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SHORT COMMUNICATION

Characteristics of The Plasmid-Mediate Colistin-Resistance Gene *Mcr-1* In *Escherichia Coli* Isolated from Pig Farm in Jiangxi

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ABSTRACT

To explore the prevalence and characteristics of MCRPEC in pig farms, 278 pig faecal samples were collected from a pig farm in Jiangxi Province. Ten MCRPEC strains were laboratory isolated by culture, PCR, and gel electrophoresis. The broth-microdilution was adopted for the drug sensitivity test. After whole-genome sequencing, the resistance genes and plasmid typing were analysed, and the transferability of *mcr-1* was verified through plasmid conjugation transfer. The results showed that all ten strains were resistant to colistin, gentamicin, florfenicol, trimethoprim, cefotaxime, and tetracycline, with a MDR rate of 100%. The ten MCRPEC strains were found to belong to seven STs, and *mcr-1* was mainly located on the IncI2 plasmid, featuring a typical plasmid coupling transfer part, type IV secretion system, and antimonite protein gene; horizontal transfer occurred in nine strains. These results indicate that MCRPEC exists on this pig farm and is MDR. Bacterial monitoring should be strengthened, the use of antibiotics on farms should be reduced, and the spread of ARGs should be controlled.

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INTRODUCTION

Chinese scholars initially identified a plasmid-mediated colistin-resistance gene mcr-1 in 2015, which could be transferred between the same and different bacterial species. Subsequently, it was widely distributed across other regions (Liu *et al.*, 2015). Currently, researchers have discovered 10 subtypes *(mcr-1~10)* of colistin-resistant genes. Despite the discovery of many *mcr* subtypes, *mcr-1* is currently the most prevalent plasmid-mediated colistin-resistance gene worldwide (Hussein *et al.*, 2021). *Mcr-1* has been reported in over 50 nations and regions, including 21 in Asia, 14 in Europe, three in North America, eight in Latin America, three in Africa, and two in Oceania (Shi *et al.*, 2020). The global prevalence of *mcr-1* is found to be 7.4% in healthy individuals and 4.2% in clinical isolates (Bastidas-Caldes *et al.*, 2022). The emergence and rapid spread of the *mcr-1* gene have threatened the last line of defence against colistin in treating human multidrug-resistant (MDR) gram-negative bacterial infections. Research suggests that livestock farms may be a source of *mcr-1* dissemination (Wang *et al.*, 2019). Due to the close relationship between livestock farms and humans, most bacteria carrying *mcr-1* are *Enterobacteriaceae*. These bacteria can spread through contaminated animal-derived foods. On the other hand, the application and discharge of animal excreta promote the enrichment of antibiotic resistance genes (ARGs) in the environment, such as soil, rivers, and groundwater. Accumulated ARGs in the soil may also contaminate crops, threatening public safety (Luo *et al.*, 2017).

Broilers and pigs are the main reservoirs of *mcr* (Bastidas-Caldes *et al.*, 2022). Therefore, this study, faecal and environmental samples were collected from a pig farm in Jiangxi Province to investigate the prevalence and characteristics of *mcr-1*-positive *Escherichia coli* (MCRPEC), providing experimental data and a reference basis for antibiotic resistance investigation and monitoring.

MATERIALS AND METHODS

Isolation and identification of bacteria: In 2022, 278 samples, including pig faeces, sewage, drinking water, and flies, were collected from a pig farm in Pingxiang City, Jiangxi Province, China. After incubation in a 37°C

constant temperature air shaker for 18 hours, the samples were subjected to isolation and purification culture using MacConkey agar containing 2mg/L colistin. Strains containing the *mcr-1~10* gene were screened using polymerase chain reaction (PCR) and agarose gel electrophoresis. After the strains using the 16S rRNA gene, glycerol was added and stored at -80°C for future use.

Antimicrobial susceptibility testing for MCRPEC: The minimum inhibitory concentration (MIC) of MCRPEC against ten antibiotics, including colistin, cefotaxime, cefepime, tetracycline, aminoglycosides, amikacin, gentamicin, ciprofloxacin, meropenem, florfenicol, and trimethoprim was determined using the broth-microdilution. *Escherichia coli* ATCC25922 was used as a quality control strain. The resistance of the test strains to the selected antibacterial drugs was determined based on the criteria of the CLSI antimicrobial susceptibility test (CLSI, 2021).

Plasmid conjugation assays: MCRPEC serves as the donor strain, while *Escherichia coli* EC600, which is resistant to rifamycin, acts as the recipient strain. The *mcr-1* gene's ability to transfer was evaluated using a plasmid conjugation experiment. In the logarithmic growth stages of both bacterial strains, a mixture of the donor and receptor was prepared in a 1:3 volume ratio. This blend was then applied onto a 0.45μm microporous filter membrane placed on a Mueller Hinton agar plate. After 12 hours of culturing at 37°C, screen for the receptor strain that has been successfully transferred on MacConkey agar supplemented with 2mg/L polymyxin and 500mg/L rifamycin. Finally ERIC-PCR fingerprinting identification was performed on *Escherichia coli* EC600 (Amin *et al.*, 2020).

Whole-genome sequencing and bioinformatics analysis: DNA extraction from the MCRPEC was performed with the TIANamp Bacteria (Tiangen Biotech, Beijing, China). Whole-genome sequencing was carried out using the Illumina HiSeq 2500 (Illumina, USA). Bacterial genomes were assembled using SPAdes version 3.14 and annotated using PATRIC 3.6.9 (Bankevich *et al.*, 2012). The STs (Sequence types) and Plasmid types in the strains were examined using the CGE server and the Phylogenetic trees were constructed with Parsnp and visualized using iTOL.

RESULTS

MCRPEC isolation rate: Ten MCRPEC strains were isolated from 278 samples, while no other subtypes of *mcr* were isolated. The isolation rate was 3.60%, with the samples derived from fecal piglet samples (n = 8) and sewage samples (n = 2).

Antimicrobial susceptibility testing: By comparing the MIC with the CLSI resistance standards, all MCRPEC strains were resistant to colistin, gentamicin, florfenicol, tetracycline, cefotaxime, and trimethoprim. 60% of the strains were resistant to ciprofloxacin, 30% to cefepime, 20% to amikacin, and all strains were sensitive to meropenem. All ten MCRPEC strains were resistant to three or more types of antibiotics, with a drug resistance rate of 100% (Table 1). Moreover, 70% of the strains exhibited moderate resistance to colistin (MIC≥8mg/L), indicating a relatively high resistance level.

Conjugative plasmid transfer: After three conjugation experiments, the results showed that 90% (n=9) of the isolates successfully transferred the plasmid carrying *mcr-1* to the recipient *Escherichia coli* EC600 (Table 2).

Antimicrobial resistance genes: Whole-genome sequencing analysis was performed on the 10 MCRPEC strains, and it was discovered that all MCRPEC carried additional ARGs. Besides *mcr-1*, there were also aminoglycosides (*aph(3)-Ia*, *aph(3'')-Ib*, *aadA1*, and *aadA2*, amphenicols (*floR*), tetracyclines (*tet(A)* and *tet(X4)*), sulphonamides and their potentiators (*sul2*, *sul3*, *dfrA12*, and *dfrA14*), β-lactams (bla_{TEM-1B} and $bla_{CTX-M-55}$), macrolides (*mph(A)* and *mef(B)*), quinolones (*OqxB* and *qnrS1*), and other ARGs for antibacterial drugs, totaling 33 types of ARGs (Fig.1).

Sequence types: The ten MCRPEC isolates were distributed among 7 STs, demonstrating genetic diversity. Among them were three strains of the ST744 type, two strains of the ST101 type, and one strain each of ST10, ST515, ST4578, ST2325, and ST11420 (Fig.1).

Plasmid replicons: Plasmid replication typing analysis was conducted on ten isolated *Escherichia coli* strains, and 19 plasmid replicons were detected (Table 2). IncI2 (9/10) and IncFIB (8/10) were identified in most isolates. The plasmid where *mcr-1* of strain P19CP was located was of the IncX4 type, and that of the remaining nine strains was of the IncI2 type.

Genetic background: The genetic environment of the 10 MCRPEC strains was highly similar to that of the MCRPEC isolated from chickens (GenBank accession number: MN746290.1; Fig 2). Most MCRPEC possessed a same structural of *nikA-nikB-mcr-1-pap2*. Insertion sequence elements (IS609), toxin genes (*hicA*), plasmid-binding transfer genes (*pilL*, *pilM*, *pilP*, *pilS*, *pilT*, and *pilV*), and genes related to the type IV secretion system (*virD4*, *virB1*, *virB2*, *virB4*, *virB8*, *virB10*, and *virB11*) were identified in almost all isolates.

DISCUSSION

The total isolation rate of colistin-resistant bacteria in this study was 31.65%. The positive rate of *mcr-1 Escherichia coli* was 3.60%, which is similar to the total isolation rate of MCRPEC (1.42%) measured by Mei et al from livestock farms in different provinces of China during 2019–2020, which is in line with the significant decline in the prevalence of colistin resistance and *mcr* genes after the ban on colistin as an animal growth promoter in China (Mei *et al.*, 2024; Wang *et al.*, 2020).

Although the detection rate of MCRPEC was relatively low, each strain harbored at least six ARGs, and the multi-drug resistance rate was as high as 100%. Notably, all MCRPEC carried at least one type of β-lactam ARGs. Some studies have found that the prevalence of *mcr-1* in ESBLs-producing *Escherichia coli*

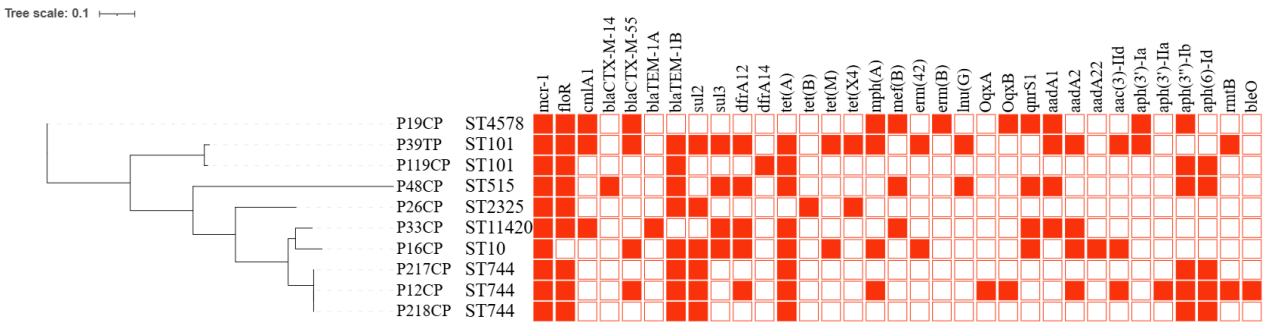

Fig. 1: Phylogenetic trees and drug-resistant genes of 10 strains of *mcr-1* positive *E. coli.*

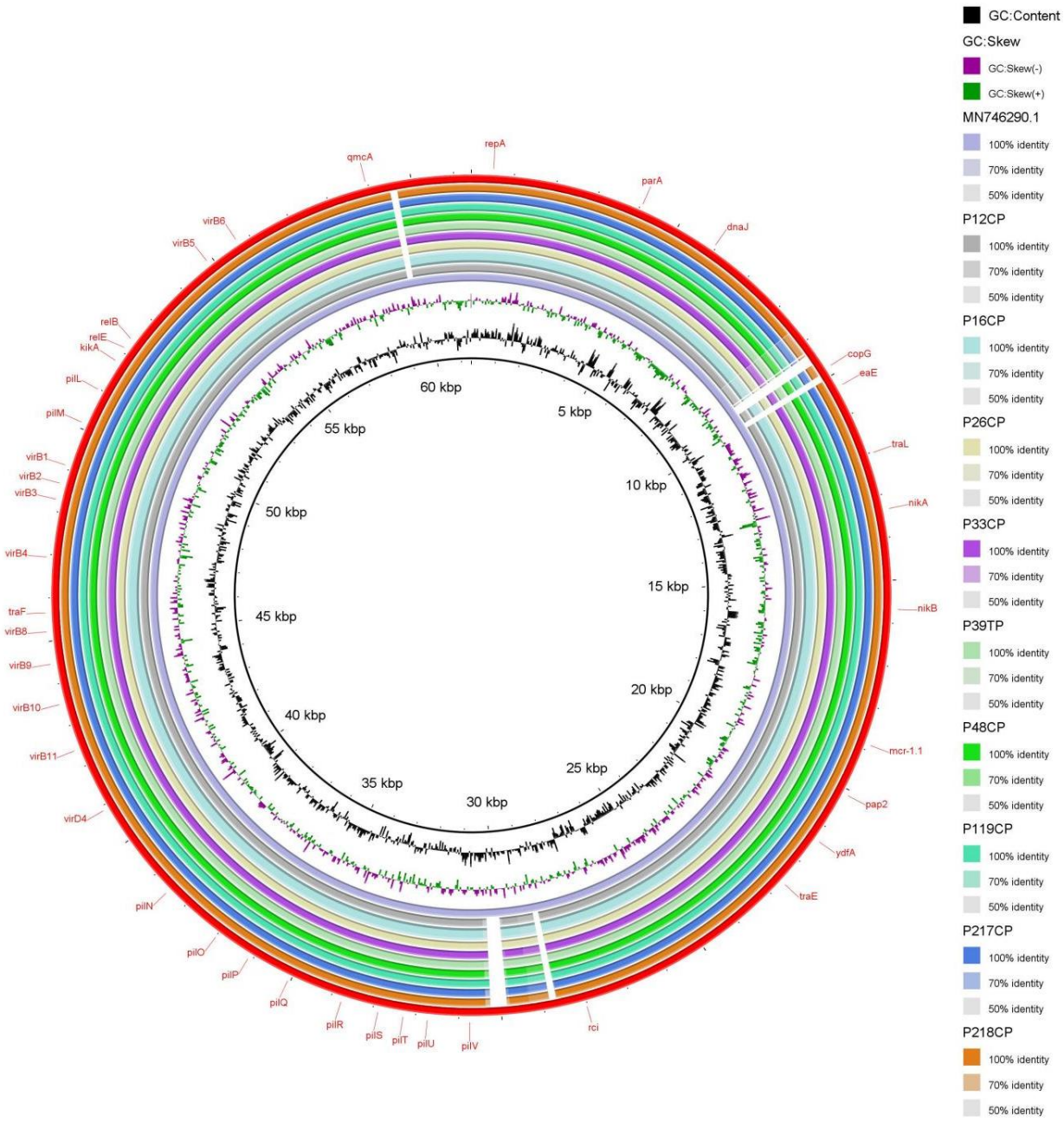

Fig. 2: Comparison of *mcr-1* carrying plasmids and plasmid GN2980 cycles.

(ESBL-EC) is higher than that in non-ESBL-EC, suggesting that ESBL-EC are more likely to recruit the *mcr-1* gene than non-ESBL-EC. This indicated that the rapid spread of ESBL-EC significantly increased the selection pressure for colistin resistance (Wu *et al.*, 2018). Thus, we infer that controlling the prevalence of β-lactam ARGs can, to a certain extent, control the emergence and prevalence of the *mcr-1* gene.

The STs of MCRPEC show diversity, and there are no major epidemic types (Wang *et al.*, 2019). This study

Table 1: Drug resistance rate and MIC (mg/L) of 10 strains of *mcr-1* positive *E. coli.*

Strain	CTX	GEN	COL	TET	FFC	TMP	CIP	FEP	AMI	MEM
p12cp	16	＞128	4	64	＞128	64	128	＞128	＞128	0.5
p16cp	8	128	8	64	64	64	1	＞128	1	0.25
p19cp	4	8	16	64	128	8	0.25	0.5	0.5	0.25
p26cp	8	8	4	64	32	16	0.25	0.5	1	0.125
p33cp	8	＞128	8	32	＞128	32	0.25	1	1	0.125
p39TP	16	＞128	16	＞128	＞128	64	16	＞128	＞128	0.25
p48cp	16	16	4	32	32	64	1	2	1	0.125
p119cp	8	＞128	8	16	＞128	16	0.25	0.125	4	0.125
p217cp	4	＞128	8	32	128	16	8	0.5	2	0.125
p218cp	4	＞128	8	32	128	16	8	0.5	2	0.125
Drug resistance rate(%)	100	100	100	100	100	100	60	30	20	0

CTX, Cefotaxime; GEN, Gentamicin; COL, Colistin; TET, Tetracycline; FFC, Florfenicol; TMP, Trimethoprim; CIP, Ciprofloxacin; FEP cefepime; AMI, Amikacin; MEM, Meropenem.

Table 2: Plasmid conjugation and plasmid replicon of 10 strains of *mcr-1* positive *E. coli.*

Strain	Transferable (+/-)	Plasmid types	*Mcr*-plasmid
P12CP	+	IncFIB(AP001918)，IncFIC(FII)，IncI2，IncP1，IncFII(pHN7A8)，IncQ1	IncI2
P16CP	-	IncFIB(AP001918)，IncFII(pHN7A8)，IncI2，IncI1-1(Alpha)，IncR，IncX1, IncQ1	IncI2
P19CP	+	IncFIB(K)，IncFIA(HI1)，IncFII，IncI1-1(Alpha)，IncX4	IncX4
P26CP	+	IncI2，IncR	IncI2
P33CP	+	IncFIB(K)，IncFIA(HI1)，IncI2，IncQ1，IncR，IncY	IncI2
P48CP	+	IncI2，ColE10，IncQ1	IncI2
P39TP	+	IncFIA，IncFIA(HI1)，IncFIB(AP001918)，IncFII，IncFII(pHN7A8)，IncHI1A，IncHI1B(R27)，IncI2，IncX1，P0111	IncI2
P119CP	+	IncFIB(AP001918)，IncI2，IncFIC(FII)	IncI2
P217CP	+	IncFIB(AP001918)，IncFIC(FII)，IncI2，IncP1	IncI2
P218CP	+	IncI2，IncFIB，IncFIC，IncP1	IncI2

identified 7 STs were identified from ten MCRPEC isolates, demonstrating sequence typing diversity. ST744 was the dominant type (30%). Currently, MCRPEC of this type have been isolated from human clinical settings (Feng *et al.*, 2023), pigs (Kompes *et al.*, 2023), and food (Kubelová *et al.*, 2021), and all are multi-drug resistant. This suggests that MCRPEC typed as ST744, is typically extensively drug-resistant and has a high transmission risk, presenting a potential threat to public health.

In the present study, the major vector of *mcr-1* was IncI2, which is in accordance with the research results of Umair et al (Umair *et al.*, 2023). Previous studies have revealed that ampicillin, amoxicillin, and cefalexin can facilitate the conjugative transfer of IncI2 plasmids among genera. Combined with the experimental outcome of this study, where all MCRPEC isolates were resistant to ceftazidime, it can be deduced that the application of β-lactam antibiotics such as amoxicillin might exert a positive selection pressure on plasmids (Liu *et al.*, 2023). These antibiotics are still extensively utilized in animals. Hence, controlling the use of β-lactam antibiotics in livestock farms can, to a certain extent, curb the prevalence of the *mcr-1* gene.

In this study, the IncI2-type plasmids contained the common fragment *nikA-nikB-mcr-1-pap2*. Previous studies have found this fragment in plasmids of human and animal origin, which is prone to horizontal transfer to different plasmids, indicating that plasmids containing this fragment are more likely to carry *mcr-1* for transmission between humans and animals (Li *et al.*, 2016 ; Kohler *et al.*, 2018). All MCRPEC used in this study contained a type IV secretion system and plasmid conjugation transfer genes. Plasmid-mediated conjugation of antibiotic resistance and virulence genes between different bacterial genera requires a type IV secretion system (Kohler *et al.*, 2018). Combined with the conjugative plasmid transfer results, it indicates that mobile genetic factors may have played significant roles in the horizontal spread of *mcr-1*. Therefore, it is necessary to strengthen the monitoring of bacteria containing type IV secretion systems.

Conclusions: The *mcr-1* gene is widely spread on this farm, and all positive bacteria carrying this gene are multi-drug resistant, suggesting that monitoring the *mcr-1* gene on the farm should be strengthened. Efforts should be made to control its spread from the source, use antibiotics rationally, strengthen the management of farm excrement discharge, reduce enrichment of the *mcr-1* gene in soil, rivers, and groundwater, reduce crop pollution, and maintain public health and safety.

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Authors' contributions: Y Wu and J Y Li conceived and designed the study. C H Wang, X Li, F Li, M L Jiang, Z K Liu and G F Liu executed the experiment. C H Wang, Y Wu and J Y Li analyzed the data.C H Wang and Y Wu wrote the article.

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