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Research Paper

Expression characteristics of the plasmid-borne *mcr-1* colistin resistance gene

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ABSTRACT

The plasmid-encoded colistin resistance gene (*mcr-1*) has recently been reported in various Gram-negative species. However, the expression profile of *mcr-1* remains unknown. Here, we investigated the expression of *mcr-1* in various plasmids and bacteria. The *mcr-1* expression levels in pMCR1_IncX4 varied from 1.81 × 10^{-5} to 1.05 × 10^{-4} (pmol per μg total RNA) in the two *K. pneumoniae* strains SZ03 and SZ04 (ST25) and the two *E. coli* strains SZ01 and CDA6 (ST2448 and ST167, respectively). The *mcr-1* expression levels of pMCR1_IncI2 in E. coli SZ02 (ST2085) and *E. coli* BJ13 (ST457) were 5.27 × 10^{-5} and 2.58 × 10^{-5}, respectively. In addition, the expression of chromosomal *mcr-1* in ST156 *E. coli* BJ10 was 5.49×10^{-5}. Interestingly, after 4μg/ml colistin treatment, mcr-1 in pMCR1_IncX4 increased 2- and 4-fold at 20 and 120 mins, respectively, in all pMCR1_IncX4-harboring strains, except for ST2448 *E. coli*, which had a lower expression after 20 mins that restored to baseline levels after 120 mins. In contrast, *mcr-1* expression of pMCR1_IncI2 in the two *E. coli* strains (SZ02, BJ13) and the chromosomal *mcr-1* in *E. coli* (BJ10) remained at baseline levels after 20 and 120 mins. In the same genetic background host strain *E. coli* E600, *mcr-1* expression of pMCR1_IncX4 and pMCR1_IncI2 were similar and were decreased after colistin treatment for 20 min. However, *mcr-1* in pMCR1_IncX4 was up-regulated after colistin treatment for 120 min, while *mcr-1* in pMCR1_IncI2 was down-regulated compared to the untreated control. Our results suggested that *mcr-1* has distinct expression profiles on different plasmids, bacterial hosts, and after antibiotic treatment.

INTRODUCTION

Multidrug-resistant Gram-negative bacteria, particularly carbapenem-resistant Enterobacteriaceae (CRE), have spread globally into hospitals and communities, and thus have become a significant public health concern [1, 2]. For clinical infections caused by CRE, the treatment options are limited and the polymixins (colistin and polymyxin B) are the last-resort antibiotic. The recent identification of a plasmid-encoded polymyxin resistance mechanism (MCR-1) in Enterobacteriaceae from both human and animal samples suggests that this last-resort antibiotic may be under jeopardy [3, 4]. To date, *mcr-1*-harboring plasmids have been identified in a number of countries with a wide

geographical distribution, and some MCR-1 producing strains were resistant to multiple antibiotics [5, 6].

Polymyxin resistance is the result of a 4′-phosphoethanolamine (PEA) or 4-amino-4-deoxy-L-arabinose (L-Arap4N) modification of bacterial lipid A, which is a component of the lipopolysaccharide (LPS), and results in a reduction in polymyxin affinity. Resistance is usually chromosomally mediated and involves modulation of two-component regulatory systems (e.g., *pmrAB*, *phoPQ*, and its negative regulator *mgrB* in *Klebsiella pneumoniae*) [7, 8]. The plasmid-transferrable mobilized colistin resistance gene *mcr-1*, encoding a novel PEA-transferase [3, 4], is able to mediate a PEA addition to the lipid A moiety at the 4′-phosphate group, thereby causing colistin resistance. So far, gene expression and transcriptomic analyses of chromosomal colistin resistance genes have been reported frequently. In contrast, the number of studies on gene regulation of *mcr-1* is limited, since most of the *mcr-1* studies have been focusing on epidemiological investigations of *mcr-1* in different Enterobacteriaceae, including *Escherichia coli*, *Salmonella enterica*, *K. pneumonia*, *Enterobacter aerogenes* and *Enterobacter cloacae* [9–11].

Different incompatibility (InC) groups of plasmids have been found to carry *mcr-1*, while among them the IncI2 and IncX4 plasmids were most commonly reported. In the previous studies, we completely sequenced *mcr-1*-harboring plasmids pMCR1_IncX4 and pMCR1_IncI2 from clinical *K. pneumoniae* and *E. coli* strains [12]. In addition, we genomically characterized one of the first chromosomally encoded *mcr-1* genes from an *E. coli* (BJ10) isolate [13]. In this study, we used quantitative reverse transcription PCR (qRT-PCR) to evaluate the expressions of *mcr-1* of different plasmids (pMCR1_IncX4 and pMCR1_IncI2) within different species (*E. coli* and *K. pneumoniae*) and their changes in response to colistin challenge.

RESULTS

Analysis of the *mcr-1* locus and its promoter

Sequence comparison of the *mcr-1* neighboring regions in pMCR1_IncX4 (accession No. KU761327), pMCR1_IncI2 (accession No. KU761326) and BJ10 (accession No. LWQZ00000000) showed that the phosphoesterase encoding gene *pap2* is universally present downstream of *mcr-1* and forms a *mcr-1-pap2* cassette, while two copies of ISA*pl1* flank the *mcr-1-pap2* cassette on the chromosome of BJ10 (Tn*Apl1*) (Figure 1A). As shown in Figure 1B, the promoter sequences of *mcr-1* in pMCR1_IncX4, pMCR1_IncI2 and BJ10 are similar to that of pAf23 and pAf48 reported by Poirel *et al.* [14].

Expression of mcr-1 in plasmids pMCR-1_InX4 and pMCR1_IncI2 in parental strains

As shown in Figure 2, *mcr-1* expressions in pMCR1_IncX4 varied from 1.81×10^{-5} to 1.05×10^{-4} (pmol per μg total RNA) in the two ST25 *K. pneumoniae* strains SZ03 and SZ04, and the two *E. coli* strains CDA6 and SZ01 (ST167 and ST2448, respectively), with the ST167 *E. coli* strain CDA6 showing the highest expression levels. The *mcr-1* expression of pMCR1_IncI2 in the ST2085 *E. coli* strain SZ02 and ST457 *E. coli* strain BJ13 was 5.27×10^{-5} and 2.58×10^{-5}, respectively. In addition, the expression of chromosomal *mcr-1* (with IS*Apl1* inserted in the two flanking regions) in the ST156 *E. coli* strain BJ10 was 5.49×10^{-5}. Our results showed that *mcr-1* expression from the same plasmid may vary significantly when expressed in the different genetic backgrounds of the different strains. In addition, we investigated the MICs for colistin in these *mcr-1* positive parental strains (Table 1) and found that the MICs for colistin were not consistent with the differences in expression of *mcr-1* in these

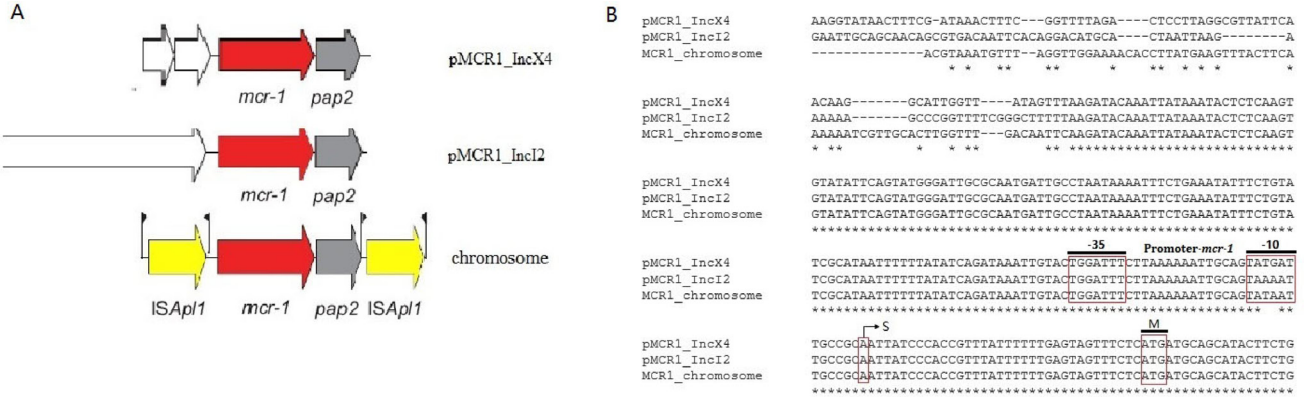

Figure 1: Schematic map of the different *mcr-1*- bearing plasmids pMCR1_IncX4 and pMCR1_IncI2. (**A**) Model of *mcr-1* locus. (**B**) Analyses of *mcr-1* promoter. S, transcription start site; M, methionine and translation initiation site; The *mcr-1* promoter sequences are indicated with the corresponding -10 and -35 boxes being underlined according to Poirel L's work.

strains. These results suggest that colistin resistance not solely depends on the expression levels of *mcr-1* and the structural modification of lipid A mediated by *mcr-1* [15].

Expression dynamics of *mcr-1* in parental strains after colistin treatment for different times

We next examined *mcr-1* expression from different plasmids in different bacterial hosts after colistin treatment. As shown in Figure 3, upon colistin treatment, the expression of *mcr-1* in pMCR1_IncX4 increased 2- and 4-fold after 20 and 120 mins, respectively, among all pMCR1_IncX4-harboirng strains, except for the ST2448 *E. coli* SZ01 strain, which showed a lower expression after 20 mins that restored to baseline levels after 120 mins. In contrast, the *mcr-1* expression levels of pMCR1_IncI2-harboring parental strains SZ02 and BJ13 remained at baseline level after 20 and 120 mins. However, the expression of chromosomal *mcr-1* in *E. coli* (BJ10) remained stable, regardless of colistin treatment. These results demonstrated various *mcr-1* expression patterns of *mcr-1*-harboring plasmids in different bacterial hosts after colistin treatment, which are likely the result of differences in bacterial backgrounds and/or the different *mcr-1*-harboirng plasmids.

Expression of *mcr-1* in *E. coli* E600 after colistin treatment for different times

To examine whether the differential expression observed above correlates with the different *mcr-1*-harboring plasmid backgrounds, we transferred plasmids pMCR1_IncX4 and pMCR1_IncI2 into the same *E. coli* host strain E600 via conjugation, and investigated the expression of *mcr-1* after colistin treatment for different times. As shown in Figure 4, the expression of *mcr-1* from the two different plasmids pMCR1_IncX4 and pMCR1_IncI2 was very similar without colistin challenge (1.31×10^{-4} and 1.49×10^{-4} pmol per μg total RNA, respectively) . However, *mcr-1* expression after colistin treatment showed variations for plasmids pMCR1_IncX4 and pMCR1_IncI2. After treatment with colistin for 20 min, *mcr-1* was significantly down-regulated in plasmid pMCR1_IncX4 and pMCR1_IncI2, while after treatment with colistin for 120 min, *mcr-1* was up-regulated in plasmid pMCR1_IncX4 but significantly down-regulated in plasmid pMCR1_IncI2.

DISCUSSION

Colistin is a cationic polypeptide antibiotic which is regarded as one of the last antibacterial agents against CRE. In general, colistin resistance

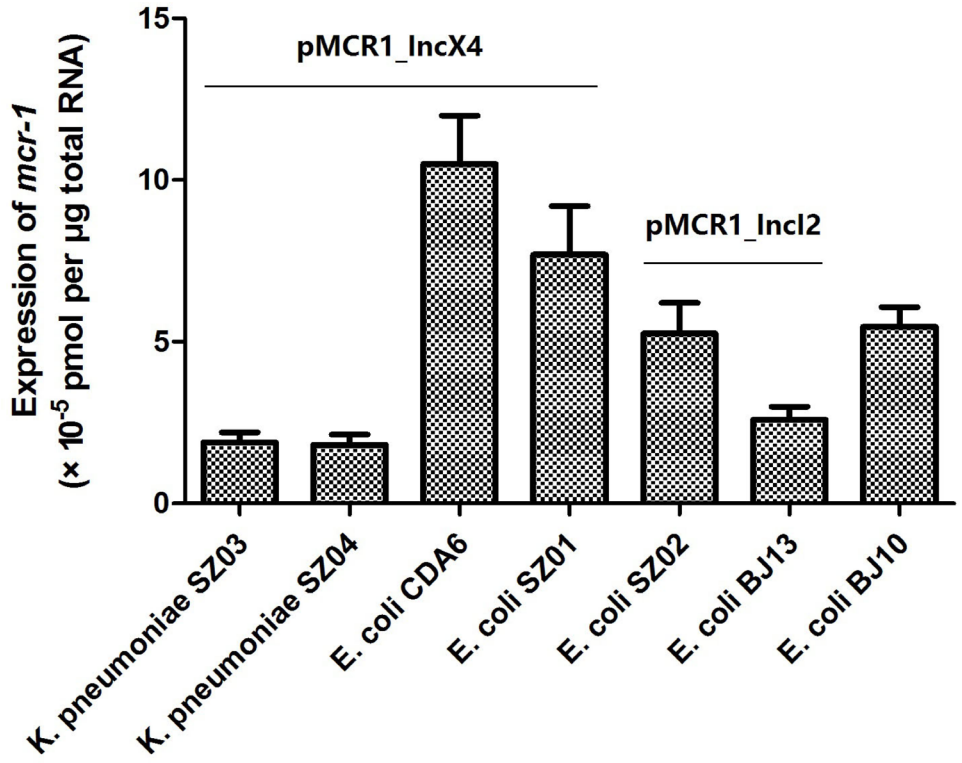

Figure 2: The *mcr-1* expression of plasmids pMCR1_IncX4 and pMCR1_IncI2 in parental strains. Bacteria were grown to an OD_{600} of 0.5 and total RNA were extracted and subsequently used to do the qRT-PCR experiments. The data were analyzed by using Student's *t*-test and shown as Mean with SEM.

Table 1: Strains and plasmids tested in this study

Strain	Background	*mcr-1*-harboring Plasmid	Colistin MIC (μg/ml)	Reference
K. pneumoniae SZ03	ST25	pMCR1_IncX4	32	[12]
*K. pneumoni*ae SZ04	ST25	pMCR1_IncX4	32	[12]
E. coli CDA6	ST167	pMCR1_IncX4	8	[13]
E. coli SZ01	ST2448	pMCR1_IncX4	4	[12]
E. coli SZ02	ST2085	pMCR1_IncI2	8	[12]
E. coli BJ13	ST457	pMCR1_IncI2	4	[13]
E. coli BJ10	ST156	$	8	[13]
E. coli E600	#	pMCR1_IncX4	4	This study
E. coli E600	#	pMCR1_IncI2	8	This study

$: *mcr-1* located in the chromosome, #: genetic engineering strain

of Gram-negative bacteria like *Klebsiella*, *E. coli* and *Salmonella enterica* has been mediated by chromosomal mutations and was thought to be non-transferable. The emergence of colistin resistance mediated by the *mcr-1* gene on a plasmid has become a matter of major concern since its first report in China. To date, the *mcr-1* gene has been detected in *Enterobacteriaceae* from almost 35 countries all of the world, including *E. coli*, *E. aerogenes*, *E. cloacae*, *K. pneumonia*, *Shigella sonnei* and *S. enterica* [16–19]. A number of different plasmids including the most common, IncI2 and IncX4, have been associated with the spread of *mcr-1*. Recently, Wang *et al.* described over 10 *mcr-1*-harboring plasmids with diversified incompatibility in *E. coli* [20], and reported that the *mcr-1* promoter of different origins exhibits similar

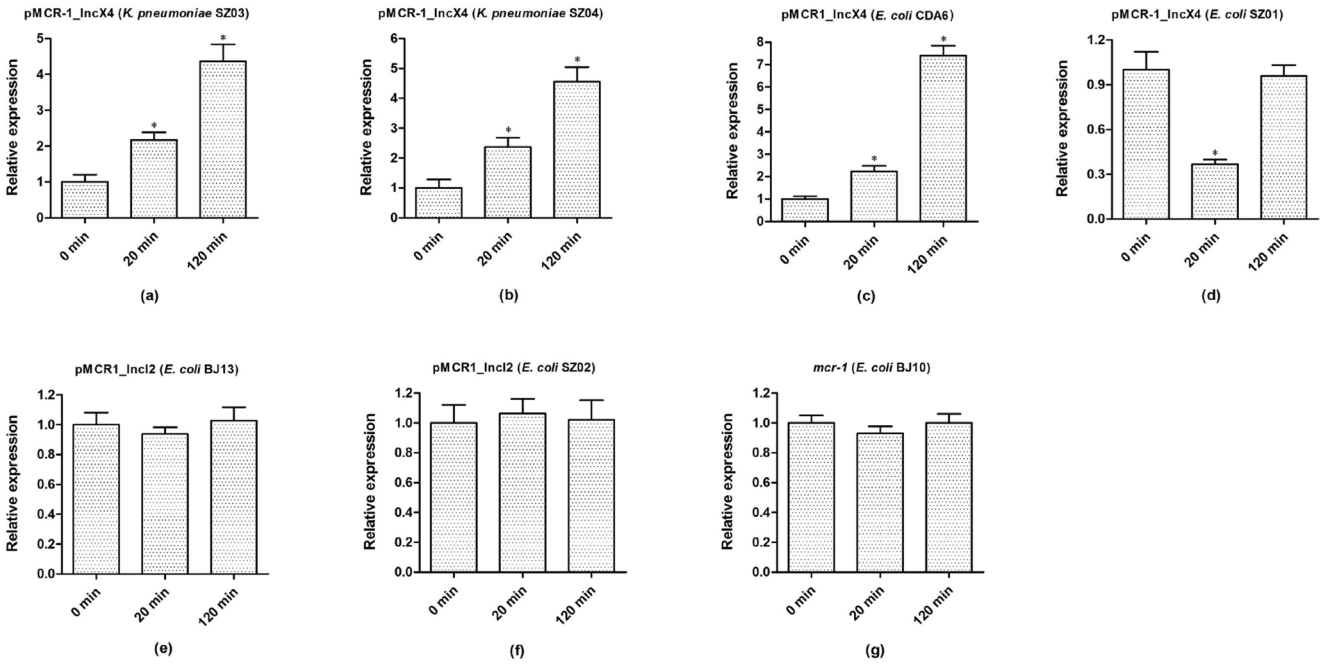

Figure 3: The expression dynamics of *mcr-1* in parental strains under colistin treatment for different time. Bacteria were grown to an OD_{600} of 0.5, and then colistin was added into the cultures with the final concentration of 4 μg/ml. After 20 mins and 120 mins treatment by colistin, bacterial total RNA were isolated and subsequently used to do the qRT-PCR experiments. The data were analyzed by using Student's *t*-test and shown as Mean with SEM. $^{*}P < 0.05$ (compared to 0 mins treatment by colistin).

activity through transcriptional analyses [21]. However, the mechanism of *mcr-1* gene regulation is still unclear.

Previously, we identified seven CRE strains carrying the *mcr-1* gene, including two *K. pneumonia* isolates and five *E. coli* isolates, which contained the two different *mcr-1*-harboring plasmids pMCR1_IncX4 and pMCR1_IncI2 [4, 12, 13]. In this study, we found that *mcr-1* expression of the plasmids pMCR1_IncX4 and pMCR1_IncI2 may vary significantly in the different genetic background of different strains, although their promoters are highly similar. In general, gene expression is controlled by its promoter and the corresponding activators and/or inhibiters. Therefore, we suspect that genes encoding activators and/or inhibiters in the host chromosome may affect the expression of *mcr-1* located on plasmids pMCR1_IncX4 and pMCR1_IncI2. In addition, we found that the phosphoesterase encoding gene *pap2* is universally present downstream of *mcr-1* and forms the *mcr-1-pap2* cassette in pMCR1_IncX4 and pMCR1_IncI2. A previous study showed that *pap2* is likely co-mobilized with the *mcr-1* gene when it transferred from its original genetic context and does not impact colistin vsusceptibility [22].

After treatment withcolistin, various *mcr-1* expression patterns of *mcr-1*-harboring plasmids from different bacterial hosts were detected, and we suspect that the differential expression changes may be due to the differences in bacterial backgrounds and/or the different *mcr-1*-harboirng plasmids. Moreover, we found that different plasmids had similar baseline *mcr-1* expression within the same *E. coli* E600 strain, suggestin that the expression of *mcr-1* from different plasmids is likely controlled by the host genetic background without colistin challenge. However, after colistin treatment, both the host bacteria and the *mcr-1*-harboring plasmids may contribute to *mcr-1* gene expression regulation, therefore a complex regulation network of *mcr-1* may be involved.

Taken together, this is one of the first studies focusing on the expression characteristics of *mcr-1* from different plasmids and in different bacterial host backgrounds. It is suggested that different genetic

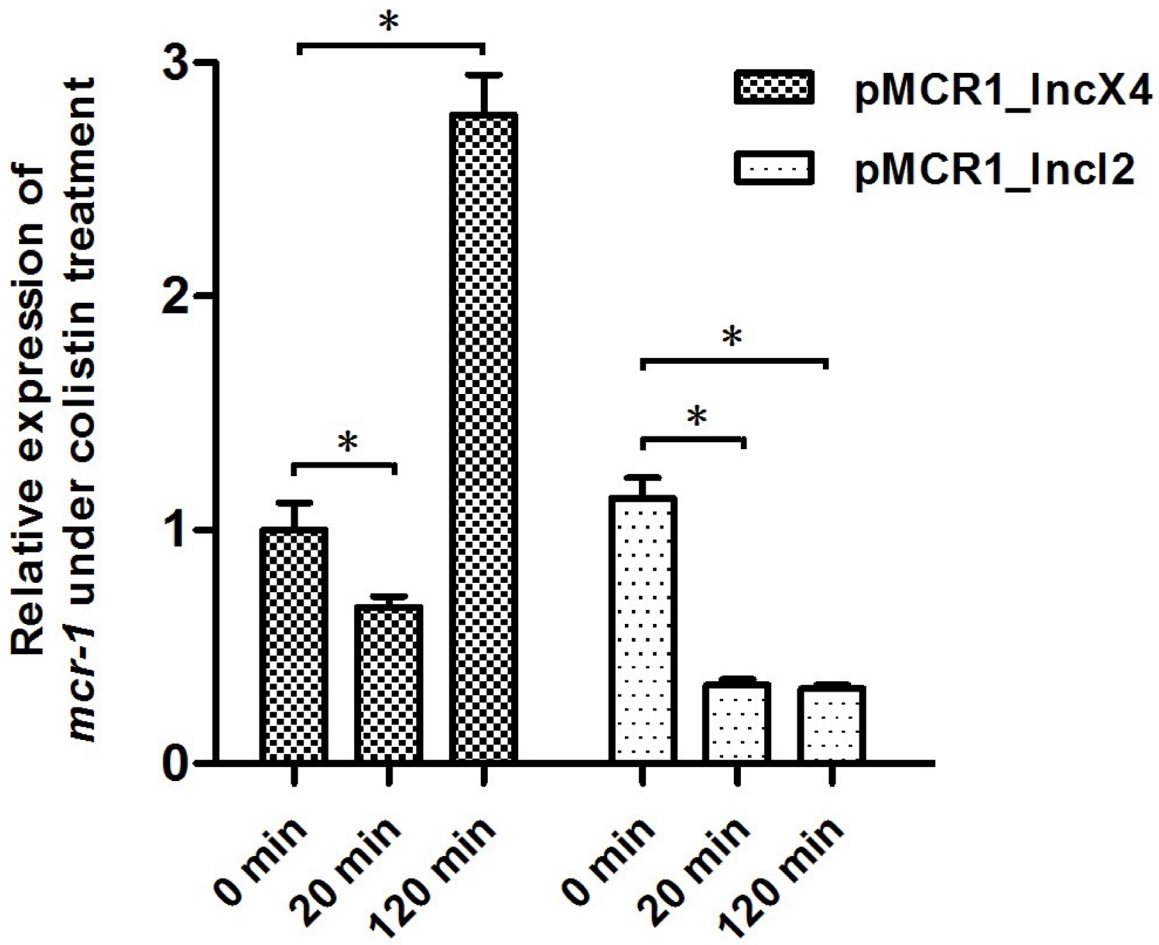

Figure 4: The *mcr-1* expression of plasmids pMCR1_IncX4 and pMCR1_IncI2 in same host strain *E. coli* E600 under colistin treatment for different time. Bacteria were grown to an OD_{600} of 0.5, and then colistin was added into the cultures with the final concentration of 4 μg/ml. After 20 mins and 120 mins treatment by colistin, bacterial total RNA were isolated and subsequently used to do the qRT-PCR experiments. The data were analyzed by using Student's *t*-test and shown as Mean with SEM. $^{*}P < 0.05$ (compared to 0 mins treatment by colistin).

signatures on the plasmids or in the bacterial strains may contribute to the observed variations, which warrant further investigations. Therefore, this study laid the foundation for further research on the regulation mechanism of *mcr-1* expression.

MATERIALS AND METHODS

Bacterial strains and MIC measurements of colistin

Seven *mcr-1* positive clinical isolates are used in this study, including two *K. pneumoniae* strains and five *E. coli* strains. All the bacterial strains used in this study are listed in Table 1. Minimal inhibitory concentrations (MICs) of colistin were determined using the agar dilution method and *E. coli* strain ATCC 25922 was used as a quality control strain.

Transfer of *mcr-1* gene to the same host strain *E. coli* E600

The *mcr-1*-harboring plasmids pMCR1_IncX4 and pMCR1_IncI2 from *E. coli* SZ01 and *E. coli* SZ02, respectively, were transferred to the same host strain, *E. coli* E600, via conjugation. *E. coli* SZ01 and *E. coli* SZ02 isolates were used as donors, and *E. coli* E600 (resistant to rifampicin) was used as the recipient strain. Conjugation was carried out by broth mating and positive strains were selected by colistin and rifampicin dual resistance.

Expression of *mcr-1* investigated by qRT-PCR

Full length *mcr-1* from pMCR1_IncX4 was cloned into a T-vector in *E. coli* DH5α, and this recombinant T-vector was used as the standard for quantitative reverse transcription PCR (qRT-PCR). The absolute expression levels of *mcr-1* of the pMCR1_IncX4 and pMCR1_IncI2 plasmids in their parental *K. pneumoniae* and *E. coli* isolates (Table 1) were investigated by qRT-PCR. In addition, the plasmid pMCR1_IncX4 and pMCR1_IncI2 were transferred into *E. coli* E600 via conjugation, and their transconjugants were evaluated by qRT-PCR. Moreover, the *mcr-1* chromosomally integrated *E. coli* (BJ10) was included in the tests [13].

In the qRT-PCR experiments, the forward primer sequence for *mcr-1* was AAATCAGCCAAACCTATCCC, and reverses primer sequence was CGTATCATAGACCGT GCCAT. The housekeeping genes *rpoD* and *gapA* were used for normalization for *E. coli* and *K. pneumoniae*, respectively. All the strains were cultured overnight at 37°C with shaking (250 rpm) in 1 ml LB broth, and then 1:100 diluted into 10 ml fresh medium. Cultures were incubated to exponential growth (OD 0.5 at 600 nm) and total RNA was extracted using an RNeasy kit (Qiagen, Germany) according to the manufacturer's instructions. Extracted RNA was treated with 1 U of RNase-free DNase I at 37°C for 10 min to remove traces of DNA and incubated at 85°C for 15 min to inactivate the DNase. Subsequently, 1 μg total RNA was subjected to qRT-PCR as described previously [23]. Each experiment was performed with three RNA samples from three independent experiments. Differences between the two groups were assessed by Student's *t*-test, $P < 0.01$ was considered to be statistically significant.

Expression dynamics of *mcr-1* under colistin treatment investigated by qRT-PCR

To describe the expression dynamics of *mcr-1* under colistin treatment, *mcr-1* positive strains were cultured to exponential growth (OD 0.5 at 600 nm), and then colistin was added into the cultures with a final concentration of 4 μg/ml. After 20 mins and 120 mins treatment with colistin, bacterial total RNA were isolated and subsequently used to do the qRT-PCR experiments as above.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

REFERENCES

1. 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, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010; 10:597–602. https://doi.org/10.1016/S1473-3099(10):70143-2.
2. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis. 2013; 13:785–96. https://doi.org/10.1016/S1473-3099(13)70190-7.

3. 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, 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; 16:161–8. https://doi.org/10.1016/S1473-3099(15)00424-7.
4. Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae. Lancet Infect Dis. 2016; 16:287–8. https://doi.org/10.1016/S1473-3099(16)00056-6.
5. Gao R, Hu Y, Li Z, Sun J, Wang Q, Lin J, Ye H, Liu F, Srinivas S, Li D, Zhu B, Liu YH, Tian GB, et al. Dissemination and Mechanism for the MCR-1 Colistin Resistance. PLoS Pathog. 2016; 12:e1005957. https://doi.org/10.1371/journal.ppat.1005957.
6. Teo JW, Chew KL, Lin RT. Transmissible colistin resistance encoded by mcr-1 detected in clinical Enterobacteriaceae isolates in Singapore. Emerg Microbes Infect. 2016; 5:e87. https://doi.org/10.1038/emi.2016.85.
7. Cannatelli A, D'Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, Gaibani P, Rossolini GM. *In vivo* emergence of colistin resistance in Klebsiella pneumoniae producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. Antimicrob Agents Chemother. 2013; 57:5521–6. https://doi.org/10.1128/AAC.01480-13.
8. Gunn JS. The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol. 2008; 16:284–90. https://doi.org/10.1016/j.tim.2008.03.007.
9. Prim N, Rivera A, Rodríguez-Navarro J, Español M, Turbau M, Coll P, Mirelis B. Detection of mcr-1 colistin resistance gene in polyclonal Escherichia coli isolates in Barcelona, Spain, 2012 to 2015. Euro Surveill. 2016; 21. https://doi.org/10.2807/1560-7917.ES.2016.21.13.30183.
10. Doumith M, Godbole G, Ashton P, Larkin L, Dallman T, Day M, Day M, Muller-Pebody B, Ellington MJ, de Pinna E, Johnson AP, Hopkins KL, Woodford N. Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales. J Antimicrob Chemother. 2016; 71:2300–5. https://doi.org/10.1093/jac/dkw093.
11. Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. Emergence of plasmid-mediated mcr-1 gene in colistin-resistant Enterobacter aerogenes and Enterobacter cloacae. Antimicrob Agents Chemother. 2016; 60:3862–3. https://doi.org/10.1128/AAC.00345-16.
12. Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X, Feng P, Tang YW, Kreiswirth BN, Chen L, Du H. Complete Sequences of mcr-1-Harboring Plasmids from Extended-Spectrum-β-Lactamase- and Carbapenemase-Producing Enterobacteriaceae. Antimicrob Agents Chemother 2016; 60:4351–4. https://doi.org/10.1128/AAC.00550-16.
13. Yu H, Qu F, Shan B, Huang B, Jia W, Chen C, Li A, Miao M, Zhang X, Bao C, Xu Y, Chavda KD, Tang YW. et al. Detection of the mcr-1 Colistin Resistance Gene in Carbapenem-Resistant Enterobacteriaceae from Different Hospitals in China. Antimicrob Agents Chemother. 2016; 60:5033–5. https://doi.org/10.1128/AAC.00440-16.
14. Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic Features of MCR-1-Producing Colistin-Resistant Escherichia coli Isolates in South Africa. Antimicrob Agents Chemother. 2016; 60:4394–7. https://doi.org/10.1128/AAC.00444-16.
15. Liu YY, Chandler CE, Leung LM, McElheny CL, Mettus RT, Shanks RMQ, Liu JH, Goodlett DR, Ernst RK, Doi Y. Structural Modification of Lipopolysaccharide Conferred by mcr-1 in Gram-Negative ESKAPE Pathogens. Antimicrob Agents Chemother 2017; 61. https://doi.org/10.1128/AAC.00580-17.
16. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016; 71:2066–70. https://doi.org/10.1093/jac/dkw274.
17. Stoesser N, Mathers AJ, Moore CE, Day NP, Crook DW. Colistin resistance gene mcr-1 and pHNSHP45 plasmid in human isolates of Escherichia coli and Klebsiella pneumoniae. Lancet Infect Dis. 2016; 16:285–6. https://doi.org/10.1016/S1473-3099(16)00010-4.
18. Yang YQ, Zhang AY, Ma SZ, Kong LH, Li YX, Liu JX, Davis MA, Guo XY, Liu BH, Lei CW, Wang HN. Co-occurrence of mcr-1 and ESBL on a single plasmid in Salmonella enterica. J Antimicrob Chemother. 2016; 71:2336–8. https://doi.org/10.1093/jac/dkw243.
19. Pham Thanh D, Thanh Tuyen H, Nguyen Thi Nguyen T, Chung The H, Wick RR, Thwaites GE, Baker S, Holt KE. Inducible colistin resistance via a disrupted plasmid-borne mcr-1 gene in a 2008 Vietnamese Shigella sonnei isolate. J Antimicrob Chemother. 2016; 71:2314–7. https://doi.org/10.1093/jac/dkw173.
20. Wang Q, Sun J, Li J, Ding Y, Li XP, Lin J, Hassan B, Feng Y. Expanding landscapes of the diversified mcr-1-bearing plasmid reservoirs. Microbiome 2017; 5:70. https://doi.org/10.1186/s40168-017-0288-0.
21. Wang Q, Li Z, Lin J, Wang X, Deng X, Feng Y. Complex dissemination of the diversified mcr-1-harbouring plasmids in Escherichia coli of different sequence types. Oncotarget 2016; 7:82112–82122. https://doi.org/10.18632/oncotarget.12621.
22. Zurfluh K, Kieffer N, Poirel L, Nordmann P, Stephan R. Features of the mcr-1 Cassette Related to Colistin Resistance. Antimicrob Agents Chemother 2016; 60:6438–9. https://doi.org/10.1128/AAC.01519-16.
23. Zhang H, Jia Y, Xie X, Wang M, Zheng Y, Xu S, Zhang W, Wang Q, Huang X, Du H. RpoE promotes invasion and intracellular survival by regulating SPI-1 and SPI-2 in Salmonella enterica serovar Typhi. Future Microbiol. 2016; 11:1011–24. https://doi.org/10.2217/fmb.16.19.