FOODBORNE PATHOGENS AND DISEASE
Volume 17, Number 11, 2020

DOI: 10.1089/fpd.2020.2810

Emergence of Transferable *mcr-9* Gene-Carrying Colistin-Resistant *Salmonella enterica* Dessau ST14 Isolated from Retail Chicken Meat in Korea

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Abstract

Colistin is an important antibiotic currently used to manage infections caused by multidrug-resistant pathogens in both humans and livestock animals. A new mobile colistin-resistance (*mcr-9*) gene was recently discovered; this discovery highlighted the need for rigorous monitoring of bacterial resistance against colistin. *Salmonella* is one of the major pathogens responsible for foodborne illnesses; however, there is minimal information regarding the presence of *mcr* genes in foodborne *Salmonella* strains. The aim of this study was to investigate the presence of *mcr* genes among 178 *Salmonella* strains isolated from chicken meat in Korea. Antimicrobial susceptibility was measured using the broth microdilution method. Bioinformatics characterization of colistin-resistant strains and genetic environment of the *mcr-9* gene were analyzed using next-generation sequencing. Transferability of the *mcr-9* carrying colistin-resistant *Salmonella* strain was tested using broth-mating conjugation. Thirteen of the 178 *Salmonella* isolates showed colistin resistance, but only one strain, *Salmonella* Dessau ST14 (KUFSE-SAL043) from a traditional chicken market in Korea, carried an *mcr* family gene, *mcr-9*. This strain also carried other acquired antimicrobial resistance genes such as $bla_{\text{TEM-1B}}$, *qnrS1*, and *aac(6′)-Iaa*. Only the IncX1 plasmid replicon type was detected in this strain. In the strain KUFSE-SAL043, the *mcr-9* gene was located between two insertion sequences, IS*903B* and IS*26*, followed by the downstream regulatory genes *qseB*-like and *qseC*-like, which were located between IS*1R* and *Δ*IS*1R*. Conjugation tests revealed that the *mcr-9* gene was successfully transferred to *Escherichia coli* J53 at a mean frequency of 2.03×10^{-7}. This is the first report of a transferable *mcr-9* gene in *Salmonella* isolated from chicken meat in Korea, highlighting the possibility of transfer of colistin resistance. Therefore, the wide use of colistin should be reconsidered, and a One Health perspective should be adopted to monitor the antimicrobial resistance of *Enterobacteriaceae* strains in humans, livestock, and the environment.

Keywords: *mcr-9*, *Salmonella*, chicken meat, colistin

Introduction

Colistin is the last therapeutic resort to treat infections by multidrug-resistant *Enterobacteriaceae* (Carroll *et al.*, 2019). Colistin has been widely used in raising livestock, especially for poultry production as a treatment or prevention of enteric diseases and growth-promoting purposes (Rau *et al.*, 2019). However, the emergence, transmission, and spread of mobile colistin-resistance (*mcr*) genes have raised concern over the continued use of colistin and its significance in spread of resistance. *mcr* encodes phosphoethanolamine transferase and induces colistin resistance by modifying the lipid A moiety of lipopolysaccharides (Kieffer *et al.*, 2019). To date, nine *mcr* genes have been

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identified, with the most recent *mcr* gene (*mcr-9*) identified in a colistin-susceptible *Salmonella* strain, highlighting the need for stringent monitoring of the potential spread of this new gene (Carroll *et al.*, 2019). The expression patterns, transferability, resistance characteristics, and molecular dynamics of the *mcr-9* gene have been described in *Enterobacter* spp. and *Escherichia coli* strains isolated in clinical settings (Börjesson *et al.*, 2019; Chavda *et al.*, 2019; Kieffer *et al.*, 2019; Yuan *et al.*, 2019); however, at present, there are no published studies that have characterized the *mcr-9* gene in foodborne strains.

Salmonella is a zoonotic bacterium and an important cause of foodborne illnesses. It represents the second most prevalent bacterial cause of foodborne illnesses in Korea from 2002 to 2020 (www.foodsafetykorea.go.kr). However, *mcr* genes are rarely reported in *Salmonella* compared with other foodborne *Enterobacteriaceae* (Lima *et al.*, 2019). Furthermore, there are no reports of *Salmonella* isolates from Korea carrying an *mcr* gene; thus, their distribution is unclear so far. Therefore, we investigated colistin resistance in *Salmonella* spp. isolated from chicken meat sold in traditional markets or hypermarkets in Korea. For bioinformatics characterization, next-generation sequencing (NGS) was performed on colistin-resistant strains. The genetic environment of the *mcr-9* gene was also investigated. Moreover, broth-mating conjugation experiments were performed to examine the potential of transferability of the *mcr-9* gene from *Salmonella*. This is the first study to verify the presence of the *mcr-9* gene in colistin-resistant *Salmonella* in Korea.

Materials and Methods

Bacterial strains and colistin resistance screening

Colistin resistance was examined in 178 strains of *Salmonella* collected from chicken meat sold in traditional markets and hypermarkets during a Korean nationwide surveillance study involving 33 markets in 22 cities between 2012 and 2017. Colistin resistance was measured in all isolates using the Trekstar Sensititre KNIHCOL custom panel (colistin test range: 0.25–128 μg/mL; Trek Diagnostic Systems, Westlake, OH) according to the manufacturer's instructions. For colistin-resistant strains, the Trekstar Sensititre KRCDCF custom panel (Trek Diagnostic Systems) was used to measure the minimum inhibitory concentration (MIC) of the following antimicrobials: ciprofloxacin (0.12–16 μg/mL), amoxicillin/clavulanate (2:1 ratio, 2–64 μg/mL amoxicillin, 1–32 μg/mL clavulanate), tetracycline (2–128 μg/mL), ampicillin (2–64 μg/mL), gentamicin (1–64 μg/mL), streptomycin (2–128 μ/mL), nalidixic acid (2–128 μg/mL), cefoxitin (2–64 μg/mL), cephalothin (2–64 μg/mL), ceftriaxone (1–32 μg/mL), cefotaxime (1–4 μg/mL), chloramphenicol (2–32 μg/mL), ampicillin/sulbactam (2:1 ratio, 2–32 μg/mL ampicillin, 1–16 μg/mL sulbactam), amikacin (4–64 μg/mL), trimethoprim/sulfamethoxazole (1–16 μg/mL trimethoprim, 19–304 μg/mL sulfamethoxazole), and imipenem (2–16 μg/mL). *Escherichia coli* ATCC 25922 (American Type Culture Collection, Manassas, VA) was used as the quality control strain. Breakpoints for colistin (MIC >2 μg/mL) were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (2019), and all other antimicrobial breakpoints followed the Clinical Laboratory Standards Institute (CLSI) guidelines (2017). Layouts of the two custom panels used in this study are provided in Supplementary Figures S1 and S2.

NGS and bioinformatics analyses

NGS was used to characterize *Salmonella* isolates and identify specific serotypes. The NGS analysis was performed using the Illumina MiSeq platform (Illumina, San Diego, CA), and the genome sequences of colistin-resistant strains were obtained. Identification of the acquired antimicrobial-resistance gene, plasmid replicon typing, serotyping, and multilocus sequence typing (MLST) were performed *in silico* using ResFinder 3.2, PlasmidFinder 2.1, SeqSero 1.2, and MLST 2.0 webserver (https://cge.cbs.dtu.dk, accessed December 20, 2019), respectively (Larsen *et al.*, 2012; Zankari *et al.*, 2012; Carattoli *et al.*, 2014; Zhang *et al.*, 2015). For the *mcr-9* gene-carrying isolate KUFSE-SAL043, further analysis was performed as described hereunder. To carry out the genetic analysis of the *mcr-9* gene, hybrid genome assembly was performed with additional long read sequence data obtained using PacBio Sequel (Pacific Biosciences of California, Menlo Park, CA). The genome was annotated using Rapid Annotation using Subsystem Technology (http://rast.theseed.org) to analyze the genetic environment of the *mcr-9* gene (Aziz *et al.*, 2008). Basic Local Alignment Search Tool (BLAST) was used to align the genetic sequences flanking the *mcr-9* gene, and the results were visualized using Easyfig version 2.2.3 (Sullivan *et al.*, 2011).

Conjugation assay

To test the transferability of the *mcr-9* gene, broth-mating conjugation experiments were performed using the *mcr-9*-containing strain isolated from the chicken meat as the donor (KUFSE-SAL043) and the sodium azide-resistant strain *Escherichia coli* J53 as the recipient (Tamang *et al.*, 2007). Transconjugants were selected on Mueller–Hinton agar (Oxoid, Basingstoke, United Kingdom) plates containing 150 μg/mL sodium azide (Sigma-Aldrich, St. Louis, MO), and 1 μg/mL colistin (Sigma-Aldrich). Additional identification of the transconjugants was performed using the VITEK-MS system (bioMerieux, Marcy l'Etoile, France). The transfer of the acquired resistance genes in the transconjugants was tested using conventional polymerase chain reaction (PCR), and the PCR products were sequenced using Sanger sequencing. Previously reported primers were used for amplification of $bla_{\text{TEM-1B}}$ and *qnrS1* genes (Kim *et al.*, 2009, 2011), and the primers for amplification of *mcr-9* and *aac(6′)-Iaa* genes were designed using the National Center for Biotechnology Information (NCBI) Primer BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast, accessed August 30, 2019) (Ye *et al.*, 2012) (Supplementary Table S1). PCR was performed using Accupower PCR premix (Bioneer, Daejeon, Korea) in accordance with the manufacturer's instructions. The PCR products were subjected to Sanger sequencing (Macrogen, Daejeon, Korea). To validate the PCR primers, KUFSE-SAL043 was used as the positive control and *Escherichia coli* J53 was used as the negative control. To confirm the transfer of colistin, ampicillin, streptomycin, and nalidixic acid resistance, susceptibility tests were performed with KUFSE-SAL043 and the obtained transconjugants using the KRNV5F and KNIHCOL custom panels (Trek Diagnostic

Systems) as described in bacterial strains and colistin resistance screening section of Materials and Methods.

Results

Antimicrobial resistance of colistin-resistant strains

Colistin resistance was tested in 178 *Salmonella* strains, and colistin did not have an MIC ≤0.5 μg/mL against any of the strains. MIC of colistin was 1 and 2 μg/mL against 106 (59.6%) and 59 (33.1%) strains, respectively. Thirteen strains (7.3%) were identified as colistin resistant, with a resistance of 4 μg/mL against 10 (5.6%) strains and 8 μg/mL against three strains (1.7%). All the colistin-resistant isolates were resistant to nalidixic acid, and most of them were resistant to ampicillin (12/13), cefotaxime (10/13), tetracycline (10/13), and gentamicin (8/13). All 13 strains exhibited multidrug resistance, which was defined as resistance to at least 3 subclasses of antimicrobials. However, none of the isolates were resistant to imipenem, chloramphenicol, or amikacin (Table 1).

Detection of mcr-9 *and in silico molecular characteristics*

Table 1 shows the Resfinder, Plasmidfinder, SeqSero, and MLST analysis results for the colistin-resistant strains. Among the 13 colistin-resistant strains, only one *Salmonella* strain was found to harbor the *mcr-9* gene and was designated KUFSE-SAL043 (NCBI GenBank Accession no. JAALJB000000000). This new strain was isolated from commercial chicken meat bought from a Korean traditional market in 2012, and contained other acquired resistance genes, such as $bla_{TEM\text{-}1B}$, *aac(6′)-Iaa*, and *qnrS1*. The serotype of this strain was identified as Dessau, and MLST determined its sequence type to be ST14.

Genetic context of mcr-9 *in KUFSE-SAL043*

Figure 1 provides the overall genetic environment of *mcr-9* in the genome sequence of KUFSE-SAL043. Sequence alignment was performed at a linear interval (∼11 kb) from the IS*903B* element upstream of the *mcr-9* gene to the *Δ*IS*1R* element downstream of the *mcr-9* gene. Sequences with GenBank accession nos. CP029248 (*Enterobacter hormaechei*, dog, United States), CP024910 (*E. hormaechei*, dog, United States), MF344582 (*Citrobacter freundii*, China), CP021137 (*Enterobacter* sp., soybean, Korea), CP030080 (*E. hormaechei*, human, China), and CP020529 (*E. cloacae*, human, United States) were identified as sequences with a query coverage of 100% and sequence identity <99.94%, and were further used for sequence comparison. In KUFSE-SAL043, IS*903B* was located upstream of *mcr-9*, whereas *wbuC*, IS*26*, IS*1R*, *qseB*-like, *qseC*-like, and *Δ*IS*1R* were located downstream. Four of the *Enterobacteriaceae* sequences (CP029248, CP024910, MF344582, and CP021137) revealed similar genetic environments, with *qseB*-like and *qseC*-like sequences found downstream of IS*26* and between two IS*1R*s, whereas the other two genomes (CP030080 and CP020529) did not contain downstream *qseB*-like and *qseC*-like sequences.

Transferability of mcr-9

Conjugation experiments demonstrated the transfer of the *mcr-9* gene from KUFSE-SAL043 to *Escherichia coli* J53 at a mean frequency of 2.03×10^{-7} ($\pm 5.42 \times 10^{-8}$). The NCBI BLAST results showed that the nucleotide sequence of the *mcr-9* PCR product of transconjugant TrECJ53-KUFSE-SAL043 was 100% identical with those of the previously deposited *mcr-9* gene in GenBank. The antimicrobial resistance genes $bla_{TEM\text{-}1B}$, *qnrS1*, and *aac(6′)-Iaa* were amplified from TrECJ53-KUFSE-SAL043 and also confirmed. MIC of colistin against TrECJ53-KUFSE-SAL043 was 2 μg/mL, which was lower than that of the donor strain KUFSE-SAL043. The transconjugant also showed resistance to ampicillin, streptomycin, and nalidixic acid, which was consistent with the results of conventional PCR and Sanger sequencing (Table 2).

Discussion

Salmonella is a major cause of foodborne illnesses, with 95 million *Salmonella* enterocolitis cases, leading to 50,771 deaths, even in 2017 (Stanaway *et al.*, 2019). Antimicrobial resistance of foodborne pathogens is a growing global health concern, complicating the treatment of these infections and contributing to this mortality rate. Colistin is a last resort antimicrobial, but its efficacy is decreasing because of the emergence of drug-resistant strains, leaving patients vulnerable. Continuous use of antimicrobials including colistin in food producing animals has increased the resistance rate of *Salmonella* isolates found in retail chicken. In this study, we investigated the colistin resistance of *Salmonella* isolated from retail chicken meat found in Korean markets. Through antimicrobial susceptibility testing, we found that the colistin resistance rate of *Salmonella* in this study (7.3%) was similar to that reported in previous studies (3–5%) in Korea (Shang *et al.*, 2018; Seo *et al.*, 2019). This resistance rate is lower than a recent study from the Sichuan province in China (13.9%) (Ma *et al.*, 2017). The difference in resistance rates in both studies suggests that the increased use of colistin in livestock industry could promote antibiotic resistance in bacteria.

mcr is a family of genes found to promote colistin resistance in bacteria, such as *E. coli.* Recently, the increasing prevalence of *mcr-1* was reported in *E. coli* isolated from fresh vegetables and livestock in Korea, which was also associated with an increase in multidrug resistance (Oh *et al.*, 2020). However, no *mcr* genes have been reported among colistin-resistant *Salmonella* strains isolated from chicken farms in Korea (Shang *et al.*, 2018; Seo *et al.*, 2019). In this study, we have identified a *Salmonella* strain harboring a member of the *mcr* gene family, and to the best of our knowledge, this is the first study in Korea to have reported this finding. This strain, KUFSE-SAL043, demonstrated an MIC to 4 μg/mL of colistin, which was higher than that previously described in a *mcr-9*-containing *Salmonella* Typhimurium strain, where the MIC of colistin was 2 μg/mL (Carroll *et al.*, 2019).

KUFSE-SAL043 was further identified to belong to the serotype Dessau and sequencing type ST14, both of which are rarely reported in *Salmonella* isolates from Korea. Only one strain of *Salmonella* Typhimurium isolated from pigs in 1995 has been confirmed to be ST14 so far (Yang *et al.*, 2002). In 2009, a study surveyed chicken meat sold in Korean grocery stores for the presence and antimicrobial susceptibility of *Salmonella* serovars and reported three strains of

Table 1. Characteristics of 13 Colistin-Resistant *Salmonella* Isolates from Chicken Meat in Korea from 2012 to 2017

Isolates	*Year of isolation*	*Origin*	*Serotype*	*MLST*	*Plasmid replicon typing*	*Antimicrobial resistance genes*	*COL MIC (μg/mL)*	*Antimicrobial phenotypic resistance*[b]
KUFSE-SAL022	2012	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S), IncX1	*aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(6)-Id*, $bla_{\text{TEM-1B}}$, *catA2*, *sul2*	4	COL, AMP, NAL, STR [128<][c]
KUFSE-SAL027	2012	Traditional market	4:-:1,2	ST19	IncFIB(S), IncFII(S)	*aac(6′)-Iaa*, *aph(6)-Id*	4	COL, TET, GEN, NAL
KUFSE-SAL034	2012	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S), IncHI2, IncHI2A, IncQ1	*aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(6)-Id*, $bla_{\text{CTX-M-15}}$, *sul2*, *tet(A)*	4	COL, TET, AMP, AXO, NAL, FOT, CEP [64<], STR [128<]
KUFSE-SAL043[a]	2012	Traditional market	Dessau	ST14	IncX1	*mcr-9*, *aac(6′)-Iaa*, $bla_{\text{TEM-1B}}$, *qnrS1*	4	COL, AMP, AXO, NAL, CEP [64<], STR [128]
KUFSE-SAL082	2012	Traditional market	Istanbul	ST33	IncFII(S), IncHI2A	*aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(6)-Id*, *tet(A)*	8	COL, TET, AMP, AXO, GEN, NAL, FOT, CEP [64<], STR [128]
KUFSE-SAL123	2013	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S)	*aac(6′)-Iaa*	4	COL, AMP, AXO, GEN, NAL, FOT, CEP [64<], STR [128<]
KUFSE-SAL132	2013	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S), IncX1	*aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(6)-Id*, $bla_{\text{TEM-1B}}$, *sul2*	4	COL, TET, AMP, AXO, A/S2, NAL, FOT, STR [128<]
KUFSE-SAL134	2013	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S), IncQ1	*aac(3)-IId*, *aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(6)-Id*, $bla_{\text{CTX-M-15}}$, *sul2*, *tet(A)*	4	COL, TET, AMP, AXO, GEN, NAL, FOT, CEP [64<], STR [128<]
KUFSE-SAL149	2013	Traditional market	Enteritidis	ST11	IncFIB(S), IncFII(S), IncQ1	*aac(3)-IId*, *aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(6)-Id*, $bla_{\text{CTX-M-15}}$, *sul2*, *tet(A)*	4	COL, TET, AMP, AXO, GEN, NAL, FOT, CEP [64<], STR [128<]
KUFSE-SAL160	2014	Hypermarket	Enteritidis	ST11	IncFIB(S), IncFII(S), IncQ1	*aac(3)-IId*, *aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(6)-Id*, $bla_{\text{CTX-M-15}}$, *sul2*, *tet(A)*	8	COL, TET, AMP, AXO, GEN, NAL, FOT, CEP [64<], STR [128<]
KUFSE-SAL161	2014	Hypermarket	Enteritidis	ST11	IncFIB(S), IncFII(S), IncQ1	*aac(3)-IId*, *aac(6′)-Iaa*, *aph(3″)-Ib*, *aph(6)-Id*, $bla_{\text{CTX-M-15}}$, *sul2*, *tet(A)*	8	COL, TET, AMP, AXO, GEN, NAL, FOT, CEP, STR [128<]
KUFSE-SAL180	2017	Hypermarket	Heidelberg	ST15	ColpVC, IncC, IncI1, IncX1	*aac(6′)-Iaa*, *aph(3″)-Ib*, $bla_{\text{CMY-2}}$, *fosA7*, *sul2*, *tet(A)*	4	COL, AUG2, TET, AMP, AXO, NAL, FOX, FOT, CEP [64<]
KUFSE-SAL187	2015	Hypermarket	Newport	ST166	No hit found	*aac(6′)-Iaa*, *floR*	4	COL, CIP, TET, AMP, AXO, GEN, NAL, SXT, FOT, CEP [64<], STR [128<]

[a]*mcr-9* gene was detected only in strain KUFSE-SAL043.
[b]None of the isolates was resistant to IMI, CHL, or AMI.
[c]There are no Clinical and Laboratory Standards Institute breakpoints for CEP and STR.
MIC value (μg/mL) of these antimicrobials were indicated in brackets.
A/S2, ampicillin/sulbactam 2:1 ratio; AMI, amikacin; AMP, ampicillin; AUG2, amoxicillin/clavulanate 2:1 ratio; AXO, ceftriaxone; CEP, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; FOT, cefotaxime; FOX, cefoxitin; GEN, gentamicin; IMI, imipenem; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

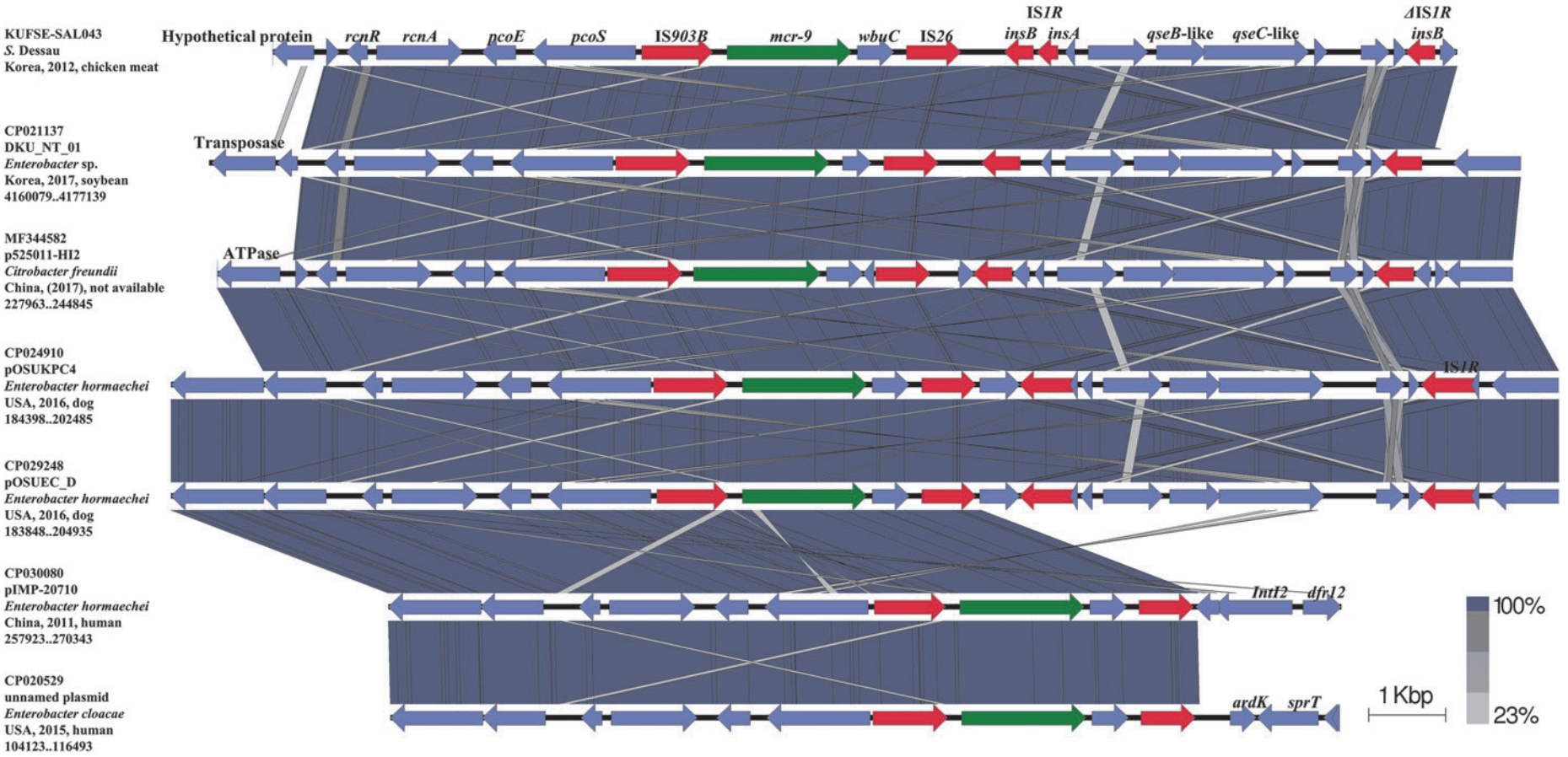

FIG. 1. Genetic environment of the *mcr-9* gene in KUFSE-SAL043 (JAALJB000000000) compared with *mcr-9*-containing regions of different plasmid reservoirs and chromosomes of *Enterobacteriaceae* strains. Arrows indicate the position and direction of the genes and *Δ* indicates truncated genes. *mcr-9* genes are indicated in green, and IS or truncated IS are highlighted in red. Gray shading denotes shared regions with a high degree of similarity. Sequence comparison was performed using BLAST and Easyfig version 2.2.3. BLAST, Basic Local Alignment Search Tool.

Table 2. Conjugation Transfer Efficiency of the *mcr-9* Gene and Antimicrobial Resistance of Transconjugant TrECJ53-KUFSE-SAL043

	Colistin MIC	*Phenotypic resistance*	*Resistance genes*	*Concentration (CFU/mL)*	*Conjugation frequency*[a]
KUFSE-SAL043 (Donor)	4 (μg/mL)	COL, AMP, STR, NAL	*mcr-9*, *aac(6′)-Iaa*, bla_{TEM-1B}, *qnrS1*	4.60×10^{9}	$2.03\times10^{-7}\pm5.42\times10^{-8}$
TrECJ53-KUFSE-SAL043 (Transconjugant)	2 (μg/mL)	COL, AMP, STR, NAL	*mcr-9*, *aac(6′)-Iaa*, bla_{TEM-1B}, *qnrS1*	9.33×10^{2}	
Escherichia coli J53 AZ^{R} (Recipient)	—	—	—	1.37×10^{9}	

[a]Conjugation frequency is presented as mean ± standard deviation.
AMP, ampicillin; CFU, colony-forming unit; COL, colistin; MIC, minimum inhibitory concentration; NAL, nalidixic acid. STR, streptomycin.

Salmonella Dessau (Hyeon *et al.*, 2011). Despite the rare incidence of *Salmonella* Dessau isolation in clinical samples, further studies should include genetic investigation of KUFSE-SAL043 because of inherent pathogenicity of *Salmonella* strains. Furthermore, as the remaining 12 colistin-resistant strains identified in this study did not carry *mcr* genes, there is a need for additional research to identify the underlying *mcr*-independent mechanisms promoting the development of colistin resistance.

We further described the genetic environment of *mcr-9* in KUFSE-SAL043 and identified the presence of IS*26* and IS*1R* in the genome, which revealed a distinct genetic environment compared with previous studies (Chavda *et al.*, 2019; Kieffer *et al.*, 2019; Yuan *et al.*, 2019). Because *mcr-9* was located between IS*903B* and IS*26*, these flanking sequences can also be potentially transferred to other bacteria along with *mcr-9*. In addition, *qseB*-like and *qseC*-like sequences, which are known to influence *mcr-9* gene function (Kieffer *et al.*, 2019), were located downstream of IS*26* and between two IS*1R*s, together with *Δ*IS*1R* in four of the *Enterobacteriaceae* sequences (CP029248, CP024910, MF344582, and CP021137). Because of this proximity, it is possible that this entire region could also be transferred with the *mcr-9* gene. However, *qseB*-like and *qseC*-like sequences were not present downstream of the *mcr-9* gene in the other two *Enterobacteriaceae* genomes (CP030080 and CP020529), indicating that this sequence between IS*903B* and IS*26* and between the two IS*1R*s may not universally exhibit the same behavior during gene transfer or loss. The origin of *mcr-9-wbuC* is not entirely known; however, a previous study suggested that these genes originated from *Buttiauxella* spp., although the *qseB-qseC* tandem was obtained from another source (Kieffer *et al.*, 2019). Our observation that IS*26* and IS*1R* are located between *wbuC* and *qseB*-like sequences strongly supports this assumption. However, further studies are required to fully understand the details of the transfer route of *mcr-9-wbuC* and *qseB-qseC*.

We also determined the transferability of *mcr-9* and other resistance genes from KUFSE-SAL043 to *E. coli*. Previously, *mcr-9* gene dissemination was found to be mediated by the IncHI2- or IncHI2A-type plasmids in human and horse isolates (Börjesson *et al.*, 2019; Carroll *et al.*, 2019; Chavda *et al.*, 2019; Kieffer *et al.*, 2019; Yuan *et al.*, 2019). However, our study detected only the IncX1-type plasmid in the KUFSE-SAL043 strain, suggesting a novel route of *mcr-9* transference. Results obtained from the conjugation assay also elucidated the functional characteristics of *mcr-9*. The MIC of colistin for the conjugated TrECJ53-KUFSE-SAL043 strain was 2 μg/mL, which was lower than that of the donor (4 μg/mL). This suggests that *mcr-9* is not the only factor driving colistin resistance. This is supported by a previous finding that colistin resistance is also affected by the expression rates of *qseB* and *qseC*, which may be transferred together with the *mcr-9* gene (Kieffer *et al.*, 2019). The findings in our study highlight the transferability of the *mcr-9* gene and the need for the reassessment of increased use of colistin in livestock industry.

Conclusions

This study confirmed the presence of the *mcr-9* gene in *Salmonella* isolated from chicken meat sold in retail markets in Korea. This is the first report of a transferable *mcr-9* gene in colistin-resistant *Salmonella* isolated from chicken meat in Korea, further suggesting the possibility of transfer of the flanking sequences IS*903B* upstream and IS*26* and IS*1R* downstream as an entire region. This finding also suggests the possibility of emergence of other colistin-resistant bacteria in chicken farms or retail chicken. The distribution of the *mcr-9* gene and its characteristics are reported in different environments, such as in clinical settings and livestock. Therefore, further studies are required to investigate the distribution, resistance mechanisms, and transmission routes of the newly described colistin-resistance gene, *mcr-9*. Future research should also take into consideration a wide range of food and clinical sources. Our results also support the growing concern that the indiscriminate use of colistin should be carefully reassessed. Therefore, it is necessary to implement a One Health perspective to better monitor the antimicrobial resistance of *Enterobacteriaceae* in humans, animals, and the environment.

Sequence Data

Sequences were deposited to GenBank under accession numbers JAALIP000000000, JAALIQ000000000, JAALIR 000000000, JAALIS000000000, JAALIT000000000, JAALIU 000000000, JAALIV000000000, JAALIW000000000, JAALIX 000000000, JAALIY000000000, JAALIZ000000000, JAALJA 000000000, and JAALJB000000000.

Disclosure Statement

No competing financial interests exist.

Funding Information

This work was supported by the Research Program funded by the Korea National Institute of Health (Grant Nos. 2017-NI41004 and 2017ER540601) and by a grant from the Korea Rural Development Administration (Grant No. PJ010500).

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Table S1

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