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Address for correspondence: Ruth R. Montgomery, Department of Internal Medicine, Yale University School of Medicine, 300 Cedar St/TAC S413, New Haven, CT 06520-8031, USA; email: ruth.montgomery@yale.edu

mcr-1 in *Enterobacteriaceae* from Companion Animals, Beijing, China, 2012–2016

Lei Lei,[1] Yang Wang,[1] Stefan Schwarz, Timothy R. Walsh, Yanran Ou, Yifan Wu, Mei Li, Zhangqi Shen

Author affiliations: China Agricultural University, Beijing, China (L. Lei, Y. Wang, Y. Ou, Y. Wu, M. Li, Z. Shen); Freie Universität Berlin, Berlin, Germany (S. Schwarz); Cardiff University, Cardiff, Wales, UK (T.R. Walsh, M. Li); Iowa State University, Ames, Iowa, USA (Z. Shen)

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[1]These authors contributed equally to this article.

To investigate the prevalence of the recently emerging colistin resistance gene *mcr-1* in *Enterobacteriaceae* among companion animals, we examined 566 isolates collected from cats and dogs in Beijing, China, during 2012–2016. Of these isolates, 49 (8.7%) were *mcr-1*–positive.

Multidrug-resistant and extensively drug-resistant gram-negative bacteria are a major threat to public health worldwide (*1*,*2*). The recent rapid dissemination of carbapenem-resistant *Enterobacteriaceae* has worsened this situation and further narrowed treatment options for infections caused by these bacteria (*3*). Colistin is a last-resort drug for treating carbapenem-resistant *Enterobacteriaceae* infections (*4*). In 2016, we identified the mobile colistin resistance gene *mcr-1* (*1*). Soon after its description, *mcr-1* was observed in *Enterobacteriaceae* from humans and food-producing animals in >30 countries on 5 continents (*5*).

A 2016 article reported that a 50-year-old man who worked in a pet store tested positive for *mcr-1*–harboring *E. coli* (*6*). Investigation identified 6 multidrug-resistant *mcr-1*–producing *E. coli* isolates in samples from 4 dogs and 2 cats in the pet store, indicating that the pathogens can be transmitted between humans and companion animals. So far, the prevalence of *mcr-1*-containing *Enterobacteriaceae* in companion animals is largely unknown. In our study, we focused on estimating the prevalence of *mcr-1* in *Enterobacteriaceae* of companion animal origin in Beijing, China, during 2012–2016, and investigated the presence of the *mcr-1* gene in pet foods purchased there.

In Beijing, the total number of registered dogs and cats is ≈1.2 million. We collected samples from both healthy and sick dogs and cats in Veterinary Teaching Hospital of China Agricultural University.

A total of 566 nonduplicate *Enterobacteriaceae* strains were isolated from 1,439 nasal and rectal swab samples collected from 1,254 dogs and 185 cats during 2012–2016. We also isolated 25 *Enterobacteriaceae* from 32 nasal swab samples from the pet owners. Because the food chain is among the main routes for humans and companion animals to acquire foodborne pathogens, we collected a small sample of pet foods (dog food, n = 30; cat food, n = 5) containing chicken as the main ingredient in Beijing during June–August 2016.

The species of all *Enterobacteriaceae* were determined by 16S rDNA sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of specimens cultured on brain–heart infusion agar plates containing 2 μg/mL colistin. A total of 79/566 (14.0%) of the *Enterobacteriaceae* isolates from companion animals were resistant to colistin: 56 *E. coli*, 16 *Klebsiella pneumoniae,* 5 *Enterobacter cloacae*, 1 *Enterobacter aerogenes,* and 1 *Shigella* spp. PCR amplification of *mcr-1* indicated that 8.7% (49/566) of *Enterobacteriaceae* and 62.0% (49/79)

of colistin-resistant isolates harbored the *mcr-1* gene, 47 *E. coli* and 2 *K. pneumoniae*. Only 1 *E. coli* isolate from a pet owner was colistin-resistant and *mcr-1*–positive. The proportions of *mcr-1*–containing *E. coli* per year ranged from 6.1% to 14.3% (Figure).

We examined the susceptibility of colistin-resistant *E. coli* to 8 other antimicrobial agents by agar dilution, according to the recommendations of Clinical and Laboratory Standards Institute (*7*). The *mcr-1*–carrying *E. coli* exhibited high resistance rates to ampicillin (97.9%), cefotaxime (91.5%), chloramphenicol (89.4%), and gentamicin (85.1%) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/23/4/16-1732-Techapp1.pdf) but were susceptible to imipenem. The *mcr-1*–positive *E. coli* were more often resistant to amoxicillin/clavulanate, ampicillin, and chloramphenicol than were the *mcr-1*–negative *E. coli* ($p<0.05$) (online Technical Appendix Table 1).

All 57 colistin-resistant *E. coli* were subjected to XbaI pulsed-field gel electrophoresis (PFGE). The 55 colistin-resistant *E. coli* strains (2 nontypeable strains were excluded) were subdivided into 33 patterns and grouped into 31 clusters (A–Z, AB–AF) (online Technical Appendix Figure). The diversity and similarity of PFGE patterns of *E. coli* from different origins suggested that the dissemination of *mcr-1* was possibly related to both clonal expansion and horizontal transmission.

Of note, the 1 *E. coli* colistin-resistant, *mcr-1*–positive isolate from a pet owner had the same PFGE pattern as 5 isolates from dogs and cats. Multilocus sequence typing linked these 6 strains to sequence type 101. These results suggest that *E. coli* strains can be exchanged between companion animals and humans.

The PCR and sequence analysis of the pet food samples showed that 7 of 35 samples were positive for *mcr-1*. Companies in China produced 5 of these foods; the other 2 were from Italy and Belgium (online Technical Appendix Table 2). These results suggest that pet foods may be a source from which intestinal bacteria of companion animals can acquire the *mcr-1* gene.

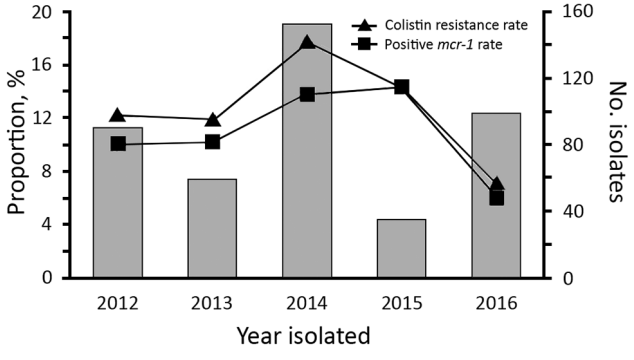

Figure. Proportion of colistin resistance and *mcr-1* in *Escherichia coli* of companion animal origin, Beijing, China, 2012–2016.

Currently, colistin is not used to treat companion animals in China. The companion animals included in this study were from an urban area of Beijing, so they had minimal or no contact with food-producing animals in which colistin may have been used. Because we found *mcr-1* in pet foods, we speculate that the pet food industry may be a source of *mcr-1* among companion animals. Because of frequent and close contact between humans and companion animals, our study proposes that opportunities exist to transmit colistin-resistant *Enterobacteriaceae* to and from both groups. Thus, colistin-resistant *Enterobacteriaceae* from companion animals may represent a potential risk to human health. Further surveillance and control efforts are needed to reduce colistin-resistant and *mcr-1*–containing *Enterobacteriaceae* in companion animals and food-producing animals.

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Ms. Lei is a PhD student in the College of Veterinary Medicine, China Agricultural University. Her main interest is the prevalence of antibiotic resistance in enteric bacteria.

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Address for correspondence: Zhangqi Shen, Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, 2 Yuanmingyuan West Rd, Beijing 100193, China; email: szq@cau.edu.cn

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mcr-1 in *Enterobacteriaceae* from Companion Animals, Beijing, China, 2012–2016

Technical Appendix

Technical Appendix Table 1. Antimicrobial drug MICs of colistin-resistant *Escherichia coli* from companion animals (n=56)*

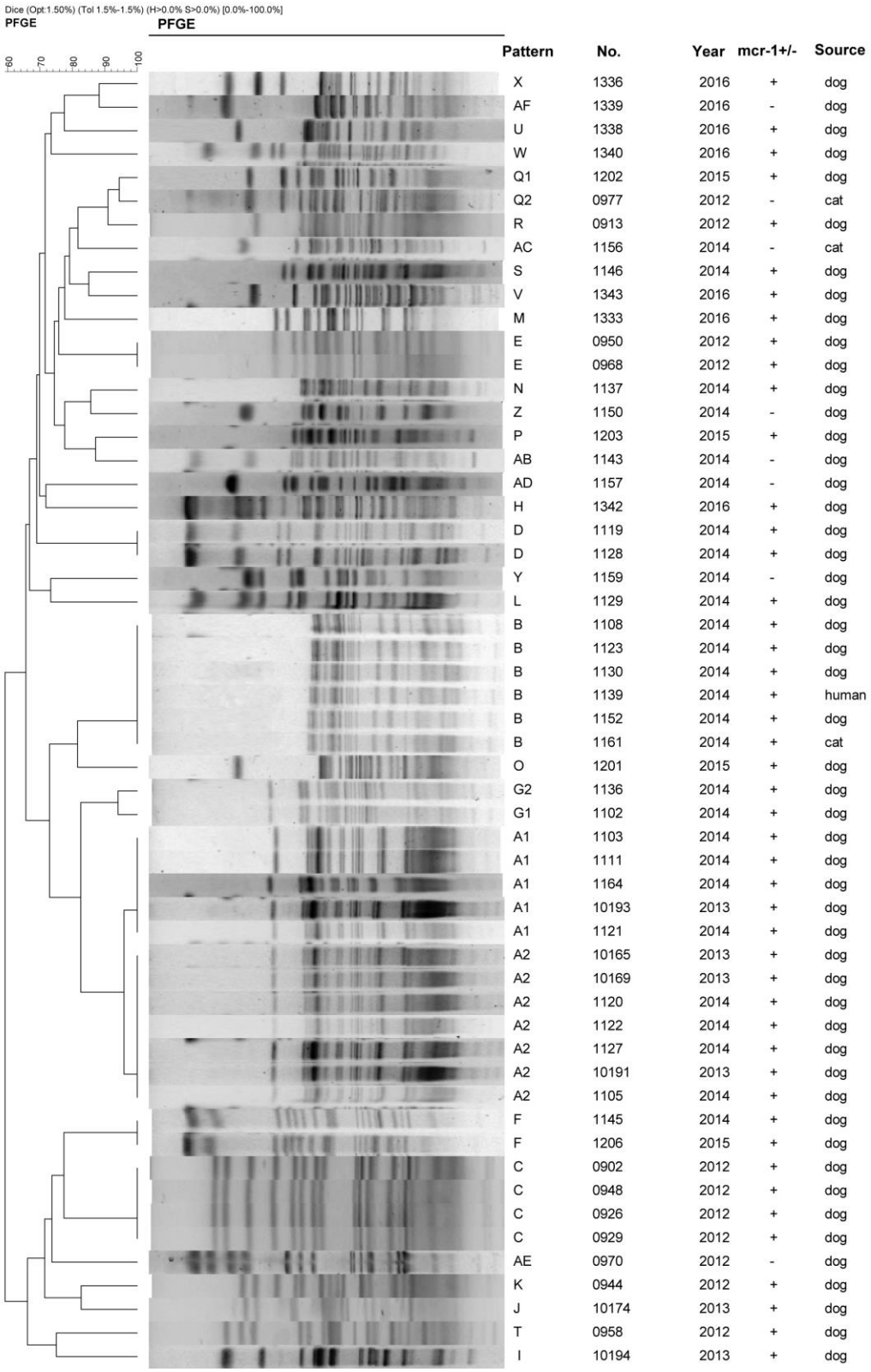
	mcr-1–positive *E. coli* (n = 47)			*mcr-1*–negative E. coli (n = 9)			
Antimicrobial agents†	MIC_{50}, µg/mL	MIC_{90}, µg/mL	Resistance, %	MIC_{50}, µg/mL	MIC_{90}, µg/mL	Resistance, %	p value‡
Colistin	8	256	100.0%	4	µg/mL	100.0%	NA
Polymyxin B	8	64	100.0%	4	16	100.0%	NA
Amoxicillin-clavulanic acid	64/32	>128/64	70.2%	16/8	16	33.3%	0.0296
Ampicillin	>256	>256	97.9%	128	64/32	66.7%	0.0111
Tigecycline	0.5	1	2.1%	0.25	>256	0.0%	NA
Enrofloxacin	32	64	74.5%	0.25	0.5	44.4%	0.1121
Cefotaxime	>256	>256	91.5%	128	128	66.7%	0.0740
Chloramphenicol	128	>256	89.4%	4	>256	22.2%	0.0002
Gentamicin	>256	>256	85.1%	64	>256	55.6%	0.0632
Imipenem	0.25	0.5	0.0%	0.25	>256	0.0%	NA

*Colistin-resistant *Escherichia coli* was isolated from 57 samples, including 1 from an owner of a companion animal that was excluded from this table; NA, not applicable.
†The MICs of amoxicillin-clavulanate, ampicillin, enrofloxacin, cefotaxime, chloramphenicol, gentamicin, and imipenum were interpreted according to the CLSI document VET01S 3rd ed. The MIC of colistin, polymixin B, and tigecycline were interpreted according to the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (version 6.0).
‡p values were determined by Fisher exact test.

Techncial Appendix Table 2. Characterization of pet foods and the presence of *mcr-1* gene

Type of pet food	Year of production	Ingredients	Pet food sample numbers*	Origin	*mcr-1*-positive pet food
Dry foods	2015	Chicken meal	15–19, 22	China	16, 19
Dry foods	2015	Chicken meal	20	Belgium	20
Dry foods	2015	Chicken meal	27	Australia	0
Dry foods	2015	Chicken, fish/pork	21, 23, 27, 29, 30, 34, 35	China	21, 34, 35
Dry foods	2016	Chicken	24–26	China	0
Dry foods	2015	Chicken	31, 32	USA	0
Dry foods	2015	Fish/chicken	33	Germany	0
Semi-moist foods	2016	Chicken	1,2,5	China	0
Semi-moist foods	2016	Chicken, bovine spleen	6–8	China	0
Semi-moist foods	2016	Chicken, pork/fish	3, 4	China	0
Canned wet foods	2016	Chicken, beef, mutton	9–11	Thailand	0
Canned wet foods	2016	Chicken	12–14	Italy	14

*Total: 35 samples of pet foods. Numbers 2, 4, 5, 33, and 34 are cat food; remaining samples are dog food



Pattern	No.	Year	mcr-1+/-	Source
X	1336	2016	+	dog
AF	1339	2016	-	dog
U	1338	2016	+	dog
W	1340	2016	+	dog
Q1	1202	2015	+	dog
Q2	0977	2012	-	cat
R	0913	2012	+	dog
AC	1156	2014	-	cat
S	1146	2014	+	dog
V	1343	2016	+	dog
M	1333	2016	+	dog
E	0950	2012	+	dog
E	0968	2012	+	dog
N	1137	2014	+	dog
Z	1150	2014	-	dog
P	1203	2015	+	dog
AB	1143	2014	-	dog
AD	1157	2014	-	dog
H	1342	2016	+	dog
D	1119	2014	+	dog
D	1128	2014	+	dog
Y	1159	2014	-	dog
L	1129	2014	+	dog
B	1108	2014	+	dog
B	1123	2014	+	dog
B	1130	2014	+	dog
B	1139	2014	+	human
B	1152	2014	+	dog
B	1161	2014	+	cat
O	1201	2015	+	dog
G2	1136	2014	+	dog
G1	1102	2014	+	dog
A1	1103	2014	+	dog
A1	1111	2014	+	dog
A1	1164	2014	+	dog
A1	10193	2013	+	dog
A1	1121	2014	+	dog
A2	10165	2013	+	dog
A2	10169	2013	+	dog
A2	1120	2014	+	dog
A2	1122	2014	+	dog
A2	1127	2014	+	dog
A2	10191	2013	+	dog
A2	1105	2014	+	dog
F	1145	2014	+	dog
F	1206	2015	+	dog
C	0902	2012	+	dog
C	0948	2012	+	dog
C	0926	2012	+	dog
C	0929	2012	+	dog
AE	0970	2012	-	dog
K	0944	2012	+	dog
J	10174	2013	+	dog
T	0958	2012	+	dog
I	10194	2013	+	dog

Technical Appendix Figure. XbaI pulsed-field gel electrophoretic analysis of colistin-resistant *Escherichia coli.*