Co-selection may explain the unexpectedly high prevalence of plasmid-mediated colistin resistance gene *mcr-1* in a Chinese broiler farm

DEAR EDITOR,

The rise of the plasmid-encoded colistin resistance gene *mcr-1* is a major concern globally. Here, during a routine surveillance, an unexpectedly high prevalence of *Escherichia coli* with reduced susceptibility to colistin (69.9%) was observed in a Chinese broiler farm. Fifty-three (63.9%) *E. coli* isolates were positive for *mcr-1*. All identified *mcr-1*-positive *E. coli* (MCREC) were multidrug resistant and carried other clinically significant resistance genes. Furthermore, the *mcr-1* genes were mainly located on the IncI2 and IncHI2 plasmids. Conjugation experiments unraveled the co-transfer of *mcr-1* with other antibiotic resistance genes ($bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-14}}$, *floR*, and *fosA3*) via the IncI2 (*n*=3) and IncHI2 (*n*=4) plasmids. The stable genetic context *mcr-1-pap2* was common in the IncI2 plasmids, whereas IS*Apl1*-*mcr-1*-*pap2*-IS*Apl1* was mainly found in the IncHI2 plasmids. The dominance of *mcr-1*-bearing IncI2 and IncHI2 plasmids and co-selection of *mcr-1* with other antimicrobial resistance genes might contribute to the exceptionally high prevalence of *mcr-1* in this broiler farm. Our results emphasized the importance of appropriate antibiotic use in animal production.

Multidrug resistant (MDR) bacteria have become a major public health concern. Colistin, the silver bullet against infections caused by MDR bacteria, was reintroduced into human clinics and hailed as an antibiotic of last resort (Nation & Li, 2009). In animal production, colistin was heavily used as a growth promoter (Casal et al., 2007), which inevitably led to colistin resistance. Since the first detection of the mobile colistin resistance gene *mcr-1* in 2015, the prevalence of colistin resistance has become worrisome (Liu et al., 2016). The *mcr-1* gene encodes phosphoethanolamine transferase MCR-1 for the modification of lipid A, which reduces the negative charge of bacterial outer membranes and causes colistin resistance (Li et al., 2019). Primarily, *mcr-1* is found in *E. coli*, as well as several other Enterobacteriaceae species and *Vibrio parahaemolyticus* (Lei et al., 2019; Nang et al., 2019). Various studies have reported on the existence of *mcr-1* in humans, animals, plants, and the environment (Liu & Liu, 2018; Nang et al., 2019; Wang et al., 2017a). In addition, an increasing number of *mcr* variants (e.g., *mcr-2* to *mcr-10*) have been identified in Enterobacteriaceae (Ling et al., 2020; Wang et al., 2020). The wide distribution of *mcr-1* is usually mediated by mobile genetic elements, with the IncI2, IncX4, and IncHI2 plasmids considered as the main culprits (Liu & Liu, 2018; Sun et al., 2018). Generally, the occurrence of colistin resistance and *mcr-1* among Enterobacteriaceae isolates from humans (0.1%–8.8%) is lower than that from livestock (0.9%–76.9%) (Liu & Liu, 2018; Liu et al., 2016; Quan et al., 2017; Wang et al., 2017b). For avian species, the detection rate of Enterobacteriaceae carrying *mcr-1* is generally below 30% (Lentz et al., 2016; Moawad et al., 2018; Perrin-Guyomard et al., 2016; Shen et al., 2016; Trung et al., 2017). In China, the prevalence of *mcr-1* and colistin resistance in *E. coli* from avians (~10%) is generally lower than that from swine (~30%) (Huang et al., 2017; Yang et al., 2017; Zhang et al., 2018). However, during routine surveillance of antimicrobial resistance in *E. coli* from food animals, an unexpectedly high prevalence (69.9%) of reduced susceptibility to colistin was found in *E. coli* from a Chinese broiler farm in 2013. Therefore, in the current study, we investigated the potential mechanism behind this phenomenon.

In July 2013, a total of 100 fresh fecal samples (~2 g per sample) were randomly collected from 100 broilers (27 days old) on a farm in eastern China. Bacterial recovery was conducted by incubating the samples in 3 mL of Luria Broth

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for 16–24 h. Then, 2 μL of bacterial solution was inoculated into MacConkey agar plates, from which non-duplicate colonies with *E. coli* morphology were selected and identified using MALDI-TOF MS (Shimadzu-Biotech Corp., Japan). Minimum inhibitory concentrations (MICs) of 14 antibiotics against *E. coli* isolates were evaluated using agar dilution. The results were interpreted according to the interpretative criteria recommended by CLSI (M100-S30) (ampicillin, cefotaxime, gentamicin, amikacin, fosfomycin, and ciprofloxacin) (Clinical and Laboratory Standards Institute, 2020) and epidemiological cut-off (ECOFF) values recommended by EUCAST (colistin, florfenicol, and neomycin) (http://www.eucast.org). Identification of MDR *E. coli* was confirmed after the bacteria showed resistance to at least three agents from different antimicrobial categories (Magiorakos et al., 2012). Polymerase chain reaction (PCR) amplification and Sanger sequencing were used to screen resistance genes, including *mcr-1*, bla_{CTX-M} (β-lactamase genes), *fosA3* (fosfomycin resistance gene), and *rmtB* (aminoglycoside resistance gene), as well as plasmids (IncHI2, IncI2, IncI1, IncX4, and IncFII) in the *E. coli* strains with the primers listed in Table S1.

In total, 83 *E. coli* strains were recovered from the broiler farm. Overall, 58 (69.9%) strains showed reduced susceptibility (MIC ≥ 2 mg/L) to colistin, among which 53 (63.9%) were positive for *mcr-1* (MCREC) (Table 1). The reason why the other five *mcr-1*-negative strains showed reduced susceptibility to colistin remains to be studied. Also, 55 (66.3%) strains showed resistance (MIC ≥ 4 mg/L) to colistin. The high prevalence of colistin resistance and circulation of *mcr-1* among the *E. coli* collected from this broiler farm was unexpected, as the occurrence of MCREC in avian farms is usually low, e.g., 10% in China (Yang et al., 2017), 8% in Egypt (Moawad et al., 2018), 2% in South Africa (Perreten et al., 2016), and 2% in France (Perrin-Guyomard et al., 2016). The exceptionally high detection rate of MCREC (63.9%) in the current study is worrying as distribution of *mcr-1* along the broiler industry chain is possible (Wang et al., 2017c).

Table 1 Antibiotic resistance profiles, resistance genes, and genetic backgrounds and locations of *mcr-1* in 53 *E. coli* isolates

Isolate[a]	Resistance profile[b]	Other resistance gene[c]	Location of *mcr-1*, size[d]	Genetic context of *mcr-1*
XCLC11	AMP, CTX, STR, TET, FFC, CL, FOS	$bla_{CTX-M-14}$,$bla_{CTX-M-64}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC12	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC16	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-55}$	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC26	AMP, CTX, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC37	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC31	AMP, STR, TET, FFC, CL, CIP	–	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC33	AMP, CTX, GEN, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC4	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC46	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, $bla_{CTX-M-65}$, *fosA3*, *floR*	IncHI2, ~244 kb	IS*Apl1-mcr-1-pap2*
XCLC52	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-55}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC54	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, $bla_{CTX-M-65}$, *fosA3*,*floR*	IncHI2, ~244 kb	IS*Apl1-mcr-1-pap2*
XCLC58	AMP, CTX, AMK, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-55}$, *fosA3*, *rmtB*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC69	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, $bla_{CTX-M-82b}$, *fosA3*,*floR*	IncHI2, ~244 kb	IS*Apl1-mcr-1-pap2*
XCLC74	AMP, CTX, STR, FFC, CL, FOS, CIP	*fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC75	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*
XCLC78	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-15}$, *fosA3*	IncHI2	IS*Apl1-mcr-1-pap2*
XCLC82	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncHI2, ~210 kb	IS*Apl1-mcr-1-pap2*
XCLC89	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*,*floR*	IncHI2, ~244 kb	IS*Apl1-mcr-1-pap2*
XCLC28	AMP, CTX, AMK, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, $bla_{CTX-M-55}$, *fosA3*, *rmtB*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC27	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC40	AMP, CTX, GEM, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-55}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC41	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC44	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-14}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC55	AMP, CAZ, CTX, STR, TET, FFC, CL, FOS, CIP	$bla_{CTX-M-55}$, $bla_{CTX-M-65}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*-IS*Apl1*(IncHI2),*mcr-1-pap2*(IncI2)

Continued

Isolate[a]	Resistance profile[b]	Other resistance gene[c]	Location of *mcr-1*, size[d]	Genetic context of *mcr-1*
XCLC6	AMP, CTX, GEN, NEO, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC73	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC8	AMP, CAZ, CTX, FOX, GEN, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncHI2, IncI2	IS*Apl1-mcr-1-pap2*(IncHI2), *mcr-1-pap2*(IncI2)
XCLC35[e]	AMP, CAZ, CTX, FOX, AMK, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-14}$, *bla*$_{CTX-M-55}$, *fosA3*, *rmtB*	IncI2, ~65 kb	*mcr-1-pap2*
XCLC5	AMP, CTX, AMK, GEN, STR, TET, FFC, CL, FOS, CIP	*rmtB*	IncI2, ~63 kb	*mcr-1-pap2*
XCLC76	AMP, CAZ, CTX, AMK, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*, *rmtB*	IncI2, ~65 kb	*mcr-1-pap2*
XCLC13	AMP, GEN, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncI2, ~63 kb	IS*Apl1-mcr-1-pap2*
XCLC15	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC21	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$	IncI2, ~63 kb	*mcr-1-pap2*
XCLC2	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-14}$, *fosA3*	IncI2, ~63kb	*mcr-1-pap2*
XCLC20	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-64}$	IncI2, ~65 kb	*mcr-1-pap2*
XCLC24	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-65}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC34	AMP, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncI2, ~63 kb	IS*Apl1-mcr-1-pap2*
XCLC39	AMP, CAZ, CTX, NEO, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncI2, ~63 kb	*mcr-1-pap2*
XCLC42	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-65}$, *fosA3*	IncI2, ~63 kb	*mcr-1-pap2*
XCLC45	AMP, CTX, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-65}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC48	AMP, CAZ, CTX, FOX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-24}$, *bla*$_{CTX-M-55}$, *fosA3*	IncI2	IS*Apl1-mcr-1-pap2*
XCLC50	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-24}$, *fosA3*	IncI2, ~63 kb	*mcr-1-pap2*
XCLC53	AMP, STR, TET, FFC, CL, FOS, CIP	*fosA3*	IncI2	IS*Apl1-mcr-1-pap2*
XCLC56	AMP, CTX, STR, TET, FFC, CL, CIP	*bla*$_{CTX-M-15}$	IncI2	*mcr-1-pap2*
XCLC60	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-65}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC64	AMP, CTX, GEN, NEO, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC65	AMP, CAZ, CTX, GEM, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-14}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC71	AMP, CAZ, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-55}$, *fosA3*	IncI2	*mcr-1-pap2*
XCLC80	AMP, CTX, STR, TET, FFC, CL, CIP	*bla*$_{CTX-M-65}$	IncI2, ~63 kb	*mcr-1-pap2*
XCLC81	AMP, CTX, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-14}$	IncI2	*mcr-1-pap2*
XCLC83	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP		IncI2	*mcr-1-pap2*
XCLC92	AMP, CTX, STR, TET, FFC, CL, FOS, CIP		IncI2	*mcr-1-pap2*
XCLC85	AMP, CTX, GEN, STR, TET, FFC, CL, FOS, CIP	*bla*$_{CTX-M-14}$	IncX4	*mcr-1-pap2*

[a]: Isolates from which *mcr-1* gene was transferred to recipient by conjugation or transformation are underlined. [b]: AMP: Ampicillin; CAZ: Ceftazidime; CTX: Cefotaxime; FOX: Cefoxitin; AMK: Amikacin; GEN: Gentamicin; NEO: Neomycin; STR: Streptomycin; TET: Tetracycline; FFC: Florfenicol; CL: Colistin; FOS: Fosfomycin; CIP: Ciprofloxacin. Resistance phenotypes transferred to recipient by conjugation are underlined. [c]: Genes co-transferred with *mcr-1* by conjugation or transformation as determined by PCR are underlined. –: Not available [d]: Replicon type of plasmid carrying *mcr-1* in transconjugant/transformant and approximate size of plasmid are underlined. [e]: Transformant was obtained from this isolate.

All 53 MCREC showed the MDR phenotype as well as very high resistance rates to tetracycline (100%), ampicillin (100%), florfenicol (98.1%), cefotaxime (92.5%), and fosfomycin (94.3%) (Supplementary Figure S1A). Of note, PCR revealed that the MCREC carried various resistance genes with clinical significance, including *fosA3* (*n*=41, 80.7%), *bla*$_{CTX-M}$ (*n*=41, 80.7%), and *rmtB* (*n*=5, 4.2%) (Figure S1b and Table 1). The *bla*$_{CTX-M}$ variants included *bla*$_{CTX-M-14}$ (*n*=19), *bla*$_{CTX-M-55}$ (*n*=16), *bla*$_{CTX-M-65}$ (*n*=8), and *bla*$_{CTX-M-64}$ (*n*=2). High frequencies of the IncHI2 (47%) and IncI2 (48%) plasmids were also observed (Supplementary Figure S1B). The high occurrence of resistance and resistance genes to third generation cephalosporines, which are used in frontline therapy, and to fosfomycin, which is effective against infection by MDR Enterobacteriaceae (Falagas et al., 2010), among these MCREC is alarming. Though the usage of colistin in this

broiler farm is not clear, the high prevalence of antimicrobial resistance among *E. coli* might result from the heavy usage of multiple antibiotics in broilers as ceftiofur, enrofloxacin, and florfenicol are routinely used in this farm (data not shown).

To elucidate the mechanism mediating the spread of *mcr-1* in the studied farm, we first investigated vertical transfer of *mcr-1* by evaluating the clonal relationships among MCREC with pulsed-field gel electrophoresis (PFGE) on a CHEF-MAPPER System (Bio-Rad, USA), as described previously (Gautom, 1997). Specifically, total DNA was digested by the *XbaI* enzyme (TaKaRa Bio Inc., Japan) and embedded in low-melting-point agarose (Bio-Rad, USA). The electrophoretic conditions were: initial switch time, 2.16 s; final switch time, 63.8 s; run time, 19 h; angle, 120°; gradient, 6.0 V/cm; temperature, 14 °C; ramping factor, linear. BioNumerics (Applied Maths, Belgium) was used to analyze the results, with the unweighted pair group method, arithmetic mean, and dice similarity index. The results were interpreted according to previous criteria (Tenover et al., 1995). PFGE was successfully performed on 45 MCREC isolates with the *XbaI* enzyme, with the remaining eight isolates not typable. Twenty-eight different *XbaI* PFGE patterns were identified (Figure 1), indicating that most MCREC were clonally unrelated.

The horizontal mobility of *mcr-1* was also investigated via conjugation using streptomycin-resistant *E. coli* C600 as the recipient (Wu et al., 2018). Twenty-seven isolates were randomly included in the conjugation. Using *E. coli* DH5α as the recipient, chemical transformation was performed on strains that failed in the conjugation assay. For the selection of transconjugants/transformants, colistin, cefotaxime, trimethoprim/sulfamethoxazole, and florfenicol were used. Subsequently, the transconjugants and transformants were subjected to PCR to confirm the existence of *mcr-1* and co-transfer of other resistance genes ($bla_{\text{CTX-M-1G}}$, $bla_{\text{CTX-M-9G}}$, *fosA3*, and *rmtB*) with *mcr-1*. S1-nuclease PFGE was performedtoconfirmthesingleplasmidswithinthetransconjugants/transformants, and to evaluate their sizes (Barton et al., 1995). The antibiotic resistance profiles of transconjugants and transformants were also determined. Plasmid replicon typing was performed with PCR and Sanger sequencing using the primers listed in Supplementary Table S1. In addition, the locations and genetic contexts of *mcr-1* in all MCREC isolates were analyzed by PCR mapping with primers targeting the region of the plasmid backbone and *mcr-1* (Supplementary Table S2).

Seventeen *mcr-1*-positive plasmids were successfully transferred from their hosts via conjugation (*n*=16) or transformation (*n*=1) (Table 1). S1-PFGE showed that only one plasmid carrying *mcr-1* was transferred to the recipients and *mcr-1* was located on the IncI2 plasmids with sizes varying from ~63 to ~65 kb (*n*=12) or IncHI2 plasmids with sizes ranging from ~210 to 244 kb (*n*=5) (Table 1). Of note, PCR revealed the co-transfer of *mcr-1* with $bla_{\text{CTX-M-64}}$/$bla_{\text{CTX-M-55}}$ via IncI2 plasmids (*n*=3, 25%), and with $bla_{\text{CTX-M-14}}$/*floR*/*fosA3* via IncHI2 plasmids (*n*=4, 80%) (Table 1). The co-transferred resistance genes were able to confer relevant antibiotic resistance to the recipients (*E. coli* C600 and DH5α). Feng et al. (2019) also reported the co-transfer of $bla_{\text{CTX-M-64}}$ with *mcr-1* via IncI2 plasmids in *E. coli* from an imported wild fox in China. In addition, *fosA3* and *floR* are frequently co-transferred with *mcr-1* via IncHI2 plasmids (Li et al., 2017; Zhi et al., 2016). These results are of concern because β-lactams (ceftiofur) and florfenicol routinely consumed in animals may select MCR-1-producing plasmids co-harboring $bla_{\text{CTX-M}}$ and/or *floR* via co-selection, and further aggravate the distribution and persistence of *mcr-1* in this broiler farm. Thus, we should not underestimate the risk that *mcr-1* may spread via a similar mechanism.

The PCR mapping results revealed that nine isolates simultaneously carried *mcr-1*-positive IncI2 and IncHI2 plasmids (Table 1). All 62 (53+9) *mcr-1* genes were located in the IncI2, IncHI2, and IncX4 plasmids, with IncI2 dominating the host profile (Table 1), in agreement with other findings (Elbediwi et al., 2019; Migura-Garcia et al., 2020; Sun et al., 2018; Wu et al., 2018). IncI2 plasmids have also been reported as the vectors of $bla_{\text{CTX-M}}$ genes, e.g., $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-64}}$ (Liu et al., 2015; Lv et al., 2013). The dominance of IncI2 (55%) may result from the low fitness cost of *mcr-1*-positive IncI2 plasmids compared with IncHI2 and IncX4 plasmids (Wu et al., 2018). Of the 62 *mcr-1* genes, three different genetic structures were detected, including *mcr-1* without IS*Apl1* (*mcr-1-pap2*) (*n*=31), *mcr-1* with IS*Apl1* upstream (IS*Apl1-mcr-1-pap2*) (*n*=24), and *mcr-1* embedded in the complete transposon Tn*6330* (IS*Apl1-mcr-1-pap2*-IS*Apl1*) (*n*=7). In addition, the frequency of these genetic contexts in IncHI2 and IncI2 plasmids was varied. In IncI2 plasmids, *mcr-1-pap2* was the most common (*n*=30), whereas the remaining four plasmids encoded IS*Apl1-mcr-1-pap2*. In IncHI2 plasmids, all *mcr-1* genes were flanked by IS*Apl1* upstream, and the complete transposon Tn*6330* was present in seven isolates. Generally, *mcr-1* was translocated into plasmid backbones via transposon Tn*6330* (IS*Apl1-mcr-1-pap2*-IS*Apl1*). Following translocation, loss of IS*Apl1* would disrupt the structure of transposon and stabilize *mcr-1* (Sun et al., 2018). Thus, the presence of the stable *mcr-1-pap2* structure in the IncI2 plasmids may also contribute to the circulation of *mcr-1* in this broiler farm.

In conclusion, this study reported on an unusually high prevalence of *mcr-1*-positive *E. coli* in a Chinese broiler farm, which may result from the co-existence of *mcr-1* with other resistance genes in the same plasmid or strain. Our findings emphasize the importance of appropriate antibiotic use in animal production as the misuse and abuse of antibiotics could facilitate the co-selection of *mcr-1*.

SUPPEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

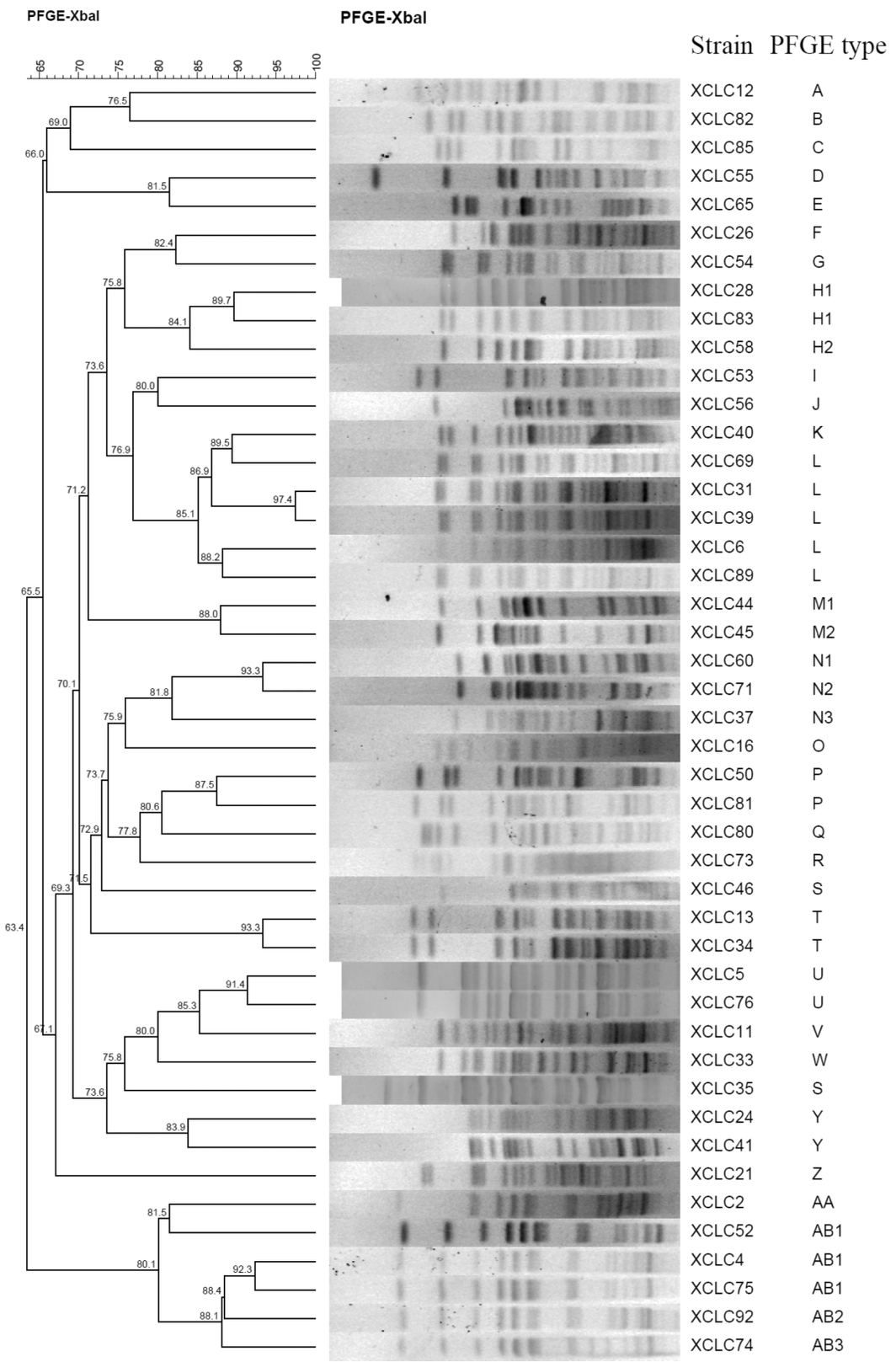

Figure 1 PFGE pattern of *mcr-1*-positive *E. coli*

AUTHORS' CONTRIBUTIONS

J.G. and J.H.L. conceived the research. Q.L., W.H., M.Y., J.W., Y.C., and L.L. collected the data. J.H.L., Q.L., Y.C., J.W., J.G., and J.Y. analyzed and interpreted the data. Y.C. drafted the manuscript. J.H.L., J.W., and J.G. revised the report. All authors read and approved the final version of the manuscript.

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