

First Detection of the *mcr-1* Gene in *Escherichia coli* Isolated from Livestock between 2013 and 2015 in South Korea

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The plasmid-mediated colistin resistance gene *mcr-1* was first reported in China in 2013 and has since been identified in both humans and livestock from many other countries (1–4). Colistin has been approved for animal use for prevention and treatment of disease in Korea. We investigated the prevalence of this gene in food animals and animal products in Korea.

In total, 10,576 *Escherichia coli* isolates were obtained from fecal and carcass samples from healthy animals at slaughterhouses and from tissue lesions or fecal samples of diseased animals at diagnostic laboratories from 2005 to 2015 in the Korean Veterinary Antimicrobial Resistance Monitoring System.

Testing for susceptibility to 15 antimicrobials in the 10,576 *E. coli* isolates and 8 transconjugants was performed by the broth microdilution method using the KRN4F Sensititre panel (Trek Diagnostic Systems) according to the manufacturer's instructions (5). The results for colistin were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoint (>2 $\mu\text{g/ml}$). *mcr-1* gene carriage was determined by PCR and DNA sequencing for colistin-resistant *E. coli* isolates (1).

Of the 10,576 *E. coli* isolates tested, 154 (1.46%) exhibited colistin resistance. The colistin-resistant isolates were obtained from cattle and cattle carcasses (1.28%, 45/3,523), pigs and pig carcasses (1.71%, 66/3,865), and chickens and chicken carcasses (1.35%, 43/3,188) (Table 1). Interestingly, isolates from diseased animals showed much higher resistance than isolates from healthy animals or carcasses at the slaughterhouse ($P < 0.01$). The annual consumption of colistin by food animals in Korea, ranging from 6 to 16 tons during 2005 to 2015, might contribute to the occurrence of colistin resistance.

Notably, the *mcr-1* gene was detected in 0.10% (11/10,576) of isolates that originated from 10 different farms in four provinces; for these isolates, colistin MICs ranged from 8 to 16 $\mu\text{g/ml}$. Sequencing revealed that the *mcr-1* amplicons were 100% identical to that reported by Liu et al. (1). Of note, *mcr-1* was found mainly in isolates collected from poultry; five each were from healthy chicken fecal samples and chicken carcasses, and one was from a diseased pig. *mcr-1* was not detected in *E. coli* isolates before 2013, but its prevalence has risen since 2013 (0.09% [1/1,078] in 2013,

0.45% [6/1,329] in 2014, and 0.34% [4/1,169] in 2015) ($P < 0.01$). This result was different from those of other studies, in which generally *mcr-1* carriage corresponded to increased colistin resistance (4). Further studies are necessary to explain the higher prevalence of *mcr-1*-carrying *E. coli* isolates in healthy chickens and carcasses than in diseased chickens and carcasses even though the resistance rate is low.

All 11 *mcr-1*-carrying isolates displayed multiple-drug resistance (≥ 3 antimicrobial subclasses), which was successfully transferred to an *E. coli* J53 recipient strain by filter mating but not to 3 other isolates; no other resistance was cotransferred. All the *mcr-1*-carrying isolates were from different farms and showed different pulsed-field gel electrophoresis (PFGE) patterns and sequence types (STs), with the exception of two ST162 isolates that carried the *bla*_{CTX-M-1} gene (Table 2). Importantly, multilocus sequence typing and XbaI PFGE results (see Fig. S1 in the supplemental material) indicated that the rise of *mcr-1*-carrying isolates may be driven by plasmid-mediated horizontal gene transfer rather than by the spread of a specific clone.

This is the first report of the *mcr-1* gene in South Korea. Although the rate of colistin resistance in healthy animals and animal products remains low, restriction of the use of colistin is essential to prevent the transmission of *mcr-1* to other bacteria in the same or different animals, to the food chain, and to the human community.

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TABLE 1 Colistin resistance and carriage of the *mcr-1* gene in *Escherichia coli* isolates obtained from animals and animal carcasses between 2005 and 2015

Yr	% resistance (no. of resistant isolates/no. of tested isolates) ^a										% of isolates with colistin resistance (no. of resistant isolates/no. of tested isolates)		% of isolates in which <i>mcr-1</i> gene was detected (no. of positive isolates/no. of tested isolates)	
	Healthy cattle	Diseased cattle	Cattle carcasses	Healthy pigs	Diseased pigs	Pig carcasses	Healthy chickens	Diseased chickens	Chicken carcasses	Isolates/no. of tested isolates	Isolates/no. of tested isolates	Isolates/no. of tested isolates	Isolates/no. of tested isolates	
2005	0.8 (1/118)	NT	0 (0/78)	0 (0/176)	NT	0 (0/89)	0 (0/113)	NT	0 (0/119)	0.14 (1/693)	0 (0/639)	0 (0/639)	0 (0/639)	
2006	0 (0/120)	NT	0 (0/82)	2.6 (5/190)	NT	0 (0/85)	0 (0/138)	NT	0 (0/129)	0.67 (5/744)	0 (0/744)	0 (0/744)	0 (0/744)	
2007	9.9 (12/121)	NT	0 (0/57)	7.1 (17/239)	NT	0 (0/62)	0 (0/169)	NT	0 (0/96)	3.90 (29/744)	0 (0/744)	0 (0/744)	0 (0/744)	
2008	12.8 (16/125)	NT	0 (0/51)	5.2 (6/116)	NT	0 (0/61)	0.9 (1/106)	NT	0 (0/100)	4.11 (23/559)	0 (0/559)	0 (0/559)	0 (0/559)	
2009	2.7 (5/184)	NT	0 (0/51)	3.8 (7/185)	NT	0 (0/48)	2.1 (2/97)	NT	0 (0/76)	2.18 (14/641)	0 (0/641)	0 (0/641)	0 (0/641)	
2010	0 (0/231)	0 (0/21)	0 (0/136)	0.9 (2/221)	1.8 (1/56)	0.8 (1/124)	5.2 (8/155)	4.0 (2/50)	0 (0/107)	1.27 (14/1,101)	0 (0/1,101)	0 (0/1,101)	0 (0/1,101)	
2011	1.2 (4/347)	0 (0/14)	1.1 (2/190)	0.9 (2/231)	5.6 (2/36)	0.7 (1/153)	2.1 (3/141)	11.6 (5/43)	3.3 (4/121)	1.80 (23/1,276)	0 (0/1,276)	0 (0/1,276)	0 (0/1,276)	
2012	0 (0/282)	0 (0/28)	0 (0/111)	0.7 (2/277)	0 (0/39)	0.7 (1/134)	0 (0/200)	0 (0/8)	0 (0/163)	0.24 (3/1,242)	0 (0/1,242)	0 (0/1,242)	0 (0/1,242)	
2013	1.0 (2/209)	0 (0/5)	0 (0/146)	0 (0/199)	10.7 (3/28)	1.4 (2/141)	1.1 (2/187)	0 (0/33)	0.8 (1/130)	0.93 (10/1,078)	0.09 (1/1,078)	0.09 (1/1,078)	0.09 (1/1,078)	
2014	0.3 (1/299)	12.5 (1/8)	0 (0/159)	0.3 (1/294)	15.8 (6/38)	0 (0/160)	1.6 (3/192)	10.3 (4/39)	3.6 (5/140)	1.58 (21/1,329)	0.45 (6/1,329)	0.45 (6/1,329)	0.45 (6/1,329)	
2015	0 (0/206)	5.3 (1/19)	0 (0/125)	0 (0/218)	4.5 (7/154)	0 (0/111)	1.1 (2/189)	0 (0/20)	0.8 (1/127)	0.94 (11/1,169)	0.34 (4/1,169)	0.34 (4/1,169)	0.34 (4/1,169)	
Total	1.8 (41/2,242)	2.1 (2/95)	1.2 (2/1,186)	1.8 (42/2,346)	5.4 (19/351)	0.4 (5/1,168)	1.2 (21/1,687)	5.7 (11/193)	0.8 (11/1,308)	1.46 (154/10,576)	0.10 (11/10,576)	0.10 (11/10,576)	0.10 (11/10,576)	

^a NT, not tested.

TABLE 2 Characteristics of the *mcr-1*-positive *Escherichia coli* isolates from animals and animal carcasses

Isolate	Sample	Province	Yr	MIC (μg/ml) of ^a :												Resistance transferred					
				GEN	NEO	STR	AMP	AmC	CEF	FOX	XNL	CIP	SXT	CHL	FFC	COL	NAL	TET	ST		
S03-109	Chicken carcass	Chungnam	2013	≤1	≤2	>128	>64	8	16	8	8	≤0.5	>16	0.25	8	4	8	>128	4	Yes	410
A03-007	Healthy chicken	Chungbuk	2014	≤1	≤2	8	>64	8	16	4	4	≤0.5	8	>4	4	4	8	>128	≤2	Yes	156
S03-009	Chicken carcass	Chungnam	2014	≤1	≤2	128	>64	8	8	2	2	≤0.5	8	≤0.12	64	>64	8	>128	32	Yes	10
A03-008	Healthy chicken	Jeonnam	2014	≤1	≤2	8	>64	8	16	4	4	≤0.5	16	0.25	>64	4	8	>128	≤2	No	101
A03-017	Healthy chicken	Chungbuk	2014	≤1	≤2	>128	>64	8	16	4	4	≤0.5	8	>4	>64	>64	8	>128	>128	Yes	226
S03-006	Chicken carcass	Chungbuk	2014	≤1	≤2	>128	>64	8	>64	2	4	8	8	0.25	64	64	8	>128	64	Yes	162
S03-031	Chicken carcass	Jeonnam	2014	≤1	≤2	>128	>64	8	8	4	4	≤0.5	0.25	0.5	>64	>64	8	128	128	No	88
S03-026	Chicken carcass	Gyeonggi	2015	64	≤2	>128	>64	8	16	4	4	≤0.5	16	>4	8	4	8	>128	128	Yes	2732
A03-003	Healthy chicken	Jeonnam	2015	≤1	≤2	8	>64	4	8	4	4	≤0.5	1	≤0.12	>64	>64	8	128	128	No	1141
A03-010	Healthy chicken	Jeonnam	2015	≤1	≤2	>128	>64	4	>64	2	2	>8	8	0.25	>64	>64	8	>128	64	Yes	162
R02-111	Diseased pig	Chungbuk	2015	16	≤2	>128	>64	8	8	4	4	≤0.5	≤0.12	>4	64	64	16	≤2	64	Yes	1

^a GEN, gentamicin; NEO, neomycin; STR, streptomycin; AMP, ampicillin; AmC, amoxicillin-clavulanic acid; CEF, cephalothin; FOX, cefoxitin; XNL, ceftiofur; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; FFC, florfenicol; COL, colistin; NAL, nalidixic acid; TET, tetracycline; ST, sequence type.

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