

Genetic Characterization of Multidrug-Resistant *Salmonella* Typhimurium Carrying Mcr-1 in China

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Keywords: Plasmid, Polymyxin, S. Typhimurium, Drug resistance

Posted Date: January 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-143500/v1>

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

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3 **Running title:** Genetic characterization of *S. Typhimurium*

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22

23 **Abstract**

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

28 **Methods:** We screened 1454 strains of *Salmonella* collected in our laboratory from 2006 to 2018 from
29 3 provinces or regions for *mcr-1* by PCR. Antimicrobial susceptibility testing was determined. Plasmid
30 conjugation assays were carried out to analysis the transferability of polymyxin resistance. Genetic
31 polymorphism analysis of *Salmonella* was performed using the PFGE, and the plasmid profiles were
32 characterized by S1-PFGE and southern blotting. The plasmids harboring *mcr-1* were sequenced and
33 compared.

34 **Results:** Eleven *S. Typhimurium* isolates harboring *mcr-1* with polymyxin resistance (MICs 4μg/ml)
35 were identified from intestinal infections and foods in China. All *S. Typhimurium* isolates were
36 multidrug-resistant to traditional antibiotics and even clinically wide-used antibiotics. Three types of
37 plasmids harboring *mcr-1* were recovered (*IncHI2*, *IncX4* and *IncI2*). Compared with the reference
38 plasmid, *IncX4* and *IncI2* plasmids had extremely similar typical backbone, and contain only *mcr-1*
39 resistance gene. However, *IncHI2* were the most diverse type of plasmid due to containing a large
40 MDR region, including *bla_{CTX-M}*, *oqxB*, *sul*, *aph*, *aadA* and *bla_{TEM}*. *IncHI2* plasmids were observed to
41 contain only one or no insertion sequence *ISAp11* around *mcr-1*, without forming a circular
42 intermediate.

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

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

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

49

50 **Introduction**

51 The emergence and evolution of multi-drug-resistant (MDR, resistance to three or more classes of
52 antimicrobials) bacteria, including gram-negative bacteria, pose a growing threat to public health [1, 2].

53 *Salmonella* is one of the common pathogens causing bacterial intestinal infections and diarrhea in both

54 developed and developing countries. In recent years, powerful fluoroquinolones and cephalosporins

55 with a broad antibacterial spectrum have been developed as the preferred drugs in the clinical treatment

56 of *Salmonella* infection [3]. In addition, azithromycin has been recommended for the clinical treatment

57 of *Salmonella* infection due to its superior curative effect on intestinal bacteria in 2017. But with the

58 increase in the MDR rate of *Salmonella* isolate, the drug sensitivity of fluoroquinolones,

59 cephalosporins and azithromycin is also decreased, and the MDR *Salmonella* causing great troubles in

60 clinical treatment have been reported in countries across six continents [4-6]. Polymyxin, as one of the

61 colistin antibiotics, is currently the last line of defense the severe infection with MDR-Gram-negative

62 bacteria [7]. A plasmid-mediated horizontally transferable colistin resistance gene, *mcr-1*, was first

63 found in China in 2015, which plays an important role in the transmission of colistin resistant strains

64 [8]. The colistin resistance gene *mcr-1*, was first detected from *E. coli* harboring a conjugative IncI2

65 plasmid in China. It encodes a phosphoethanolamine transferase which is modification of the lipid A.

66 The propagation of polymyxin resistance gene *mcr-1* is closely related to these types of plasmids,

67 which include IncHI1, IncHI2, IncI2, IncX4, IncF, IncFI, IncFII and IncP[3, 9]. It may provide

68 the insight about potential of multiple drug resistance genes transfer between *Salmonella* species in

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

71 **MATERIALS AND METHONDS**

72 ***Bacterial Isolation, Serotyping, mcr-1 Gene Screening***

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

83 ***Antimicrobial Susceptibility Testing***

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

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

93 ***Plasmid conjugation assays***

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

101 ***Pulsed-Field Gel Electrophoresis (PFGE), S1-PFGE and Southern*** 102 ***blotting***

103 Genomic polymorphism analysis of *Salmonella* strains was performed using the pulsed field gel
104 electrophoresis (PFGE) after a slight modification of the pulseNet standardized PFGE protocol for
105 *Salmonella* [11]. These isolates were digested with *XbaI* (Takara, Dalian, China) at 37°C, and the
106 *salmonella* enterica var. Braendrup H9812 strain was used as the reference. Electrophoresis running on
107 a CHEF MAPPER variable angle system (Bio-Rad, California, America) with the parameters set at
108 2.16s-63.8s for 19h were performed following a previously methods [12]. The plasmid profiles were
109 characterized by S1-PFGE. The endonuclease S1 nuclease (Takara, Dalian, China) was used to digest
110 at 37°C, and electrophoresis running set at 0.22s-26.29s for 15h. Following this, the southern blotting
111 with digoxigenin-labelled *mcr-1* probe sequences by using the published primers [8] was performed to
112 step of membrane transfer, molecular hybridization, and probe detection following a previously

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

115 ***Whole Genome Sequencing and Bioinformatic Analysis***

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

128 **RESULT**

129 ***Characterization of MDR salmonella harboring mcr-1***

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

135 4µg/ml), and were resistance to multiple antimicrobial agents, including gentamicin, ampicillin,
136 sulfamethoxazole, streptomycin, tetracycline and chloramphenicol. Moreover, six of eight strains
137 isolated from patients were resistant to ceftriaxone and cefotaxime. Among three strains isolated from
138 pork and pork products, one was resistant to ceftriaxone and cefotaxime, and another one was
139 insensitive to azithromycin (Table 1).

140 ***PFGE, plasmid profiling and southern blotting***

141 Eleven *mcr-1* positive *S. Typhimurium* and four *mcr-1* negative *Salmonella* were analyzed by PFGE.
142 Based on the PFGE results of fifteen isolates of *Salmonella* strains, four clusters were formed among
143 the strains, which were divided according to about 85% homology. In Shanghai, the similarity of five
144 *mcr-1* positive *Salmonella* strains isolated from pork and humans in 2015 was above 95% with the
145 *mcr-1* negative *Salmonella* isolated from humans in 2012. The *mcr-1* negative *Salmonella* strains
146 isolated from pork in 2014 had high homology with *mcr-1* positive *Salmonella* isolated from human in
147 2015 and lower similarity with *mcr-1* negative *Salmonella* isolated from pork in Guangdong. Further
148 analysis of plasmid characteristics, S1-PFGE and southern blotting showed that all isolates carried
149 different plasmids, of which two strains carried two plasmids, others carried one plasmid (Figure S1).

150 ***Plasmid conjugation assays***

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

156 ***Genetic characterization of plasmids harboring mcr-1***

157 We have obtained the plasmid sequence of eleven *S. Typhimurium* strains. Through the plasmid
158 comparison on NCBI, we downloaded five highly homologous plasmids containing *mcr-1* from
159 *Salmonella* strains as a reference (at above 95% coverage and at above 99% identity). Through the
160 analysis of plasmids, three IncX4, two IncI2 and six IncHI2 plasmids were identified to contain *mcr-1*
161 resistance genes among 11 strains. Three IncX4 plasmids were 33kb in size, and were extremely
162 similar to pNG14043 from *Salmonella* in Taiwan. These plasmids were typical IncX4 backbone which
163 varied only by single nucleotide polymorphism (SNP) (at above 99% homology). The insertion
164 sequence IS26 was upstream of *mcr-1* in our isolates, and IS15 was on these plasmids but absent
165 around *mcr-1*. Those plasmids had only *mcr-1* genes and no other identifiable resistance genes. (Fig. 2,
166 Fig. 5). Two IncI2 plasmids were 50kb, and were similar to pHNSHP45 by *E. coli* strains from
167 Shanghai in July, 2013. We found that these two IncI2 plasmids have a common plasmid backbone.
168 However, compared with the two IncI2 plasmids, the region around *mcr-1* were located in an inverted
169 orientation. The insertion sequence ISAp11 and IS683 were missing in two IncI2 plasmids in this study
170 by comparing with the reference plasmid sequence (Fig. 3, Fig. 5). Unlike the IncX4 and IncI2 types of
171 plasmids, six IncHI2 plasmids were the most diverse type of plasmid due to containing a large MDR
172 region. The MDR region is a region in the IncHI2 plasmid that contains a wide range of resistance
173 genes, integrons and ISs. These IncHI2 plasmids that share a common backbone were 250kb, used
174 pHNSHP45-2 as a reference plasmid with high homology. The MDR region of the reference plasmid
175 contained a variety of resistance genes, including *bla_{CTX-M}*, *oqxA*, *oqxB*, *oqxR*, *sul*, *aph*, *aadA*, *dfrA*,
176 *floR*, *aac*, *fosA*, *hph*, *intl*. Some drug resistance genes and gene element can be observed in that region
177 of the IncHI2 plasmids in our study, distinguishing them from the reference plasmid sequence (Table
178 1). Compared to the reference plasmid, part of drug-resistant genes in the MDR region has missed in

179 our IncHI2 plasmids (pS49, pS54, pS70), including *oqxA*, *oqxB*, *oqxR*, *sul*, *aadA*; The drug-resistant
180 gene *dfrA12* is missing in the MDR region of five IncHI2 plasmids except pS51 (Fig. 4). Among six
181 IncHI2 plasmids, five IncHI2 plasmids were observed to contain the insertion sequence ISAp11 which
182 was upstream of *mcr-1*, whereas another plasmid lacked ISAp11 around *mcr-1* (Fig. 5).

183 **Discussion**

184 *Salmonella* is a common zoonotic pathogen that can cause bromatoxism and diarrhea of human beings
185 [5, 10]. For some special populations, antibiotic therapy is necessary for *Salmonella* infection.
186 According to the *Salmonella* monitoring report, the MDR of *Salmonella* increased 40 percent in the last
187 decade of 20th century [18, 19]. Among the many serotypes of *Salmonella*, the MDR of *S.*
188 *Typhimurium*, has drawn global concern in particular. However, the effects of traditional antibiotics
189 and even clinically wide-used antibiotics to *S. Typhimurium* have been reduced, which include
190 fluoroquinolones, cephalosporins and even azithromycin [20].

191 Polymyxin has been used as the ultimate means of combating multidrug-resistant Gram-negative
192 bacterial infections [21]. Since 2015, polymyxin has been listed to be one of the crucially important
193 antibiotics by WHO, while it has been approved for treatment of bacterial infections since 2017 [22].
194 But plasmid mediated polymyxin resistant strains with *mcr-1* positive were first reported in China in
195 2015[8]. Eleven *S. Typhimurium* strains harbored *mcr-1* gene were found in a retrospective study from
196 1454 strains in our laboratory. Under the analysis, we found that multi-drug resistance existed in all
197 these eleven *mcr-1* positive *S. Typhimurium* strains. These eleven polymyxin resistant *S. Typhimurium*
198 strains from different sources have multi-drug resistance, resisting nalidixic acid, gentamicin,
199 ampicillin, sulfisoxazole, streptomycin, tetracycline, and chloramphenicol, of which eight were from
200 the stools of patients with diarrhea and three were from pork and pork products. Even the

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

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

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

237 The insertion sequence (IS) family in the bacterial genome, a widely variable DNA sequence in nature,
238 is also one of the important ways for the transmission of resistance genes between bacterial pathogens
239 [12]. The eleven plasmids are divided into different patterns according to the presence or absence of IS
240 elements and the relationship with *mcr-1* sites. The different types and positions of IS sequences in
241 these plasmids are both exposed the potential threat presented by *Salmonella* strains carrying *mcr-1*
242 plasmids via their role in transmitting the polymyxin resistance gene. ISAp11 possesses significant role
243 in the transmission of *mcr-1*. In our study, ISAp11 element is closely related to the IncHI2 type plasmid.
244 This suggests that under the pressure of various antibiotics in the natural environment, strains may

245 obtain *mcr-1* and other resistance elements distributed in different plasmids through a circular
246 intermediate with the aid of ISAp11 [12]. The stark truth is that the *mcr-1* is general associated with the
247 *mcr-1* gene cassettes, designated as Tn6330. Tn6330 containing ISAp11-*mcr-1*-orf- ISAp11 structure
248 can insert *mcr-1* into different types of plasmids by forming a circular intermediate. In our research,
249 five of six IncHI2 plasmids were observed to contain the insertion sequence ISAp11 which was
250 upstream of *mcr-1*, whereas another plasmid lacked ISAp11 around *mcr-1*. The *mcr-1* translocation
251 could be mediated the insertion of the *mcr-1* gene into the IncHI2 plasmid backbone, and possibly
252 other plasmids through *mcr-1* gene cassettes. Indeed, these two types of insertion sequences in our
253 IncHI2 plasmids cannot detect Tn6330. It should be noted that the plasmids like these two types are
254 more stable in *Salmonella* strains.

255 **Conclusion**

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

263 **Accession number**

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

266 **Acknowledgements**

267 The present study was funded by grants from the National Key R&D Program of China (no.

268 2018YFC1603801 and 2017YFC1600105), the National Science and Technology Major Project (nos.
269 2018ZX10101003 and 2018ZX10714002), and the National Nature Science Foundation of China (nos.
270 81803296, 81872678 and 81673237).

271 **Disclosure**

272 The authors report no conflicts of interest in this work.

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

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

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405 **Figure legends**

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

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411

412 **Fig2.** Sequencing alignment of *mcr-1* harbouring IncX4 plasmids. The *mcr-1* harbouring plasmid
413 pNG14043 with GenBank no.KY120364 which was isolated from *S. Typhimurium* in Taiwan was used
414 as reference plasmid (black circle). The outmost circle in red arrows denotes the annotations of reference
415 plasmid. The figure shows the extremely high degree of homology of the four *mcr-1* harbouring IncX4
416 plasmids. Detailed information of *mcr-1* location of plasmids is provided in figure5.

417

418 **Fig3.** Sequencing alignment of *mcr-1* harbouring IncI2 plasmids. The first *mcr-1* harbouring plasmid,
419 pHNSHP45 with GenBank no.KP347127 which was isolated from *E. coli* strains from Shanghai in July,
420 2013 was used as reference plasmid (black circle). The outmost circle in red arrows denotes the
421 annotations of reference plasmid. The IS683 and ISAp11 are absent in two IncI2 plasmids in this study.
422 Detailed information of *mcr-1* location of plasmids is provided in figure5.

423

424

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

430 location of plasmids is provided in figure5.

431

432 **Fig5.** Genetic context of eleven plasmids surrounding the *mcr-1* gene. In CDS, red and blue arrows

433 represent *mcr-1* and IS respectively, black arrows present plasmid bone. The light blue and orange

434 shaded regions indicate genetic regions that show the direct and reverse nucleotide ideatityhomology

435 between different segments(>99%).

Figures

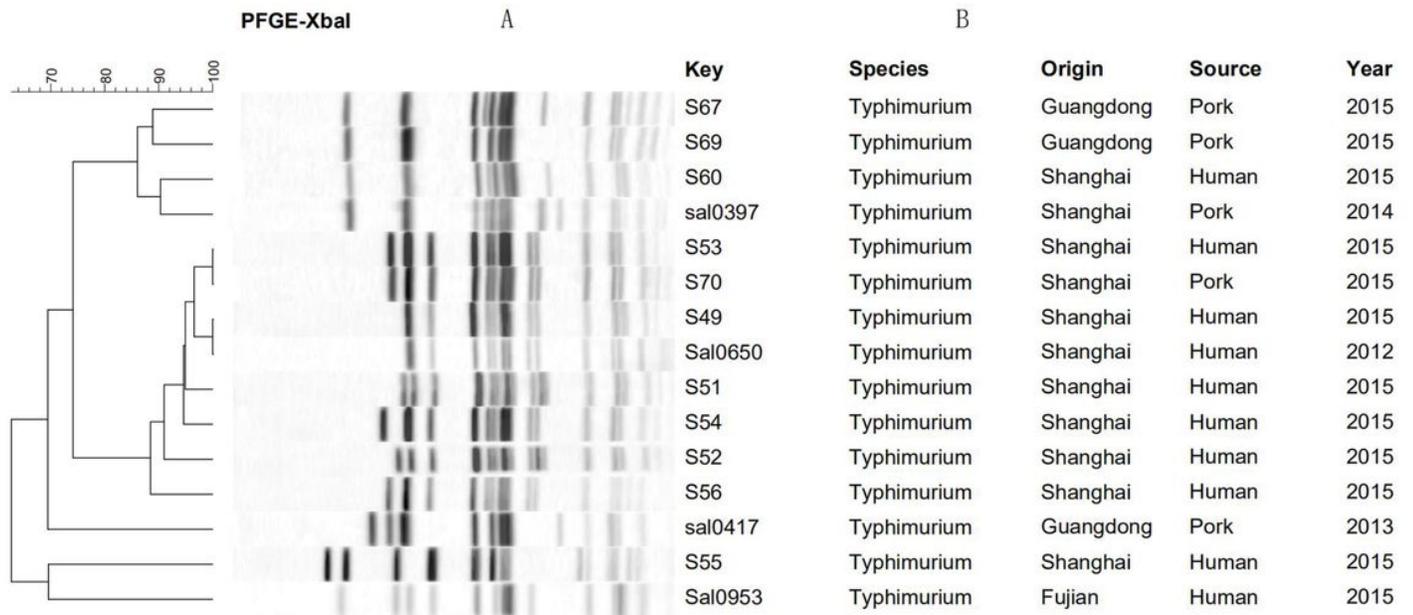


Figure 1

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.

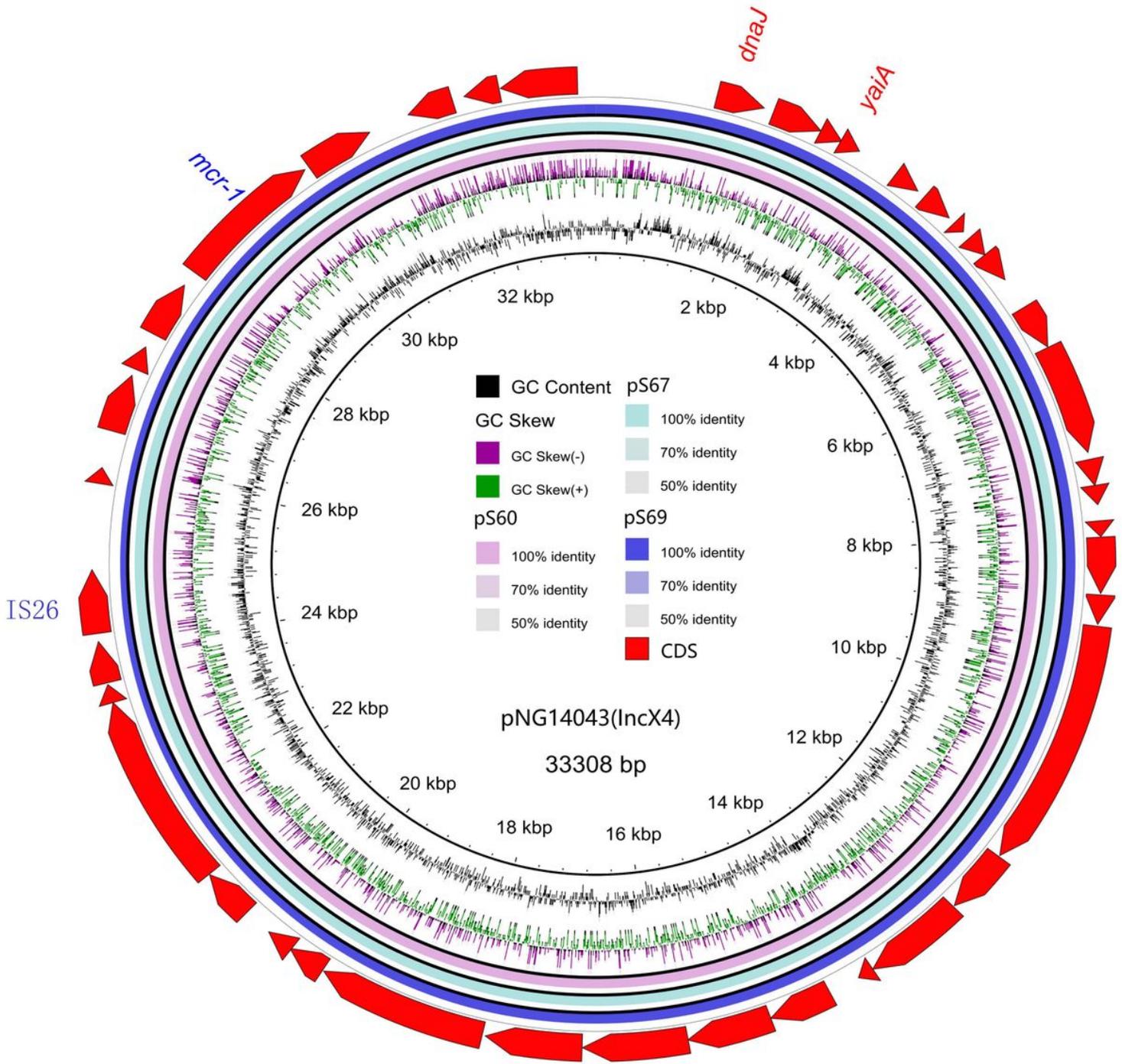


Figure 2

Sequencing alignment of *mcr1* harbouring IncX4 plasmids. The *mcr1* harbouring plasmid pNG14043 with GenBank no. KY120364 which was isolated from *S. Typhimurium* in Taiwan was used as reference plasmid (black circle). The outermost circle in red arrows denotes the annotations of reference plasmid. The figure shows the extremely high degree of homology of the four *mcr1* harbouring IncX4 plasmids. Detailed information of *mcr1* location of plasmids is provided in figure 5.

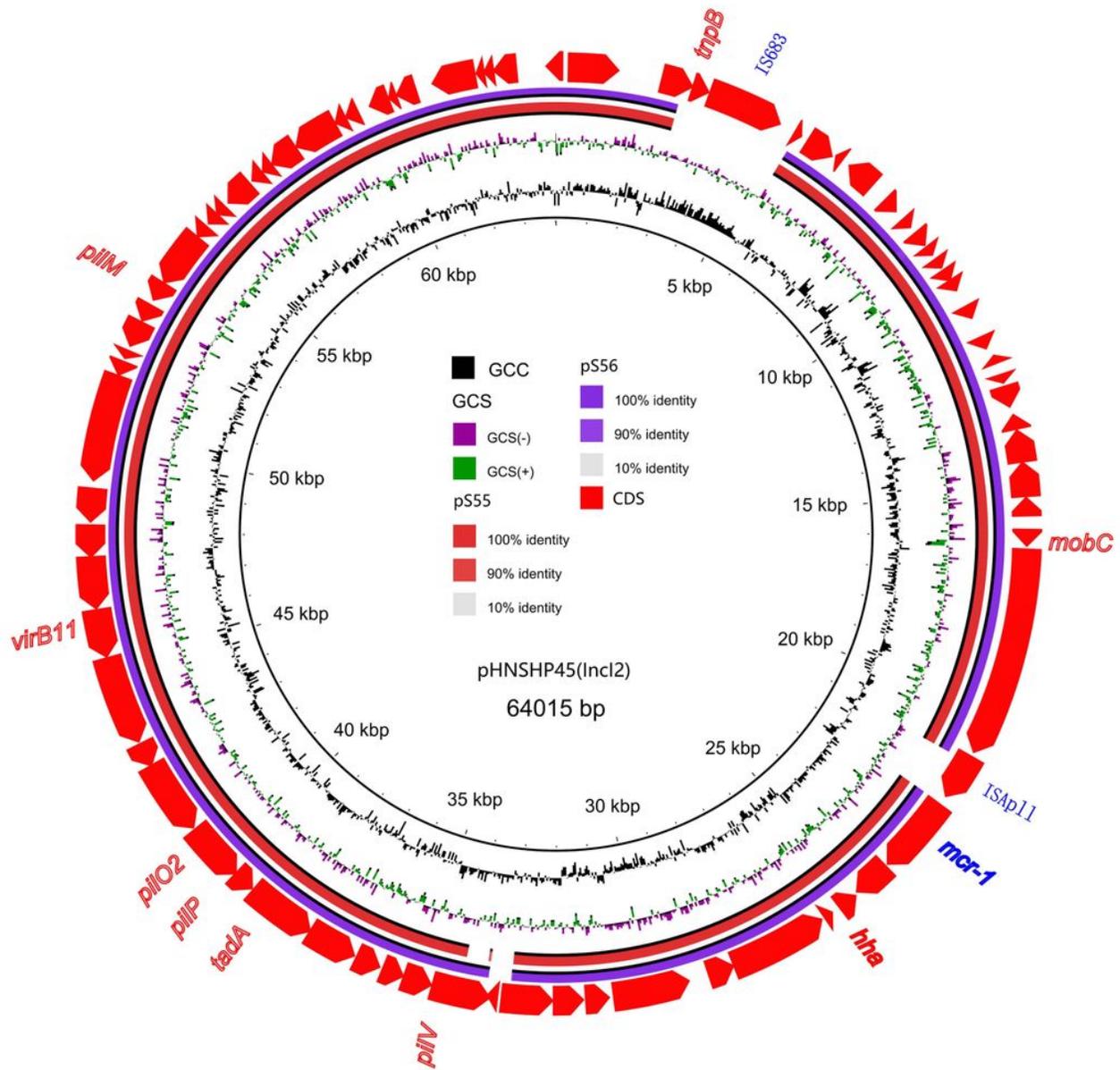


Figure 3

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 reference plasmid (black circle). The outmost circle in red arrows denotes the annotations of reference plasmid. The IS 683 and ISAp11 are absent in two IncI2 plasmids in this study. Detailed information of *mcr 1* location of plasmids is provided in figure 5.

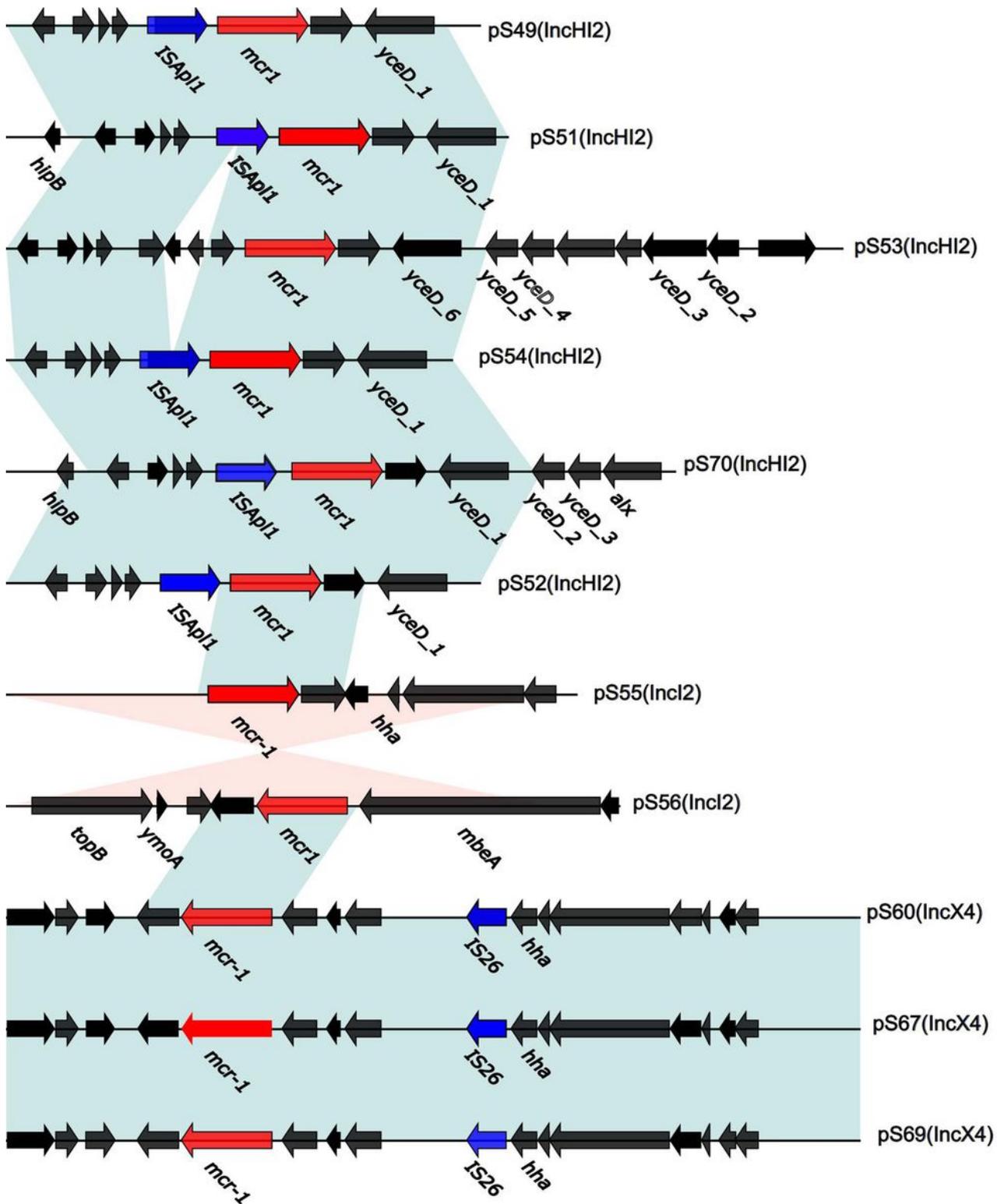


Figure 5

Genetic context of eleven plasmids surrounding the *mcr1* gene. In CDS, red and blue arrows represent *mcr1* and IS respectively, black arrows present plasmid genes. The light blue and orange shaded regions indicate genetic regions that show the direct and reverse nucleotide identity homology between different segments (>99%)

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