Genomic characterization of carbapenem resistant *Escherichia coli* from multiple hospitals in Nanjing, China: focusing on frequent co-occurrence of *bla*NDM and *bla*KPC-2

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Research

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Abstract

Background: The increasing emergence of carbapenem resistant *Escherichia coli* (CREC) poses a potential threat to public health, hence genomic characterization of isolates is needed for a better understanding of its transmission and implementation of infection control measures.

Materials and methods: Eleven CREC isolates were collected in 2015 from 6 hospitals in Nanjing, China, and analyzed using whole genome sequencing. Resistance determinants, virulence elements, multi-locus sequence type (MLST), serotypes, phylogeny and fimH types were determined.

Results: All of the CREC carried at least one carbapenemase. NDM-5 (n=9) was the most frequent carbapenemase, followed by KPC-2 (n=3) and NDM-1 (n=2); three isolates produced NDM-5 and KPC-2. Ten out of the 11 isolates co-carried *bla*CTX-M variants. MLST analysis found 7 distinct STs, including ST410 (n=2), ST3489 (n=1), ST156 (n=1), ST683 (n=1), ST297 (n=1), ST167 (n=1), and ST361 (n=1). Six distinct serotypes and 8 Fim types were identified. A great diversity of plasmid profiles was observed with plasmid replicon IncX3 being the most frequent (n=11). Phylogenetic analysis showed great diversity between the 11 CREC isolates and also between 6 additional isolates co-carrying *bla*NDM and *bla*KPC which were selected from the strains collection of Nanjing Drum Tower Hospital for comparison. Conjugation assays demonstrated that *bla*NDM was transferable.

Conclusion: NDM is the major carbapenemase among CREC, with NDM-5 being the main variant which can be horizontally disseminated by IncX3 plasmids. These isolates displayed genetic diversity by MLST, Fim typing and serotyping. We herein provided the first report on emergence of NDM-5 producing *E. coli* ST297, ST683, ST3489, and NDM-1 producing *E. coli* ST361.

Introduction

Escherichia coli mainly inhabits the lower intestinal tract of warm-blooded animals. It is a major pathogen for numerous types of infections, such as intestinal, urinary, and respiratory tract infections in humans and other animals (1) . In recent years, carbapenems have been increasingly used as the most effective antibiotic in clinical therapy for infections caused by multidrug-resistant (MDR) strains, due to production of extended spectrum β-lactamases (ESBLs) or AmpC-type β-lactamases(2). Therefore, the frequent occurrence of carbapenem-resistant *Escherichia coli* (CREC) worldwide has been posing a threat to public health (3, 4),

Production of carbapenemases has been so far the main mechanism for carbapenem resistance in *Enterobacterales*(5), and New Delhi metallo-β-lactamase (NDM) is the major carbapenemase in *E. coli* all over the world (6). It is worthy to note that the co-occurrence of multiple ß-lactamases among single bacteria species (7), especially carbapenemase, such as co-production of NDM-1 and *Klebsiella pneumoniae* carbapenemase 2 (KPC-2)(8), co-occurrence of KPC-2 and OXA-48, have so far been frequently described in multiple clinical *Enterobacterales,* such as *K. pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundi*(8-10). Thus, such a frequent co-occurrence of carbapenemase in one isolate are causing rapidly rising carbapenem resistance, leading to increasing CRE-associated morbidity and mortality (11)

Report from the China CRE Network showed that the overall CREC infection incidence differed significantly by region, with the highest in Shandong (9.1%) and the lowest in Qinghai (0 %) during January to December 2015 (12); another study showed a 4.6% prevalent rate of CREC in in Northern Jiangsu Province, China during September 2015 to August 2016 (13). However, strains analyzed were predominantly collected from tertiary hospitals. Specialized hospitals, Children's hospital and level II hospitals were less involved.

Moreover, multiple studies have shown that that *bla*NDM is often located on IncX3 plasmids, (14, 15), but little information on virulence genes, serotyping and fim typing is available for such strains.

In this study, we tried to characterize the genomic epidemiology of CREC strains including resistance determinants, virulence factors, serotyping, fim typing and plasmid replicons. Furthermore, the molecular characterization of strains co-producing NDM-5 and KPC-2 screened from clinical CREC in our hospital during 2013-2017 were investigated.

Materials And Methods

Isolates collections

The isolates in our study included two parts. Firstly, eleven non-repetitive CREC isolates collected from 6 hospitals during the period June-December 2015 in Nanjing city were analyzed for genomic characterization by whole genome sequencing. These strains were isolated from urine (n=4), sputum (n=3), blood (n=1), bile (n=1), secretion of uterus neck (n=1), and the source of one strain was unknown. The following hospitals were involved: Nanjing Mingji Hospital I (n=1), Nanjing Children's Hospital (n=2), Nanjing Lishui Hospital (n=1), Nanjing Drum Tower Hospital (n=4), Nanjing jinyu Hospital (n=1), Nanjing Maternal and Child Health Hospital (n=1), and Nanjing First Hospital (n=1).

Secondly, considering the high prevalence of *E. coli* isolates co-producing KPC-2 and NDM among the 11 CREC strains from 6 hospitals, we tried to investigate the distribution of these strains among clinical CREC isolates. Therefore, a total of 43 consecutive non-duplicate isolates collected in Nanjing Drum Tower hospital during 2013-2017 were further analyzed for isolates co-producing KPC-2 and NDM by PCR and DNA sequencing. Among them, 4 strains were isolated in 2013, 10 in 2014, 2 in 2015, and 11 from 2016 and 16 in 2017. The source of the samples was as follows: urine (n=18), blood (n=9), sputum (n=6), secretion (n=3), bile (n=3), abdominal dropsy (n=2), and pus (n=1).

All the CREC strains were identified by Vitek 2.0 (BioMérieux. Marcy l'Etoile, France) or ATB 32E Semi-auto identification machine (Bio-Mérieux, France). Isolates resistant to at least one carbapenem (imipenem, meropenem, ertapenem) were included in the study.

Antimicrobial Susceptibility testing

Susceptibility of the 11 CREC isolates towards antimicrobial agents were tested by micro-broth method. The antimicrobial agents tested included ertapenem, imipenem, meropenem, cefepime, ceftazidime, cefotaxime, cefuroxime, cefazolin, piperacillin/tazobactam, amikacin, gentamicin, funantuoyin, trimethoprim and sulphame-thoxazole, aztreonam, piperacillin, ciprofloxacin, levofloxacin, aztreonam/avibactam, ceftazidime/avibactam, tigecycline, and colistin B. *E. coli* ATCC 25922 was used as quality control. The results were interpreted according to the CLSI 2019 guideline (16). Whereas, for tigecycline and colistin, the European Committee on Antimicrobial Susceptibility Testing breakpoints were referred (http://www.eucast.org/clinical_breakpoints).

DNA Extraction

The Ultraclean Microbial DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, US) was used to extract genomic DNA. The NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used for measuring the DNA concentration and purity for whole genome sequencing.

Whole Genome Sequencing, denovo Assembly, Scaffolding, and Annotation

The prepared pair-end DNA library was sequenced on the MiSeq (Illumina, SanDiego, CA, USA). Denovo assembly of the paired-end reads was performed by CLC Genomics Workbenchv7.0.4 (QIAGEN, Hilden, Germany) after quality trimming (Qs ≥ 20). Scaffolding was finished using SSPACE standard version 3.0 and the gaps within scaffolds were further closed by GapFiller (17, 18). Then genomes were then submitted to NCBI for annnotation.

Analysis of genomic epidemiology

The antimicrobial resistance determinants and virulence factors were identified using Resfinder v2.1 (http://cge.cbs.dtu.dk/services/ResFinder-2.1/) and Virulence Finder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/); the 11 CREC were typed by multi-locus sequence typing (MLST) 2.0 (https://cge.cbs.dtu.dk/services/MLST/), Plasmid Finder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/), Serotype Finder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/), and FimTyper 1.0 (https://cge.cbs.dtu.dk/services/FimTyper/).

Phylogenetic relationship of 11 CREC

The core-genome phylogeny of the 11 CREC was constructed by using single-nucleotide polymorphisms (SNP)-sites detected from 1010 core genes (identity > 95%; coverage = 100%) derived from 502 ST11 strains (19). A maximum-likelihood tree was calculated using RAxML version 8.2.8, with general time-reversible model and 100 bootstraps (20). Interactive Tree Of Life (https://itol.embl.de) was used to produce the phylogenetic tree (21).

The screening of *Escherichia coli* co-producing NDM and KPC-2 carbapenemases

In order to investigate the prevalence of *E. coli* isolates co-producing NDM-5 and KPC-2 in our hospital during 2013-2017, genes encoding carbapenemases (KPC and NDM) were detected by PCR and DNA sequencing (22). The positive products were sent to the Qingke Biotechnology Co., Ltd (Nanjing, China) for purification and sequencing. Sequences were further analyzed by using the Chromas-Pro application and BLAST (www.ncbi.nlm.nih.gov/BLAST).

Pulsed-field gel electrophoresis

Six *E. coli* isolates co-producing NDM-5 and KPC-2 including 3 ones from 11 CREC and 3 ones selected from the 43 CREC were further analyzed for genetic relatedness by PFGE, which was performed according to the protocol as previously described (23). Briefly, fresh colonies were mixed with proteinase K (Merck Sharp & Dohme Ltd, Germany) into plugs. After the plugs were digested by restriction endonuclease XbaI (Fermentas, ABI, Germany), the resultant DNA fragments were separated in a PFGE CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA) in 0.5×Tris-borate-EDTA buffer at 120 V for 19 h. The pulse times ranged from 2.2 s to 54.2 s. Finally, the BioNumerics software (Applied Math, Sint-Maten-Latem, Belgium) was used to analyze the banding patterns.

Conjugation assay

For the 6 isolates co-carrying *bla*KPC and *bla*NDM, broth mating was performed in order to analyze the transferability of these genes according to the protocol prescribed previously(24). Azide resistant *E. coli* J53 was used as the recipient. Briefly, fresh colonies were inoculated into 5 ml LB broth and incubated at 37°C, 200 rpm. After 5 hours, 500 µl recipient cells and 100 ul donor were suspended in 5 ml LB broth for overnight culture at 37°C, 200 rpm, then 100 µl were plated onto the LB plates containing 30 mg/L cefoxitin and 100 mg/L sodium azide for *E. coli* J53. PCR (amplification for *bla*NDM and *bla*KPC) and Eric-PCR were used to verify conjugants.

Results

The susceptibilities of the 11 CREC.

All the 11 CREC isolates displayed resistance toward β-lactams (including carbapenems), β-lactamase inhibitors (including ceftazidime/avibactam) and fluoroquinolones tested in our study. Resistance to trimethoprim and sulphame-thoxazole (72.7, 8/11), gentamicin (36.4, 4/11), amikacin (18.1, 2/11) as well as funantuoyin (9.1%, 1/11) were also observed. All the 11 isolates were susceptible to tigecycline, colistin and aztreonam/avibactam (Table 1). Compared to the minimum inhibitory concentrations (MICs) of strains co-carrying *bla*KPC and *bla*NDM, the isolates only carrying *bla*NDM did not show obviously higher MICs toward all the antimicrobial agents.

Genomic epidemiology of 11 CREC

The genomic features including the sizes of genome, GC content and accession number of the 11 CREC isolates were shown in Table 2. Genomic analysis showed that all the 11 CREC strains carried NDM carbapenemases, including 2 NDM-1 and 9 NDM-5, while three strains co-carried NDM-5 and KPC-2; ten out of the 11 isolates carried CTX-M variants, including CTX-M-55 (n=4), CTX-M-65 (n=2), CTX-M-14 (n=2) and CTX-M-15 (n=2). AmpC enzyme including 2 CMY-2 and 1 CMY-43, plasmid mediated quinolone resistance (PMQRs), including *oqxAB* (n=2), *qnrA1* (n=1), *qnrS1* (n=4), *aac(6')Ib-cr* (n=4), and *qepA* (n=1), as well as plasmid-mediated glutathione S-transferase (PMGST) genes *fosA3* (n=4) were also identified (Figure 1).

Virulence genes analysis showed that 12 VFs were detected, with *gad* (n=9) being the most frequent one, followed by *lpfA* (n=7) and *iss* (n=7), other VFs including *astA* (n=3), *cma* (n=3), *capU* (n=3), *iroN* (n=2), *sat* (n=1), *senB* (n=1), *sepA* (n=1), *iha* (n=1) and *cnf1* (n=1) were also found (Table 3).

MLST analysis found 7 distinct STs, including ST410 (N=2), ST3489 (n=1), ST156 (n=1), ST683 (n=1), ST297 (n=1), ST167 (n=1) and ST361 (n=1), in addition, 3 new STs were identified (Figure 1);

The 11 strains were typed into 5 *E. coli* O groups (O8, O9, O25, O30, O89) and 8 H groups (H9, H10, H4, H21, H40, H45, H30 and H26). Six distinct serotypes were found including O8:H9 (n=3), O9:H4 (n=1), O89:H9 (n=1), O30:H21 (n=1), O25:H26 (n=1) and O9:H30 (n=1).

Analysis of FimH identified 8 types, including Fim24 (n=2), Fim23 (n=2), Fim34 (n=1), Fim38 (n=1), Fim121 (n=1), Fim276 (n=1), Fim54 (n=1) and Fim31 (n=1).

A great diversity of plasmid profiles was observed. Among them, plasmid replicon IncX3 was found among all the 11 strains followed by IncFII (n=7) and IncFIB (n=6), additionally, IncI1 (n=3), IncFIA (n=3), IncY (n=2) and IncFIC (n=2) were also detected.

Phylogenetic characterization of 11 CREC strains

The phylogenetic tree showed that 11 CREC evolve into 2 main clades albeit a great diversity was observed (Figure 1). Two isolates from Nanjing Children's hospital displayed close evolutionary relationship.

Prevalence of isolates co-producing NDM-5 and KPC-2

Among the 43 CREC collected from our hospital during 2013-2017, 6 KPC-2 and 23 NDM were identified, two strains co-producing NDM-5 and KPC-2 and one strain co-carrying NDM-1 and KPC-2 were found. One was isolated from the urine of an inpatient in ICU in 2014, the other two strains were isolated from abdominal dropsy and sputum of the different patients in 2017.

Genetic relatedness of strains co-carrying *bla*KPC and *bla*NDM

PFGE displayed a high diversity of the 6 strains co-producing *bla*KPC and *bla*NDM (Figure 2), indicating that these strains were not from the same clone.

Transferability of *bla*KPC and *bla*NDM

Conjugation assay revealed that the *bla*NDM of all the 6 isolates was transferable to *E. coli* J53. However, we could not isolate any conjugants with *bla*KPC, suggesting that the *bla*KPC and *bla*NDM were not on the same plasmid.

Discussion

In this study, we provided data on genomic epidemiology of 11 CREC strains from 6 hospitals in Nanjing city, Jiangsu province. Based on the high co-occurrence of *bla*NDM-5 and *bla*KPC-2, 43 CREC strains collected from a tertiary hospital during 2013-2017 were further screened to investigate the prevalence of such strains in our hospital. This is the first study that provided the genomic epidemiology of the CREC from multiple hospitals in Nanjing.

The high resistance toward the commonly used antibiotics in clinical therapy displayed by the CREC from 6 hospitals were consistent with the previous report (25), leading to a quite limited choice of antimicrobial agents for infections caused by such strains. Fortunately, tigecycline, colistin and aztreonam/avibactam showed the best sensitivity. Of note, the co-occurrence of KPC and NDM among single isolate seems not confer higher resistance to β-lactams when the MICs of β-lactams were compared between the strains carrying *bla*NDM and the ones co-carrying *bla*KPC and *bla*NDM. In addition, it was reported that MICs of ertapenem against strains producing NDM-5 are 4- or 8-fold higher than those against strains producing NDM-1(26), however, we did not observe such a phenomenon, we therefore speculate that the existence of other resistant mechanism such as the production of ESBLs and AmpCs, overexpressed efflux pumps, as well as decreased outer membrane permeability may contribute to the resistance towards β-lactams.

The high prevalence of NDM among 11 CREC in our study is in accordance with the previous report (6), indicating that NDM is the major carbapenemase for carbapenem resistance in *E. coli,* which may result from the low fitness burden of the plasmid harboring *bla*NDM in *E. coli* (27). We found that NDM-5 is the most common variant, this is also in agreement with the present epidemiological data (28), demonstrating that *NDM-5* is the predominant determinant conferring carbapenem resistance in CREC. Noteworthily, NDM-5 has been predominantly found in high-risk clone ST167, ST410 and ST101 in the hospital (29).This may result from successful expansion of *E. coli* clonal groups and frequent horizontal gene transfer of NDM-5 expressing plasmids. Notetaceblily, ST131 as a multidrug clone has spread extensively throughout the world (30). However, ST131 was not detected in our study. The multiple distinct STs identified in our study indicated the diversity of these CREC, which was also confirmed by the phylogenetic relationship. As known, NDM-5 has been reported in *E. coli* ST410, ST156, and ST167 (24). However, to the best of our knowledge, NDM-5 producing *E. coli* ST297, ST683 and ST3489, as well as NDM-1 producing *E. coli* ST361 has not been reported previously.

Moreover, we found a high co-occurrence of KPC-2 and NDM-5, 5 out of the 6 strains that were isolated from our hospital which is a comprehensive tertiary hospital with 3000 beds. The more worse is that multiple resistance determinants including *bla*OXA-1, *bla*CMY, *bla*CTX-M, and *fosA3*, *rmtB*, *qnr* and *aac(6')-Ib-cr* were also identified in these NDM-5 and/or KPC-2 producing strains, representing a significant challenge for clinical management and public health.

Despite multiple plasmid replicons were found among our CREC, most *bla*NDM -carrying plasmids belong to limited replicon types (IncX3, IncFII, or IncC)(4)

Considering that plasmid replicon InX3 was found among all the NDM-producing *E. coli*, we speculate that IncX3 is the main host for *bla*NDM (31). Note worthily, the conjugation assay in our study showed that the spread of *bla*NDM was not accompanied by transfer of *bla*KPC-2, indicating that *bla*NDM and *bla*KPC-2 were not harbored by the same plasmid.

Virulence gene analysis revealed several major VFs in CREC, among them, *gad* encodes glutamate decarboxylase, which is the structural component of the major acid resistance system that protects *E. coli* from strong acid stress (pH < 3), typically encountered in the mammalian gastrointestinal tract (32), *lpfA* (Long polar fimbriae) is a putative adhesion gene, encoding one of the few fimbrial adhesions of enterhemorrhagic *E. coli* O157:H7 associated with colonization on host intestine, which play essential roles during the bacterial infection process(32). *iss* (increased serum survival*)* is the most common avian pathogenic *E. coli* encoding gene. It has been identified as a virulence trait associated with the virulence of *E. coli*, causing colibacillosis in poultry (33). The high prevalence of these VFs among CREC may suggest that CREC mainly colonize the host intestine, and they might have a lower potential to cause human disease. Noteworthy, a strain isolated from urine belong to a new ST, which not only carried *gad* and *iss*, *lpfA*, but also *sat* (secreted autotransporter toxin), *senB* (Plasmid-encoded enterotoxin), *sepA* (Shigella extracellular protein A), *iha* (Adherence protein) and *cnf1* (Cytotoxic necrotizing factor). It is known that *sat* can promote cytotoxic effects in several lines of undifferentiated epithelial cells and is highly prevalent in certain *E. coli* pathogenic groups responsible for urinary and intestinal infections(34). Cnf1 is frequently expressed in clinical UPEC isolates, CNF1-producing and β-hemolytic *E. coli* strains most notably cause urinary tract and meningeal infections in humans(35). Altogether, the wide presence of these VFs among the urinary CREC may indicate a higher pathogenicity of this strain.

O8:H9 as the most common serotype in our study was consistent with previous report, indicating that O8:H9 is a clinically-relevant serotype correlated with multidrug resistance(36)

Altogether, the multiple fimH types, the diverse STs and serotypes in our study, indicates a high diversity of these CREC strains. Although further PFGE from the strains co-carrying KPC and NDM excludes an epidemic dissemination of the frequent occurrence of these strains, the emergence of these strains poses a potential public health threat.

In conclusion, genomic analysis found that NDM is the main carbapenemase for 11 CREC strains, with a frequent occurrence of KPC. These CREC strains displayed genetic diversity by MLST, Fim typing and serotyping, as well as the phylogenetic relationship. IncX3 may be the main plasmid for NDM, and *gad*, *iss* and *lpfA* were the main VFs; Albeit a frequent occurrence of strains co-carrying *bla*NDM and *bla*KPC-2 were detected, no clonal dissemination was found; based on the potential public health threat posed by these strains, infection control measures should be further strengthened.

Declarations

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Conflict of interest statement

None declared

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Tables

Table 1. The minimum inhibitory concentrations of the 11 carbapenem resistant *Escherichia coli* isolates from 6 hospitals in Nanjing city, Jiangsu province

Strains	ETP	IPM	MEM	FEP	CAZ	CTX	CXM	CZL	TZP	AMK	GEN	NIT	SXT	ATM	PIP	CIP	LVX	A
NJMJYY4	32	>16	>16	>32	>32	>32	>64	>32	>256/4	<1	<1	64	<0.25	32	256	>8	16	<
NJSETYYY11	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	<1	<1	16	>32	>128	>256	>8	>16	1
NJSETYYY 14	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	<1	<1	32	>32	>128	>256	>8	16	1
NJSFYYY17	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	1	2	>128	>32	>128	>256	>8	>16	1
NJLSYY40	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	<1	64	8	>32	64	>256	>8	>16	<
NJSDYYY42	16	>16	>16	>32	>32	>32	>64	>32	>256/4	>128	>128	16	>32	64	>256	2	4	1
NJJYYY51	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	>128	>128	32	>32	>128	>256	>8	16	0
NJGLYY3610	8	16	>16	>32	>32	>32	>64	>32	128/4	2	16	8	320	64	>256	4	8	1
NJGLYY 3940	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	<1	<1	16	>32	32	>256	>8	16	<
NJGLYY 4197	>32	>16	>16	>32	32	>32	>64	>32	256/4	<1	<1	32	<0.25	>128	>256	2	>16	1
NJGLYY 4673	>32	>16	>16	>32	>32	>32	>64	>32	>256/4	1	2	8	<0.25	>128	>256	>8	>16	0

ETP, ertapenem; IPM, imipenem; MEM, meropenem; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CXM, cefuroxime; CZL, cefazolin; TZP, piperacillin/tazobactam; AMK, amikacin; GEN, gentamicin; NIT, funantuoyin; SXT, trimethoprim and sulphame-thoxazole; ATM, aztreonam; PIP, piperacillin; CIP, ciprofloxacin; LVX, levofloxacin; AZA, aztreonam/avibactam; CZA, ceftazidime/avibactam; TGC, tigecycline; COL, colistin B.

Table 2. The genome features of the 11 carbapenem resistant *Escherichia coli* isolates from 6 hospitals in Nanjing city, Jiangsu province.

Sample	Number of contigs	Total length (bp)	GC%	Accession number
NJJYYY51	148	5308317	50.34	JABEWN000000000
NJLSYY40	126	4964668	50.65	JABEWL000000000
NJMJYY4	105	5024932	50.45	JABEWH000000000
NJSDYYY42	139	5054242	50.76	JABEWL000000000
NJSETYYY11	90	4884617	50.56	JABEWM000000000
NJSETYYY14	112	4998406	50.60	JABEWJ000000000
NJSFYBJY17	127	4768034	50.56	JABEWK000000000
NJGLYY3610	194	5105893	50.73	RZMI00000000
NJGLYY3940	210	5874804	50.85	RZMM00000000
NJGLYY4197	3342	5786687	51.08	JACKXC000000000
NJGLYY4673	66	5013086	50.69	JACKXB000000000

The Whole Genome Shotgun BioProject for these CREC isolates has been deposited at GenBank,

Table 3. The distribution of plasmid mediated quinolone genes, Virulence factors and plasmid replicons among the 11 carbapenem resistant *Escherichia coli* isolates from 6 hospitals in Nanjing.

Strains	PMQRs	Virulence factors	Plasmid replicons
NJMJYY4	*oqxAB*	*astA, gad*	IncFII, IncI1, IncX3, p0111
NJSETYYY11	*qnrS1*	*lpfA*	IncFIA, IncFIB, IncX3
NJSETYYY 14		*lpfA*	IncFII, Col(BS512), IncFIA, IncX3，IncB/O/K/Z, IncFIB
NJSFYYY17		*gad*	IncX3
NJLSYY40		*gad*，*cma*，*iss*，*lpfA*，*astA*	IncFIB, IncFIC(FII)，IncX3
NJSDYYY42	*qnrS2, aac(6')Ib-cr*	*iroN*，*iss*，*cma*，*lpfA*，*gad*	IncFII, IncFIB, IncFIC, IncX1, IncX3，IncN
NJJYYY51	*qnrS1*, oqxAB, *aac(6')Ib-cr*	*Iss, gad, lpfA*	IncFII, IncFII(K), IncHI2, IncHI2A, IncX3
NJGLYY3610	*qnrS1*	*iss, gad, capU*	IncFII, IncFII, IncFIB, IncI1, IncX3, IncY
NJGLYY 3940	*qepA, qnrA1, aac(6')Ib-cr*	*gad, iroN, gad, cma, capU, iss*	IncFII, IncFIB, IncY, IncX3
NJGLYY 4197	*qnrS1, aac(6')Ib-cr*	*sat, senB, gad, iss, sepA, iha, cnf1, lpfA*	IncFII, IncFIB, IncFII, ColpVC, IncHI1B, IncX1, IncX3, Col156
NJGLYY 4673	*oqxAB*	*astA*，*gad*，*iss*，*lpfA*，*capU*	IncFII, IncFIA, IncFIB, IncHI2A, IncHI2, IncI1, IncX3

PMQRs: plasmid mediated quinolone genes.

Figures

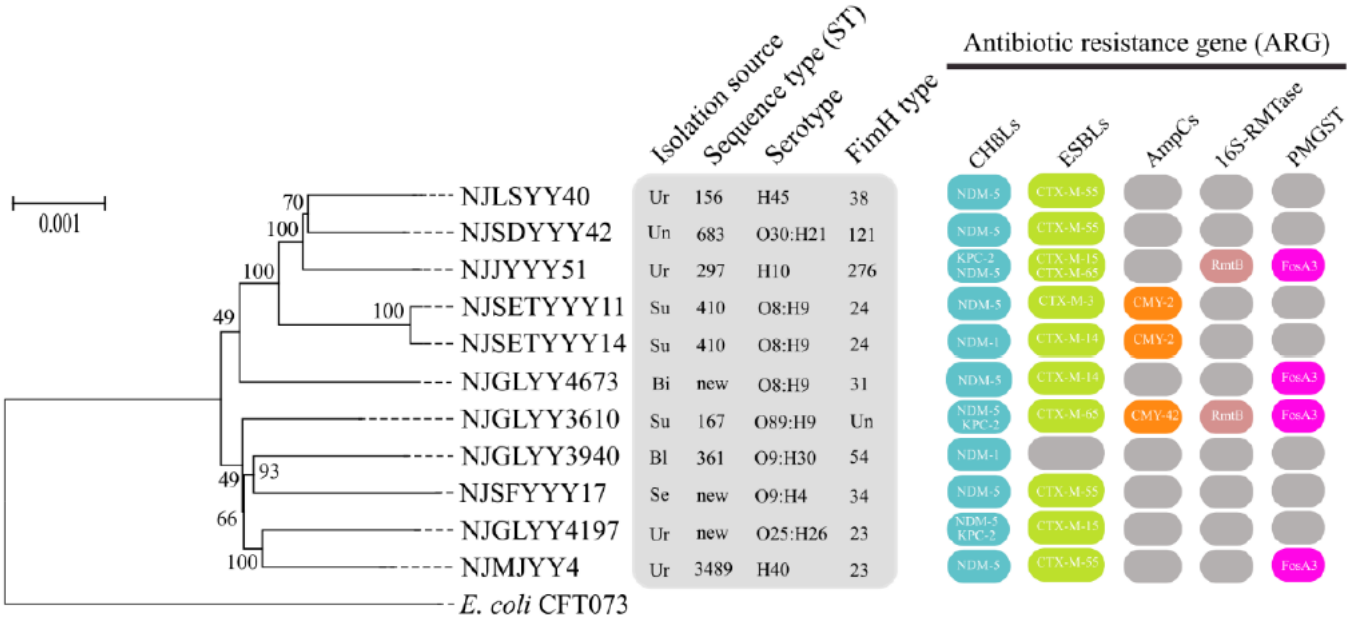

Figure 1

The heatmap of the 11 carbapenem resistant Escherichia coli isolates from 6 hospitals in Nanjing City. CHβLs: Carbapenem hydrolysis β-lactamase; ESBLs: extended-spectrum β-lactamase; pAmpCs: plasmid-mediated AmpC enzyme; 16S-RMTase: exogenously acquired 16S rRNA methyltransferase; PMGST: plasmid-mediated glutathione S-transferase.

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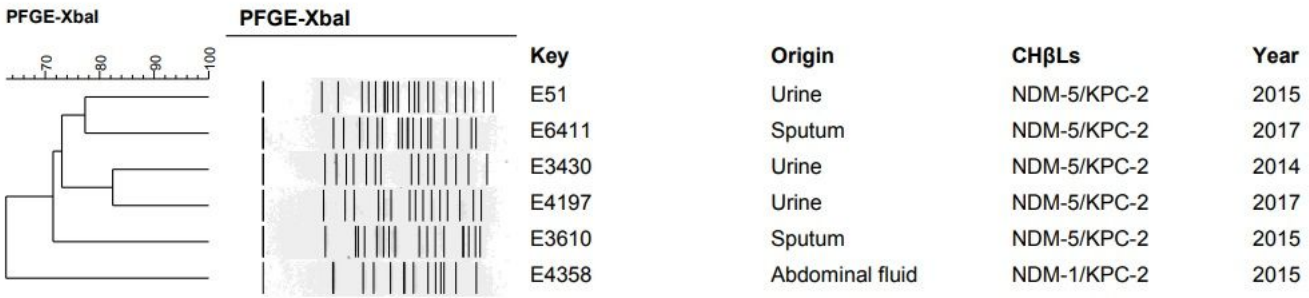

Figure 2

Dendrogram based on PFGE profiles of 6 KPC-2 and NDM co-producing Escherichia coli isolates. The dendrogram was produced by the UPGMA algorithm based on the Dice similarity coefficient included five PFGE groups as defined based on 85% similarity of PFGE profiles. CHßLs, carbapenem hydrolyzing ß-lactamase.