



# Prevalence of Colistin Resistance Gene *mcr-1* and Absence of *mcr-2* in *Escherichia coli* Isolated from Healthy Food-Producing Animals in Japan

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**ABSTRACT** We screened *mcr-1* and *mcr-2* genes in 9,306 *Escherichia coli* strains isolated from healthy animals in the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) system. *mcr-1* was detected in 39 strains (5, 20, and 14 strains isolated from cattle, swine, and broilers, respectively), whereas *mcr-2* was not detected. *mcr-2* was also not detected with the investigation sequence homology search against our curated GenEpid-J database.

**KEYWORDS** GenEpid-J, Japanese Veterinary Antimicrobial Resistance Monitoring system, colistin resistance, food-producing animals, *mcr-1*, *mcr-2*

Colistin has been used as a veterinary drug for the treatment of Gram-negative gastrointestinal infections and as a feed additive to promote healthy development in food-producing animals for more than 5 decades. Colistin is currently considered the last-resort antibiotic for the treatment of infections caused by multidrug-resistant Gram-negative bacteria worldwide in human medicine. The resistance mechanism to colistin was only known to involve chromosomal mutations. However, in 2015, Liu et al. reported the first plasmid-mediated colistin resistance gene, *mcr-1*, in *Enterobacteriaceae* isolated from food-producing animals, retail meat, and humans in China (1).

In a previous study, after analyzing data from the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) system, we showed that only two isolates were *mcr-1* positive in 90 colistin-resistant *Escherichia coli* isolates (MIC,  $\geq 8$  mg/liter), and the plasmids carrying *mcr-1* from animal isolates were identified in the GenEpid-J database (2). However, other studies have shown that *E. coli* strains with an MIC of  $\geq 2$  mg/liter had *mcr-1* genes (3, 4). Kusumoto et al. reported that more than 13% of the isolates were *mcr-1* positive, with MICs of  $\geq 4$  mg/liter in swine-pathogenic *E. coli* (5). Furthermore, Xavier et al. reported the presence of another plasmid-mediated colistin resistance gene, *mcr-2*, which encodes the MCR-2 protein and has 76.7% nucleotide identity to *mcr-1* (6). In response to these findings, we screened the *mcr-1* and *mcr-2* genes in *E. coli* isolates with colistin MICs of  $\geq 2$  mg/liter, part of which were colistin-susceptible isolates according to the breakpoint of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

We screened 9,306 *E. coli* isolates isolated from healthy animals from JVARM (3,134 isolates from cattle, 2,052 isolates from swine, 2,017 isolates from broilers, and 2,103 isolates from layers) between 2000 and 2014. MICs of colistin were determined by the agar dilution method in the isolates from 2000 to 2009 and broth dilution method in the isolates from 2010 to 2014, according to the recommendation of the Clinical and

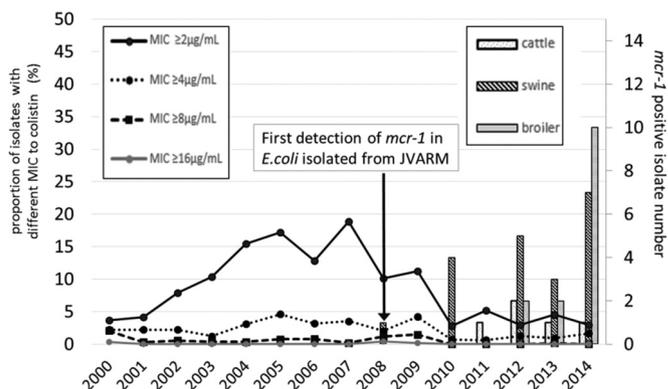
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**FIG 1** Proportion of *Escherichia coli* isolates that showed different MICs to colistin, and *mcr-1*-positive strain numbers in *E. coli* isolates from healthy animals between 2000 and 2014 in the Japanese Veterinary Antimicrobial Resistance Monitoring system. Filled black circle and solid line shows proportion of isolates with a colistin MIC of  $\geq 2$  mg/liter. Filled black circle and dashed line shows proportion of isolates with a colistin MIC of  $\geq 4$  mg/liter. Filled black square and dashed line shows proportion of isolates with a colistin MIC of  $\geq 8$  mg/liter. Filled gray circle and solid line shows proportion of isolates with a colistin MIC of  $\geq 16$  mg/liter. The dark gray bar shows the number of *mcr-1*-positive isolates from broilers, the hatched bar shows the number of *mcr-1*-positive isolates from swine, and the light gray bar shows the number of *mcr-1*-positive isolates from cattle.

Laboratory Standards Institute (7). A total of 732 (7.9%) isolates showed an MIC of  $\geq 2$  mg/liter for colistin and were examined for the presence of *mcr-1* and *mcr-2* by PCR, as described by Liu et al. (1) and Xavier et al. (6), respectively. Additionally, we searched a plasmid carrying *mcr-2* against the GenEpid-J database. By November 2015, 1,747 plasmid sequences from 671 Gram-negative bacteria were analyzed using next-generation sequencing and curated in the GenEpid-J database; these sequences represented 431, 184, and 56 isolates from patients admitted to hospitals, animals, and the environment, respectively.

A total of 39 isolates, including 12 isolates of MIC of 2 mg/liter, were *mcr-1* positive, corresponding to 5.3% (39/732) of the isolates of colistin MIC of  $\geq 2$  mg/liter and 0.42% (39/9,306) of the total collected isolates. The numbers of *mcr-1*-positive isolates from cattle, swine, and broilers were 5, 20, and 14, respectively, and overall, the number of positive isolates increased slightly over the years (Fig. 1). By using the broth mating method (8), 9 (3 cattle, 3 swine, and 3 broilers) of 39 (23.0%) isolates were transferred to *E. coli* DH5 $\alpha$ . All the transconjugants had a size of roughly 60 kb and incompatibility type of IncI2 plasmid by using pulsed-field gel electrophoresis (PFGE) after S1 nuclease digestion (9), a PCR-based replicon-typing assay (10, 11), and hybridization (12). These results suggested that they were similar to the plasmid reported by Liu et al. in China (1). However, the number of *mcr-1*-positive isolates was limited in healthy animals, and none of the layer isolates were *mcr-1* positive. From 2000 to 2014, colistin-resistant isolates (MIC,  $> 2$  mg/liter by EUCAST criterion) were consistently low, and even after taking into account isolates with an MIC of 2 mg/liter, the proportion of colistin-resistant and reduced-susceptibility isolates in *E. coli* did not increase since the first *mcr-1* detection in 2008 (Fig. 1).

Compared with the swine-pathogenic *E. coli* strains isolated from diseased swine in Japan (5), the *mcr-1*-positive (healthy swine, 20/2,052 [0.97%] versus diseased swine, 90/684 [13.16%]) and resistance ( $\geq 4$  mg/liter; healthy swine, 92/2,052 [4.48%] versus diseased swine, 309/684 [45%]) rates of isolates from healthy animals were much lower. Colistin is the common treatment option of swine diarrheal diseases in Japan. The difference in the selection pressure of colistin between diseased and healthy swine may explain the discrepancy in the prevalence of *mcr-1* between *E. coli* isolated from diseased and healthy swine.

The prevalence of *mcr-2* in swine colistin-resistant *E. coli* in Belgium was higher than that of *mcr-1* (6), necessitating an immediate introduction of *mcr-2* screening in

ongoing molecular epidemiological surveillance of colistin-resistant Gram-negative pathogens; however, *mcr-2* was not detected in this investigation by using Xavier's method. Moreover, we could not detect the *mcr-2* gene in the GenEpid-J database. It is speculated that the distribution of *mcr-2* between European countries and Japan is different and might be related to geography or differences in veterinary practices between these regions, but further research is needed.

In conclusion, the prevalence of *mcr-1* in *E. coli* from healthy animals increased slightly over the years, but the prevalence remained very low, and *mcr-2* was not detected in this study. The proportion of strains with an MIC of  $\geq 2$  mg/liter colistin has not increased since the first *mcr-1* detection. In Japan, there have been no reports of *mcr-1*-positive bacteria isolated from food and humans. However, continuous surveillance and monitoring and ensuring the prudent use of antibiotics in veterinary medicine are essential to prevent or reduce the transfer of resistant bacteria or resistance determinants within animal populations and the environment, and between animals and humans.

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