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Genetic environment of colistin resistance genes *mcr-1* and *mcr-3* in *Escherichia coli* from one pig farm in China

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ABSTRACT

The aim of this study was to assess the presence of mobile colistin resistance in bacteria isolated from the swine production environment and to analyze the genomic environment of the new colistin resistance gene *mcr-3*. Anal swabs and environmental samples were collected from a commercial pig farm. Direct sample testing (DST) for *mcr* genes and isolation of colistin-resistant isolates was performed. The *mcr-3*-positive isolates were subjected to whole genome sequencing (WGS). Transferability and genomic location analyses of *mcr-3* gene were performed using conjugation and S1 nuclease-PFGE with Southern blotting assays, respectively. The antimicrobial susceptibility profiles of the *mcr*-carrying isolates were determined using the agar dilution method. A total of 65 samples were collected. The DST rates of *mcr-1* (64.6%, 42/65) and *mcr-3* (40.0%, 26/65) were considerably higher than the rates of *mcr-1*-positive *E. coli* (49.2%, 32/65) and *mcr-3*-positive *E. coli* (7.7%, 5/65) isolated from these samples, respectively. The five *mcr-3*-positive isolates were derived from different sources (pig, fly and soil) and four of the five isolates were also positive for *mcr-1*. The *mcr-3* genes were located on IncP-1 plasmids in three isolates or IncHI2 plasmids in two isolates. Several mobile elements, including IS*4321*, ΔTn*As2* or IS*Kpn40*, were identified in the flanking regions of *mcr-3* in the *E. coli* isolates. In conclusion, the mobile colistin resistance genes *mcr-1* and *mcr-3* are prevalent in the monitored pig farm and its surrounding environment. Due to their location on broad-host range IncP-1 plasmids and their proximity to different IS sequences, *mcr-3* gene might have excellent opportunities for transmission.

1. Introduction

The emergence of the mobile colistin resistance genes *mcr-1* and *mcr-2* has attracted significant attention worldwide (Liu et al., 2016; Xavier et al., 2016). Recently, six novel plasmid-mediated colistin resistance genes, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7* and *mcr-8* were identified mainly in Enterobacteriaceae in China or European countries (Yin et al., 2017; Partridge et al., 2018; Carattoli et al., 2017; Borowiak et al., 2017; AbuOun et al., 2018; Yang et al., 2018; Wang et al., 2018). Among these *mcr* genes, *mcr-1* and *mcr-3* have spread around the world (Shen et al., 2018), whereas *mcr-2* has only been detected in European countries (Garcia-Graells et al., 2018). By searching the GenBank database, *mcr-3* and *mcr-3*-related genes, whose deduced amino acids exhibited > 70%-100% identity to MCR-3, were also found in *E. coli*, *Klebsiella pneumoniae*, *Salmonella enterica* serovar Typhimurium and 10 different *Aeromonas* species from humans, animals, and aquatic environment in at least 12 countries across four continents. Soon after the report of *mcr-3* in *E. coli*, it was also found in *Salmonella* isolates from human infections in Denmark (Litrup et al., 2017), and two variants of the *mcr-3* gene, *mcr-3.2* and *mcr-3.3*, have been identified in *E. coli* of cattle in Spain and chicken meat in China (Hernandez et al., 2017; Ling et al., 2017), respectively. Moreover, several novel *mcr-3* gene variants were identified in *Aeromonas* isolates from fish, turkey, chicken meat and river water (Eichhorn et al., 2018; Shen et al., 2018). This observation indicated that this type of mobile colistin resistance gene had already been disseminated globally (Yin et al., 2017).

In this study, we did an extensive sampling strategy from one commercial pig farm in Henan province, China, to determine the prevalence of *mcr* genes and *mcr*-carrying isolates within this farm. Isolates carrying both, *mcr-3* and *mcr-1* genes, were further characterized.

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Table 1
DST of *mcr* genes and isolation of *mcr*-carrying isolates from the pig farm.

Origin of samples	*mcr-1*		*mcr-3*	
	DST/samples (% positive samples)	Isolates/samples (% positive isolates)	DST/samples (% positive samples)	Isolates/samples (% positive isolates)
Cloacal swabs	30/30 (100%)	26/30 (86.7%)	18/30 (60.0%)	3/30 (10.0%)
Soil samples	2/8 (25.0%)	0	1/8 (12.5%)	1/8 (12.5%)
Sewage samples	7/7 (100%)	5/7 (71.4%)	6/7 (85.7%)	0
Fly samples	3/20 (15.0%)	1/20 (5.0%)	1/20 (5.0%)	1/20 (5.0%)
Σ	42/65 (64.6%)	32/65 (49.2%)	26/65 (40.0%)	5/65 (7.7%)

2. Materials and methods

2.1. Sample collection and isolation of colistin-resistant strains from a pig farm

We carried out an extensive sampling and screening strategy for colistin resistance genes in various samples collected from a commercial pig farm with an annual output of 3000 pigs in Henan province, China, in December 2016. Colistin has been used in this farm for the purpose of both, prevention and treatment of diarrheal diseases in pigs during the past three years. A total of 30 anal swabs from fattening pigs and 35 environmental samples including eight soil samples, seven sewage samples and 20 flies were collected. Environmental samples were pretreated as previously described (Zhao et al., 2016; Wang et al., 2017a). Then, all 65 samples were inoculated on MacConkey plates with 2 mg/L colistin. At least three suspected enterobacterial isolates per sample were selected, purified and tested by PCR for the presence of *mcr* genes. Species identification was performed with both, 16S rDNA sequencing and MALDI-TOF MS (Bruker Daltonik Gmbh, Bremen, Germany).

2.2. Detection of mcr genes and pulsed-field gel electrophoresis (PFGE) analysis

In our study, we investigated the presence of all *mcr* gene classes known to date, *mcr-1* to *mcr-8*, using the primer pairs previously described (Wang et al., 2018). At the same time, direct sample testing (DST) for the eight *mcr* genes was performed as described earlier (Wang et al., 2017a). All the *mcr*-positive isolates were subjected to XbaI-PFGE before further analysis.

2.3. Genomic DNA sequencing and analysis

To further analyze the *mcr-3*-positive isolates, total DNA was extracted using a Wizard genomic DNA purification kit (Promega, Beijing, China) and used for whole genome sequencing (WGS) on an Illumina Hiseq 2500 platform (Berry Genomics Company, Beijing, China). A draft assembly of the sequences was conducted using the CLC Genomics Workbench 9 (CLC Bio-Qiagen, Aarhus, Denmark), and the annotation of the contigs was further conducted by using RAST. Detection of the multi locus sequence type (MLST), plasmid replicon types and resistance genes was performed in silico using online tools.

2.4. Molecular methods

The *mcr-3*-positive isolates were subjected to S1 nuclease-PFGE and Southern blot hybridization with both *mcr-1* and *mcr-3* probes. The *Salmonella* Braenderup H9812 digested with XbaI was used as the molecular weight marker. To investigate the transferability of plasmid-borne *mcr* genes, conjugation by filter mating was performed using the *mcr-3*-positive isolates as donors and *E. coli* J53 AzR as the recipient. Transconjugants were selected on Brain Heart Infusion agar supplemented with 2 mg/L colistin and 200 mg/L sodium azide, and confirmed further by PCR detection and PFGE analysis. Antimicrobial susceptibility of all *mcr-3*-positive isolates and the corresponding J53 transconjugants was determined by agar dilution according to CLSI recommendations (CLSI, 2016). The MIC results were interpreted according to the CLSI clinical breakpoints except for colistin, which was interpreted according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing breakpoints. The reference strain *E. coli* ATCC 25922 served as a quality control strain in MIC determinations.

3. Results and discussion

3.1. Isolation of mcr-positive isolates

In total, 33 *mcr-1*- and/or *mcr-3*-positive isolates were identified. They included 28 isolates carrying only *mcr-1*, four isolates carrying *mcr-1* and *mcr-3*, and a single isolate carrying only *mcr-3*. All samples were negative for the other six *mcr* genes. The 32 *mcr-1*-positive isolates comprised one *Klebsiella pneumoniae* from an anal swab, one *Kluyvera ascorbata* and one *Enterobacter cloacae*, both from sewage samples. All remaining *mcr-1*-positive isolates were *E. coli* from anal swabs (n = 25) and environmental samples (n = 4). Among these latter isolates, four *mcr-1*- and *mcr-3*-positive *E. coli* isolates were identified (three from pigs and one from a fly). The *mcr-3* only carrying *E. coli* isolate originated from a soil sample. The DST rates of *mcr-1* (64.6%, 42/65) and *mcr-3* (40.0%, 26/65) were higher than the rates for *mcr-1*-positive (49.2%, 32/65) and/or *mcr-3*-positive isolates (7.7%, 5/65) obtained from the same samples, respectively (Table 1). This observation implies that the extent of dissemination of these two mobile colistin resistance genes among pig farms might be underestimated as most of the published studies or monitoring efforts only focus on the screening of bacteria and not on DST (Li et al., 2015; Munk et al., 2018). Interestingly, the DST rate for *mcr-3* in sewage (85.7%, 6/7) is significantly higher than the *mcr-3*-positive isolates (0%, 0/7) from the same source, suggesting that species other than Enterobacteriaceae, for instance, the water-borne *Aeromonas* spp. (Eichhorn et al., 2017; Yin et al., 2017), which is unculturable on MacConkey plates used in this study, may harbor *mcr-3*.

The five *mcr-3*-positive isolates were derived from three different sample sources: WZR5, WZR10, and WZR12 from pigs, WZR78 from a fly and WZR481 from soil. All of them, except for WZR481, were also positive for the *mcr-1* gene. All *mcr-1* genes in four *mcr-3*-positive strains were identical to the original *mcr-1.1* gene (Partridge et al., 2018) according to our blast result. All five *mcr-3*-positive isolates showed individual PFGE patterns and four ST types, including ST1437 for two isolates, ST10, ST101 and ST7601 for one isolate each (Table 2). *E. coli* of both, ST10 and ST101, have been reported among *mcr-1*-harbouring isolates of human, animal and environmental origin (Wang et al., 2017b; Zhou et al., 2017; Yang et al., 2017). The *mcr-3* gene in two (WZR5 and WZR10) and three (WZR12, WZR78, and WZR481) isolates presented 100% and 99.8% nucleotide sequence identity to the original *mcr-3.1* gene in plasmid pWJ1 from *E. coli* WJ1 (Yin et al., 2017), respectively. The *mcr-3* gene variant in the latter three isolates encodes a phosphoethanolamine transferase enzyme that differed from MCR-3 by three amino acid substitutions: Met23 to Val, Ala457 to Glu and Thr488 to Ile. This *mcr-3* gene variant was designated as *mcr-3.5* by

Table 2
Background information on the five isolates and MIC profiles of the *mcr-3* positive isolates and their transconjugants.

Isolate and source	MLST	Inc groups and sizes (kb) of *mcr*-carrying plasmids		MIC of original strains and its transconjugants (mg/L)[a]											
		mcr-3.1 or *mcr-3.5*	*mcr-1.1*	COL	PMB	GEN	FFC	AMP	TET	CIP	IMI	AZT	CRO	AMC	SXT
WZR5, pig	ST1437	*mcr-3.1*, IncHI2, ~250	IncX4, ~40	4	8	> 32	> 128	> 128	> 128	> 4	0.25	0.25	> 16	16/8	2/38
J53-5-mcr-1			IncX4, ~40	4	8	1	8	4	4	0.008	0.25	0.25	0.06	8/4	0.25/4.75
WZR10, pig	ST1437	*mcr-3.1*, IncHI2, ~250	IncX4, ~40	4	8	> 32	> 128	> 128	> 128	> 4	0.25	0.25	> 16	16/8	2/38
J53-10-mcr-1			IncX4, ~40	4	8	1	8	4	8	0.008	0.25	0.25	0.06	8/4	0.25/4.75
WZR12, pig	ST10	*mcr-3.5*, IncP-1, ~250	IncX4, ~40	4	8	> 32	> 128	> 128	> 128	> 4	0.25	0.25	> 16	8/4	2/38
J53-12-mcr-1		–	IncX4, ~40	4	8	1	8	4	8	0.008	0.25	0.25	0.06	8/4	0.25/4.75
J53-12-mcr-1/3		*mcr-3.5*, IncP-1, ~250	IncX4, ~40	4	8	1	8	2	8	0.008	0.25	0.25	0.06	8/4	0.25/4.75
WZR78, fly	ST101	*mcr-3.5*, IncP-1, ~220	P0111, > 90	4	8	2	> 128	> 128	> 128	> 4	0.25	0.25	2	8/4	2/38
J53-78-mcr-3		*mcr-3.5*, IncP-1, ~220	–	4	8	1	8	4	4	0.008	0.25	0.25	0.06	4/2	0.25/4.75
WZR481, soil	ST7601	*mcr-3.5*, IncP-1, ~220	–	4	8	1	> 128	8	64	0.25	0.25	0.25	0.06	4/2	2/38
J53-481-mcr-3		*mcr-3.5*, IncP-1, ~220	–	4	8	1	8	2	8	0.008	0.25	0.25	0.06	4/2	0.25/4.75
J53, recipient	–	–	–	0.5	1	1	8	4	4	0.008	0.25	0.25	0.06	4/2	0.25/4.75

[a] COL: Colistin, PMB: Polymyxin B, GEN: gentamicin, FFC: florfenicol, AMP: ampicillin, TET: tetracycline, CIP: ciprofloxacin, IMI: imipenem, AZT: aztreonam, CRO: Ceftriaxone, AMC: amoxicillin-clavulanic acid, SXT: trimethoprim/sulfamethoxazole.

Liu with the GenBank accession no: MF489760.1 (Partridge et al., 2018).

3.2. Plasmids carrying mcr-1 and mcr-3

S1 nuclease-PFGE and Southern blot hybridization revealed that the *mcr-3.1* or *mcr-3.5* genes were located on plasmids ranging in size from 220 to 250 kb (Table 2 and Fig. S1). Transconjugants carrying only *mcr-3.5* gene were obtained when WZR78 and WZR481 were used as donors, and transconjugants harboring only *mcr-1.1* were detected when WZR5 and WZR10 served as donors. The transconjugants carrying both *mcr-3.5* and *mcr-1.1*, or only *mcr-1.1* were obtained when WZR12 was used as donor. The three conjugative plasmids carrying *mcr-3.5* belonged to the incompatibility group IncP-1, a broad-host-range plasmid type which is widely spread in Gram-negative pathogens, including *Pseudomonas aeruginosa* and *Klebsiella aerogenes*, and is also present in various environments (Adamczyk and Jagura-Burdzy, 2003). All original isolates and transconjugants exhibited identical MICs of colistin (4 mg/L) and polymyxin B (8 mg/L), but all transconjugants were susceptible to all other tested antimicrobial agents. However, all original isolates were classified as resistant or intermediate to ciprofloxacin, tetracycline, and florfenicol, and presented elevated MICs to β-lactams as compared with the transconjugants (Table 2).

3.3. Genomic environment of mcr-1

Studies of the genetic environment of *mcr-1.1* from four *mcr-3*-positive isolates, WZR5, WZR10, WZR12, WZR78, identified IS*Apl1*-*mcr-1.1*on the p0111-type plasmid pZR78_mcr1. The 91,281 bp *mcr-1.1*-positive contig in pZR78_mcr1 (GenBank accession no. MF455226) showed 99.9% nucleotide sequence identity to the *mcr-1.1*-carrying plasmid pHYEC7-mcr1 (KX518745.1) that was described as phage-like plasmid and may also play an important role in the dissemination of the *mcr-1.1* gene (Li et al., 2017). The flanking sequences of *mcr-1* in the other two IncX4 plasmids, pZR5_mcr1 and pZR10_mcr1, showed 99.9% nucleotide sequence identity to pECMCR-1011 (KX570748.1) from *E. coli* of pig origin in which the IS*Apl1* was absent. The contig carrying *mcr-1.1* in WZR12 was about 3 kb and those in WZR5 and WZR10 were about 9 kb in size. No further information could be obtained through WGS analysis.

3.4. Genomic environment of mcr-3

Two types of *mcr-3*-carring plasmids, including IncP-1 plasmids carrying *mcr-3.5* and IncHI2 plasmids carrying *mcr-3.1*, were identified in the five *mcr-3*-positive isolates by WGS analysis. Apart from these plasmids, other incompatibility (Inc) groups were also detected in the respective isolates, including IncX1, IncX4 carrying *mcr-1.1*, as well as IncF type plasmids. In three IncP-1 plasmids, the *mcr-3.5*-carrying contigs from pZR12_mcr3 (53.7 kb, GenBank accession no. MF455227), pZR78_mcr3 (27.3 kb) and pZR481_mcr3 (49.9 kb) showed > 99.9% nucleotide sequence identity to the corresponding region of the 57.3 kb *mcr-1*-positive plasmid pMCR_1511 of *K. pneumoniae* from a Chinese hospital sewage sample (KX377410) with the coverage of 90.4%–97.5% (Fig. 1). The homologous regions between the *mcr-3.5*-carrying contigs and the *mcr-1.1*-carrying plasmid encoded mainly the genes for conjugative gene transfer. A core segment Δ*Tn*A*s2-mcr-3-dgkA*, the conjugative transfer genes *trb* and *tra*, and the toxin-antitoxin genes *higB-higA* were presented in the three *mcr-3.5*-carrying plasmids. In plasmids pZR78_mcr3 and pZR12_mcr3, an additional insertion sequence IS*4321*, originating from *Enterobacter aerogenes* (meanwhile reclassified as *K. aerogenes*) (Thorsted et al., 1998), was present immediately downstream of the Δ*Tn*A*s2-mcr-3-dgkA* segment (Fig. 2).

The two IncHI2 plasmids pZR5_mcr3 and pZR10_mcr3 carrying the *mcr-3.1* gene (GenBank accession no. MF461273) had a similar backbone and *mcr-3.1*-carrying region when compared with the original *mcr-3.1*-carrying plasmid pWJ1 (KY924928, Figs. 3 and 4). An intact *nimC/nimA* gene, which was located between Δ*Tn*A*s2* and Δ*ble* in the $bla_{CTX\text{-}M\text{-}55}$-carrying plasmid pSCE516-3 from *E. coli* (KX023260), was interrupted by a 3564-bp fragment harboring *mcr-3-dgkA* and a novel IS element, assigned as IS*Kpn40* by ISfinder, in plasmids pZR5_mcr3 and pZR10_mcr3 (Fig. 4). IS*Kpn40* is 1213 bp in size and contains two ORFs of 315 bp and 981 bp, respectively. The intact first ORF (104 aa) and the partial sequence (273/326 aa, 83.7%) of the second ORF showed 100% and 99.9% identity to a transpose from *Aeromonas* spp. (WP_076577685) and an integrase from *Aeromonas caviae* (KGY67519.1), respectively. This observation suggested that this IS element might originate from *Aeromonas* spp. and play a role in the spread of the *mcr-3.1* gene between aeromonads and *Enterobacteriaceae*. Four base pair direct repeats (5'-CACC-3') were observed immediately upstream and downstream of this *mcr-3-dgkA*-IS*Kpn40* segment in both plasmids pZR5_mcr3 and pZR10_mcr3 (Fig. 4), suggesting that this segment was inserted into the *nimC/nimA* gene.

4. Conclusion

Our study revealed that only the *mcr-1* and *mcr-3* genes spread widely in pig farms. The co-existence and co-transfer of two plasmids carrying different *mcr* genes further facilitates the dissemination of *mcr* genes. Unlike most *mcr-1*-carrying plasmids which belong mainly to narrow-host range plasmid types, such as IncI2 and IncX4 (Sun et al., 2017; Chen et al., 2013), three conjugative *mcr-3*-carrying plasmids

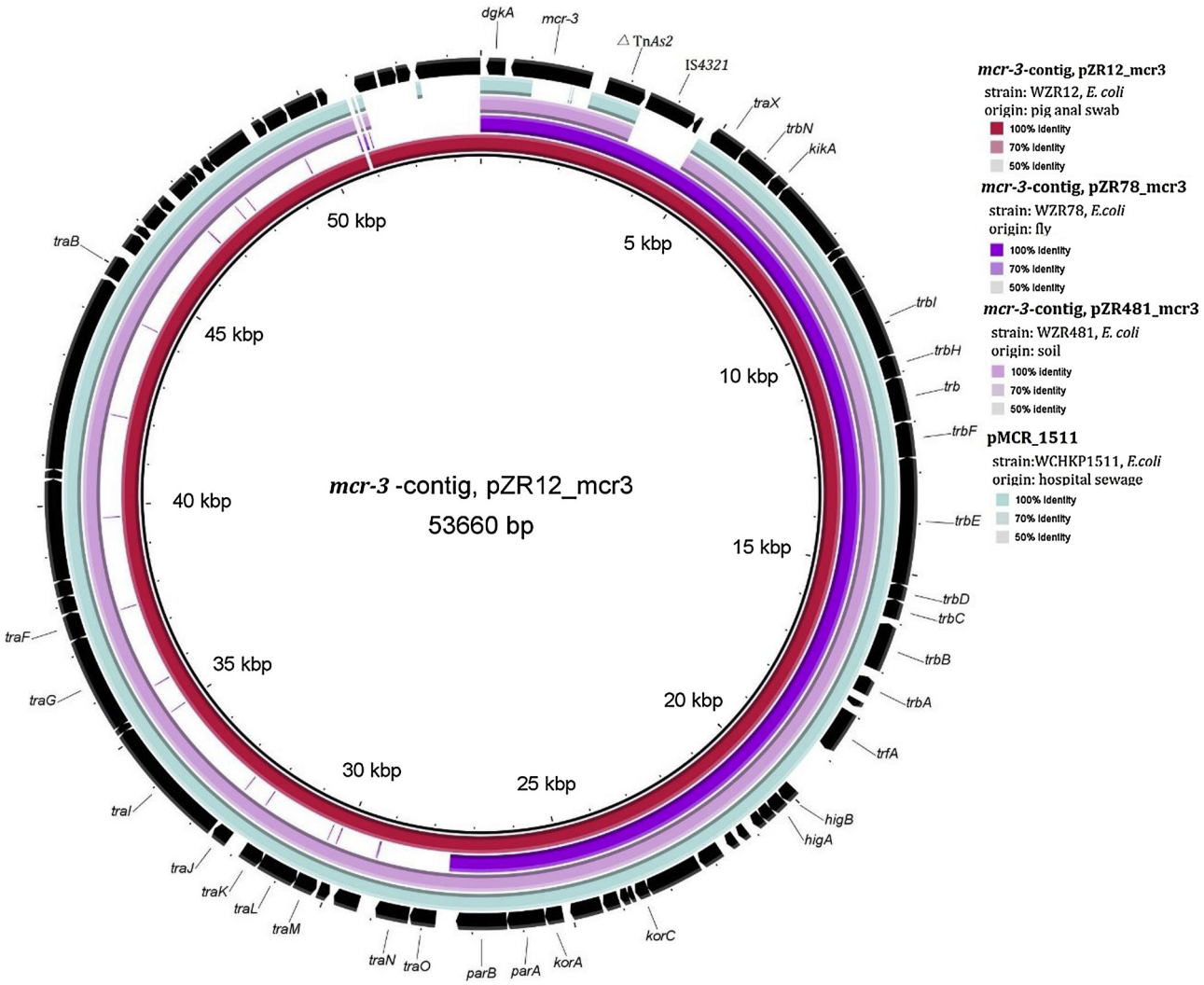

Fig. 1. Comparative analysis of *mcr-3*-carrying contigs obtained from plasmids pZR12_mcr3, pZR78_mcr3, and pZR481_mcr3 with the corresponding region of the *mcr-1*-positive plasmid pMCR_1511 from *E. coli* isolates using the BLAST Ring Image Generator. The concentric rings display similarity between the reference sequence in the inner ring and the other sequences in the outer rings. The varying color levels indicate a BLAST result with matched degree of shared regions, as shown to the right of the ring.

belonged to the IncP-1 group, which represents broad-host range plasmids identified in various environments (Heuer and Smalla, 2012). This might cause a wider dissemination and a faster spread of *mcr-3* than *mcr-1*. In contrast to the relatively conserved flanking regions of the *mcr-1* gene, in which only the IS*Apl1* element plays a vital role in the spread of *mcr-1* (Snesrud et al., 2017, 2016b), several transposases and IS elements, including IS*4321*, ΔTn*As2* or the novel IS*Kpn40*, were identified in the flanking regions of *mcr-3* in all *E. coli* isolates in this study. All these differences imply that the new mobile colistin resistance gene *mcr-3* might have a stronger capacity of transmission than *mcr-1*. However, whether all of these IS elements and transposons or only specific ones play a role in the mobility of *mcr-3* gene among

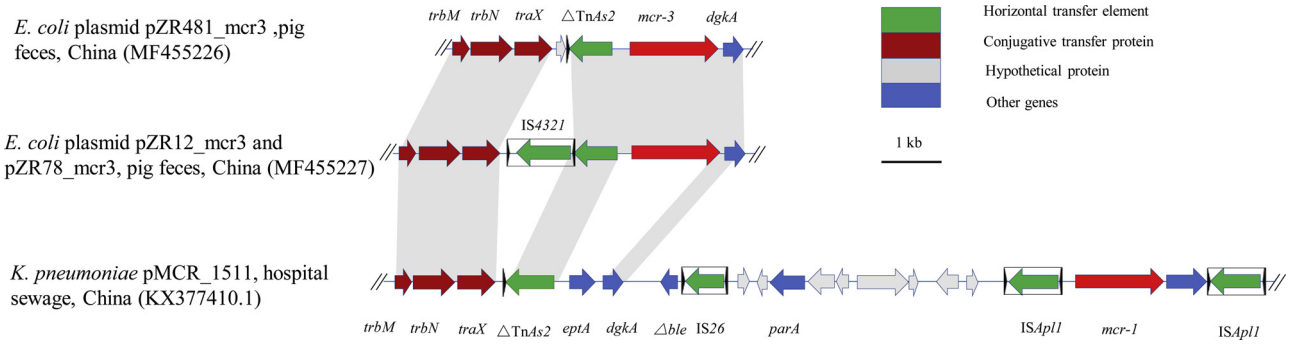

Fig. 2. Comparison of genetic environment of *mcr-3* gene in pZR12_mcr3, pZR78_mcr3, and pZR481_mcr3 and pMCR1511. Arrows indicate the positions and directions of the genes, Δ indicates a truncated gene. Regions with > 99% homology are indicated by grey shading, with homology > 85% shown by a lighter grey shading.

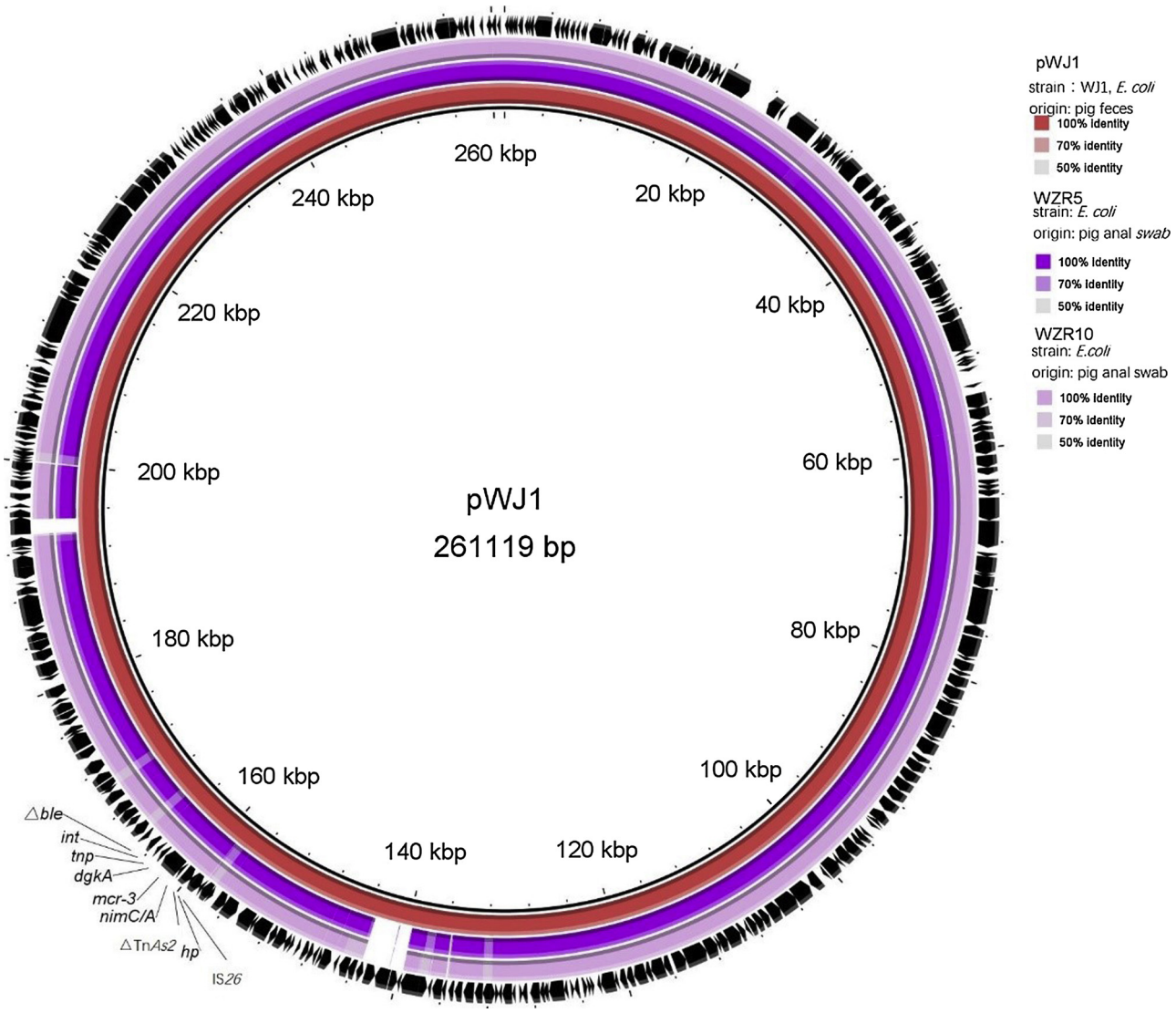

Fig. 3. Comparative analysis of the *mcr-3*-carrying plasmids pZR5_mcr3 and pZR10_mcr3 with the original *mcr-3* positive plasmid pWJ1 from *E. coli* isolate using the BLAST Ring Image Generator.

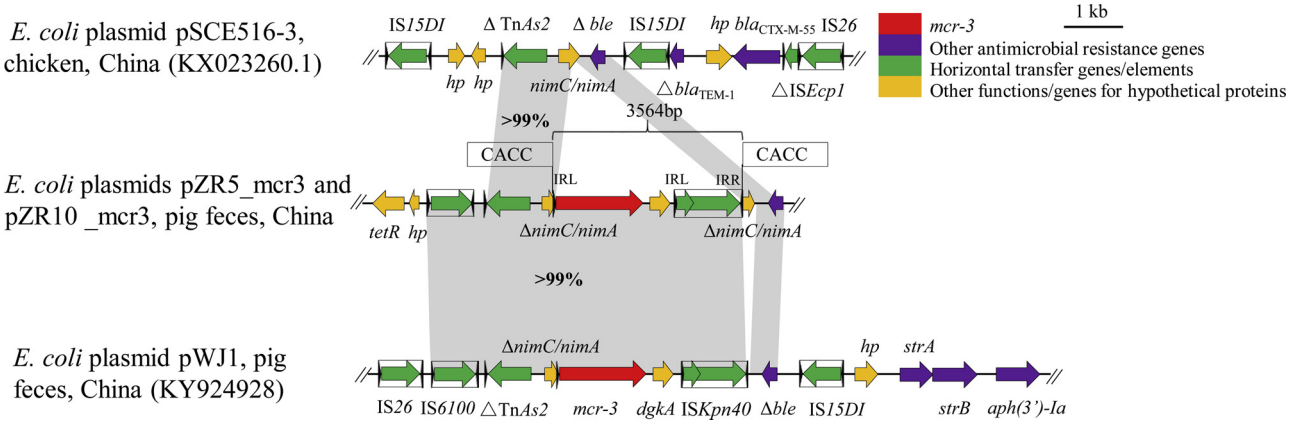

Fig. 4. Comparison of the genetic environment of *mcr-3* gene in pZR5_mcr3 and pZR10_mcr3 with pWJ1 and pSCE516-3. Arrows indicate the positions and directions of the genes, Δ indicates a truncated gene. Regions with > 99% homology are indicated by grey shading. Inverted repeat nucleotide sequences (IRL – left IR; IRR – right IR) of IS are marked by triangles.

different species or genera of bacteria remains to be elucidated.

Nucleotide sequence accession numbers: The sequence of pZR78_mcr1, pZR12_mcr3, pZR10_mcr3 has been deposited into GenBank under accession numbers: MF455226, MF455227, MF461273.

Conflict of interest statement

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.01.011.

References

AbuOun, M., Stubberfield, E.J., Duggett, N.A., Kirchner, M., Dormer, L., Nunez-Garcia, J., Randall, L.P., Lemma, F., Crook, D.W., Teale, C., Smith, R.P., Anjum, M.F., 2018. *mcr-1* and *mcr-2* (*mcr-6.1*) variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J. Antimicrob. Chemother. 73, 2904.

Adamczyk, M., Jagura-Burdzy, G., 2003. Spread and survival of promiscuous IncP-1 plasmids. Acta Biochim. Pol. 50, 425–453.

Borowiak, M., Fischer, J., Hammerl, J.A., Hendriksen, R.S., Szabo, I., Malorny, B., 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene,*mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J. Antimicrob. Chemother. 72, 3317–3324.

Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., Pezzotti, G., Magistrali, C.F., 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill. 22.

Chen, L., Chavda, K.D., Al, L.N., Melano, R.G., Jacobs, M.R., Bonomo, R.A., Kreiswirth, B.N., 2013. Complete nucleotide sequence of a bla_{KPC}-harboring IncI2 plasmid and its dissemination in New Jersey and New York hospitals. Antimicrob. Agents Chemother. 57, 5019–5025.

CLSI, 2016. Performance Standards for Antimicrobial Susceptibility Testing, 26th ed. Clinical and Laboratory Standards Institute, Wayne, PA CLSI supplement M100S.

Eichhorn, I., Feudi, C., Wang, Y., Kaspar, H., Fessler, A.T., Lubke-Becker, A., Michael, G.B., Shen, J., Schwarz, S., 2018. Identification of novel variants of the colistin resistance gene*mcr-3* in *Aeromonas* spp. from the national resistance monitoring programme GERM-Vet and from diagnostic submissions. J. Antimicrob. Chemother. 73, 1217–1221.

Garcia-Graells, C., De Keersmaecker, S., Vanneste, K., Pochet, B., Vermeersch, K., Roosens, N., Dierick, K., Botteldoorn, N., 2018. Detection of plasmid-mediated colistin resistance,*mcr-1* and *mcr-2* genes, in *Salmonella* spp. isolated from food at retail in Belgium from 2012 to 2015. Foodborne Pathog. Dis. 15, 114–117.

Hernandez, M., Iglesias, M.R., Rodriguez-Lazaro, D., Gallardo, A., Quijada, N., Miguela-Villoldo, P., Campos, M.J., Piriz, S., Lopez-Orozco, G., de Frutos, C., Saez, J.L., Ugarte-Ruiz, M., Dominguez, L., Quesada, A., 2017. Co-occurrence of colistin-resistance genes *mcr-1* and *mcr-3* among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. Euro Surveill. 22.

Heuer, H., Smalla, K., 2012. Plasmids foster diversification and adaptation of bacterial populations in soil. FEMS Microbiol. Rev. 36, 1083–1104.

Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J.M., Zhang, T., 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J. 9, 2490–2502.

Li, R., Xie, M., Lv, J., Wai-Chi, C.E., Chen, S., 2017. Complete genetic analysis of plasmids carrying *mcr-1* and other resistance genes in an *Escherichia coli* isolate of animal origin. J. Antimicrob. Chemother. 72, 696–699.

Ling, Z., Yin, W., Li, H., Zhang, Q., Wang, X., Wang, Z., Ke, Y., Wang, Y., Shen, J., 2017. Chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat. Antimicrob. Agents Chemother. 61.

Litrup, E., Kiil, K., Hammerum, A.M., Roer, L., Nielsen, E.M., Torpdahl, M., 2017. Plasmid-borne colistin resistance gene *mcr-3* in *Salmonella* isolates from human infections, Denmark, 2009-17. Euro Surveill. 22.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. 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. 16, 161–168.

Munk, P., Knudsen, B.E., Lukjancenko, O., Duarte, A., Van Gompel, L., Luiken, R., Smit, L., Schmitt, H., Garcia, A.D., Hansen, R.B., Petersen, T.N., Bossers, A., Ruppe, E., Lund, O., Hald, T., Pamp, S.J., Vigre, H., Heederik, D., Wagenaar, J.A., Mevius, D., Aarestrup, F.M., 2018. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat. Microbiol. 3, 898–908.

Partridge, S.R., Di Pilato, V., Doi, Y., Feldgarden, M., Haft, D.H., Klimke, W., Kumar-Singh, S., Liu, J.H., Malhotra-Kumar, S., Prasad, A., Rossolini, G.M., Schwarz, S., Shen, J., Walsh, T., Wang, Y., Xavier, B.B., 2018. Proposal for assignment of allele numbers for mobile colistin resistance (*mcr*) genes. J. Antimicrob. Chemother. 73, 2625–2630.

Shen, Y., Xu, C., Sun, Q., Schwarz, S., Ou, Y., Yang, L., Huang, Z., Eichhorn, I., Walsh, T.R., Wang, Y., Zhang, R., Shen, J., 2018. Prevalence and genetic analysis of *mcr-3*-positive aeromonas species from humans, retail meat, and environmental water samples. Antimicrob. Agents Chemother. 62.

Snesrud, E., He, S., Chandler, M., Dekker, J.P., Hickman, A.B., McGann, P., Dyda, F., 2016. A model for transposition of the colistin resistance gene*mcr-1* by IS*Apl1*. Antimicrob. Agents Chemother. 60, 6973–6976.

Snesrud, E., Ong, A.C., Corey, B., Kwak, Y.I., Clifford, R., Gleeson, T., Wood, S., Whitman, T.J., Lesho, E.P., Hinkle, M., McGann, P., 2017. Analysis of serial isolates of *mcr-1*-positive *Escherichia coli* reveals a highly active IS*Apl1* transposon. Antimicrob. Agents Chemother. 61.

Sun, J., Fang, L.X., Wu, Z., Deng, H., Yang, R.S., Li, X.P., Li, S.M., Liao, X.P., Feng, Y., Liu, Y.H., 2017. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the *mcr-1* acquisition. Sci. Rep. 7, 424.

Thorsted, P.B., Macartney, D.P., Akhtar, P., Haines, A.S., Ali, N., Davidson, P., Stafford, T., Pocklington, M.J., Pansegrau, W., Wilkins, B.M., Lanka, E., Thomas, C.M., 1998. Complete sequence of the IncP beta plasmid R751: implications for evolution and organisation of the IncP backbone. J. Mol. Biol. 282, 969–990.

Wang, Y., Tian, G.B., Zhang, R., Shen, Y., Tyrrell, J.M., Huang, X., Zhou, H., Lei, L., Li, H.Y., Doi, Y., Fang, Y., Ren, H., Zhong, L.L., Shen, Z., Zeng, K.J., Wang, S., Liu, J.H., Wu, C., Walsh, T.R., Shen, J., 2017a. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect. Dis. 17, 390–399.

Wang, Y., Zhang, R., Li, J., Wu, Z., Yin, W., Schwarz, S., Tyrrell, J.M., Zheng, Y., Wang, S., Shen, Z., Liu, Z., Liu, J., Lei, L., Li, M., Zhang, Q., Wu, C., Zhang, Q., Wu, Y., Walsh, T.R., Shen, J., 2017b. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat. Microbiol. 2, 16260.

Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., Wang, Y., 2018. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerg. Microbes Infect. 7, 122.

Xavier, B.B., Lammens, C., Ruhal, R., Kumar-Singh, S., Butaye, P., Goossens, H., Malhotra-Kumar, S., 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill. 21.

Yang, Y.Q., Li, Y.X., Song, T., Yang, Y.X., Jiang, W., Zhang, A.Y., Guo, X.Y., Liu, B.H., Wang, Y.X., Lei, C.W., Xiang, R., Wang, H.N., 2017. Colistin resistance gene*mcr-1* and Its variant in *Escherichia coli* isolates from chickens in China. Antimicrob. Agents Chemother. 61.

Yang, Y.Q., Li, Y.X., Lei, C.W., Zhang, A.Y., Wang, H.N., 2018. Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. J. Antimicrob. Chemother.

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., Zhang, R., Walsh, T.R., Shen, J., Wang, Y., 2017. Novel plasmid-mediated colistin resistance gene*mcr-3* in *Escherichia coli*. MBIO 8.

Zhao, Q., Wang, Y., Wang, S., Wang, Z., Du, X.D., Jiang, H., Xia, X., Shen, Z., Ding, S., Wu, C., Zhou, B., Wu, Y., Shen, J., 2016. Prevalence and abundance of florfenicol and linezolid resistance genes in soils adjacent to swine feedlots. Sci. Rep. 6, 32192.

Zhou, H.W., Zhang, T., Ma, J.H., Fang, Y., Wang, H.Y., Huang, Z.X., Wang, Y., Wu, C., Chen, G.X., 2017. Occurrence of plasmid- and chromosome-carried *mcr-1*in waterborne enterobacteriaceae in China. Antimicrob. Agents Chemother. 61.