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

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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, \( \text{bla}_{\text{CMH}} (n = 130, 47.8\%) \) and \( \text{bla}_{\text{ACT}} (n = 126, 46.3\%) \) were the most predominant ones, 9 genotypes of carbapenem-hydrolyzing β-lactamase (CHβLs) were identified with \( \text{bla}_{\text{VIM}} (n = 29, 10.7\%) \) and \( \text{bla}_{\text{KPC}} (n = 24, 8.9\%) \) being the most dominant ones. In addition, 115 distinct STs for the 272 β-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 those among non-CHβLs-producing strains (p < 0.05).

Conclusion: Almost all the E. cloacae contained β-lactamase encoding gene. Among the global E. cloacae, \( \text{bla}_{\text{CMH}} \) and \( \text{bla}_{\text{ACT}} \) were main \( \text{bla}_{\text{AmpC}} \) genes. \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{CTX-M}} \) were the predominant ESBLs. \( \text{bla}_{\text{KPC}}, \text{bla}_{\text{VIM}} \) and \( \text{bla}_{\text{NDM}} \) 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 Enterobacteraceae [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
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-lactamasem, the more groupings in clinical laboratory generally correlate with broadly based molecular classification, where β-lactamasem 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-lactamasem (pAmpCs) with blaACT-like ampC genes being highly prevalent [6], extended-spectrum β-lactamasem (ESBLs) with blaSHV and blaCTX-M being widely distributed [7], and CHβLs, all of which are challenging antibiotic effectiveness.

Globally, blaCHβLs such as blaSEC (class A), blaNDM/VIM/IMP (class B) and blaOXA-48 (class D) are of grave clinical concern and proliferating [8]. It was reported that blaNDM-1 and blaNDM-5 were the main blaCHβLs, 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, blaIMP-1 was the dominant blaCHβLs conferring carbapenem resistance [10], and blaVIM was the main blaCHβLs 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 blaIMP-4-carrying strains [13], however, the prevalence of plasmid replicons among these bacteria were unknown. Notably, the association between IncN plasmid encoding blaCMY-2 β-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-lactamasem 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, blaESBLs and blaCHβLs 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 16th, 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 β-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<sub>CHβLs</sub>-carrying strains and strains without bla<sub>CHβLs</sub> was analyzed by Chi-square test. Distribution difference on resistant determinants and plasmid replicons among all the β-lactamase-producing and among the bla<sub>CHβLs</sub>-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 β-lactamase being found, bla<sub>CMH</sub> (n = 130, 47.8%) and bla<sub>ACT</sub> (n = 126, 46.3%) were the most predominant ones. Other β-lactamase encoding genes included bla<sub>TEM</sub> (n = 90, 33.1%), bla<sub>OXA</sub> (n = 51, 18.8%), bla<sub>CTX-M</sub> (n = 48, 17.6%), bla<sub>VIM</sub> (n = 29, 10.7%), bla<sub>KPC</sub> (n = 24, 8.8%), bla<sub>SHV</sub> (n = 23, 8.5%), bla<sub>NDM</sub> (n = 22, 8.1%), bla<sub>IMI</sub> (n = 17, 6.3%), bla<sub>MIR</sub> (n = 11, 4.0%), bla<sub>LAP-2</sub> (n = 10, 3.7%), bla<sub>IMP</sub> (n = 7, 2.6%), bla<sub>DIHA</sub> (n = 7, 2.6%), bla<sub>GES</sub> (n = 4, 1.5%), bla<sub>CMY</sub> (n = 3, 1.1%), bla<sub>FOX-5</sub> (n = 3, 1.1%), bla<sub>VEB-3</sub> (n = 2, 0.7%), bla<sub>NMC-A</sub> (n = 2, 0.7%), bla<sub>CARB</sub> (n = 2, 0.7%), bla<sub>FLC-1</sub> (n = 1, 0.4%), bla<sub>ORN-1</sub> (n = 1, 0.4%) and bla<sub>SCO-1</sub> (n = 1, 0.4%).

In detail, the variants of pAmpCs including bla<sub>CMH</sub>, bla<sub>ACT</sub> and bla<sub>MIR</sub> were shown in Fig. 1, with bla<sub>CMH</sub>-6 (n = 41, 15.1%) and bla<sub>ACT</sub>-59 (n = 34, 12.5%) being the most frequent ones. Multiple variants of bla<sub>ESBLs</sub> including bla<sub>CTX</sub>, bla<sub>TEM</sub>, bla<sub>OXA</sub> and bla<sub>SHV</sub> were also found (Fig. 2). Among them, bla<sub>CTX</sub>-M-15 (n = 33, 12.1%) and bla<sub>SHV</sub>-12 (n = 19, 7.0%) were the most common ones. Overall, 9 genotypes of bla<sub>CHβLs</sub> including bla<sub>NDM</sub>, bla<sub>IMP</sub>, bla<sub>OXA</sub>, bla<sub>KPC</sub>, bla<sub>VIM</sub>, bla<sub>FLC-1</sub>, bla<sub>NMC-A</sub>, bla<sub>GES</sub> and bla<sub>IMI</sub> were found among 106 strains (Fig. 3). Besides the bla<sub>CHβLs</sub> in the Fig. 3, other ones including bla<sub>OXA-48</sub> (n = 3, 2.9%) and bla<sub>OXA-181</sub> (n = 2, 1.9%), bla<sub>NMC-A</sub> (n = 2, 1.9%) and bla<sub>FLC-1</sub> (n = 1, 1.0%) were also identified.

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**Fig. 1** Variants of the predominant plasmid-mediated AmpC β-lactamases (pAmpCs) among *Enterobacter cloacae*. 1A, variants of bla<sub>CMH</sub>-18, variants of bla<sub>MIR</sub>-1C, variants of bla<sub>ACT</sub>-1D, variants of other pAmpCs
The distribution of bla\textsubscript{ACT}, bla\textsubscript{SHV} and bla\textsubscript{TEM} were obviously higher among bla\textsubscript{CHβLs}-carrying E. cloacae comparing to the prevalence of these genes among the strains without bla\textsubscript{CHβLs} (p<0.05), whereas bla\textsubscript{CMH} and oxacillin-hydrolyzing-bla\textsubscript{OXA} were much more prevalent among E. cloacae strains without bla\textsubscript{CHβLs} than bla\textsubscript{CHβLs}-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\textsubscript{CHβLs}-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\textsubscript{CHβLs}-carrying E. cloacae (Fig. 6). Interestingly, all the 23 bla\textsubscript{VIM-4} carrying E. cloacae, and 3 out of 6 bla\textsubscript{VIM-1} carrying E. cloacae isolates were assigned into ST873 (Fig. 6A). Whereas 19 bla\textsubscript{KPC-2} ones were assigned to 13 STs (Fig. 6B), and 17 bla\textsubscript{NDM-1} ones were assigned into 14 STs (Fig. 6C). Furthermore, 9 distinct STs for 17 bla\textsubscript{IMI} carrying strains (Fig. 6D), 7 different STs for 7 bla\textsubscript{IMP} carrying ones (Fig. 6E) and 2 STs for 5 strains carrying carbapenem-hydrolyzing bla\textsubscript{OXA} (Fig. 6F) were identified. Of note, 27 out of 34 bla\textsubscript{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%), IncFIA (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%), IncHI1A (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. 2 Variants of the predominant extended-spectrum β-lactamases (ESBLs) among Enterobacter cloacae. 2A, Variants of bla\textsubscript{SHV}; 2B, Variants of bla\textsubscript{CTX-M}; 3B, Variants of bla\textsubscript{OXA}; 4B, Variants of bla\textsubscript{TEM}](image-url)
(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

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

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

CHβLs: Carbenem-hydrolyzing β-lactamase

* Continuity correction
were observed on the distribution of \( \text{bla}^{\text{IMP}} \), \( \text{bla}^{\text{NDM}} \) 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^{CMH}*-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^{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^{CMH-2}* and *bla^{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^{CMH-6}*, *bla^{CMH-4}*, *bla^{CMH-5}*, and *bla^{CMH-3}* were not identified among *E. cloacae* at all. Moreover, *bla^{ORN-1}*,
identified in the chromosome of *Raoultella ornitholytica* in 2004 [24, 25], has never been reported in *E. cloacae*. Interestingly, *bla*$_{\text{CARB-2}}$ 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_{CHBLS} positive and bla_{CHBLS} negative Enterobacter cloacae

|                | bla_{CHBLS} positive strains (n = 106) | bla_{CHBLS} negative 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_{AP-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 carbencillinase 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 worthy, 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_{CHBLS} 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 little different from previous report showing bla_{KPC-2} and bla_{IMP-8} was the main bla_{CHBLS} within E. cloacae in China [36]. Noteworthy, 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_{CHBLS}, 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_{CHBLS} has been frequently detected in E. cloacae [39, 40] [41, 42], albeit we just found 2 bla_{NMC-A} in this study. As bla_{CHBLS}, 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) | bla_{ACT} (n = 126) | bla_{OXA} (n = 43) | bla_{CMH-24} (n = 47) | bla_{SMV} (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             |

*Oxacillin-hydrolyzing-OXA

Table 4 The differences on the distribution of plasmid replicons and resistant determinants among the bla_{CHBLS} -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 \textit{bla}_{\text{TEM}} and \textit{bla}_{\text{SHV}} among \textit{bla}_{\text{CHβLs}}-carriers in our study was in accordance with a previous report to some degree, which showed that \textit{bla}_{\text{CTX-M}}, \textit{bla}_{\text{TEM}} and \textit{bla}_{\text{SHV}} were mostly detected concurrently with \textit{bla}_{\text{CHβLs}} [43]. Albeit no distribution difference of \textit{bla}_{\text{CTX-M}} was observed. Notably, the significantly higher distribution of \textit{bla}_{\text{CMH}} among non-\textit{CHβLs}-producers may indicate that \textit{bla}_{\text{CMH}} may be the predominant gene conferring β-lactams among the strains without \textit{bla}_{\text{CHβLs}}.

Furthermore, the multiple STs identified in our study displayed a genetic diversity of β-lactamase producing \textit{E. cloacae}. It seemed that clonal dissemination for such strain was rare except for \textit{bla}_{\text{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 \textit{bla}_{\text{CHβLs}}-carrying strains may suggest association between \textit{bla}_{\text{CHβLs}} 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 \textit{bla}_{\text{KPC}} and \textit{bla}_{\text{VIM}} may indicate a good cost fitness between them.

There were several limitations in this study. First, the number of \textit{E. cloacae} was relatively small which may result from the reason that \textit{E. cloacae} was only little part of \textit{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 \textit{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 \textit{E. cloacae} contained β-lactamase encoding gene. Among the global \textit{E. cloacae}, \textit{bla}_{\text{CMH}} and \textit{bla}_{\text{ACT}} were main \textit{bla}_{\text{AmpC}} genes. \textit{bla}_{\text{TEM}} and \textit{bla}_{\text{CTX-M}} were the predominate ESBLs. \textit{bla}_{\text{KPC}}, \textit{bla}_{\text{VIM}} and \textit{bla}_{\text{NDM}} were the major \textit{CHβLs}. Additionally, diversely distinct STs and different replicons were identified.

\begin{center}
\textbf{Abbreviations}
\begin{tabular}{ll}
CRE: Carbapenem-resistant Enterobacteriaceae; ESBLs: Extended-spectrum β-lactamases; \textit{CHβLs}: Carbapenem-hydrolyzing β-lactamase; pAmpCs: Plasmid-mediated AmpC β-lactamases; CREL: Carbapenem resistance.
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\end{center}
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