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

Eun-Jeong Yoon, R.Ph., Ph.D.[1], Jun Sung Hong, B.S.[1,2], Ji Woo Yang, B.S.[3], Kwang Jun Lee, Ph.D.[3], Hyukmin Lee, M.D., Ph.D.[1], and Seok Hoon Jeong, M.D., Ph.D.[1]

[1]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea; [2]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea; [3]National Institute of Health, Centers for Disease Control and Prevention, Cheongju, Korea

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

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

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

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

Key Words: *mcr-1*, Colistin resistance, *Enterobacteriaceae*, IncI2 plasmid

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Corresponding author: Seok Hoon Jeong
https://orcid.org/0000-0001-9290-897X
Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea
Tel: +82-2-2019-3532
Fax: +82-2-2057-8926
E-mail: kscpjsh@yuhs.ac

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, *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: IncI2, 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 IS*Apl1* [13].

The co-harboring of resistance genes for third generation cephalosporins and carbapenems adds further complications. Additional plasmids carrying $bla_{\text{CTX-M}}$ encoding extended-spectrum beta-lactamases (ESBLs) [14, 15], bla_{NDM} [14, 16] and bla_{IMP} [15] encoding metallo-beta-lactamases, and bla_{KPC} [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 $bla_{\text{CTX-M-55, -14, -65}}$, and $bla_{\text{NDM-5}}$ 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 $bla_{\text{CTX-M-55}}$ and two *E. coli* strains possessing a *mcr-1*-carrying IncI2 plasmid along with plasmids containing multiple-drug resistant genes, including $bla_{\text{CTX-M-55}}$ and $bla_{\text{NDM-9}}$, 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 $\geq$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′-CTTATGGCACGGTCTATGA-3′) and *mcr-12*-93R (5′-CACATTTTCTTGGTATTTGG-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-

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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 incI2_63-86 (5′-GATTTGTAAATGCAGAAAACGAGG-3′) and incI2_273-250 (5′-GAGTTGATATTTCCTTCTCATGGA-3′) targeting the *incI2* gene were used. The plasmid copy number was normalized to that of the *gyrB* gene using gyrB-F_1466-1489 (5′-GTTATCACAGCATCATCATCATGA-3′) and gyrB-R_1650-1627 (5′-TTCGTCGTCTTTAATGTACTGTTC-3′) and that of *rpoD* using rpoD-F_883-906 (5′-C-3′) and rpoD-R_1085-1062 (5′-TTGATATCTTTAACCTGCTCGATG-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 *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 9,396 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		Resistant to [33]
	City	Year	
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-RifR	0.5
E. coli EJ53-RifR / pUSU-ECO-12704_4	4
Klebsiella pneumoniae Kpn-RifR	2
K. pneumoniae Kpn-RifR / pUSU-ECO-12704_4	8
Enterobacter cloacae Ecl-RifR	0.5
E. cloacae Ecl-RifR / 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-icd-mdh-purA-recA*, 6-4-159-44-112-1-17) consisted of a 5.0-Mb chromosome containing seven drug resistance genes (bla_{TEM-1}, *dfrA*, *aadA*, *sul1*, *mphA*, *aac(3)-II*, and $bla_{CTX-M-55}$) and five plasmids: a 94,115-bp p0111-type plasmid possessing a *tet(A)* gene; a 89,509-bp IncFIB 1/IncFII 34 plasmid carrying *oqxA/oqxB* genes; a 70,600-bp IncFII plasmid containing the bla_{TEM-1}, $bla_{CTX-M-55}$, 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-1}, *dfrA*, *tet(A)*, *aph(3')-1*, *mph(A)*, and *catA* drug resistance genes and five plasmids: a 118,328-bp p0111-type plasmid carrying *fosA*, *dfrA*, *aadA2*, *sul1*, *mphA*, and bla_{NDM-9}; a cryptic 96,521-bp p0111-type plasmid; a 79,911-bp IncFIB plasmid carrying *strA* and $bla_{CTX-M-27}$; 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 $bla_{CTX-M-55}$ 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 IS*Ecp1*-$bla_{CTX-M-55}$ downstream of the *parA* gene, IS*903B* 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 (TAAAAT 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, 2.8×10^{-5} on the membrane surface and 1.1×10^{-5} in liquid. Plasmid pCREC-527_4 transfer frequency was 9.6×10^{-7} on the membrane surface and $<1.0\times10^{-10}$ in liquid. Plasmid pCRENT-301_1 transfer frequency was below the 1.0×10^{-10} detection threshold, both on the membrane surface and in liquid. For *E. coli* USU-ECO-12704, which had the high-

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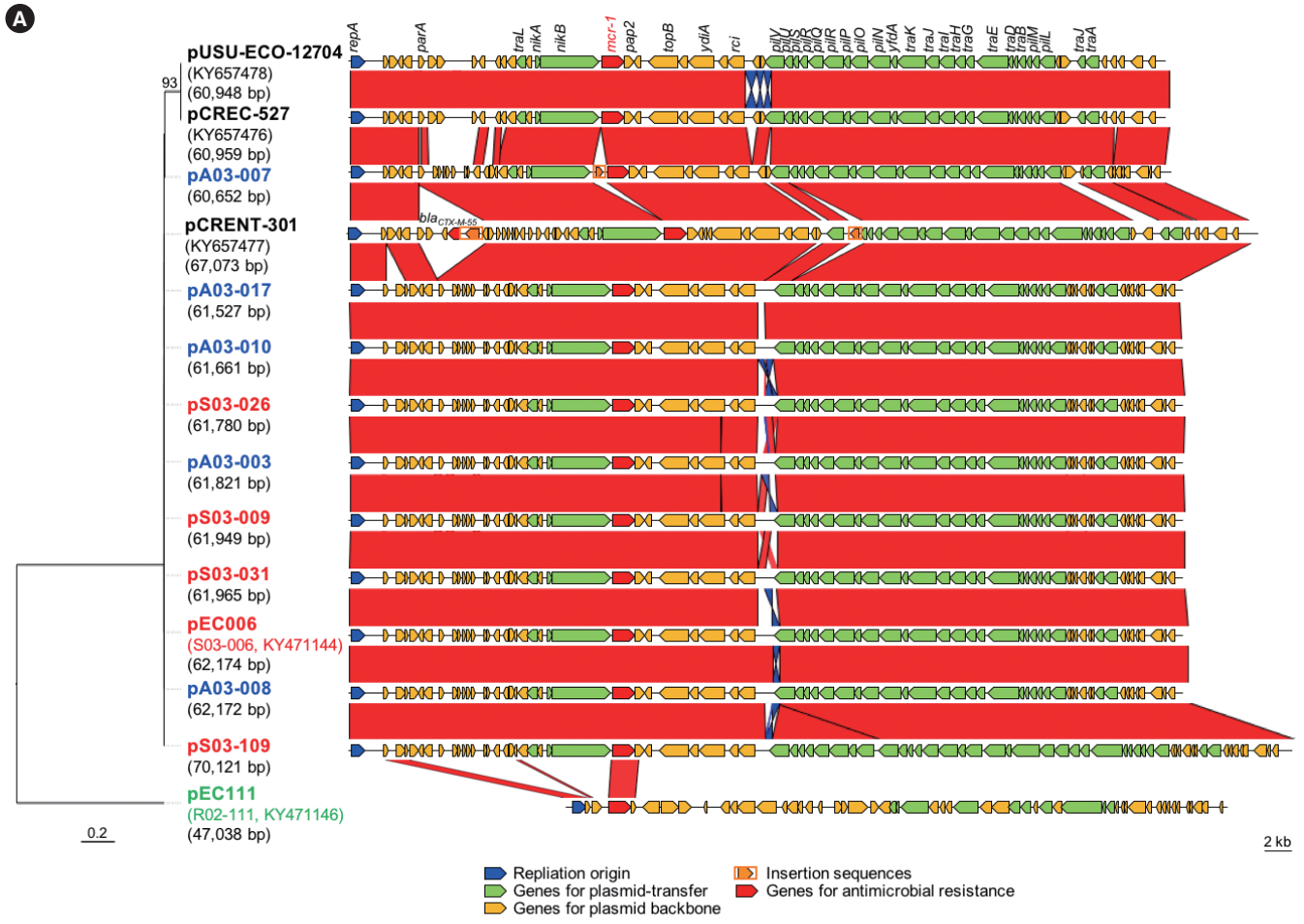

Fig. 1. Comparative analyses of the *mcr-1*-plasmids from clinical *Enterobacteriaceae* strains and livestock-origin *Escherichia coli* strains. (A) Left, molecular phylogeny was conducted by neighbor joining analysis of nucleotide sequences (324 bp) of the replication origin of the *mcr-1*-plasmids. 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 *pilV* gene. Black arrowheads represent the six 19-bp repeats. Open reading frames in the direction of translation are indicated by arrows.

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 3.0×10^{-9} in *K. pneumoniae* and 1.1×10^{-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-

quence IS*679* 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 IncI2 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 IncI2-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 shuffle 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 IS*Apl1* 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 IncI2 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 IncI2-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 $bla_{\text{CTX-M-55}}$ 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 $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-27}}$ encoding ESBLs and $bla_{\text{NDM-9}}$ 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 IncI2. The representative IncI2 plasmid, R721, possesses *pil* and *tra* gene clusters assembling thin and thick conjugal pili, respectively [28], necessary for plasmid transfer [29]. Among the three IncI2 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 IncI2 *mcr-1*-plasmids in livestock isolates [21].

The notably higher colistin MIC of *E. aerogenes* CRENT-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 IncI2, 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]. IS*Apl1* 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-

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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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REFERENCES

1. Giamarellou H and Poulakou G. Multidrug-resistant gram-negative infections: what are the treatment options? Drugs 2009;69:1879-901.
2. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism *MCR-1* in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16:161-8.
3. Skov RL and Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro Surveill 2016;21:30155.
4. Di Pilato V, Arena F, Tascini C, Cannatelli A, Henrici De Angelis L, Fortunato S, et al. *mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512. Antimicrob Agents Chemother 2016;60:5612-5.
5. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 2016;21:30280.
6. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. MBio 2017;8:e00543-17.
7. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, et al. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 2017;22:30589.
8. Shen Z, Wang Y, Shen Y, Shen J, Wu C. Early emergence of *mcr-1* in *Escherichia coli* from food-producing animals. Lancet Infect Dis 2016;16:293.
9. von Wintersdorff CJ, Wolffs PF, van Niekerk JM, Beuken E, van Alphen LB, Stobberingh EE, et al. Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in faecal metagenomes of Dutch travellers. J Antimicrob Chemother 2016;71:3416-9.
10. Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, et al. Antibiotic resistance is the quintessential One Health issue. Trans R Soc Trop Med Hyg 2016;110:377-80.
11. Gao R, Hu Y, Li Z, Sun J, Wang Q, Lin J, et al. Dissemination and mechanism for the *MCR-1* colistin resistance. PLoS Pathog 2016;12:e1005957.
12. Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Kasbohrer A, Roesler U, et al. Colistin resistance gene *mcr-1* in extended-spectrum beta-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis 2016;16:282-3.
13. Snesrud E, Ong AC, Corey B, Kwak YI, Clifford R, Gleeson T, et al. Analysis of Serial Isolates of *mcr-1*- positive *Escherichia coli* reveals a highly active IS*Apl1* transposon. Antimicrob Agents Chemother 2017;61:e00056-17.
14. Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the *mcr-1* colistin resistance gene in carbapenem-resistant *Enterobacteriaceae*. Lancet Infect Dis 2016;16:287-8.
15. Zhang XF, Doi Y, Huang X, Li HY, Zhong LL, Zeng KJ, et al. Possible transmission of *mcr-1*-harboring *Escherichia coli* between companion animals and human. Emerg Infect Dis 2016;22:1679-81.
16. Yang RS, Feng Y, Lv XY, Duan JH, Chen J, Fang LX, et al. Emergence of NDM-5- and *MCR-1*-producing *Escherichia coli* clones ST648 and ST156 from a single muscovy duck (*Cairina moschata*). Antimicrob Agents Chemother 2016;60:6899-902.
17. Teo JQ, Ong RT, Xia E, Koh TH, Khor CC, Lee SJ, et al. *mcr-1* in multidrug-resistant bla_{KPC-2}-producing clinical *Enterobacteriaceae* isolates in Singapore. Antimicrob Agents Chemother 2016;60:6435-7.
18. Sun J, Li XP, Yang RS, Fang LX, Huo W, Li SM, et al. Complete nucleotide sequence of an IncI2 plasmid coharboring $bla_{CTX-M-55}$ and *mcr-1*. Antimicrob Agents Chemother 2016;60:5014-7.
19. Quan J, Li X, Chen Y, Jiang Y, Zhou Z, Zhang H, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. Lancet Infect Dis 2017;17:400-10.
20. Moellering RC Jr. NDM-1—a cause for worldwide concern. N Engl J Med 2010;363:2377-9.
21. Lim SK, Kang HY, Lee K, Moon DC, Lee HS, Jung SC. First detection of the *mcr-1* gene in *Escherichia coli* isolated from livestock between 2013 and 2015 in South Korea. Antimicrob Agents Chemother 2016;60:6991-3.
22. The Joint CLSI– European Committee on Antimicrobial Susceptibility Testing (EUCAST) Polymyxin Breakpoints Working Group. Recommendations for MIC determination of colistin (polymyxin E). Wayne, PA: Clinical and Laboratory Standards Institute. 2016. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf (Updated on Mar 2016).
23. Hong JS, Yoon EJ, Lee H, Jeong SH, Lee K. Clonal Dissemination of *Pseudomonas aeruginosa* sequence type 235 isolates carrying bla_{IMP-6} and emergence of bla_{GES-24} and bla_{IMP-10} on novel genomic islands PAGI-15 and -16 in South Korea. Antimicrob Agents Chemother 2016;60:7216-23.
24. Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic features of *MCR-1*-producing colistin-resistant *Escherichia coli* isolates in South Africa. Antimicrob Agents Chemother 2016;60:4394-7.
25. Kim ES, Chong YP, Park SJ, Kim MN, Kim SH, Lee SO, et al. Detection and genetic features of *MCR-1*-producing plasmid in human *Escherichia coli* infection in South Korea. Diagn Microbiol Infect Dis 2017;89:158-60.
26. Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, et al. First report of the globally disseminated IncX4 plasmid carrying the *mcr-1* gene in a colistin-resistant *Escherichia coli* Se-

quence Type 101 isolate from a human infection in Brazil. Antimicrob Agents Chemother 2016;60:6415-7.
27. Elnahriry SS, Khalifa HO, Soliman AM, Ahmed AM, Hussein AM, Shimamoto T, et al. Emergence of plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate from Egypt. Antimicrob Agents Chemother 2016;60:3249-50.
28. Bradley DE and Coetzee JN. The determination of two morphologically distinct types of pilus by plasmids of incompatibility group I2. J Gen Microbiol 1982;128:1923-6.
29. Yoshida T, Kim SR, Komano T. Twelve pil genes are required for biogenesis of the R64 thin pilus. J Bacteriol 1999;181:2038-43.
30. Akahane K, Sakai D, Furuya N, Komano T. Analysis of the pilU gene for the prepilin peptidase involved in the biogenesis of type IV pili encoded by plasmid R64. Mol Genet Genomics 2005;273:350-9.
31. Komano T, Kim SR, Yoshida T, Nisioka T. DNA rearrangement of the shufflon determines recipient specificity in liquid mating of IncI1 plasmid R64. J Mol Biol 1994;243:6-9.
32. Sun J, Fang LX, Wu Z, Deng H, Yang RS, Li XP, et al. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the *mcr-1* acquisition. Sci Rep 2017;7:424.
33. Yoon EJ, Yang JW, Kim JO, Lee H, Lee KJ, Jeong SH. Carbapenemase-producing *Enterobacteriaceae* in South Korea: a report from the National Laboratory Surveillance System. Future Microbiol 2018;13:771-83.
34. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32:1792-7.

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