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Short Communication

Colistin-Resistant *mcr*-Positive Enterobacteriaceae in Fresh Vegetables, an Increasing Infectious Threat in China

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ABSTRACT

The presence of mobilized colistin resistance (*mcr*) genes is a global concern. However, data concerning *mcr* in fresh vegetables, a reservoir for antibiotic resistance genes, are still rare. In this study, *mcr* genes were analysed in 528 vegetable samples from 53 supermarkets or farmer's markets in 23 cities of 9 provinces in China, and the *mcr*-positive Enterobacteriaceae were characterized. Nineteen (3.6%) samples carried one or more *mcr*-positive isolates, and the highest three detection rates were found in carrot, pak choi and green pepper. Twenty-four *mcr-1*-positive isolates (23 *Escherichia coli* and one *Enterobacter cloacae*) were obtained, and *E. coli* isolates showed high genetic diversity. Different multilocus sequence type (MLST) isolates were also observed within the same sample. All 24 isolates showed multidrug resistance, and 14 carried bla_{CTX-M} genes. Most isolates harbored similarly conjugative IncX4-type (~33 kb) or IncI2-type (~60 kb) *mcr-1*-bearing plasmids. The sequenced prevalent IncX4 plasmid and IncI2 plasmid from tomato were similar to the relevant plasmids from animals and clinical isolates in various countries. *mcr-1*-bearing IncHI2/ST3 plasmid highly similar to that carrying 14 resistance genes from *E. coli* of chicken was also observed. In conclusion, a high prevalence of *mcr-1* in fresh vegetables was found in China, and the dissemination of *mcr-1* was mediated by similar IncX4 or IncI2 plasmids. The plasmids from vegetables showed high similarity to plasmids from clinical isolates, indicating MCR-1-producers in ready-to-eat vegetables may pose a huge threat to public health and measures need to be taken to ensure food safety.

1. Introduction

Colistin has attracted much attention as the treatment of last resort for carbapenem-resistant Enterobacteriaceae (CRE), which is increasing globally. However, the clinical usefulness of colistin has been challenged by the emergence of colistin resistance gene, *mcr-1* [1]. Several mobilized colistin resistance determinants (*mcr*), including *mcr-1* to *mcr-8*, have been identified in various Enterobacteriaceae species from samples of different origins around the world [2,3]. Recently, *mcr-1* was found in one *E. coli* from lettuce in Portugal [4].

Contaminated fresh vegetables eaten raw have often been linked with outbreaks of foodborne diseases [5], and are also prone to transmit antibiotic resistant microorganisms or resistance genes to humans. Therefore, studies on screening *mcr* in bacteria from fresh vegetables are urgently needed. However, there are few reports focusing on *mcr*-positive Enterobacteriaceae in vegetables. *mcr-1* was found in one lettuce sample in Portugal [4], and was also reported in two imported vegetable samples in Switzerland at around the same time [6]. Recently, *mcr-1*-positive *Raoultella ornithinolytica* and *E. coli* have been found in vegetables from city Guangzhou, China [7]. There is a paucity of large-scale study data regarding the prevalence of *mcr* in fresh vegetables.

Considering the high prevalence and wide distribution of *mcr* in China [2], there is an urgent need to investigate the presence of *mcr* in vegetables in more cities in China. This can help provide effective strategies to control the dissemination of colistin-resistant bacteria in food. In this study, we conducted a surveillance of the prevalence of *mcr* in fresh vegetables from 23 cities of 9 provinces in China and analysed the characteristics of *mcr*-bearing plasmids.

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2. Material and methods

A total of 528 fresh vegetable samples belonging to 18 types were purchased from 53 supermarkets and farmer's markets in 23 cities or districts of 9 provinces in China, between May 2017 and April 2018 (Table S1). Sampled vegetables were processed with trypticase soy broth (TSB) containing vancomycin (8 mg/L) and colistin (2 mg/L), according to the previously reported protocol [8]. The total DNA of half the survival bacteria in the TSB was screened for *mcr* genes (*mcr-1* to *mcr-8*) as previously described [3]. The remaining bacteria harboring *mcr* were diluted and spread onto eosin methylene blue (EMB) agar plates containing colistin (2 mg/L). Three colonies with the same Enterobacteriaceae appearance of each sample were selected for screening the *mcr* genes. *mcr*-positive isolates were identified by 16S rRNA sequencing, and were compared by multilocus sequence type (MLST) to identify different colonies in the same sample [9].

mcr-positive isolates were also screened for PMQR, 16S rRNA methyltransferase genes, carbapenemase-encoding genes, $bla_{\text{CTX-M}}$ and *fosA3* as previously described [8]. The minimum inhibitory concentrations (MICs) of cefotaxime, ceftiofur, meropenem, ampicillin, enrofloxacin, ciprofloxacin, levofloxacin, nalidixic acid, amikacin, gentamicin, kanamycin, doxycycline, tetracycline, tigecycline, and fosfomycin were determined by the agar dilution method, and the results (except for tigecycline) were analysed according to the CLSI criteria of 2015 [10]. The MIC method for colistin and breakpoints for colistin and tigecycline were recommended by the 2017 EUCAST (available at http://www.eucast.org/clinical_breakpoints/).

For *mcr*-positive isolates, conjugation experiments were performed using the broth-mating method [11]. The replicons of the *mcr*-positive transconjugants were determined using the protocol provided in the Plasmid MLST Database (http://pubmlst.org/plasmid/). To analyse the location of *mcr-1*, S1-pulsed-field gel electrophoresis (PFGE) and Southern Hybridization were performed twice on the transconjugants carrying *mcr-1* and isolates without transconjugants as previously described [11]. *mcr*-bearing plasmids in the transconjugants harboring one plasmid were digested with the endonuclease *EcoR*I to analyse the restriction fragment length polymorphism (RFLP) profiles of plasmids with the same replicon. Representative plasmids belonging to different replicon types were selected for sequencing. Briefly, total genomic DNA from transconjugants T-TO89, T-CTX148 and T-SQB-1-1 were extracted, and small fragments (350 bp) of DNA libraries were sequenced using Illumina HiSeq PE150. After assembling the sequence reads and filtering the sequence data of *E. coli* C600 chromosomal DNA, plasmid contigs were obtained. Functional annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline server and Rapid Annotation using Subsystem Technology (RAST) [12]. To determine the resistance genes and replicon types, plasmid contigs were subjected to ResFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder-2.0/). Sequence comparison and map generation were performed using the BLASTn implemented in BRIG [13].

3. Results

3.1. Prevalence of mcr-positive Enterobacteriaceae

When screening the total DNA, 19 (3.6%) fresh vegetable samples belonging to 10 types carried *mcr-1*, and no other *mcr* genes were found. From the 19 samples, 24 *mcr-1*-positive isolates were retrieved and 23 were identified as *E. coli* and isolate CTX145B was *Enterobacter cloacae*. MLST subtyping identified 16 types along with a new ST type (6-7-4-1-8-222-2 for isolate CTX148) not previously registered in the *E. coli* MLST database, and the most prevalent sequence types (STs) were ST744 (4) and ST224 (3). Of note, different *mcr-1*-positive *E. coli* from the same sample (sample 79, 173 and 174) had different MLST types (Table S2). The highest detection rate of *mcr-1* was found in carrot (14.3%), followed by pak choi (13.3%), green pepper (7.7%), leaf lettuce (5.6%), leaf rape (4.9%), romaine lettuce (4.3%), tomato (3.5%), spinach (3.2%), cucumber (3.1%), and curly endive (2.4%) (Table S1). The 19 *mcr-1*-positive samples were all from Beijing, Shanghai and Shandong provinces. Beijing (37.0%) carried the highest detection rate of *mcr-1*, followed by Shandong (3.3%) and Shanghai (2.3%) (Table S1).

3.2. Antimicrobial susceptibility and resistance genes

All 24 isolates showed multidrug resistance, but were susceptible to meropenem and tigecycline. Resistance rates to other antibiotics were as follows: colistin (24/24), ampicillin (24/24), gentamicin (22/24), nalidixic acid (21/24), enrofloxacin (19/24), tetracycline (18/24), kanamycin (18/24), ciprofloxacin (16/24), levofloxacin (14/24), doxycycline (14/24), cefotaxime (14/24), ceftiofur (14/24) and fosfomycin (8/24). Isolates CTX148 and LL166 were also resistant to amikacin and carried *rmtB*. Fourteen isolates carried $bla_{\text{CTX-M}}$ genes and all were found in the 14 isolates resistant to cefotaxime. Eleven and five isolates harbored $bla_{\text{CTX-M-9G}}$ and $bla_{\text{CTX-M-1G}}$, respectively, including two isolates (CA79Z and CTX171) carrying both types of genes. The eight fosfomycin-resistant isolates harbored *fosA3*. *oqxAB* was the most prevalent PMQR gene, followed by *qnrS* and *qnrB* (Table S2).

3.3. Analysis of plasmids carrying mcr-1

Seventeen transconjugants carrying *mcr-1* were obtained and all showed resistance to colistin (Table 1). IncX4 replicon was detected in nine transconjugants and the number of transconjugants harboring IncI2 and IncHI2 replicon was seven and one, respectively. S1 nuclease-PFGE showed that seven transconjugants with IncX4 replicon carried only one plasmid and two transconjugants carried two plasmids (Fig. 1 and Table 1). Southern blot hybridization indicated that *mcr-1* was on an IncX4 plasmid of ~33 kb in size in all nine transconjugants (Fig. 1). Notably, the seven IncX4 plasmids in transconjugants harboring single plasmid shared highly similar RFLP profiles, although they were from different fresh vegetables or cities (Figure S1 and Table 1).

S1-PFGE and Southern blot hybridization revealed that six transconjugants with IncI2 replicon carried single plasmid and all the seven IncI2 plasmids (~60 kb) carried *mcr-1* (Fig. 1). The two IncI2 plasmids in CTX148 and PA175 from different types of vegetables from two markets shared identical RFLP profiles (Figure S1 and Table 1). Notably, the two ST744 isolates (CU173Z and CU173) from the same cucumber sample carried IncI2 and IncX4 *mcr-1*-bearing plasmid, respectively (Table 1). In T-SQB-1-1, co-transfer of resistance to the β-lactams, gentamycin and fosfomycin was observed, and single IncHI2/ST3 plasmid (~250 kb) carrying *mcr-1*, $bla_{\text{CTX-M-14}}$, and *fosA3* was identified (Fig. 1 and Table 1). In the seven isolates without transconjugants, *mcr-1* was on ~140 kb plasmids in three isolates, whereas CTX101, CTX145B and CTX171 carried ~220 kb, ~250 kb and ~70 kb plasmids, respectively. Notably, *mcr-1* was on the chromosome of SG-2-1 (Fig. 1).

Among the three sequenced plasmids, IncX4 pTO89 and IncI2 pCTX148 carried only *mcr-1*, accounting for the phenotypes of their transconjugants. Alignment of contig 38 of pTO89 (SRMK00000000) to other reported plasmids showed that it aligned very well to pCSZ4 (KX711706) (99% in both identity and coverage) from pork *E. coli* and pHNGDF49 (MF978387) from *E. coli* of fish in China (Fig. 2A). Notably, pTO89 was also highly similar to pKP15450-MCR-1 (MH715959) from clinical *Klebsiella pneumoniae* in Taiwan, pmcr1_IncX4 (KU761327) from clinical *K. pneumoniae*

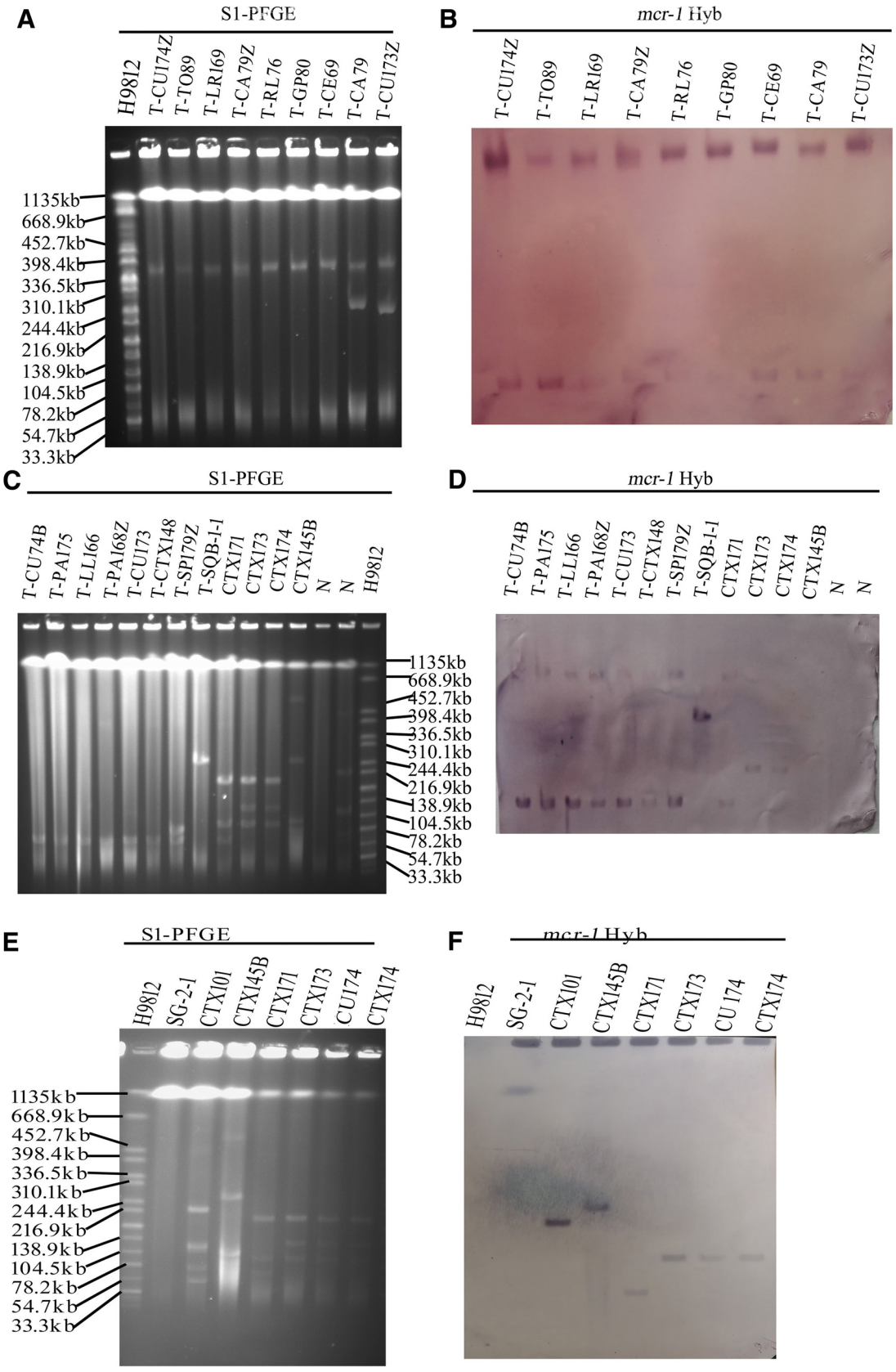

Fig. 1. Analysis of the location of *mcr-1* among transconjugants and isolates without transconjugants. (A) S1 nuclease-PFGE of transconjugants carrying IncX4 plasmids. (B) Southern blot hybridization with the *mcr-1* probe. (C) S1 nuclease-PFGE of transconjugants carrying IncI2 plasmids. (D) Southern blot hybridization with the *mcr-1* probe. (E) S1-PFGE of *mcr-1*-positive isolates without transconjugants. (F) Southern blot hybridization with the *mcr-1* probe. Lane H9812: chromosomal DNA of *Salmonella enterica* serotype Braenderup H9812 digested with *Xba*I serving as size markers; Lane N: *E. coli* C600 lacking plasmid; Lane N: *E. coli* isolate harboring plasmids lacking *mcr-1*.

Table 1
Characteristics of the 17 transconjugants harboring *mcr-1*

Transconjugants	Origin of donor	MLST types of donors	MICs (mg/L)		Other resistance profiles	Plasmid replicon type (size kb)	Plasmid RFLP profiles[a]
			COL	NAL			
T-CU174Z	cucumber	744	8	8	STR	X4 (~33)	A1
T-TO89	tomato	713	8	4	STR	X4 (~33)	A1
T-LR169	leaf rape	744	8	4	STR	X4 (~33)	A1
T-CA79Z	carrot	5539	8	8	TET, STR	X4 (~33)	A2
T-RL76	romaine lettuce	10	4	4	STR	X4 (~33)	A3
T-GP80	green pepper	5873	8	4	STR	X4 (~33)	A4
T-CE69	curly endive	13	8	4	STR	X4 (~33)	A4
T-CA79	carrot	13	4	8	TET, STR	X4 (~33)	NA
T-CU173Z	cucumber	744	4	4	STR	X4 (~33)	NA
T-CU74B	cucumber	1115	8	4	STR	I2 (~60)	B3
T-PA175	pak choi	1196	8	4	STR	I2 (~60)	B1
T-LL166	leaf lettuce	6397	8	4	STR	I2 (~60)	B4
T-PA168Z	pak choi	648	8	4	STR	I2 (~60)	C
T-CU173	cucumber	744	8	4	STR	I2 (~60)	B2
T-CTX148	tomato	UT	8	4	STR	I2 (~60)	B1
T-SP179Z	spinach	2253	4	4	STR	I2 (~60)	NA
T-SQB-1-1	romaine lettuce	2705	8	4	AMP, CTX, CTF, KAN, GEN, FOS, STR	HI2 (~250)	NA
C600			0.25	4	STR		

MIC, minimum inhibitory concentration; MLST, multilocus sequence type; NA, not analysed; UT. untypable; [a]RFLP profiles differing by only a few bands (n=1 ~ 3) were assigned to the same profile.
COL, colistin; NAL, nalidixic acid; AMP, ampicillin; CTX, cefotaxime; CTF, ceftiofur; KAN, kanamycin; GEN, gentamicin; FOS, fosfomycin; STR, streptomycin;

in China and pNG14043 (KY120364) from clinical *Salmonella Typhimurium* in Taiwan (Fig. 2A). Contig 63 of pCTX148 (MK754161) aligned well to pHNGDF93 (MF978388) from fish *E. coli* and pD90-2 (CP022452) from *S. Indiana* of chicken in China. Additionally, the three IncI2 plasmids mentioned above were highly similar (99%) to p5CRE51-MCR-1 (CP021176) and pJIE3685-1 (KY795978) from clinical *E. coli* in Taiwan and Australia, respectively (Fig. 2B). For IncHI2/ST3 plasmid pSQB-1-1 (SRML00000000), 20 contigs were obtained and the multiresistance region contained resistance genes *aph(3')-Ia, aac(3)-IV, aph(4)-Ia, aadA1, aadA2, cmlA, floR, tet(M), fosA3,bla*$_{CTX-M-14}$, *sul2, sul3*, and *mph(A). mcr-1* was also present on this plasmid and all resistance genes were surrounded by insertion sequences and transposons (Fig. 2C). Notably, *mcr-1*-bearing pTBMCR421 (NZ_CP034788) from chicken *E. coli* in China showed the highest similarity (100%) to pSQB-1-1 (Fig. 2C).

4. Discussion

mcr-positive bacteria have been found in fresh vegetables in Portugal [4], Switzerland [6] and China [7]; however, the number of samples and geographical area studied in the three previous reports were limited. In the current study, 19 of the 528 vegetable samples (3.60%) from 23 cities of 9 provinces in China carried *mcr-1*, which was significantly higher than that (0.98%) in vegetables in Guangzhou [7] and that (1.35%) in vegetables in Portugal [4]. This indicated that the contamination rate of *mcr*-positive Enterobacteriaceae among vegetables in China might have been increasing in recent years. Besides lettuce and tomato, which are often found to carry *mcr* [4,7], another eight types of fresh vegetables, including green pepper (7.7%) and cucumber (3.1%), also harbored *mcr*-positive isolates, indicating that these types of vegetables should attract more attention because they are often consumed raw.

Besides *E. coli* reported in vegetables [4,6,7], one *mcr-1*-positive *E. cloacae* isolate from green pepper was also identified in this study. *E. cloacae* is a prevalent clinical pathogen around the world [14,15], and *mcr-1* has been reported in clinical *E. cloacae* isolates in China [16] and France [17]. Recently, an *E. cloacae* isolate from Jinghang Grand Canal, China, was found to carry *mcr-1* [18]. These results indicated *E. cloacae* in vegetables might be from irrigation water and should be monitored in the future. Sixteen MLST types were identified in the 23 *E. coli* isolates, indicating the genetic diversity of these strains. The most prevalent STs in this study were ST744 and ST224, which were also prevalent in *mcr-1*-positive *E. coli* from animals [11]; this indicates the MCR-1-producers in vegetables might originate from animals through manure fertilization. Isolate RL76 belonged to ST10, a prevalent ST of clinical isolates harboring *mcr-1* in China [19]. More than one MCR-1-producer of different STs was found in three vegetable samples, indicating that fresh vegetables were important reservoirs of MCR-1-producers and the sampling method of one isolate from each sample would miss out such isolates. Fourteen isolates also carried *bla*$_{CTX-M}$ in this study, further proving the tight association of *mcr-1* and *bla*$_{CTX-M}$, as found in *E. coli* from other origins [11].

Among the 24 isolates in this study, 17 *mcr-1*-positive transconjugants were obtained, similar to the rate in *E. coli* from vegetables [7], but significantly higher than that (35/109) in *E. coli* from retail food [20]. The location of *mcr-1* on the chromosome of SG-2-1 resulted in its failure in conjugation and the non-transference of the remaining six plasmids might be due to the limitation of the conjugation method. Our results showed that the dissemination of *mcr-1* among Enterobacteriaceae in fresh vegetables was mainly mediated by IncX4 and IncI2 conjugative plasmids, and both types of plasmids could confer the spread of *mcr-1* in Enterobacteriaceae from humans and animals [2]. In this study, the same type plasmids shared highly similar RFLP profiles, although they were from isolates of different ST types, different types of fresh vegetables or different cities, which indicates the horizontal transfer of similar plasmids is responsible for the transmission of *mcr-1* in vegetables in China. Notably, pTO89 was 99% similar to other IncX4 plasmids from animals and clinical patients in various countries, and there were similar results for IncI2 plasmid pTCTX148 (Fig. 2). We also obtained IncHI2/ST3 *mcr-1*-bearing pSQB-1-1 carrying 14 resistance genes, which showed 100% similarity to that from *E. coli* of chicken in China. All these findings indicated similar IncX4, IncI2 and IncHI2/ST3 plasmids have disseminated *mcr-1* around the world and the plasmids in vegetables can be also spread to clinical isolates, representing a threat to human health.

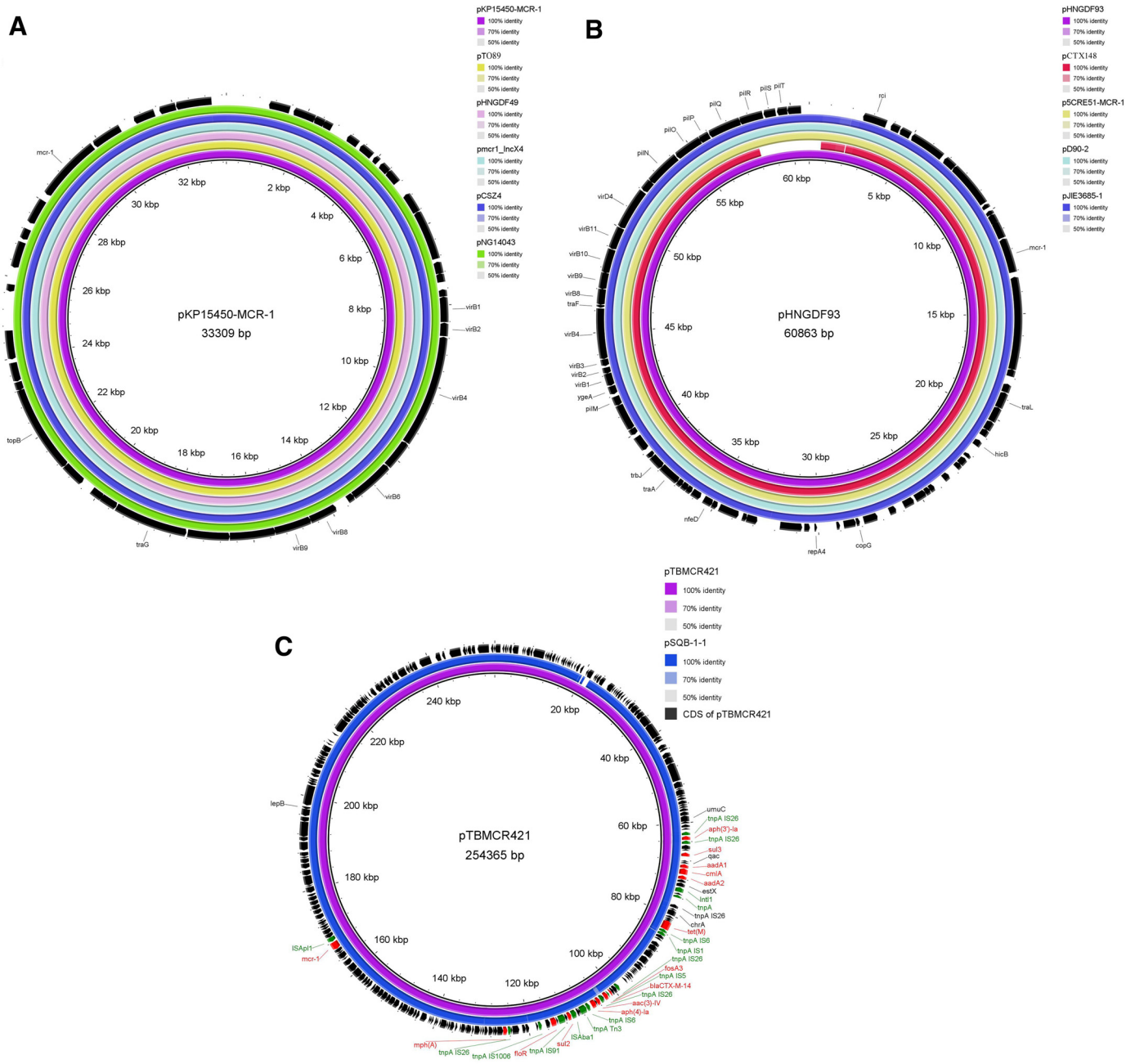

Fig. 2. Sequence alignment of IncX4, IncI2, and IncHI2/ST3 type *mcr-1*-bearing plasmids. (A) The plasmid pKP15450-MCR-1 (MH715959) from clinical *Klebsiella pneumoniae* in Taiwan was used as a reference to compare with the IncX4 plasmids. The outer circle with black arrows signifies annotation of the reference sequence. The yellow, light purple, light blue, dark blue and green ring represents pT-89 (SRMK00000000) from *E. coli* of tomato in this study, pHNGDF49 (MF978387) from *E. coli* of fish in China, pmcr1_IncX4 (KU761327) from *Klebsiella pneumoniae* of clinical peritoneal fluid in China, pCSZ4 (KX711706) from *E. coli* of pork in China and pNG14043 (KY120364) from clinical *Salmonella Typhimurium* in Taiwan, respectively. (B) The plasmid pHNGDF93 (MF978388) from fish *E. coli* in China was used as a reference to compare with the IncI2 plasmids. The purple, red, yellow, light blue, and dark blue ring represents the reference plasmid, pT-CTX148 (MK754161) from *E. coli* of tomato in this study, p5CRE51-MCR-1 (CP021176) from clinical *E. coli* in Taiwan, pD90-2 (CP022452) from *Salmonella Indiana* of chicken in China and pJIE3685-1 (KY795978) from clinical *E. coli* in Australia, respectively. (C) The plasmid pTBMCR421 (NZ_CP034788) from chicken *E. coli* in China was used as a reference to compare with the IncHI2/ST3 plasmids. The purple and blue ring represents the reference plasmid and pSQB-1-1 (SRML00000000) from *E. coli* of romaine lettuce in this study, respectively. The outer circle with black arrows shows the annotation of plasmid pTBMCR421 and the resistance genes are marked with red.

In summary, we reported a high prevalence of *mcr-1* in fresh vegetables in China, and the dissemination of *mcr-1* was mediated by similar IncX4 or IncI2 plasmids. The sequenced prevalent IncX4 plasmid and IncI2 plasmid from tomato in this study were similar to the relevant plasmids from animals and clinical isolates in various countries. IncHI2/ST3 *mcr-1*-bearing plasmid similar to the plasmid carrying 14 resistance genes from *E. coli* of chicken in China was also observed in romaine lettuce. Multiple *mcr-1*-positive *E. coli* isolates with different STs were found in the same sample in this study and *E. cloacae* isolate carrying *mcr-1* was also identified in green pepper. The MCR-1-producers in ready-to eat vegetables may pose a huge threat to public health and further investigations are required to ensure food safety.

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Competing Interests

None declared.

Ethical Approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.04.013.

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;16:161–8.
[2] Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance. Trends Microbiol 2018;26:794–808.
[3] Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect 2018;7:122.
[4] Jones-Dias D, Manageiro V, Ferreira E, Barreiro P, Vieira L, Moura IB, et al. Architecture of Class 1, 2, and 3 integrons from Gram negative bacteria recovered among fruits and vegetables. Front Microbiol 2016;7:1400.
[5] Jung Y, Jang H, Matthews KR. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb Biotechnol 2014;7:517–27.
[6] Zurfuh K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in river water and imported vegetable samples in Switzerland. Antimicrob Agents Chemother 2016;60:2594–5.
[7] Luo J, Yao X, Lv L, Doi Y, Huang X, Huang S, et al. Emergence of *mcr-1* in *Raoultella ornithinolytica* and *Escherichia coli* isolates from retail vegetables in China. Antimicrob Agents Chemother 2017;61.
[8] Liu BT, Zhang XY, Wan SW, Hao JJ, Jiang RD, Song FJ. Characteristics of carbapenem-resistant *Enterobacteriaceae* in ready-to-eat vegetables in China. Front Microbiol 2018;9:1147.
[9] Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol 2006;60:1136–51.
[10] , Wayne, PA: CLSI; 2015. Twenty-Fifth Informational Supplement. CLSI document M100-S25.
[11] Liu BT, Song FJ, Zou M. Characterization of highly prevalent plasmids coharboring *mcr-1*, *oqxAB*, and bla_{CTX-M} and plasmids harboring *oqxAB* and bla_{CTX-M} in *Escherichia coli* isolates from food-producing animals in China. Microb Drug Resist 2019;25:108–19.
[12] Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 2014;42:D206–14.
[13] Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 2011;12:402.
[14] Fernandez J, Montero I, Martinez O, Fleites A, Poirel L, Nordmann P, et al. Dissemination of multiresistant *Enterobacter cloacae* isolates producing OXA-48 and CTX-M-15 in a Spanish hospital. Int J Antimicrob Agents 2015;46:469–474.
[15] Liu C, Qin S, Xu H, Xu L, Zhao D, Liu X, et al. New Delhi metallo-beta-lactamase 1(NDM-1), the dominant carbapenemase detected in carbapenem-resistant *Enterobacter cloacae* from Henan Province, China. PLoS One 2015;10:e0135044.
[16] Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. Emergence of plasmid-mediated *mcr-1* gene in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. Antimicrob Agents Chemother 2016;60:3862–3.
[17] Baron S, Bardet L, Dubourg G, Fichaux M, Rolain JM. *mcr-1* plasmid-mediated colistin resistance gene detection in an *Enterobacter cloacae* clinical isolate in France. J Glob Antimicrob Resist 2017;10:35–6.
[18] Zhou HW, Zhang T, Ma JH, Fang Y, Wang HY, Huang ZX, et al. Occurrence of Plasmid- and chromosome-carried *mcr-1* in water-borne *Enterobacteriaceae* in China. Antimicrob Agents Chemother 2017;61.
[19] Shen Y, Wu Z, Wang Y, Zhang R, Zhou HW, Wang S, et al. Heterogeneous and flexible transmission of *mcr-1* in hospital-associated *Escherichia coli*. MBio 2018;9.
[20] Liu X, Li R, Zheng Z, Chen K, Xie M, Chan EW, et al. Molecular characterization of *Escherichia coli* isolates carrying *mcr-1*, *fosA3*, and extended-spectrum-beta-lactamase genes from food samples in China. Antimicrob Agents Chemother 2017;61.