AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

LETTER TO THE EDITOR

Detection of Colistin Resistance Gene *mcr-1* in Hypervirulent *Klebsiella pneumoniae* and *Escherichia coli* Isolates from an Infant with Diarrhea in China

Dan-xia Gu,[a] Yong-lu Huang,[a] Ji-hua Ma,[b] Hong-wei Zhou,[a] Ying Fang,[a] Jia-chang Cai,[a] Yan-yan Hu,[a] Rong Zhang[a]

Department of Clinical Laboratory, Second Affiliated Hospital of Zhejiang University, School of Medicine, Hangzhou, China[a]; Department of Laboratory Medicine, The Children's Hospital, Zhejiang University, School of Medicine, Hangzhou, China[b]

In recent years, antimicrobial resistance has increasingly been recognized as a significant problem that transcends international boundaries. With the global increase in the prevalence of carbapenem-resistant *Enterobacteriaceae*, polymyxins have been resurrected as a last-resort treatment option (1). Thus, the first decade of the 21st century witnessed a dramatic increase in the consumption of colistin (2). Heavy use of colistin in agriculture has been reported in China and other countries, and, inevitably, colistin resistance has been increasing (3). Resistance to polymyxins mainly depends on modification of lipopolysaccharide (LPS), which is often chromosomally mediated (4). Recently, Liu et al. identified the plasmid-mediated colistin resistance gene *mcr-1* in animals and humans in China (5), which was followed by reports from several other countries (6–8). In this report, we describe the detection of *mcr-1* in *Klebsiella pneumoniae* (belonging to capsular serotype K1) and *Escherichia coli* isolates from a stool specimen of an infant in China.

The patient displayed diarrhea of undetermined origin for 7 days and cough and fever for 5 days. He was transferred to a hospital in Hangzhou, China, in December 2015 after therapy at a local hospital failed to alleviate symptoms. After exclusion of dysentery and intussusception, the patient was diagnosed with acute enteritis and acute bronchitis. Tests for rotavirus, adenovirus, *Campylobacter jejuni*, and respiratory viruses were all negative. On the second day following admission, stool samples were collected and submitted for etiological diagnosis. *K. pneumoniae* 15451-1 and *E. coli* 15451-2 were isolated simultaneously. Following treatment with piperacillin-tazobactam (100 mg given every 8 h [q8h]) for 7 days, the cough had been alleviated but the diarrhea continued. The therapeutic regimen was changed to imipenem (50 mg q6h), and the patient improved and was discharged after 10 days of hospitalization.

Isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and antimicrobial susceptibility profiles were determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) (9). The MIC of colistin was determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (10). Both of the isolates presented polymyxin-resistant phenotypes (16 μg/ml for 15451-1 and 8 μg/ml for 15451-2) and were confirmed to be positive for *mcr-1* while negative for resistant mutations in *pmrAB*, *PhoPQ*, or *mgrB* by PCR and sequencing using previously reported primers (5). In addition, strain 15451-1 was positive for AmpC-type beta-lactamase gene $bla_{\mathrm{DHA\text{-}1}}$ and demonstrated a hypermucoviscous phenotype, as confirmed by a string test (11). Further detection of the K1 capsular serotype indicated that 15451-1 was a hypervirulent *K. pneumoniae* (hvKP) isolate. Conjugation experiments were conducted with rifampin-resistant *E. coli* EC600 (LacZ^{-} Nal^{r} Rif^{r}) as the recipient. Transconjugants were successfully obtained using both *K. pneumoniae* 15451-1 and *E. coli* 15451-2 (referred to here as 15451-1-C and 15451-2-C, respectively). Both the parental isolates and transconjugants were colistin resistant and carried *mcr-1*, as shown in Table 1. Plasmid analysis was carried out by S1-nuclease digestion and pulsed-field gel electrophoresis (PFGE) as previously described (12), and probing with the *mcr-1* DNA fragment was subsequently performed. The *mcr-1* gene was located on an ~75-kb IncF plasmid in *E. coli* 15451-2; however, the identity of the *mcr-1*-carrying plasmid in *K. pneumoniae* remained unknown, as the band of this isolate always diffused during S1-PFGE analysis. Multilocus sequence type (MLST) analysis of *K. pneumoniae* and *E. coli* was conducted according to previous reports (13, 14). Strain 15451-1 and strain 15451-2 were identified as ST661 and ST3176, respectively; ST661 and ST3176 have seldom been reported in the literature, and neither belongs to the popular sequence types.

Previous reports (5, 7) of high rates of detection of *mcr-1* in animals and human strongly suggested an underlying route of food chain transmission of *mcr-1*. Interestingly, the patient in this study was about 1 year of age, fed on breast milk and rice flour, and had no history of contact with poultry or treatment with polymyxins. Therefore, the origin of the *mcr-1*-carrying isolates is unknown. Further inquiries revealed that, prior to the infant's hospitalization, the grandparents had suffered transient diarrhea, although because the family members declined to supply stool samples, further research was not possible.

Detection of *mcr-1*-carrying hvKP and *E. coli* isolates from an infant patient is of significant importance. First, hvKP isolates are increasingly reported, as they can cause life-threatening infections (15). The emergence of *mcr-1* in hvKP isolates limits the available therapeutic options and presents an enormous challenge in the battle against infections. Second, the human gut is regarded as a reservoir for antibiotic resistance genes and plays a vital role in horizontal gene transfer. Simultaneous detection of *mcr-1* in *K.*

Accepted manuscript posted online 6 June 2016

Citation Gu D-X, Huang Y-L, Ma J-H, Zhou H-W, Fang Y, Cai J-C, Hu Y-Y, Zhang R. 2016. Detection of colistin resistance gene *mcr-1* in hypervirulent *Klebsiella pneumoniae* and *Escherichia coli* isolates from an infant with diarrhea in China. Antimicrob Agents Chemother 60:5099–5100. doi:10.1128/AAC.00476-16.

Address correspondence to Rong Zhang, brigitte_zr@163.com.

D.-X.G. and Y.-L.H. contributed equally to this article.

TABLE 1 Characteristics of *Klebsiella pneumoniae* 15451-1, *Escherichia coli* 15451-2, corresponding transconjugants (15451-1-C and 15451-1-C), and the recipient *E. coli* isolate EC600

Isolate	Antimicrobial susceptibility (μg/ml)[a]																Resistance gene(s)
	AMP	AMC	CZO	FOX	CRO	FEP	ATM	ETP	IPM	AMK	GEN	TOB	CIP	TIG	F	CL	
15451-1	≥32	≥32	≥64	≥64	≤1	≤1	≤1	≤0.5	≤1	≤2	≤1	8	2	2	128	16	*mcr-1*, bla_{DHA-1}
15451-1-C	≥32	8	≤4	16	≤1	≤1	≤1	≤0.5	≤1	≤2	≤1	≤1	≤0.25	≤0.5	≤16	16	*mcr-1*, bla_{DHA-1}
15451-2	16	8	≤4	32	≤1	≤1	≤1	≤0.5	≤1	≤2	≤1	≤1	0.5	≤0.5	≤16	8	*mcr-1*
15451-2-C	≥32	8	≤4	16	≤1	≤1	≤1	≤0.5	≤1	≤2	≤1	≤1	≤0.25	≤0.5	≤16	16	*mcr-1*
EC600	≤4	≤2	≤4	≤4	≤1	≤1	≤1	≤0.5	≤1	≤2	≤1	≤1	≤0.25	≤0.5	≤16	≤1	

[a] AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CZO, cefazolin; FOX, cefoxitin; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; ETP, ertapenem; IPM, imipenem; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; TIG, tigecycline; F, nitrofurantoin; CL, colistin.

pneumoniae and *E. coli* isolates from one patient indicates horizontal transmission of *mcr-1* between different species, leading to the likelihood of wide dissemination of colistin-resistant strains, and is thus a potential threat to the effective use of colistin. To address the global problem of polymyxin resistance, a comprehensive understanding of the emergence and epidemiology of these pathogens is needed. In addition, particular strategies, such as banning the use of polymyxins in agriculture and cutting off the transmission of resistant bacteria, should be promoted.

ACKNOWLEDGMENTS

We declare that we have no conflicts of interest.

This work was supported by National Natural Science Foundation of China (no. 81401720), Science Technology Department of Zhejiang Province (no. 2014C33191), and Health and Family Planning Commission of Zhejiang Province (no. 2016137891).

FUNDING INFORMATION

This work, including the efforts of Rong Zhang, was funded by Science Technology Department of Zhejiang Province (2014C33191). This work, including the efforts of Dan-xia Gu, was funded by Health And Family Planning Commission of Zhejiang Province (2016137891). This work, including the efforts of Hong-wei Zhou, was funded by National Natural Science Foundation of China (NSFC) (81401720).

REFERENCES

1. **Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM.** 2012. Colistin: an update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther **10:**917–934. http://dx.doi.org/10.1586/eri.12.78.
2. **Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R.** 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis **14:** 742–750. http://dx.doi.org/10.1016/S1473-3099(14)70780-7.
3. **Bradford PA, Kazmierczak KM, Biedenbach DJ, Wise MG, Hackel M, Sahm DF.** 2015. Correlation of beta-Lactamase production and colistin resistance among Enterobacteriaceae isolates from a global surveillance program. Antimicrob Agents Chemother **60:**1385–1392.
4. **Olaitan AO, Morand S, Rolain JM.** 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol **5:**643. http://dx.doi.org/10.3389/fmicb.2014.00643.
5. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J.** 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis **16:**161–168. http://dx.doi.org/10.1016/S1473-3099(15)00424-7.
6. **Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agerso Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas RS, Cavaco LM, Hansen DS, Aarestrup FM, Skov RL.** 2015. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro Surveill **20**(49). http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085.
7. **Hu Y, Liu F, Lin IY, Gao GF, Zhu B.** 2016. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis **16:**146–147. http://dx.doi.org/10.1016/S1473-3099(15)00533-2.
8. **Olaitan AO, Chabou S, Okdah L, Morand S, Rolain JM.** 2016. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis **16:**147. http://dx.doi.org/10.1016/S1473-3099(15)00540-X.
9. **CLSI.** 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **EUCAST.** 2016. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0. The European Committee on Antimicrobial Susceptibility Testing. http://www.eucast.org.
11. **Pomakova DK, Hsiao CB, Beanan JM, Olson R, MacDonald U, Keynan Y, Russo TA.** 2012. Clinical and phenotypic differences between classic and hypervirulent *Klebsiella pneumoniae*: an emerging and underrecognized pathogenic variant. Eur J Clin Microbiol Infect Dis **31:**981–989. http://dx.doi.org/10.1007/s10096-011-1396-6.
12. **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N.** 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis **10:**597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2.
13. **Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol **43:**4178–4182. http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005.
14. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol **60:** 1136–1151. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x.
15. **Siu LK, Yeh K-M, Lin J-C, Fung C-P, Chang F-Y.** 2012. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lancet Infect Dis **12:** 881–887. http://dx.doi.org/10.1016/S1473-3099(12)70205-0.