Dissemination of *mcr-1*-Carrying Plasmids among Colistin-Resistant *Salmonella* Strains from Humans and Food-Producing Animals in Taiwan

Chien-Shun Chiou,^a Ying-Tsong Chen,^{b,c} You-Wun Wang,^a Yen-Yi Liu,^a Hung-Chih Kuo,^d Yeuh-Hua Tu,^a Ann-Chi Lin,^c Ying-Shu Liao,^a Yu-Ping Hong^a

Centers for Disease Control, Taichung, Taiwan^a; Institute of Genomics and Bioinformatics, National Chung Hsing University, Taichung, Taiwan^b; Institute of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Taiwan^c; Department of Veterinary Medicine, National Chiayi University, Chiayi City, Taiwan^d

ABSTRACT We detected the colistin resistance gene *mcr-1* in four *Salmonella* serovars isolated from humans and animals with diarrhea. The resistance gene was carried on different plasmids. One *mcr-1*-carrying conjugative plasmid, a variant of pHNSHP45, was disseminated among *Salmonella* isolates recovered from humans, pigs, and chickens.

KEYWORDS *Salmonella enterica*, zoonosis, antimicrobial drug resistance, colistin, plasmid

Colistin is considered the last resort for treatment of multidrug-resistant Gram-negative bacterial infections. Colistin resistance in bacteria is mediated by several subtle mechanisms that mainly result from mutations in chromosomal genes (1). Recently, Liu et al. (2) found a plasmid-borne colistin resistance gene—*mcr-1*, which encodes a phosphoethanolamine transferase—in *Escherichia coli* from humans and animals (pigs and chickens) in China. The resistance gene was soon detected worldwide in several *Enterobacteriaceae* species, including *E. coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, and several *Salmonella* serovars, either carried by various plasmids (3–5) or located in the chromosomes (6). However, few reports show the dissemination of *mcr-1*-carrying plasmids in zoonotic pathogens between humans and animals. We investigated *mcr-1* and the plasmids that carry it in *Salmonella* isolates from humans and food-producing animals, collected between 2010 and 2015 in Taiwan.

We conducted antimicrobial susceptibility testing on 6,386 *Salmonella* isolates from humans (5,178) and diseased animals (1,208), namely, chickens (450), pigs (279), ducks (206), turkeys (170), geese (88), and other animals (15) (see Table S1 in the supplemental material), using a custom-made 96-well Sensititre MIC panel (Trek Diagnostic Systems Ltd., West Grinstead, England). The isolates from humans were collected from 52 hospitals across the country, and the isolates from animals were recovered by the Veterinary Teaching Hospital of National Chiayi University from sick animals in central and southern Taiwan. *Salmonella* serotypes were determined by use of the typing scheme developed by Chiou et al. (7). Colistin resistance was identified in 1,917 isolates, grouped into 28 *Salmonella* serovars, with MIC values of ≥ 4 mg/liter (8). Of the colistin-resistant isolates, 1,792 (93.4%) were *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S. Enteritidis*), and 125 belonged to the other 27 serovars (see Table S2 in the supplemental material). Among the 1,917 colistin-resistant isolates, we selected 556 (431 colistin-resistant *S. Enteritidis* isolates and all resistant isolates of the other 27 serovars) for detection of *mcr-1* using the PCR primers designed by Liu et al. (2). The tests identified 19 *mcr-1*-carrying isolates (*S. enterica* subsp. *enterica* serovars Typhimu-

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Address correspondence to Chien-Shun Chiou, nipmcsc@cdc.gov.tw.

FIG 1 Genetic relationships among 19 *mcr-1*-carrying *Salmonella* isolates built using PFGE patterns, their corresponding antimicrobial resistance patterns, and relative information. Antimicrobials: FTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; AMP, ampicillin; CHL, chloramphenicol; STR, streptomycin; SUL, sulfamethoxazole; TCY, tetracycline; SXT, trimethoprim-sulfamethoxazole; COL, colistin; EPT, ertapenem; FOX, ceftiofur. Red boxes, resistance; yellow boxes, intermediate resistance; green boxes, susceptibility. The plasmid types are defined on the basis of the BglII and AclI restriction profiles of the *mcr-1*-carrying conjugative plasmids from the isolates. ND, plasmid type not determined.

rium [14], Anatum [3], Albany [1], and Newport [1]) recovered from 10 humans, 7 pigs, and 2 chickens between 2012 and 2015 (Fig. 1). Notably, *S. Enteritidis* displayed a high colistin resistance rate, but *mcr-1* was not detected in all of the 431 colistin-resistant isolates tested. The resistance mechanism for these colistin-resistant *S. Enteritidis* isolates has not been identified. The *mcr-1*-carrying isolates, except for one, were multidrug resistant, with resistance to three or more classes of antimicrobial agents (Fig. 1). The 9 *mcr-1*-carrying isolates from animals were collectively more resistant than the 10 isolates from humans (see Table S3 in the supplemental material). We conducted whole-genome sequencing of 3 cefotaxime-resistant isolates (C215, P111, and P165) and found a *bla*_{CMY-2} gene in P165. No other β -lactamase gene was found in C215 or P111. The *bla*_{CMY-2} gene was then detected by PCR in P163, P164, NG14.043, and C214 but not in P110. These 3 cefotaxime-resistant isolates (C215, P110, and P111) should have different resistance mechanisms from the 5 *bla*_{CMY-2}-carrying isolates.

We characterized *Salmonella* isolates, using the standardized PulseNet pulsed-field gel electrophoresis (PFGE) protocol with XbaI (9), and investigated the genetic relatedness among *Salmonella* isolates by comparing PFGE patterns, using the cluster analysis program provided in BioNumerics version 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). The PFGE analysis identified 10 patterns for the 14 *mcr-1*-carrying *S. Typhimurium* isolates and 5 patterns for the 5 remaining *mcr-1*-carrying isolates. Cluster analysis of PFGE patterns for the *mcr-1*-carrying and 3,084 non-*mcr-1*-carrying *S. Typhimurium* isolates collected between 2010 and 2015 revealed that the 14 *mcr-1*-carrying isolates fell into 2 major clusters, and each clonal cluster contained isolates recovered from humans, pigs, and chickens. These studies indicated that the *mcr-1* resistance gene in the strains should have derived from multiple origins.

We conducted conjugation experiments to transfer the *mcr-1*-carrying plasmids to *E. coli* C600 recipients and used LB medium with 1 mg/liter colistin and 2,000 mg/liter streptomycin for the selection of transconjugants. The *mcr-1*-carrying plasmids in *S. Albany*, *S. Newport*, and *S. Typhimurium* were transferable, but transfer was not achieved in *S. Anatum*. We extracted plasmids of transconjugants, using the Qiagen Plasmid Midi kit (Hilden, Germany), and treated purified plasmids with BglII and AclI. Four restriction patterns were identified for the plasmids (see Fig. S1 in the supplemental material). Plasmid type I (~60 kb) was identified in 5 *S. Typhimurium* isolates

FIG 2 Genetic structures surrounding the *mcr-1* gene in pP111, pR150626, pC214, and pNG14043 compared with that in pHNSHP45. Open reading frames and functions: red arrow, antimicrobial resistance and metal transporter; yellow arrow, transposition of mobile element; green arrow, virulence; blue arrow, plasmid replication; white arrow, unknown function.

from humans, pigs, and chickens and 1 *S. Albany* and 1 *S. Newport* isolate from humans (Fig. 1). Three isolates (P110, P111, and C215) with the type I plasmid recovered from pigs and chickens shared an indistinguishable PFGE pattern. Plasmid type II (~43 kb) was carried by 2 *S. Typhimurium* isolates from pigs and chickens, and type III (~33 kb) and type IV (~60 kb) plasmids were harbored in 7 *S. Typhimurium* isolates from humans.

We determined the sequences of the plasmids derived from strains P111 (type I), C214 (type II), NG14.043 (type III), and R15.0626 (type IV) using the Illumina MiSeq platform (2 × 300 bp), and we assembled the sequence reads and analyzed the complete sequences using the CLC Genomics Workbench (Qiagen Bioinformatics). The BglII, AclI, and AflII restriction profiles predicted for the complete plasmid sequences were concordant with those generated from purified plasmids digested with each of the enzymes. We determined the plasmid incompatibility types using the Plasmid-Finder tool provided on the website of the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) and annotated the four plasmid complete sequences with the Prokka program (10). The genetic maps for the four plasmids are listed in Fig. S2 in the supplemental material. pP111 (60,960 bp, type I) and pR150626 (59,651 bp, type IV) belonged to IncI2; pC214 (42,941 bp, type II) and pNG14043 (33,308 bp, type III) belonged to IncX4. All of the plasmids contained one copy of *mcr-1*, and the sequence of *mcr-1* on these plasmids shared 100% sequence identity with the first one published by Liu et al. (2). pP111 had 89% sequence sharing with pR150626, and both had the same genetic structure surrounding *mcr-1* (Fig. 2). pR150626 was a close variant of the first reported *mcr-1*-carrying plasmid, pHNSHP45, in China (2); its whole sequence could be found in pHNSHP45. However, compared to pHNSHP45, pR150626 and pP111 lacked an ISAp1 insertion element upstream of *mcr-1*. pC214 was a novel *mcr-1*-carrying plasmid; it contained two copies of ISAp1 elements surrounding *mcr-1*, harbored additional *sulII* and *floR* resistance genes (see Fig. S2B), and shared a 25-kb backbone with a non-*mcr-1*-carrying plasmid identified in an *S. enterica* subsp. *diarizonae* strain (accession no. CP011293.1). pNG14043 displayed a genetic structure surrounding *mcr-1* that was distinct from that of the other 3 plasmids, and nearly 100% of its whole sequence was found in 6 plasmids (accession no. KX772777.1, KX236309.1, KU761327.1, KU743383.1, CP015977.1, and CP016550.1) in the NCBI nucleotide database, which were recovered from geographically dispersed countries, including China, Italy, the Netherlands, Estonia, and Brazil.

In conclusion, *mcr-1* was found in diverse *Salmonella* strains of four serovars causing infections in humans and food-producing animals in Taiwan. The resistance gene was carried on distinctly different plasmids. Among the four *mcr-1*-carrying conjugative plasmids characterized, pP111, a variant of pHNSHP45 (2), had disseminated in four

Salmonella serovars recovered from humans, pigs, and chickens. *mcr-1* was recently reported in *E. coli* isolates from humans and retail beef, chicken, and pork in Taiwan (11). Thus, *mcr-1* may have been widespread and become prevalent in zoonotic pathogens in this country.

Accession number(s). The new plasmid complete sequences were submitted to the GenBank of the National Center for Biotechnology Information under accession numbers [KY120363](#) to [KY120366](#).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00338-17>.

SUPPLEMENTAL FILE 1, PDF file, 1.8 MB.

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