Detection of \textit{mcr-1} Plasmids in \textit{Enterobacteriaceae} Isolates From Human Specimens: Comparison With Those in \textit{Escherichia coli} Isolates From Livestock in Korea

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Background: The emerging mobile colistin resistance gene, \textit{mcr-1}, is an ongoing worldwide concern and an evaluation of clinical isolates harboring this gene is required in Korea. We investigated \textit{mcr-1}-possessing \textit{Enterobacteriaceae} among \textit{Enterobacteriaceae} strains isolated in Korea, and compared the genetic details of the plasmids with those in \textit{Escherichia coli} isolates from livestock.

Methods: Among 9,396 \textit{Enterobacteriaceae} clinical isolates collected between 2010 and 2015, 1,347 (14.3%) strains were resistant to colistin and those were screened for \textit{mcr-1} by PCR. Colistin minimum inhibitory concentrations (MICs) were determined by microdilution, and conjugal transfer of the \textit{mcr-1}-harboring plasmids was assessed by direct mating. Whole genomes of three \textit{mcr-1}-positive \textit{Enterobacteriaceae} clinical isolates and 11 livestock-origin \textit{mcr-1}-positive \textit{E. coli} isolates were sequenced.

Results: Two \textit{E. coli} and one \textit{Enterobacter aerogenes} clinical isolates carried carried IncI2 plasmids harboring \textit{mcr-1}, which conferred colistin resistance (\textit{E. coli} MIC, 4 mg/L; \textit{E. aerogenes} MIC, 32 mg/L). The strains possessed the complete conjugal machinery except for \textit{E. aerogenes} harboring a truncated prepilin peptidase. The \textit{E. coli} plasmid transferred more efficiently to \textit{E. coli} than to \textit{Klebsiella pneumoniae} or \textit{Enterobacter cloacae} recipients. Among the three bacterial hosts, the colistin MIC was the highest for \textit{E. coli} owing to the higher \textit{mcr-1}-plasmid copy number and \textit{mcr-1} expression levels. Ten \textit{mcr-1}-positive chicken-origin \textit{E. coli} strains also possessed \textit{mcr-1}-harboring IncI2 plasmids closely related to that in the clinical \textit{E. aerogenes} isolate, and the remaining one porcine-origin \textit{E. coli} possessed an \textit{mcr-1}-harboring IncX4 plasmid.

Conclusions: \textit{mcr-1}-harboring IncI2 plasmids were identified in clinical \textit{Enterobacteriaceae} isolates. These plasmids were closely associated with those in chicken-origin \textit{E. coli} strains in Korea, supporting the concept of \textit{mcr-1} dissemination between humans and livestock.

Key Words: \textit{mcr-1}, Colistin resistance, \textit{Enterobacteriaceae}, IncI2 plasmid

INTRODUCTION

One of the few remaining options for the treatment of infectious diseases caused by multiple-drug resistant gram-negative bacilli is colistin [1]. Accordingly, the emerging mobile colistin resistance gene, \textit{mcr-1}, encoding a phosphoethanolamine transfer-
ase has become a critical threat to public health [2]. Following its initial report [2], studies examining the global dissemination of this gene have rapidly emerged. Various Enterobacteriaceae carrying mcr-1-plasmids from humans, animals, and environments have been identified in Asia, Europe, Africa, and North and South America [3]. The subsequently detected mcr-1.2 derivative [4] and other subtypes (mcr-2 [5], mcr-3 [6], and mcr-4 [7]) are an additional threat. Extensive colistin usage in farm animals [8] and unrestricted international migration of humans [9], livestock, and agricultural products have facilitated the rapid spread of the mcr genes [10].

The mcr-1 gene is carried by conjugative plasmids belonging to various incompatibility groups: IncI1, IncX4, IncHI1B, IncHI2A, IncFII, and IncFIB [11]. The genomic environment of the mcr-1 gene differs by plasmid type [12], frequently being bracketed by one or two copies of ISApl1 [13].

The co-harbouring of resistance genes for third generation cephalosporins and carbapenems adds further complications. Additional plasmids carrying bla<sub>CTX-M</sub> encoding extended-spectrum beta-lactamases (ESBLs) [14, 15], bla<sub>NDM</sub> [14, 16] and bla<sub>IMP</sub> [15] encoding metallo-beta-lactamases, and bla<sub>SHV</sub> [17] encoding serine carbapenemases are often found in mcr-1-possessing strains. Moreover, the mcr-1 gene is co-carried by a plasmid containing the bla<sub>CTX-M-55</sub> [14, 16], and bla<sub>NDM</sub> genes [18, 19]. Plasmids carrying multiple resistance genes are of further concern because bacteria could become extensively- or pan-drug resistant by a single event of horizontal gene transfer. An E. coli strain carrying a mcr-1 plasmid was first reported in 2015 in livestock and humans [2]; however, based on a retrospective analysis, the emergence of the gene dates back to the 1980s in E. coli from chicken specimens [8]. Similar to the case of NDM-1 [20], the mcr-1 gene was reported as a novel emerging resistance gene following worldwide dissemination [3]. The origin of this problematic gene is still controversial; however, based on the fact that the gene is predominant in bacteria from food animals and that colistin is extensively used in the livestock industry [8], food animals are considered responsible for spreading the mcr-1 gene.

We investigated mcr-1-possessing Enterobacteriaceae among Enterobacteriaceae strains isolated in Korea. We identified one Enterobacter aerogenes strain harboring an IncI2 plasmid co-carrying mcr-1 and bla<sub>CTX-M-55</sub> and two E. coli strains possessing a mcr-1-carrying IncI2 plasmid along with plasmids containing multiple-drug resistant genes, including bla<sub>CTX-M-55</sub> and bla<sub>NDM-9</sub>, from clinical specimens. The mcr-1-carrying plasmids were examined in terms of horizontal gene transfer, plasmid copy number, and gene expression, and the genetic details of the plasmids were compared with those in E. coli strains from livestock.

**METHODS**

1. Bacterial strains

We retrospectively analyzed a total of 5,206 clinical isolates, including 2,547 Klebsiella pneumoniae and 2,659 E. coli strains, collected between 2011 and 2015 through the Korean Antimicrobial Resistance Monitoring System, and 4,190 carbapenemase-producing Enterobacteriaceae (CPE) clinical isolates, including 2,738 K. pneumoniae, 565 E. coli, and 887 Enterobacter spp. strains, collected between 2010 and 2015 at the National Laboratory Surveillance of CPE. Eleven E. coli strains from livestock (10 chicken and one porcine) [21] were included in the study for whole genome sequencing.

2. Determination of the minimum inhibitory concentration (MIC) of colistin

From the 9,396 Enterobacteriaceae clinical isolates, colistin-resistant strains were first obtained using 1 mg/L colistin media. For the selected putative colistin-resistant strains, the MIC of colistin was determined using the microdilution method with Mueller–Hinton (MH) broth, following the recommendations of the Joint CLSI-EUCAST Polymyxin Breakpoints Working Group [22], and the confirmed colistin-resistant strains exhibiting colistin MIC ≥2 mg/L were used for further analysis.

3. DNA manipulation and conventional PCR

Genomic DNA of colistin-resistant strains was extracted by the boiling method, and PCR was performed using the AccuPower Taq PCR Premix (Bioneer, Daejeon, Korea). Using the genomic DNA as a template, conventional PCR was carried out to amplify the mcr-1 [2], mcr-3 [6], and mcr-4 [7] genes, as previously described. The mcr-2 gene was amplified using newly designed primers mcr-12-281F (5′-CTTATGGCCAGGCTATAGA-3′) and mcr-12-93R (5′-CACATTTTCTGATTGTTG-3′) under the following conditions: 30 seconds at 97°C for pre-denaturation; 30 cycles of 10 seconds at 97°C, 20 seconds at 53°C, and 30 seconds at 72°C for amplification; and 5 minutes at 72°C for final amplification. Amplified products were subjected to direct sequencing.

4. Whole genome sequencing and comparative genomics

The whole bacterial genomes of the three clinical Enterobacteriaceae isolates harboring the mcr-1 gene underwent single-mol-
ecule real-time (SMRT) sequencing using a PacBio RSII instrument (Pacific Biosciences, Menlo Park, CA, USA), as previously described [23]. The 11 mcr-1 possessing plasmids in E. coli strains from livestock isolated between 2013 and 2015 in Korea [21] were sequenced by SMRT. Further, these plasmids were comparatively analyzed with the three pUSU-ECO-12704_4, pCREC-527_4, and pCRENT-301_1 plasmids from clinical isolates. The 324-bp sequences of the replication origin were obtained from the 11 plasmids, and molecular phylogeny was analyzed together with the three plasmids in the clinical Enterobacteriaceae strains.

5. Determination of gene copy number by quantitative PCR (qPCR)
For the three clinical Enterobacteriaceae isolates, the total DNA was extracted by boiling and used for qPCR. The CLR5-F and -R primers targeting the mcr-1 gene and primer pair incl2_6386 (5′-GATTGTAAATGCAGAAAACGAGG-3′) and incl2_273-250 (5′-GAGTTGATTTTTCTTCATGGA-3′) targeting the incl2 gene were used. The plasmid copy number was normalized to that of the gyrB gene using gyrB-F_1466-1489 (5′-GTATTCAAGACTCATATCATGTA-3′) and gyrB-R_1650-1627 (5′-TTGCTGCTTTAAAAGTACTGGTC-3′) and that of rpoD using rpoD-F_883-906 (5′-CTGTCGTTCTTTAATGTACTGTTC-3′) and rpoD-R_1085-1062 (5′-TTGATTTGTAAATGCAGAAAACGAGG-3′). Standard curves were generated using five 10-fold serial dilutions of DNA for each target gene. All experiments were carried out in duplicate and at least twice independently.

6. RNA isolation and reverse transcription (RT) qPCR
Total RNA was extracted at the exponential phase using the RNeasy plus mini kit (Qiagen, Hilden, Germany). The mcr-1 transcriptome was determined using the LightCycler RNA amplification kit with SYBR green I (Roche Diagnostics, Basel, Switzerland) and normalized to that of the rpoD and gyrB using the primer sets mentioned above. Experiments were carried out in duplicate at least twice independently.

7. Plasmid transfer by bacterial conjugation
Spontaneous rifampin-resistant mutants of E. coli J53, K. pneumoniae ATCC 13883, and E. cloacae ATCC 23355 were used as recipients. K. pneumoniae ATCC 13883 possesses undisturbed IncFII and IncFIA plasmids, and E. cloacae ATCC 23355 does not contain marked plasmids, as determined by plasmid mini prep. Equal amounts of exponential cultures of the donor and the recipient strains were mixed, incubated either in MH broth or on a membrane filter (Merck Millipore, Darmstadt, Germany) for 12 hours, and then spread on Brain Heart Infusion agar containing rifampin (40 mg/L) and colistin (2 mg/L). Each colony was tested by the disk diffusion method and confirmed by PCR. The plasmid transfer frequency was calculated based on the number of transconjugants per donor.

8. Accession numbers
Nucleotide sequence data are available in the GenBank nucleotide database under accession numbers KY657478 (pUSU-ECO-12704_4), KY657476 (pCREC-527_4), and KY657477 (pCRENT-301_1).

RESULTS
1. Identification of three Enterobacteriaceae clinical isolates carrying the mcr-1 gene
Of the 9,936 clinical Enterobacteriaceae strains tested, 14.3% (1,347/9,396) strains, including 15.3% (810/5,285) K. pneumoniae, 10.5% (340/3,224) E. coli, and 22.2% (197/887) Enterobacter spp. strains, exhibited colistin MIC ≥2 mg/L. Among these, the mcr-1 gene was identified by PCR in two E. coli isolates, USU-ECO-12704 and CREC-527, collected in 2012 and 2015, respectively, and in one E. aerogenes strain, CRENT-301, isolated in 2013. These strains were all isolated from urine specimens collected in different provinces of Korea (Table 1). The mcr-2, mcr-3, and mcr-4 genes were not detected in any of the isolates. No known amino acid substitution in PmrABC conferring colistin resistance was observed in the three chromosomes. The E. aerogenes CRENT-301 strain had the highest colistin MIC (32 mg/L), and the E. coli USU-ECO-12704 and CREC-527 strains exhibited identical colistin MICs of 4 mg/L (Table 2).

Table 1. Clinical isolates used in the study

| Strain                  | Isolated* in City | Year | Resistant to [33]                                      |
|------------------------|-------------------|------|-------------------------------------------------------|
| Escherichia coli USU-ECO-12704 | Ulsan             | 2012 | Gentamicin, tobramycin, tetracycline, ciprofloxacin, ampicillin, ceftazidime, colistin |
| Enterobacter aerogenes CRENT-301 | Incheon          | 2013 | Cefotaxime, colistin                                    |
| E. coli CREC-527       | Seoul             | 2015 | Tetracycline, ampicillin, ceftazidime, imipenem, colistin |

*All isolates were from urine specimens.
**Table 2.** Colistin MICs in the mcr-1 harboring Enterobacteriaceae

| Strain                          | Colistin MIC (mg/L) |
|--------------------------------|---------------------|
| *Escherichia coli* USU-ECO-12704 | 4                   |
| *Enterobacter aerogenes* CRENT-301 | 32                  |
| *E. coli* CREC-257              | 4                   |
| *E. coli* ATCC 25922            | 0.5                 |
| *E. coli* EJ53-RiR              | 0.5                 |
| *E. coli* EJ53-RiR / pUSU-ECO-12704_4 | 4                   |
| *Klebsiella pneumoniae* Kpn-RiR | 2                   |
| *K. pneumoniae* Kpn-RiR / pUSU-ECO-12704_4 | 8                   |
| *Enterobacter cloacae* Ecl-RiR  | 0.5                 |
| *E. cloacae* Ecl-RiR / pUSU-ECO-12704_4 | 1                   |

Abbreviation: MIC, minimal inhibitory concentration.

2. Colistin MICs conferred by mcr-1

Transconjugants carrying the mcr-1-possessing pUSU-ECO-12704_4 exhibited various MICs of colistin according to the bacterial host. The *E. coli* transconjugant carrying the plasmid exhibited an 8-fold-elevated colistin MIC (4 mg/L), while the *K. pneumoniae* transconjugant had a 4-fold higher MIC (8 mg/L), and the *E. cloacae* transconjugant presented a 2-fold higher colistin MIC (1 mg/L; Table 2). The transconjugant plasmid copy number was the highest (11 copies/genome equivalent [GE]) in the *E. coli* host, six copies/GE in *K. pneumoniae*, and the lowest (one copy/GE) in *E. cloacae*. mcr-1 gene expression corresponded to the plasmid copy number of the bacterial hosts.

3. Genomic insights into the three mcr-1-positive Enterobacteriaceae strains

The genome of *E. coli* USU-ECO-12704 sequence type (ST) 1011 (adk-fumC-gyrB-ica-mdh-purA-recA, 6-4-159-44-112-1-17) consisted of a 5.0-Mb chromosome containing seven drug resistance genes (*bla*TEM, *dfrA*, *aadA*, *sul1*, *mphA*, *aac(3)-II*, and *blaCTX-M-55*), and five plasmids: a 94,115-bp p0111-type plasmid possessing a *tet(A)* gene; an 89,509-bp IncFIB 1/IncFII 34 plasmid carrying *oxaAoxB* genes; a 70,600-bp IncFII plasmid containing the *bla*TEM, *blaCTX-M-55*, and *fosA* genes; a 60,948-bp IncI2 plasmid carrying the *mcr-1* gene; and a 20,902-bp cryptic plasmid of unidentified incompatibility group.

The *E. coli* CREC-527 strain belonged to ST101 (43-41-15-18-11-7-6). Its genome consisted of a 4.8-Mb chromosome possessing the *bla*TEM, *dfrA*, *tet(A)*, *aph(3’)-1*, *mphA*, and *catA* drug resistance genes and five plasmids: a 118,328-bp p0111-type plasmid carrying *fosA*, *dfrA*, *aadA2*, *sul1*, *mphA*, and *blaNDM-9*; a cryptic 96,521-bp p0111-type plasmid; a 79,911-bp IncFIB plasmid carrying *stra* and *blaCTX-M-27*; a 60,959-bp IncI2 plasmid carrying the *mcr-1* gene; and a cryptic 8,678-bp plasmid of unidentified incompatibility group.

The genome of *E. aerogenes* CRENT-301 consisted of a 5.3-Mb chromosome without any acquired resistance determinants and two plasmids: a 67,073-bp IncI2 plasmid carrying *mcr-1* and *blaCTX-M-55* genes and a 33,722-bp cryptic plasmid.

4. IncI2 plasmids carrying the mcr-1 gene in clinical *E. coli* isolates

The three *mcr-1*-plasmids were designated as pUSU-ECO-12704_4 in *E. coli* USU-ECO-12704, pCREC-527_4 in *E. coli* CREC-527, and pCRENT-301_1 in *E. aerogenes* CRENT-301. The structure of the three IncI2 plasmids differed from that of the first identified pHNSHP45 containing a type IV secretion system and a relaxase [2]. pCRENT-301_1 shared the most similarity with pHNSHP45 (Fig. 1A); compared with pHNSHP45, pCRENT-301_1 contains IScep1-blaCTX-M-55 downstream of the parA gene, IS903B interrupting a pilU gene resulting in a 93-aa-premature 211-aa PilU protein, and a differently arranged shufflon. The other two plasmids in *E. coli* strains were indistinguishable (99% nucleotide identity) and have a replication initiation protein sharing 97.95% aa identity (97.57% nucleotide identity) with that in pHNSHP45. Interestingly, pCREC-527_4 has nucleotide substitutions in the 5-region of pilS, resulting in a shortened PilS protein, a truncated hypothetical protein following a one nucleotide deletion of the heptameric adenine, and a rearranged shufflon (Fig. 1B).

The -35 and -10 sequences of the mcr-1 promoter region were identified by 5´ rapid PCR amplification of cDNA ends and compared with the pAf23 plasmid from a clinical *E. coli* strain isolated in South Africa [24]. The sequences in pUSU-ECO-12704_4 and pCREC-527_4 were identical to those of pAf23, while that in pCRENT-301_1 contained a substitution in the -10 sequence generating a weaker consensus sequence (TAAAT vs TATAAT) relative to the other two plasmids. The putative ribosomal binding site (RBS) of *mcr-1* in pCRENT-301_1 was GAGTAG, identical to that in pAf23, while the RBS of the other two plasmids had a one nucleotide difference, GATTAG.

The frequency of pUSU-ECO-12704_4 plasmid transfer was the highest, 2.8×10⁵ on the membrane surface and 1.1×10⁵ in liquid. Plasmid pCREC-527_4 transfer frequency was 9.6×10⁴ on the membrane surface and <1.0×10¹⁰ in liquid. Plasmid pCRENT-301_1 transfer frequency was below the 1.0×10¹⁰ detection threshold, both on the membrane surface and in liquid. For *E. coli* USU-ECO-12704, which had the high-
est conjugal efficiency, mating was carried out with K. pneumoniae and E. cloacae recipients, and plasmid transfer was observed in only liquid media with a frequency of \(3.0 \times 10^{-9}\) in K. pneumoniae and \(1.1 \times 10^{-10}\) in E. cloacae.

An IncI2 plasmid possessing the mcr-1 gene found in an E. coli isolate from the blood culture of a patient with cholangitis identified in Korea [25] exhibits a nearly identical structure to that of pCRENT-301_1, except for one copy of insertion se-

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Fig. 1. Comparative analyses of the mcr-1-plasmids from clinical Enterobacteriaceae strains and livestock-origin Escherichia coli strains. (A) Left, molecular phylogeny was conducted by neighbor joining analysis of nucleotide sequences (324 bp) of the replication origin of the mcr-1-plasmids. Multiple sequence alignments were performed with MUSCLE v3.8, and the phylogenetic tree was reconstructed using the distance method implemented in the BioNJ program [34]. Plasmid names are indicated in each taxon along with the size in brackets. Strain name and GenBank accession number are indicated below the plasmid name, if available. Color codes: Black, clinical Enterobacteriaceae strains; blue, E. coli from healthy chickens; red, E. coli from chicken carcasses; and green, E. coli from diseased pig. Right, schematic representation of plasmid structures. The sequence of each plasmid was aligned using BlastN and compared using the Artemis Comparison Tool. Highly-conserved regions (>96% nucleic acid identity) are indicated in red, and moderately conserved regions (>92% nucleic acid identity) are indicated in blue. Open arrows, open reading frames; blue, the replication origin; red, antimicrobial resistance; yellow, plasmid backbone; orange, transposases; and green, plasmid transfer. (B) The yellow arrow indicates the site-specific recombinase rci gene, and the green arrow indicates the pilus assembly pV gene. Black arrowheads represent the six 19-bp repeats. Open reading frames in the direction of translation are indicated by arrows.
quence IS679 downstream of the replication origin (Fig. 1A). The promoter sequences of the two plasmids are also identical.

5. Comparison of mcr-1-plasmids in E. coli strains from those in livestock

Ten of the 11 mcr-1-plasmids in E. coli strains from either healthy chickens or chicken carcasses were Incl2 type, the same as the three plasmids in clinical Enterobacteriaceae strains. Interestingly, one E. coli plasmid from a diseased pig belonged to type IncX4. The 10 Incl2-type plasmids were 60,652 to 70,121 bp in length, and the plasmid backbone was indistinguishable from that of pCRENT-301_1, except the shufflon region (Fig. 1A). The longest pS03-109 plasmid had a 7,758-bp duplication of the region including conjugative elements resulting in a length of 70,121 bp. Remarkably, pA03-007 possessed a copy of insertion sequence ISAp1 upstream of the mcr-1 gene. The only IncX4-type plasmid was 46,931 bp in length. This plasmid shared only the mcr-1 gene and the downstream partial pap2 with the ten Incl2 plasmids. None of the 11 plasmids in livestock isolates possessed known antimicrobial resistance determinants except for mcr-1.

The phylogeny corresponded to plasmid structure analysis. The pCRENT-301_1 plasmid in clinical E. aerogenes was included in the clade of 11 plasmids in livestock isolates, while the other two plasmids in clinical E. coli strains belonged to a clade split from the other Incl2-type plasmids.

DISCUSSION

Following a report on livestock-origin mcr-1-positive E. coli strains isolated between 2013 and 2015 in Korea [21], we report three clinical Enterobacteriaceae strains carrying the gene, identified in 2012, 2013, and 2015. The three strains were well-prepared in terms of fitness in clinical settings. pCRENT-301_1 in E. aerogenes co-carried the blaCTX-M-35 gene encoding ESBL, which could provide a great advantage when encountering the third generation cephalosporins; the two mcr-1-positive E. coli strains harbored secondary plasmids carrying multiple genes for antimicrobial resistance, including blaCTX-M-35 and blaCTX-M-27 encoding ESBLs and blaqom3 encoding a carbapenemase.

In terms of molecular epidemiology, the three strains, one E. aerogenes and two E. coli belonging to either ST1011 or ST101, were clearly distinct. Among the 11 livestock isolates from Korea, only one E. coli strain was ST101 [21]. An E. coli ST101 clinical isolate from Brazil harboring an IncX4 mcr-1-plasmid has been identified [26], and an E. coli ST1011 strain possessing an IncX4 mcr-1-plasmid has been isolated from a sputum specimen in Egypt [27].

Despite the divergent hosts, the incompatibility type of all mcr-1-plasmids in clinical isolates was Incl2. The representative Incl2 plasmid, R721, possesses pil and tra gene clusters assembling thin and thick conjugal pili, respectively [28], necessary for plasmid transfer [29]. Among the three Incl2 plasmids, pCRENT-301_1 completely lost conjugal capacity, possibly owing to a truncated PilU prepilin peptidase, which is essential for thin pilus formation [30]. The pCREC-527_4 plasmid produces a premature PilS pilin precursor resulting in diminished plasmid transfer efficiency. In addition, the rearrangement of the pilV gene due to rci-derived DNA reshuffling allowed recipient-specificity [31]; pUSU-ECO-12704_4 exhibited higher conjugal efficiency in E. coli compared with the other species recipients. Interestingly, the pUSU-ECO-12704_4 plasmid conferred a different level of colistin resistance in each of the bacterial hosts, which was compatible with plasmid copy number. The relatively lower plasmid transfer rates compared with that of the first identified mcr-1 plasmid, pHNShP45 [2], could be because of bacterial host background, corresponding with the diverse transfer efficiency of Incl2 mcr-1-plasmids in livestock isolates [21].

The notably higher colistin MIC of E. aerogenes CREN-301 compared with the other two E. coli strains could also be associated with bacterial host background. Enterobacteriaceae strains harboring the mcr-1 plasmid varied in colistin susceptibilities by species (Table 2). In addition, plasmid copy number, which was likely responsible for the elevated colistin MIC from the recipient strain, was dependent on bacterial species.

The 11 mcr-1-plasmids in livestock isolates belonged to two incompatibility groups and differed according to the isolate origin: the plasmids in chicken isolates were Incl2, similar to pHNShP45 and the plasmids in clinical isolates, while the plasmid in the pig isolate was type IncX4. Except for incompatibility type, no specific trait differences were identified in the pig isolates [21]. ISAp1 was likely involved in mcr-1 gene acquisition [13, 32] in the plasmids, and the insertion sequence was identified in one of the 11 plasmids. Unfortunately, only the nucleotide sequences of the mcr-1-possessing plasmids were available for the 11 livestock isolates; thus, no further analyses were possible.

This study has an obvious limitation concerning the clarification of mechanisms involved in colistin resistance. Among the subjected isolates, 1,347 were resistant to colistin; however, only three of those conferred resistance by harboring the mcr-1 gene, and the others remained unknown. The mechanism of resistance, moreover, a novel type of mcr gene, should be in-
vestigated in the future.

The mcr-1–possessing strains identified in this study support this concept. Better stewardship for the proper usage of antimicrobials, as well as collaborative surveillance in terms of One Health, is essential.

**Authors’ Disclosures of Potential Conflicts of Interest**

No potential conflicts of interest relevant to this article were reported.

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