Emergence of *mcr-1* in *Raoultella ornithinolytica* and *Escherichia coli* Isolates from Retail Vegetables in China

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ABSTRACT The presence of *mcr-1* among *Enterobacteriaceae* isolates collected from retail vegetables in China between 2015 and 2016 was investigated. Two *Raoultella ornithinolytica* and seven *Escherichia coli* strains recovered from lettuce and tomato samples were identified as MCR-1-producers. Similar to isolates from animals and humans, the *mcr-1* gene was located on the IncHI2/ST3, IncI2, or IncX4 plasmids. The presence of MCR-1-producing organisms in ready-to-eat food samples represents a serious risk for human health.

KEYWORDS *Escherichia coli*, *Raoultella ornithinolytica*, *mcr-1*, plasmid, vegetable

Colistin is generally considered the last resort antibiotic for treating infections caused by extensively drug-resistant (XDR) *Enterobacteriaceae* (1, 2). The emergence of the plasmid-mediated colistin resistance gene, *mcr-1*, has therefore gained a lot of attention (3). This gene has spread worldwide and been detected in several genera of *Enterobacteriaceae* (*Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, *Enterobacter*, *Kluyvera*, and *Citrobacter*) (4–6). Although MCR-1-producing bacteria are mainly detected in animal sources, environmental samples (river water and sewage) with strains harboring *mcr-1* have also been reported (4–9). Vegetables, which might be contaminated with bacteria via sewage irrigation, have also been reported to carry bacteria harboring *mcr-1* (8). We investigated the occurrence of *mcr-1* in *Enterobacteriaceae* isolated from retail vegetables in Guangzhou, China.

Between May 2015 and August 2016, 916 fresh vegetable samples (239 lettuce heads, 218 tomatoes, 176 carrots, 264 cucumbers, and 19 bean sprouts) were collected from 41 farmers' markets and 12 supermarkets in Guangzhou, China. Chromogenic Brilliance extended-spectrum β -lactamase (ESBL) agar plates (CHROMagar Microbiology, Paris, France) were used for isolation of ESBL producers which were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Shimadzu, Japan) and some were confirmed by 16S rRNA sequencing. A total of 244 ESBL-producing *Enterobacteriaceae* isolates (175 *Klebsiella pneumoniae*, 23 *E. coli*, and 46 others) were recovered from 216 samples. The MICs of 10 antibiotics were determined by an agar dilution or a broth microdilution method. The presence of *mcr-1* and *mcr-2* in this strain collection was determined by PCR and sequencing using the primers described previously (1, 10). Our results showed that six of the 244 (2.5%) ESBL-producing isolates (four *E. coli* and two *Raoultella ornithinolytica*) were positive for *mcr-1* (Table 1). The prevalence of *mcr-1* in the 23 ESBL-producing *E. coli* isolates was 17.4%. Four ESBL producers (one *Klebsiella pneumoniae* isolate and three *Enterobacter species* isolates) were resistant to colistin but were negative for *mcr-1* and *mcr-2*.

In addition, 26 *E. coli* isolates were obtained from the 916 samples by using

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TABLE 1 Characteristics of *mcr-1*-positive Enterobacteriaceae isolates from vegetables in China, 2015-2016^a

Organism and isolate	Origin	Farmers' market	Isolation time	MLST	PFGE	Location	Plasmid size (kb)	Colistin MIC (μg/ml)	Other resistance phenotype(s) ^b	Other resistance gene(s) ^c
<i>Raoultella ornithinolytica</i> TS48CTX TS53CTX	Lettuce	B	2015.10.15	NA	E	IncHI2/ST3	~244	4	AMP, CTX, FFC, FOS, GEN, TET, SXT	<i>bla</i> _{CTX-M-14^r} , <i>floR</i> , <i>fosA3</i> , <i>oqxAB</i>
	Lettuce	B	2015.10.15	NA	E	IncHI2/ST3	~244	8	AMP, CTX, FFC, FOS, GEN, TET, SXT	<i>bla</i> _{CTX-M-14^r} , <i>floR</i> , <i>fosA3</i> , <i>oqxAB</i>
<i>Escherichia coli</i> 6HS20eCTX 6BS21eCTX 6BF21eCTX TS62CTX	Lettuce	A	2016.8.11	ST795	A	IncHI2/ST3	~244	4	AMP ^b , CTX ^b , FFC ^b , FOS ^b , GEN ^b , TET, SXT ^b	<i>bla</i> _{CTX-M-14^r} , ^c <i>floR</i> , ^c <i>fosA3</i> , ^c <i>oqxAB</i>
	Lettuce	C	2016.8.11	ST2505	B	IncI2	~60	8	AMP, FFC, TET, SXT	<i>bla</i> _{CTX-M-55^r} , <i>floR</i>
	Tomato	C	2016.8.11	ST69	C	IncHI2/ST3	~244	8	AMP ^b , CTX ^b , FFC ^b , FOS ^b , GEN ^b , TET, SXT ^b	<i>bla</i> _{CTX-M-14^r} , ^c <i>floR</i> , ^c <i>fosA3</i> , ^c <i>oqxAB</i>
	Lettuce	D	2015.10.17	ST156	D	IncI2	~60	8	AMP, CTX, AMK, GEN, FFC, FOS, TET, CIP, SXT	<i>bla</i> _{CTX-M-55^r} , <i>rmtB</i> , <i>floR</i> , <i>fosA3</i>
	Lettuce	A	2016.8.11	ST48	F	IncX4	~33	4	AMP, FFC, TET, SXT	<i>floR</i>
6HS20E	Lettuce	A	2016.8.11	ST48	F	IncX4	~33	4	AMP, FFC, TET, SXT	<i>floR</i>
TF33E	Tomato	D	2015.10.15	ST206	G	chromosome	NA	8	AMP, FFC, STR, TET, SXT	<i>floR</i>
TF10E	Tomato	D	2015.10.15	ST206	G	chromosome	NA	8	AMP, FFC, STR, TET, SXT	<i>floR</i>

^aAccording to recommendations of the Joint Clinical and Laboratory Standards Institute (CLSI)–European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Polymyxin Breakpoints Working Group (<http://www.eucast.org/>), the International Organization for Standardization standard broth microdilution method (20776-1) was used to determine colistin MICs for these strains. Antimicrobial susceptibility was determined and evaluated according to CLSI document no. M100-S25. Resistance to florfenicol was interpreted according to EUCAST clinical breakpoints and epidemiological cutoff values (>16 mg/liter) (<http://mic.eucast.org/Eucast2/>). Abbreviations: AMK, amikacin; AMP, ampicillin; CIP, ciprofloxacin; CTX, cefotaxime; FFC, florfenicol; FOS, fosfomicin; GEN, gentamicin; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline; MLST, multilocus sequence type; NA, not applicable; PFGE, pulsed-field gel electrophoresis.

^bIndicates resistance phenotypes transferred to a recipient by conjugation.

^cIndicates resistance genes transferred to a recipient by conjugation.

MacConkey agar plates without antibiotics and were identified by biochemical methods and 16S rRNA sequencing. Three (11.5%) of them were positive for *mcr-1*.

All nine *mcr-1*-carrying isolates showed resistance to colistin and florfenicol (Table 1). In addition, 6 and 5 isolates exhibited resistance to cefotaxime and fosfomycin, respectively. By PCR, all *mcr-1*-positive isolates carried the *floR* gene, and 6, 5, and 4 of them carried the *bla*_{CTX-M} (4 *bla*_{CTX-M-14} and 2 *bla*_{CTX-M-55}), *fosA3*, and *oqxAB* genes, respectively (Table 1).

The clonal relatedness among the *mcr-1*-positive isolates was determined using pulsed-field gel electrophoresis (PFGE) and multilocus sequence type (MLST) analysis (the second only for *E. coli*) (<http://www.warwick.ac.uk/mlst/>). Six of the *mcr-1*-positive *E. coli* isolates were clonally unrelated, while two *E. coli* and the two *R. ornithinolytica* strains were related (Table 1). PFGE after S1 nuclease digestion and hybridization revealed that *mcr-1* was located on 30- to 250-kb plasmids in 7 isolates (five *E. coli* and 2 *R. ornithinolytica*), but was located on the chromosome in the two ST206 *E. coli* strains (Table 1). A conjugation experiment using streptomycin-resistant *E. coli* C600 as recipient and selection with colistin (2 µg/ml) and streptomycin (3000 µg/ml) yielded 5 transconjugants from five of the seven *mcr-1*-positive *E. coli* isolates. In 2 transconjugants, *fosA3*, *floR*, and *bla*_{CTX-M-14} were cotransferred with *mcr-1*. The replicon types of the *mcr-1*-harboring plasmids were determined using the protocol provided in the Plasmid MLST Database (<http://pubmlst.org/plasmid/>); two IncHI2/ST3, two IncI2, and one IncX4 plasmids were identified (Table 1). The genetic context of *mcr-1* was investigated using primers: CLR3-F (CGAAGCACCAAGACATCA) and CLR3-R (CCACAAG ACAAACGGACT); Mhp-F (TTGCCAGATTGCTACTGT) and ISAp-R (TTTCTCGCTCGTTAT TGTA). ISAp1 was located upstream of *mcr-1* in all the isolates except *E. coli* TS62CTX and 6HS20E. Three isolates (6BS21eCTX, TF33E, and TF10E) also carried ISAp1 downstream of *mcr-1*.

Recent studies have implied that fresh produce might be a possible route for the spread of resistance genes in the community (8, 11). This study documented the presence of *mcr-1* in *Enterobacteriaceae* isolates from lettuce and tomato. Although the occurrence of *mcr-1* in isolates from vegetables was lower than that in isolates from food animals and animal foods in China (3), the presence of MCR-1-producing bacteria in vegetables represents a threat to human health, as fresh vegetables such as tomato and lettuce are often consumed raw. Of note, the plasmids (IncHI2/ST3, IncX4, and IncI2) accounting for the spread of *mcr-1* found in this study were similar to those found in food animals from China (5, 12–14) and all *mcr-1*-positive isolates carried the florfenicol (an antimicrobial agent used only in animals) resistance gene *floR*. Vegetables are often in direct contact with the soil and water, which are known to be important reservoirs of antimicrobial resistance genes shared between animals, humans, and the environment (15). In addition, vegetables might be fertilized with manure and wastewater from antimicrobial-treated livestock. Thus, the MCR-1-producers found in vegetables might have originated from animals.

Here, we reported the emergence of *mcr-1* and *fosA3* in *R. ornithinolytica*, belonging to a genus in *Enterobacteriaceae* that is closely related to *Klebsiella* (16). *R. ornithinolytica* is usually found in aquatic, soil, and botanical environments. It is also an unusual pathogen associated with community-acquired infections. However, cases of *R. ornithinolytica* infection might have been underrecognized due to its misidentification as a *Klebsiella* species in clinical laboratories that use conventional phenotypic methods (17). Recently, the number of reported *R. ornithinolytica* infections in humans has grown, probably due to the introduction of mass spectrometry and the use of molecular identification techniques in clinical microbiology laboratories (16, 17). Carbapenem resistance genes have been detected in *R. ornithinolytica* in China (18); thus, the presence of *mcr-1* in *R. ornithinolytica*, as well as *fosA3* and *bla*_{CTX-M-14}, will lead to the emergence of multidrug-resistant *R. ornithinolytica* strains.

In summary, the results of this study indicated that fresh vegetables constituted a possible route for the spread of MCR-1-producing *Enterobacteriaceae* species. The presence of MCR-1-producing organisms in the food supply and ready-to-eat foods is

alarming and represents a serious risk for human health. To improve food safety and consumer health, appropriate measures such as proper disposal of animal excrement before use as fertilizers and improvement of the quality of irrigation water need to be taken. Further studies are required for evaluating the prevalence of antimicrobial resistance genes in vegetables in China and other countries.

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