

Prevalence of avian-origin *mcr-1*-positive *Escherichia coli* with a potential risk to humans in Tai'an, China

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ABSTRACT Multidrug-resistant (MDR) *Escherichia coli* are responsible for difficult-to-treat infections. We sought to determine the prevalence and characteristics of MDR *E. coli* strains isolated from poultry and clinical patients in the same geographical region. Eighty-seven *E. coli* strains were isolated from poultry with perihepatitis lesions at different slaughterhouses, and 356 nonrepetitive *E. coli* strains were isolated from clinical patients. All samples were continuously collected from October to December 2017 in Tai'an, China. The presence of the *mcr-1* gene in the strains was assessed by PCR. The genetic relationships of the polymyxin (POL)-resistant *E. coli* strains were analyzed by pulsed-field gel electrophoresis and multilocus sequence typing. The results indicate that the POL resistance rate for the *E. coli* isolates from poultry was 31.03% (27 of 87), whereas the human-origin *E. coli* isolates were 100% sensitive to POL. The *mcr-1* gene and extended-spectrum β -lactamase *bla*_{CTX-M-14} genes were identified in all 27

POL-resistant avian-origin *E. coli* isolates. Our pulsed-field gel electrophoresis analysis suggested that the 27 strains were represented by 14 pulsotypes, among which there were 3 strains each with A, E, I, and K pulsotypes, and 1 to 2 strains represented by the other 10 pulsotypes. Furthermore, multilocus sequence typing molecular typing identified 16 sequence types, including 4 ST156 strains, 3 ST533 strains, and 1 to 2 strains represented by the remaining 14 sequence types. In summary, the *E. coli* strains isolated in the Tai'an area all showed the MDR phenotype, the rate of which for poultry was higher than that for humans. No POL-resistant human-origin *E. coli* strains were identified in the clinical patients. Our study reveals that poultry-derived MDR *mcr-1*-positive *E. coli* strains may pose a potential risk to humans, and the surveillance findings presented herein will be conducive to our understanding of the prevalence and characteristics of *mcr-1*-positive *E. coli* strains in the Tai'an area.

Key words: *Escherichia coli*, *mcr-1* gene, antibiotic resistance, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST)

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INTRODUCTION

The global increase in antibiotic-resistant bacteria is a concern for human and animal health worldwide because these bacteria can spread between food-producing animals and humans (Roth et al., 2019). In the era of

widespread antimicrobial resistance, the prevalence of multidrug-resistant (MDR) gram-negative bacteria continues to increase (Hawkey, 2015; Rodrigo-Troyano and Sibila, 2017; Roth et al., 2019; Song et al., 2020a,b). The main pathogen responsible for poultry epidemics in China, *Escherichia coli*, is also an important zoonotic foodborne bacterium capable of endangering human health (Roth et al., 2019; Zhuge et al., 2019).

The emergence of pandrug-resistant pathogenic bacteria, which are resistant to all available clinical antibiotics, is not only a cause of serious morbidity and increased mortality but severely restricts therapeutic options (de Kraker et al., 2011; Huang et al., 2018). Polymyxins (POL), fluoroquinolones, cephalosporins, macrolides,

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and third-generation antibiotics, which are considered to be the “highest priority critically important” antibiotics for human use as per the World Health Organization, are approved for use in large poultry-producing regions (Roth et al., 2019).

The recent emergence of *mcr-1*-positive *E. coli* (MCRPEC) strains is a serious concern around the world. Because poultry-origin *E. coli* has zoonotic potential, improving our current understanding of the population structure of MCRPEC strains and antimicrobial resistance is critical for public health (Zhuge et al., 2019). *E. coli* can readily develop antibiotic resistance and also displays a wide drug-resistance spectrum with complex underlying resistance mechanisms. The situation pertaining to carbapenem resistance is becoming increasingly serious, with colistin being the last line of defense against Enterobacteriaceae infections (Shen et al., 2018). Gram-negative bacilli with resistance to POL have been successively reported around the world, a situation that has attracted much attention, although this has not seriously affected their clinical use (Gales et al., 2006). The drug-resistance mechanism for POL is mainly chromosomally mediated (Olaitan et al., 2014). The POL-resistance gene *mcr-1*, which was discovered in 2015, was found to mediate low levels of POL resistance by virtue of its transmissibility among different bacterial strains (Liu et al., 2016). Therefore, it is now important to investigate whether the mechanism of drug resistance is related to the transmission of carbapenems and/or POL-resistant *E. coli* in humans and other animals.

Therefore, in the present study, we conducted a comprehensive analysis of drug resistance and carriage of the *mcr-1* gene. We also looked for correlations among drug-resistant bacteria carrying the *mcr-1* gene in *E. coli* strains isolated from poultry (chickens and ducks) and from clinical patients in the same geographical region (Tai'an, China). Our isolation and analysis of *E. coli* from poultry and humans will provide a foundation for uncovering the epidemiology of *E. coli* in this Chinese region. The ability to investigate and monitor drug resistance in *E. coli*, especially POL resistance, is important for assessing the economic and public health implications of such resistance. Our findings will provide a scientific basis for the clinical treatment and prevention of *E. coli* infections in poultry and humans in the Tai'an region.

MATERIAL AND METHODS

Ethics Statement

The study protocol and poultry studies were approved by the Animal Care and Use Committee of Shandong Agricultural University, Tai'an, China. Human sample collection was carried out in accordance with the approved guidelines of the Ethics Committee of Tai'an City Central Hospital during routine checkups by medical professionals. All the subjects gave written informed consent in accordance with the Declaration of Helsinki.

Bacterial Isolates

From October to December 2017, 87 *E. coli* strains were isolated from chickens and ducks with perihepatitis lesions at slaughterhouses in Tai'an, China. During this time, we also collected 356 strains of nonrepeating *E. coli* isolates from urine, sputum, and blood samples from clinical hospital patients at Tai'an City Central Hospital, Tai'an, China.

Bacterial Identification and Drug Sensitivity Testing

All samples were seeded into MacConkey or eosin methylene blue media. After 3 to 5 rounds of purification, putative *E. coli* isolates were selected based on bacterial colony morphology and confirmed using a BD Phoenix-100 automated microbiology system (BD Diagnostic Systems, Sparks, MD). The drug sensitivity tests were conducted using the BD Phoenix 100 NMIC/ID-4 composite board (Becton Dickinson Co., Franklin Lake, NJ) and the Siemens WalkAway 96 plus MicroScan NC61 composite board (Siemens Healthcare Diagnostics (Shanghai), Shanghai, China). The following antibiotics were examined: amoxicillin/clavulanic acid, amikacin, ampicillin, aztreonam, ceftazidime, cefepime, cefoxitin, ceftriaxone, ceftazidime, cefuroxime, cefotaxime (CTX), ciprofloxacin, ertapenem, gentamicin, imipenem (IPM), levofloxacin, meropenem (MEM), and trimethoprim/sulfamethoxazole. The sensitivity of the strains to POL B was determined via broth microdilution in accordance with the European Commission on Antimicrobial Susceptibility Testing. The drug sensitivity results were assessed by reference to the European Commission on Antimicrobial Susceptibility Testing (<http://www.eucast.org/clinical.breakpoints>).

Detection of the *mcr-1* Gene and β -Lactamase Genes

DNA templates were prepared by the boiling method of DNA extraction, and the *mcr-1* gene was PCR amplified with forward 5'-CGGTCAGTCCGTTTGTTC-3' and reverse 5'-CTTGGTCGGTCTGTAGGG-3' primers (Liu et al., 2016). The genes encoding extended-spectrum β -lactamases (ESBL) and β -lactamases (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CMY}) were identified and characterized by DNA sequencing as previously described (Liao et al., 2015; Bado et al., 2016; Song et al., 2020b). PCR products were sent to Shanghai Sunny Biotech Co., Ltd. (China) for sequencing, and the sequences were compared with those in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>).

Pulsed-Field Gel Electrophoresis

These experiments were designed with reference to the unified method of pulsed-field gel electrophoresis

(PFGE) for *E. coli* segments developed by the US Center for Disease Control. Enzyme digestion was performed with the *Xba* I restriction endonuclease. The PFGE results from 27 strains were analyzed using BioNumerics software (version 5.1, Applied Mathematics, Inc.) with uniform marker normalization to record the strip position. A threshold of 85% homology was set to define clonal clustering of PFGE types.

Multilocus Sequence Typing Analysis

The 7 housekeeping genes, namely *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*, were used to perform multilocus sequence typing (MLST) analysis (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/documents/primersColi_html). These housekeeping genes were PCR amplified and sequenced, the results of which were compared with the *E. coli* MLST database using the Basic Local Alignment Search Tool. By a comparison with the existing housekeeping gene spectrum in the MLST database, the corresponding sequence type (ST) of each bacterial strain can be obtained (Zhou et al., 2020). When a ST could not be completely compared with the existing housekeeping gene spectrum in the database, this ST was confirmed to be a new type.

Conjugation Experiments

Plasmid transferability was evaluated by conjugation experiments using *E. coli* J53 (J53AZR). Each *mcr-1*-positive isolate was incubated with *E. coli* J53 on a cellulose membrane in a Luria–Bertani agar plate (Rau et al., 2020). After incubation at 37°C for 24 h, POL-resistant transconjugants were selected on Luria–Bertani agar plates containing sodium azide (100 µg/mL) and POL (0.5 µg/mL). Antimicrobial susceptibility in each transconjugant was determined by the E-test method. The presence of the *mcr-1* gene in the transconjugants was confirmed by PCR.

RESULTS

Antimicrobial Susceptibility Testing

Among the 356 *E. coli* strains isolated from the clinical patients, 16 were found to be resistant to IPM, and no POL-resistant *E. coli* isolates were detected. Of the 87 *E. coli* strains isolated from the poultry slaughterhouses (chickens and ducks), 27 were resistant to POL and one was resistant to IPM. Figure 1 shows the resistance rates of the *E. coli* isolates to the different antibiotics. The lowest resistance rates were to IPM and MEM (1%), whereas 83% of the human-derived and 99% of the poultry-derived isolates showed resistance to ampicillin, and more than half of the human- and poultry-derived isolates were resistant to cefazolin, cefuroxime, ciprofloxacin, ceftriaxone, and CTX.

The 27 POL-resistant *E. coli* strains from poultry were sensitive to IPM, MEM, and ertapenem and resistant to CTX, ceftiofloxacin, levofloxacin, trimethoprim/

sulfamethoxazole, and POL (Figure 2). Importantly, all of the POL-resistant avian-origin *E. coli* isolates were resistant to at least six antibiotics, and 21 (77.8%) of them were resistant to at least 10 antibiotics.

Pulsed-Field Gel Electrophoresis Results for POL-Resistant Avian-Origin *E. coli*

The PFGE and cluster analyses on the POL-resistant avian-origin *E. coli* are shown in Figure 3. Using 85% as the boundary, the bacterial types can be divided into 14 pulsotypes, some of which can be further divided into different groups of subtypes with genetic correlation. Strains C13, C22, and C23 were found to be type A (A1, A2, and A3); strain C15 was type B; strain C3 was type C; strains C16 and C17 were type D (D1 and D2); strains C19, C21, and C20 were type E (E1, E2, and E3); strain C18 was type F; strains C6 and C7 were type G; strains C10 and C1 were type H (H1 and H2); strains Y26, Y25, and C12 were type I (I1, I2, and I3); strain C2 was type J, strains C8, C9, and C11 were type K (K1, K2, and K3); strains C4 and C5 were type L (L1 and L2); strains Y27 and Y24 were type M (M1 and M2); and strain C14 was type N. Epidemic strains A, E, I, and K were also identified from this analysis.

Multilocus Sequence Typing Molecular Typing of POL-Resistant Avian-Origin *E. coli*

Multilocus sequence typing was performed on the 27 POL-resistant avian-origin *E. coli* strains. The main ST were ST156 and ST533 comprising 4 and 3 isolates, respectively, followed by ST23, ST767, ST1968, ST4204, ST5912, and ST48, each comprising 2 isolates. Each of the other ST (6,395, 4,408, 4,408, 117, 1,638, 101, 155, 457, and 10) had one isolate. Multilocus sequence typing demonstrated that each of the *E. coli* PFGE pulsotype was associated with a unique ST. The 27 strains belonging to 14 pulsotypes (A ~ N) were assigned to 16 ST (Figure 3).

Prevalence of Antibiotic Resistance Genes in POL-Resistant Avian-Origin *E. coli*

Various antibiotic resistance genes were identified among the 27 POL-resistant avian-origin *E. coli* isolates (Table 1), including some β-lactam resistance genes (*bla*_{TEM}, *bla*_{CTX-M}, *bla*_{CMY}), and the *mcr-1* gene. The target fragments from these genes were successfully PCR amplified, and the sequences were confirmed in the 27 strains. All 27 *E. coli* isolates contained at least 2 of the ESBL genes and the plasmid-mediated AmpC β-lactamase genes. All of the 27 *E. coli* isolates (100%) carried *mcr-1* and *bla*_{CTX-M-14} genes, and 21 of 27 (77.8%) also carried *bla*_{CTX-M-15}. Overall, 18 of 27 (66.7%) *mcr-1*-positive isolates carried *bla*_{TEM}, 4 of 27 (14.8%) carried

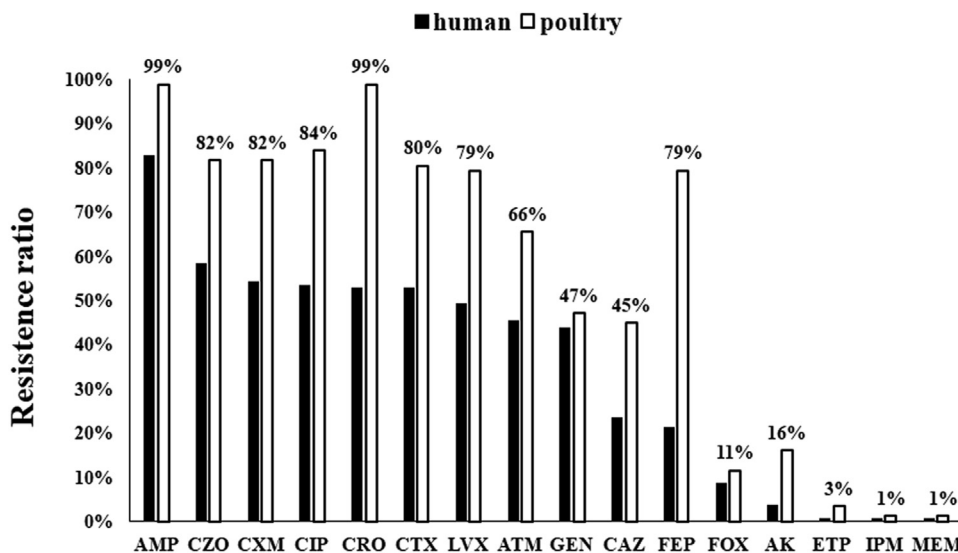


Figure 1. Drug resistance of *Escherichia coli* to 16 commonly used antibiotics. The lowest resistance rates were to IPM and MEM (1%), whereas 83% of the human-derived and 99% of the poultry-derived isolates showed resistance to AMP, and more than half of the human- and poultry-derived isolates were resistant to CZO, CXM, CIP, CRO, and CTX. Abbreviations: AK, amikacin; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxime; CZO, ceftazolin; ETP, ertapenem; FEP, ceftazidime; FOX, ceftazidime; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole.

*bla*_{CMY-2}, and none of the isolates contained *bla*_{SHV} (Table 1).

Transferability of the *mcr-1* and β -Lactamase Genes

To investigate the transfer potential of the plasmids harboring the *mcr-1* gene, 13 of the 27 *E. coli* isolates were subjected to conjugation experiments using *E. coli* J53 as the recipient strain. The results showed that all the plasmids were able to conjugate, and the transconjugants were all confirmed to harbor *mcr-1* and *bla*_{CTX-M-14} genes by PCR. All the transconjugants

were found to be resistant to CTX and POL, whereas 10 of 13 (76.9%) transconjugants showed resistance to aztreonam and 8 of 13 (61.5%) showed resistance to gentamicin (Table 1).

DISCUSSION

E. coli is a common pathogenic bacterium of poultry and humans. As a class of long-serving, reliable, β -lactam antibiotics, carbapenems are potently active against *E. coli* and other gram-negative bacteria and are regarded as the last-line treatment in clinics for MDR bacterial infections (Bi et al., 2018; Cheng et al., 2019).

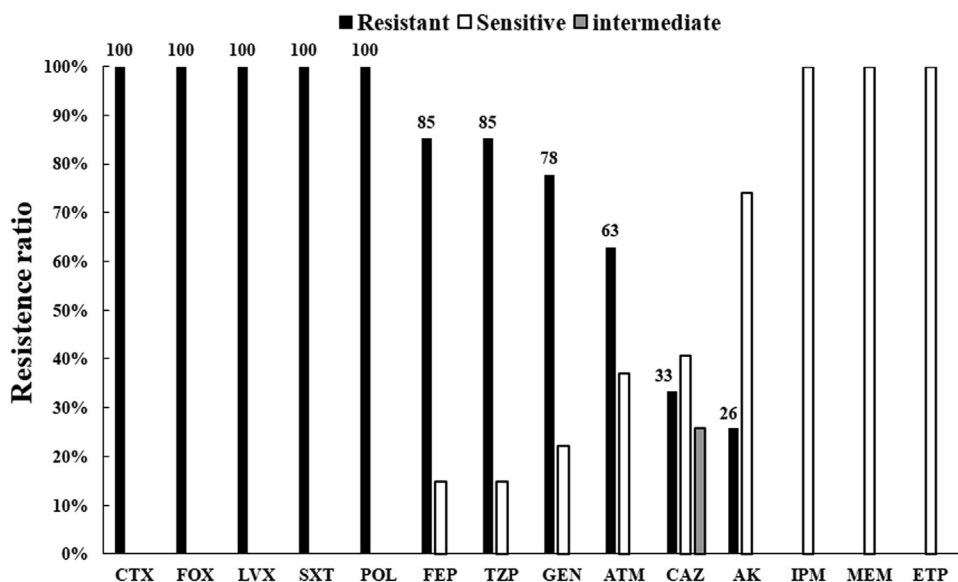


Figure 2. Susceptibility of POL-resistant *Escherichia coli* to 14 commonly used antibiotics. The 27 POL-resistant *E. coli* strains from poultry were sensitive to IPM, MEM, and ETP, and resistant to CTX, FOX, LVX, SXT, and POL. Abbreviations: AK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; ETP, ertapenem; FEP, ceftazidime; FOX, ceftazidime; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; POL, polymyxin; SXT, trimethoprim/sulfamethoxazole.

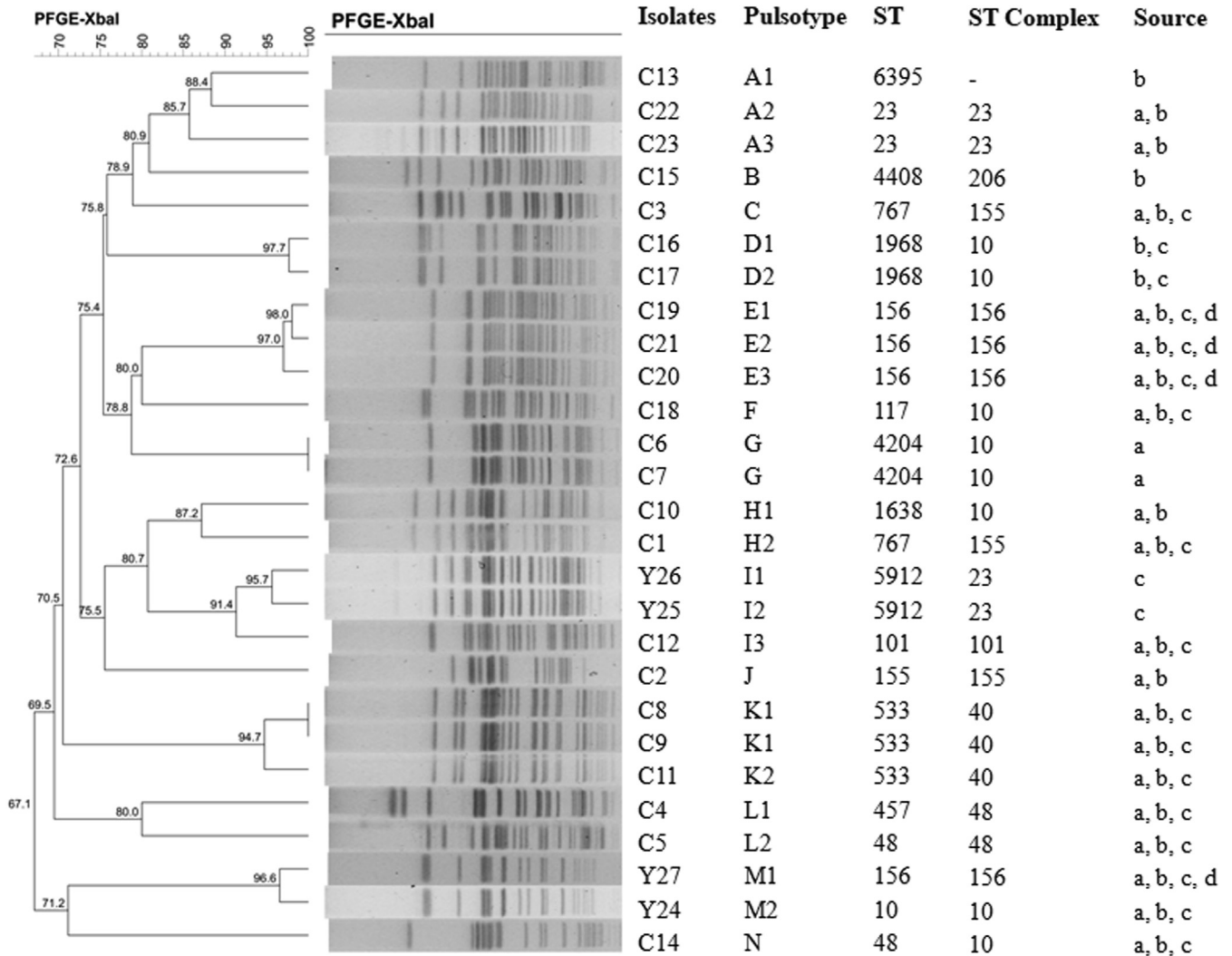


Figure 3. The PFGE and cluster analyses on the POL-resistant avian-origin *Escherichia coli*. Using 85% as the boundary, the bacterial types can be divided into 14 pulstypes, among which strains C13, C22, and C23 were found to be type A; strain C15 was type B; strain C3 was type C, strains C16 and C17 were type D; strains C19, C20, and C21 were type E; strain C18 was type F; strains C6 and C7 were type G; strains C1 and C10 were type H; strains Y26, Y25, and C12 were type I; strain C2 was type J; strains C8, C9, and C11 were type K; strains C4 and C5 were type L; strains Y27 and Y24 were type M; and strain C14 was type N. Epidemic strains A, E, I, and K were identified in this analysis. “-” denotes no ST Complex number. The lower case letters a, b, c, and d represent human-, poultry-, livestock-, and domesticated (companion animal)-derived bacteria ever reported in the MLST database, respectively. Abbreviations: PFGE, pulsed-field gel electrophoresis; POL, polymyxin.

The emerging carbapenemase-resistant Enterobacteriaceae, New Delhi metallo-lactamase (NDM), can mediate resistance to all β -lactams except for monobactams. Since 2009, infections with MDR bacteria harboring *bla*_{NDM} have been reported on almost all continents and are responsible for a global health crisis (Johnson and Woodford, 2013).

Before 2015, sporadic cases of POL-resistant *E. coli* infections did not attract much attention (Gao et al., 2016). However, discovery of the plasmid-mediated POL resistance *mcr-1* gene in *E. coli* isolates from Chinese patients and animals represents a mechanism for the transmission of POL resistance (Liu et al., 2016). The location of *mcr-1* on a plasmid means that it is more easily replicated and can spread horizontally between bacteria, leading to pan-resistant or even superbug strains. Since their initial discovery in China, *mcr-*

1-containing plasmids with various Inc-types have been discovered in gram-negative bacteria worldwide (Malhotra-Kumar et al., 2016; Li et al., 2017; Trung et al., 2017; Cyويا et al., 2018; Brillhante et al., 2019; Vounba et al., 2019).

Food-producing animals, especially poultry, serve as resistance gene “reservoirs” (Liu et al., 2017; Song et al., 2020b; Xiu et al., 2020). Alarming, spread of the plasmid-mediated POL resistance *mcr-1* gene into NDM-producing bacterial isolates can cause untreatable infections (Xu et al., 2019). Recently, isolates of extensively drug-resistant *E. coli* harboring both the *bla*_{NDM} and *mcr* genes were reported, which poses a potential threat to public health (Mediavilla et al., 2016; Yang et al., 2016; Liu et al., 2017; Wang et al., 2017; Lv et al., 2018).

In the present study, the sensitivity of POL was determined, and we found that the *E. coli* isolated from

Table 1. The phenotypes and genes of the POL-resistant *Escherichia coli* strains.

Strain	ST	MIC	Resistance genes	Resistance phenotype
C 1	767	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMP-CZO-FEP-CTX-CRO-CXM-GEN-TE-POL /CTX-POL
C 2	155	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE
C 3	767	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMP-CZO-FEP-CTX-CRO-CXM-GEN-TE-POL /CTX-POL
C 4	457	8/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/ CTX-CAZ-ATM-POL
C 5	48	4	<i>bla</i> _{CTX-M-14} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE
C 6	4,204	8/8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMC-AMP-ATM-CZO-FEP-CTX-FOX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-AK-GEN-POL
C 7	4,204	8/8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMC-AMP-ATM-CZO-FEP-CTX-FOX-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-AK-GEN-POL
C 8	533	8/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMC-AMP-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-GEN-POL
C 9	533	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE
C 10	1,638	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMC-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/ CTX-ATM-GEN-POL
C 11	533	16/8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMC-AMP-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-GEN-POL
C 12	101	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE
C 13	6,395	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE
C 14	48	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-FOX-CAZ-CRO-CXM-CIP-ETP-LVX-TE
C 15	4,408	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-CIP-GEN-LVX-TE
C 16	1,968	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-LVX-TE-POL/CTX-ATM-POL
C 17	1,968	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-LVX-TE-POL/CTX-ATM-POL
C 18	117	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AK-AMP-CIP-GEN-LVX-TE
C 19	156	8/8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMC-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-AK-GEN-POL
C 20	156	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMC-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-AK-GEN-POL
C 21	156	4/4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>bla</i> _{CTX-M-14} , <i>mcr-1</i>	AK-AMC-AMP-ATM-CZO-FEP-CTX-CAZ-CRO-CXM-CIP-GEN-LVX-TE-POL/CTX-ATM-AK-GEN-POL
C 22	23	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-CZO-FEP-CTX-CRO-CXM-CIP-LVX
C 23	23	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-CZO-FEP-CTX-CRO-CXM-CIP-LVX
Y24	10	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{CMY-2} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-FOX-CAZ-CRO-CXM-CIP-LVX-TE
Y25	5,912	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{CMY-2} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-FOX-CAZ-CRO-CXM-CIP-LVX-TE
Y26	5,912	8	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{CMY-2} , <i>mcr-1</i> / <i>not transconjugated</i>	AMP-ATM-CZO-FEP-CTX-CRO-CXM-CIP-GEN-LVX-TE
Y27	156	4	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{CMY-2} , <i>mcr-1</i> / <i>not transconjugated</i>	AMC-AMP-ATM-CZO-FEP-CTX-FOX-CAZ-CRO-CXM-CIP-GEN-LVX-TE

Abbreviations: AK, amikacin; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxime; CZO, cefazolin; ETP, ertapenem; FEP, cefepime; FOX, ceftazidime; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; POL, polymyxin; ST, strain type; SXT, trimethoprim/sulfamethoxazole.

Note: Among these POL-resistant non-repeating *E. coli* strains, C1–C23 strains were isolated from chicken lung tissue samples and Y24–Y27 strains were isolated from duck lung tissue samples.

A breakpoint of >2 µg/mL was considered POL-resistant following EUCAST guidelines http://www.eucast.org/clinical_breakpoints/.

On the left and right sides of the "/" are the phenotypes of the donor *E. coli* and the positive transconjugants, respectively.

poultry had a higher POL-resistance rate than that of *E. coli* from humans. Among the 356 *E. coli* strains isolated from clinical patients, no POL-resistant human-origin *E. coli* was detected. Of the 87 *E. coli* strains isolated from sick poultry (chickens and ducks), 27 were POL-resistant. We detected only one IPM-resistant avian-origin *E. coli* isolate and 2 IPM-resistant human-origin isolates. Therefore, we comprehensively analyzed the *mcr-1* gene carriage status of POL-resistant *E. coli* in poultry and explored the genetic relationships among the bacteria carrying this resistance gene. All of the POL-resistant avian-origin *E. coli* isolates from poultry (27 of 27) carried the *mcr-1* and *bla*_{CTX-M-14}, and 21 of 27 (77.8%) also carried *bla*_{CTX-M-15}.

Horizontal transfer of plasmid-encoded MDR determinants is a major risk to human health and has therefore attracted much public attention (Chen et al., 2016). In the present study, conjugative assays revealed that all plasmids harboring *mcr-1* were successfully transferred to *E. coli* J53 from the 13 donors by conjugation. Among the 13 *mcr-1*-positive transconjugants, all of them showed additional resistance to CTX and harbored *bla*_{CTX-M-14}. This indicates that the high efficiency of transfer of mobile resistance elements facilitates the spread of *mcr-1*-positive ESBL *E. coli*.

In the Tai'an area, *mcr-1* and *bla*_{NDM} were both detected in the poultry and human isolates, although the colocalization of *mcr-1* and *bla*_{NDM} did not occur in 1 *E. coli* strain. With the recognition of *bla*_{NDM} and *mcr-1* as emerging and very serious threats for the health of production animals and humans (Wang et al., 2017), *E. coli* strains coharboring *mcr-1* and *bla*_{NDM} will inevitably accelerate the horizontal transmission of carbapenem and POL resistance among Enterobacteriaceae species (Liu et al., 2017), making it important to reduce antibiotic use, as this is conducive to the development of antibiotic resistance (Roth et al., 2019). Our data and those from previous reports indicate that POL treatments in poultry must be monitored and limited to prevent *mcr-1*-*bla*_{NDM} emergence and restrict its dissemination in Tai'an.

China is one of the countries in the world where POL has always been used for large-scale production operations in farmed animals (Wang et al., 2017). Worryingly, animals treated with POL have become a transmission source of POL-resistant strains (Roth et al., 2019). The emergence and spread of *E. coli* and POL-resistant *E. coli* make antimicrobial treatment challenging and disease outbreaks hard to prevent. Further attention should be directed at reducing the misuse of such drugs in farmed animals and to work harder to avoid the emergence of POL-resistant strains capable of causing a fulminant epidemic. Therefore, current epidemiological investigations should be strengthened to identify the extent of POL-resistant *E. coli* in production animals and in the environment in the Tai'an region of China. We should also ensure the effective monitoring of POL resistance and improve the monitoring methodology to provide accurate antimicrobial sensitivity information for timely clinical control and treatment of infections.

Pulsed-field gel electrophoresis analysis revealed that the 27 POL-resistant avian-origin *E. coli* strains comprised 14 pulsotypes (Figure 3). Pulsotypes A, E, I, and K (the epidemic strains) were distributed and disseminated among poultry in the Tai'an area. Multilocus sequence typing provides a scalable typing system that reflects the population and evolutionary biology of *E. coli*, thereby enabling valid comparisons of the results from different laboratories (Qiu et al., 2019). Multilocus sequence typing of these 27 poultry-origin POL-resistant *E. coli* strains also showed diversity among the isolates. Based on the MLST online database, 11 of 16 (68.8%) ST were found in both human- and poultry-origin *E. coli* (Figure 3). The POL-resistant *E. coli* from poultry was mainly found to be the sporadic ST, suggesting substantial horizontal dissemination of the *mcr-1* gene through poultry-origin *E. coli* populations (Zhuge et al. 2019). Most of the ST were both found in human- and poultry-origin *E. coli*, which indicates that these MDR MCRPEC strains are a potential threat to human health via food chain transmission.

In conclusion, our study documents a high incidence of poultry-origin *E. coli* strains harboring *mcr-1* and *bla*_{CTX-M-14}. *mcr-1*-positive *E. coli* strains were not found in the clinical patients from this study. The coexistence of *mcr-1* and ESBL-encoding genes along with the extremely high rates of MDR amongst the poultry-origin MCRPEC isolates from this study is a major concern. Our data have revealed that MDR MCRPEC strains in poultry are a potential risk to humans. The surveillance findings presented herein will augment our understanding of the prevalence and characteristics of MCRPEC strains in the Tai'an area.

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Conflict of Interest Statement: No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.

Ethics Statement: The study protocol and the poultry studies were approved by the Animal Care and Use Committee of Shandong Agricultural University, Tai'an, China. Human sample collection was carried out in accordance with the approved guidelines of the Ethics Committee of Tai'an City Central Hospital during routine checkups by medical professionals. All the subjects gave written informed consent in accordance with the Declaration of Helsinki.

Data Availability: The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.06.054>.

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