Molecular Characterization of Co-Existence of MCR-1 and NDM-1 in Extended-Spectrum β-Lactamase-Producing *Escherichia coli* ST648 Isolated from a Colonized Patient in China

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1. Introduction

Polymyxin B (colistin) has been used in agriculture and veterinary medicine since the 1960s. The recent discovery of the plasmid-mediated colistin-resistance gene *mcr-1* in China (1) has grabbed the attention of medical science. Colistin is the last available drug to treat infections caused by multidrug-resistant bacteria, particularly carbapenem-resistant *Enterobacteriaceae* (2). Since 2015, more than 10 distinct alleles of *mcr-1* have been reported in *Escherichia coli* (*E. coli*), *Klebsiella*, and *Salmonella* globally (3). The incidence and spread of the plasmid-mediated colistin-resistance gene *mcr-1* in *E. coli* pose a global concern to the community. Of our particular concern is the dissemination of *mcr-1* into carbapenemase-producing or extended-spectrum β-lactamases (ESBLs)-producing *E. coli*, which results in highly resistant strains, e.g., pandrug-resistant strains that are potentially untreatable (4). Reports are still infrequent on *E. coli* isolates co-harboring *mcr-1* and *bla*NDM-1 from clinical cases. We report the first case of *E. coli* pandemic clone sequence type 648 co-harboring colistin-resistance-encoding *mcr-1*, carbapenemase-encoding *bla*NDM-1, and ESBLs-encoding *bla*CTX-M-15 from a clinical case in Shenzhen, China.

2. Method

An 83-year-old male presented to the Medicine Department of Shenzhen hospital with the main complaint of diarrhea in 2015. Initially, *Enterobacter* species from fecal samples were cultivated on a Columbia blood agar plate. The acquired bacteria were routinely subjected to biochemical tests as well as API 20 system (BioMerieux, Marcy l’Etoile, France) with and further confirmed by using 16S rDNA PCR and sequences. Extended-spectrum β-lactamases production was confirmed using the combination disc diffusion method and carbapenemase production was confirmed using a carbapenem inactivation method (CIM), followed by antimicrobial susceptibility testing. The antibiogram testing was performed with 22 routinely used antimicrobial agents using the VITEK2 compact system (Ref. No. 27530/275660) according to the Clinical and Laboratory Standard Institute guidelines (CLSI guidelines, 2010).

The standard PCR method was performed to detect the presence of ESBLs-producing genes; *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*GES, and *bla*VEB using specific primers as previously described. Additionally, carbapenemase genes (*bla*KPC and *bla*NDM) and colistin-resistance *mcr-1* were determined in ESBL-producing *E. coli* by the PCR assay and sequencing. The specific primers were used as described in our previous study (Table 1). The purified PCR products were sequenced commercially (Sangon Biotech-Shanghai, China). DNA sequences were analyzed by the NCBI-BLAST program. Multi-locus sequence typing (MLST) was determined by amplying the internal portions of seven housekeeping genes of *E. coli* (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) with specific primers as described in the *E. coli* MLST.
database (http://mlst.ucc.ie/mlst/dbs/Ecoli). The phylogenetic group was determined by multiplex PCR assays, using 18 pairs of primers that are recognized as Inc (incompatibility) replicon types: FIA, FIB, FIC, HII, HI2, II-IC, IM, N, P, W, T, A/C, K, B/O, X, Y, F, and FIH. Moreover, IncX typing was determined as reported by Timothy J. et al. (2007).

3. Results and Discussion

We confirmed the E. coli SP-17 isolate in the stool sample of a diarrhea patient by biochemical tests. The API 20 system (BioMerieux, Marcy l’Etoile, France) further confirmed 165 rDNA sequences. The antibiotic susceptibility test results showed that the E. coli SP-17 isolate was most resistant to colistin [minimum inhibitory concentrations (MICs) = 8 mg/mL], followed by ampicillin, cefotaxime, cefepime, aztreonam, ciprofloxacin, levofloxacin, trimethoprim, and ertapenem but sensitive to piperacillin, ceftoxime, ampicillin, and tigecycline (Table 2). Escherichia coli SP-17 showed a pandrug-resistant phenotype known as “superbug”. Based on the PCR assay and sequencing, we confirmed E. coli SP-17 carrying mcr-1, blaNDM1, and blaCTX-M-15. In addition, blaSHV, blaTEM, blaGES, mphA, strA, and dfrA were detected in the same isolate. We did not find other β-lactamase genes including blaGES and blaVEB. The housekeeping gene sequences and phylogenetic group analysis showed that the E. coli SP17 isolate belonged to the ST648 type.

Carbapenem and colistin-resistant C600 transconjugants were successfully obtained from this isolate. The PCR-based replicon type assay showed that plasmids carrying mcr-1 and blaNDM1 belonged to IncX3, the size of which was confirmed in 0.7% agarose gel electrophoresis. The co-existence of blaNDM1 and mcr-1 has been reported in the specimen of cases with bloodstream infection and urinary tract infection (5). To the best of our knowledge, this is the first study from Shenzen, China, that reports such an occurrence in the fecal specimen of a colonized individual. The MLST and phylogenetic group results showed that the E. coli SP-17 isolate belonged to the ST648 type group A (Table 3).

Conjugation experiments were performed to analyze the horizontal gene transfer of blaCTX-M, blaNDM1, and mcr-1 using streptomycin-resistant E. coli C600 as the recipient strain. We used the liquid mating assay as described in our earlier study. Transconjugants were selected on Luria Bertani agar containing streptomycin 2000 (µg/mL) and cefotaxime (32 µg/mL). The transconjugants were further tested by the PCR assay, followed by sequencing. PCR-based replicon typing was performed to detect the plasmid type using 18 pairs of primers that are recognized as Inc (incompatibility) replicon types: FIA, FIB, FIC, HII, HI2, II-IC, IM, N, P, W, T, A/C, K, B/O, X, Y, F, and FIH. Moreover, IncX typing was determined as reported by Timothy J. et al. (2007).
Table 2. Antibiotic Susceptibility of Escherichia coli Strain SP-E-15-17 and Its Transconjugants

| Antibiotics            | Minimum Inhibitory Concentrations, mg/L | Isolates            |
|------------------------|----------------------------------------|---------------------|
|                        |                                        | SP-E-15-17 | SP-M-1517 | C600   | ATCC2599 |
| Colistin               |                                        | 8          | 6         | < 0.25 | < 0.25  |
| Ampicillin             |                                        | > 32       | > 32      | < 2    | < 2     |
| Ampicillin Sulbactam   |                                        | > 32       | > 32      | < 2    | < 2     |
| Piperacillin/Tazobactam|                                        | < 4        | < 4       | < 4    | < 4     |
| Cefazolin              |                                        | > 64       | > 64      | < 4    | < 4     |
| Cefotetan              |                                        | < 4        | < 4       | < 4    | < 4     |
| Ceftazidime            |                                        | > 64       | > 64      | < 1    | < 1     |
| Ceftriaxone            |                                        | > 64       | > 64      | < 1    | < 1     |
| Cefepime               |                                        | 16         | 8         | < 1    | < 1     |
| Aztreonam              |                                        | > 64       | > 64      | < 1    | < 1     |
| Ertapenem              |                                        | < 0.5      | < 0.5     | < 0.5  | < 0.5   |
| Imipenem               |                                        | < 1        | < 1       | < 1    | < 1     |
| Tobramycin             |                                        | 8          | < 4       | < 1    | < 1     |
| Amikacin               |                                        | < 2        | < 2       | < 2    | < 2     |
| Gentamicin             |                                        | > 16       | < 1       | < 1    | < 1     |
| Ciprofloxacin          |                                        | > 4        | < 0.5     | < 0.25 | < 0.25  |
| Levofloxacin           |                                        | > 8        | < 0.05    | < 0.25 | < 0.25  |
| Nitrofurantoin         |                                        | < 16       | < 16      | < 16   | < 16    |
| Trimethoprim           |                                        | < 20       | < 20      | < 20   | < 20    |
| Doxycycline            |                                        | > 16       | < 4       | < 4    | < 4     |
| Minocycline            |                                        | > 16       | < 4       | < 4    | < 4     |
| Tigecycline            |                                        | < 0.5      | < 0.5     | < 0.5  | < 0.05  |

Table 3. Details of MLST and Phylogenetic Analysis

| Isolate    | adk | fumC | gyrB | icd | mdh | purA | recA | ST  | STCLPX | Phylogenetic Group |
|------------|-----|------|------|-----|-----|------|------|-----|--------|-------------------|
| SP-E-15-17 | 92  | 4    | 87   | 96  | 70  | 58   | 2    | ST648| ST648  | A                 |

reported globally. The PCR-based replicon typing indicated that the resistance determinants were located on the commonly reported IncX3 plasmid. One limitation of this study is the lack of performing St-PFGE, followed by Southern blotting to determine the location of the gene or even the whole genome sequencing.

4. Conclusions

Overall, to the best of our knowledge, this is the first report of co-harboring mcr-1 and blaNDM-1, and blaCTX-M-15 in E. coli ST648 isolated from a diarrhea patient in Shenzhen, China. The occurrence of the multidrug-resistance enzyme-encoding genes in E. coli ST648 (group A) pandemic clone is quite alarming to society. The constant surveillance of the field strains is essentially required.

Footnotes

Authors’ Contribution: Sandip Patil designed the study, executed the laboratory experiments, and wrote the manuscript; Jiang Min provided the isolate; Wen Feiqiu validated the results and wrote the manuscript.

Conflict of Interests: No competing interest is reported.

Ethical Approval: The present study was approved by the
Research Ethics Committee of Shenzhen Children Hospital (No. 2018013).

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**Patient Consent:** The clinical isolates used in this study were part of the routine hospital laboratory procedure. Oral consent was obtained from the patient.

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