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Molecular characteristics of global β-lactamase-producing *Enterobacter cloacae* by genomic analysis

Jincao Hu[1†], Jia Li[1†], Chang Liu[1], Yan Zhang[1], Hui Xie[1], Chuchu Li[2], Han Shen[1*] and Xiaoli Cao[1*]

Abstract

Objective: To analyze the characteristics of global β-lactamase-producing *Enterobacter cloacae* including the distribution of β-lactamase, sequence types (STs) as well as plasmid replicons.

Methods: All the genomes of the *E. cloacae* were downloaded from GenBank. The distribution of β-lactamase encoding genes were investigated by genome annotation after the genome quality was checked. The STs of these strains were analyzed by multi-locus sequence typing (MLST). The distribution of plasmid replicons was further explored by submitting these genomes to the genome epidemiology center. The isolation information of these strains was extracted by Per program from GenBank.

Results: A total of 272 out of 276 strains were found to carry β-lactamase encoding genes. Among them, 23 varieties of β-lactamase were identified, bla_{CMH} ($n=130$, 47.8%) and bla_{ACT} ($n=126$, 46.3%) were the most predominant ones, 9 genotypes of carbapenem-hydrolyzing β-lactamase (CHβLs) were identified with bla_{VIM} ($n=29$, 10.7%) and bla_{KPC} ($n=24$, 8.9%) being the most dominant ones. In addition, 115 distinct STs for the 272 ß-lactamase-carrying *E. cloacae* and 48 different STs for 106 CHβLs-producing *E. cloacae* were detected. ST873 ($n=27$, 9.9%) was the most common ST. Furthermore, 25 different plasmid replicons were identified, IncHI2 ($n=65$, 23.9%), IncHI2A ($n=64$, 23.5%) and IncFII ($n=62$, 22.8%) were the most common ones. Notably, the distribution of plasmid replicons IncHI2 and IncHI2A among CHβLs-producing strains were significantly higher than theat among non-CHβLs-producing strains ($p<0.05$).

Conclusion: Almost all the *E. cloacae* contained β-lactamase encoding gene. Among the global *E. cloacae*, bla_{CMH} and bla_{ACT} were main bla_{AmpC} genes. Bla_{TEM} and bla_{CTX-M} were the predominant ESBLs. Bla_{KPC}, bla_{VIM} and bla_{NDM} were the major CHβLs. Additionally, diversely distinct STs and different replicons were identified.

Keywords: *Enterobacter cloacae*, β-lactamase, Sequence type, Carbapenem-hydrolyzing β-lactamase

Introduction

Enterobacter (E. cloacae) belongs to facultative anaerobic Gram-negative bacilli, grouping into the *E. cloacae* complex group, the family *Enterobacterale* [1]. Generally, such bacteria colonize soil and water as well as the animal and human gut, representing one of the most leading species described in clinical infections, particularly in vulnerable patients [2]. It has been reported that *E. cloacae* is frequently associated with a multidrug resistance (MDR) phenotype, due to the inducible overproducing

†Jincao Hu and Jia Li contributed equally to this work.

*Correspondence: shenhan10366@sina.com; cao-xiao-li@163.com

[1] Department of Laboratory Medicine, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Zhongshan Road 321, GulouJiangsu Province, Nanjing, People's Republic of China
Full list of author information is available at the end of the article

AmpC β-lactamases and acquisition of numerous genetic mobile elements containing resistance [3]. More worrisome, the production of carbapenem-hydrolyzing β-lactamase (CHβLs) rendering ineffective almost all β-lactams families have been continually acquired, resulting in the production of super-resistant bacteria carbapenem-resistant *Enterobacter cloacae* (CREL) [4].

β-lactamase is a predominant resistance determinant for β-lactam antibiotics in *E. cloacae*. To date, there are two classification schemes for β-lactamases, the more groupings in clinical laboratory generally correlate with broadly based molecular classification, where β-lactamases are divided into class A, B, C and D enzymes based on the amino acid sequence [5]. Currently, the most problematic enzymes are plasmid-mediated AmpC β-lactamases (pAmpCs) with $bla_{\text{ACT-like}}$ *ampC* genes being highly prevalent [6], extended-spectrum β-lactamases (ESBLs) with bla_{SHV} and $bla_{\text{CTX-M}}$ being widely distributed [7], and CHβLs, all of which are challenging antibiotic effectiveness.

Globally, $bla_{\text{CHβLs}}$ such as bla_{KPC} (class A), $bla_{\text{NDM/VIM/IMP}}$ (class B) and $bla_{\text{OXA-48}}$ (class D) are of grave clinical concern and proliferating [8]. It was reported that $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$ were the main $bla_{\text{CHβLs}}$, ST93, ST171 and ST145 was the predominant sequence types (STs) for CREL in a tertiary Hospital in Northeast China during 2010–2019 [9]. Whereas in Japan, $bla_{\text{IMP-1}}$ was the dominant $bla_{\text{CHβLs}}$ conferring carbapenem resistance [10], and bla_{VIM} was the main $bla_{\text{CHβLs}}$ in France between 2015–2018 [11]. However, the whole distribution of β-lactamase among global *E. cloacae* is unclear, and information on the clones of *E. cloacae* spreading internationally remains unknown. As we know that plasmids play an important role in horizontal gene transfer of antimicrobial resistance genes (ARG), and the identification of replicon types is helpful to analyze plasmid characteristics. Further, the association between plasmid replicons and different resistant determinants is essential to understand the role of plasmids in transmission of ARG [12]. For instance, IncN plasmids have been reported to be the predominant replicon types for $bla_{\text{IMP-4}}$-carrying strains [13], however, the prevalence of plasmid replicons among these bacteria were unknown. Notably, the association between IncIγ plasmid encoding $bla_{\text{CMY-2}}$ ß-lactamase and the international ST19 was observed in multidrug-resistant *Salmonella Typhimurium* [14]. Whether or not this phenomenon could be observed in *E. cloacae* needs to be confirmed.

With the extensive use and development of antibacterial drugs, β-lactamases have evolved rapidly. Meanwhile, due to the rapid development of whole-genome sequencing (WGS) technology, the number of sequenced bacterial genomes has grown enormously, new β-lactamase variants continue to be described. As a common opportunistic pathogen [15], the information on the distribution of β-lactamase among *E. cloacae* was limited.

In this study, we first explored the distribution of β-lactamase including pAmpCs, bla_{ESBLs} and $bla_{\text{CHβLs}}$ among *E. cloacae* isolates based on a global database. For β-lactamase positive strains, the sequence types (STs) and the distribution of plasmid replicons were further investigated. Furthermore, the prevalent characteristics of β-lactamase-producing *E. cloacae* were analyzed.

Materials and methods

Acquisition of *E. cloacae* genomes and strain information

A total of 296 *E. cloacae* genomes were downloaded in batches from NCBI using Aspera software on 16$^{\text{th}}$, Dec 2021 [16]. The genomic quality of these 296 strains was further filtered by Checkm and Quast software [17, 18]. The high-quality genome was defined as "completeness > 90% and containment < 5%". Meanwhile, the quantity of contigs is required to be " ≤ 500, and N50 ≥ 40,000". Twenty genomes that did not meet the above conditions were filtered out. The investigated strains were collected from different years shown in Figure S1A, the collected dates of 58 strains were "blank" meaning that the information was missing. These strains were submitted by 32 countries, mainly from USA ($n = 58$), France ($n = 30$), United Kingdom ($n = 27$), China ($n = 24$), Japan ($n = 18$), Singapore ($n = 13$) and Nigeria ($n = 12$), other countries were also involved (Figure S1B). The countries of 26 strains remained unknown. Notably, 158 out of 272 strains were hosted by Homo sapiens ($n = 158$, 58.1%), mainly from gastrointestinal tract ($n = 57$, 21.0%).

Investigation of β-lactamase among global *E. cloacae*

To avoid differences in genome gene prediction by different annotation methods. All the 276 genomes were annotated by Prokka software [19], which is a fast prokaryotic genome annotation software. All the strains containing β-lactamase encoding genes were further analyzed.

Analysis on the sequence type of β-lactamase carrying *E. cloacae*

The self-made Perl program was used to extract the nucleotide coding sequence of genes from each genome sequence file (GBK format) [20]. The allele sequences and allelic profiles of 7 conserved genes of *E. cloacae* were downloaded from website https://pubmlst.org/. The sequence of the genome was set as "query", the seven conserved gene sequence files were set as "subject "(database). Blastn alignment analysis was then implemented between query and subject. The thresholds set were as follows: E-value = 1e-5, identity = 100%, matching length = subject gene length.

Investigation of plasmid replicons among ß-lactamase positive *E. cloacae*

To analyze the distribution of plasmid replicons among β-lactamase-carrying *E. cloacae*. The genomes were submitted into the website and PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) was used to analyze the presence of plasmid replicons (Identity: 90%; Coverage: 90%).

Statistical analysis

The differences on the distribution of major resistant determinants and plasmid replicons among $bla_{CH\beta Ls}$-carrying strains and strains without $bla_{CH\beta Ls}$ was analyzed by Chi-square test. Distribution difference on resistant determinants and plasmid replicons among all the β-lactamase-producing and among the $bla_{CH\beta Ls}$-carrying strains were checked by McNemar test. The distribution rates were statistically different when p value was less than 0.05.

Results

The distribution of β-lactamase among global *E. cloacae*

In total, 272 out of 276 strains were found to carry β-lactamase encoding genes. There were 23 varieties of ß-lactamase being found, bla_{CMH} ($n=130$, 47.8%) and bla_{ACT} ($n=126$, 46.3%) were the most predominant ones. Other ß-lactamase encoding genes included bla_{TEM} ($n=90$, 33.1%), bla_{OXA} ($n=51$,18.8%), bla_{CTX-M} ($n=48$, 17.6%), bla_{VIM} ($n=29$, 10.7%), bla_{KPC} ($n=24$, 8.8%), bla_{SHV} ($n=23$, 8.5%), bla_{NDM} ($n=22$, 8.1%), bla_{IMI} ($n=17$, 6.3%), bla_{MIR} ($n=11$, 4.0%), bla_{LAP-2} ($n=10$, 3.7%), bla_{IMP} ($n=7$, 2.6%), bla_{DHA} ($n=7$, 2.6%), bla_{GES} ($n=4$, 1.5%), bla_{CMY} ($n=3$,1.1%), bla_{FOX-5} ($n=3$,1.1%), bla_{VEB-3} ($n=2$, 0.7%), bla_{NMC-A} ($n=2$, 0.7%), bla_{CARB} ($n=2$, 0.7%), bla_{FLC-1} ($n=1$, 0.4%), bla_{ORN-1} ($n=1$, 0.4%) and bla_{SCO-1} ($n=1$, 0.4%).

In detail, the variants of pAmpCs including bla_{CMH}, bla_{ACT} and bla_{MIR} were shown in Fig. 1, with bla_{CMH-6} ($n=41$, 15.1%) and bla_{ACT-59}($n=34$, 12.5%) being the most frequent ones. Multiple variants of bla_{ESBLs} including bla_{CTX}, bla_{TEM}, bla_{OXA} and bla_{SHV} were also found (Fig. 2). Among them, $bla_{CTX-M-15}$ ($n=33$, 12.1%) and bla_{SHV-12} ($n=19$, 7.0%) were the most common ones.

Overall, 9 genotypes of $bla_{CH\beta Ls}$ including bla_{NDM}, bla_{IMP}, bla_{OXA}, bla_{KPC}, bla_{VIM}, bla_{FLC-1}, bla_{NMC-A}, bla_{GES} and bla_{IMI} were found among 106 strains (Fig. 3). Besides the $bla_{CH\beta Ls}$ in the Fig. 3, other ones including bla_{OXA-48} ($n=3$, 2.9%) and $bla_{OXA-181}$ ($n=2$, 1.9%), bla_{NMC-A} ($n=2$, 1.9%) and bla_{FLC-1} ($n=1$, 1.0%) were also identified.

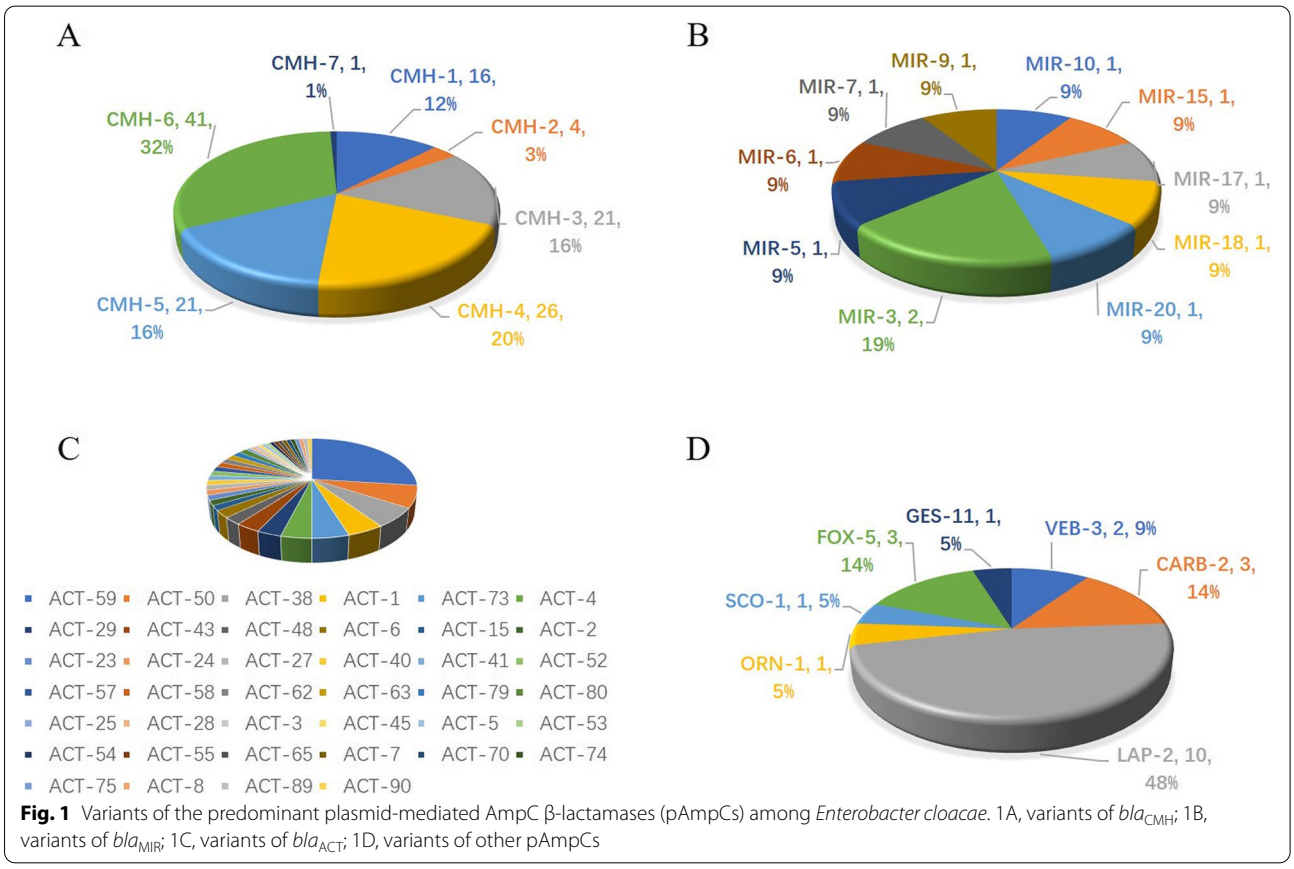


Fig. 1 Variants of the predominant plasmid-mediated AmpC β-lactamases (pAmpCs) among *Enterobacter cloacae*. 1A, variants of bla_{CMH}; 1B, variants of bla_{MIR}; 1C, variants of bla_{ACT}; 1D, variants of other pAmpCs

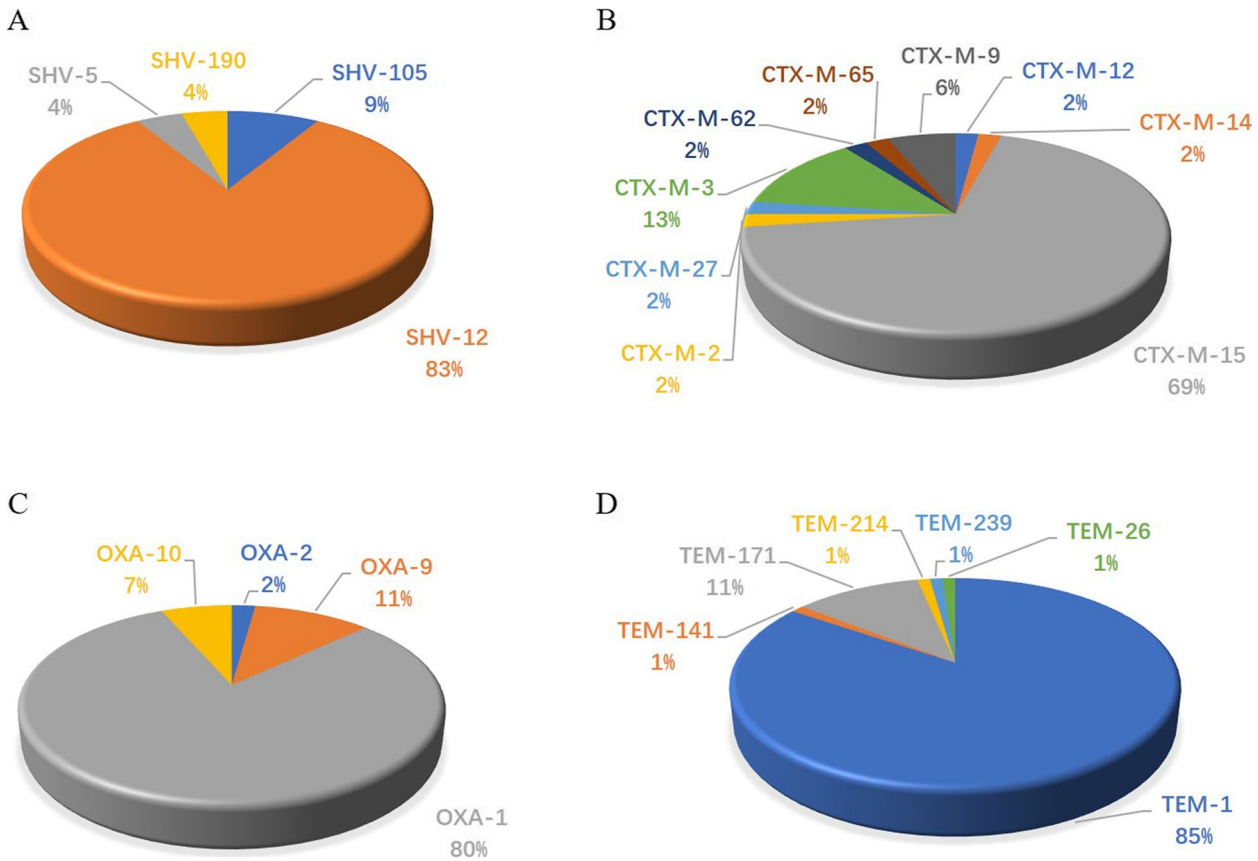


Fig. 2 Variants of the predominant extended-spectrum β-lactamases (ESBLs) among *Enterobacter cloacae*. 2A, Variants of bla_{SHV}; 2B, Variants of bla_{CTX-M}; 3B, Variants of bla_{OXA}; *4B,* Variants of bla_{TEM}

The distribution of bla_{ACT}, bla_{SHV} and bla_{TEM} were obviously higher among $bla_{CH\beta Ls}$-carrying *E. cloacae* comparing to the prevalence of these genes among the strains without $bla_{CH\beta Ls}$ ($p<0.05$), whereas bla_{CMH} and oxacillin-hydrolyzing-bla_{OXA} were much more prevalent among *E. cloacae* strains without $bla_{CH\beta Ls}$ than $bla_{CH\beta Ls}$-carrying ones ($p<0.05$) (Table 1).

The sequence types of β-lactamase-carrying *E. cloacae*

Totally, there were 115 distinct STs for the 272 β-lactamase-carrying *E. cloacae* (Fig. 4). ST873 ($n=27$, 23.5%) was the most frequent one followed by ST456 ($n=11$, 9.6%). ST1 ($n=9$, 7.8%), ST93 ($n=5$, 4.3%) and ST976 ($n=5$, 4.3%) were less common. The STs of 41 strains remained unknown and 12 strains belonged to novel STs. Other 110 STs were scattered (Fig. 4).

Furthermore, 48 different STs were identified for $bla_{CH\beta Ls}$-carrying *E. cloacae* (Fig. 5). And ST873 ($n=27$, 25.7%) and ST456 ($n=11$,10.5%) was the most common ones. Diverse STs were identified for $bla_{CH\beta Ls}$-carrying *E. cloacae* (Fig. 6). Interestingly, all the 23 bla_{VIM-4}-carrying *E. cloacae*, and 3 out of 6 bla_{VIM-1}- carrying *E. cloacae* isolates were assigned into ST873 (Fig. 6A). Whereas 19 bla_{KPC-2} ones were assigned to 13 STs (Fig. 6B), and 17 bla_{NDM-1} ones were assigned into 14 STs (Fig. 6C). Furthermore, 9 distinct STs for 17 bla_{IMI}- carrying strains (Fig. 6D), 7 different STs for 7 bla_{IMP}-carrying ones (Fig. 6E) and 2 STs for 5 strains carrying carbapenem-hydrolyzing bla_{OXA} (Fig. 6F) were identified. Of note, 27 out of 34 bla_{ACT-59} were found to be carried by ST873 strains.

The plasmid replicons of CHβLs-carrying *E. cloacae*

Totally, 25 different plasmid replicons were identified. IncHI2 ($n=65$, 23.9%), IncHI2A ($n=64$, 23.5%) and IncFII ($n=62$, 22.8%) were the most common ones followed by IncCol ($n=48$, 17.6%), IncFII ($n=41$, 15.1%) and IncR ($n=28$, 10.3%). IncFIA ($n=20$, 7.4%), IncN ($n=18$, 6.6%), IncX3 ($n=12$, 4.4%), IncC ($n=8$, 2.9%), IncHI1B ($n=8$, 2.9%), IncM1 ($n=7$, 2.6%), IIncHI1A ($n=6$, 2.2%), IncP6 ($n=5$, 1.8%), pKPC-CAV1193 ($n=4$, 1.5%), IncQ1 ($n=3$, 1.1%), IncL ($n=3$, 1.1%), IncX5 ($n=2$, 0.7%), IncX4 ($n=1$, 0.4%), IncM2 ($n=1$, 0.4%), IncN2 ($n=1$, 0.4%), IncP1 ($n=1$, 0.4%), IncA

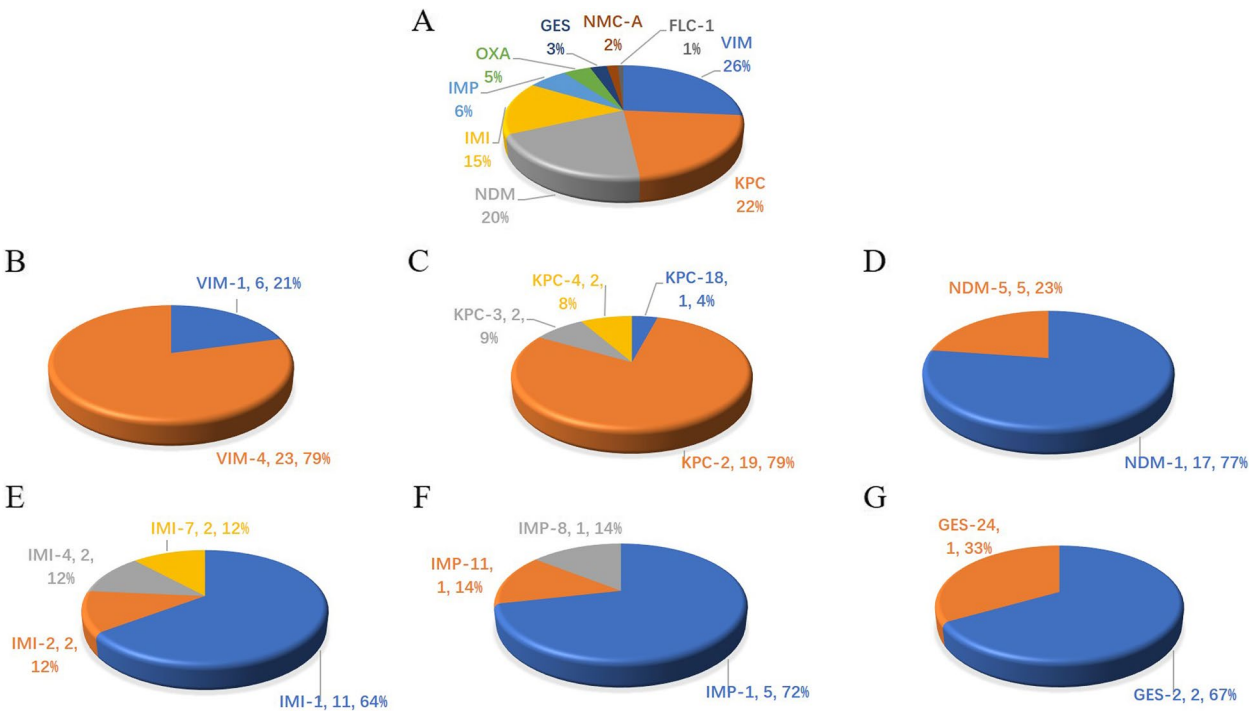


Fig. 3 Variants of the predominant carbapenem-hydrolyzing β-lactamase (CHβLs) among *Enterobacter cloacae*. 3A, carbapenemase detected in this study. 3B, Variants of bla_{VIM}; 3C, Variants of bla_{KPC}; 3D, Variants of bla_{NDM}; 3E, Variants of bla_{IMI}; 3F, Variants of bla_{IMP}; 3G, Variants of bla_{GES}

Table 1 The differences on the distribution of resistant determinants among $bla_{CHβLs}$ positive and $bla_{CHβLs}$ negtive *Enterobacter cloacae*

	$bla_{CHβLs}$ positive strains ($n=106$)	$bla_{CHβLs}$ negative strains ($n=166$)	Chi-square value	*P* value
bla_{CMH} ($n=130$)	41 (38.7%)	89 (53.6%)	5.873	0.016
bla_{ACT} ($n=126$)	60 (56.7%)	66 (39.8%)	7.382	0.007
bla_{OXA} ($n=51$)	3 (2.8%)	27 (16.3%)	10.569[a]	0.001
bla_{CTX-M} ($n=48$)	18 (17.0%)	29 (17.5%)	0.011	0.917
bla_{SHV} ($n=23$)	16 (15.1%)	5 (3.0%)	13.255	0.000
bla_{TEM} ($n=90$)	61 (57.5%)	29 (17.5%)	46.932	0.000

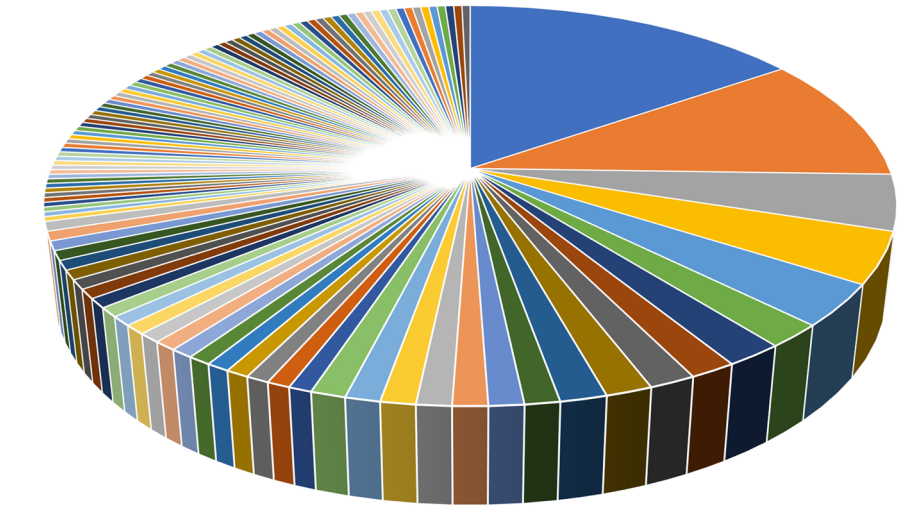
CHβLs Carbapenem-hydrolyzing β-lactamase

[a] Continuity correction

($n=1$, 0.4%), repA ($n=1$, 0.4%) and repB ($n=1$, 0.4%) were also found. It was worth mentioning that no plasmid replicons were found among 97 strains, 62 (22.8%) out of which only contained one bla_{CMH}, 21 (7.7%) ones carried $bla_{CHβLs}$.

Notably, the prevalence of replicons IncHI2 and IncHI2A among $bla_{CHβLs}$ -carrying strains were significantly higher than that among the strains without $bla_{CHβLs}$ ($p<0.05$), whereas no significant difference on the prevalence of plasmid replicons IncCOI, IncFII, IncFIB and IncR among these two groups were observed (Table 2).

The distribution of bla_{SHV} was consistent with plasmid replicon IncR, and prevalence of bla_{CTX-M} was in accordance with the prevalence of IncFII, IncFIB and IncHI2A ($p>0.05$). Additionally, the prevalence of oxacillin-hydrolyzing-bla_{OXA} and IncFIB as well as IncCOI was accordant (Table 3). Moreover, the prevalence of bla_{KPC} and bla_{VIM} were consistent with the distribution of IncCOI, IncFII, IncFIB, IncHI2 and IncHI2A, and no differences



Unknown ST873 Novel ST456 ST1 ST93 ST976 ST24 ST519
ST836 ST1421 ST373 ST432 ST513 ST658 ST712 ST820 ST932
ST41 ST50 ST78 ST84 ST113 ST156 ST477 ST524 ST608
ST609 ST669 ST719 ST721 ST738 ST780 ST995 ST1065 ST1067
ST1385 ST1513 ST1516 ST25 ST28 ST32 ST88 ST90 ST97
ST108 ST131 ST137 ST167 ST175 ST187 ST191 ST200 ST231
ST232 ST254 ST267 ST286 ST316 ST382 ST412 ST413 ST414
ST421 ST435 ST462 ST484 ST525 ST595 ST610 ST627 ST647
ST657 ST673 ST674 ST680 ST681 ST690 ST700 ST717 ST736
ST765 ST767 ST771 ST774 ST789 ST807 ST813 ST837 ST922
ST927 ST928 ST948 ST982 ST984 ST997 ST1010 ST1028 ST1059
ST1066 ST1312 ST1392 ST1510 ST1511 ST1515 ST1517 ST1519 ST1520
ST1521 ST1522 ST1523 ST1524 ST1525 ST1551 ST1552 ST1576 ST1615

Fig. 4 The sequence types of 272 β-lactamase-producing *Enterobacter cloacae*

were observed on the distribution of bla_{IMI}, bla_{NDM} and those of IncCOI, IncFII and IncFIB ($p > 0.05$) (Table 4).

Discussion

β-lactamase is a primary resistance determinant, widely disseminating on mobile genetic elements across the opportunistic pathogens including *E. cloacae* [21]. Exploring the spread characteristics of β-lactamase among *E. cloacae* based on the global genome database of GenBank is quite important for illustrating the resistance characteristics of such strains and guiding rational drug use in clinic.

Our analysis showed that the number of *E. cloacae* has been continuously increasing since the genome of first one was submitted in 2003. More than 32 countries all over the world submitted the genomes, indicating the representativeness of these strains. To note, the host of these β-carrying *E. cloacae* strains were predominantly Homo sapiens, with the gastrointestinal tract being the major isolation resource, suggesting that Homo sapiens were the dominant host and gastrointestinal tract was predilection seat. Importantly, 106 $bla_{\text{CHβLs}}$-carrying *E. cloacae* strains isolated during 2010–2020 were scattered among global 27 countries and 5 continents, indicating a rapid emergence and wide distribution of such strain, which alerts us the urgency of implementation of prevention and control measures.

Our analysis showed that bla_{CMH} was the most frequent β-lactamase gene. However, literature search with bla_{CMH} as the key word showed that $bla_{\text{CMH-1}}$ was first detected in *E. cloacae* as a novel bla_{AmpC} gene at a Medical Center in Southern Taiwan [22]. Since then, $bla_{\text{CMH-2}}$ and $bla_{\text{CMH-3}}$ were sequentially identified in India and Europe [22, 23]. Thereafter, no bla_{CMH} was reported in PubMed database albeit genomic analysis showed the widest distribution of these enzyme. To our surprise, the most prevalent $bla_{\text{CMH-6}}$, $bla_{\text{CMH-4}}$, $bla_{\text{CMH-5}}$ and $bla_{\text{CMH-3}}$ were not identified among *E. cloacae* at all. Moreover, $bla_{\text{ORN-1}}$,

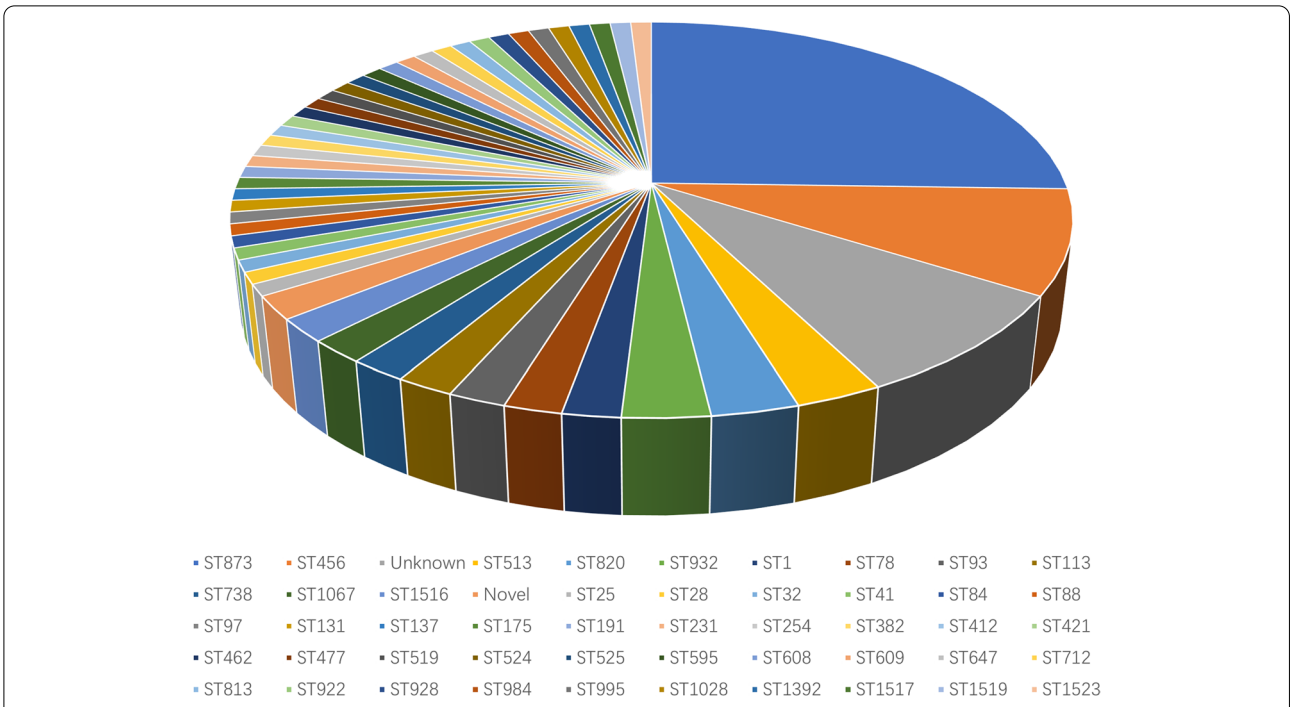


Fig. 5 The sequence types of 106 carbapenem-hydrolyzing β-lactamase-producing *Enterobacter cloacae*

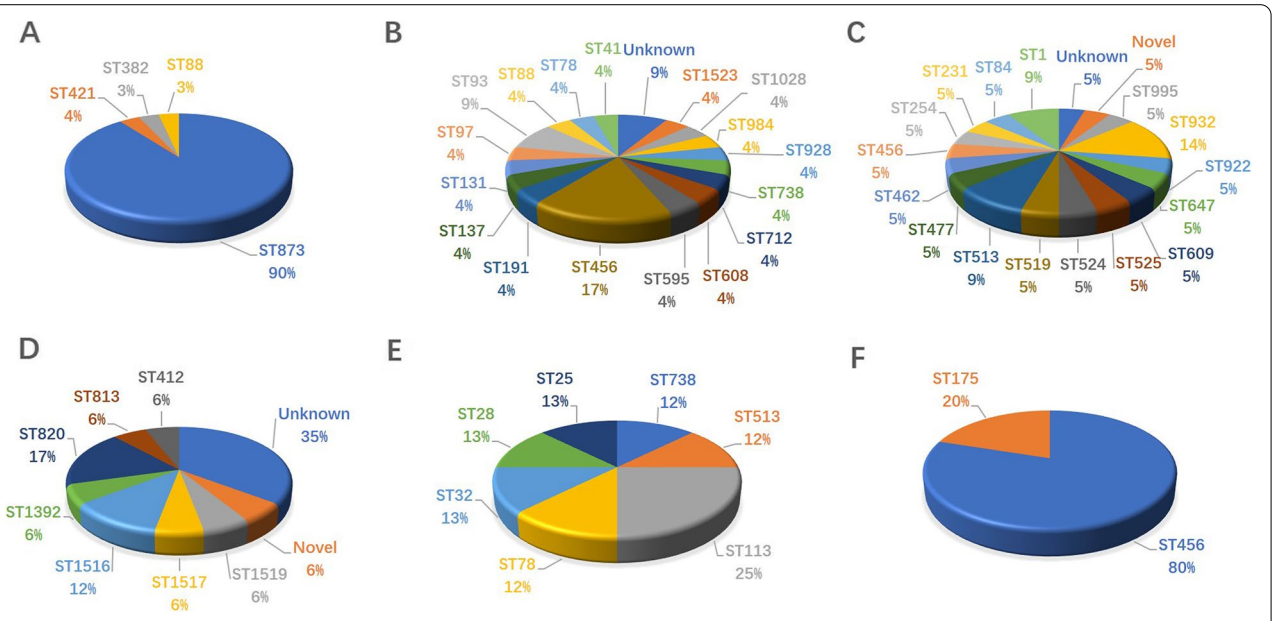


Fig. 6 The sequence types of dominant carbapenem-hydrolyzing β-lactamase-producing *Enterobacter cloacae*. 6A, the sequence types (STs) of bla_{VIM}-carrying strains; 6B, The STs of bla_{KPC}-carrying strains; 6C, STs of bla_{NDM}-carrying strains; 6D, STs of bla_{IMI}-carrying strains; 6E, STs of bla_{IMP}-carrying strains; 6F, STs of bla_{OXA}-carrying strains

identified in the chromosome of *Raoultella. ornithinolytica* in 2004 [24, 25], has never been reported in *E. cloacae*. Interestingly, bla_{CARB-2} as a carbenicillin-hydrolyzing enzyme, has been identified within multiple strains including *Klebsiella. pneumonia* [26], *Achromobacter xylosoxidan* [27], *Escherichia. coli* [28], *Acinetobacter pittii* [29] and *E. cloacae* [30] in a variety of countries, however, was quite rare in our study. Which may be related

Table 2 The differences on the distribution of plasmid replicons among $bla_{CH\beta Ls}$ positive and $bla_{CH\beta Ls}$ negtive *Enterobacter cloacae*

	$bla_{CH\beta Ls}$ positive strains ($n=106$)	$bla_{CH\beta Ls}$ negtive strains ($n=166$)	Chi-square	*P* value
IncCOI ($n=41$)	21 (19.8%)	20 (12.4%)	3.046	0.081
IncFII ($n=62$)	28 (26.4%)	34 (37.3%)	1.294	0.255
IncFIB ($n=58$)	29 (27.4%)	29 (17.5%)	3.771	0.052
IncHI2 ($n=65$)	37 (34.9%)	28 (16.9%)	11.574	0.001
IncHI2A ($n=64$)	37 (57.5%)	27 (16.3%)	12.493	0.000
IncR ($n=27$)	9 (8.5%)	19 (11.4%)	0.612	0.434

CHβLs Carbapenem-hydrolyzing β-lactamase

to its clinical importance. bla_{LAP-2} as a narrow-spectrum β-lactamase was also rare in our study, albeit it has been reported [31] [32]. Furthermore, bla_{SCO-1} was a novel plasmid-mediated class A β-lactamase with carbenicillinase characteristics in *E. coli* [33], has not been reported in *E. cloacae* until now. As we know that bla_{ACT} was also a plasmid-encoded *ampC* gene [34]. Although the prevalence of bla_{ACT} was secondary to bla_{CMH} in our study, distribution of exact bla_{ACT}-variants was not so high. Note worthily, the most common bla_{ACT-59} in our study has never been reported. Which may be due to the limitation of screening methods. It was reported that bla_{VEB-3} was encoded by the chromosome and located in an integron, and only 2 bla_{VEB-2} genes were detected in our study. However, outbreak of infection caused by bla_{VEB-3}-carrying-*E. cloacae* has been reported in China [35],

Additionally, bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMI} and bla_{IMP} were the major $bla_{CH\beta Ls}$ accounting for carbapenem resistance in global *E. cloacae*. Among them, bla_{KPC-2} and bla_{VIM-4} were the most predominant ones. Which is a light different from previous report showing bla_{KPC-2} and bla_{IMP-8} was the main $bla_{CH\beta Ls}$ within *E. cloacae* in China [36]. Noteworthily, 28 bla_{VIM-4}-carrying *E. cloacae* ST873 were only found in Homo sapiens in France, indicating that there was a clonal dissemination of such strain among Homo sapiens in France during 2010–2020, which was not reported previously, albeit nosocomial infections caused by *E. cloacae* ST873 in 2 hospitals in France has been reported [37]. As a novel $bla_{CH\beta Ls}$, bla_{FLC-1} belongs to Ambler class A β-lactamases, has been identified an *E. cloacae* Complex isolated from food products [38]. Interestingly, such enzyme displayed a distinctive substrate profile, hydrolyzing penicillin, narrow- and broad-spectrum cephalosporins, aztreonam, and carbapenems but not extended-spectrum cephalosporin. In addition, bla_{NMC-A}, a class A $bla_{CH\beta L}$, has been frequently detected in *E. cloacae* [39, 40] [41, 42], albeit we just found 2 bla_{NMC-A} in this study. As $bla_{CH\beta Ls}$, bla_{GES-24} seems to have a broader host than bla_{GES-2} although we only found 2 bla_{GES-2} and 1 bla_{GES-24} in this study. To date, all reports on bla_{IMI-1} focus on *E. cloacae*, indicating that *E. cloacae* may be the best host for bla_{IMI}.

Table 3 The differences on the distribution of plasmid replicons and resistant determinants among the β-lactamase producing *Enterobacter cloacae*

	bla_{CMH} ($n=130$)	$blaA_{CT}$ ($n=126$)	bla_{OXA}[a] ($n=43$)	bla_{CTM-M} ($n=47$)	bla_{SHV} ($n=21$)	bla_{TEM} ($n=90$)
IncCOI ($n=48$)	0.000	0.000	0.609	1.000	0.000	0.000
IncFII ($n=62$)	0.000	0.000	0.037	0.137	0.000	0.012
IncFIB ($n=58$)	0.000	0.000	0.146	0.272	0.000	0.041
IncHI2 ($n=65$)	0.000	0.000	0.023	0.038	0.000	0.005
IncHI2A ($n=64$)	0.000	0.000	0.031	0.053	0.000	0.003
IncR ($n=28$)	0.000	0.000	0.006	0.007	0.347	0.000

[a] Oxacillin-hydrolyzing-OXA

Table 4 The differences on the distribution of plasmid replicons and resistant determinants among the $bla_{CH\beta Ls}$ -carrying *Enterobacter cloacae*

Plasmid replicons	bla_{KPC} ($n=24$)	bla_{IMI} ($n=17$)	bla_{VIM} ($n=29$)	bla_{NDM} ($n=22$)
IncCOI ($n=21$)	0.736	0.627	0.268	1.000
IncFII ($n=28$)	0.652	0.080	1.000	0.451
IncFIB ($n=29$)	0.551	0.058	1.000	0.337
IncHI2 ($n=37$)	0.085	0.008	0.200	0.036
IncHI2A ($n=37$)	0.085	0.008	0.200	0.036

CHβLs Carbapenem-hydrolyzing β-lactamase

The higher prevalence of bla_{TEM} and bla_{SHV} among $bla_{CH\beta Ls}$-carriers in our study was in accordance with a previous report to some degree, which showed that bla_{CTX-M}, bla_{TEM} and bla_{SHV} were mostly detected concurrently with $bla_{CH\beta Ls}$ [43]. Albeit no distribution difference of bla_{CTX-M} was observed. Notably, the significantly higher distribution of bla_{CMH} among non-CHβLs-producers may indicate that bla_{CMH} may be the predominant gene conferring β-lactams among the strains without $bla_{CH\beta Ls}$.

Furthermore, the multiple STs identified in our study displayed a genetic diversity of β-lactamase producing *E. cloacae*. It seemed that clonal dissemination for such strain was rare except for bla_{VIM-4}-carrying ST873 ones, suggesting that the spread of CREL was mainly mediated by mobile elements such as plasmids.

Additionally, variously distinct plasmid replicons detected in our study indicate their dissemination potential for resistant determinants. Noteworthily, the obviously higher prevalence of IncHI2 and IncHI2A among $bla_{CH\beta Ls}$-carrying strains may suggest association between $bla_{CH\beta Ls}$ and IncHI2. It was reported that IncHI2 widely detected in global CRE genomes, was termed as 'super-plasmids' resulting from the large size and prolific carriage of resistance determinants [44]. And the consistent distribution of such plasmid and bla_{KPC} and bla_{VIM} may indicate a good cost fitness between them.

There were several limitations in this study. First, the number of *E. cloacae* was relatively small which may result from the reason that *E. cloacae* was only little part of *E. cloacae* complex. Second, the resistance profiles of these strains were not available for us to compare the difference between the genotypes and phenotypes. Some of the strain information were missing, which was not beneficial for us to fully illustrate the characterization of *E. cloacae*. Third, some new enzymes are devoid of further phenotypic descriptions because they were directly obtained from whole-genome sequencing studies. Anyway, it is currently difficult to draw an accurate global picture of this bacteria, highlighting the need for more comprehensive genome sequence data and genomic analysis.

In summary, almost all the *E. cloacae* contained β-lactamase encoding gene. Among the global *E. cloacae*, bla_{CMH} and bla_{ACT} were main bla_{AmpC} genes. Bla_{TEM} and bla_{CTX-M} were the predominant ESBLs. Bla_{KPC}, bla_{VIM} and bla_{NDM} were the major CHβLs. Additionally, diversely distinct STs and different replicons were identified.

Abbreviations

CRE: Carbapenem-resistant Enterobacteriaceae; ESBLs: Extended-spectrum β-lactamases; CHβLs: Carbapenem-hydrolyzing β-lactamase; pAmpCs: Plasmid-mediated AmpC β-lactamases; CREL: Carbapenem resistance *Klebsiella pneumoniae*; KPC: *Klebsiella pneumoniae* Carbapenemase; NDM: New Delhi metallo-beta-lactamase; ESBLs: Extended-spectrum β-lactamases; MLST: Multi-locus sequence typing; STs: Sequence types.

Supplementary Information

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Additional file 1.

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Disclaimer

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Authors' contributions

HJC performed the Bioinformatics analysis and writing; LJ sorted the data and help with the writing; LC, LCC and ZY interpreted the data regarding the resistant determinants and plasmid replicons. XH performed statistical analysis. SH and CXL designed the work and were a major contributor in revising the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from GenBank and the accession number and web link to datasets for the provided name of these strains were shown in table S1.xlsx.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Laboratory Medicine, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Zhongshan Road 321, GulouJiangsu Province, Nanjing, People's Republic of China. [2]Department of Acute Infectious Disease Control and Prevention, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

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References

1. Wu W, Wei L, Feng Y, Xie Y, Zong Z. Precise species identification by whole-genome sequencing of enterobacter bloodstream infection. China Emerg Infect Dis. 2021;27(1):161–9.
2. Stokes W, Peirano G, Matsumara Y, Nobrega D, Pitout JDD. Population-based surveillance of Enterobacter cloacae complex causing blood

stream infections in a centralized Canadian region. Eur J Clin Microbiol Infect Dis. 2022;41(1):119–25.
3. Ito A, Nishikawa T, Ota M, Ito-Horiyama T, Ishibashi N, Sato T, et al. Stability and low induction propensity of cefiderocol against chromosomal AmpC beta-lactamases of Pseudomonas aeruginosa and Enterobacter cloacae. J Antimicrob Chemother. 2018;73(11):3049–52.
4. Davin Regli A, Lavigne JP, Pages JM. Enterobacter spp.:update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev. 2019;32(4):e00002-19.
5. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. beta-Lactamases and beta-Lactamase Inhibitors in the 21st Century. J Mol Biol. 2019;431(18):3472–500.
6. Peymani A, Naserpour-Farivar T, Yeylagh-Beygi M, Bolori S. Emergence of CMY-2- and DHA-1-type AmpC β-lactamases in *Enterobacter cloacae* isolated from several hospitals of Qazvin and Tehran. Iran Iran J Microbiol. 2016;8(3):168–74.
7. Garza-Gonzalez E, Bocanegra-Ibarias P, Bobadilla-Del-Valle M, Ponce-de-Leon-Garduno LA, Esteban-Kenel V, Silva-Sanchez J, et al. Drug resistance phenotypes and genotypes in Mexico in representative gram-negative species: Results from the infivar network. PLoS ONE. 2021;16(3): e0248614.
8. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, et al. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant enterobacteriaceae isolated from adult and children patients in China. Front Cell Infect Microbiol. 2020;10:314.
9. Chen J, Tian S, Nian H, Wang R, Li F, Jiang N, et al. Carbapenem-resistant Enterobacter cloacae complex in a tertiary Hospital in Northeast China, 2010–2019. BMC Infect Dis. 2021;21(1):611.
10. Kananizadeh P, Oshiro S, Watanabe S, Iwata S, Kuwahara-Arai K, Shimojima M, et al. Emergence of carbapenem-resistant and colistin-susceptible Enterobacter cloacae complex co-harboring blaIMP-1 and mcr-9 in Japan. BMC Infect Dis. 2020;20(1):282.
11. Emeraud C, Petit C, Gauthier L, Bonnin RA, Naas T, Dortet L. Emergence of VIM-producing Enterobacter cloacae complex in France between 2015 and 2018. J Antimicrob Chemother. 2022;77(4):944–51.
12. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother. 2018;73(5):1121–37.
13. Liu W, Dong H, Yan T, Liu X, Cheng J, Liu C, et al. Molecular Characterization of bla IMP - 4 -Carrying Enterobacterales in Henan Province of China. Front Microbiol. 2021;12:626160.
14 Dong N, Li Y, Zhao J, Ma H, Wang J, Liang B, et al. The phenotypic and molecular characteristics of antimicrobial resistance of Salmonella enterica subsp. enterica serovar Typhimurium in Henan Province, China. BMC Infect Dis. 2020;20(1):511.
15. Chen Q, Lin Y, Li Z, Lu L, Li P, Wang K, et al. Characterization of a new transposon, Tn6696, on a bla NDM- 1-carrying plasmid from multidrug-resistant enterobacter cloacae ssp. dissolvens in China. Front Microbiol. 2020;11:525479.
16. Kim T, Seo HD, Hennighausen L, Lee D, Kang K. Octopus-toolkit: a workflow to automate mining of public epigenomic and transcriptomic next-generation sequencing data. Nucleic Acids Res. 2018;46(9):e53.
17. Khezri A, Avershina E, Ahmad R. Hybrid assembly provides improved resolution of plasmids, antimicrobial resistance genes, and virulence factors in escherichia coli and klebsiella pneumoniae clinical isolates. Microorganisms. 2021;9(12):2560.
18. Cornet L, Meunier L, Van Vlierberghe M, Leonard RR, Durieu B, Lara Y, et al. Consensus assessment of the contamination level of publicly available cyanobacterial genomes. PLoS ONE. 2018;13(7):e0200323.
19. Marchi E, Jones M, Klenerman P, Frater J, Magiorkinis G, Belshaw R. BreakAlign: a Perl program to align chimaeric (split) genomic NGS reads and allow visual confirmation of novel retroviral integrations. BMC Bioinformatics. 2022;23(1):134.
20. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, 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):S196–205.
21. Ku YH, Lee MF, Chuang YC, Yu WL. Detection of Plasmid-Mediated beta-Lactamase Genes and Emergence of a Novel AmpC (CMH-1) in Enterobacter cloacae at a Medical Center in Southern Taiwan. J Clin Med. 2018;8(1):8.
22. Ingti B, Laskar MA, Choudhury S, Maurya AP, Paul D, Talukdar AD, et al. Molecular and in silico analysis of a new plasmid-mediated AmpC beta-lactamase (CMH-2) in clinical isolates of Klebsiella pneumoniae. Infect Genet Evol. 2017;48:34–9.
23. López Hernández I, García Barrionuevo A, Díaz de Alba P, Clavijo E, Pascual A. Characterization of NDM-1- and CMH-3-producing Enterobacter cloacae complex ST932 in a patient transferred from Ukraine to Spain. Enferm Infecc miCrobiol cli (English ed). 2020;38(7):327–30.
24. Bush K. Past and Present Perspectives on beta-Lactamases. Antimicrob Agents Chemother. 2018;62(10).
25. Walckenaer E, Poirel L, Leflon-Guibout V, Nordmann P, Nicolas-Chanoine MH. Genetic and biochemical characterization of the chromosomal class a beta-lactamases of Raoultella (formerly Klebsiella) planticola and Raoultella ornithinolytica. Antimicrob Agents Chemother. 2004;48(1):305–12.
26. Zhu LC, Lu JW, Wang J, Xu T, Xu JH. Analyses on distribution and structure of bla(CARB-2) in Klebsiella pneumoniae. Yi Chuan. 2018;40(7):593–600.
27. Zhu Z, Xu J, He F. Genomic and phylogenetic analysis of multidrug-resistant Achromobacter xylosoxidans ST273 strain MTYH1 co-carrying blaOXA-114g and blaCARB-2 recovered from a wound infection in China. J Glob Antimicrob Resist. 2021;25:110–3.
28. Atlaw NA, Keelara S, Correa M, Foster D, Gebreyes W, Aidara-Kane A, et al. Identification of CTX-M Type ESBL E. coli from sheep and their Abattoir environment using whole-genome sequencing. Pathogens. 2021;10(11):1480.
29. Kamolvit W, Derrington P, Paterson DL, Sidjabat HE. A case of IMP-4-, OXA-421-, OXA-96-, and CARB-2-producing Acinetobacter pittii sequence type 119 in Australia. J Clin Microbiol. 2015;53(2):727–30.
30. Rodriguez-Martinez JM, Nordmann P, Poirel L. Group IIC intron with an unusual target of integration in Enterobacter cloacae. J Bacteriol. 2012;194(1):150–60.
31. Huang Z, Mi Z, Wang C. A novel beta-lactamase gene, LAP-2, produced by an Enterobacter cloacae clinical isolate in China. J Hosp Infect. 2008;70(1):95–6.
32. Kocsis B, Kocsis E, Fontana R, Cornaglia G, Mazzariol A. Identification of blaLAP-$_2$ and qnrS1 genes in the internationally successful Klebsiella pneumoniae ST147 clone. J Med Microbiol. 2013;62(Pt 2):269–73.
33. Papagiannitsis CC, Loli A, Tzouvelekis LS, Tzelepi E, Arlet G, Miriagou V. SCO-1, a novel plasmid-mediated class A beta-lactamase with carbenicillinase characteristics from Escherichia coli. Antimicrob Agents Chemother. 2007;51(6):2185–8.
34. Reisbig MD, Hossain A, Hanson ND. Factors influencing gene expression and resistance for Gram-negative organisms expressing plasmid-encoded ampC genes of Enterobacter origin. J Antimicrob Chemother. 2003;51(5):1141–51.
35. Jiang X, Ni Y, Jiang Y, Yuan F, Han L, Li M, et al. Outbreak of infection caused by Enterobacter cloacae producing the novel VEB-3 beta-lactamase in China. J Clin Microbiol. 2005;43(2):826–31.
36. Dai W, Sun S, Yang P, Huang S, Zhang X, Zhang L. Characterization of carbapenemases, extended spectrum β-lactamases and molecular epidemiology of carbapenem-non-susceptible Enterobacter cloacae in a Chinese hospital in Chongqing. Infect Genet Evol. 2013;14:1–7.
37. Beyrouthy R, Barets M, Marion E, Dananché C, Dauwalder O, Robin F, et al. Novel Enterobacter Lineage as Leading Cause of Nosocomial Outbreak Involving Carbapenemase-Producing Strains. Emerg Infect Dis. 2018;24(8):1505–15.
38. Brouwer MSM, Tehrani K, Rapallini M, Geurts Y, Kant A, Harders F, et al. Novel Carbapenemases FLC-1 and IMI-2 Encoded by an Enterobacter cloacae Complex Isolated from Food Products. Antimicrob Agents Chemother. 2019;63(6):e02338-18.
39. Mariotte-Boyer S, Nicolas-Chanoine MH, Labia R. A kinetic study of NMC-A beta-lactamase, an Ambler class A carbapenemase also hydrolyzing cephamycins. FEMS Microbiol Lett. 1996;143(1):29–33.
40. Swarén P, Maveyraud L, Raquet X, Cabantous S, Duez C, Pédelacq JD, et al. X-ray analysis of the NMC-A beta-lactamase at 1.64-A resolution, a class A carbapenemase with broad substrate specificity. J Biol Chem. 1998;273(41):26714–21.
41 Boyd DA, Mataseje LF, Davidson R, Delport JA, Fuller J, Hoang L, et al. Enterobacter cloacae Complex Isolates Harboring bla(NMC-A) or bla(IMI)-Type Class A Carbapenemase Genes on Novel Chromosomal Integrative Elements and Plasmids. Antimicrob Agents Chemother. 2017;61(5):e02578-16.

42. Antonelli A, D'Andrea MM, Di Pilato V, Viaggi B, Torricelli F, Rossolini GM. Characterization of a Novel Putative Xer-Dependent Integrative Mobile Element Carrying the bla(NMC-A) Carbapenemase Gene, Inserted into the Chromosome of Members of the Enterobacter cloacae Complex. Antimicrob Agents Chemother. 2015;59(10):6620–4.
43. Kopotsa K, Osei Sekyere J, Mbelle NM. Plasmid evolution in carbapenemase-producing Enterobacteriaceae: a review. Ann N Y Acad Sci. 2019;1457(1):61–91.
44. Macesic N, Blakeway LV, Stewart JD, Hawkey J, Wyres KL, Judd LM, et al. Silent spread of mobile colistin resistance gene mcr-9.1 on IncHI2 "superplasmids" in clinical carbapenem-resistant Enterobacterales. Clin Microbiol Infect. 2021;27(12):1856.e7-e13.

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