Distribution of Fluoroquinolone Resistance Determinants in Carbapenem-Resistant *Klebsiella Pneumoniae* Clinical Isolates Associated with Bloodstream Infections in China

Qing Zhan
Jiangxi Provincial Key Laboratory of Preventive Medicine, School of Public Health, Nanchang University, Nanchang 330006

Yanlei Xu
Jiangxi Provincial Key Laboratory of Preventive Medicine, School of Public Health, Nanchang University, Nanchang 330006

Bingjie Wang
Department of Clinical Laboratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200082

Jingyi Yu
Department of Laboratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000

Xiaofei Shen
Department of Respiratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000

Li Liu
Department of Laboratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000

Xingwei Cao
Jiangxi Provincial Key Laboratory of Preventive Medicine, School of Public Health, Nanchang University, Nanchang 330006

Yinjuan Guo
Department of Clinical Laboratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200082

Fangyou Yu (✉ wzjxyfy@163.com)
Department of Clinical Laboratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200082

Research Article

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Abstract

Background The rate of fluoroquinolone (FQ) resistance among the carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is considerably high. The present study aimed to investigate the distribution of fluoroquinolone resistance determinants in CRKP clinical isolates associated with bloodstream infections (BSIs).

Result A total of 149 carbapenem-resistant *Klebsiella pneumoniae* clinical isolates causing bloodstream infections (BSIs) collected from 11 Chinese teaching hospitals from 2015 to 2018 were investigated for the prevalence of fluoroquinolone resistance determinants including plasmid-mediated quinolone resistance (PMQR) genes and spontaneous mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes. Among 149 CRKP clinical isolates, 117 (78.5%) exhibited resistance to ciprofloxacin. The substitutions (Ser83→Ile/phe) and (Asp87→Gly/Ala) of GyrA were found among 112 (75.2%) of 149 isolates. And the substitution (Ser80→Ile) of ParC was found in 111 (74.5%) of 149 isolates. Seventy point five percent (105/149) CRKP isolates had at least two mutations within *gyrA* as well as a third mutation in *parC*. No mutation of QRDRs was found in 31 sensitive CRKP isolates. Eighty-nine (56.9%) of 149 were found to carry PMQR genes including *qnrS1* (43.0%), *aac(6')-Ib-cr* (16.1%), *qnrB4* (6.0%), *qnrB2* (2.7%) and *qnrB1* (1.3%). Nine isolates contained two or more PMQR genes, with one carrying four PMQR genes including *aac(6')-Ib-cr*, *qnr-S1*, *qnrB2* and *qnrB4*). The co-existence rate of PMQR determinants and mutations of QRDRs in *gyrA* and *parC* reached 68.5% (61/89). Seventy-four (83.1%, 74/89) PMQR-positive isolates harbored ESBL genes. Multilocus sequence typing (MLST) analysis found that the ST types of PMQR-negative isolates were more concentrated relative to PMQR-positive isolates.

Conclusion Mutations in QRDRs of *gyrA* and *parC* were the key factor leading to the high prevalence of fluoroquinolones resistance among CRKP causing BSIs. PMQR genes can promote QRDRs and increase the resistance level of CRKP to fluoroquinolones in clinical settings. ST11 CRKP isolates with the same substitution patterns in QRDRs spread throughout hospitals in China.

Introduction

Klebsiella pneumoniae is a common pathogen causing nosocomial and community-acquired infections, which involved lung, urinary tract, surgical sites, soft tissue infections and bacteremia[1]. Carbapenem-resistant *K. pneumoniae* (CRKP) has emerged as a worldwide problem, posing severe challenges for its clinical management and public health, as they can cause healthy individuals to suffer from severe and untreatable infections[2, 3]. Bloodstream infections (BSIs) caused by CRKP is a more serious situation for ineffective antibacterial and high mortality[4]. CRKP usually shows high levels of resistance to many types of antibiotics[5]. The most optimal treatment options for CRKP infections have not been well defined. Current treatment options include the use of some older agents either in monotherapy or in combination therapy, such as fluoroquinolones and other agents[6, 7].

Fluoroquinolone (FQ) agents are important synthetic antimicrobial agents widely used in clinical and veterinary medicine since FQs exhibit broad-spectrum activity against a range of important clinical pathogens and excellent tissue permeation[8–10]. What's more, FQs have been proposed as the first choice to treat FQs susceptible ESBL-producing *Enterobacterial* organisms in pyelonephritis to reduce the use of carbapenems[11]. It is also used carbapenems with FQ to treat carbapenem non-susceptible *K. pneumoniae* infections. However, resistance to FQs has increased rapidly due to their overuse that limiting available treatment options or leading to treatment failure[12, 13]. Fluoroquinolones target DNA gyrase A and topoisomerase IV, which are encoded by *gyrA* and *parC*, respectively. The biological mechanisms of resistance to FQ include impermeability, active efflux, target modification, and antibiotic neutralization. Two major mechanisms for quinolone resistance are the acquisition of plasmid-mediated quinolone resistance (PMQR) genes (such as *aac(6')-Ib-cr* and *qnr*) and spontaneous mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes[14–16]. The PMQR mechanisms have recently been shown to induce low-level resistance to FQs and can be transferred horizontally. The coexistence of mutations in QRDRs and PMQR genes carriage can occur together have been reported in clinical isolates of *Enterobacteriaceae* with high-level quinolone resistance[16–18]. Alterations in both *gyrA* and *parC* often confer high-level resistance and are reported more frequently than alterations in *gyrB* or *parE*[14]. However, there are few studies about the prevalence of PMQR determinants and the diversity of DNA gyrase and topoisomerase IV mutations in clinical isolates of CRKP associated with BSIs in China. Accordingly, the current study aimed to investigate the prevalence, molecular characteristics, and distribution of PMQR determinants and mutations in the QRDRs of *gyrA* and *parC* among CRKP clinical isolates associated with bloodstream infections (BSIs) from 11 hospitals in China.

Materials And Methods

Collection and identification of *K. pneumoniae* clinical isolates

From April 2015 to November 2018, a total of 149 CRKP isolates were cultured from the blood of patients with bloodstream infections in 11 hospitals, including Zhejiang (n=22), Fujian (n=9), Shandong (n=26), Hubei (n=10), Henan (n=16), Shanghai (n=18), Jiangxi (n=40), and Hunan(n=8). These *K. pneumoniae* isolates were identified by Gram-staining and a VITEK-2 automated platform (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions, as well as additional biochemical testing. CRKP isolates were selected based on resistance to imipenem or meropenem according to Clinical and Laboratory Standards Institute (CLSI) guidelines[19]. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as control isolates for the identification and antimicrobial susceptibility test of bacterial clinical isolates.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MIC) of carbapenem (imipenem, (meropenem), fluoroquinolones (ciprofloxacin), aminoglycosides (amikacin and gentamicin), β-lactams/β-lactamase inhibitor complexes

(ceftazidime-avibactam and piperacillin-tazobactam), cephalosporin (ceftazidime, cefepime, cefotaxime, and cefoxitin), folate metabolic pathway inhibitors (sulfamethoxazole), polymyxin B, tetracyclines (tigecycline, minocycline, and tetracycline) and monocyclic β-lactam (aztreonam) were determined by the broth microdilution method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines[19]. The results were interpreted according to CLSI breakpoints. *E. coli* ATCC 25922 was used as a control isolate for antimicrobial susceptibility testing.

PCR detection and DNA sequence analyses of QRDR and PMQR

Genomic DNA (gDNA) of the 149 CRKP isolates was extracted using the Ezup Column Bacteria Genomic DNA Purification Kits (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. The Qubit and Nanodrop were used to determine the concentrations and purity of extracted gDNA. The nucleotide mutations in QRDRs of *gyrA* and *parC* were further tested by PCR and nucleotide sequencing with the primers described previously[20]. The nucleotide mutations were identified based on the available nucleotide sequences of *gyrA* and *parC* of *K. pneumoniae* ATCC 13833. Sequence alignment and analysis were performed online using the BLAST program (http://www.ncbi.nlm.nih.gov). Plasmids of 149 CRKPs were extracted using a Plasmid Midi Kit (Qiagen, Germany), and all isolates were screened for the presence of the PMQR genes, including *qnrA, qnrB, qnrS, qepA,* and *aac(6')-Ib-cr*, by PCR and DNA sequencing.

Detection of carbapenem resistance genes and ESBLs genes

Carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}) and ESBLs genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) of all CRKP isolates were detected by PCR with specific primers for each one, as previously reported[21, 22].

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed on all 149 CRKP isolates using primers of seven standard housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB* and *tonB*) listed in the PubMLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) according to previously published methods, and the sequence types (STs) were determined using the MLST database[23].

Statistical analysis

All CRKP isolates harboring different FQR mechanisms were analyzed by SPSS statistical software (version 20, IBM SPSS Statistics). We used the chi-square test for categorical variables. P-value <0.05 was considered significant.

All methods were carried out following relevant guidelines and regulations.

Results

The resistance of CRKP isolates to ciprofloxacin

Among 149 *K. pneumoniae* isolates, 117 (79.4%) exhibited resistance to ciprofloxacin (3 with ciprofloxacin MICs of 4 μg/ml, 70 with ciprofloxacin MICs of 8 μg/ml, 30 with ciprofloxacin MICs of 16 μg/ml, 14 with ciprofloxacin MICs of 32 μg/ml), whereas only 31 (20.8%) showed susceptibility to ciprofloxacin (MICs of ≦1 μg/ml), and one showed intermediate resistance to ciprofloxacin (MIC of 2 μg/ml).

Prevalence of mutations in the QRDRs of *gyrA* and *parC* among CRKP clinical isolates

Among 149 CRKP isolates, the nucleotide mutations of QRDRs were detected in 112 (75.2%) isolates (2 with ciprofloxacin MICs of 4 μg/ml, 65 with ciprofloxacin MICs of 8 μg/ml, 30 with ciprofloxacin MICs of 16 μg/ml, 14 with ciprofloxacin MICs of 32 μg/ml and one with ciprofloxacin MIC of 2 μg/ml). The substitutions of QRDRs were observed at position 83 (102 isolates with Ser83→Ile and 10 isolates with Ser83→phe) and position 87 (97 isolates with Asp87→Gly and 10 isolates with Asp87→Ala) of GyrA. The substitutions of Ser83→phe and Asp87→Ala co-existed in 10 ciprofloxacin-resistant isolates. Mutations in *parC* were only found at position 80 (Ser80→Ile) among 111 (74.5%) of 149 isolates. No *gyrB* and *parE* mutations were observed in any of the CRKP isolates. 105 ciprofloxacin-resistant CRKP isolates had at least two mutations within *gyrA* as well as a third mutation in *parC*, of which 95 had two mutations in *gyrA* (Ser83→Ile, Asp87→Gly) and one mutation in *parC* (Ser80→Ile) simultaneously, and 10 isolates had two mutations in *gyrA* (Ser83→phe, Asp87→Ala) and one mutation in *parC* (Ser80→Ile) simultaneously (table 1).

The 105 isolates with multiple mutations in QRDRs were distributed in 8 provinces, including Jiangxi (n = 38), Shandong (n = 10), Hubei (n = 7), Henan (n = 13), Shanghai (n = 16), Zhejiang (n = 9), Fujian (n = 9) and Hunan (n = 3).

Prevalence of PMQR determinants among 149 CRKP isolates

Among 149 CRKP isolates tested, 89 (56.9%, 89/149) including 73.0% (65/89) of ciprofloxacin-resistant isolates were found to carry at least one PMQR gene, including *qnrS1* (71.9%, 64/89), *aac(6')-Ib-cr* (27.0%,24/89), *qnrB4* (10.1%, 9/89), *qnrB2* (4.5%, 4/89) and *qnrB1* (3.4%, 3/89). Nine isolates contained two or more PMQR genes, including 3 with *aac(6')-Ib-cr* and *qnr-B2*, 2 with *aac(6')-Ib-cr* and *qnr-S1*, 1 with four PMQR genes (*aac(6')-Ib-cr*, *qnr-S1*, *qnrB2*, *qnrB4)*, 1 with *aac(6')-Ib-cr*, *qnr-S1* and *qnrB1,* 1 with *aac(6')-Ib-cr, qnrB1 and qnrB4* and *1 with aac(6')-Ib-cr, qnr-S1 and qnrB4.* However, *oqxAB*, *qepA*, *qnrA*, *qnrC*, and *qnrD* were not detected in these isolates tested. The *qnr* genes (59.7%, 74/149) were the major PMQR determinants, including 2 *qnr* families (*qnrB* and *qnrS)*. Twenty-eight (31.5%) of 89 with PMQR lwere not found the mutations of QRDRs, among which 4 were resistant to ciprofloxacin. Among 31 ciprofloxacin-susceptible isolates, 8 with no PMQR genes had ciprofloxacin MICs of ≦0.25 μg/ml and 23 carrying PMQR genes had increased ciprofloxacin MICs (10 with ciprofloxacin MICs of 0.5 μg/ml and one with ciprofloxacin MICs of 1 μg/ml) (Table 2). Ciprofloxacin-susceptible isolates with PMQR genes had higher MICs than those without PMQR genes.

Eighty-nine isolates carrying the PMQR genes were distributed in 8 provinces, including Jiangxi (n = 29), Shandong (n = 13), Hubei (n = 3), Henan (n = 8), Shanghai (n = 10), Zhejiang (n = 16), Fujian (n = 8) and Hunan (n = 2). Sixty-six isolates with the *qnr* family genes mainly distributed in Jiangxi and Zhejiang and 14 with *aac(6')-Ib-cr* mainly in Shandong.

The co-existence of PMQR andmutations in QRDRs

The co-existence rate of PMQR determinants and mutations in *gyrA* and *parC* of QRDRs was relatively high (68.5%, 61/89), with 60 being resistant to ciprofloxacin, and one being intermediary resistant to ciprofloxacin. Among 61 isolates with both PMQR and mutations in QRDRs, 45 (73.7%, 45/61) carried *qnrS1* and mutations in *gyrA* (Ser83→Ile, Asp87→Gly) and *parC* (Ser80→Ile), 3 carried *qnrB4* and mutations in *gyrA* (Ser83→Ile, Asp87→Gly) and *parC* (Ser80→Ile), 2 carried *qnrB4* and mutations in *gyrA* (Ser83→phe, Asp87→Ala) and *parC* (Ser80→Ile) (Table 1). These 61 isolates were distributed in 7 provinces, including Jiangxi (n = 29), Hubei (n = 3), Henan (n = 6), Shanghai (n = 8), Zhejiang (n = 6), Fujian (n = 8) and Hunan (n = 1).

Molecular characteristics of 149 CRKP clinical isolates

Six sequence types (STs) were identified among 112 CRKP isolates with mutations in QRDRs of *gyrA* and *parC*. ST11 being the most prevalent ST (86.6%, 97/112), followed by ST15 (7.1%, 8/112), ST2237 (1.8%, 2/112), and ST438 (1.8%, 2/112). ST485 and ST395 were found only one isolate. Among 89 CRKP isolates carrying PMQR genes, 51 were ST11 accounting for 57.7% (51/89), followed by ST45 (12.4%, 11/89). Furthermgore, among 61 CRKP isolates with co-existence of PMQR and mutation in QRDRs, ST11 was the most prevalent ST (83.6%, 51/61), followed by ST15 (6.5%, 4/61). Ninety-five ST11 CRKP isolates with complete same mutations in QRDRs were distributed in 8 provinces, including Jiangxi (n = 38), Shandong (n = 10), Hubei (n = 7), Henan (n = 10), Shanghai (n = 15), Zhejiang (n = 3), Fujian (n = 9) and Hunan (n = 3).

Molecular characteristics, carbapenem resistance genes profiles, and antimicrobial resistance among PMQR-positive and PMQR-negative CRKP isolates

Among 89 PMQR-positive isolates, a total of 14 ST types were identified. ST11 was the most prevalent ST (57.3%, 51/89), followed by ST45 (12.4%, 11/89), ST290 (10.1%, 9/89), ST15 (4.5%, 4/89), ST438 (2.2%, 2/89), ST1319 (2.2%, 2/89), ST2237 (2.2%, 2/89). ST37, ST107, ST395, ST462, ST485, ST1692, ST2236 were found only one isolate. Among 60 PMQR-negative isolates, 8 ST types were identified that included ST11 (78.3%, 47/60), ST15 (6.7%, 4/60), ST307 (5.0%, 3/60), ST35 (3.3%, 2/60), ST45, ST375, ST485 and ST2390 were found only one isolate.

Among 89 PMQR-positive CRKP isolates, bla_{KPC-2} (61 isolates) was the most frequent, then followed by bla_{NDM-5} (18 isolates), bla_{NDM-1} (17 isolates), bla_{IMP-4} (1 isolate). Among 60 PMQR-negative CPKP isolates, bla_{KPC-2} (53 isolates) was the most frequent, then followed by bla_{NDM-5} (4 isolates), bla_{NDM-1} (4 isolates),

bla$_{IMP-30}$ (3 isolates). Compared to PMQR-negative isolates, PMQR-positive isolates harbored fewer *bla*$_{KPC}$ genes but carried more *bla*$_{NDM}$ genes (Table 3).

Among 149 CRKP isolates, relative to PMQR-negative isolates, PMQR-positive isolates are more sensitive to gentamicin and amikacin but have higher resistance rates to ceftazidime/avibactam, tetracycline, minocycline, and sulfamethoxazole. PMQR-negative isolates are more resistant to ciprofloxacin due to mutation at QRDRs.

Discussion

Over the past few decades, the emergence of CRKP has caused an increasing threat to public health worldwide[2]. We collected a total of 149 CRKP isolates from clinical patients with bloodstream infections from 11 teaching hospitals across China. FQs have broad-spectrum antimicrobial activities against both gram-positive and gram-negative bacteria and have been used widely since the 1980s[24]. In our study, 78.5% (117/149) exhibited resistance to ciprofloxacin, it is noteworthy that the majority of CRKP isolates tested showed high-level resistance to ciprofloxacin and were distributed in eight provinces surveyed of China.

The most prevalent mechanisms of FQs resistance in *K. pneumoniae* involve mutations in QRDRs. Resistance to FQs has been shown to be associated with alterations in the GyrA subunit of DNA gyrase and the ParC subunit of DNA topoisomerase IV[25]. Besides, Georgiou et al. reported that some key mutations identified in *gyrA* and *parC* were associated with high-level resistance to ciprofloxacin in 1996[26]. In our study, the mutations in QRDRs among 117 ciprofloxacin-resistant isolates reached 94.8%, and all high resistance levels (MICs of 16 and 32) are caused by QRDR mutations. Ser80→Ile in ParC (111/149, 74.5%) is the most common substitution in the 149 CRKP isolates. Ser 83→Ile/Phe and Asp87→Ala/Gly in GyrA was also observed frequently. These GyrA and ParC substitutions observed in this study have already been reported[14]. All CRKP isolates containing mutations in QRDRs were not sensitive to ciprofloxacin, it suggests that QRDRs mutations are the key cause of FQs resistance. Multiple amino acid substitutions in QRDRs are needed to acquire high-level resistance to FQs[9, 27]. In the present study, the isolates possessing double or more amino acid substitutions in QRDRs were highly prevalent, there are 105 FQ-resistant CRKP isolates had at least two mutations within *gyrA* as well as a third mutation in *parC*. The rate of FQs resistance in *K. pneumoniae* has become very high in some parts of Europe, CRKP usually belongs to several genetic lineages, such as the high prevalence of ST11[28]. ST11 is the most frequent ST-types in 117 ciprofloxacin-resistant isolates in our results. Almost all ST11 isolates had complete identical mutation patterns at QRDRs and were distributed in 8 provinces in China, indicating the distribution of ST11 isolates which suggests that these isolates with the same FQs resistant genes profiles may be disseminated vertically by clonal and multiclonal expansion. Mutations of the same pattern have been found in other studies, but not such large accumulations in CRKP clinical isolates [29].

PMQR determinants (*qnr* and *aac(6')-Ib-cr* genes) have been found in plasmids and are generally thought to confer only low levels of FQ resistance[30]. The PMQR gene can not only provide the topoisomerase protection proteins (*qnr) and* the acetylation enzyme variant (*aac(6')-Ib-cr* genes) but also act as a mobile genetic element to cause the spread of resistance mechanism because antibiotics are used more frequently in clinical infections of CRKP. A higher PMQR carrying rate (59.7% 89/149) was detected with *K. pneumoniae* donors in the current study, which may explain the predominance of PMQR genes among *K. pneumoniae* isolates[29]. The most frequently detected PMQR gene was *qnrS1*, followed by *aac(6')-Ib-cr*, *qnrB4*, *qnrB2* and *qnrB1* in all isolates, this was not consistent with other people's reports of *aac(6')-Ib-cr* dominance[31]. Nine isolates contained two or more PMQR genes, of which 1 carried four PMQR genes (*aac(6')-Ib-cr*, *qnr-S1*, *qnrB2, qnrB4*). To the best of our knowledge, the pattern of co-existence of four plasmid genes (*aac(6')-Ib-cr*, *qnr-S1*, *qnrB2*, *qnrB4*) in CRKP was firstly reported in the current study. In our study, among 31 fluoroquinolone-sensitive CRKP isolates, the PMQR-positive isolates had an increased MICs value than PMQR-negative isolates, suggesting that PMQR can indeed mediate low levels of drug resistance or increase the MICs of sensitive isolates to fluoroquinolones. It seems notable that 4 ciprofloxacin-resistant isolates investigated in the present study had no amino acid substitutions in their QRDRs, but all of them had at least one PMQR gene, and the 4 isolates showed considerably resistance levels to FQs (ciprofloxacin MICs of 8 μg/L) by possessing wild-type gyrA and parC, which suggesting that PMQR can also mediate drug resistance, although the MIC value is not as high as some CRKP isolates with mutations in QRDRs. Furthermore, it notable that 1 ciprofloxacin-resistant isolate investigated in the present study had no mutations in QRDRs and without PMQR genes. We speculate that in addition to the resistance increase caused by PMQR genes, there may be other undetected mechanisms like altered permeability or efflux pump systems.

Yukiko Nagasaka et al. mentioned cephalosporin-resistant *K. pneumoniae* isolates, including ESBL-producing ones, tend to demonstrate resistance to FQs[29]. It was reported that double-serine residues were often observed to mutate in the isolates of the major international sequence types of ESBL-producing *K. pneumonia* (*gyrA* Ser83→Phe/Ile; *parC* Ser80→Ile)[9]. What's more, ESBL genes can be co-transferred on the same mobile genetic elements with PMQR genes and they are very related[32, 33]. In our results, 91.8% FQR isolates with PMQR genes harbored ESBL genes and are predominantly bla_{CTX-M}, and 90.6% FQ-r CRKP isolates with mutations in QRDRs also harbored ESBL genes. Furthermore, compared to PMQR-negative isolates, PMQR-positive isolates harbored fewer bla_{KPC} genes but carried more bla_{NDM} genes ($p < 0.01$). It indicated that there may be a co-transfer phenomenon of PMQR with NDM in CRKP isolates. This result is similar to the study of Shravani Mitra et. al[34].

In conclusion, we characterized 149 CRKP clinical isolates associated with BSIs from 11 hospitals located in 8 different provinces of China. In our study, mutations in QRDRs and PMQR genes are highly prevalent in CRKP clinical isolates in China. Mutations in QRDRs of *gyrA* and *parC* were the vital factor leading to the resistance of CRKP to fluoroquinolones, PMQR genes can also increase the resistance level of CRKP to fluoroquinolones. Furthermore, their co-existence leads to high levels of FQs resistance that are also common in this study. ST11 with the same resistant mechanism is the most prevalent, spread

across the eight provinces we investigated, so we should remain vigilant to prevent its further spread. It is recommended that antibiotics, especially quinolone antibiotics, be used reasonably in the treatment of CRKP clinical infections.

Declarations

Ethical approval and consent to participate.

Written informed consent was obtained from the patients involved in the current study. The Ethics Committee of the Shanghai Pulmonary Hospital of Tongji University School of Medicine approved our study. All experiments in our study strictly adhere to the guideline of the Ethics Committee of the Shanghai Pulmonary Hospital of Tongji University School of Medicine.

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Authors' contributions

QZ, XS, YX, LL, JY, XC, YG isolated bacteria and performed the laboratory measurements. QZ, YX collated and analyzed the data. QZ drafted the article. FY made substantial contributions to conception and work design. BW made a critical revision of the article. All authors read and approved the final article.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request. Most of the data is included in this article.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interest in this work.

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Tables

Table 1. Patterns and distribution of GyrA and ParC substitutions and PMQR in 149 CRKP clinical isolates

CIP(MIC)	Mutation	Carrying PMQR (Frequency)	Frequency	MLST (Number of isolates)
S	No substitution	*aac(6')-Ib-cr* (12); *qnr-S1*(9); *qnrB1*(1); *aac(6')-Ib-cr*, *qnr-S1*(1); *aac(6')-Ib-cr*, *qnr-S1, qnr-B4*(1)	31	ST107(1); ST1319(2); ST2390(1); ST290(7); ST307(3); ST35(2); ST37(1); ST375(1); ST45(12); ST462(1).
I	ParC-80I; GyrA-83I; GyrA-87G	*qnr-S1*(1)	1	ST11(1)
R	No substitution	*aac(6')-Ib-cr* (1); *qnr-S1*(1); *qnrB4*(1); *aac(6')-Ib-cr*, *qnr-S1*(1); *aac(6')-Ib-cr*, *qnr-S1, qnr-B2*(1)	6	ST1692(2); ST290(1); ST290(1); ST2236(1)
R	ParC-80I; GyrA-83F; GyrA-87A	-	4	ST15
R	ParC-80I; GyrA-83F; GyrA-87A	*aac(6')-Ib-cr*(2); *qnrB4*(2): *aac(6')-Ib-cr,qnr-S1*, *qnrB1*(1); aac(6')-Ib-cr,qnrB2(1)	6	ST15(4); ST2237(2)
R	ParC-80I; GyrA-83I; GyrA-87G	-	47	ST11(47)
R	ParC-80I; GyrA-83I; GyrA-87G	*qnrS1*(44); *qnrB4*(3); *aac(6')-Ib-cr, qnrB1, qnrB4*(1)	48	ST11(48)

R	GyrA-83I; GyrA-87G	*qnrS1*(1)	1	ST11
R	ParC-80I; GyrA-83I	*aac(6')-Ib-cr*(1)*; qnrS1(2);* *aac(6')-Ib-cr, qnr-S1, qnrB2, qnrB4*(1) *aac(6')-Ib-cr qnrB2*(1)	5	ST438(2); ST485(1); ST11(1); ST395(1)

CIP: Ciprofloxacin; MIC, minimum inhibitory concentration; R: Resistance; I: Intermediate; S: Sensitive

Table 2. The role of PMQR in 31 FQ-sensitive CRKP isolates

Number of isolates	Carrying PMQR (Frequency)	Mutation in QRDRs	CIP MIC (μg/ml)
8	-	No substitution	≦0.25(8 isolates)
23	*aac(6')-Ib-cr*(11)*;* *qnr-S1*(9); *qnrB1*(1); *aac(6')-Ib-cr*,*qnr-S1*(11); *aac(6')-Ib-cr*, *qnr-S1*, *qnrB4*(1)	No substitution	0.5(10 isolates) 1(1 isolates) ≦0.25(12 isolates)

CIP: Ciprofloxacin; MIC, minimum inhibitory concentration

Table 3 Antibiotic resistance gene profiles and antimicrobial resistance profiling in PMQR-positive and PMQR-negative isolates

Antimicrobial resistance profiling		CRKPs(n=149)				P-values
		PMQR+ (n=89)	□	PMQR- (n=60)	□	
Carbapenem resistance genes	bla_{NDM}	26	29.2	3	5	<0.01
	bla_{KPC}	51	57.3	48	80	<0.01
	bla_{IMP}	0	0	3	5	>0.05
	bla_{NDM}+bla_{KPC}	9	10.1	5	8.3	>0.05
	bla_{IMP}+bla_{KPC}	1	1.1	0	0	1
ESBL genes	bla_{CTX-M}	41	46.1	31	51.7	>0.05
	bla_{SHV}	5	5.6	5	8.3	>0.05
	bla_{CTX-M}+bla_{SHV}	26	29.2	21	35	>0.05
Antimicrobial	Imipenem	88	98.9	58	96.7	>0.05
	Meropenem	89	100.0	60	100	>0.05
	Cefoxitin	86	96.6	58	96.7	>0.05
	Cefotaxime	88	98.9	60	100	>0.05
	Cefepime	88	98.9	59	98.3	>0.05
	Ceftazidime	87	97.8	59	98.3	>0.05
	Aztreonam	79	88.8	58	96.7	>0.05
	Gentamicin	52	58.4	45	75.0	<0.05
	Amikacin	35	39.3	40	66.7	<0.01
	Ceftazidime/avibactam	28	31.5	9	15.0	<0.05
	Polymyxin B	2	2.2	3	5.0	>0.05
	Tigecycline	4	4.5	0	0	>0.05
	Piperacillin/tazobactam	80	89.9	54	90.0	>0.05
	Ciprofloxacin	65	73.0	52	86.7	<0.05
	Tetracycline	68	76.4	12	20.0	<0.01
	Minocycline	52	58.4	12	20.0	<0.01
	Sulfamethoxazole	65	73.0	17	28.3	<0.01

PMQR+: Represents the CRKP isolates with PMQR gene

PMQR−: Represents the CRKP isolates without PMQR gene.

+: Represents one CRKP isolate harboring two antibiotic resistance genes simultaneously.

P <0.05 was considered statistically significant.