Fecal Carriage and Molecular Epidemiology of mcr-1-Harboring Escherichia Coli from Children in Southern China.

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Research

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Abstract

Objective: The increase of multidrug resistant *Enterobacteriaceae* bacteria has led to reintroduction of colistin for clinical treatments, and colistin has become a last resort for infections caused by multidrug resistant bacteria. *Enterobacteriaceae* bacteria carrying the *mcr-1* gene are majorly related to colistin resistance, which may be the main reason for continued increase in the colistin resistance rate of *Enterobacteriaceae*. The purpose of this study was to investigate the sequence type and prevalence of bacteria harboring *mcr-1* gene in the gut flora of children in Southern China.

Method: Fecal samples (n=2632) of children from 3 medical centers in Guangzhou were cultured for *Escherichia coli* (*E. coli*). The *mcr-1*-harboring isolates were screened by Polymerase chain reaction (PCR). The colistin resistance transfer frequency was studied by conjugation experiments. DNA sequencing of seven housekeeping genes were used for multi locus sequence typing analysis (MLST).

Result: PCR indicated that 21 isolates from the 2632 *E. coli* (0.80%) were positive for *mcr-1*; these strains were resistant to colistin. Conjugation experiment indicated that 18 of the *mcr-1*-harboring isolates could transfer colistin resistance phenotypes to *E. coli* J53. MLST analysis revealed that the 21 isolates were divided into 18 sequence types (STs); ST69 was the most common (14.3%), followed by ST58 (9.5%).

Conclusion: These results demonstrate the colonisation dynamics and molecular epidemiology of *mcr-1*-harboring *E. coli* in the gut flora of children in Southern China, and the *mcr-1* gene can be horizontally transmitted within species, it is necessary to monitor the *mcr-1*-harboring bacteria in children.

Introduction

The rapid increase of carbapenem-resistant *Enterobacteriaceae* has raised serious concern in clinical settings. Colistin is considered to be one of the last resort antibiotics for the treatment of widespread drug-resistant gram-negative infections [1]. However, as the clinical application of colistin has increased, strains resistant to colistin have inevitably appeared. In the past, colistin resistance was caused by mutations in chromosome-related genes, such as mutations in genes involved in bicomponent regulatory systems such as *phoP/phoQ* or inactivation of the negative feedback regulatory gene *mgrB* [2]; in 2015, Liu et al. [3] first discovered that the plasmid that carried the *mcr-1* gene, which can mediate resistance of colistin. MCR-1 is a phosphoethanolamine transferase that catalyzes the binding of phosphoethanolamine to the bacterial outer membrane lipopolysaccharide. Subsequently, the *mcr-1* gene was reported in many countries, such as United States, South Africa, and Italy, from different genera, such as *Klebsiella pneumoniae*, *E. coli* and *Salmonella* [4]. The plasmid-mediated *mcr-1* gene discovery indicated that colistin resistance could be transmitted horizontally in different genera, resulting in increased colistin resistance. The *mcr-1* gene has been detected in agricultural animals and adult humans worldwide [5]. However, few studies have investigated the presence of *mcr-1* in children.

Colonization of multidrug resistant bacteria in the gastrointestinal tract may be a risk factor for bacterial translocation, leading to endogenous infections in immunocompromised children [6]. Colistin resistance

is considered of special clinical significance in the *Enterobacteriaceae*. Therefore, we set out to investigate the fecal carriage of *mcr-1* carrying *E. coli* from three children's hospital in Guangzhou, China. All the *mcr-1*-carrying isolates were characterized by MLST, antimicrobial susceptibility testing, and conjugation experiment in this study.

Materials And Methods

Samples

Fecal samples (n = 2632) were collected from children during November 2018 and February 2019 from three hospitals in Guangzhou, i.e., Guangzhou Children's hospital in Yuexiu, west of Guangzhou; Zengcheng Children's hospital in Zengcheng, north of Guangzhou; Guangzhou Women and Children's Medical Center in Tianhe, central Guangzhou. Patient information was acquired from the laboratory information system.

mcr-1 **detection**

Fecal samples were incubated on MacConkey agar plates at 35 ℃ for 18–24 h. Suspected *E. coli* colonies were sorted from each sample and sub-cultured on fresh blood agar plates (Detgerm Microbiology Technology, Guangzhou, China). All isolates were further identified for their species assignment by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (bioMérieux, Marcy l'Étoile, France).

PCR was performed using *mcr-1* specific primers, as previously described [7]. The PCR amplicons were sent for sequencing (Beijing Genomics Institute, Shenzhen, China), and the results were confirmed by the BLAST analysis on the NCBI website (www.ncbi.nlm.nih.gov).

Antibiotic susceptibility tests

Susceptibility tests of all the *mcr-1* positive *E. coli* isolates to 15 antibiotics (aztreonam, imipenem, meropenem, piperacillin/tazobactam, cefoperazone/sulbactam, cefepime, ceftazidime, tobramycin, amikacin, levofloxacin, ciprofloxacin, compound sulfamethoxazole, minocycline, tigecycline, doxycycline) were performed by broth microdilution using an automated VITEK2 compact system (bioMérieux). The MICs were interpreted according to the Clinical and Laboratory Standards Institute breakpoints, except for colistin, which was interpreted according to the guidelines established by the European Committee on Antimicrobial Susceptibility Testing. A resistant breakpoint of greater than 2 mg/L and a susceptible breakpoint of 2 mg/L or lower [8].

Conjugation experiments

The colistin resistance transfer frequency was studied by conjugation experiments with azide-resistant *E. coli* J53 as a recipient [9]. The donor and recipient strains were inoculated into Chinese blue plates for 18–24 h. Five to nine fresh single colonies were picked and inoculated into 5 mL Lysogeny broth (LB)

medium and placed on a 37 ° C shaker for 3.5-4 h. The donor bacteria (100 μL) and recipient bacteria (100 μL) were added into 1 mL of fresh LB medium and co-incubated for 18–24 h at 37 °C. The transconjugants were screened on MH agar plates supplemented with colistin (2 mg/L) and sodium azide (150 mg/L). The presence of colistin resistance *mcr-1* gene in transconjugant strains was identified by PCR.

MLST typing

MLST genotyping of 21 *mcr-1*-producing *E. coli* isolates was performed by amplifying and sequencing 7 housekeeping genes as previously reported [10]. ST were confirmed by searching against the MLST database (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). MEGA5 was used to draw unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on ST types.

Results

mcr-1 **gene carrying isolates**

Twenty-one *mcr-1* positive strains (0.80%) were detected in 2632 *E. coli* isolated from 2632 different fecal samples, the 21 *mcr-1*-carrying isolates were isolated from 21 different samples, Table 1 summarizes the clinical characteristics of these 21 patients. The ages of these 21 patients ranged from 1 day to 8 years and 3 months. Only 3 patients used broad-spectrum antibiotics within 3 months, and all patients did not use colistin. The correlation between the carrying rate of *E. coli* mcr-1 in feces and different clinical features was analyzed. As shown in Table 2, there was no significant correlation between the mcr-1 fecal carrying rate and the patient's gender, hospitalization, age group, district.

Antimicrobial Susceptibility Tests results of isolated E. coli

Twenty-one *mcr-1*-carrying *E. coli* strains were tested for susceptibility to colistin. The results showed that all the strains were resistant to colistin (MIC ≧ 4 μg/mL) (Fig. 1). The antimicrobial susceptibility results showed that *mcr-1*-carrying isolates were sensitive to piperacillin/tazobactam, imipenem, meropenem, and tigecycline, and the resistance rates to levofloxacin, ciprofloxacin, tobramycin, and doxycycline were 23.8%, 23.8%, 33.3%, 42.8% respectively. For aztreonam, cefoperazone/sulbactam, ceftazidime, cefepime, amikacin, sulfamethoxazole, and minocycline, the resistant rates were less than 10%. One of the strains isolated from neonatal feces (ST 453)were sensitive to all the antibiotics tested except colistin, the other strain from neonatal feces (ST48) had similar susceptibility expect doxycycline(intermediate).

Conjugation experiments analysis

Conjugation experiments were conducted on the 21 *mcr-1* carrying *E. coli* isolates. The result showed that 18 *E. coli* isolates (85.7%) transferred their colistin resistance phenotypes to the *E. coli* J53 recipient, demonstrating horizontal transfer of *mcr-1* genes.

Multilocus sequence typing

The MLST typing of each region in Guangzhou is shown in Fig. 1. A total of 18 MLST types were detected in the 21 strains of *E. coli*, of which ST69 was the most common (3 strains, 14.3%), followed by ST58 (2 strains, 9.5%). The MLST classification was generally scattered. No new MLST typing was found in this study. Based on ST types, UPGMA dendrogram showed two major groups. It can be seen from the figure that the MLST classification is generally dispersed, but with ST69 as a core source (Fig. 1).

Discussion

Multidrug resistant *Enterobacteriaceae* bacterial infections have increased worldwide, leading to an increased clinical application of colistin; thus, colistin resistance has recently been reported globally [3]. In the present study, 21 colistin resistance *mcr-1*-carrying *E. coli* strains were detected from 2632 *E. coli* (0.80%; 21/2632) isolated from fecal samples, which was close to the 0.1-1% *mcr-1* prevalence in *Enterobacteriaceae* isolates from clinical samples in previous investigations [11–13]. Compared with other fecal carriage data, the prevalence of colistin resistance in the present study was lower than that reported in Hongkong (2.08%), Guangzhou (6.2%), and Singapore (8.0%) [14–16]. This may be because the stool samples included in our study were obtained only from children. Further research is needed with larger cohorts from different geographic locations, representative populations, and demographics to fully understand the prevalence of *mcr-1* in our local context. The 21 *mcr-1* positive strains detected in this study had MICs of only 4–8 mg/L, which was lower than that of previously reported *mcr–c*arrying *Enterobacteriaceae* bacteria (MICs of 4–16 mg/L), which showed moderate level of colistin resistance [17]. These results indicate that the resistance level of colistin in *E. coli* from children was different from that of adults. The 21 *mcr-1* positive strains had higher resistance rates to tobramycin and doxycycline, 33.3% and 42.8%, respectively, than the other antibiotics, particularly carbapenem antibiotics; all isolates were sensitive to imipenem and meropenem, which is similar to observation in several other studies [11, 18, 19].

It was reported that ST10 *E. coli* played an important role in *mcr-1* gene dissemination in France and Argentina [5, 20]. In addition, ST10 is a common MLST classification in extended spectrum beta-lactamases (ESBLs) *E. coli* [21, 22]. In this study, ST10 was not the predominant sequence type (1/21), but ST69 was the predominant sequence type (3/21). ST69 has been reported in previous studies wherein the *mcr-1*-carrying *E. coli* strain was isolated from raw milk in Egypt [23] and a broiler chicken in Germany [24].

The *mcr-1* gene is widely spread in Enterobacteriaceae, especially in *K. pneumoniae* and *E. coli* isolated from food sources, environment, and humans [3, 25] In the present study, 18 strains successfully transmitted the *mcr-1* gene to *E. coli* J53. Previous studies have shown that *mcr-1* is present on many types of plasmids, such as IncI2, IncHI2, IncX4, etc. [14]. These plasmids often carry multiple drug resistance genes, such as β-lactamase gene (bla_{CTX-M}, bla_{SHV-2}), bla_{CMY-2}, etc., fosfomycin resistance gene fosA3, and quinolone resistance gene oqxAB [26, 27], and can be transferred to a variety of clinically common host bacteria, such as *Pseudomonas aeruginosa*, *K. pneumoniae*, and *E. coli*, etc., threatening the treatment of drug-resistant gram-negative bacteria [3]. Therefore, due to increasing supply and use of

polymyxin in China, plasmids harboring *mcr-1* and other drug resistance genes can be horizontally transmitted under their selective pressure, resulting in an increase in multidrug resistance of β-lactams, polymyxins, and fosfomycin. Therefore, the detection of *mcr-1* gene in isolates and the drug sensitivity of the strain carrying the gene should be continuously monitored.

Colistin resistance is increasingly being identified in members of the *Enterobacteriaceae*. This study analyses the presence of *mcr-1*-carrying *E. coli* in fecal samples of child patients in Southern China. The study showed that 18 out of the 21 *mcr-1*-carrying *E. coli* strains were able to transfer the colistin resistance phenotype to a donor strain. Thus, this study is significant as it sheds light on the vast potential of horizontal transfer of this gene. Due to the limited funding, only *E. coli was* investigated in this study, other *mcr-1*-carrying species should also be studied, and further study is necessary to investigate the molecular mechanisms mediating colistin resistance.

Declarations

Ethics approval and consent to participate

Guardians of all the patients provided informed consent, and the study was approved by the Guangzhou women and children's medical center research ethics committee (No.201903600).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author s' Contributions

ZZ designed the study. MJ, XZ and LB performed PCR, MLST, and antibiotic sensitivity test. YS, GF, LY, LZ, GS, GX conducted bacterial culture and isolation. CX, HZ and AX prepared the samples. MJ wrote the

manuscript. ZZ revised the manuscript.

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Not applicable.

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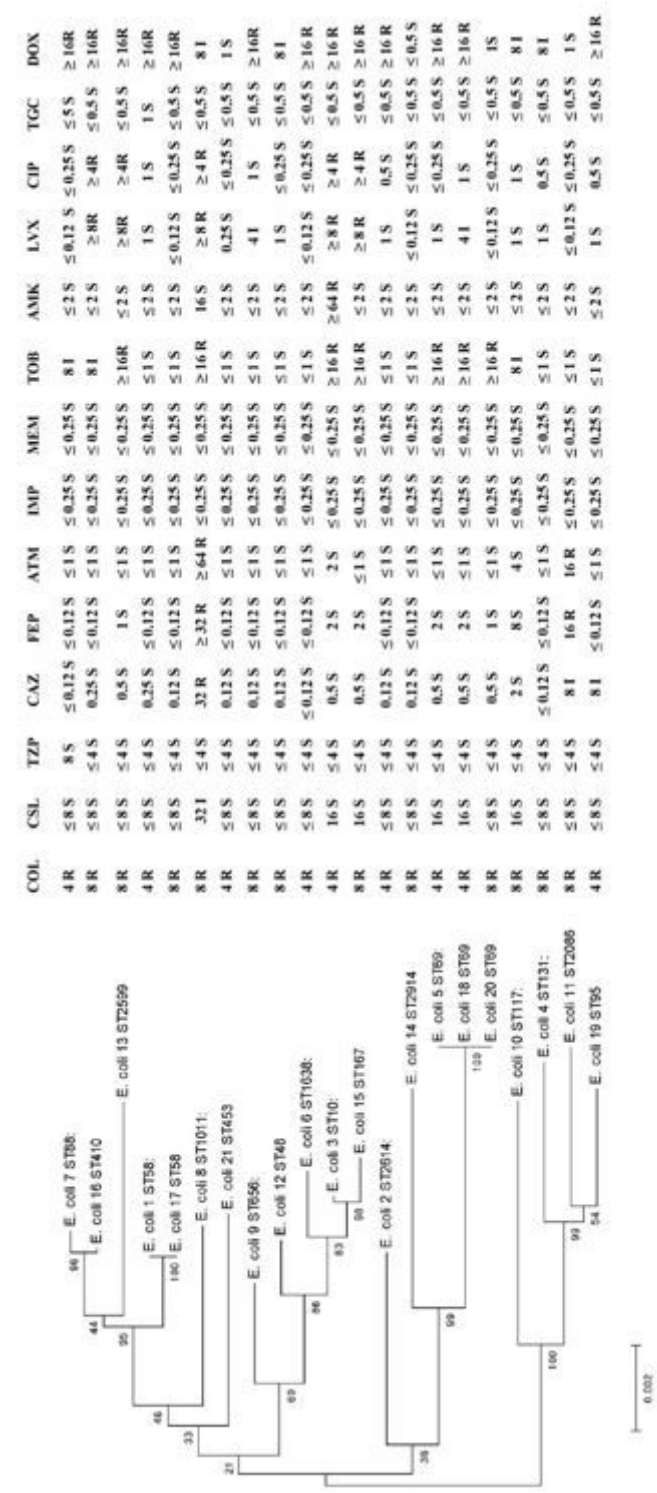
Figures



COL	CSL	TZP	CAZ	FEP	ATM	IMP	MEM	TOB	AMK	LVX	CIP	TGC	DOX
4 R	≤ 8 S	8 S	≤ 0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	8 I	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 5 S	≥ 16 R
8 R	≤ 8 S	≤ 4 S	0.25 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	8 I	≤ 2 S	≥ 8 R	≥ 4 R	≤ 0.5 S	≥ 16 R
8 R	≤ 8 S	≤ 4 S	0.5 S	1 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≥ 16 R	≤ 2 S	≥ 8 R	≥ 4 R	≤ 0.5 S	≥ 16 R
4 R	≤ 8 S	≤ 4 S	0.25 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	1 S	1 S	1 S	≥ 16 R
8 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 0.5 S	≥ 16 R
8 R	32 I	≤ 4 S	32 R	≥ 32 R	≥ 64 R	≤ 0.25 S	≤ 0.25 S	≥ 16 R	16 S	≥ 8 R	≥ 4 R	≤ 0.5 S	8 I
4 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	0.25 S	≤ 0.25 S	≤ 0.5 S	1 S
8 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	4 I	1 S	≤ 0.5 S	≥ 16 R
8 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	1 S	≤ 0.25 S	≤ 0.5 S	8 I
4 R	≤ 8 S	≤ 4 S	≤ 0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 0.5 S	≥ 16 R
4 R	16 S	≤ 4 S	0.5 S	2 S	2 S	≤ 0.25 S	≤ 0.25 S	≥ 16 R	≥ 64 R	≥ 8 R	≥ 4 R	≤ 0.5 S	≥ 16 R
8 R	16 S	≤ 4 S	0.5 S	2 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	≥ 8 R	≥ 4 R	≤ 0.5 S	≥ 16 R
4 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	1 S	0.5 S	≤ 0.5 S	≤ 0.5 S
8 R	≤ 8 S	≤ 4 S	0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 0.5 S	≥ 16 R
4 R	16 S	≤ 4 S	0.5 S	2 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≥ 16 R	≤ 2 S	1 S	≤ 0.25 S	≤ 0.5 S	≥ 16 R
8 R	≤ 8 S	≤ 4 S	0.5 S	1 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≥ 16 R	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 0.5 S	1 S
8 R	16 S	≤ 4 S	2 S	8 S	4 S	≤ 0.25 S	≤ 0.25 S	8 I	≤ 2 S	1 S	1 S	≤ 0.5 S	8 I
8 R	≤ 8 S	≤ 4 S	≤ 0.12 S	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	1 S	0.5 S	≤ 0.5 S	8 I
8 R	≤ 8 S	≤ 4 S	8 I	16 R	16 R	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	≤ 0.12 S	≤ 0.25 S	≤ 0.5 S	1 S
4 R	≤ 8 S	≤ 4 S	8 I	≤ 0.12 S	≤ 1 S	≤ 0.25 S	≤ 0.25 S	≤ 1 S	≤ 2 S	1 S	0.5 S	≤ 0.5 S	≥ 16 R

Figure 1

MEGA analysis, genotypes and drug resistances of 21 mcr-1 E. coli isolated from children in Guangzhou, China. ST, sequence type. COL colistin, CSL cefoperazone-sulbactam, TZP piperacillin-tazobactam, CAZ ceftazidime, FEP cefepime, ATM aztreonam, IMP imipenem, MEM meropenem, TOP tobramycin, AMK amikacin, LVX levofloxacin, CIP ciprofloxacin, TGC tetracycline, DOX doxycycline