

Molecular characteristics of carbapenem-resistant Enterobacteriaceae isolated from clinical infection and fecal survey samples in Southern China

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Research

Keywords: carbapenem-resistant Enterobacteriaceae, carbapenemase, mcr, KPC-2, fecal, molecular characteristics

Posted Date: August 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52967/v1>

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Abstract

Background: The identified rate of carbapenem-resistant Enterobacteriaceae (CRE) have been increasingly in the clinical infections. Here, a study of analysing the relationship of clinical infectious CRE and fecal carried CRE was performed.

Methods: Clinical CRE and fecal CRE were collected from hospital in China. Polymerase chain reaction (PCR)-based amplification and sequencing were performed to analyse the carriage of drug-resistant genes and mobile genetic elements (MGEs), Enterobacterial Repetitive Intergenic Consensus (ERIC) technology and whole genome sequencing (WGS) were used to analysis the characteristic of genetic structure of CRE isolates.

Results: 99 clinical CRE and 30 fecal CRE were collected, respectively. The top three strains present in the highest proportions were *K. pneumoniae* (86; 66.67%), *E. cloacae* (22; 17.05%), and *E. coli* (11; 8.53%). Most of the isolates were susceptible to colistin (98.45%) and tigecycline (98.45%). *bla*_{KPC-2} (96.03%) was the dominant carbapenemase gene in clinical CRE and fecal CRE, followed by *bla*_{NDM} (52.7%); co-existence of the *bla*_{KPC-2} and *bla*_{NDM} genes was detected in 63 (50.00%) strains. One *K. pneumoniae* isolate co-producing NDM-5 and mcr-1, and one *E. coli* isolate co-producing KPC-2, IMP-4, and mcr-1 were detected. Two novel gene cassettes of intI2 were detected. ERIC genotyping and genomic analysis revealed that *K. pneumoniae* isolated from clinical infections and fecal survey samples were same clone.

Conclusions: This is the first report of comparing the molecular characteristics of CRE isolated from clinical infection and fecal survey samples. we found that fecal carried CRE were closely related to CRE which caused infections.

Background

Carbapenem-resistant Enterobacteriaceae (CRE) have been listed by the World Health Organization (WHO) as high priority pathogens owing to their association with high mortality and morbidity^[1]. CRE have spread rapidly around the world, posing great challenges to human health. The China Antimicrobial Resistance Surveillance Report (<http://www.carss.cn/>), the largest survey of antimicrobial resistance in China, reported that the rate of CRE is increasing every year; in particular, the rate of carbapenem-resistant *K. pneumoniae* increased from 4.9% in 2013 to 10.1% in 2018. Carbapenem resistance in *K. pneumoniae* has been reported in all WHO regions and exceeded 50% of all isolates in two regions^[2]. CRE can cause multiple infections, most frequently pneumonia, followed by urinary tract infections, primary bloodstream infections, skin and soft tissue infections, and surgical site infections^[3]. Patients infected with CRE are at an increased risk of death due to the lack of appropriate antibiotics. CRE, especially carbapenem-resistant *K. pneumoniae* (CRKP), have a high potential to cause outbreaks in healthcare settings. Such outbreaks have been reported from several EU member states; e.g., the Czech Republic, France, Germany, Greece, Italy, Spain, the Netherlands, and the UK^[4-10]. Therefore, the timely and accurate detection of CRE is important; investigating the mechanism of transmission and the route of drug resistance will also be necessary.

Intestinal flora forms a major reservoir of various bacteria, including multidrug resistant (MDR) bacteria, which serves as an important and large reservoir of resistance genes. It may greatly increase the transmission of resistance genes and the probability of nosocomial infections caused by MDR bacteria^[11]. In particular, CRE can colonize the intestines of patients or individuals without underlying disease, greatly increasing the risk of clinical infection. Thus, studying the drug-resistant and molecular characteristics of CRE between clinical infection and fecal survey samples will also be important.

CRE can lead to clinical infection, which will increase the risk of standard antimicrobial therapy failing and increase the difficulty of treatment. CRE can also become part of the intestinal flora of healthy individuals who have not been exposed to antimicrobials. There is a risk of hidden transmission of CRE, and possibly even CRE outbreaks. The aim of this study was to compare the molecular characteristics of CRE isolated from clinical infection and fecal survey samples in Southern China, and provide information for the prevention and control of CRE infections.

Methods

Bacterial isolates and species identification

Between July 2016 and June 2019, 99 CRE were obtained from clinical infection samples (including sputum, puncture fluid, urine, instrument, and blood) collected from Nanfang Hospital, a large general and teaching hospital in Guangzhou, China.

A total of 2,000 fecal survey samples were collected from July 2016 to June 2019, followed by incubation on MacConkey agar plates with 2 µg/mL meropenem. All isolates were confirmed as Enterobacteriaceae by biochemical methods, and a total of 30 CRE were collected. The study was approved by the Medical Ethics Committee of Nanfang Hospital Southern Medical University and conducted in compliance with the Declaration of Helsinki.

Antimicrobial susceptibility testing

Analysis of susceptibility to antimicrobial compounds was performed using the automated BD Phoenix 100 Microbiology System (Becton Dickinson and Co., Franklin Lakes, NJ, USA) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M100-S29 criteria. In addition, minimum inhibitory concentration (MIC) breakpoints of susceptibility to tigecycline were determined by E-test according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for antimicrobial susceptibility testing.

Molecular detection of carbapenemase genes

The following carbapenemase-encoding genes were detected: the class A β-lactamase gene *bla*_{KPC}^[12]; class B metallo-β-lactamase genes: *bla*_{IMP}^[12], *bla*_{VIM}^[12], *bla*_{SPM}^[12], and *bla*_{NDM}^[12]; and class D oxacillinase genes: *bla*_{OXA-48-like}^[12]. The common ESBL genes *CTX-M*^[13], *SHV*^[13], and *TEM*^[13] were also

detected.

Molecular detection of colistin-related resistance genes

The colistin-related resistance genes *mcr-1*^[14], *mcr-2*^[14], *mcr-3*^[14], *mcr-4*^[14], and *mcr-5*^[14] were detected. There are substantial differences in the nucleotide sequences of the five subtypes of the *mcr* gene, so each subtype had a specific pair of primers.

Detection of MGEs

MGE-related resistance genes, including integrons (*int1*^[15,16], *int2*^[15,17], and *int3*^[15]) and ISCRs^[18], including the variable region of class 1 integron, class 2 integron, and ISCR1, were also analyzed.

The primers including the IncFII plasmid replicon^[19] used for PCR are shown in Supplementary Table 1.

Whole genome sequencing and analysis

Three KPC-producing *K. pneumoniae* 156070, 158590, and NFYY0065 were submitted for WGS. The whole genome was sequenced using the Single Molecule Real-Time sequencing platform with the PacBio sequencer and Illumina HiSeq at the Health Time Gene Institute (Shenzhen, China).

ERIC-PCR

ERIC-PCR was performed on all isolates depending on the species^[20]. Band comparisons were performed by clustering analysis based on the unweighted pair-group method with arithmetic mean of isolates using NTSYS.

Results

Identification of isolates

During the study period, clinical infection samples were collected from 20 departments of Nanfang hospital; the top four departments were hematology (37.4%), intensive care unit (ICU) (22.2%), rehabilitation (7.1%), and the surgical department (4.0%). A total of 99 CRE from clinical infection samples and 30 CRE from fecal survey samples were isolated. There was no obvious difference in the types of CRE between clinical and fecal samples. The species present in the highest proportions among the six different species isolated from clinical infection samples were *K. pneumoniae* (73.74%), *E. cloacae* (15.15%), and *E. coli* (7.07%); the species present in the highest proportions among the seven different species isolated from fecal survey samples were *K. pneumoniae* (43.33%), *E. cloacae* (23.33%), and *E. coli* (13.33%) (Table 1).

Antimicrobial susceptibility testing

According to the MIC values of tested antibiotics (Supplementary Tables 2 and 3). All isolates were resistant to cepheems and carbapenems, and most of the isolates were sensitive to colistin and tigecycline. Among clinical CRE, the sensitivities to amikacin, tetracycline, and sulfamethoxazole were 35.35%, 35.35%, and 32.32%, respectively. Among fecal CRE, the sensitivities to amikacin, tetracycline, and sulfamethoxazole were 56.67%, 36.67%, and 40.00%, respectively. We found that the sensitivities of different species to amikacin and tetracycline vary widely, and CRE isolated from feces were generally more sensitive to antibiotics than clinical strains with the exception of *K. pneumoniae* (Table 2).

Carbapenemase-encoding genes

Strains with carbapenemase genes accounted for 126 (97.67%) of all the isolates; 98 (98.99%) CRE isolated from clinical samples and 28 (93.33%) CRE isolated from fecal samples were positive for carbapenemase genes. Among isolates containing carbapenemase genes, the most prominent carbapenemase gene was *blaKPC-2* (121; 93.8%) and *blaNDM* was the second most frequent (68 isolates, 52.7%). *blaNDM-1* and *blaNDM-5* were detected in 40 clinical strains and 11 clinical strains, respectively, and fecal strains carried four types of *blaNDM* genes: *blaNDM-1* (11; 39.29%), *blaNDM-5* (5; 17.86%), and *blaNDM-4* (1; 3.57%). Co-existence of the *blaKPC* and *blaNDM* genes was detected in 47 clinical strains, and co-existence of the *blaKPC* and *blaNDM* genes was detected in 15 fecal strains. It is notable that 20 strains simultaneously carried the *blaKPC* genes and two types of metallo- β -lactamases genes, including 17 clinical and 3 fecal isolates. For other carbapenemase genes in all strains, 38 (29.46%) isolates were positive for *blaIMP*, 4 (3.10%) for *blaVIM*, and 125 (96.90%) for ESBL genes. *mcr-1* was only detected in fecal CRE, including one *K. pneumoniae* isolate co-producing NDM-5 and *mcr-1*, and one *E. coli* isolate co-producing KPC-2, IMP-4, and *mcr-1*. *blaOXA-48* and *blaSPM* were not identified (Table 3).

MGE-related resistance genes

Class I and II integrons and ISCR1 were detected in 101 (78.29%), 6 (4.65%), and 90 (69.77%) strains, respectively, while class III integrons were absent. Class I and II integrons and ISCR1 were detected in 77 (77.77%), 2 (2.02%), and 71 (71.71%) strains isolated from clinical samples, and class I and II integrons and ISCR1 were detected in 24 (80.00%), 4 (13.33%), and 19 (63.33%) strains isolated from feces, respectively. The prevalence of *int1*, *int2*, and ISCR1 varied in different species (Supplementary Table 4); the gene cassettes of *int1*, *int2*, and ISCR1 were detected in 29 (28.71%), 3 (50%), and 4 (4.44%), respectively. Sequencing results revealed that the variant region of class 1 integron has eight distinct gene cassettes, and the variant region of the class 2 integron had two novel gene cassettes (*dfrA1-sat2-aadA1-ybeA-ybfA-ybfB-ybgA-ORF(5)-Tra*, *dfrA1-sat2-aadA1-ybeA-ybfA-ybfB-ybgA-IS3-IS110-IS3-Tra-ybgA*) (Table 4).

Replicon of IncFII plasmids

Most of the strains (60.5%, 78/129) were positive for the replicon of IncFII plasmids. In the clinical and fecal strains, the IncFII replicon was observed in 69.23% (54/78) and 30.77% (24/78) of strains, and the IncFII replicon (84.62%, 66/78) was carried from *K. pneumoniae*.

Comparative genomic analysis of two KPC-CRKP

Using WGS, we determined that the complete genome of 156070-KPC-CRKP consists of a 5,415,677 bp circular chromosome (chr156070) and a 138,959 bp plasmid (p156070-KPC). The complete genome of 158590-KPC-CRKP consisted of a 5,439,711 bp circular chromosome (chr158590), a 138,959 bp plasmid (p158590-KPC), and a 10,060 bp plasmid (data not shown). Both 156070-KPC-CRKP and 158590-KPC-CRKP contained 89 drug-resistant genes, and there were four genes related to β -lactamase antibiotic resistance: *SHV-11*, *KPC-2*, *CTX-M-65*, and *TEM-1b*.

The complete sequence analysis showed that chr156070 contained 5227 genes with a G + C content of 57.40%, and chr158590 contained 5262 genes with a G + C content of 57.37%. Both chr156070 and chr158590 contained *blaSHV-11*. BLAST analysis revealed that similarities in the chromosomes of the two KPC-CRKP were greater than 95%. Comparative genomic analysis showed that the core genes of chr156070 and chr158590 accounted for 89% of all genes, and chr156070 had no specific resistant genes compared with chr158590. Co-linear analysis found that although there were structural differences between chr156070 and chr158590, they had a high degree of similarity and high coverage for one another (Fig. 1). They are ST11 by multilocus sequence typing. chr156070 and chr158590 were similar. BLASTn analysis revealed that chr156070 and chr158590 were highly similar to 18CPO060 and KPNH45, and phylogenetic analysis showed that chr156070 and chr158590 had a close evolutionary relationship and high affinity to KPNH45, which was derived from CRKP isolated from a US hospital in 2018.

The p156070-KPC is a circular plasmid containing 184 putative open reading frames (ORFs) with a G + C content of 53.95%, and p158590-KPC was a circular plasmid containing 184 putative ORFs with a G + C content of 53.95%. Both p156070-KPC and p158590-KPC had the same replication protein RepA1 of IncFII plasmids. The p156070-KPC had one unique gene relative to p158590-KPC, and p158590-KPC had two unique genes relative to p156070-KPC. Both p156070-KPC and p158590-KPC co-carried the *blaKPC-2*, *blaCTX-M-65*, and *blaTEM-1b* genes. BLAST analysis showed that p156070-KPC was highly similar to p158590-KPC at the nucleotide level (100% identities and 100% query coverage), and colinearity analysis revealed that the plasmid was divided into three parts: the first segment plus chain of p156070-KPC and p158590-KPC were performed collinear comparison from 1 bp to 32195 bp, with a similarity of 99%; the second segment of p156070-KPC and p158590-KPC plasmid were performed translocation comparison from 31,374 bp to 106,077 bp, with a similarity of 100%, the phenomenon might be result from the rearrangement of small fragments that made up the A fragment and B fragment due to MGEs; the third segment 105,256 bp to 138,959 bp of p156070-KPC and p158590-KPC were collinear comparison, with a similarity of 100% (Fig. 2).

The resistant gene structure of the two plasmids showed that *blaKPC-2* and *blaCTX-M-65* existed in the prophage sequence, with surrounding MGEs, which suggested that the *blaKPC-2* and *blaCTX-M-65* genes of the two plasmids were from the insertion of foreign genes (Supplementary Fig. 1). BLASTN analysis showed that p156070-KPC and p158590-KPC were 96% similar to the previously reported IncFII plasmid p283747-KPC (MF168406.1), which harbored *blaKPC-2* between ISKpn27 and ISKpn6 (Fig. 3), the structures of *blaCTX-M-65* and *blaTEM-1* were basically identical to pKSH203-KPC (CP034324.1).

Phylogenetic analysis showed that p156070-KPC and p158590-KPC are closely genetically related to most of the IncFII plasmids previously reported (Fig. 4). The 17 IncFII plasmids were selected from the database, and the pNFY0Y11 plasmids were the IncFII plasmids carrying the *blaKPC* gene sequenced by us (Supplementary Table 5).

ERIC-PCR

Genotyping by ERIC-PCR demonstrated greater than 90% similarity of *Klebsiella pneumoniae* in different samples, including clinical infection and fecal survey samples (Supplementary Fig. 2), suggesting they may have originated from the same clone. However, *E. cloacae* and *E. coli* detected in clinical and fecal samples had a high genetic diversity.

Discussion

In all isolates, the top three species were *K. pneumoniae* (66.67%), *E. cloacae* (17.05%), and *E. coli* (8.53%); thus, CRKP was more abundant than other species. This is consistent with Spiliopoulou *et al.*^[21], which showed that *K. pneumoniae* was present in 1,137 (77.9%) of 1,460 clinical infectious samples of hospitalized patients around the world between 2015 and 2017. The number of clinical infections caused by *K. pneumoniae* is increasing; in particular, the number of CRKP is increasing^[22]. In our study, the species present in the highest proportions in fecal survey samples were also *K. pneumoniae*, *E. cloacae*, and *E. coli*. The fecal samples were primarily obtained from individuals who had not previously used related antibiotics; however, they carried a high proportion of CRE similar to clinical infection samples, indicating that CRE can also exist in the intestines of normal individuals without a history of antibiotic use and that fecal CRE may be associated with CRE causing clinical infection.

The sensitivity of CRE to antibiotics has reduced, which is a challenge for clinical treatment. In our study, clinical and fecal CRE were resistant to most of the antibiotics tested and only showed a high sensitivity to tigecycline and colistin. The susceptibilities of clinical CRE to tigecycline and colistin were 100% and 100%, and the susceptibilities of fecal CRE were 93.33% and 93.33%, respectively. Spiliopoulou *et al.*^[21] reported that the susceptibilities of meropenem-non-susceptible Enterobacteriaceae to colistin and tigecycline were 92.4% and 77.4%, respectively; colistin and tigecycline were shown to be active against MBL-positive isolates (susceptibilities of 92.1% and 71.9%, respectively) in Asia. Wang *et al.*^[23] reported that 1801 CRE isolates showed high susceptibility to colistin (96.9%), followed by tigecycline (89.7%). Thus, CRE are still highly sensitive to colistin and tigecycline; in support of this, we found that colistin has high activity against CRE in Southern China, which will help in the choice of treatment of clinical infections. The susceptibility of different CRE species to amikacin varied, with a lower rate in *K. pneumoniae* (24.4%) than in *E. coli* and *E. cloacae* (>72%). This result provides important data for selecting specific drug and aminoglycoside combinations for empiric therapy of infections caused by these species.

CRE is resistant to carbapenem antibiotics for different reasons, but the high rate of carbapenemase genes detected in this study is likely the primary mechanism. Our data showed that the prevalent carbapenemases in Southern China primarily encoded KPC and NDM, and especially KPC, which may be related to a high proportion of CRKP. Notably, *blaKPC* is also commonly found in other species. KPC has already spread widely to different species, mainly KPC-2 in Southern China. We found a similar proportion of *blaKPC* between clinical and fecal CRE, especially CRKP, indicating that fecal KPC-CRKP may be closely related to KPC-CRKP isolated from clinical infection samples. In addition, we found that one *K. pneumoniae* isolate contained *blaNDM-5* and *mcr-1*, and one *E. coli* isolate contained *blaKPC-2*, *blaIMP-4*, and *mcr-1*, which have not been reported previously. The *mcr* and carbapenemase-positive strains were isolated from fecal survey samples from individuals who had not previously used carbapenem and colistin, indicating that *mcr-1* and carbapenemase genes may also be present in the gastrointestinal tract of patients who have not used related antibiotics. If individuals expressing resistance genes have infections, it will lead to the failure of antibiotic treatment, and increase the risk of transmission to other patients. Therefore, strengthening the monitoring of feces is necessary to prevent the spread of drug-resistant bacteria and drug-resistant genes.

Our data showed that clinical and fecal CRE carry MGE-related genes, which is consistent with the high prevalence of carbapenemase genes. Carbapenemase-producing CRE strains carry resistance genes on mobile plasmids or MGEs that can shuttle between resistant and susceptible strains^[24, 25], especially those encoding KPC and NDM. This suggested that MGEs were important for the spread of carbapenemase genes and multidrug resistance of strains. In addition, we identified two novel gene cassette structures of class II integrons, which have not been previously reported.

We found a high genetic diversity of *E. cloacae* and *E. coli* in different samples by ERIC genotyping; however, *K. pneumoniae* detected in clinical and fecal samples showed greater than 90% similarity, indicating that fecal CRE were closely related to CRE isolated from clinical infection samples, and that they may have originated from the same clone. Clinical and fecal samples were obtained from different places in South China, suggesting that KPC-CRKP has spread widely. However, all strains were isolated from samples collected between 2016 and 2019, indicating that there was no outbreak in the short term.

Conclusions

This was the first study to compare the molecular characteristics of CRE isolated from clinical infection and fecal survey samples in Southern China. We found that CRKP isolated from fecal survey samples were closely related to CRKP isolated from clinical infections samples. CRE can spread between human, animals and the environment by fecal contamination and especially outbreak. Thus, Strengthening the monitoring and management of feces is essential for the prevention and control of CRE.

Abbreviations

CRE carbapenem-resistant Enterobacteriaceae

CRKP carbapenem-resistant *Klebsiella pneumoniae*

MDR multidrug-resistant

ERIC enterobacterial repetitive intergenic consensus

WGS whole genome sequencing

Declarations

Ethics approval and consent to participate:

Verbal informed consent was obtained from all participants rather than written consent for personal reasons. The samples used were the specimens remaining after the patient's clinical examination. The study was approved by the Medical Ethics Committee of Nanfang Hospital Southern Medical University and conducted in compliance with the Declaration of Helsinki (No. NFEC-2014-002).

Consent to publish:

Not applicable.

Availability of data and materials:

All data generated or analyzed in this study are included in this published article and its supplementary information files.

Competing interests:

The authors declare that they have no competing interests.

Funding:

This study was supported by grants from Guangdong Province Science and Technology Project (Nos. 2014A010107011 and 2015A020211011) and Guangzhou City Science and Technology (No. 201510010167). The funding body provides financial support in designing research and collecting, analyzing, interpreting data and writing articles.

Authors' contributions:

DXN carried out the experiments, data organization and analysis, and contributed to writing the manuscript and annotating the results. QYC collected all of the isolates and clinical data. PY participated in a subset of the experiments. RYY contributed to the design of the study and assisted in the drafting of the manuscript. All authors have read and approved the manuscript.

Acknowledgments:

We thank International Science Editing.

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Tables

Table 1 Numbers of isolated Carbapenem-resistant Enterobacteriaceae

Species	clinical samples	fecal samples	Total
<i>K.pneumoniae</i>	73(73.74)	13(43.33)	86(66.67)
<i>K.oxytoca</i>	2(2.02)	2(6.67)	4(3.10)
<i>E.coli</i>	7(7.07)	4(13.33)	11(8.53)
<i>E. aerogenes</i>	15(15.15)	7(23.33)	22(17.05)
<i>E.cloacac</i>			
	1(1.01)	1(3.33)	2(1.55)
<i>E.asburiaac</i>	1(1.01)	0(0.00)	1(0.78)
<i>C.freundii</i>	0(0.00)	1(3.33)	1(0.78)
<i>S.marcescens</i>	0(0.00)	2(6.67)	2(1.55)
Total	99	30	129

Table 2 Antimicrobial susceptibility testing of Carbapenem-resistant Enterobacteriaceae

Antimicrobial agent	No.(%) of isolates:								
	clinical samples				fecal samples				Total
	<i>K.pneumoniae</i> (n=73)	<i>E.cloacae</i> (n=15)	<i>E.coli</i> (n=7)	Total* (n=99)	<i>K.pneumoniae</i> (n=13)	<i>E.cloacae</i> (n=7)	<i>E.coli</i> (n=4)	Total* (n=30)	Enter (n=12)
Aztreonam	5(6.85)	3(20.00)	2(28.57)	10(10.10)	1(7.69)	2(28.57)	2(50.00)	6(20.00)	16(12.3)
Amikacin	18(24.66)	12(80.00)	4(57.14)	35(35.35)	3(23.08)	6(85.71)	4(100)	17(56.67)	52(40.3)
Imipenem	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Meropenem	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
^a Colistin	73(100)	15(100)	7(100)	99(100)	12(92.31)	7(100)	3(75.00)	28(93.33)	127(95.4)
Levofloxacin	3(4.11)	1(6.67)	0(0.00)	4(4.04)	1(7.69)	1(14.29)	0(0.00)	5(16.67)	9(6.9)
Ciprofloxacin	1(1.37)	1(6.67)	0(0.00)	2(2.02)	0(0.00)	1(14.29)	1(25.00)	2(6.67)	4(3.1)
Tetracycline	27(36.99)	5(33.33)	0(0.00)	35(35.35)	4(30.77)	3(42.86)	1(25.00)	11(36.67)	46(35.4)
Trimethoprim-Sulfamethoxazole	25(34.25)	5(33.33)	0(0.00)	32(32.32)	6(46.15)	2(28.57)	1(25.00)	12(40.00)	44(34.1)
^b Tigecycline	73(100)	15(100)	7(100)	99(100)	11(84.62)	7(100)	4(100)	28(93.33)	127(95.4)

*shows the number of all isolates which are susceptibility to antimicrobial compounds, only three kinds of Carbapenem-resistant Enterobacteriaceae with the highest separation ratios are shown in the table.

^a Antimicrobial susceptibility to colistin according to Clinical and Laboratory Standards Institute (CLSI) M100-S29 criteria

^b Antimicrobia susceptibility to tigecycline according to European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 3 Carbapenemase-encoding genes and colistin-related resistance gene of Carbapenem-resistant Enterobacteriaceae

	No.(%) of isolates:								
	clinical samples				fecal samples				Total
	<i>K.pneumoniae</i> (n=73)	<i>E.cloacae</i> (n=15)	<i>E.coli</i> (n=7)	Total* (n=99)	<i>K.pneumoniae</i> (n=13)	<i>E.cloacae</i> (n=7)	<i>E.coli</i> (n=4)	Total* (n=30)	<i>Enter</i> (n=12)
Carbapenemase-encoding genes									
class A β-lactamases									
<i>blaKPC</i>	66(90.41)	14(93.33)	7(100)	94(94.94)	12(92.31)	5(71.43)	4(100)	27(90.00)	121(95.83)
class B metalloβ-lactamases									
<i>blaIMP</i>	18(24.66)	6(40.00)	2(28.57)	29(29.29)	3(23.08)	2(28.57)	2(50.00)	9(30.00)	38(29.23)
<i>blaVIM</i>	2(2.74)	2(13.33)	0(0.00)	4(4.04)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	4(3.11)
<i>blaSPM</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
<i>blaNDM</i>	35(47.95)	7(46.67)	5(71.43)	51(51.51)	11(84.62)	2(28.57)	2(50.00)	17(56.67)	68(52.31)
class D oxacillinases									
<i>blaOXA-48-like</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
ESBL genes									
<i>blaCTX-M</i>	18(24.66)	1(6.67)	3(42.86)	24(24.24)	4(30.77)	2(28.57)	1(25.00)	9(30.00)	33(25.38)
<i>blaTEM</i>	59(80.82)	10(66.67)	6(85.71)	78(78.79)	11(84.62)	7(100)	4(100)	27(90.00)	105(80.77)
<i>blaSHV</i>	68(93.15)	11(73.33)	5(71.43)	87(87.88)	11(84.62)	6(85.71)	4(100)	26(86.67)	113(86.54)
colistin-related resistance gene									
<i>mcr-1</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(7.69)	0(0.00)	1(25.00)	2(6.67)	2(1.54)
<i>mcr-2</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
<i>mcr-3</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
<i>mcr-4</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
<i>mcr-5</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

*shows the number of all isolates which are susceptibility to antimicrobial compounds, only three kinds of Carbapenem-resistant Enterobacteriaceae with the highest separation ratios are shown in the table.

Table 4 Characterization of integrons and ISCR in Carbapenem-resistant Enterobacteriaceae

Mobile genetic elements	Gene cassette array	No.(%) of isolates ^a
<i>intl1</i>	aadB-aadA2	1(3.12)
	dfrA1-aadA1	9(28.12)
	dfrA17-aadA5	1(3.12)
	arr-3-dfrA27	2(6.25)
	aadA8b-orfF-dhfrxII	3(9.37)
	ORFIII-ORFII-orfF-dfrA12	4(12.50)
	aadA3c-orfF-dfrA12	1(3.12)
	aadA2-orfF-dfrA12	8(25.00)
<i>intl2</i>	dfrA1-sat2-aadA1-ybeA-ybfA-ybfB-ybgA-ORF(5)-Tra	2(6.25)
	dfrA1-sat2-aadA1-ybeA-ybfA-ybfB-ybgA-IS3-IS110-IS3-Tra-ybgA	1(3.12)
<i>ISCR1</i>	qnrB6-qacE	1(25.00)
	SapA-qnrB91-qacE	1(25.00)
	qnrB19-qacE	1(25.00)
	IS630-hypA-qacE	1(25.00)

Figures

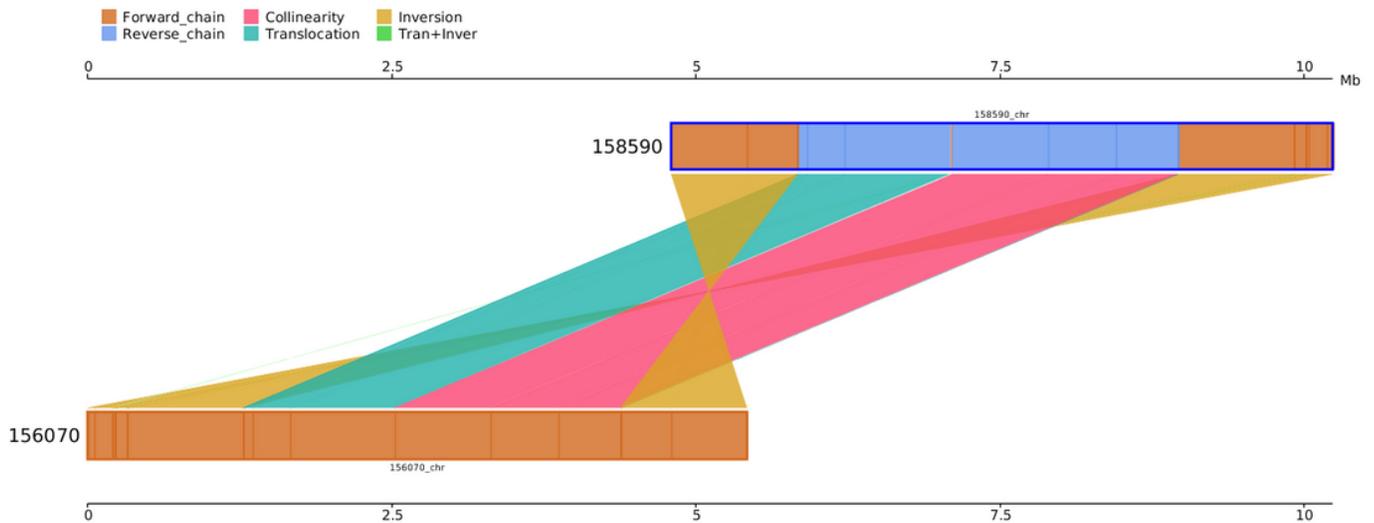


Figure 1

Co-linear analysis found that although there were structural differences between chr156070 and chr158590, they had a high degree of similarity and high coverage for one another.

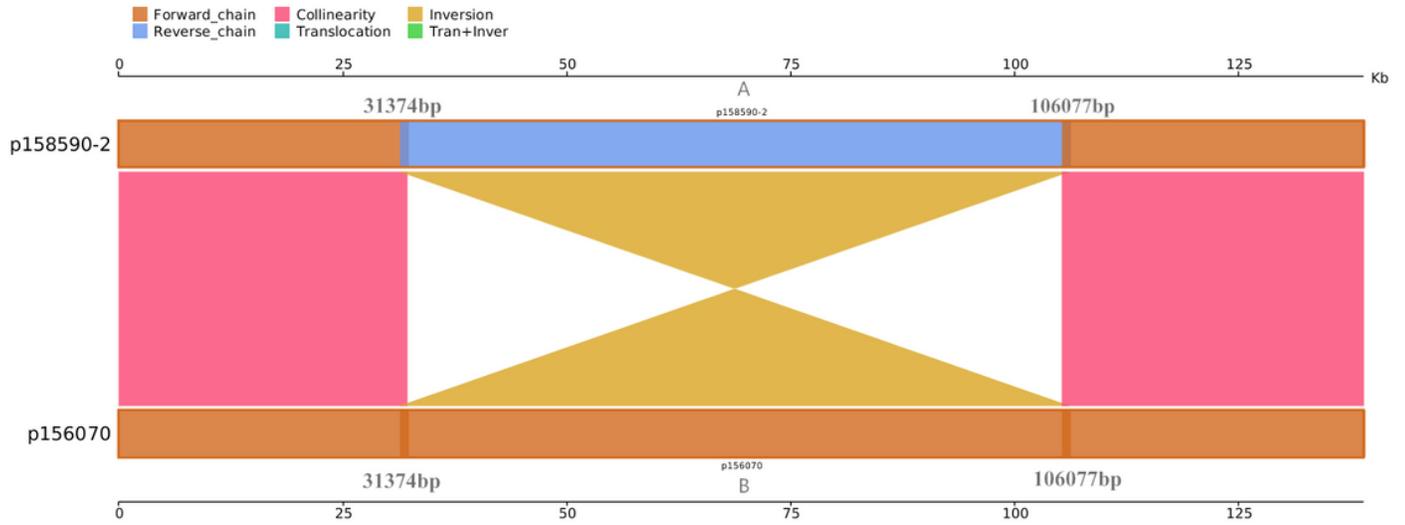


Figure 2

The p156070-KPC had one unique gene relative to p158590-KPC, and p158590-KPC had two unique genes relative to p156070-KPC. Both p156070-KPC and p158590-KPC co-carried the blaKPC-2, blaCTX-M-65, and blaTEM-1b genes. BLAST analysis showed that p156070-KPC was highly similar to p158590-KPC at the nucleotide level (100% identities and 100% query coverage), and collinearity analysis revealed that the plasmid was divided into three parts: the first segment plus chain of p156070-KPC and p158590-KPC were performed collinear comparison from 1 bp to 32195 bp, with a similarity of 99%; the second segment of p156070-KPC and p158590-KPC plasmid were performed translocation comparison from 31,374 bp to 106,077 bp, with a similarity of 100%, the phenomenon might be result from the rearrangement of small fragments that made up the A fragment and B fragment due to MGEs; the third segment 105,256 bp to 138,959 bp of p156070-KPC and p158590-KPC were collinear comparison, with a similarity of 100%.

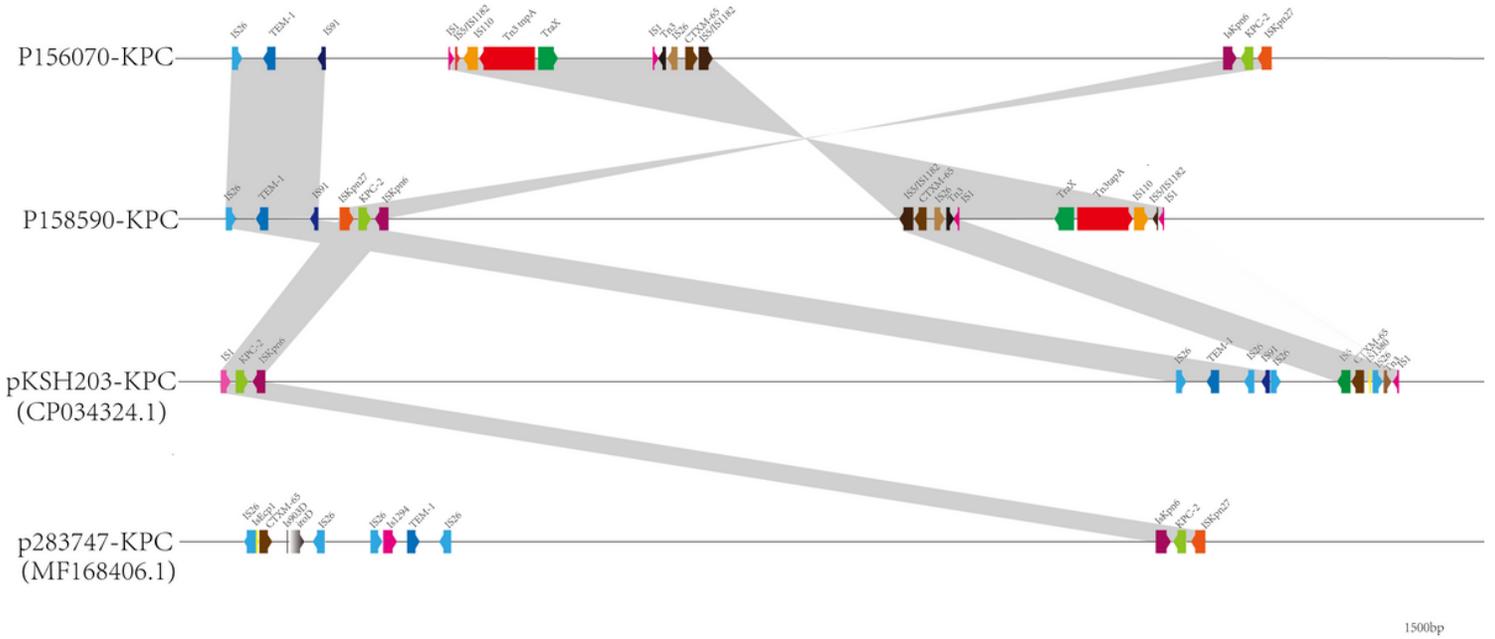


Figure 3

BLASTN analysis showed that p156070-KPC and p158590-KPC were 96% similar to the previously reported IncFII plasmid p283747-KPC (MF168406.1), which harbored blaKPC-2 between ISKpn27 and ISKpn6

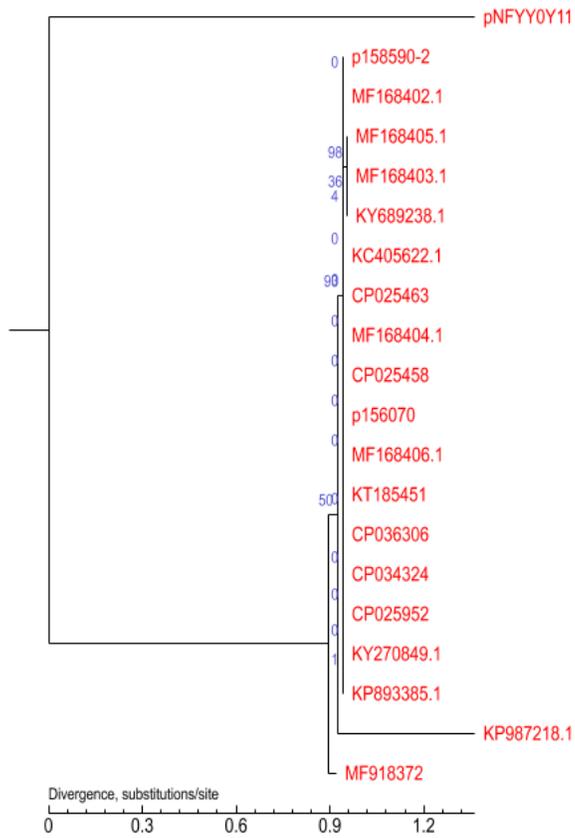


Figure 4

Phylogenetic analysis showed that p156070-KPC and p158590-KPC are closely genetically related to most of the IncFII plasmids previously reported

Supplementary Files

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