

# Epidemiology and molecular characterization of *mcr-1* in *Escherichia coli* recovered from patients with bloodstream infections in Changsha, central China

This article was published in the following Dove Press journal:  
*Infection and Drug Resistance*

Yi-Ming Zhong<sup>1</sup>  
Wen-En Liu<sup>1</sup>  
Zhao-Feng Zheng<sup>2</sup>

<sup>1</sup>Department of Clinical Laboratory, Xiangya Hospital, Central South University, Changsha, People's Republic of China; <sup>2</sup>Faculty of Laboratory Medicine, Xiangya School of Medicine, Central South University, Changsha, People's Republic of China

**Objectives:** The main aim of this study was to investigate the prevalence and molecular characteristics of the *mcr-1* gene in *Escherichia coli* isolates obtained from all patients with bloodstream infections over a year in a Chinese teaching hospital. We also assessed the susceptibility profiles of the *mcr-1*-positive strains and prognostic impact of this gene on the patients.

**Methods:** A total of 144 consecutive, non-repetitive *E. coli* isolates causing bloodstream infections were collected at a teaching hospital in Changsha, China from January to December 2016. The presence of the *mcr-1* gene was assessed by PCR. All *mcr-1*-positive *E. coli* isolates were characterized by antimicrobial susceptibility testing, multilocus sequence typing (MLST), a conjugation experiment, and plasmid replicon typing. Clinical data were obtained from medical records.

**Results:** The *mcr-1* gene was detected in three (2.1%) of the 144 *E. coli* isolates. The three *mcr-1*-positive *E. coli* isolates were resistant to colistin. All three isolates showed a lower resistance to other classes of antibacterials, with all three being susceptible to carbapenems. The MLST results indicated that the three *E. coli* isolates were assigned to three different sequence types: ST457, ST101, and ST1413, respectively. The conjugation experiment showed that the *mcr-1* gene was successfully transferred to the recipient (*E. coli* EC600) from two isolates, one of which possessed IncII replicons and the other of which carried IncHI2 and IncN replicons. The patients with bloodstream infections caused by *mcr-1*-positive isolates had severe underlying diseases and were cured after antibacterial treatment.

**Conclusion:** The prevalence of the *mcr-1* gene in patients with *E. coli* bloodstream infection was 2.1% in Changsha, China. The *mcr-1*-positive *E. coli* isolates had varied susceptibility profiles, although all three were susceptible to carbapenems. This therapeutic window is crucial given the risk of rapid deterioration in high-incidence areas worldwide.

**Keywords:** *E. coli*, *mcr-1*, colistin resistance, bloodstream infection, clinical characteristics

## Introduction

Multidrug-resistant Gram-negative bacteria such as carbapenem-resistant Enterobacteriaceae have led to the resurgent use of polymyxins (including polymyxin B and colistin) as “last-line” treatment options.<sup>1–3</sup> However, the increased use of polymyxin antibacterials has been accompanied by more frequent reports of emerging polymyxin-resistant isolates around the world.<sup>1,3,4</sup> The mechanisms of polymyxin resistance have been found to involve alterations in the PmrAB or PhoPQ two-component regulatory systems caused by chromosome-mediated

Correspondence: Wen-En Liu  
Department of Clinical Laboratory,  
Xiangya Hospital, Central South  
University, Changsha, Hunan Province  
410008, People's Republic of China  
Tel +86 731 8432 7437  
Fax +86 731 8432 7332  
Email wenenliu@163.com

mutations.<sup>1,4</sup> Although the earliest reports of the emerging plasmid-mediated colistin resistance gene *mcr-1* originated from China, subsequent studies from other countries reported similar results.<sup>1,5–7</sup> In China, the prevalence rate of the *mcr-1* gene in Enterobacteriaceae in samples from humans ranged from 0.6% among consecutive clinical infection isolates in central China to 6.2% among fecal samples of patients in southern China.<sup>8,9</sup> Several studies have focused on clinical *Escherichia coli* isolates carrying the *mcr-1* gene from patients with bloodstream infection.<sup>7,10,11</sup> In a recent Chinese study, Quan et al showed that 1% of *E. coli* isolates causing bloodstream infections carried *mcr-1*.<sup>1</sup> More worryingly, the co-presence of the *mcr-1* gene with carbapenem and extended-spectrum beta-lactamase (ESBL) resistance genes has been reported in several studies,<sup>1,5,11</sup> indicating the possibility of pan-drug resistance strains. Many plasmids, such as IncHI2 and IncI2, have been involved in the spread of *mcr-1*. China's high population density makes the country vulnerable to uncontrolled spread of *mcr-1*-positive *E. coli* unless vigorous surveillance measures are implemented immediately.

The aims of this study were to investigate the prevalence and molecular characteristics of the *mcr-1* gene in *E. coli* isolates obtained from all patients with bloodstream infection over a year in a Chinese teaching hospital. The susceptibility profiles of the *mcr-1*-positive strains and the prognostic impact of this gene were also assessed.

## Materials and methods

### Bacterial isolates

From January to December 2016, 144 consecutive, non-repetitive *E. coli* isolates causing bloodstream infections, one from each patient, were collected at Xiangya Hospital, a general teaching hospital affiliated with Central South University (Changsha, Hunan Province, China) that has 3500 beds and 7500–10,000 patient visits every day. Isolates were identified using the Vitek 2-system (bioMérieux, Marcy-l'Étoile, France). The *mcr-1*-positive control strain was provided by Prof. Jianzhong Shen, who was the first to report the *mcr-1* gene. *E. coli* ATCC 25922 was used as a negative quality control strain.

### Screening for the *mcr-1* gene in clinical strains

Screening for the *mcr-1* gene was performed as previously described.<sup>5</sup> The PCR products were bidirectionally

sequenced. Sequence type (ST) determination was performed according to the *E. coli* multilocus sequence typing (MLST) database.<sup>1</sup> The primers used for all PCR reactions are listed in Table S1.

### Clinical data collection

For the cases of bloodstream infection caused by *mcr-1*-positive *E. coli*, we reviewed the medical records and collected patient data, including data on demographic characteristics, underlying disease, clinical manifestations, treatments, and clinical outcomes. The study was approved by the Ethical Committee of Xiangya Hospital of Central South University.

### Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) of colistin for the *mcr-1*-positive isolates was determined using the broth microdilution method.<sup>12</sup> The antimicrobial susceptibility of other antibacterials, including ampicillin, ceftazidime, cefepime, aztreonam, piperacillin–tazobactam, imipenem, ertapenem, gentamicin, amikacin, ciprofloxacin, and trimethoprim/sulfamethoxazole, was determined using the Vitek 2 system (bioMérieux). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint recommendations.<sup>13</sup>

### Conjugation experiment

The conjugation experiment was carried out in mixed broth cultures with rifampin-resistant *E. coli* EC600 as the recipient strain. Briefly, purified donor strains (*mcr-1*-positive strains) and recipient strains (*E. coli* EC600) were separately inoculated in Luria–Bertani (LB) broth at 37 °C for 4 h. A 20 µl aliquot of mixture containing 100 µl of each of the above cultures was then added onto a microporous membrane that was pre-placed on Mueller–Hinton agar. After overnight culture at 37 °C, all strains were collected and the *E. coli* transconjugants were then selected on the basis of growth on Mueller–Hinton agar containing colistin (2 mg/L) and rifampicin (600 mg/L).<sup>1</sup> Transfer frequencies were calculated as the number of transconjugants obtained per recipient. The presence of *mcr-1* was confirmed by PCR. The transconjugants' colistin MIC values were also determined.

### Plasmid replicon typing

Plasmid replicons were determined using the PCR-based replicon typing scheme.<sup>14</sup>

## Results

### Screening for the *mcr-I* gene

Among the 144 *E. coli* strains recovered from patients with bloodstream infection, three (2.1%) were positive for the *mcr-I* gene.

### Antimicrobial susceptibility and molecular characteristics of *mcr-I* genes

As indicated in Table 1, the *mcr-I*-positive *E. coli* strains in this study, herein designated #1, #2, and #3, all exhibited an unfavorable antimicrobial susceptibility profile in addition to exhibiting colistin resistance conferred by the *mcr-I* gene. The MLST results indicated that the three isolates were assigned to three different STs: ST457, ST101, and ST1413, respectively.

Strain #1 carried IncII replicons. Strain #2 carried IncHI2 and IncN replicons. Strain #3 was not typed (Table 1). In the conjugation experiment, the *mcr-I* gene was successfully transferred to the *E. coli* EC600 standard recipient from strains #1 and #2, but not from strain #3. The transfer frequency of the *mcr-I* gene for strains #1 and #2 was  $6.4 \times 10^{-6}$  and  $2.2 \times 10^{-6}$ , respectively. The two resulting transconjugants both exhibited elevated colistin MIC values.

### Clinical information on the three patients infected with *mcr-I*-positive isolates

The demographic and clinical characteristics of the three patients are summarized in Table 2. All three patients infected with *mcr-I*-positive *E. coli* had severe underlying diseases. Two patients were hospitalized within 3 months prior to the onset of bloodstream infection. Patient #1 was a 72-year-old woman with a history of right hemicolectomy because of colon cancer. She did not follow clinical recommendations regarding receiving postoperative chemotherapy and she was later diagnosed with colon cancer recurrence and metastasis. Patient #2 was a 10-year-old male child. He was diagnosed with acute leukemia and had undergone chemotherapy on three occasions. He was admitted to the Emergency Department due to septic shock. Patient #3 was a 47-year-old man with several underlying diseases, including coronary heart disease, type 2 diabetes mellitus, diabetic nephropathy, and stage 5 chronic kidney disease. After hemodialysis, he was admitted to hospital after developing signs of septic shock. All three patients with bloodstream infection were cured after treatment with antibacterials that they had been found to be susceptible to.

**Table 1** Antibiotic susceptibility, ST and replicon type of the three *mcr-I*-positive isolates

Strain	Susceptibility categorization											ST	Replicon type
	CST	AMP	CAZ	FEP	ATM	TZP	IPM	ETM	GEN	AMK	CIP		
#1	4 (R)	2 (S) <sup>a</sup>	16 (R)	2 (I)	16 (R)	8 (S)	≤1 (S)	≤0.5 (S)	≤1 (S)	≤2 (S)	2 (R)	457	II
#2	4 (R)	4 (R) <sup>a</sup>	16 (R)	16 (R)	>64 (R)	≤4 (S)	≤1 (S)	≤0.5 (S)	>16 (R)	≤2 (S)	>4 (R)	101	HI2, N
#3	4 (R)	>32 (R)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤1 (S)	≤0.5 (S)	>16 (R)	≤2 (S)	≤0.25 (S)	1413	<sup>b</sup>

Notes: <sup>a</sup>transconjugants; <sup>b</sup>not detected.

Abbreviations: CST, colistin; AMP, ampicillin; CAZ, cefazidime; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; IPM, imipenem; ETM, eropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; R, resistant; I, intermediate; S, susceptible.

**Table 2** Clinical characteristics of the three patients infected with *mcr-1*-positive isolates

Clinical characteristic	Patient #1	Patient #2	Patient #3
Age (years)	72	10	47
Gender	F	M	M
Hospital admission date	06/15/2016	01/12/2016	11/29/2016
Underlying disease	Colon cancer	Acute leukemia	Diabetes mellitus, coronary heart disease, hypertension, chronic hepatitis B, diabetic nephropathy, stage 5 chronic kidney disease
Surgery	Right hemicolectomy	No	No
Invasive procedures	No	Peripherally inserted central catheter	Hemodialysis
Hospitalization within 3 months	Yes	Yes	No
Prior broad-spectrum antibacterial use within 3 months	Ceftazidime	Cefoperazone-sulbactam	No
Previous colistin use	No	No	No
Previous animal exposure	No	No	No
Source of infection	Primary bacteremia	Pneumonia	Hemodialysis
Acquisition of bacteremia	Healthcare-associated	Healthcare-associated	Healthcare-associated
Maximum temperature (°C)	39.2	40.2	40
Antibacterial use after isolation	Imipenem	Piperacillin-tazobactam, imipenem	Meropenem, piperacillin-tazobactam
Treatment duration (days)	10	5	22
Outcome	Survived	Survived	Survived

## Discussion

In our study, 144 clinical *E. coli* isolates were collected from patients treated for bloodstream infection in a teaching hospital in China. Of these isolates, three (2.1%) carried *mcr-1* genes. Our data showed that the low prevalence of the *mcr-1* gene among *E. coli* isolates from human bloodstream infections was slightly higher than that observed (0–1%) from patients in other countries and regions,<sup>1,11,15,16</sup> whereas Lai et al detected *mcr-1* carriage in 75% of *E. coli* strains from pigs.<sup>11</sup> Hence, the reservoir for *mcr-1*-positive *E. coli* seems to be larger among animals than among humans. Some studies have reported that *mcr-1*-positive *E. coli* might have a susceptibility profile that is generally favorable for antibacterials other than colistin.<sup>17,18</sup> On the other hand, Lai et al have shown that *mcr-1*-positive *E. coli* can be highly resistant, thus suggesting that the potential for *mcr-1* gene dissemination is not limited to community strains with a low baseline level of antimicrobial resistance.<sup>11</sup> In this study, the *mcr-1*-positive *E. coli* strains #1 and #2 were resistant to cephalosporins, while all three strains were susceptible to carbapenems. This highlights the risk that

the *mcr-1* gene might spread even in carbapenem-susceptible *E. coli* isolates. This may be due to the fact that not all plasmids with the *mcr-1* gene carry other genes conferring resistance to clinically relevant antibacterials such as carbapenemase.<sup>17,19</sup> Therefore, it seems imperative that testing for colistin susceptibility should be part of the clinical routine in all microbiological laboratories in China.

A recent study involving an MLST analysis reported the extreme divergence of *E. coli* strains carrying *mcr-1* in different provinces and even in different hospitals in China.<sup>20</sup> In the current study, the high genetic diversity among the *mcr-1*-positive *E. coli* strains #1, #2, and #3, which belonged to STs ST457, ST101, and ST1413, respectively, indicates that the isolates were unrelated. *E. coli* ST101 is considered as having an environmental lineage (related to water, sewage, and poultry), while many clinical isolates in multiple countries are also classified as *E. coli* ST101.<sup>20–22</sup> It is also regarded as a reservoir for antibiotic resistance genes. The *mcr-1*-positive *E. coli* ST101 strains have been detected in chickens, public transportation, and one hospital in

China.<sup>20,22,23</sup> Thus, more attention should be focused on *E. coli* ST101 as it might promote the spread of *mcr-1*. In contrast, *mcr-1*-positive *E. coli* ST457 is very rare and we are the first to report ST1413 carrying *mcr-1* in China.

In this study, we found that strain #1 carried one replicon (IncI1) and strain #2 carried two replicons (IncHI2 and IncN). Several studies have reported that the *mcr-1* gene is primarily located on two prevalent plasmids (IncHI2 and IncI2).<sup>1,10,24–26</sup> Consequently, these plasmids might play an important role in driving the spread of the *mcr-1* gene. The conjugation experiment in this study, in which the *mcr-1* gene was successfully transferred from strains #1 and #2 to the *E. coli* EC600 standard recipient, clearly demonstrates the potential for horizontal transfer of the *mcr-1* gene. In contrast, in an experiment using the same methodology, strain #3 did not transfer the gene. This indicates that the *mcr-1* gene of strain #3 might be located on a yet unknown non-conjugative plasmid. Further studies are required to elucidate and monitor the spread of the *mcr-1* gene in China.

Previous reports on the epidemiology of *mcr-1*-positive members of the Enterobacteriaceae bacterial family have suggested a narrow variety of sources for bloodstream infection.<sup>7,17,18,27</sup> Contrary to these findings, we have showed that *mcr-1*-positive *E. coli* might invade the bloodstream, not only from the urinary and biliary organs,<sup>7,17,18,27</sup> but also from the respiratory tract and as primary, idiopathic infection. Some of our findings are in line with previous studies,<sup>7,17,18</sup> however, since unhealthy patients might be vulnerable to *mcr-1*-positive *E. coli* bloodstream infection. Previous studies have established an association between infection and both kidney disease and diabetes mellitus,<sup>7,17,18</sup> and, of the three patients in our study, two had cancer and one had diabetes mellitus. Of the three patients, two were treated with antibacterials in the 3-month period before admission to hospital. Interestingly, none of the patients received previous treatment with colistin or had close contact with farm animals. We postulate that considering the occurrence of *mcr-1*-positive Enterobacteriaceae in the environment, vegetables, and meat in China,<sup>28–30</sup> the patients might have been exposed to the *mcr-1* gene via these sources. Similar to previous reports,<sup>7,17,18,27</sup> all three patients with *mcr-1*-positive *E. coli* bloodstream infection had a favorable outcome after treatment with antibacterials that they had been found to be susceptible

to. However, Lai et al have reported that four patients out of ten (40%) succumbed to bloodstream infection despite being intensively treated (often with more than one antibacterial preparation).<sup>11</sup> The inconsistent findings regarding the outcomes in these studies might be due to the small sample sizes.<sup>7,11,17,18,27</sup>

A limitation of our study is that the *mcr-1*-positive strains were not analyzed by whole-genome sequencing and thus further studies are required to better clarify the molecular characteristics of this resistance gene. However, this systematic investigation of all bloodstream infections caused by *E. coli* over a year in a Chinese hospital is noteworthy.

## Conclusion

In an examination of 144 consecutive, non-repetitive, clinical samples of invasive *E. coli* isolates retrieved from a hospital in China over one year, we found three invasive *E. coli* isolates that were *mcr-1*-positive (2.1% of all samples). Patients, especially those with underlying diseases, may be infected with *mcr-1*-positive *E. coli* even if they have not received previous treatment with colistin. The three *mcr-1*-positive *E. coli* isolates had varied susceptibility profiles, although all three were susceptible to carbapenems. This therapeutic window is crucial given the risk of rapid deterioration in high-incidence areas of the world. We call for immediate inclusion of colistin in routine susceptibility testing in all microbiological laboratories in China.

## Ethical approval

This study was approved by the Ethics Committee of the Xiangya Hospital of Central South University, and the requirement for informed consent from patients was waived because the study was retrospective and used a database that ensured confidentiality.

## Acknowledgments

We thank Prof. Jianzhong Shen for offering the *mcr-1*-positive control strain. This work was supported by National Natural Science Foundation of China (81672066).

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Quan J, Li X, Chen Y, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. *Lancet Infect Dis.* 2017;17(4):400–410. doi:10.1016/S1473-3099(16)30528-X



2. Long W, Yuan J, Liu J, et al. Multidrug resistant brain abscess due to *Acinetobacter baumannii* Ventriculitis cleared by intraventricular and intravenous tigecycline therapy: a case report and review of literature. *Front Neurol*. 2018;9:518. doi:10.3389/fneur.2018.00518
3. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol*. 2014;5:643. doi:10.3389/fmicb.2014.00643
4. Halaby T, Al Naiemi N, Kluytmans J, et al. Emergence of colistin resistance in Enterobacteriaceae after the introduction of selective digestive tract decontamination in an intensive care unit. *Antimicrob Agents Chemother*. 2013;57(7):3224–3229. doi:10.1128/AAC.02634-12
5. Liu YY, Wang Y, Walsh TR, et al. 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*. 2016;16(2):161–168. doi:10.1016/S1473-3099(15)00424-7
6. Brennan E, Martins M, McCusker MP, et al. Multidrug-resistant *Escherichia coli* in bovine animals, Europe. *Emerg Infect Dis*. 2016;22(9):1650–1652. doi:10.3201/eid2209.160140
7. Hasman H, Hammerum AM, Hansen F, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill*. 2015;20(49):30085. doi:10.2807/1560-7917.ES.2015.20.49.30085
8. Cao L, Li X, Xu Y, et al. Prevalence and molecular characteristics of *mcr-1* colistin resistance in *Escherichia coli*: isolates of clinical infection from a Chinese University Hospital. *Infect Drug Resist*. 2018;11:1597–1603. doi:10.2147/IDR.S166726
9. Zhong LL, Phan HTT, Shen C, et al. High rates of human fecal carriage of *mcr-1*-positive multidrug-resistant Enterobacteriaceae emerge in China in association with successful plasmid families. *Clin Infect Dis*. 2018;66(5):676–685. doi: 10.1093/cid/cix885
10. Macesic N, Green D, Wang Z, et al. Detection of *mcr-1*-Carrying *Escherichia coli* causing bloodstream infection in a New York City Hospital: avian origins, human concerns? *Open Forum Infect Dis*. 2017;4(3):ofx115. doi:10.1093/ofid/ofx115
11. Lai CC, Lin YT, Lin YT, et al. Clinical characteristics of patients with bacteraemia due to the emergence of *mcr-1*-harbouring Enterobacteriaceae in humans and pigs in Taiwan. *Int J Antimicrob Agents*. 2018;52(5):651–657. doi:10.1016/j.ijantimicag.2018.08.015
12. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-seventh Informational Supplement M100-S27*. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
13. European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters*. European Committee on Antimicrobial Susceptibility Testing; 2017. Available from: [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_tables/v\\_7.1\\_Breakpoint\\_Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf). Accessed July 05, 2019.
14. Johnson TJ, Wannemuehler YM, Johnson SJ, et al. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. *Appl Environ Microbiol*. 2007;73(6):1976–1983. doi:10.1128/AEM.02171-06
15. Juhász E, Iván M, Pintér E, et al. Colistin resistance among blood culture isolates at a tertiary care centre in Hungary. *J Glob Antimicrob Resist*. 2017;11:167–170. doi:10.1016/j.jgar.2017.08.002
16. Nordmann P, Assouvie L, Prod'Hom G, et al. Screening of plasmid-mediated MCR-1 colistin-resistance from bacteremia. *Eur J Clin Microbiol Infect Dis*. 2016;35(11):1891–1892. doi:10.1007/s10096-016-2739-0
17. Corbella M, Mariani B, Ferrari C, et al. Three cases of *mcr-1*-positive colistin-resistant *Escherichia coli* bloodstream infections in Italy, August 2016 to January 2017. *Euro Surveill*. 2017;22(16):30517. doi:10.2807/1560-7917.ES.2017.22.16.30517
18. Nordmann P, Lienhard R, Kieffer N, et al. Plasmid-mediated colistin-resistant *Escherichia coli* in bacteremia in Switzerland. *Clin Infect Dis*. 2016;62(10):1322–1323. doi:10.1093/cid/ciw124
19. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev*. 2017;30(2):557–596. doi:10.1128/CMR.00064-16
20. Wang Y, Tian GB, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. *Lancet Infect Dis*. 2017;17(4):390–399. doi:10.1016/S1473-3099(16)30527-8
21. Toleman MA, Bugert JJ, Nizam SA. Extensively drug-resistant New Delhi metallo- $\beta$ -lactamase-encoding bacteria in the environment, Dhaka, Bangladesh, 2012. *Emerg Infect Dis*. 2015;21(6):1027–1030. doi:10.3201/eid2106.141578
22. Shen C, Feng S, Chen H, et al. Transmission of *mcr-1*-producing multidrug-resistant Enterobacteriaceae in public transportation in Guangzhou, China. *Clin Infect Dis*. 2018;67(Suppl 2):S217–S224. doi:10.1093/cid/ciy661
23. Wu C, Wang Y, Shi X, et al. Rapid rise of the ESBL and *mcr-1* genes in *Escherichia coli* of chicken origin in China, 2008–2014. *Emerg Microbes Infect*. 2018;7(1):30. doi:10.1038/s41426-018-0033-1
24. Zheng B, Yu X, Xu H, et al. Complete genome sequencing and genomic characterization of two *Escherichia coli* strains co-producing MCR-1 and NDM-1 from bloodstream infection. *Sci Rep*. 2017;7(1):17885. doi:10.1038/s41598-017-18273-2
25. Mohsin J, Pál T, Petersen JE, et al. Plasmid-mediated colistin resistance gene *mcr-1* in an *Escherichia coli* ST10 bloodstream isolate in the Sultanate of Oman. *Microb Drug Resist*. 2018;24(3):278–282. doi:10.1089/mdr.2017.0131
26. Xu L, Wang P, Cheng J, et al. Characterization of a novel blaNDM-5-harboring IncFII plasmid and an *mcr-1*-bearing IncI2 plasmid in a single *Escherichia coli* ST167 clinical isolate. *Infect Drug Resist*. 2019;12:511–519. doi:10.2147/IDR.S192998
27. Rossi F, Girardello R, Morais C, et al. Plasmid-mediated *mcr-1* in carbapenem-susceptible *Escherichia coli* ST156 causing a blood infection: an unnoticeable spread of colistin resistance in Brazil? *Clinics (Sao Paulo)*. 2017;72(10):642–644. doi:10.6061/clinics/2017(10)09
28. Ji X, Zheng B, Berglund B, et al. Dissemination of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* carrying *mcr-1* among multiple environmental sources in rural China and associated risk to human health. *Environ Pollut*. 2019;251:619–627. doi:10.1016/j.envpol.2019.05.002
29. Liu BT, Li X, Zhang Q, et al. Colistin-resistant *mcr*-positive Enterobacteriaceae in fresh vegetables, an increasing infectious threat in China. *Int J Antimicrob Agents*. 2019;54(1):89–94. doi:10.1016/j.ijantimicag.2019.04.013
30. Liu BT, Song FJ, Zou M. Characterization of highly prevalent plasmids coharboring *mcr-1*, *oxqAB*, and *blaCTX-M* and plasmids harboring *oxqAB* and *blaCTX-M* in *Escherichia coli* isolates from food-producing animals in China. *Microb Drug Resist*. 2019;25(1):108–119. doi:10.1089/mdr.2017.0391

## Supplementary material

**Table S1** The primers used for all PCR reactions in this study

Name	Direction	DNA sequence (5'-3')
<i>mcr-I</i>	mcr-I-F mcr-I-R	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG
MLST	adk-F adk-R adk-FI adk-RI fumC-F fumC-R fumC-RI gyrB-F gyrB-R gyrB-RI icd-F icd-R mdh-F Mdh-R mdhF-I mdhR-I purA-F purA-R purA-FI recA-F recA-R recA-FI recA-RI	ATTCTGCTTGGCGCTCCGGG CCGTCAACTTTCGCGTATTT TCATCATCTGCACTTCCGC CCAGATCAGCGCGAACTTCA TCACAGGTCGCCAGCGCTTC GTACGCAGCGAAAAAGATT TCCCGGCAGATAAGCTGTGG TCGGCGACACGGATGACGGC ATCAGGCCTTCACGCGCATC GTCCATGTAGGCGTTACAGG ATGGAAAGTAAAGTAGTTGTTCCGGCACA GGACGCAGCAGGATCTGTT ATGAAAGTCGCAGTCTCGGCGCTGCTGGCGG TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT AGCGCGTTCTGTTCAAATGC CAGGTTCAGAACTCTCTCTGT CGCGCTGATGAAAGAGATGA CATACGGTAAGCCACGCAGA TCGGTAACGGTGTTGTGCTG CGCATTGCTTTACCCTGACC TCGTGCGAAATCTACGGACCGGA ACCTTTGTAGCTGTACCACG AGCGTGAAGGTAAAACCTGTG
Plasmid replicon typing	B/O-F B/O-R FIC-F FIC-R A/C-F A/C-R P-F P-R T-F T-R K/B-F K/B-R W-F W-R FIIA-F FIIA-R FIA-F FIA-R FIB-F FIB-R Y-F Y-R	GCGGTCCGGAAAGCCAGAAAAC TCTGCGTTCGCCAAGTTCTGA GTGAACTGGCAGATGAGGAAGG TTCTCCTCGTCGCCAACTAGAT GAGAACCAGAACAAAGACCTGGA ACGACAAACCTGAATTGCCTCCTT CTATGGCCCTGCAAACGCGCCAGAAA TCACGCGCCAGGGCGCAGCC TTGGCCTGTTTGTGCCTAAACCAT CGTTGATTACACTTAGCTTTGGAC GCGGTCCGGAAAGCCAGAAAAC TCTTTCACGAGCCCCGCCAAA CCTAAGAACAACAAAGCCCCCG GGTGCGCGGCATAGAACCGT CTGTCGTAAGCTGATGGC CTCTGCCACAACTTCAGC CCATGCTGGTTCTAGAGAAGGTG GTATATCCTTACTGGCTTCCGCAG GGAGTTCTGACACACGATTTTCTG CTCCCGTCGCTTCAGGGCATT AATTCAAACAACACTGTGCAGCCTG GCGAGAATGGACGATTACAAAACCTT

(Continued)

**Table S1** (Continued).

Name	Direction	DNA sequence (5'-3')
	II-F	CGAAAGCCGGACGGCAGAA
	II-R	TCGTCGTTCCGCCAAGTTTCGT
	Frep-F	TGATCGTTTAAGGAATTTTG
	Frep-R	GAAGATCAGTCACACCATCC
	X-F	AACCTTAGAGGCTATTTAAGTTGCTGAT
	X-R	TGAGAGTCAATTTTTATCTCATGTTTTAGC
	HII-F	GGAGCGATGGATTACTTCAGTAC
	HII-R	TGCCGTTTCACCTCGTGAGTA
	N-F	GTCTAACGAGCTTACCGAAG
	N-R	GTTTCAACTCTGCCAAGTTC
	HI2-F	TTTCTCCTGAGTCACCTGTTAACAC
	HI2-R	GGCTCACTACCGTTGTCATCCT
	L/M-F	GGATGAAAACATCAGCATCTGAAG
	L/M-R	CTGCAGGGGCGATTCTTTAGG

## Infection and Drug Resistance

Dovepress

### Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>