

Emergence of the Plasmid-Mediated *mcr-1* Gene in Colistin-Resistant *Enterobacter aerogenes* and *Enterobacter cloacae*

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The gene *mcr-1* was reported as the first plasmid-mediated colistin resistance gene in *Escherichia coli* isolates from food animals, food, and patients in China (1). Since then, detection of *mcr-1*-positive strains has been reported in *Enterobacteriaceae* worldwide (2–4). The emergence of *mcr-1* has the potential to pose a major therapeutic challenge in the treatment of infections caused by *Enterobacteriaceae*. Here, we report the identification of *mcr-1* in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae* isolates.

E. aerogenes strain GB68 was recovered from the vaginal secretion of a 37-year-old pregnant female in Guangzhou, China, in August 2014. She was admitted to a hospital for cervical cerclage. Ten days after the surgery, she was diagnosed with *E. coli* vaginal infection by vaginal culture. In addition, she had mycoplasma coinfection in the reproductive tract. The vaginal infection resolved after treatment with azithromycin and amoxicillin-clavulanic acid. *E. cloacae* strain GB38 was isolated from the urine of a 70-year-old male with urinary tract infection, who was admitted to the same hospital in September 2014.

The species were identified using the API 20E system. Suscep-

tibility to various antimicrobial agents was tested by the agar dilution method (5). *E. aerogenes* GB68 was resistant to polymyxins, including colistin and polymyxin B with a MIC of 16 µg/ml each, cephalosporin, and ciprofloxacin. *E. cloacae* GB38 was resistant to all agents tested, including colistin and polymyxin B with a MIC of >32 µg/ml each, carbapenems, and tigecycline (Table 1). PCR analyses were performed to identify various resistance genes (6). The *mcr-1* gene was detected, as well as genes *bla*_{CTX-M-15}, *bla*_{TEM-1}, *qnrS*, and *aac(6')-Ib-cr* in both isolates and *armA* in *E. cloacae* GB38 (Table 1).

Accepted manuscript posted online 14 March 2016

Citation Zeng K-J, Doi Y, Patil S, Huang X, Tian G-B. 2016. Emergence of the plasmid-mediated *mcr-1* gene in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. *Antimicrob Agents Chemother* 60:3862–3863. doi:10.1128/AAC.00345-16.

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TABLE 1 Characteristics of *mcr-1*-harboring *Enterobacter aerogenes* and *Enterobacter cloacae* isolates

Characteristic	<i>Enterobacter aerogenes</i> GB68	<i>E. coli</i> C600 (transconjugant of <i>E. aerogenes</i> GB68)	<i>Enterobacter cloacae</i> GB38	<i>E. coli</i> C600 (transconjugant of <i>E. cloacae</i> GB38)	<i>E. coli</i> C600
Isolation date	August 2014		September 2014		
Inpatient or outpatient	Inpatient		Inpatient		
Isolation site	Vaginal secretion		Urine		
Resistance gene(s)	<i>mcr-1</i> , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>qnrS</i> , <i>aac(6')-Ib-cr</i>	<i>mcr-1</i> , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1}	<i>mcr-1</i> , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>armA</i> , <i>qnrS</i> , <i>aac(6')-Ib-cr</i>	<i>mcr-1</i>	
MIC (µg/ml)					
Colistin	16	16	>32	16	<0.25
Polymyxin B	16	16	>32	16	<0.25
Tigecycline	2	0.25	4	0.5	0.5
Ampicillin	>256	>256	>256	16	16
Amoxicillin-clavulanic acid	64	4	256	<2	<2
Cefotaxime	256	>256	>256	<1	<1
Ceftazidime	32	32	>256	2	<1
Cefepime	16	16	>256	<0.5	<0.5
Gentamicin	64	4	>256	4	<1
Amikacin	4	4	>256	4	<2
Ertapenem	<0.25	<0.25	>16	<0.25	<0.25
Imipenem	<0.25	<0.25	>16	<0.25	<0.25
Meropenem	<0.25	<0.25	>16	<0.25	<0.25
Fosfomycin	<16	<16	128	<16	<16
Nitrofurantoin	64	32	64	32	<16
Ciprofloxacin	16	0.016	64	0.032	<0.03

These two isolates were subjected to conjugation assays to assess the transferability of the *mcr-1*-harboring plasmid. The plasmid replicon type was determined by PCR (7). Colistin resistance in both isolates was successfully transferred to recipient strain *E. coli* C600. Additional PCR and sequencing analysis for resistance genes showed that transconjugants of *E. aerogenes* GB68 were positive for *bla*_{CTX-M-15} and *bla*_{TEM-1} as well. The results of S1 nuclease digestion followed by pulsed-field gel electrophoresis (S1-PFGE) and hybridizations for the transconjugants indicated that *mcr-1* was located on an ~65-kDa IncFI plasmid together with the two β -lactamase genes in *E. aerogenes* GB68 and on an ~70-kDa IncFI plasmid in *E. cloacae* GB38.

Our study indicates that the *mcr-1* gene can spread in *Enterobacteriaceae* species other than those initially reported, which include *E. coli*, *Klebsiella pneumoniae*, and *Salmonella enterica*. Broader surveillance of *Enterobacteriaceae* is warranted in areas with high prevalence of *mcr-1* in these species.

The patients gave written consent for publication. This study was approved by the ethics committee at Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China.

ACKNOWLEDGMENTS

We sincerely thank the patients for giving written consent for publication. We declare no competing interests.

FUNDING INFORMATION

This work, including the efforts of Guo-Bao Tian, was funded by The 111 Project (B13037). This work, including the efforts of Guo-Bao Tian, was funded by Guangdong Natural Science Foundation (S2013010015810). This work, including the efforts of Guo-Bao Tian, was funded by Program

of Science and Technology New Star of Guangdong (2014J2200038). This work, including the efforts of Guo-Bao Tian, was funded by National Natural Science Foundation of China (NSFC) (81471988).

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