Extensive antimicrobial resistance and plasmid-carrying resistance genes in mcr-1-positive E. coli sampled in swine, in Guangxi, South China

Jingzhi Yuan
Guangxi University

Xiaoye Wang (✉ xywang@gxu.edu.cn)
GuangXi University  https://orcid.org/0000-0003-0355-6859

Dali Shi
Guangxi University

Qiang Ge
Guangxi University

Xingxing Song
Guangxi University

Wen Hu
Guangxi University

Deyuan Wei
Guangxi University

Chenling Ge
Guangxi University

Xun Li
Guangxi University

Chuanhuo Hu
Guangxi University

Research article

Keywords: swine-origin multi-drug resistance MCRPEC, antimicrobial resistance, extensively drug-resistant, acquired antimicrobial resistance genes

DOI: https://doi.org/10.21203/rs.2.13699/v4

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background:** The discovery of the superbug mcr-1-positive *Escherichia coli* (MCRPEC) has attracted worldwide attention. Swine-origin multi-drug resistant MCRPEC is a potential threat to public health and safety. To date, few detailed studies have been reported on swine MCRPEC in Guangxi, South China.

**Results:** In this study, thirty-three MCRPEC strains were identified from 142 *E. coli* strains isolated from 116 samples in Guangxi in 2018. All MCRPEC isolates were classified into eight unique STs and a total of six incompatibility plasmid groups (IncFI, IncHI1, IncY, IncN, IncI1 and IncX1) were found. Then, susceptibility of MCRPEC isolates to 27 antimicrobial agents belonging to 17 antimicrobial categories was tested. There were nineteen 3rd and 4th generation cephalosporins resistant *E. coli* and twelve carbapenem resistant *E. coli* among the 33 MCRPEC strains. Importantly, the MCRPEC were highly resistant to two carbapenem antibiotics, imipenem and meropenem, which were not permitted for use in livestock production. Three MCRPEC strains were further identified to be extensively drug-resistant (XDR), and the other isolates were recognized as multi-drug-resistant (MDR). Moreover, we detected whether the plasmid-carrying resistance genes coexist with the mcr-1 gene of the MCRPEC isolates. At last, β-lactamase antimicrobial resistance genes such as ESBL genes (*bla*<sub>CTX-M14</sub>, *bla*<sub>CTX-M24</sub>, *bla*<sub>CTX-M123</sub>, *bla*<sub>OXA-1</sub>), plasmid-mediated AmpC (pAmpC) gene (*bla*<sub>CMY-2</sub>), and the carbapenem gene *bla*<sub>NDM-5</sub> were detected. In addition, non-β-lactamase antimicrobial resistance genes such as *qnrA*, *qnrB*, *qnrS*, *aac(6')-Ib-cr*, *tetA*, *tetB*, *sul1*, *sul2*, *floR*, *aadA* were also detected.

**Conclusion:** Thirty-three mcr-1-positive *E. coli* isolates in Guangxi had a wide range of antimicrobial resistance. Plasmid-carrying resistance genes might be the main cause of MCRPEC multidrug resistance. This study highlighted the necessity for long-term surveillance of mcr-1-positive *E. coli* in pigs.

**Background**

Superbug infections are one of the most serious threats to public and animal health. The emergence and rapid spread of multi-drug-resistant (MDR), extensively drug-resistant (XDR) and pan-drug-resistant (PDR) bacteria is a major public health problem worldwide[1]. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories[2]. The transmissibility of antimicrobial resistance mediated by mobile plasmids is an important reason for the generation of XDR and PDR bacteria[3].

In China, colistin is the last line of defense against carbapenem-resistant *Escherichia coli* (CREC)[4, 5]. However, colistin has been used in animal production in China for decades as a treatment and feed additive[6]. In 2015, the plasmid-mediated mcr-1 gene was first discovered in food animals in South China[7]. Since then, a total of nine different mcr alleles, ranging from mcr-1 to mcr-9, have been detected in different bacteria in many countries and regions[7-15].
Due to the widespread use of β-Lactam antimicrobial in human and veterinary medicine, the number of extended-spectrum β-lactamase (ESBL)-producing E. coli is rising rapidly worldwide\[16, 17\]. In addition, relevant studies on CREC have been increased in worldwide scale, while CREC is multi-drug resistant microorganism that is difficult to treat and has a high fatality rate on infection\[18, 19\]. The emergence of colistin resistant CREC worsens the situation from a public health perspective, especially from the perspective of antimicrobial resistance and drug-resistant genes transfer\[20, 21\].

Thus, we aimed at describing antimicrobial resistance phenotype and plasmid-carrying resistance genes of mcr-1-positive E. coli from pigs in Guangxi, South China.

**Results**

**Identification of mcr-1 positive E. coli (MCRPEC) isolates**

A total of 142 E. coli isolates were isolated from pigs with diarrhea/dyspnea in Guangxi in 2018. Seventy-two (50.7%, 72/142) E. coli isolates were tested with MICs of colistin $\geq 3.5$ mg/L. To investigate the proportion of mcr genes in E. coli, PCR amplification was performed to test mcr-1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7, and mcr-8. A total of 33 mcr-1-positive E. coli strains were detected. While no other mcr genes were detected. The MCRPEC strains accounted for 45.8% (33/72) in colistin resistant E. coli strains and 23.2% (33/142) in all isolated E. coli strains. The thirty-three MCRPEC isolates were used for subsequent study.

The full-length 16S rRNA nucleotide sequences of the 33 MCRPEC strains were used to generate a phylogenetic tree by means of Neighbor Joining method in MEGA-X (Fig. 1). Thirty-three MCRPEC strains were classified into eight distinct STs, including ST10, ST224, ST361, ST410, ST641, ST1408, ST3345, and an unknown ST. ST10 and ST224 were the dominant STs, which accounted for 69.7% (23/33) (Fig. 1). More information about MLST was included in the supplementary materials (supplementary materials Table 2).

Multiple PCR was used to identify incompatibility plasmid groups in MCRPEC by using plasmid DNA of MCRPEC isolates. As is shown in Figure 1, six incompatibility plasmid groups were detected, including IncFI (97.0%, 32/33), IncHI (12.1%, 4/33), IncY (48.5%, 16/33), IncN (15.2%, 5/33), IncI1 (3.0%, 1/33) and IncX1 (6.1%, 2/33). Ten (30.3%, 10/33) MCRPEC isolates were detected to carry one incompatibility plasmid group (IncFI). Sixteen (16/33, 48.5%) MCRPEC isolates were detected to carry two incompatibility plasmid groups, of which eleven (11/16, 68.8%) isolates were the combination of IncFI and IncY, four (4/16, 25%) isolates were the combination of IncHI and IncFI, and one (1/16, 6.2%) isolate was the combination of IncFI and IncX1. Six (6/33, 18.2%) MCRPEC isolates were detected to carry three incompatibility plasmid groups, of which four (4/6, 66.6%) isolates were the combination of IncFI, IncN and IncY, one (1/6, 16.7%) isolate was the combination of IncFI, IncI1 and IncY, and one (1/16, 16.7) isolate was the combination of IncFI, IncN and IncX1. In addition, one (1/33, 3.0%) MCRPEC isolate was not detected to carry incompatibility plasmid group.
Antimicrobial resistance in MCRPEC

The antimicrobial resistance proportion of thirty-three MCRPEC isolates were as follows: gentamicin (72.7%, 24/33), amikacin (48.5%, 16/33), ceftaroline (69.7%, 23/33), piperacillin-tazobactam (24.2%, 8/33), imipenem (36.4%, 12/33), meropenem (24.2%, 8/33), cefalexin (69.7%, 23/33), ceftiofurxime (57.6%, 19/33), cefotaxime (57.6%, 19/33), ceftriaxone (57.6%, 19/33), cefepime (39.4%, 13/33), cefoxitin (0%, 0/33), ciprofloxacin (75.8%, 25/33), sulfadiazine (24.2%, 8/33), trimethoprim-sulphamethoxazole (0%, 0/33), aztreonam (24.2%, 8/33), ampicillin (97.0%, 32/33), amoxicillin-clavulanic acid (0%, 0/33), ampicillin-sulbactam (24.2%, 8/33), chloramphenicol (84.8%, 28/33), fosfomycin (78.8%, 26/33), tetracycline (100%, 33/33), doxycycline (72.7%, 24/33), azithromycin (57.6%, 19/33), polymyxin B (100%, 33/33) and colistin (100%, 33/33) (Fig. 2a). In addition, each MCRPEC isolate showed significant antimicrobial resistance (Fig. 2b). According to the results of cephalosporin susceptibility test, nineteen strains (57.6%, 19/33) were resistant to the 3rd and 4th generation cephalosporins and twelve strains (36.4%, 12/33) were resistant to carbapenem (Fig. 2a). According to the definition criteria of MDR, XDR, and PDR bacteria, all 33 MCRPEC isolates were identified as MDR (Fig. 2b)[2]. Moreover, three of the MDR MCRPEC isolates were identified as XDR (Fig. 2c).

Coexistence of \textit{mcr-1} gene in plasmids with \textit{β}-lactamase antimicrobial resistance genes and non-\textit{β}-lactamase antimicrobial resistance genes in the MCRPEC isolates

For the finding of the detection, there were 22 MCRPEC isolates harbored ESBL genes, including two \textit{bla_{OXA-1}} and thirty-two \textit{bla_{CTX-M}}. The dominate \textit{bla_{CTX-M}} gene was \textit{bla_{CTX-M-14}} (59.4%, 19/32), followed by \textit{bla_{CTX-M-123}} (37.5%, 12/32) and \textit{bla_{CTX-M-24}} (3.1%, 1/32) (Fig. 3a, Fig. 3b). Additionally, there were two and eight MCRPEC isolates identified to harbor \textit{bla_{CMY-2}} and \textit{bla_{NDM-5}} respectively. (Fig. 3a, Fig. 3c).

Furthermore, many non-\textit{β}-lactamase antimicrobial resistance genes were also investigated in MCPEC isolates, including fluoroquinolone resistance gene, tetracycline resistance genes, sulfanilamide resistance genes, aminoglycoside resistance genes and chloramphenicol resistance genes. As is shown in Fig 3a, the detection rates of fluoroquinolone resistance related genes \textit{qnrA}, \textit{qnrB}, \textit{qnrS}, and \textit{aac(6\textprime )-Ib-cr} were respectively 36.4% (12/33), 36.4% (12/33), 33.3% (11/33), and 24.2% (8/33). Tetracycline resistance related genes \textit{tetA}, \textit{tetB}, and \textit{tetX} accounted for 100% (33/33), 18.2% (6/33), and 0% (0/33), respectively. Sulfanilamide resistance related genes \textit{sul1} and \textit{sul2} accounted for 90.0% (30/33) and 78.8% (26/33), respectively. Aminoglycoside resistance related gene \textit{aadA} (100%, 33/33) and chloramphenicol resistance related gene \textit{floR} (100%, 33/33) both have 100% detection rates.

In addition, virulence genes (Enterotoxigenic \textit{E. coli} and Shigatoxin-pruducing \textit{E. coli}) of thirty-three MCRPEC isolates from clinical diagnostic were detected. The results were shown in Table 3 of supplementary materials. Twenty-six (26/33, 78.8%) MCRPEC isolates were identified as pathogenic \textit{E. coli}, of which twenty-two (22/26, 84.6%) isolates were identified as Enterotoxigenic \textit{E. coli} (ETEC), three (3/26, 11.5%) isolates were identified as Shigatoxin-pruducing \textit{E. coli} (STEC), and one (1/26, 3.9%) isolate was both ETEC and STEC. Fifteen (15/22, 68.2%) ETEC isolates only carried \textit{STb} gene, another part of
ETEC isolates (7/22, 31.8%) carried STb and LT genes. Three STEC isolates only carried stx2e gene. The ETEC/STEC isolate carried STb, LT and stx2e genes.

Discussion

China raises and consumes about 500 million pigs a year, nearly half the world’s total, and is the world’s largest consumer of antibiotics[22]. Overall consumption of antibiotics has been high for a long time, which may contribute to the spread of antibiotic resistance[23]. Faced with the serious problem of antibiotic resistance, the Chinese government formulated the veterinary drug prescription management measures in 2013[24]. In recent years, plasmid-mediated colistin resistant genes mcr-7 to mcr-9 have been found worldwide[7-15]. Although the Chinese government began to tighten regulation of colistin in 2017, MCRPEC remains a chronic problem in pig farms[25-27]. Thus, we report extensive antimicrobial resistance and plasmid-carrying resistance genes in MCRPEC from pigs in Guangxi, China.

In this study, MCRPEC was found to be highly resistant to some of the β-lactam and non-β-lactam antibiotics commonly used in human medicine and veterinary medicine, such as penicillin, cephalosporins, fluoroquinolones, aminoglycosides, quinolones, sulfonamides and tetracycline. In addition, rare resistance to carbapenems in animal production such as imipenem and meropenem was also found in MCRPEC. According to veterinary prescription list, imipenem and meropenem are not permitted to use in livestock production. Recently, studies have found that MCRPEC contains a “new Delhi metallo-lactam resistance gene” that is resistant to almost all β-lactam antibiotics but monobactam[28, 29]. Thirty-three MCRPEC isolates were susceptible to tigecycline and cefoxitin. According to veterinary prescription list, tigecycline and cefoxitin are not permitted to use in livestock production[24, 25]. As the same time, Guangxi government published the notice explained tigecycline was special use level and cefoxitin was restricted use level (i.e. ordinary people is difficul to acquire)[30]. The presence of these multidrug-resistant phenotypes suggests that MCRPEC coexists with other drug-resistant genes.

Interestingly, there is evidence that ESBL E. coli has a higher level of mcr-1 than non-ESBL E. coli, and the rapid rise in ESBL also significantly increases the selective pressure for colistin resistance[17]. In this study, 63.6% (21/33) of MCRPEC strains were detected to contain different ESBL genes simultaneously. Among them, blaCTX-M (19/21, 90.5%) was dominant in MCRPEC plasmid mediated drug resistance genes. Among the 32 blaCTX-M sequences of 19 MCRPEC isolates, blaCTX-M-14 gene had the highest proportion blaCTX-M gene (59.4%, 19/32), followed by blaCTX-M-123 (37.5%, 12/32) and blaCTX-M-24 (3.1%, 1/32). Notably, all MCRPEC isolates carrying the blaCTX-M-14 genes were resistant to ciprofloxacin (Fig. 3a and Fig. 3c), consistent with another study[31]. Recently, several reports have shown that pig waste not only frequently carries mcr-1 and blaNDM but also transfers these genes by affecting the environment around farms and contaminating the food chain[22, 27, 32]. Therefore, the co-existence of mcr-1 gene with carbapenem-resistant gene blaNDM-5 (8/33, 24.2%) in E. coli has drawn our attention to the spread of such superbugs in Guangxi.
E. coli is by nature sensitive to almost all clinically relevant antimicrobial agents, but this bacterium has a great capacity to accumulate resistance genes, mainly through horizontal gene transfer[33]. In this study, 11 plasmids carrying non-lactam genes were found in MCRPEC, including fluoroquinolone (qnrA, qnrB, qnrS, aac(6’)-Ib-cr), tetracycline (tetA, tetB), sulfonamide (sul1, sul2), aminoglycoside (aadA), and chloramphenicol (floR), suggesting that the effect of MCRPEC on spreading non-β-lactam genes should not be underestimated.

It has previously been reported that mcr-1 gene was found in the conjugative plasmids, IncI2, IncFII, IncX4, IncHI1, IncHI2, IncP, IncF, and IncY[34, 35]. We also detected these incompatibility types by PCR typing. The 33 MCRPEC isolates showed six different Inc plasmid groups including IncHI, IncI1, IncN, IncFI, IncY, IncX1. Meanwhile, we found that IncFIA and IncFrepB were prevalent in 33 MCRPEC in this study. MLST results reflected that ST10 (13/33, 39.3%) was the most common ST among the 33 MCRPEC isolates, ST224 (10/33, 30.3%) and another ST (10/33, 30.3%) followed (Fig. 1). Recently, a study analyzed 616 whole genomes of mcr-1-positive E. coli isolates from NCBI online database, among them ST10 was the most abundant MCRPEC strains[36].

**Conclusions**

The study showed that thirty-three mcr-1-positive E. coli isolates in Guangxi had a wide range of antimicrobial resistance. Plasmid-carrying resistance genes might be the main cause of MCRPEC multidrug resistance. The results indicated that many ESBL genes (blaCTX-M, blaOXA-1) coexisted with mcr-1. The carbapenemase gene blaNDM-5 was detected in 8 MCRPEC strains. Furthermore, a number of non-β-lactam genes also coexisted with mcr-1 gene. Food animals and their feces are important sources of bacterial drug resistance transfer, our study highlights the necessity for long-term surveillance of mcr-1-positive E. coli in pigs.

**Methods**

**Sample collection and detection of MCRPEC isolates**

A total of 116 samples were collected from 44 pig farms that include 37 family farms and 7 swine breeding farms distributed in different towns of Guangxi, China in 2018. In which, 51 samples were collected from family farms and 65 samples were collected from swine breeding farms. These samples were collected from June 2018 to December 2018 and taken from rectal swabs or lung, intestinal tract, or lymph gland tissue collected from dead or unhealthy pigs with diarrhea or dyspnea. Before this study, these samples were sent to clinical veterinary laboratory of College of Animal Science and Technology of Guangxi University for molecular diagnosis. These farms managed about 27.8 thousand fattening pigs and 30.8 thousand breeding pigs during this study period. All fattening pigs belonged to family farms which were companies plus farmers model and small in size (number of pigs were between 400 to 1000 in one farm). Swine breeding farms adopted closed management and bigger in size (number of pigs were
between 1000 to 5000 in one farm). The figure 4 showed distribution of pig farms and proportion of \textit{mcr-1} positive \textit{E. coli} in different city (compare with number of samples).

First, the collected samples were inoculated with MacConkey agar for 24h at 37°C. Then all single colonies of different forms on MacConkey agar were inoculated with eosin-methylene blue agar to screen suspected \textit{E. coli} isolates. Colonies with a purplish black color or a metallic dark green color on the eosin-methylene blue agar were considered as suspected \textit{E. coli} and further inoculated into LB Broth (Luria-Bertani Broth) for 8-10h at 37°C. Colistin resistance isolates were isolated by self-made SuperPolymyxin medium (i.e. a mixture of 10ml Eosin-methylene blue agar and 35μg colistin) as previous reported[37]. The genome DNA of Colistin resistance isolates was extracted with TIANamp Bacteria DNA Kit. Colistin resistance \textit{E. coli} strains were determined by 16S rRNA gene sequencing and BLAST analysis (i.e. First, DNA and primers were used to amplify target fragment largely. PCR amplicons were purified by agarose gel electrophoresis and gel extraction from TIAN Gel Extraction Kit. Purified PCR products were sequenced by ABI 3730xl DNA Analyzer. SeqMan was used for sequence analysis. Finally, sequences were BLAST in NCBI.)[38]. Phylogenetic tree was generated by Neighbor joining statistical method in MEGA-X software. Thirty-three MCRPEC isolates would be tested in follow-up experiments. These isolates were from 116 pigs (un-weaned piglets, nursery piglets and sows) of 25 pig farms of different sizes distributed in 18 different towns of Guangxi, China in 2018 (Table 1). All \textit{E. coli} isolates were stored in glycerol medium at -80°C.

The MCRPEC isolates were preliminarily screened by PCR amplification using genome DNA and special primer pairs for the \textit{mcr-1}, \textit{mcr-2}, \textit{mcr-3}, \textit{mcr-4}, \textit{mcr-5}, \textit{mcr-6}, \textit{mcr-7}, and \textit{mcr-8} genes (supplementary material Table 1)[39, 40]. To identify MCRPEC strains, the \textit{mcr-1} gene sequences in the \textit{E. coli} strains were determined by direct sequencing from the PCR products and BLAST analysis[38].

**Detection of multilocus sequence typing (MLST) and incompatibility plasmid groups**

MLST analysis was performed by PCR amplicons of seven housekeeping genes, namely \textit{adk}, \textit{fumC}, \textit{gyrB}, \textit{icd}, \textit{mdh}, \textit{purA}, and \textit{recA} using genome DNA (supplementary material Table 1). PCR amplicons were sequenced after purified by agarose gel electrophoresis and gel extraction by TIAN Gel Extraction Kit. The gene sequences for seven housekeeping genes were uploaded to the EnteroBase database to obtain the sequence type (ST) of corresponding \textit{E. coli} isolate[41].

Incompatibility plasmid groups were assigned by PCR-based replicon types (HI1, HI2, I1, L/M, N, FIA, FIB, FIC, Frep, W Y, P, A/C, T, FIIS, K/B, B/O)[42]. Additional PCRs were performed for the IncX (X1, X2, X3, X4) replicon types[43]. All house genes and replicon primers, and PCR reaction conditions were included in supplementary material (supplementary material Table 1).

**Antimicrobial susceptibility testing**

According to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) consensus, a total of 27 commonly used human antimicrobials from 18 antimicrobial categories were selected in this
study, including gentamicin, amikacin, ceftaroline, piperacillin-tazobactam, imipenem, meropenem, cefalexin, cefuroxime, cefotaxime, ceftriaxone, cefepime, cefoxitin, ciprofloxacin, sulfadiazine, trimethoprim-sulphamethoxazole, aztreonam, ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, chloramphenicol, fosfomycin, tetracycline, doxycycline, azithromycin, polymyxin B and colistin (Table 2)[2]. Minimum inhibitory concentrations (MICs) were determined by using the agar microdilution (Mueller-Hinton Agar) method according to the Clinical and Laboratory Standards Institute[44]. The MICs of each drug were measured and recorded. E. coli ATCC25922 was used as a quality control. Resistant breakpoints of other antimicrobial abided by the CLSI-M100 document[45]. CLSI breakpoints are not available for colistin and cefalexin. So, in this study, we adopted the European Committee on Antimicrobial Susceptibility Testing Resistant/Susceptible breakpoints for determine colistin and cefalexin MICs. MICs of ≤2 mg/L and ≤16 mg/L are considered as susceptible (S) for colistin and cephalaxin, respectively, according to the EUCAST guidelines[46].

Molecular identification of ESBL, pAmpC, and carbapenem resistance genes

The ESBL, plasmid-mediated AmpC (pAmpC), and carbapenem genes were detected by multiplex PCR in plasmid DNA of MCRPEC isolates. The ESBL genes (bla\textsubscript{CTX-M}, bla\textsubscript{TEM}, bla\textsubscript{OXA-1}, and bla\textsubscript{SHV}), plasmid-mediated AmpC (pAmpC) genes (bla\textsubscript{CMY}, bla\textsubscript{FOX}, bla\textsubscript{DHA}), and carbapenem resistance genes (bla\textsubscript{NDM}, bla\textsubscript{KPC}, bla\textsubscript{OXA-48}, and bla\textsubscript{IMP}) were amplified using specific primers, as previously reported[47]. The DNA sequences for the ESBL, plasmid-mediated AmpC (pAmpC), and carbapenemase genes were determined by using BLAST analysis[38]. All β-lactamase primers and PCR reaction conditions were included in supplementary material (supplementary material Table 1).

Detection of non-β-lactamase antimicrobial resistance genes

The non-β-lactamase antimicrobial resistance genes were detected by PCR in plasmid DNA of MCRPEC isolates. The special primers included plasmid-encoded fluoroquinolone resistance genes (qnrA, qnrB, qnrS, aac(6\textsuperscript{-}I)-lb-cr)[48], tetracycline resistance genes (tetA, tetB, tetX)[49], sulfonamide resistance genes (sul1, sul2), aminoglycoside resistance genes (aadA), and chloramphenicol resistance genes (floR), respectively. All non-β-lactamase primers and PCR reaction conditions were included in supplementary material (supplementary material Table 1).

Abbreviations

MCRPEC: mcr-1-positive Escherichia coli; MDR: multi-drug-resistance; XDR: extensively-drug-resistant; PDR: pan-drug-resistant; CREC: carbapenem resistant Escherichia coli, ESBL: extended-spectrum β-lactam, pAmpC: plasmid-mediated AmpC

Declarations

Ethics approval and consent to participate
This study was approved by Animal Experimental Ethical Committee of Guangxi University. This study was approved by College of Animal Science and Technology of Guangxi University and all relevant companies. This study conformed to the legal requirements of Guangxi Zhuang Autonomous Region. Animal Experimental Ethical Inspection Form (GXU2018-053) were added in supplementary material Fig. 1.

Consent for publication

Not applicable

Availability of data and material

The raw data generated during the current study are available in the [Baidu Netdisk] repository, [https://pan.baidu.com/s/1ZQDr0k1FP3dZW8J7BU8T-w], code is MCR1.

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no conflict of interest.

Funding

This research was supported by grant from the National Natural Science Foundation of China (31502079 and 31660700), National Natural Science Foundation of Guangxi (2017GXNSFAA198071) and Science and Technology Development Program of Nanning (20180526). The Purchase of experimental consumables in this study were supported by Grant 31502079. The sample collections and gene sequence were supported by Grant 2017GXNSFAA198071. The data analysis were aided by Grant 31660700. And the writing of this manuscript was supported by Grant 20180526.

Authors’ contributions

JY carried out the experiment, analyzed samples and statistical data and wrote the manuscript. DS, QG and XS participated in experiment. WH, DW and CG isolated samples. XL and CH contributed to revise the manuscript. XW was corresponding authors, who mainly designed the study and supervised the whole program. All authors have read and approved the final manuscript.

Acknowledgements

Not applicable.

References
1. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, Zhang Y, Jin L, Chen H, Wang Z et al. Phenotypic and Genotypic Characterization of Carbapenem-resistant Enterobacteriaceae: Data From a Longitudinal Large-scale CRE Study in China (2012-2016). Clin Infect Dis 2018, 67(suppl_2):S196-S205.

2. Huang YH, Chou SH, Liang SW, Ni CE, Lin YT, Huang YW, Yang TC. Emergence of an XDR and carbapenemase-producing hypervirulent Klebsiella pneumoniae strain in Taiwan. J Antimicrob Chemother 2018, 73(8):2039-2046.

3. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012, 18(3):268-281.

4. Dolejska M, Papagiannits CC. Plasmid-mediated resistance is going wild. Plasmid 2018, 99:99-111.

5. Shen Z, Hu Y, Sun Q, Hu F, Zhou H, Shu L, Ma T, Shen Y, Wang Y, Li J et al. Emerging Carriage of NDM-5 and MCR-1 in Escherichia coli From Healthy People in Multiple Regions in China: A Cross Sectional Observational Study. EclinicalMedicine 2018, 6:11-20.

6. Collignon P, Voss A. China, what antibiotics and what volumes are used in food production animals? Antimicrob Resist Infect Control 2015, 4:16.

7. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infectious Diseases 2016, 16(2):161-168.

8. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro Surveill 2016, 21(27).

9. Roer L, Hansen F, Stegger M, Sönksen UW, Hasman H, Hammerum AM. Novel mcr-3 variant, encoding mobile colistin resistance, in an ST131 Escherichia coli isolate from bloodstream infection, Denmark, 2014. Eurosurveillance 2017, 22(31).

10. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. 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 2017, 72(12):3317-3324.

11. Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmid-mediated colistin resistance gene mcr-7.1 in Klebsiella pneumoniae. J Antimicrob Chemother 2018, 73(7):1791-1795.

12. AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, Randall LP, Lemma F, Crook DW, Teale C et al. mcr-1 and mcr-2 variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother 2017, 72(10):2745-2749.

13. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z, Wang Y. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg
14. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. **Novel Plasmid-Mediated Colistin Resistance Gene mcr-3 in Escherichia coli.** MBio 2017, 8(3).

15. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. **Identification of Novel Mobilized Colistin Resistance Gene mcr-9 in a Multidrug-Resistant, Colistin-Susceptible Salmonella enterica Serotype Typhimurium Isolate.** MBio 2019, 10(3).

16. Dandachi I, Chabou S, Daoud Z, Rolain JM. **Prevalence and Emergence of Extended-Spectrum Cephalosporin-, Carbapenem- and Colistin-Resistant Gram Negative Bacteria of Animal Origin in the Mediterranean Basin.** Front Microbiol 2018, 9:2299.

17. Wu C, Wang Y, Shi X, Wang S, Ren H, Shen Z, Wang Y, Lin J, Wang S. **Rapid rise of the ESBL and mcr-1 genes in Escherichia coli of chicken origin in China, 2008-2014.** Emerg Microbes Infect 2018, 7(1):30.

18. Alotaibi FE, Bukhari EE, Al-Mohizea MM, Hafiz T, Essa EB, AlTokhais YI. **Emergence of carbapenem-resistant Enterobacteriaceae isolated from patients in a university hospital in Saudi Arabia.** Epidemiology, clinical profiles and outcomes. J Infect Public Health 2017, 10(5):667-673.

19. Zhang R, Liu L, Zhou H, Chan EW, Li J, Fang Y, Li Y, Liao K, Chen S. **Nationwide Surveillance of Clinical Carbapenem-resistant Enterobacteriaceae (CRE) Strains in China.** EBioMedicine 2017, 19:98-106.

20. van Duin D, Lok JJ, Earley M, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins RR, Doi Y *et al.* **Colistin Versus Ceftazidime-Avibactam in the Treatment of Infections Due to Carbapenem-Resistant Enterobacteriaceae.** Clin Infect Dis 2018, 66(2):163-171.

21. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, Dever LL, Kreiswirth BN. **Colistin- and Carbapenem-Resistant Escherichia coli Harboring mcr-1 and blaNDM-5, Causing a Complicated Urinary Tract Infection in a Patient from the United States.** mBio 2016, 7(4).

22. Larson C. **Pharmaceuticals. China's lakes of pig manure spawn antibiotic resistance.** Science 2015, 347(6223):704.

23. Hvistendahl M. **Public health. China takes aim at rampant antibiotic resistance.** Science 2012, 336(6083):795.

24. **Announcement No. 1997 [2013] of the Ministry of agriculture of China.**
   http://www.moa.gov.cn/gk/tzgg_1/gg/201612/t20161201_5386856.htm. 1 Dac 2019.

25. **Announcement No. 2471 [2016] of the Ministry of agriculture of China.**
   http://www.moa.gov.cn/gk/tzgg_1/gg/201612/t20161201_5386856.htm. 1 Dac 2019.

26. Tong H, Liu J, Yao X, Jia H, Wei J, Shao D, Liu K, Qiu Y, Ma Z, Li B. **High carriage rate of mcr-1 and antimicrobial resistance profiles of mcr-1-positive Escherichia coli isolates in swine faecal samples collected from eighteen provinces in China.** Vet Microbiol 2018, 225:53-57.

27. Xia X, Wang Z, Fu Y, Du XD, Gao B, Zhou Y, He J, Wang Y, Shen J, Jiang H *et al.* **Association of colistin residues and manure treatment with the abundance of mcr-1 gene in swine feedlots.** Environ Int 2019, 127:361-370.
28. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 2009, 53(12):5046-5054.

29. Kong LH, Lei CW, Ma SZ, Jiang W, Liu BH, Wang YX, Guan R, Men S, Yuan QW, Cheng GY et al. Various Sequence Types of Escherichia coli Isolates Coharboring blaNDM-5 and mcr-1 Genes from a Commercial Swine Farm in China. Antimicrob Agents Chemother 2017, 61(3).

30. Announcement No. 80 [2012] of the Health Commission of Guangxi Zhuang Autinomous Region. http://wsjkw.gxzf.gov.cn/jgxx/cszc/yzc/zhengcefagui/2012/0801/1995.html. 13 May 2020.

31. Patil S, Chen X, Lian M, Wen F. Phenotypic and genotypic characterization of multi-drug-resistant Escherichia coli isolates harboring blaCTX-M group extended-spectrum beta-lactamases recovered from pediatric patients in Shenzhen, southern China. Infect Drug Resist 2019, 12:1325-1332.

32. Atterby C, Osbjer K, Tepper V, Rajala E, Hernandez J, Seng S, Holl D, Bonnedahl J, Borjesson S, Magnusson U et al. Carriage of carbapenemase- and extended-spectrum cephalosporinase-producing Escherichia coli and Klebsiella pneumoniae in humans and livestock in rural Cambodia; gender and age differences and detection of blaOXA-48 in humans. Zoonoses Public Health 2019.

33. Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S. Antimicrobial Resistance in Escherichia coli. Microbiol Spectr 2018, 6(4).

34. Wang Q, Sun J, Li J, Ding Y, Li XP, Lin J, Hassan B, Feng Y. Expanding landscapes of the diversified mcr-1-bearing plasmid reservoirs. Microbiome 2017, 5(1):70.

35. Zhang C, Feng Y, Liu F, Jiang H, Qu Z, Lei M, Wang J, Zhang B, Hu Y, Ding J et al. A Phage-Like IncY Plasmid Carrying the mcr-1 Gene in Escherichia coli from a Pig Farm in China. Antimicrob Agents Chemother 2017, 61(3).

36. Zhuge X, Ji Y, Tang F, Sun Y, Jiang M, Hu W, Wu Y, Xue F, Ren J, Zhu W et al. Population structure and antimicrobial resistance traits of avian-origin mcr-1-positive Escherichia coli in Eastern China, 2015 to 2017. Transbound Emerg Dis 2019, 66(5):1920-1929.

37. Nordmann P, Jayol A, Poirel L. A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates. J Clin Microbiol 2016, 54(5):1395-1399.

38. National Center of Biotechnology Information. http://www.ncbi.nlm.nih.gov. 15 May 2019.

39. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill 2018, 23(6).

40. Yang F, Shen C, Zheng X, Liu Y, El-Sayed Ahmed MAE, Zhao Z, Liao K, Shi Y, Guo X, Zhong R et al. Plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli and Klebsiella pneumoniae isolated from market retail fruits in Guangzhou, China. Infect Drug Resist 2019, 12:385-389.

41. Zhou Z, Alikhan NF, Mohamed K, Fan Y, Agama Study G, Achtman M. The EnteroBase user’s guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core
genomic diversity. *Genome Res* 2020, 30(1):138-152.

42. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005, 63(3):219-228.

43. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. *Plasmid* 2012, 68(1):43-50.

44. CLSI. Performance standards for antimicrobial susceptibility testing. In. Wayne, PA: CLSI Supplement M100 Clinical and Laboratory Standards Institute; 2018.

45. CLSI-M100. [http://www.clsi.org/m100/](http://www.clsi.org/m100/). 20 Apr 2019.

46. European Committee on Antimicrobial Susceptibility Testing. [http://www.eucast.org/clinical_breakpoints/](http://www.eucast.org/clinical_breakpoints/). 20 Apr 2019.

47. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011, 70(1):119-123.

48. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007, 60(2):394-397.

49. He T, Wang R, Liu D, Walsh TR, Zhang R, Lv Y, Ke Y, Ji Q, Wei R, Liu Z *et al*. Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. *Nat Microbiol* 2019, 4(9):1450-1456.

**Tables**
| Number  | Town/County/City                  | Date          | Usage/Scale | Clinical symptom  |
|---------|----------------------------------|---------------|-------------|------------------|
| GXEC-A1 | Ning Wu, Wu Ming, Nan Ning      | June 11       | Porker/800  | Diarrhea         |
| GXEC-A3 | Gan Xu, Wu Ming, Nan Ning       | June 19       | Porker/600  | Diarrhea         |
| GXEC-A6 | Jin Ling, Xi Xiang Tang, Nan Ning| July 05       | Porker/570  | Diarrhea         |
| GXEC-A7 | Shuang Ding, Xi Xiang Tang, Nan Ning | July 14    | Porker/400  | Diarrhea         |
| GXEC-B2 | Shuang Ding, Xi Xiang Tang, Nan Ning | July 23    | Porker/700  | Diarrhea/Dyspnea |
| GXEC-B4 | Jin Ling, Xi Xiang Tang, Nan Ning | August 02    | Porker/800  | Diarrhea/Dyspnea |
| GXEC-B10| Shuang Ding, Xi Xiang Tang, Nan Ning | August 19    | Porker/530  | Diarrhea         |
| GXEC-B11| Fu cheng, Wu Ming, Nan Ning     | August 27     | Porker/1019 | Diarrhea         |
| GXEC-C4 | Shuang Qiao, Wu Ming, Nan Ning  | September 28  | Porker/750  | Diarrhea         |
| GXEC-C6 | Ling Tian, Ling Chuan, Gui Lin  | October 15    | Porker/1000 | Diarrhea/Dyspnea |
| GXEC-C12| Shuang Qiao, Wu Ming, Nan Ning  | October 15    | Porker/600  | Diarrhea         |
| GXEC-C13| Sha Tian, Ping Gui, He Zhou     | November 01   | Porker/900  | Diarrhea/Dyspnea |
| GXEC-C15| Shuang Ding, Xi Xiang Tang, Nan Ning | October 29   | Porker/800  | Diarrhea         |
| GXEC-C17| He Jie, Ping Gui, He Zhou       | November 17   | Porker/900  | Diarrhea         |
| GXEC-C18| Ren Yi, Ba Bu, He Zhou          | December 01   | Porker/900  | Diarrhea/Dyspnea |
| GXEC-D3 | Sha Tian, Ping Gui, He Zhou     | November 18   | Porker/700  | Diarrhea         |
| GXEC-D4 | Kui Yang, Xing Ye, Yu Lin       | December 16   | Porker/830  | Diarrhea         |
| GXEC-D6 | Long Meng, Pu Bei, Qin Zhou     | December 20   | Porker/750  | Diarrhea/Dyspnea |
| GXEC-E1 | Shuang Qiao, Wu Ming, Nan Ning  | October 11    | Un-weaned piglet/700 | Diarrhea |
| GXEC-E5 | Shuang Qiao, Wu Ming, Nan Ning  | October 11    | Un-weaned piglet/700 | Diarrhea |
| GXEC-E6 | Shuang Qiao, Wu Ming, Nan Ning  | October 11    | Sow/700     | Diarrhea         |
| GXEC-F2 | Da Hua, He Chi                  | November 03   | Un-weaned piglet/5000 | Diarrhea |
| GXEC-F9 | Da Hua, He Chi                  | November 03   | Un-weaned piglet/5000 | Diarrhea |
| GXEC-G8 | Fu Cheng, Wu Ming, Nan Ning     | November 11   | Un-weaned piglet/1500 | Diarrhea |
| GXEC-H1 | Lu Zhai, Liu Zhou               | November 22   | Un-weaned piglet/1500 | Diarrhea |
| GXEC-H2 | Lu Zhai, Liu Zhou               | November 22   | Un-weaned piglet/1500 | Diarrhea |
| GXEC-H10| Lu Zhai, Liu Zhou               | November 22   | Sow/1500    | Diarrhea         |
| GXEC-I5 | Feng Huang, Xing Bin, Lai bin   | December 06   | Sow/5000    | Diarrhea         |
| GXEC-I6 | Feng Huang, Xing Bin, Lai bin   | December 06   | Sow/5000    | Diarrhea         |
| GXEC-J8 | Xing An, Xing An, Gui Lin       | December 15   | Un-weaned piglet/1500 | Diarrhea |
| GXEC-K2 | San Jie, Ling Chuan, Gui Lin    | December 22   | Un-weaned piglet/1000 | Diarrhea |
| GXEC-K3 | San Jie, Ling Chuan, Gui Lin    | December 22   | Un-weaned piglet/1000 | Diarrhea |
| GXEC-K5 | San Jie, Ling Chuan, Gui Lin    | December 22   | Sow/1000    | Diarrhea         |
Note: Un-weaned piglets were concentrated on less than 20 days. Nursery piglets were concentrated on 40 to 60 days. Sows were less than 100 days old.

## TABLE 2

The antimicrobial agents for 17 antimicrobial categories used to define the *E. coli* antimicrobial resistance

| Antimicrobial category                                      | Antimicrobial agent                                      |
|-------------------------------------------------------------|----------------------------------------------------------|
| Aminoglycosides                                             | Gentamicin (GEN)                                         |
| Anti-MRSA cephalosporin                                     | Amikacin (AMK)                                           |
| Anti-MRSA cephalosporin                                     | Ceftaroline (CPT)                                        |
| Antipseudomonal penicillin + β-lactamase inhibitor          | Piperacillin/tazobactam (TZP)                            |
| Carbapenem                                                  | Imipenem (IMP)                                           |
| Non-extended spectrum cephalosporins                        | Meropenem (MEM)                                          |
| 3rd and 4th generation cephalosporins                      | Cefalexin (LEX)                                          |
| 3rd and 4th generation cephalosporins                      | Cefuroxime (CXM)                                         |
| Cephamycin                                                  | Cefepime (FEP)                                           |
| Fluoroquinolone                                             | Cefotaxime (CTX)                                         |
| Folate pathway inhibitor                                    | Ceftriaxone (CRO)                                        |
| Glycylcycline                                               | Trimethoprim/sulfamethoxazole (SXT)                     |
| Monobactam                                                  | Tigecycline (TGC)                                        |
| Penicillin                                                  | Aztreonam (ATM)                                          |
| Penicillin + β-lactamase inhibitors                        | Ampicillin (AMP)                                         |
| Penicillin + β-lactamase inhibitors                        | Amoxicillin/clavulanicacid (AMC)                         |
| Phenicol                                                    | Ampicillin-sulbactam (SAM)                               |
| Phosphonic acid                                             | Chloramphenicol (CHL)                                    |
| Polymyxins                                                  | Fosfomycin (FOS)                                         |
| Polymyxins                                                  | PolymyxinB(PB)                                           |
| Tetracycline                                                | Colistin (COL)                                           |
| Tetracycline                                                | Tetracycline (TET)                                       |
| Macrolides                                                  | Doxycycline (DOX)                                        |
| Macrolides                                                  | Azithromycin (AZM)                                       |

## Figures
Figure 1

STs, incompatibility plasmid groups and phylogenetic tree of the 33 swine-origin MCRPEC isolates. Phyllogenetic tree was generated by Neighbor joining method in MEGA-X, and toggled scaling of tree.
Figure 2

Antimicrobial resistance characteristics of the 33 swine-origin MCRPEC isolates from swine E. coli. (a) Antimicrobial resistance proportion, the bar chart showed the percentages of the 33 MCRPEC isolates that were sensitive (green), intermediate (yellow), or resistant (red) to 27 commonly used antimicrobials. (b) Statistics of the multi-antimicrobial category. (c) The first panel consists of 27 columns representing the sensitivity (green), intermediates (yellow), or resistance (red) of 27 antimicrobial agents. The next panel contains 18 columns indicating the antimicrobial category of the 33 MCRPEC isolates, including A: aminoglycosides, B: anti-MRSA cephalosporins, C: antipseudomonal penicillins + β-lactamase inhibitors, D: carbapenems, E: 1st and 2nd generation cephalosporins, F: 3rd and 4th generation cephalosporins, G: cephemycins, H: fluoroquinolones, I: folate pathway inhibitors, J: glycyclcyclines, K: monobactams, L: penicillins, M: penicillins + β-lactamase inhibitors, N: phenicsols, O: phosphonic acids, P: polymyxins, Q: tetracyclines, and R: Macrolides. The colors indicate resistance to all kinds agents of one antimicrobial category (black), resistant to some of the agents of one antimicrobial category (gray), no agent (white). The rightmost section are judgments for MDR, XDR, or PDR. Multidrug resistant (MDR) is the acquired (but not natural) insensitivity (mediated or resistant) to three or more antimicrobial agents (at least one in each category). Extensive resistant (XDR) means that it is insensitive to all antimicrobial species (at least one in each category) except for those in the 1-2 category. PDR is defined as non-susceptibility to all agents in all antimicrobial categories. The MICs of antimicrobial resistance test were putted in supplementary material Table 4.