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ORIGINAL RESEARCH

Plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* isolated from market retail fruits in Guangzhou, China

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Background: As a result of the growing prevalence of the plasmid-mediated mobile colistin resistance gene *mcr-1* among Gram-negative bacteria, the surveillance of *mcr-1* has been globally applied. In our study, we aimed to shed light on the possibility of transmission of *mcr-1*-resistant isolates through market retail fruits.

Methods and results: Herein, 133 different fruit surface samples were collected and screened for the different MCR variants (*mcr-1* to *mcr-8*) using PCR and confirmed with sequencing. We identify for the first time *mcr-1*-carrying *Escherichia coli* and *Klebsiella pneumoniae* from market retail fruits in Guangzhou, China. Minimum inhibitory concentrations were detected by the broth microdilution method. Liquid mating was performed to check the transferability of the *mcr-1* gene. Pulsed field gel electrophoresis analysis of S1 nuclease-digested DNA and Southern blotting were performed to check the location of the *mcr-1* gene. Then, whole-genome sequencing and in silico multilocus sequence typing analysis were performed.

Conclusion: We showed that *E. coli* GB110 can mediate the spreading of antibiotic resistance genes through the food chain, while *K. pneumoniae* GB015 was considered to be the progenitor of the most successful multidrug-resistant clone. Since fruits are usually consumed fresh, this may serve as a direct source of *mcr-1*-producing bacteria in humans that requires prompt surveillance and intervention to limit the spread of resistance.

Keywords: colistin, *mcr-1*, *Escherichia coli*, *Klebsiella pneumoniae*, fruit

Introduction

Colistin is a polymyxin antibiotic that has been used for many years in veterinary medicine. Nowadays, a need for using colistin in human medicine has evolved as the last resort drug for the treatment of infections caused by multidrug-resistant bacteria, especially carbapenem-resistant Enterobacteriaceae. However, the use of colistin as a last resort antibiotic is seriously threatened by the rise of plasmid-borne mobile colistin resistance (*mcr* family) genes (*mcr-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7* and *-8*),[1–3] which can spread rapidly via horizontal gene transfer between bacterial strains and species.[4] In August 2016, a survey reported detection of *mcr-1*-positive Enterobacteriaceae from farming soil in Shandong province, China, suggesting that Enterobacteriaceae harboring *mcr-1* can contaminate agricultural products.[5] Here, we report the identification of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* isolated from market retail fruits in Guangzhou, China.

Methods and results

A total of 133 fruit surface samples were collected from market retail fruits in Guangzhou, China, from June to November 2016, at various intervals. Different fruits were

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collected, including apples (n=52), oranges (n=31), tangerines (n=16), pears (n=15), bananas (n=11), pomegranates (n=6) and grapes (n=2). Initially, surface samples from the fruits were wiped with sterile cotton swabs moistened with saline. Each swab contained one fruit surface sample and was transported to the laboratory immediately. The swabs were cultured on to lysogeny broth (LB) liquid medium (Oxoid, Basingstoke, UK), then DNA extraction was performed using the boiling method. All samples were screened for *mcr-1* to *mcr-8* using PCR and confirmed by Sanger sequencing. The primers and conditions used for the PCR assays are listed in Table S1.[3]

Out of 133 fruit samples, *mcr-1* was detected in two samples (1.5%). No *mcr-2* to *mcr-8* genes were identified among these samples. To screen for colistin resistance, the two *mcr-1*-positive samples were directly seeded on LB agar plates containing 4 μg/mL colistin and incubated at 37°C for 24 hours. The species of selected colonies from the LB agar plates were identified using the API 20E system (bioMérieux, Marcy l'Etoile, France) and confirmed by 16S rRNA sequencing. As a result, *mcr-1*-harboring *E. coli* GB110 and *K. pneumoniae* GB015 were identified from apple and orange samples, respectively. Minimum inhibitory concentrations (MICs) were detected by the broth microdilution method and interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). It was found that *E. coli* GB110 was susceptible to all tested agents except colistin and polymyxin B; and *K. pneumoniae* GB015 was resistant to colistin, polymyxin B and ampicillin (Table 1).

A conjugation experiment was performed using streptomycin-resistant *E. coli* C600 as the recipient. The *mcr-1*-producing isolates and recipient were mixed (ratio of 1:9) in LB and incubated overnight at 37°C. The mixture was then spread on LB agar plates containing colistin (2 μg/mL) and streptomycin (2,000 μg/mL). The transconjugants were confirmed for *mcr-1* by PCR and Sanger sequencing. The results showed that *mcr-1* was successfully transferred to streptomycin-resistant *E. coli* C600 through conjugation in both isolates, suggesting that *mcr-1* was located on transferable plasmids. Plasmid incompatibility groups were identified by PCR assay as described previously.[6] Pulsed field gel electrophoresis analysis of S1 nuclease-digested DNA (S1-PFGE), followed by Southern blotting, showed that *mcr-1* was located on ~62.9 kb IncFIA and ~204.2 kb IncHI1 plasmids for the transconjugants of *E. coli* GB110 and *K. pneumoniae* GB015, respectively (Figure 1).

The genome DNA of *E. coli* GB110 and *K. pneumoniae* GB015 were extracted using a Qiagen Blood & Tissue kit (Qiagen, Hilden, Germany) and DNA libraries were constructed with 350 bp paired-end fragments. In total, 9,367,660 and 10,515,584 paired-end 150 bp reads were produced by the Illumina Hiseq2000 platform for *E. coli* GB110 and *K. pneumoniae* GB015, respectively. Reads were assembled using SPAdes

Table 1 Characteristics of *mcr-1*-producing *Escherichia coli* GB110, *Klebsiella pneumoniae* GB015 and their transconjugants isolated from market retail fruits in China

Isolate code	Source	Isolation site	MLST	Plasmid replicon type	Encoding genes of resistance	MICs
E. coli GB110	Apple	Market retail fruits	ST189	IncHI1, IncFIA	*aadA2*, *aadA1*, *mcr-1*, *floR*, *cmlA1*, *sul2*, *sul3*, *tetA*, *tetM*, *dfrA12*, *mdfA*	CL (16), PB (8), TGC (≤1), AMP (4), AMC (8), CTX (≤0.125), CAZ (≤0.125), FEP (0.25), GEN (≤1), AMK (4), ETP (≤0.125), IPM (≤0.125), MEM (≤0.125), FOS (≤8), NIT (≤8), CIP (≤0.03)
K. pneumoniae GB015	Orange	Market retail fruits	ST442	IncHI1, IncFIB	$bla_{SHV-110}$, *mcr-1*, *qnrS1*, *oqxA*, *oqxB*, *fosA6*, *sul1*, *tetA*, *dfrA1*	CL (64), PB (64), TGC (≤1), AMP (64), AMC (8), CTX (≤0.125), CAZ (0.5), FEP (≤0.125), GEN (≤1), AMK (4), ETP (≤0.125), IPM (0.5), MEM (≤0.125), FOS (16), NIT (64), CIP (1)
E. coli C600 (transconjugant of *E. coli* GB110)	–	–	–	IncFIA	*mcr-1*	CL (16), PB (8), TGC (≤1), AMP (4), AMC (8), CTX (≤0.125), CAZ (≤0.125), FEP (≤0.125), GEN (≤1), AMK (4), ETP (≤0.125), IPM (≤0.125), MEM (≤0.125), FOS (≤8), NIT (8), CIP (≤0.03)
E. coli C600 (transconjugant of *K. pneumoniae* GB015)	–	–	–	IncHI1	*mcr-1*	CL (16), PB (16), TGC (≤1), AMP (4), AMC (8), CTX (≤0.125), CAZ (≤0.125), FEP (≤0.125), GEN (≤1), AMK (4), ETP (≤0.125), IPM (≤0.125), MEM (≤0.125), FOS (≤8), NIT (≤8), CIP (≤0.03)

Abbreviations: AMC, amoxicillin–clavulanic acid; AMK, amikacin; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CL, colistin; CTX, cefotaxime; ETP, ertapenem; FEP, cefepime; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; NIT, nitrofurantoin; PB, polymyxin B; TGC, tigecycline.

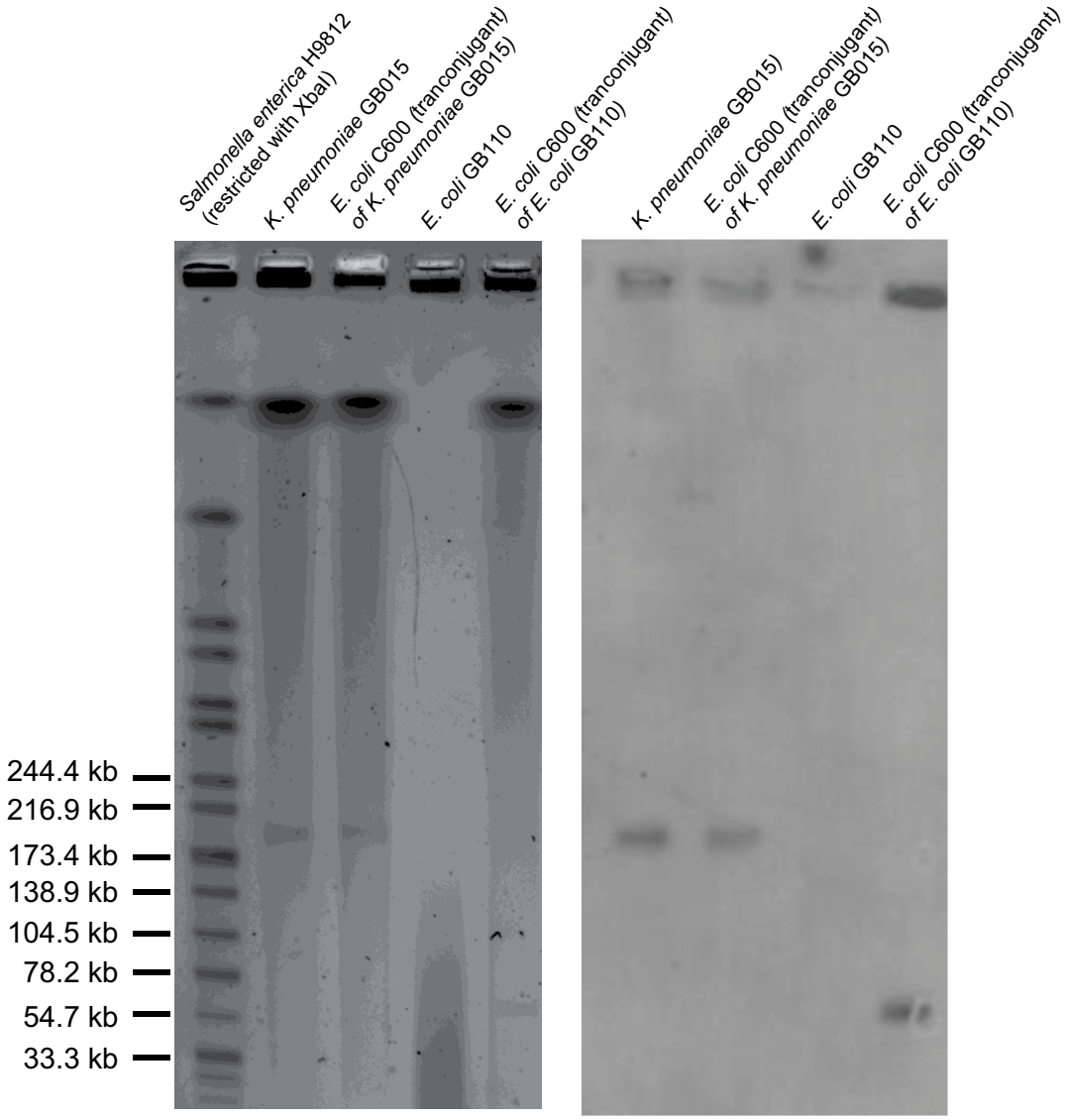

Figure 1 S1-PFGE and Southern hybridization with the *mcr-1* probe.
Abbreviations: *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; S1-PFGE, pulsed field gel electrophoresis analysis of S1 nuclease-digested DNA.

v3.12.0.[7] This Whole Genome Shotgun project has been deposited at GenBank under the accession number PRJNA482733. The genome annotation was performed using PATRICK (https://www.patricbrc.org/) and ISFinder (https://www-is.biotoul.fr/). The assembled genomes showed that IS*Apl1* was located upstream of *mcr-1* for *E. coli* GB110 and downstream of *mcr-1* for *K. pneumoniae* GB015, consistent with reported variability in the location of IS*Aba1*, which was probably involved in the original mobilization of *mcr-1* from *Moraxella* spp.[4,8]

Multilocus sequence typing (MLST), serotyping, antimicrobial resistance genes and virulence factors were annotated by the Center for Genomic Epidemiology website (http://www.genomicepidemiology.org/).

MLST analysis of *E. coli* GB110 (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) showed that it belonged to sequence type 189 (165 cplx), which was reported from poultry retail meat mediating the spread of extended-spectrum β-lactamase (ESBL) genes in Colombia,[9] while *K. pneumoniae* GB015 belonged to ST442. ST442 and ST11, the most common carbapenem-resistant clones in China, are considered to be the progenitors of *K. pneumoniae* ST258, which is widespread worldwide as the most successful multidrug-resistant clone of *K. pneumoniae*.[10]

The serotype of *E. coli* GB110 was identified as O38:H26, which can cause diarrhea in humans.[11] The *E. coli* heat-stable enterotoxin-1 (EAST-1)-encoding gene *astA* was found in *E.*

coli GB110, which is associated with human diseases. These results indicate the risk that eating contaminated fruits could lead to diarrheal diseases.

The *E. coli* GB110 was found to harbor ten resistance genes, including *mcr-1* and aminoglycoside resistance genes, *aadA2* and *aadA1*, while *K. pneumoniae* GB015 was found to possess nine resistance genes, including *mcr-1*, $bla_{SHV-110}$, *qnrS1* and *fosA6* (Table 1).

Conclusion

In our study, we reported the detection of *mcr-1* from market retail fruits, which is significant since, unlike meat and vegetables, fruits are usually consumed without cooking or processing, making them a potentially high-risk source of *mcr-1* acquisition and infection in humans. This work sheds light on the urgent need for continued surveillance and prompt intervention in China to guard against the worldwide distribution of *mcr-1*, which threatens the use of colistin as a last resort antibiotic.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Primers and PCR conditions used in this study

Purpose	Primer	Nucleotide sequence (5′→3′)	Amplicon size (bp)	Annealing temperature (°C)
Amplification of *mcr-1*	MCR-1F	ATCAGCCAAACCTATCCCATCG	1,257	55
	MCR-1R	GCAGACGCACAGCAATGCCTAT		
Amplification of *mcr-2*	MCR-2F	GCGATGGCGGTCTATCCTGTAT	378	55
	MCR-2R	TGCGATGACATGGGGTGTCAGC		
Amplification of *mcr-3*	MCR-3F	TATGGGTTACTATTGCTGG	814	55
	MCR-3R	CTACCCTGATGCTCATCG		
Amplification of *mcr-4*	MCR-4F	GTCATAGTGGTATAAAAGTACAG	669	55
	MCR-4R	CCACCGTCTATCAGAGCCAAC		
Amplification of *mcr-5*	MCR-5F	GCGGTTGTCTGCATTTATCAC	1,042	50
	MCR-5R	CTTTGAAAACCTGTCTTCGGCA		
Amplification of *mcr-6*	MCR-6F	GTCCGGTCAATCCCTATCTGT	556	55
	MCR-6R	ATCACGGGATTGACATAGCTAC		
Amplification of *mcr-7*	MCR-7F	TGCTCAAGCCCTTCTTTTCGT	892	55
	MCR-7R	TTCATCTGCGCCACCTCGT		
Amplification of *mcr-8*	MCR-8F	AACCGCCAGAGCACAGAATT	667	60
	MCR-8R	TTCCCCCAGCGATTCTCCAT		