Molecular characteristic of mcr-1 producing Escherichia coli in a Chinese university hospital

Qing-wen He1,2†, Xiao-hong Xu1†, Fang-jun Lan1,2, Zhi-chang Zhao3, Zhi-yun Wu1,2, Ying-ping Cao1 and Bin Li1*

Abstract
Background: Colistin has been considered as a last-line treatment option in severe infections caused by multidrug-resistant (MDR) gram-negative pathogens. However, the emergence of the mobile colistin resistance gene (mcr-1) has challenged this viewpoint. The aim of this study is to explore the prevalence of mcr-1 in Escherichia coli (E. coli) in a Chinese teaching hospital, and investigate their molecular characteristics.

Methods: A total of 700 E. coli isolates were used to screen mcr-1 by PCR and sequencing in a Chinese university hospital from August 2014 to August 2015. Susceptibility test of mcr-1-producing isolates was determined by Vitek-2 Compact system. 26 virulence factors (VFs), phylogenetic groups, Multi-locus sequence typing (MLST), and DNA Fingerprinting (ERIC-PCR) of strains were investigated by PCR.

Results: Four (0.6%) mcr-1 producing E. coli isolates were found in this study. The results of antibiotic susceptibility test showed that all four isolates were resistant to colistin, ciprofloxacin, levofloxacin, cefazolin, and trimethoprim/sulfamethoxazole, and were susceptible to amikacin, ertapenem and imipenem. In addition, all 4 isolates exhibited high-level resistance to aztreonam, cefotaxime and gentamicin. The numbers of VFs contained in mcr-1 positive isolates were no more than 4 in our study. MLST result demonstrated that these isolates were assigned to two sequence types: ST156 and ST167. The result of phylogenetic analysis showed that four mcr-1-positive isolates belong to two phylogenetic groups: A and B1 group. ERIC-PCR showed that four mcr-1 positive strains were categorized into three different genotypes.

Conclusions: Our study demonstrated a low prevalence of mcr-1 in E. coli clinical isolates in a Chinese teaching hospital, and we have gained insights into the molecular characteristics of these mcr-1-positive strains. Increasing the surveillance of these infections, as well as taking effective infection control measures are urgently needed to take to control the transmission of mcr-1 gene.

Keywords: E. coli, mcr-1, Colistin, Multidrug-resistant

Background
In recent years, colistin has been considered as an effective therapeutic option for the rapid increasing of multidrug-resistant (MDR) gram-negative pathogens [1, 2]. However, the prevalence of the mobile colistin resistance gene (mcr-1) in animals and human beings worldwide has challenged this viewpoint [3, 4]. Resistance to polymyxins is mainly caused by the modification to bacterial outer membrane, which was usually considered as chromosomally mediated resistance [5, 6]. Since it was initially found, plasmid-mediated mcr-1 has been detected widely [3, 7]. Nowadays, mcr-1-producing bacteria have been reported in many regions in China [4, 8]. Mcr-1 was firstly found in Escherichia coli (E. coli), and now it has been spreading to other Enterobacteriaceae [9]. Several reports showed that the mcr-1 gene could coexist with other resistance genes (such as...
CRE/ESBL) in *E. coli* and *Klebsiella pneumoniae*, which probably lead to the emergence pan-drug resistant and increase the difficulty of treatment [8, 10]. Therefore, the emergence and spread of *mcr-1* gene among human beings should be given close attention. The aim of this study was to evaluate the prevalence of *mcr-1* in *E. coli* clinical isolates in a Chinese teaching hospital, and to investigate the molecular characteristics of these strains.

**Methods**

**Bacterial strains**
A total of 700 *E. coli* clinical isolates were collected from the clinical laboratory of Fujian Medical University Union Hospital (Fuzhou, Fujian province, China) from August 2014 to August 2015. It is a 2200-bed tertiary care teaching hospital with approximately 95,000 hospital admissions per year, located in southeastern China. All isolates were identified by GNI card of the Vitek system (BioMérieux, Missouri, France).

**Antibiotic susceptibility testing**
Antimicrobial susceptibility testing was performed using the AST-GN16 of Vitek-2 Compact system (Bio Mérieux, France). The antimicrobial agents tested included: tigecycline (glycylcycline); ertapenem and imipenem (carbapenems); cefazolin; cefoxitin; cefepime, and cefotaxime (cephalosporins); aztreonam (monobactam); amikacin and gentamicin (aminoglycosides); ciprofloxacin and levofloxacin (quinolone); piperacillin/tazobactam; trimethoprim/sulfamethoxazole. The results were interpreted by the Clinical and Laboratory Standards Institute (CLSI) [11]. The MIC of colistin was determined using agar dilution method, and the result was interpreted according to European Committee On Antimicrobial Susceptibility Testing (EUCAST) guidelines [12]. *E. coli* ATCC 25922 was used as a quality control.

**DNA extraction**
Several colonies were suspended in 50 µl of sterile distilled water for preparing genomic DNA of the isolates, and then the bacterial suspension was heated at 100 °C for 10 min as described previously [13].

**MCR-1 detection**
*mcr-1* gene was screened in *E. coli* clinical isolates by PCR using primers as previously described [4]. All of the PCR products were sequenced and then compared with known sequences listed in the GenBank (http://www.ncbi.nlm.nih.gov/blast/).

**Detection of virulence factor genes**
Twenty six virulence factors (VFs) genes associated with extraintestinal virulence [14, 15] were detected using a multiplex PCR method as previously described [15]. These genes were as follows: adhesions (*papAH, papEF, papC, papG* allele I, *papG II/III, papG* allele II, *sfa/focDE, afa/draBC, fimH, gafD, sfaS, focG* and *nfaE*), toxins (*hlyA, cnf1* and *cdtB*), siderophores (*fyuA* and *iutA*), protections and invasions (*kpsMTI, kpsMTIII, traT, cvaC, kpsMT* and *K1/K5*), miscellaneous (*rfc* and *PAI*). The PCR products were sequenced and then compared with known sequences listed in the GenBank (http://www.ncbi.nlm.nih.gov/blast/).

**Phylogenetic analysis**
The phylogenetic groups (A, B1, B2, and D) of *mcr-1* producing *E. coli* isolates were identified by a triplex PCR as previously described [16].

**Multi-locus sequence typing (MLST)**
*Mcr-1* positive strains were analyzed by multilocus sequence typing (MLST), which was based on 7 standard housekeeping genes (*adk, fimC, gyrB, icd, mdh, purA, recA*) (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli) [17].

**DNA fingerprinting**
Enterobacterial Repetitive Intergenic Consensus Sequences PCR (ERIC-PCR) was applied to typing *mcr-1* producing *E. coli* isolates with the primers ERIC-1 and ERIC-2 [18]. DNA fingerprints were compared by visual inspection, ERIC profiles were regarded as different if there were different bands on visual inspection [19].

**Results and discussion**
In this study, four isolates (0.6%) were confirmed to carry *mcr-1* gene, which is lower than previous study [4]. The age of the patients ranged between 38 and 80 years. These *mcr-1* producing strains were isolated from two different wards (Table 1). Two strains were isolated from the same patient. The clinical data of patients with *mcr-1* positive *E. coli* infection were shown in Table 2.

*Mcr-1* was usually found to be co-localized with other resistance genes on plasmids, such as ESBL genes and carbapenemase genes [20], which might increase the emergence of pan-drug resistance. In our study, the results of antimicrobial susceptibility test showed a high drug resistance in the *mcr-1*-producing isolates. All of the *mcr-1* positive isolates were resistance to at least 3 different kinds of antibiotics (Table 1).

All four *mcr-1* positive strains detected in our study were resistant to colistin and the MICs ranged from 4 to 16 µg/ml. It will be worrisome once *mcr-1* coexists with other resistant genes, especially carbapenemase genes because of limited therapeutic options [20]. Previous studies revealed that *mcr-1* co-produced with carbapenem-resistant genes in *E. coli* [8, 21]. Fortunately, all of
them were susceptible to carbapenems (IPM and ETP), which probably indicated that no carbapenem-resistant genes coexisted with mcr-1. Result of ERIC-PCR (Fig. 1) showed that four mcr-1 positive strains were categorized into three different genotypes, one of which contained 2 strains (from the same patient). These isolates which have different patterns suggest that they were non-clonal transmission. In a previous study, two mcr-1 positive E. coli isolates from a single fowl were belonging to phylogenetic B1 and D group [22]. The mcr-1-producing isolates in this study were belonged to phylogenetic groups A and B1, which were mainly distributed among human commensal E. coli isolates [23]. The mcr-1 producing isolates were assigned by MLST to two different sequence types: ST156 and ST167 (Table 1), which was similar to previous reports in other studies from China [8, 22]. E. coli ST156 has been found that it has connection with different ESBL genes [24, 25]. ST167 was belonged to ST10 complex and regarded as prevalent ST among ESBL-producing E. coli from human and animal sources [26]. In addition, E. coli ST167 was reported to be closely related to blaNDM, which needed closely concern of spreading [27]. The similar molecular characterizations illustrated that mcr-1 positive isolates detected from the same department in our study were clonally related.

Table 1  Main characteristics of the mcr-1 E. coli

| Isolates | Data    | Ward             | Specimen       | Phylogenetic groups | MLST | ERIC pattern | VFs             | Antibiotic resistance |
|----------|---------|------------------|----------------|--------------------|------|--------------|-------------------|-----------------------|
| E321     | 2014.8  | Colorectal surgery | Drainage-fluid | B1                 | ST156| 1            | traT, iutA        | COL, CFZ, FOX, CIP,  |
|          |         |                   |                |                    |      |              |                   | LVX, SXT, TGC        |
| E684     | 2015.1  | Colorectal surgery | Secretion      | B1                 | ST156| 2            | fimH, traT, iutA   | COL, CFZ, CTX, FEP, |
|          |         |                   |                |                    |      |              |                   | ATM, GEN, CIP, LVX, |
|          |         |                   |                |                    |      |              |                   | SXT                   |
| E921     | 2015.4  | Hepatobiliary surgery | Secretion | A                   | ST167| 3            | fyuA, traT, iutA   | COL, CFZ, CTX, ATM, |
|          |         |                   |                |                    |      |              |                   | GEN, CIP, LVX, SXT   |
| E1005    | 2015.5  | Hepatobiliary surgery | Drainage-fluid | A                   | ST167| 3            | fyuA, cvaC, traT, iutA | COL, CFZ, CTX, ATM, |
|          |         |                   |                |                    |      |              |                   | GEN, CIP, LVX, SXT   |

Table 2  Clinical data of patients with mcr-1 positive E. coli infection

| Isolates | Patients | Gender | Age (years) | Underlying diseases | Length of hospital stay (days) | Treatments used | Outcomes |
|----------|----------|--------|-------------|--------------------|-----------------------------|-----------------|----------|
| E321     | Patient 1| Female | 80          | Malignancy, hypertension, pulmonary tuberculosis | 51              | TZP             | Survived |
| E684     | Patient 2| Female | 57          | Perineal infection, hypertension | 37              | TZP             | Survived |
| E921     | Patient 3| Male   | 38          | Hypertension, pancreatitis, diabetes | 26              | MEM             | Survived |
| E1005    | Patient 3| Male   | 38          | Hypertension, pancreatitis, diabetes | 26              | MEM             | Survived |

TZP piperacillin/tazobactam, MEM meropenem

VF s in E. coli were associated with colonization, bacterial fitness and virulence [28]. VF s include five main groups: (1) adhesions; (2) toxins; (3) siderophores; (4) capsule production and (5) protections and invasions.

**Fig. 1** ERIC-PCR products from four mcr-1 positive isolates. M mark, lane 1 E321, lane 2 E684, lane 3 E921, lane 4 E1005
Clinical *E. coli* strains often carry multiple VFs, and isolates belonging to groups A and B1 often have less VFs than those belonging to phylogroups B2 and D [28]. To the best of our knowledge, there is no study concerning about VFs in *mcr-1* producing *E. coli*. In our study, *mcr-1* producing isolates contained less than 4 different VFs (Table 1). Only five different kinds of VFs had been detected in our *mcr-1* positive isolates, which included *fimH*, *fyuA*, *traT*, *iutA* and *cvaC*. *fimH* is one of the most commonly VFs present in *E. coli*, which encodes the adhesion subunit of type 1 fimbriae and related to colonization [15]. Lee et al. reported that *fyuA*, *traT*, and *iutA* were found to be independent predictors for pathogenicity. Meanwhile, *traT* and *iutA* were thought to be closely related to ESBL genes [29]. Pitout et al. found that *cvaC* was only present in non-CTX-M-producing isolates [30]. Previous reports suggested that antibiotic resistance has negative association with virulence factors [31], which could be interpreted by the loss of VSs associated with mutation to resistance [32].

It is noteworthy that two *mcr-1* positive *E. coli* strains were isolated from the same patient but at different time (Table 1). Results of MLST and ERIC-PCR revealed that these isolates had identical genetic background. Result of antimicrobial susceptibility test showed that they had similar antibiograms. We speculate that the two isolates probably originated from a same source.

In conclusion, we have revealed a low prevalence of *mcr-1* in *E. coli* clinical isolates in a Chinese teaching hospital, and presented detailed molecular characteristics of these isolates. The presence of *mcr-1* in *E. coli* clinical isolates suggests that it will pose a threat to public healthcare. Effective infection control measures are urgently needed to take to control the transmission of *mcr-1* gene.

**Abbreviations**

MCR-1: mobile colistin resistance gene; MDR: multi-drug resistant; PCR: polymerase chain reaction; CLSI: Clinical and Laboratory Standards Institute; MLST: multi-locus sequence typing; CRE: carbapenem-resistant Enterobacteriaceae; ESBL: extended-spectrum β-lactamase; ERIC-PCR: Enterobacterial Repetitive Intergenic Consensus Sequences PCR; VFs: virulence factors.

**Authors’ contributions**

QH, XX, FL, ZZ and YC conducted laboratory assays. ZW collected clinical data. QH wrote the paper. BL designed the study and reviewed the manuscript. All authors read and approved the final manuscript.

**Author details**

1 Department of Clinical Laboratory, Fujian Medical University Union Hospital, 299 Xinquan Road, Fuzhou 350001, Fujian, China. 2 The Union Clinical Medical College of Fujian Medical University, Fuzhou 350004, Fujian, China. 3 Department of Pharmacy, Fujian Medical University, Union Hospital, Fuzhou 350001, Fujian, China.

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**Competing interests**

The competing interests declare that they have no competing interests.

**Availability of data and materials**

There is no additional data and materials, except those in the sections of “Methods” and “Results and discussion”.

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