (Figure S1).

Plasmid conjugation assays

To assume the transfer ability of the *mcr-1* positive plasmid, plasmid conjugation assays were performed using *S. Typhimurium* harbored *mcr*-1 gene as a donor and *E. coli j53* as a recipient. Five (S49, S51, S52, S55, S56) of eleven recipients in this experiment tested positive for the *mcr-1* gene via PCR amplification and sequence analysis. The MIC of colistin for the transconjugants (the *E. coli j53* harboring *mcr-1* gene) increased to 4 μg/ml.

Genetic characterization of plasmids harboring mcr-1

We have obtained the plasmid sequence of eleven *S. Typhimurium* strains. Through the plasmid comparison on NCBI, we downloaded five highly homologous plasmids containing *mcr-1* from *Salmonella* strains as a reference (at above 95% coverage and at above 99% identity). Through the analysis of plasmids, three IncX4, two IncI2 and six IncHI2 plasmids were identified to contain *mcr-1* resistance genes among 11 strains. Three IncX4 plasmids were 33kb in size, and were extremely similar to pNG14043 from *Salmonella* in Taiwan. These plasmids were typical IncX4 backbone which varied only by single nucleotide polymorphism (SNP) (at above 99% homology). The insertion sequence IS26 was upstream of *mcr-1* in our isolates, and IS15 was on these plasmids but absent around *mcr-1*. Those plasmids had only *mcr-1* genes and no other identifiable resistance genes. (Fig. 2, Fig. 5). Two IncI2 plasmids were 50kb, and were similar to pHNSHP45 by *E. coli* strains from Shanghai in July, 2013. We found that these two IncI2 plasmids have a common plasmid backbone. However, compared with the two IncI2 plasmids, the region around *mcr-1* were located in an inverted orientation. The insertion sequence ISApl1 and IS683 were missing in two IncI2 plasmids in this study by comparing with the reference plasmid sequence (Fig. 3, Fig. 5). Unlike the IncX4 and IncI2 types of plasmids, six IncHI2 plasmids were the most diverse type of plasmid due to containing a large MDR region. The MDR region is a region in the IncHI2 plasmid that contains a wide range of resistance genes, integrons and ISs. These IncHI2 plasmids that share a common backbone were 250kb, used pHNSHP45-2 as a reference plasmid with high homology. The MDR region of the reference plasmid contained a variety of resistance genes, including $bla_{CTX\text{-}M}$, *oqxA*, *oqxB*, *oqxR*, *sul*, *aph*, *aadA*, *dfrA*, *floR*, *aac*, *fosA*, *hph*, *intl*. Some drug resistance genes and gene element can be observed in that region of the IncHI2 plasmids in our study, distinguishing them from the reference plasmid sequence (Table 1). Compared to the reference plasmid, part of drug-resistant genes in the MDR region has missed in

our IncHI2 plasmids (pS49, pS54, pS70), including *oqxA, oqxB, oqxR, sul, aadA*; The drug-resistant gene *dfrA12* is missing in the MDR region of five IncHI2 plasmids except pS51 (Fig. 4). Among six IncHI2 plasmids, five IncHI2 plasmids were observed to contain the insertion sequence ISApl1 which was upstream of *mcr-1*, whereas another plasmid lacked ISApl1 around *mcr-1* (Fig. 5).

Discussion

Salmonella is a common zoonotic pathogen that can cause bromatoxism and diarrhea of human beings [5, 10]. For some special populations, antibiotic therapy is necessary for *Salmonella* infection. According to the *Salmonella* monitoring report, the MDR of *Salmonella* increased 40 percent in the last decade of 20th century [18, 19]. Among the many serotypes of *Salmonella*, the MDR of *S. Typhimurium*, has drawn global concern in particular. However, the effects of traditional antibiotics and even clinically wide-used antibiotics to *S. Typhimurium* have been reduced, which include fluoroquinolones, cephalosporins and even azithromycin [20].

Polymyxin has been used as the ultimate means of combating multidrug-resistant Gram-negative bacterial infections [21]. Since 2015, polymyxin has been listed to be one of the crucially important antibiotics by WHO, while it has been approved for treatment of bacterial infections since 2017 [22]. But plasmid mediated polymyxin resistant strains with *mcr-1* positive were first reported in China in 2015[8]. Eleven *S. Typhimurium* strains harbored *mcr-1* gene were found in a retrospective study from 1454 strains in our laboratory. Under the analysis, we found that multi-drug resistance existed in all these eleven *mcr-1* positive *S. Typhimurium* strains. These eleven polymyxin resistant *S. Typhimurium* strains from different sources have multi-drug resistance, resisting nalidixic acid, gentamicin, ampicillin, sulfisoxazole, streptomycin, tetracycline, and chloramphenicol, of which eight were from the stools of patients with diarrhea and three were from pork and pork products. Even the

third-generation antibiotics such as cephalosporins and azithromycin, which are clinically common-used to treat Salmonella infections, have become noneffective. It is important to note that all eight patients infected by *mcr-1* positive MDR *S. Typhimurium* are all children under 4 years of age (Table S1). This finding is consistent with the results reported by Luo et al [23], which most of the *Salmonella* infected people are children under 5 years old and patients with low immunity. At the same time, the results of PFGE prompted us, five strains of polymyxin resistant *S. Typhimurium* isolated from children under 4 years and pork in Shanghai in 2015 had high homology. These isolates were highly homologous with the *mcr-1* negative *Salmonella* in the same region in 2012 and were not in the same cluster with the strains from Guangdong and Fujian. Both the above results and plasmid conjugation assays suggest that the *mcr-1* gene can be transmitted between local strains by horizontal transfer of plasmids, and these plasmids may simultaneously carry a variety of different resistance genes, resulting in an increase of MDR *S. Typhimurium* strains. The emergence of *mcr-1* positive MDR *S. Typhimurium* strains may lead to serious consequences such as the increasing difficulty of future treatment, therapeutic failure and even death [24, 25].

In the *Salmonella* strains, plasmids play a vital role in the acquisition of drug resistance caused by drug resistance genes [26]. The *mcr-1* and other resistance genes are usually located on the same or different plasmids. These genes are widely spread in animals, environment and food in many countries and regions around the world through horizontal transfer of plasmids [27]. It is demonstrated that *mcr-1* and other resistance genes with the spread of *Salmonella* strains have potential to transfer from food product animal strains to human strains [28, 29]. During plasmid transfer, the *mcr*-1 positive plasmids display significant diversity in terms of antibiotic resistance patterns, incompatibility groups, and genetic contents [3]. IncI2 and IncX4 plasmids which promote *Salmonella* resistance are the two main

types of plasmids spreading globally [14]. In the study, eleven strains of *S. Typhimurium* found that the plasmids containing *mcr-1* were mainly located in IncI2, IncX4 and IncHI2. According to reports in *Enterobacteriaceae* [30], IncHI2 plasmid (216-280kb), containing a multi-drug resistance region, is the fifth most common plasmid family, and is one of the main plasmid groups carrying *mcr-1* gene variants. We observed the coexistence of plasmids containing *mcr-1* and multiple drug resistance genes in this work. The reduced sensitivity to multiple antibiotics is attributed to the fact that *mcr-1* positive *S. Typhimurium* also coharbored *oqxB*, bla_{TEM} and bla_{CTX} resistance genes. These genes are co-located in drug-resistant plasmids, resulting in decreased antibiotic susceptibility to polymyxin, quinolones and the third-generation cephalosporins. The transfer of IncHI2 plasmids carrying multiple drug resistance genes between bacteria caused the rapid spread of antibiotic resistance in a certain area. MDR IncHI2 plasmids containing *mcr-1* are widely distributed in human pathogens, and are the most efficient vectors for the transmission of *mcr-1* and other drug-resistant genes. The worrying assumption is that MDR IncHI2 plasmids in the dissemination of *mcr-1* and other drug-resistant genes cause the change of *Salmonella* resistance phenotype and spread within a short period of time.

The insertion sequence (IS) family in the bacterial genome, a widely variable DNA sequence in nature, is also one of the important ways for the transmission of resistance genes between bacterial pathogens [12]. The eleven plasmids are divided into different patterns according to the presence or absence of IS elements and the relationship with *mcr-1* sites. The different types and positions of IS sequences in these plasmids are both exposed the potential threat presented by *Salmonella* strains carrying *mcr-1* plasmids via their role in transmitting the polymyxin resistance gene. ISApl1 possesses significant role in the transmission of *mcr-1*. In our study, ISApl1 element is closely related to the IncHI2 type plasmid. This suggests that under the pressure of various antibiotics in the natural environment, strains may

obtain *mcr-1* and other resistance elements distributed in different plasmids through a circular intermediate with the aid of ISApl1 [12]. The stark truth is that the *mcr-1* is general associated with the *mcr-1* gene cassettes, designated as Tn6330. Tn6330 containing ISApl1-*mcr-1*-orf- ISApl1 structure can insert *mcr-1* into different types of plasmids by forming a circular intermediate. In our research, five of six IncHI2 plasmids were observed to contain the insertion sequence ISApl1 which was upstream of *mcr-1*, whereas another plasmid lacked ISApl1 around *mcr-1*. The *mcr-1* translocation could be mediated the insertion of the *mcr-1* gene into the IncHI2 plasmid backbone, and possibly other plasmids through *mcr-1* gene cassettes. Indeed, these two types of insertion sequences in our IncHI2 plasmids cannot detect Tn6330. It should be noted that the plasmids like these two types are more stable in *Salmonella* strains.

Conclusion

This study describes in detail the genetic characteristics of *mcr-1*-postive *S.* Typhimurium strains from patients, pork and pork products in China. Three types of plasmids sharing a common plasmid backbone have their own characteristics regarding IS and resistance genes. These prevalent plasmids are responsible for resistance to polymyxin, traditional antibiotics and even clinically wide-used antibiotics resulting from transmission of mcr-1 and other resistance genes. Therefore, these findings emphasize the necessity of assessing the potential of mcr-1 to spread and jointly monitoring its international epidemic and preemptive further upgrade.

Accession number

The complete sequences of plasmids in this study have been deposited in GenBank under accession numbers MW264504- MW264516, respectively.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Jain P, Chowdhury G, Samajpati S, Basak S, Ganai A, Samanta S *et al.*: **Characterization of non-typhoidal Salmonella isolates from children with acute gastroenteritis, Kolkata, India, during 2000-2016**. 2020, **51**(2):613-627.doi:10.1007/s42770-019-00213-z.
2. Tang B, Chang J, Zhang L, Liu L, Xia X, Hassan BH *et al.*: **Carriage of Distinct mcr-1-Harboring Plasmids by Unusual Serotypes of Salmonella**. *Adv Biosyst* 2020, **4**(3):e1900219.doi:10.1002/adbi.201900219.
3. Touati A, Mairi A: **Plasmid-Determined Colistin Resistance in the North African Countries: A Systematic Review**. *Microb Drug Resist* 2020.doi:10.1089/mdr.2019.0471.
4. Wang Y, Xu C, Zhang R, Chen Y, Shen Y, Hu F *et al.*: **Changes in colistin resistance and mcr-1 abundance in Escherichia coli of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study**. *The Lancet Infectious Diseases* 2020.doi:10.1016/s1473-3099(20)30149-3.
5. Ling Z, Yin W, Shen Z, Wang Y, Shen J, Walsh TR: **Epidemiology of mobile colistin resistance genes mcr-1 to mcr-9**. *J Antimicrob Chemother* 2020.doi:10.1093/jac/dkaa205.
6. Anyanwu MU, Jaja IF, Nwobi OC: **Occurrence and Characteristics of Mobile Colistin Resistance (mcr) Gene-Containing Isolates from the Environment: A Review**. *Int J Environ Res Public Health* 2020, **17**(3).doi:10.3390/ijerph17031028.
7. Lu X, Zeng M, Xu J, Zhou H, Gu B, Li Z *et al.*: **Epidemiologic and genomic insights on mcr-1-harbouring Salmonella from diarrhoeal outpatients in Shanghai, China, 2006-2016**. *EBioMedicine* 2019, **42**:133-144.doi:10.1016/j.ebiom.2019.03.006.
8. Liu Y, Wang Y, Walsh T, Yi L, Zhang R, Spencer J *et al.*: **Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study**. 2016, **16**(2):161-168.doi:10.1016/s1473-3099(15)00424-7.
9. Zurfluh K, Nuesch-Inderbinen M, Klumpp J, Poirel L, Nordmann P, Stephan R: **Key features of mcr-1-bearing plasmids from Escherichia coli isolated from humans and food**. *Antimicrob Resist Infect Control* 2017, **6**:91.doi:10.1186/s13756-017-0250-8.
10. Wang J, Li Y, Xu X, Liang B, Wu F, Yang X *et al.*: **Antimicrobial Resistance of Salmonella enterica Serovar Typhimurium in Shanghai, China**. *Front Microbiol* 2017, **8**:510.doi:10.3389/fmicb.2017.00510.
11. Ribot E, Fair M, Gautom R, Cameron D, Hunter S, Swaminathan B *et al.*: **Standardization of**

pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. 2006, **3**(1):59-67.doi:10.1089/fpd.2006.3.59.

12. Liu H, Zhu B, Liang B, Xu X, Qiu S, Jia L *et al.*: **A Novel mcr-1 Variant Carried by an IncI2-Type Plasmid Identified From a Multidrug Resistant Enterotoxigenic Escherichia coli**. *Front Microbiol* 2018, **9**:815.doi:10.3389/fmicb.2018.00815.
13. Zou D, Huang Y, Zhao X, Liu W, Dong D, Li H *et al.*: **A novel New Delhi metallo-β-lactamase variant, NDM-14, isolated in a Chinese Hospital possesses increased enzymatic activity against carbapenems**. 2015, **59**(4):2450-2453.doi:10.1128/aac.05168-14.
14. Arredondo-Alonso S, Rogers M, Braat J, Verschuuren T, Top J, Corander J *et al.*: **mlplasmids: a user-friendly tool to predict plasmid- and chromosome-derived sequences for single species**. 2018, **4**(11).doi:10.1099/mgen.0.000224.
15. O'Connell J, Schulz-Trieglaff O, Carlson E, Hims M, Gormley N, Cox AJB: **NxTrim: optimized trimming of Illumina mate pair reads**. 2015, **31**(12):2035-2037.doi:10.1093/bioinformatics/btv057.
16. Bortolaia V, Kaas R, Ruppe E, Roberts M, Schwarz S, Cattoir V *et al.*: **ResFinder 4.0 for predictions of phenotypes from genotypes**. 2020.doi:10.1093/jac/dkaa345.
17. Roberts A, Chandler M, Courvalin P, Guédon G, Mullany P, Pembroke T *et al.*: **Revised nomenclature for transposable genetic elements**. 2008, **60**(3):167-173.doi:10.1016/j.plasmid.2008.08.001.
18. Elbediwi M, Pan H, Biswas S, Li Y, Yue M: **Emerging colistin resistance in Salmonella enterica serovar Newport isolates from human infections**. *Emerg Microbes Infect* 2020, **9**(1):535-538.doi:10.1080/22221751.2020.1733439.
19. Osei Sekyere J, Maningi NE, Modipane L, Mbelle NM: **Emergence of mcr-9.1 in Extended-Spectrum-beta-Lactamase-Producing Clinical Enterobacteriaceae in Pretoria, South Africa: Global Evolutionary Phylogenomics, Resistome, and Mobilome**. *mSystems* 2020, **5**(3).doi:10.1128/mSystems.00148-20.
20. Huang H, Dong N, Shu L, Lu J, Sun Q, Chan E *et al.*: **mcrColistin-resistance gene in clinical carbapenem-resistant strains in China, 2014-2019**. 2020, **9**(1):237-245.doi:10.1080/22221751.2020.1717380.
21. Bai F, Li X, Niu B, Zhang Z, Malakar PK, Liu H *et al.*: **A mcr-1-Carrying Conjugative IncX4 Plasmid in Colistin-Resistant Escherichia coli ST278 Strain Isolated From Dairy Cow Feces in Shanghai, China**. *Front Microbiol* 2018, **9**:2833.doi:10.3389/fmicb.2018.02833.
22. Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X *et al.*: **Genetic characterization of mcr-1-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant**. *J Antimicrob Chemother* 2017, **72**(2):393-401.doi:10.1093/jac/dkw411.
23. Luo Q, Wan F, Yu X, Zheng B, Chen Y, Gong C *et al.*: **MDR Salmonella enterica serovar Typhimurium ST34 carrying mcr-1 isolated from cases of bloodstream and intestinal infection in children in China**. *J Antimicrob Chemother* 2020, **75**(1):92-95.doi:10.1093/jac/dkz415.
24. Folster J, Grass J, Bicknese A, Taylor J, Friedman C, Whichard JJMdr: **Characterization of Resistance Genes and Plasmids from Outbreaks and Illness Clusters Caused by Salmonella Resistant to Ceftriaxone in the United States, 2011-2012**. 2017,

23(2):188-193.doi:10.1089/mdr.2016.0080.

25. Wang Y, Cao C, Alali W, Cui S, Li F, Zhu J *et al.*: **Distribution and Antimicrobial Susceptibility of Foodborne Salmonella Serovars in Eight Provinces in China from 2007 to 2012 (Except 2009)**. 2017, **14**(7):393-399.doi:10.1089/fpd.2016.2237.

26. McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R *et al.*: **Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First Report of mcr-1 in the United States**. *Antimicrob Agents Chemother* 2016, **60**(7):4420-4421.doi:10.1128/AAC.01103-16.

27. Huang H, Dong N, Shu L, Lu J, Sun Q, Chan EW *et al.*: **Colistin-resistance gene mcr in clinical carbapenem-resistant Enterobacteriaceae strains in China, 2014-2019**. *Emerg Microbes Infect* 2020, **9**(1):237-245.doi:10.1080/22221751.2020.1717380.

28. Ma Y, Xu X, Gao Y, Zhan Z, Xu C, Qu X *et al.*: **Antimicrobial resistance and molecular characterization of Salmonella enterica serovar Corvallis isolated from human patients and animal source foods in China**. *Int J Food Microbiol* 2020, **335**:108859.doi:10.1016/j.ijfoodmicro.2020.108859.

29. Pornsukarom S, van Vliet AHM, Thakur S: **Whole genome sequencing analysis of multiple Salmonella serovars provides insights into phylogenetic relatedness, antimicrobial resistance, and virulence markers across humans, food animals and agriculture environmental sources**. *BMC Genomics* 2018, **19**(1):801.doi:10.1186/s12864-018-5137-4.

30. Zingali T, Chapman TA, Webster J, Roy Chowdhury P, Djordjevic SP: **Genomic Characterisation of a Multiple Drug Resistant IncHI2 ST4 Plasmid in Escherichia coli ST744 in Australia**. *Microorganisms* 2020, **8**(6).doi:10.3390/microorganisms8060896.

393 **Table 1** Characteristic of eleven *mcr-1*-positive MDR *S. Typhimurium*

Strain no.	Antibiogram	Results of sequencing for eleven *mcr-1*-positive plasmids				
		Plasmid name	Size of *mcr-1* plasmid(b)	Type of *mcr-1* plasmid	Drug-resistant gene	IS Types
S49	CRO,EFT,AMP,GEN,SM,SX,C,COL	pS49	222291	IncHI2	*mcr-1*, bla_{CTX-M}, *aac*, *floR*, *aph*, *fosA*	ISApl1
S51	CRO,EFT,AMP,GEN,SM,SX,SXT,C,TE,COL	pS51	249475	IncHI2	*mcr-1*, bla_{CTX-M}, *sul*, *oqxA*, *oqxR*, *dfrA*, *floR*, *oqxB*, *aadA*, *aph*, *aac*, *fosA*, *cml*	ISApl1
S52	CRO,EFT,AMP,GEN,SM,SX,C,TE,COL	pS52	249043	IncHI2	*mcr-1*, bla_{CTX-M}, *oqxB*, *sul*, *aph*, *aadA*, *fosA*, *floR*, *aac*, *cml*, *oqxR*	ISApl1
S53	CRO,EFT,AMP,GEN,SM,SX,C,COL	pS53	228926	IncHI2	*mcr-1*, bla_{CTX-M}, *oqxA*, *sul*, *oqxR*, *aadA*	ISApl1
S54	CRO,EFT,AMP,GEN,SM,SX,C,COL	pS54	222880	IncHI2	*mcr-1*, bla_{CTX-M}, *aac*, *sul*, *aph*, *floR*, *fosA*	ISApl1
S55	FOX,AMC,CRO,EFT,AMP,SM,SX,SXT,AZ,C,TE,COL	pS55	59233	IncI2	*mcr-1*	none
S56	COL	pS56	60454	IncI2	*mcr-1*	none
S60	AMP,NAL,GEN,SM,SX,SXT,C,TE,COL	pS60	33308	IncX4	*mcr-1*	IS15, IS26
S67	AMP,NAL,SM,SX,TE,COL	pS67	33308	IncX4	*mcr-1*	IS15, IS26
S69	AMP,NAL,GEN,SX,SXT,C,TE,COL	pS69	33308	IncX4	*mcr-1*	IS15, IS26
S70	CRO,EFT,AMP,GEN,SM,SX,C,TE,COL	pS70	223256	IncHI2	*mcr-1*, bla_{CTX-M}, *aac*, *sul*, *aph*, *floR* , *fosA*	ISApl1

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Figure legends

Fig1. Pulsed field gel electrophoresis (PFGE) of the fifteen *Salmonella* isolates in 2015. (A) Results of PFGE profiles of *Salmonella* were clustered. (B) The key, species, origin, source and isolation year of the fifteen *Salmonella* are shown.

Fig2. Sequencing alignment of *mcr-1* harbouring IncX4 plasmids. The *mcr-1* harbouring plasmid pNG14043 with GenBank no.KY120364 which was isolated from *S. Typhimurium* in Taiwan was used as refrence plasmid (black circle). The outmost circle in red arrows denots the annotations of refrence plasmid. The figure shows the extremly high degree of homology of the four *mcr-1* harbouring IncX4 plasmids. Detailed information of *mcr-1* location of plasmids is provided in fugure5.

Fig3. Sequencing alignment of *mcr-1* harbouring IncI2 plasmids. The first *mcr-1* harbouring plasmid, pHNSHP45 with GenBank no.KP347127 which was isolated from E. coli strains from Shanghai in July, 2013 was used as refrence plasmid (black circle). The outmost circle in red arrows denots the annotations of refrence plasmid. The IS683 and ISApl1 are absent in two IncI2 plasmids in this study. Detailed information of *mcr-1* location of plasmids is provided in fugure5.

Fig4. Sequencing alignment of *mcr-1* harbouring IncHI2 plasmids. pHNSHP45-2 with GenBank no.KU341381 which was isolated from E. coli strains was used as refrence plasmid (black circle). The outmost circle in red arrows denots the annotations of refrence plasmid. Among the six IncHI2 plasmids, pSH51, pS52 and pS53 exhibit sequence with the refrence sequence, others are low sequence homology. An MDR region is exhibieted in the five IncHI2 plasmids. Detailed information of *mcr-1*

location of plasmids is provided in fugure5.

Fig5. Genetic context of eleven plasmids surrouding the *mcr-1* gene. In CDS, red and blue arrows represent *mcr-1* and IS respectively, black arrows present plasmid bone. The light blue and orange shaded regions indicate genetic regions that show the direct and reverse nucleotide ideatityhomology between different segments(>99%).