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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

Sandip Patil [1, 2, 3], Jiang Min [4] and Wen Feiqiu [1, 2, *]

[1] Department of Haematology and Oncology, Shenzhen Children's Hospital, Shenzhen, China
[2] Paediatric Research Institute, Shenzhen Children's Hospital, Shenzhen, China
[3] Shenzhen Institute of Advanced Technology, Chinese Academy of Science, Shenzhen, China
[4] Centre for Disease Control and Prevention, Shenzhen, China

[*] *Corresponding author*: Shenzhen Children's Hospital, 7019 Yitian Road, District Futian, Shenzhen, 518038, China. Tel: +86-18938690333, Fax: +86-75583009888, Email: fwen62@163.com

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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 -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 antibiotic susceptibility 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-1}) 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 amplifying 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

Table 1. Primers Used in This Study

Resistance Genes	Primer Pair Sequences	Amplicon Size, bp	Annealing Temperature, °C
mcr-1	ATGATGCAGCATACTTCTGTG	1626	56
	TCAGCGGATGAATGCGGTG		
bla_{NDM}	TGCGGGGTTTTTAATGCTG	785	53
	TGGCTCATCACGATCATGC		
bla_{KPC}	ATGTCACTGTATCGCCGTC	883	54
	TTACTGCCCGTTAACGCC		
bla_{TEM}	AGGAAGAGTATGATTCAACA	531	57
	CTCGTCGTTTGGTATGGC		
bla_{SHV}	GGTTATGCGTTATATTCGCC	866	57
	TTAGCTTTGCCAGTGCTC		
bla_{OXA48}	TTGGTGGCATCGATTATCGG	745	55
	GAGCACTTCTTTTGTGATGC		
bla_{SME}	AACGGCTTCATTTTTGTTTAG	831	55
	GCTTCCGCAATAGTTTTATC		
bla_{CMY}	CTGACAGCCTCTTTCTCCA	504	56
	GCCAAACAGACCAATGCT		
bla_{VIM}	GTTAAAAGTTATTAGTAGTTTATTG	799	60
	CTACTCGGCGACTGAGC		
bla_{IMP}	ATGAGCAAGTTATCTGTATTC	741	60
	TTAGTTGCTTGGTTTTGATG		
bla_{GES}	ATGCGCTTCATTCACGCAC	864	57
	CTATTTGTCCGTGCTCAGG		
bla_{CARB}	AAAGCAGATCTTGTGACCTATTC	588	56
	TCAGCGCGACTGTGATGTA1		
bla_{PER}	AGTCAGCGGCTTAGATA	978	56
	CGTATGAAAAGGACAATC		
bla_{VEB}	GCGGTAATTTAACCAGA	961	57
	GCCTATGAGCCAGTGTT		
bla_{CTX-M}	TTTGCGATGTGCAGTACCAGTAA	544	57
	CGATATCGTTGGTGGTGCC/		

database (http://mlst.ucc.ie/mlst/dbs/Ecoli). The phylogenetic group was determined by multiplex PCR assays, using a combination of three DNA marker genes (*chuA, yjaA*, and *TspE4.C2*) as described by Clermont et al. (2000).

Conjugation experiments were performed to analyze the horizontal gene transfer of bla_{CTX-M}, bla_{NDM-1}, 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, HI1, HI2, I1-Ic, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA. 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 16S 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, ceftazidime, cefepime, aztreonam, ciprofloxacin, levofloxacin, doxycycline, minocycline, ceftriaxone, gentamicin, nitrofurantoin, trimethoprim, and ertapenem but sensitive to piperacillin, cefotetan, imipenem, amikacin, and tigecycline (Table 2). *Escherichia coli* SP17 showed a pandrug-resistant phenotype known as "superbug". Based on the PCR assay and sequencing, we confirmed *E. coli* SP 17 co-harboring *mcr-1*, bla_{NDM-1}, and $bla_{CTX-M-15}$. In addition, bla_{SHV}, bla_{TEM}, bla_{aac}, *mphA, strA,* and *dfrA* were detected in the same isolate. We did not find other β-lactamase genes including bla_{GES} and bla_{VEB}. The housekeeping gene sequences and phylogenetic group analysis showed that the *E. coli* SP17 isolate belonged to the ST648 type group A (Table 3).

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 bla_{NDM-1} belonged to IncX3, the size of which was confirmed in 0.7% agarose gel electrophoresis. The co-existence of bla_{NDM-1} 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 phylogenic group results showed that the *E. coli* SP17 isolate belonged to ST648 type group A, which is the most pandemic clone combining multidrug resistance and virulence (6). The *E. coli* ST648 clone has been observed globally in humans, companion animals, livestock, and wild birds and is commonly allied with various β-lactamases, including ESBLs, NDM, and KPC (7, 8).

Conjugation experiments showed that the plasmids harboring the *mcr-1* and bla_{NDM-1} genes were successfully transferred to *E. coli* EC600, indicating that *mcr-1* and bla_{NDM-1} were located on conjugative plasmids but $bla_{CTX-M-15}$ was located on another plasmid. Similar results have been

Table 2. Antibiotic Susceptibility of *Escherichia coli* Strain SP-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	< .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	Housekeeping Genes							ST	STCLPX	Phylogenic Group
	adk	*fumc*	*gyrb*	*icd*	*mdh*	*purA*	*recA*			
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 S1-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 bla_{NDM-1}, and $bla_{CTX-M-15}$ 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

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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.

References

1. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, 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):161–8. doi: 10.1016/S1473-3099(15)00424-7. [PubMed: 26603172].
2. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment options for carbapenem-resistant enterobacteriaceae infections. *Open Forum Infect Dis*. 2015;**2**(2):ofv050. doi: 10.1093/ofid/ofv050. [PubMed: 26125030]. [PubMed Central: PMC4462593].
3. Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, et al. The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nat Commun*. 2018;**9**(1):1179. doi: 10.1038/s41467-018-03205-z. [PubMed: 29563494]. [PubMed Central: PMC5862964].
4. Dandachi I, Chabou S, Daoud Z, Rolain JM. Prevalence and emergence of extended-spectrum cephalosporin-, carbapenem- and colistin-resistant gram negative bacteria of animal origin in the mediterranean basin. *Front Microbiol*. 2018;**9**:2299. doi: 10.3389/fmicb.2018.02299. [PubMed: 30323797]. [PubMed Central: PMC6172473].
5. Li X, Mu X, Zhang P, Zhao D, Ji J, Quan J, et al. Detection and characterization of a clinical Escherichia coli ST3204 strain co-producing NDM-16 and MCR-1. *Infect Drug Resist*. 2018;**11**:1189–95. doi: 10.2147/IDR.S175041. [PubMed: 30147347]. [PubMed Central: PMC6101002].
6. Ewers C, Bethe A, Stamm I, Grobbel M, Kopp PA, Guerra B, et al. CTX-M-15-D-ST648 Escherichia coli from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? *J Antimicrob Chemother*. 2014;**69**(5):1224–30. doi: 10.1093/jac/dkt516. [PubMed: 24398338].
7. Gao R, Li Y, Lin J, Tan C, Feng Y. Unexpected complexity of multidrug resistance in the mcr-1-harbouring Escherichia coli. *Sci China Life Sci*. 2016;**59**(7):732–4. doi: 10.1007/s11427-016-5070-1. [PubMed: 27178474].
8. Chakraborty A, Saralaya V, Adhikari P, Shenoy S, Baliga S, Hegde A. Characterization of Escherichia coli phylogenetic groups associated with extraintestinal infections in south Indian population. *Ann Med Health Sci Res*. 2015;**5**(4):241–6. doi: 10.4103/2141-9248.160192. [PubMed: 26229711]. [PubMed Central: PMC4512115].