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# Low prevalence of colistin-resistant *Escherichia coli* from companion animals, China, 2018–2021

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## Abstract

China banned colistin as growth promoter for animals in the year of 2017. A decrease of colistin-resistant *Escherichia coli* (COREC) and *mcr-1*-positive *Escherichia coli* (MCRPEC) were observed in livestock (pigs and chickens) and humans after the ban policy. However, the prevalence of COREC among Chinese companion animals after the ban policy has not been investigated. Here, we recovered 771 *E. coli* isolates from the China Antimicrobial Resistance Surveillance Network for Pets (CARPet) surveillance system (19 provinces/municipalities) from 2018 to 2021. We identified 12 COREC from eight dogs and four cats, among which one feline and three canine isolates were MCRPEC. The prevalence of COREC and MCRPEC in pets from 2018–2021 (1.1%–2.2% and 0.8%–1.1%) were lower than those from 2012–2016 (7.1%–17.8% and 6.1%–14.3%). The phylogenetic analysis revealed that the four MCRPEC isolates displayed genetic diversity, while one canine isolate exhibited only 26 SNPs difference with one human MCRPEC isolate in the same city, suggesting the exchange of MCRPEC isolates between companion animals and humans. In three MCRPEC isolates, *mcr-1* was located on an IncI2 plasmid, which exhibited 99.5%–99.9% nucleotide sequence identity with plasmid pHNSD133-MCR from *E. coli* of chicken origin. In the remaining MCRPEC, *mcr-1* was chromosomally located flanked by intact IS*Apl1* elements forming a unit of IS*Apl1-mcr-1-pap2*-IS*Apl1*. Despite the low prevalence of COREC and MCRPEC observed in companion animals after the ban policy, the association of pet-derived MCRPEC and *mcr*-carrying plasmids with those from humans and farm animals suggest that annual surveillance of colistin resistance in bacteria of pet origin is essential.

**Keywords** Colistin, *mcr-1*, Companion animals

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## Introduction

Antimicrobial resistance has become a serious threat to public health. The emergence of multidrug-resistant (MDR) bacteria further narrowed treatment options for infections and reintroduced last-line antimicrobial agents, such as colistin, as therapeutics [1]. Since the first identification of a mobile colistin resistance gene, *mcr-1*, in 2015, this gene has been reported predominantly in colistin-resistant *Escherichia coli* (COREC) from animals, humans, and the environment in more than 60 countries [2, 3]. Although the overall detection rate of *mcr-1*-positive *Escherichia coli* (MCRPEC) in clinical samples from patients is low (0.3%–2.9%), higher MCRPEC carriages (0.6%–15.0%) have been identified in healthy humans across China from 2007 to 2016 [4–8]. Similarly, the average detection rate of MCRPEC in companion animals from 2015 to 2021 in China is 9.9%, ranging from 1.0% to 16.0% in different studies [9–14]. Moreover, phylogenic studies have implicated potential transmission of MCRPEC between companion animals and humans due to their close contact [9–11, 15].

Given the highest priority of colistin for human medicine in the WHO list, with the rising risk of the dissemination of *mcr* genes between humans and animals, China has approved the ban of colistin as a growth promoter in 2017 [16, 17]. After the implementation of the ban, the prevalence of COREC has been reported to decrease in pigs, chickens, and humans in China [18–20]. However, the prevalence of COREC and MCRPEC in companion animals had never been investigated in China after the ban. Based on the "One Health" concept, this study aims at investigating the prevalence of COREC from companion animals in pet clinics in China since the ban of colistin as a growth promoter in food-producing animals and performing a molecular characterization of canine and feline MCRPEC isolates.

## Results

### Prevalence of colistin-resistant *E. coli* in companion animals

A total of 771 *E. coli* isolates from pet clinical specimens were recovered from the CARPet system. These isolates were collected from dogs ($n=547$) and cats ($n=224$) in clinics in 19 Chinese provinces/municipalities from 2018 to 2021 (Fig. 1A). Antimicrobial susceptibility testing identified 12 COREC isolates (12/771, 1.6%, Fig. 1B), among which eight and four were derived from dogs (8/547, 1.5%, from six urinary tract samples as well as single skin and reproductive system samples) and cats (4/224, 1.8%, from two urinary tract samples as well as single skin and pleural effusion samples, Fig. 1A), respectively. All COREC isolates originated from Beijing, except for one from Shanghai (Fig. 1A). There was no remarkable alteration in the overall trends of colistin resistance rates from 2018 to 2021, with slightly higher rates in 2018 (2/92, 2.2%) and 2021 (5/238, 2.1%) than those in 2019 (3/253, 1.2%) and 2020 (2/188, 1.1%, Fig. 1B). Only four COREC isolates, three from canine urinary tract samples and one from a feline urinary tract sample, were positive for the *mcr-1.1* gene (4/771, 0.5%, M-1 to M-4), and the remaining eight COREC isolates were negative for all known *mcr* genes (C-1 to C-8). These four MCRPEC were collected in 2020 and 2021, with the detection rates being 1.1% (2/188) and 0.8% (2/238, Fig. 1B). The colistin minimal inhibitory concentration (MIC) values of the four MCRPEC and the eight *mcr*-negative COREC isolates were 4–8 μg/mL and 4–32 μg/mL, respectively (Table S1). In addition, all 12 COREC isolates showed resistance or high MIC values to ampicillin, florfenicol levofloxacin, enrofloxacin, ceftriaxone, and cefquinome (63.6%–75.0%, Table S1) and susceptibility to amikacin, meropenem, and tigecycline (75.0%–91.7%).

### Molecular epidemiology of MCRPEC

WGS and MLST analysis revealed that the four MCRPEC isolates were assigned to three known sequence types (STs). Two MCRPEC from dogs and cats in Shanghai and Beijing belonged to the same ST (ST1011), and the other two MCRPEC from dogs in Beijing belonged to ST224 and ST453, respectively (Fig. 2). The core genome-based phylogenetic analysis with Bayesian analysis of the population structure was performed to define lineages within our four pet-derived MCRPEC isolates and 161 NCBI-downloaded MCRPEC isolates from humans ($n=67$), farm animals ($n=60$), environmental sources ($n=26$), and companion animals ($n=8$) in China. A total of 11 lineages were observed among the 165 MCRPEC isolates (Fig. 2). The four pet MCRPEC isolates fell into lineage 3 (isolates M-1 and M-4), lineage 6 (isolate M-2) and lineage 7 (isolate M-3). Within the same corresponding lineages, the canine isolate M-2 exhibited high similarity (26 SNPs) to one clinical MCRPEC isolate from human drainage fluid in Beijing [21]. The corresponding dog owner had no apparent contact with the inpatients. Another canine isolate, M-3, also showed high similarity with MCRPEC isolates from companion (145 SNPs) and farm animals (140 SNPs, Fig. 2).

The *mcr-1* gene could be transferred from three MCRPEC isolates (isolates M-3, M-1, and M-2) to the recipient *E. coli* J53 in conjugation assays with transfer rates ranging from $2.53 \times 10^{-6}$ to $1.49 \times 10^{-4}$. The transconjugants showed colistin MICs of 4–8 μg/mL. In addition, the three transconjugants also exhibited resistance or high MICs to ampicillin (2/3, 66.7%), cefquinome (1/3, 33.3%), ceftriaxone (1/3, 33.3%), enrofloxacin (3/3, 100.0%), florfenicol (2/3, 66.7%), levofloxacin (2/3,

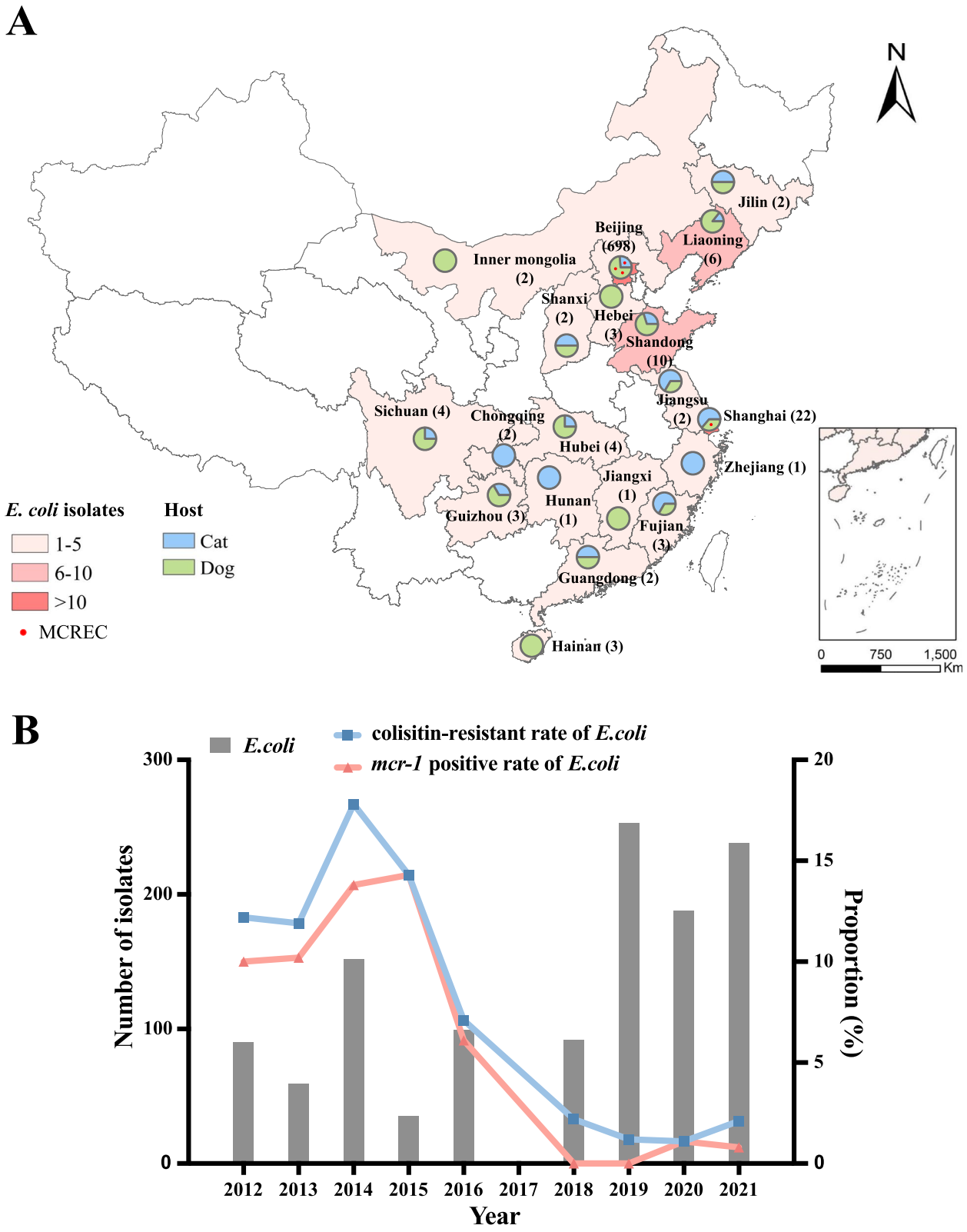


**Fig. 1** Characterization of the COREC and MCRPEC isolates. **A** The geographical distribution of *E. coli* and MCRPEC isolates from dogs and cats. The numbers in brackets indicate the number of *E. coli* isolates in the corresponding province. **B** The prevalence of COREC and MCRPEC isolates among companion animals in China from 2012 to 2021, and the data in 2012 to 2016 was referenced in our previous study [10]

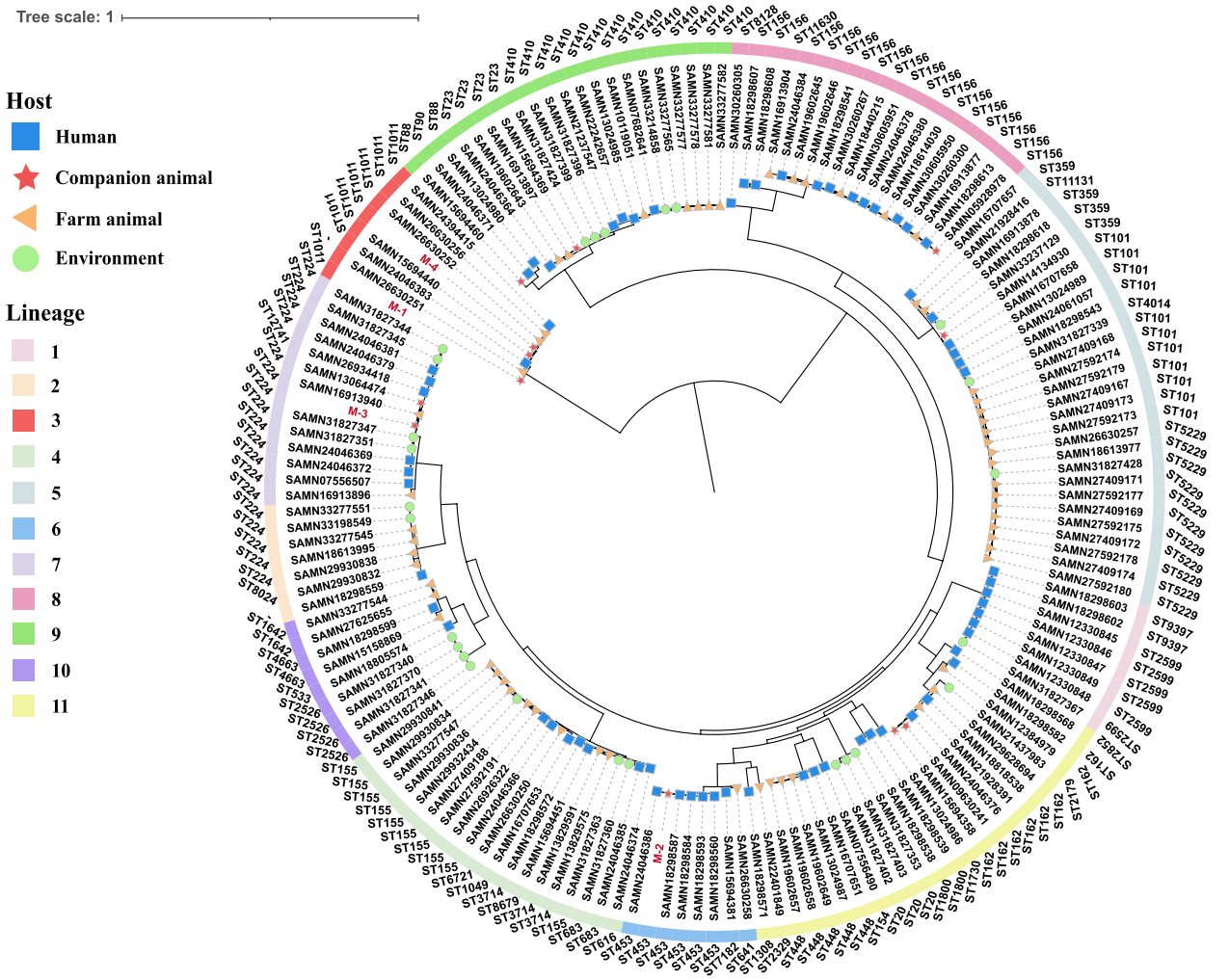


**Fig. 2** Phylogenetic tree of the MCRPEC isolates from different origins. The MCRPEC isolates were collected from companion animals in this study (in red designations) and from humans, companion animals, farm animals and environment in China downloaded from the NCBI databases (in black designations). The hosts of isolates are indicated by different symbols at end of the branches. The lineages are indicated in the outside circle with different colors. Each isolate is labeled with its ST type in the outermost circle

66.7%) and trimethoprim-sulfamethoxazole (1/3, 33.3%). In addition to the *mcr-1* gene, three MCRPEC isolates harbored the corresponding genes conferring resistance to β-lactams ($bla_{\text{TEM-32}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-1B}}$), tetracyclines (*tet*(A)), phenicols (*floR*), aminoglycosides (*aac(3)-IId*, *aadA2*, *aph(3')-Ia*, *aph(3")-Ib*, *aph(6)-Id*), and sulfamethoxazole (*sul1*, *sul2*) (Table S2). Notably, the canine MCRPEC isolate M-3 collected in 2021 only carried the *mcr-1* gene without additional resistance genes, but showed resistance to fluoroquinolones (enrofloxacin and levofloxacin, Table S2).

WGS revealed that the four MCRPEC isolates only carried a few virulence genes, which were associated with adherence (*aatA*, *eaeH*, *ehaA*, *eaeX*, *fimE*, *fimB*), effector delivery systems (*hbp*, *espX4*, *espY4*, *tsh*), invasion (*ibeC*, *traJ*), and nutritional/metabolic factors (*iucA*, *iucC*, *iutA*, *entF*, *chuS*, *chuA*, *chuW*, *iutA*, *iroN*, *iroC*, *irp*) (Table S2).

### Identification of the location of *mcr-1* gene

WGS analysis revealed that the *mcr-1* gene was located on IncI2 plasmids in the three isolates M-3 (61.0 kb), M-1 (64.3 kb), and M-2 (65.4 kb). These plasmids showed 99.5% to 99.9% nucleotide sequence identity to the plasmid pHNSD133-MCR (GenBank: MG725031) from *E. coli* of chicken origin in China (Fig. 3A). Similar to the MCRPEC isolates from pets [11], the *mcr-1* gene in these three IncI2 plasmids was flanked by genes *nikB* and *pap2*, and no IS*Apl1* element was observed in the flanking regions of *mcr-1* (Fig. 3B). The coexistence of the *mcr-1* gene and the extended-spectrum β-lactamase

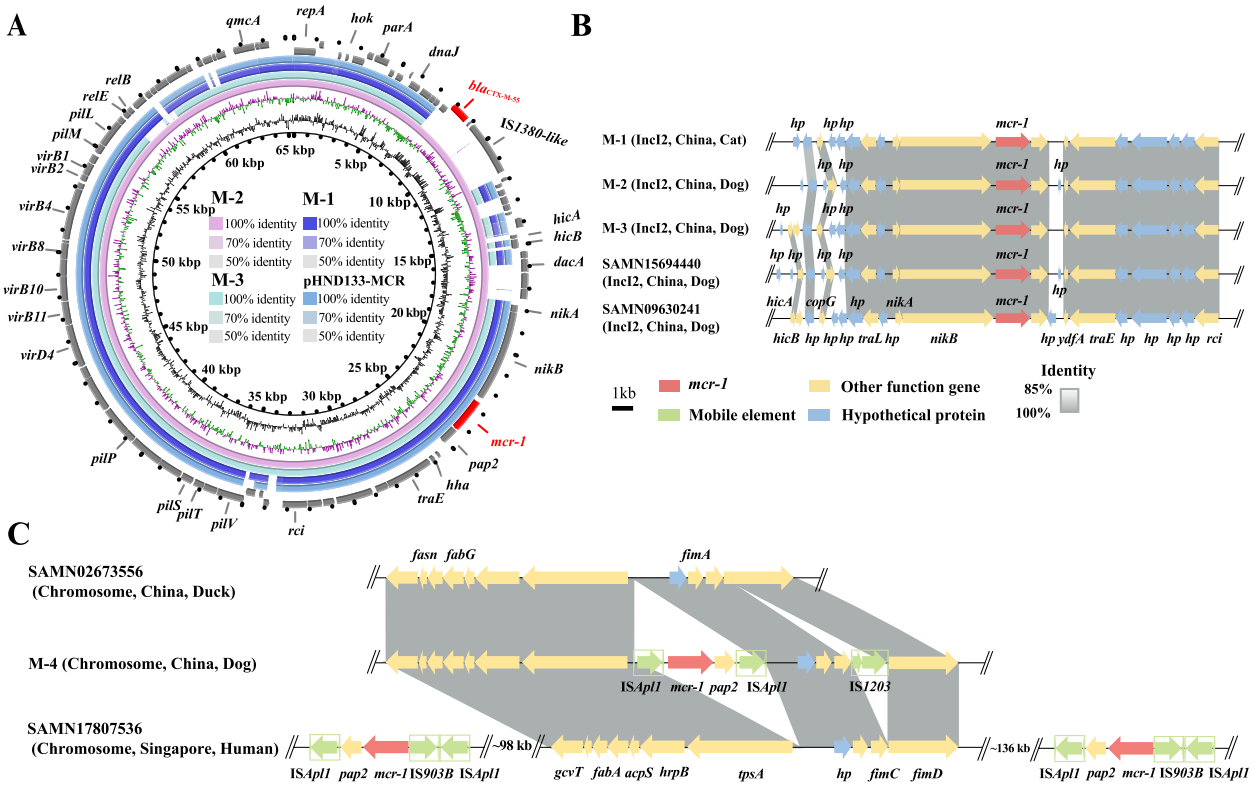


**Fig. 3** Genetic characterization of the MCRPEC isolates. **A** Sequence comparison of three reconstructed *mcr-1*-positive plasmids from the whole genome sequencing data of MCRPEC (M-1, M-2, M-3) in this study with plasmid pHNSD133-MCR from *E. coli* (MG725031.1) of chicken origin. **B** Comparison of the genetic environment of the plasmid-borne *mcr-1* gene in MCRPEC isolates in this study and in isolates (SAMN15694440 and SAMN09630241) from dogs. **C** Comparison of the genetic environment of the chromosomal *mcr-1* gene in MCRPEC isolates in this study and an isolate of duck origin in China (SAMN02673556) and another isolate of human origin in Singapore (SAMN17807536)

(ESBL) gene $bla_{\text{CTX-M-55}}$ in the IncI2 plasmid was observed in isolate M-2 (Fig. 3A), which was responsible for the resistance of the transconjugant of M-2 to ampicillin, ceftriaxone and cefquinome in addition to colistin (Table S2). A chromosomal *mcr-1* gene was found in the remaining isolate M-4. In this isolate, the *mcr-1* gene was flanked by IS*Apl1* elements, forming a unit of IS*Apl1*-*mcr-1*-*pap2*-IS*Apl1*, namely, transposon Tn*6330* (Fig. 3C). The integration site of the *mcr-1*-bearing transposon Tn*6330* was located between genes associated with fatty acid biosynthetic process and cell adhesion. The 15 kb segment bracketing Tn*6330* in M-4 showed 100% nucleotide sequence identity and 94.0% query coverage to the corresponding region of one duck *E. coli* from China in 2011 and one human *E. coli* from Singapore in 2019. The isolate of duck origin was negative for *mcr* genes, while the human isolate carried two *mcr-1* genes located about 98 kb upstream and 136 kb downstream of this segment (Fig. 3C).

## Discussion

During the years 2018 to 2021, a total of 771 isolates from companion animals in China were investigated for COREC and MCRPEC with 12 and four isolates, respectively, being tested positive. The detection rates of COREC (1.1%–2.2%) and MCRPEC (0.8%–1.1%) isolates in companion animals from 2018 to 2021 in China in this study were significantly lower than those of COREC (7.1%–17.8%) and MCRPEC (6.1%–14.3%) isolates among companion animals collected from 2012 to 2016 in our previous study [10]. This observation suggested that the ban of colistin in livestock in China, not only led to a decline in the prevalence among pigs (34.0% in 2015–2016 *vs* 5.1% in 2017–2018), chickens (18.1% in 2015–2016 *vs* 5.0% in 2017–2018) and humans (14.3% in 2016 *vs* 6.3% in 2019) [19], but also had a positive impact on reducing colistin resistance among bacteria from pets. The detection rate of MCRPEC among pets in this study is higher than that in Argentina, Korea and Thailand (0.1%–0.5%), while it is lower than that in Ecuador (2.0%) and France (8.3%) [22–26]. However, it should be noted that two thirds of the COREC isolates were negative for

all *mcr* genes (*mcr-1* to *mcr-10*), implying that either mutations in two-component system genes or unknown colistin resistance determinants might be present in isolates from companion animals which needs further investigation.

Although the four MCRPEC isolates exhibited three ST types and diversity in the phylogenetic tree, these isolates showed close genetic relationship with MCRPEC isolates from humans and farm animals. The 26 SNPs difference between one canine isolate and one human isolate in Beijing suggested a MCRPEC transfer between pets and humans. The high similarity of MCRPEC isolates between companion animals and humans had been reported previously [9, 11]. In addition, the transmission of extended-spectrum β-lactamase (ESBL)-producing *E. coli*, carbapenemase-producing *E. coli* and methicillin-resistant *Staphylococcus pseudintermedius* between companion animals and humans were also observed [11, 27–29]. The transmission of antimicrobial-resistant bacteria between companion animals and humans is probably due to the close contact between them and the sharing of the same living areas. Besides, the *mcr-1*-carrying plasmids in the three pet MCRPEC isolates in our study showed ≥ 99.5% nucleotide sequence identity to a plasmid of chicken-derived *E. coli* in China. Together with the evidence that the *mcr-1* gene had been detected in pet foods containing chicken as the main ingredient in other studies [10, 30], this may suggest that the *mcr-1* gene in *E. coli* from companion animals could also have originated from farm animals through the meat-processing pet food along the food chain. These above observations suggested MCRPEC from pets can be associated with the *mcr-1*-carrying plasmids or MCRPEC from either humans or food-producing animals.

In MCRPEC from both humans and farm animals, the diversity of plasmids carrying the *mcr-1* decreased after the ban of colistin, with the IncI2 and IncX4 being the dominant plasmid types [18, 31]. A similar trend was also observed in pet isolates. A comparison revealed a distribution of *mcr-1* on IncI2, IncX4 and IncHI2 plasmids from different studies in China before 2018 [11, 12], whereas the *mcr-1* was later on only located on IncI2 plasmids in this study and one other report in 2021 [14]. Several reports revealed that Tn*6330* or incomplete Tn*6330* with only one IS*Apl1* are still present on most IncHI2 type plasmids, while both IS*Apl1* copies were lost in most IncI2 type plasmids of MCRPEC isolates in humans and farm animals after the ban of colistin [18, 31]. Similarly, the *mcr-1*-harboring IncI2 type plasmids in the three pet MCRPEC isolates lost the IS*Apl1* elements, which resembles the IncI2 type plasmid in MCRPEC from another report in 2021 [14]. The loss of IS*Apl1* may result in a more stable genetic context for *mcr-1* on plasmids or in the chromosomal DNA [32]. Besides, it could pose threats to public health when the *mcr-1* gene is co-transferred with other antimicrobial resistance genes. The coexistence of *mcr-1* and $bla_{\text{CTX-M-55}}$ genes on the same plasmid of isolate M-2 and co-transfer of resistance to colisin, ampicillin, ceftriaxone and cefquinome in the transconjugant of M-2 in this study were also observed in another study [33], which indicated the co-transmission and co-selection of these two genes. Therefore, strengthening the surveillance of antimicrobial resistance and optimizing the rational use of antimicrobial agents in pet clinics are both essential.

## Material and methods

### Screening for colistin-resistant *E. coli* and testing for *mcr* genes

A total of 771 *E. coli* isolates included in this study were obtained from the China Antimicrobial Resistance Surveillance Network for Pets (CARPet) [34]. These 771 isolates were identified from clinical samples of dogs and cats from animal hospitals in 19 Chinese provinces/municipalities from 2018 to 2021. The routine clinical samples and the associated background information were collected from dogs and cats across China by the medical microbiology laboratories of the China Agricultural University Veterinary Teaching Hospital. The bacteria were recovered and isolated from the clinical samples using Brain Heart Infusion Agar containing 5% defibrinated sheep blood and species were confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Autobio, Zhengzhou, China) and 16S rRNA gene sequencing. Antimicrobial susceptibility testing of the identified isolates was performed by the broth microdilution method with custom-made broth microdilution panels (Thermo Fisher Scientific) according to the Clinical and Laboratory Standard Institute (CLSI) documents VET01S-Ed5 [35]. The *E. coli* isolates were tested for their susceptibility to 14 antimicrobial agents including ampicillin, amoxicillin-clavulanate, ceftriaxone, cefquinome, meropenem, gentamicin, amikacin, levofloxacin, enrofloxacin, trimethoprim-sulfamethoxazole, tigecycline, florfenicol, doxycycline and colistin in the CARPet system. *E. coli* ATCC 25922 served as the quality control strain. The MIC values were interpreted according to the clinical breakpoints listed in the CLSI documents VET01S-Ed5 [35] and M100-Ed31 [36]. We selected and recovered all the colistin-resistant *E. coli* isolates by culturing on MacConkey agar plates containing 2 μg/mL of colistin at 37 °C for 24 h. The obtained colistin-resistant isolates were screened for *mcr* genes by PCR [37].

### Transferability of *mcr* genes

The transferability of *mcr* genes in MCRPEC isolates was investigated by conjugation assays with the tested MCRPEC isolates used as the donor and the sodium-azide-resistant *E. coli* J53 as the recipient. The donor and recipient cells were mixed at a 1:3 ratio and then spread on a filter on the LB agar plate and incubated at 37 °C overnight. Transconjugants were selected on LB agar plates containing sodium azide (150 μg/mL) and colistin (2 μg/mL). The transfer frequency of *mcr* genes was calculated as transconjugants per donor cell.

### Whole genome sequencing and bioinformatics analysis

Genomic DNA of MCRPEC isolates was extracted by using HiPure Bacterial DNA Kit (Magen, Guangzhou, China). The DNA libraries were constructed using KAPA HyperPrep Kit (Kapa Biosystems) and sequenced on the Illumina HiSeq X Ten platform with a 150-bp paired-end strategy. To obtain the complete plasmid information, we used the Rapid Barcoding Sequencing kit (SQK-RBK004) to construct the MinION library and loaded the library into the Flow Cell R9.4 (FLO-MIN106) on the MinION device. We used guppy 3.2.4 to do the De-multiplexing and basecalling, and then used Unicycler v0.4.7 [38] or SPAdes [39] to generate de novo hybrid assemblies or draft genomes. All genome assemblies of MCRPEC isolates were registered under BioProject accession no. PRJNA942464. Sequence types, antimicrobial resistance genes, and virulence genes were identified by SRST2 [40]. Phylogenetic clusters were determined by hierarchical Bayesian analysis by using fastBAPS software [41]. The *mcr-1*-carrying plasmids were compared using BLAST Ring Image Generator (BRIG) [42].

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-023-00015-x.

**Additional file 1: Table S1.** Antimicrobial susceptibility testing results of studied *E. coli*. **Table S2.** Profiles of antimicrobial resistance genes, virulence genes and plasmid replicon typing of MCRPEC isolates.

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#### Authors' contributions

Y.W. and Z.X. designed the study, J.J., Y.C., X.D. and Z.Z. collected the data. J.J., S.M., S.C., S.S., W.Z., and Y.L. analyzed and interpreted the data. J.J., S.M. and Y.W. wrote the manuscript. All authors reviewed, revised, and approved the final report.

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#### Availability of data and materials

All the data supporting the conclusions of this article is included within the article.

## Declarations

#### Ethics approval and consent to participate

Ethical approval was approved and given by China Agricultural University Animal Ethics Committee document (No. AW01017102-2).

#### Consent for publication

Not applicable.

#### Competing interests

All authors declare that they have no conflicts of interest.

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