



RESEARCH ARTICLE



## Epidemiological characteristics and molecular features of carbapenem-resistant *Enterobacter* strains in China: a multicenter genomic study

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### ABSTRACT

Epidemiological characteristics and molecular features of carbapenem-resistant *Enterobacter* (CR-Ent) species remain unclear in China. In this study, we performed a genomic study on 92 isolates from *Enterobacter*-caused infections from a multicenter study in China. Whole genome sequencing (WGS) was used to determine the genome sequence of 92 non-duplicated CR-Ent strains collected from multiple tertiary health centres. The precise species of *Enterobacter* strains were identified by average nucleotide identity (ANI) and in silico DNA–DNA hybridization (isDDH). Molecular features of high-risk CR-Ent sequence type (ST) lineages and carbapenemase-encoding plasmids were determined. The result revealed that the most common human-source CR-Ent species in China was *E. xiangfangensis* (66/92, 71.93%), and the proportion of carbapenemase-producing *Enterobacter* (CP-Ent) in CR-Ent was high (72/92, 78.26%) in comparison to other global regions. Furthermore, ST171 and ST116 *E. xiangfangensis* were the major lineages of CP-Ent strains, and ST171 *E. xiangfangensis* was more likely to cause infections in older patients. Genomic analysis also highlighted the likelihood of intra-hospital/inter-hospital clonal transmission of ST171 and ST116 *E. xiangfangensis*. In addition, the *bla*<sub>NDM</sub>-harbouring IncX3-type plasmid was identified as the prevalent carbapenemase-encoding plasmid carried by CR-Ent strains, and was experimentally confirmed to be able to self-transfer with high frequency. This study detailed the genomic and clinical characteristics of CR-Ent in China in the form of multicenter for the first time. The high risk of carbapenemase-producing ST171 and ST116 *E. xiangfangensis*, and the *bla*<sub>NDM</sub>-harbouring IncX3-type plasmid were detected and emphasized.

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The genus *Enterobacter* is the third most common pathogenic *Enterobacteriaceae* species in human after *Escherichia* and *Klebsiella*, which can cause a variety of clinical infections, including bloodstream and intra-abdominal infections [1,2]. Carbapenems are the main drugs for the treatment of multidrug-resistant *Enterobacter* infections, but the rise of carbapenem resistance in *Enterobacter* spp., primarily caused by the acquisition of carbapenemase-encoding genes (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, etc.), has significantly compromised the choice of clinical antimicrobial treatment [3,4].

Identification of *Enterobacter* is still a challenging process. Although automated microbial identification systems and mass spectrometers are reliable for bacterial species identification, they are not specific enough for detecting *Enterobacter* species [1]. Clinical laboratories usually only classify *Enterobacter* species as *E. cloacae* complex, resulting in the inability to correlate antimicrobial resistance to *Enterobacter* at the species level. The combination of average nucleotide identity (ANI) and in silico DNA–DNA hybridization (isDDH) based on whole-genome sequencing analysis

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was recently recommended for more precise identification of *Enterobacter* species [5]. These methods provide high resolution to further divide *Enterobacter* into different species.

Carbapenemase-encoding genes have been increasingly detected in *Enterobacter* strains. Recently, Zong et al. examined 4899 high-quality *Enterobacter* genome sequences within GenBank and carried out the precise species identification and preliminary resistance gene screening [3]. The result showed that *E. xiangfangensis* was the most common species globally, and *bla*<sub>NDM</sub> gene was mostly encountered in China. Several high-risk *Enterobacter* clones associated with carbapenem resistance have also been described. The carbapenemase-producing ST171 *E. xiangfangensis* has been recognized as a high-risk lineage in the USA [6,7]. Additionally, ST90, ST93 and ST114 *E. xiangfangensis*, and ST78 *E. hoffmannii* are also globally distributed and are associated with multiple carbapenemases (VIM, NDM, KPC, and OXA-48) [8]. However, our understanding of precise species and genomic features of clinical carbapenem-resistant *Enterobacter* (CR-Ent) in China is still limited.

Herein, we collected 92 non-duplicated CR-Ent strains causing clinical infections from nine hospitals in China. Whole genome sequencing (WGS) and genomic analysis were conducted to characterize the precise species and genomic features of these strains.

## Materials and methods

### Bacterial strains

The multicenter study covered nine tertiary health care grade hospitals from Beijing, Chongqing, Guangdong, Jiangsu, Ningxia, Sichuan, and Yunnan. These provinces or municipalities are located in North China (Beijing), East China (Jiangsu), South China (Guangdong), Southwest China (Chongqing, Sichuan, and Yunnan), and Northwest China (Ningxia), respectively. All archived carbapenem-resistant Enterobacterales (CRE) strains from nosocomial infections were collected from the above nine hospitals between January 2017 and March 2021 (data not shown), and 92 CR-Ent strains were further selected (Table S1). Detailed bacterial strain collection was described in the Supplemental Text 1. Clinical data were obtained from medical chart using the same standardized questionnaire in each hospital.

All high-quality *Enterobacter* genome sequences within GenBank database were acquired for further analysis (details in Supplemental Text 1).

### Sequencing and assembly

Bacterial genomic DNA of 92 screened CR-Ent strains was isolated using the Omega Bio-Tek Bacterial DNA

Kit (Doraville, GA, USA). Further WGS and assembly were performed (details in Supplemental Text 1).

### Identification of bacterial species

Precise species identification was performed by calculating the pairwise isDDH and ANI value between the genome sequence of the query strain and those of type strains of *Enterobacter* spp. described recently [3] (details in Supplemental Text 1).

### Multi-locus sequence typing (MLST) analysis and phylogenetic analysis

The sequence types (STs) of *Enterobacter* strains were identified according to the online MLST scheme (<https://pubmlst.org/>). Further phylogenetic analysis was performed on the ST116 and ST171 *E. xiangfangensis* strains (details in Supplemental Text 1).

### Sequence annotation and comparison

Open reading frames (ORFs) and pseudogenes were predicted using RAST 2.0 [9] combined with BLASTP/BLASTN searches against the UniProtKB/Swiss-Prot database [10] and the RefSeq database [11]. Annotation of resistance genes, virulence genes, mobile elements, and other features was carried out using the online databases, including ResFinder 4.1 [12], VFDB (core dataset) [13], ISfinder [14], and Tn Number Registry [15]. Multiple and pairwise sequence comparisons were performed using BLASTN.

### Conjugal transfer

A conjugal transfer experiment was carried out using sodium azide-resistant *E. coli* J53 as the recipient and the *E. xiangfangensis* strains HD7405, HD8705, and HD2956 as the donor (details in Supplemental Text 1).

### Growth curve analysis

Strains were grown overnight in 3 ml of LB with shaking (200 rpm) at 37°C, and were then diluted to an OD<sub>600</sub> of 0.25. Next, 2 µl of the solution was added to 200 µl LB in a 96-well plate in triplicate. Culture densities were determined every 10 min by measuring the OD<sub>600</sub> for 12 h with shaking (200 rpm) at 37 °C via FLUOstar Omega (BMG Labtech, Germany). Growth curves were estimated via GraphPad Prism 5.0 (GraphPad Software, Inc.) using a two-way analysis of variance (ANOVA), and *p*-value < 0.05 was considered to be statistically significant.

## Statistical analysis

Unpaired two-tailed Student's *t*-tests or Mann–Whitney *U* test were performed to analyse the statistical significance of measurement data, while the chi-square test or Fisher exact test were performed to analyse the statistical significance of categorical data. All data were analysed using *GraphPad Prism* 5.0 (GraphPad Software, Inc.), and *p*-value <0.05 was considered to be statistically significant.

## Nucleotide sequence accession numbers

The complete sequences of chromosome and plasmids from HD8642 were submitted to GenBank under BioProject PRJNA824643, while the sequences of the rest 91 *Enterobacter* strains were submitted to GenBank under BioProject PRJNA824645. In addition, the sequences of 28 mapped plasmids were submitted to GenBank under accession number ON209123–ON209150.

## Results

### Identification and STs of *Enterobacter* strains

A total of 92 nonduplicated CR-*Ent* strains were identified in this study (Table S1), including 64 from Chengdu, 13 from Yinchuan, 6 from Suzhou, 5 from Chongqing, 3 from Kunming and 1 from Guangzhou. Most of these strains were initially reported as *Enterobacter cloacae* or *E. cloacae* complex in clinical laboratories. Precise species identification revealed that the most common CR-*Ent* species was *E. xiangfangensis* (*n* = 66), while the next common *Enterobacter* species was *E. hoffmannii* (*n* = 16) (Table 1). The remaining strains were classified into six *Enterobacter* species: *E. chengduensis* (*n* = 4), *E. mori* (*n* = 2), *E. hormaechei* (*n* = 1), *E. kobei* (*n* = 1), *E. roggenkampii* (*n* = 1), and *E. chuandaensis* (*n* = 1). Additionally, *E. xiangfangensis* was also the most prevalent *Enterobacter* species in each region where CR-*Ent* was collected (66.67–100%).

These 92 *Enterobacter* strains belong to 38 STs (including six unnamed novel STs), and the most prevalent lineages were ST116 (*n* = 15) and ST171 (*n* = 14) *E. xiangfangensis*. Except for Chengdu and Yinchuan, the distribution of CR-*Ent* was very diverse, without a predominant clone. The most prevalent lineages in Chengdu were ST171 (12/64, 18.75%) and ST116 (8/64, 12.50%) *E. xiangfangensis*, while ST116 (5/13, 38.41%) in Yinchuan.

### Carbapenemase-producing *Enterobacter* (CP-*Ent*) strains

Most of the 92 CR-*Ent* strains carried carbapenemase-encoding genes (72/92, 78.26%) (Table 1), which

included 71 carrying a sole carbapenemase-encoding gene (*bla*<sub>NDM-1</sub> [*n* = 45], *bla*<sub>NDM-5</sub> [*n* = 18], *bla*<sub>IMP-26</sub> [*n* = 5], *bla*<sub>IMP-4</sub> [*n* = 2] and *bla*<sub>VIM-1</sub> [*n* = 1]) and one harbouring two genes (*bla*<sub>NDM-1</sub> and *bla*<sub>VIM-1</sub>). The *bla*<sub>NDM</sub> was the most prevalent carbapenemase-encoding gene in each region (66.67–100%), except Guangzhou.

According to the method described in the method section, all high-quality *Enterobacter* genome sequences within GenBank database were acquired (dated 4 June 2021). We further determined 1402 human-source *Enterobacter* genome sequences carrying carbapenemase-encoding genes and having geographic information (Table S2). These genomes and the 72 genomes sequenced in this study were further analysed. The results showed that globally *E. xiangfangensis* remained to be the most common CP-*Ent* species from human sources (Table S3) and the prevalence of CP-*E. xiangfangensis* was high (>37%) in Asia, Africa, Europe, Oceania, and North or South America. CP-*E. hoffmannii*, *E. cloacae*, and *E. asburiae* were also commonly detected, but their prevalence was lower than that of *E. xiangfangensis*.

Nevertheless, the prevalent CP-*Ent* lineages in different regions vary significantly (Table S3). The result showed that ST171 *E. xiangfangensis* was the most common in Asia (including China) and North America, while ST114 *E. xiangfangensis* was common in Europe, ST90 *E. xiangfangensis* in Oceania, ST510 *E. xiangfangensis* in South America, and ST78 *E. hoffmannii* and ST456 *E. cloacae* in Africa. Additionally, some lineages were only prevalent in one region, such as ST116 *E. xiangfangensis* in China and ST830 *E. xiangfangensis* in Oceania, the prevalence rates of which were second in their respective regions.

A total of 1505 carbapenemase-encoding genes were identified in the above 1474 genome sequences (Table S4). For class A carbapenemase-encoding genes, the *bla*<sub>KPC</sub> was most common in South America (mainly *bla*<sub>KPC-2</sub>) and North America (mainly *bla*<sub>KPC-3</sub>), while the *bla*<sub>IMI</sub> (mainly *bla*<sub>IMI-1</sub>) was most common in the Asia (except China). Among class B carbapenemase-encoding genes, the *bla*<sub>NDM</sub> was prevalent in Asia, Africa, and Europe (mainly *bla*<sub>NDM-1</sub>). Besides *bla*<sub>NDM-1</sub>, *bla*<sub>NDM-5</sub> was also frequently detected in China. In addition, *bla*<sub>IMP</sub> was mostly encountered in Asia (mainly *bla*<sub>IMP-1</sub>) and Oceania (mainly *bla*<sub>IMP-4</sub>), and *bla*<sub>VIM</sub> was typically detected in Europe (mainly *bla*<sub>VIM-1</sub>). For class D carbapenemase-encoding genes, *bla*<sub>OXA-48</sub>-like gene was most common in Asia and Europe (mainly *bla*<sub>OXA-48</sub>).

In general, the most common CP-*Ent* species from human sources in the world was *E. xiangfangensis*, while the specific prevalent *Enterobacter* lineages and carbapenemase-encoding genes showed large geographical variations. Since ST171 and ST116

**Table 1.** Proportion of *Enterobacter* species and STs of carbapenem-resistant *Enterobacter* strains sequenced in this study.

Species	No. (%)	ST	Lineage	
			No. (%)	Carbapenemase-producing strains [No. (%)]
<i>E. xiangfangensis</i>	66 (71.74)	ST116	15 (16.30)	15 (20.83)
		ST171	14 (15.22)	14 (19.44)
		ST50	4 (4.35)	2 (2.78)
		Other	33 (35.87)	20 (27.78)
<i>E. hoffmannii</i>	16 (17.39)	ST158	7 (7.61)	7 (9.72)
		ST78	5 (5.43)	4 (5.56)
		Other	4 (4.35)	2 (2.78)
<i>E. chengduensis</i>	4 (4.35)	ST414	4 (4.35)	4 (5.56)
<i>E. mori</i>	2 (2.17)	ST308	1 (1.09)	1 (1.39)
		N4*	1 (1.09)	1 (1.39)
<i>E. hormaechei</i>	1 (1.09)	ST528	1 (1.09)	1 (1.39)
<i>E. kobei</i>	1 (1.09)	N2	1 (1.09)	0
<i>E. roggenkampii</i>	1 (1.09)	ST466	1 (1.09)	0
<i>E. chuandaensis</i>	1 (1.09)	N6	1 (1.09)	1 (1.39)
Total	92 (100)	–	92 (100)	72 (100)

\*N, novel. There were six unnamed novel STs of *Enterobacter* identified in this study, designed N1–N6.

*E. xiangfangensis* were the most prevalent lineages in China, we then performed a detailed genetic dissection analysis on the genome sequences of the two lineages.

### Phylogenetic analysis of ST171 and ST116 strains

The STs of all high-quality *Enterobacter* genome sequences within GenBank database described above were further identified. We detected 264 human-source ST171 *E. xiangfangensis* genome sequences and 41 human-source ST116 *E. xiangfangensis* genome sequences, which had geographic information, including both carbapenemase gene producers and non-producers (Table S5). Phylogenetic analysis based on core SNPs was performed on 278 ST171 *E. xiangfangensis* genome sequences [including 14 sequenced in this study, and 264 from GenBank], and 56 ST116 *E. xiangfangensis* strains [including 15 sequenced in this study, and 41 from GenBank], respectively.

The phylogenetic tree of ST171 *E. xiangfangensis* strains revealed that 278 ST171 *E. xiangfangensis* strains were divided into four major separately clustering clades (Figure 1). Most of the ST171 *E. xiangfangensis* strains carried carbapenemase-encoding genes (265/278, 95.32%), which included 264 carrying a sole carbapenemase-encoding gene (*bla*<sub>KPC-3</sub> [*n* = 161], *bla*<sub>NDM-1</sub> [*n* = 53], *bla*<sub>KPC-4</sub> [*n* = 21], *bla*<sub>NDM-5</sub> [*n* = 16], *bla*<sub>KPC-2</sub> [*n* = 6], *bla*<sub>OXA-48</sub> [*n* = 4], *bla*<sub>VIM-1</sub> [*n* = 2] and *bla*<sub>VIM-4</sub> [*n* = 1]) and one harbouring two genes (*bla*<sub>KPC-4</sub> and *bla*<sub>IMP-13</sub>). The *bla*<sub>KPC</sub>-carrying ST171 *E. xiangfangensis* strains were the most common (189/278, 67.99%). Among these strains, *bla*<sub>KPC-3</sub>-carrying strains were mainly classified into clade IV (160/161, 99.38%) and mainly distributed in the USA (160/161, 99.38%), while all *bla*<sub>KPC-4</sub>-carrying ones (including the strain co-harboring *bla*<sub>KPC-4</sub> and *bla*<sub>IMP-13</sub>) were all classified into clade II and were evenly distributed in the USA (11/22, 50.00%) and the UK (11/22, 50.00%). Furthermore, most *bla*<sub>NDM</sub>-carrying ST171 *E. xiangfangensis* strains

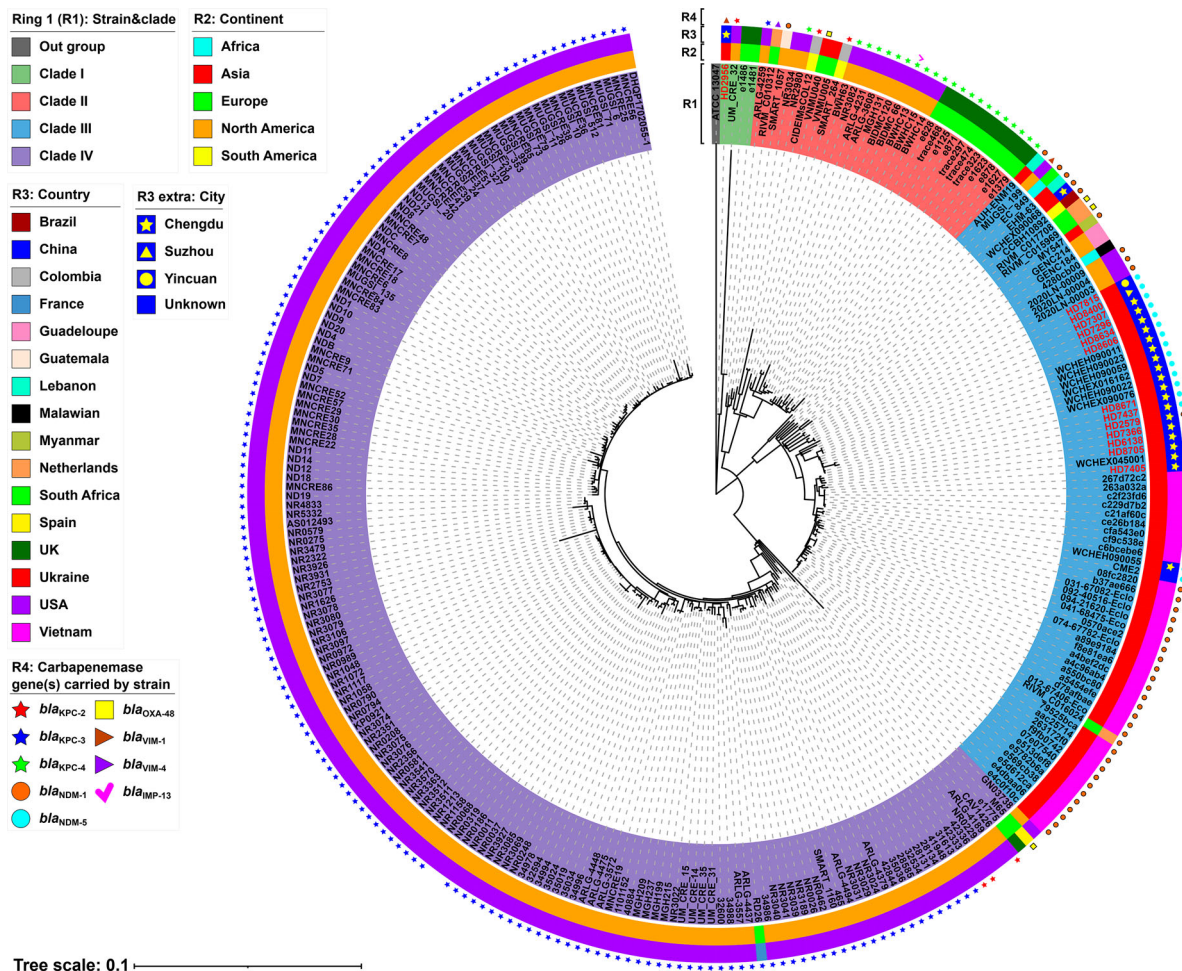
were classified into clade III (68/69, 98.56%) and mainly distributed in Asia (62/69, 89.86%). These results showed that *bla*<sub>NDM</sub>-carrying ST171 *E. xiangfangensis* most likely originated and was predominant in Asia and had a tendency to spread to other continents. Additionally, the strains carrying *bla*<sub>KPC-2</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM-1</sub>, *bla*<sub>VIM-4</sub> or *bla*<sub>IMP-13</sub> had no obvious geographical distributions due to the small quantity.

The core SNPs of 74 ST171 *E. xiangfangensis* strains from clade III were further pairwise compared to determine their clonal relatedness (Table S6). In the 22 ST171 strains from China, there were 0–112 SNPs difference, suggesting that they did not belong to a single strain. However, the sporadic clonal transmission of ST171 strains was still detected; for instance, HD7296, HD8634, and HD8606 had 0–1 SNP difference and were collected from different patients in the same ward in a hospital in Chengdu. Additionally, the inter-hospital clonal transmission of ST171 strains was also detected. For example, WCHEH090011, WCHEH090023, WCHEH090059, and WCHEX016162 were collected from another hospital in Chengdu [5] and shared 2–4 SNPs difference with HD8671 and HD7437 from our study, supporting the inter-hospital transmission of NDM producing ST171 strains in China.

For ST116 *E. xiangfangensis*, 56 strains were divided into two major separately clustering clades (Figure 2). Among them, only 17 strains carried carbapenemase-encoding genes, including 8 *bla*<sub>NDM-1</sub>, 7 *bla*<sub>NDM-5</sub>, 1 *bla*<sub>KPC-4</sub>, and 1 *bla*<sub>IMP-4</sub>. Of note, all 15 *bla*<sub>NDM</sub>-carrying strains were from China and were evenly distributed in the two clades (Figure 2). These results showed that *bla*<sub>NDM</sub>-carrying ST116 *E. xiangfangensis* was also an important lineage in China, and the carbapenem resistance in this ST was driven by the independent acquisition of the *bla*<sub>NDM</sub> gene in two major phylogenetic clades.

The core SNPs of 56 ST116 *E. xiangfangensis* strains were also pairwise compared (Table S7). In the 15 ST116 strains from China, there were 1–3604 SNPs





**Figure 1.** A maximum-likelihood phylogenetic tree of ST171 *E. xiangfangensis* strains. A total of 278 ST171 *E. xiangfangensis* strains (including 14 sequenced in this study, and 264 from GenBank) were performed with phylogenetic analysis, while *E. cloacae* strain ATCC 13047 (GenBank accession number NC\_014121) was used as the out group. Bar corresponds to scale of sequence divergence.

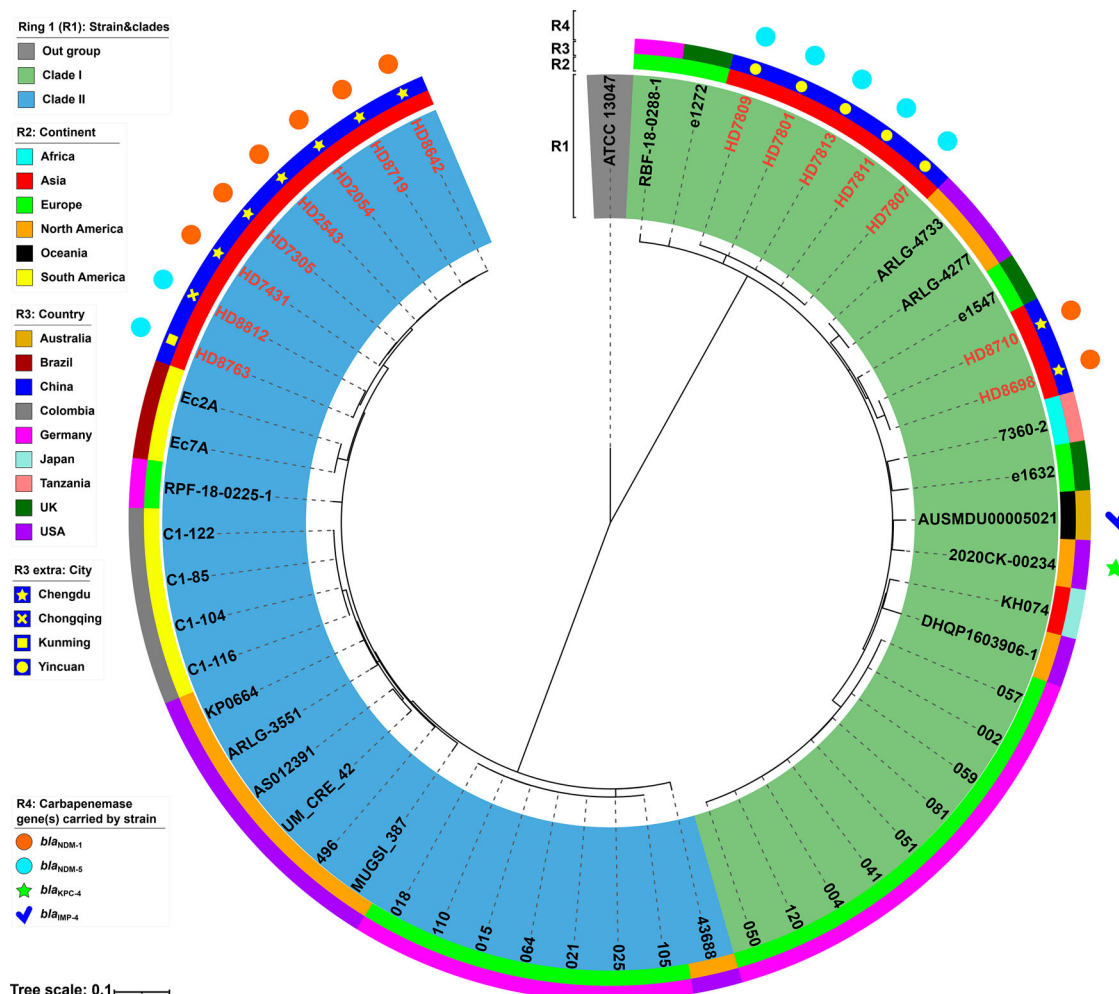
difference. Similarly, the regional clonal transmission of ST116 strains was found in Chengdu and Yinchuan. For example, HD7801, HD7807, HD7809, HD7811, and HD7813 had 1–5 SNPs difference and were collected from different patients in the same ward in a hospital in Yinchuan, while HD7305 and HD7431 had 2 SNPs difference, and were from different patients in the same ward in a hospital in Chengdu.

### Clinical and genetic features of different *Enterobacter* groups

The clinical data of patients with CR-*Ent* were collected (Table S1). Further statistical analysis was conducted between ST116 *E. xiangfangensis* ( $n=15$ ), ST171 *E. xiangfangensis* ( $n=14$ ), and the other CR-*Ent* strains ( $n=63$ ), including age, sex, isolation source, time to positive culture, community or hospital onset, admission route, length of hospital stay, post-culture length of hospital stay, and 30-day outcome of patient after positive culture (Table 2). The results showed that compared with patients with the other CR-*Ent* strains, patients with ST171

*E. xiangfangensis* strain were older ( $p < 0.05$ ), while other factors did not significantly differ among groups. However, the proportion of poorer 30-day outcomes (worsened/died/withdrawing treatment) of patients with ST116 (6/15, 40.00%) and ST171 (6/14, 42.86%) *E. xiangfangensis* strains after positive culture was relatively higher compared with patients with the other CR-*Ent* strains (16/63, 25.40), respectively.

The virulence genes and acquired antimicrobial resistance genes carried by 92 CR-*Ent* strains were identified through VFDB (core dataset) [13] and ResFinder 4.1 [12] (Table S8 and S9). Further statistical analysis was also conducted between three groups (Table 2). Genes that were statistically different among groups were further listed. For virulence genes, the result showed that compared with the other CR-*Ent* strains, ST116 *E. xiangfangensis* strain carried significantly more virulence genes ( $p < 0.001$ ) while ST171 *E. xiangfangensis* strain carried fewer virulence genes ( $p < 0.05$ ). ST116 *E. xiangfangensis* strain was more likely to carry *iroDCBNE*, *csgG*, *cgsD*, *vipA/tssB*, *vipB/tssC*, *hcp/tssD*, *tssF*, and *acrA* genes ( $p < 0.05$ ). Additionally, though ST171 *E. xiangfangensis* strain carried fewer virulence



**Figure 2.** A maximum-likelihood phylogenetic tree of ST116 *E. xiangfangensis* strains. A total of 56 ST171 *E. xiangfangensis* strains (including 15 sequenced in this study, and 41 from GenBank) were performed with phylogenetic analysis, while *E. cloacae* strain ATCC 13047 (GenBank accession number NC\_014121) was used as the out group. Bar corresponds to scale of sequence divergence.

genes, it was more likely to carry *entE*, *fepD*, *cgsD*, *vipB*/*tssC*, and *ugd* genes ( $p < 0.05$ ). For antimicrobial resistance genes, the number of antimicrobial resistance genes carried by ST116 *E. xiangfangensis* strain and ST171 *E. xiangfangensis* strain had no significant difference compared with the other CR-*Ent* strain. However, ST116 *E. xiangfangensis* strain and ST171 *E. xiangfangensis* strain were more likely to carry *bla*<sub>NDM</sub> gene ( $p < 0.05$ ) and were less likely to carry *mcr-9* gene ( $p < 0.05$ ) compared with the other CR-*Ent* strains.

### Growth curves of ST171 and ST116 strains

The growth assay was performed to compare the proliferative capability of ST171 and ST116 *E. xiangfangensis* with other ST carbapenem-resistant *E. xiangfangensis*. The 15 ST116 *E. xiangfangensis* strains sequenced in this study were divided into three groups according to the phylogenetic analysis (Figure S1a), while 14 ST171 *E. xiangfangensis* strains sequenced in this study were divided into two groups (Figure S1b). One randomly selected strain from each of the five groups, and three randomly selected strains

from other STs of *E. xiangfangensis* strains, were included. The growth of ST116 *E. xiangfangensis* and ST171 *E. xiangfangensis* was slower ( $p < 0.001$ ) in LB without antibiotic, as evidenced by decreases in optical density compared with the optical densities of other ST *E. xiangfangensis*, respectively (Figure 3a). However, the growth curves of ST116 *E. xiangfangensis* and ST171 *E. xiangfangensis* showed no significant difference ( $p > 0.05$ ) with other ST carbapenem-resistant *E. xiangfangensis* strains in LB with 2 µg/mL meropenem (Figure 3b). The results showed that the fitness cost of ST171 and ST116 *E. xiangfangensis* was not obvious under carbapenem selection pressure.

### Plasmid analysis of ST171 and ST116 strains

The complete sequences of carbapenemase-encoding plasmids carried by 14 ST171 *E. xiangfangensis* strains and 15 ST116 *E. xiangfangensis* strains sequenced in this study were obtained. Fifteen carried *bla*<sub>NDM-5</sub>, 13 carried *bla*<sub>NDM-1</sub>, and one carried *bla*<sub>VIM-1</sub> (Table S10). All *bla*<sub>NDM-5</sub> genes ( $n = 15$ ) were carried by IncX3-type plasmid, while *bla*<sub>NDM-1</sub> genes were

**Table 2.** Statistical analysis of *Enterobacter* strains sequenced in this study.

	(A) ST116 strains	(B) ST171 strains	(C) Other strains	<i>p</i> -value <sup>a</sup> (between A and C)	<i>p</i> -value (between B and C)
Number	15 (16.30)	14 (15.21)	63 (68.48)	–	–
Age, years	53 (30–92)	80 (71–83)	61 (45–77)	0.8339	<b>0.0136</b>
Sex	–	–	–	0.1688	0.7496
Male	14 (93.33)	10 (71.43)	47 (74.60)	–	–
Female	1 (6.67)	4 (28.57)	16 (25.40)	–	–
Source	–	–	–	0.1126	0.0632
Blood	2 (13.33)	0	11 (17.46)	–	–
Respiratory tract	8 (53.33)	7 (50.00)	14 (22.22)	–	–
Urinary tract	1 (6.67)	4 (28.57)	8 (12.70)	–	–
Wound	4 (26.67)	1 (7.14)	13 (20.63)	–	–
Intraperitoneal	0 (0)	0 (0)	8 (12.70)	–	–
Other	0 (0)	2 (14.29)	9 (14.29)	–	–
Time to positive culture, days <sup>b</sup>	19 (11–36)	12 (7–30)	17 (8–30)	0.5259	0.8018
Community onset <sup>c</sup>	1 (6.67)	1 (7.14)	8 (12.70)	1.000	1.000
Admitted from	–	–	–	0.7214	0.4448
Home	13 (86.67)	13 (92.86)	49 (77.78)	–	–
Hospital transfer	2 (13.33)	1 (7.14)	14 (22.22)	–	–
Length of hospital stay, days	41 (27–70)	34 (23–57)	30 (23–65)	0.4985	0.9657
Post-culture length of hospital stay, days	14 (7–43)	18 (8–30)	16 (7–28)	0.8250	0.8146
30-day outcome after positive culture	–	–	–	0.3391	0.2059
Cured/improved	9 (60.00)	8 (57.14)	47 (74.60)	–	–
Worsened/died/withdrawing treatment	6 (40.00)	6 (42.86)	16 (25.40)	–	–
Virulence genes <sup>d</sup>	24 (24–24)	17 (17–17)	18 (17–21)	<b>&lt; 0.0001</b>	<b>0.0227</b>
Salmocheilin ( <i>iroDCBNE</i> )	15 (100)	0 (0)	22 (34.92)	<b>&lt; 0.0001</b>	<b>0.0075</b>
Agf ( <i>csgG</i> )	15 (100)	0 (0)	39 (61.90)	<b>0.0036</b>	<b>&lt; 0.0001</b>
Enterobactin ( <i>entE</i> )	0 (0)	14 (100)	35 (55.56)	<b>&lt; 0.0001</b>	<b>0.0013</b>
Enterobactin ( <i>fepD</i> )	0 (0)	14 (100)	30 (47.62)	<b>0.0003</b>	<b>0.0002</b>
Curli fibres ( <i>csgD</i> )	15 (100)	14 (100)	30 (47.62)	<b>&lt; 0.0001</b>	<b>0.0002</b>
T6SS ( <i>vipA/tssB</i> )	15 (100)	2 (14.29)	47 (74.60)	<b>0.0318</b>	<b>&lt; 0.0001</b>
T6SS ( <i>vipB/tssC</i> )	15 (100)	13 (92.86)	34 (53.97)	<b>0.0006</b>	<b>0.0066</b>
T6SS ( <i>hcp/tssD</i> )	15 (100)	0 (0)	38 (60.32)	<b>0.0018</b>	<b>&lt; 0.0001</b>
T6SS ( <i>tssF</i> )	15 (100)	0 (0)	23 (36.51)	<b>&lt; 0.0001</b>	<b>0.0074</b>
Capsule ( <i>ugd</i> )	0 (0)	13 (92.86)	5 (7.94)	0.5768	<b>&lt; 0.0001</b>
AcrAB ( <i>acrA</i> )	15 (100)	0 (0)	28 (44.44)	<b>&lt; 0.0001</b>	<b>0.0013</b>
Antimicrobial resistance genes <sup>d</sup>	13 (7–13)	10 (7–13)	12 (6–15)	0.7075	0.7860
Aminoglycoside ( <i>strAB</i> )	9 (60.00)	0 (0)	24 (38.10)	0.1518	<b>0.0036</b>
Colistin ( <i>mcr-9</i> )	0 (0)	0 (0)	16 (25.40)	<b>0.0318</b>	<b>0.0337</b>
Quinolone ( <i>qnrB</i> )	0 (0)	5 (35.71)	21 (33.33)	<b>0.0079</b>	1.000
Quinolone ( <i>qepA</i> )	2 (13.33)	0 (0)	0 (0)	<b>0.035</b>	–
Phenicol ( <i>catII</i> )	7 (46.67)	0 (0)	26 (41.27)	0.7753	<b>0.0017</b>
Phenicol ( <i>floR</i> )	6 (40.00)	9 (64.29)	4 (6.35)	<b>0.0026</b>	<b>0.0018</b>
Tetracycline [ <i>tet(D)</i> ]	7 (46.67)	0 (0)	19 (30.16)	0.2382	<b>0.0163</b>
Narrow-Spectrum β-Lactamase ( <i>bla<sub>DHA</sub></i> )	0 (0)	0 (0)	16 (25.40)	<b>0.0318</b>	<b>0.0337</b>
Extended-Spectrum β-Lactamase ( <i>bla<sub>CTX-M</sub></i> )	0 (0)	9 (64.29)	16 (25.40)	<b>0.0318</b>	<b>0.0096</b>
Carbapenemase ( <i>bla<sub>NDM</sub></i> )	15 (100)	13 (92.86)	36 (57.14)	<b>0.0008</b>	<b>0.0134</b>

Note: Data are *n* (%) or median (IQR). *P* value < 0.05 was in bold.

<sup>a</sup>*p* value comparing distributions where applicable.

<sup>b</sup>Time to first positive culture indicates the number of days from admission to the collection date of the index culture, with 0 indicating that the index culture was obtained on the day of admission.

<sup>c</sup>Community onset defined as home origin with first positive culture date less than 3 days from date of admission.

<sup>d</sup>The virulence genes and antimicrobial resistance genes which have statistical difference between groups are listed in this table.

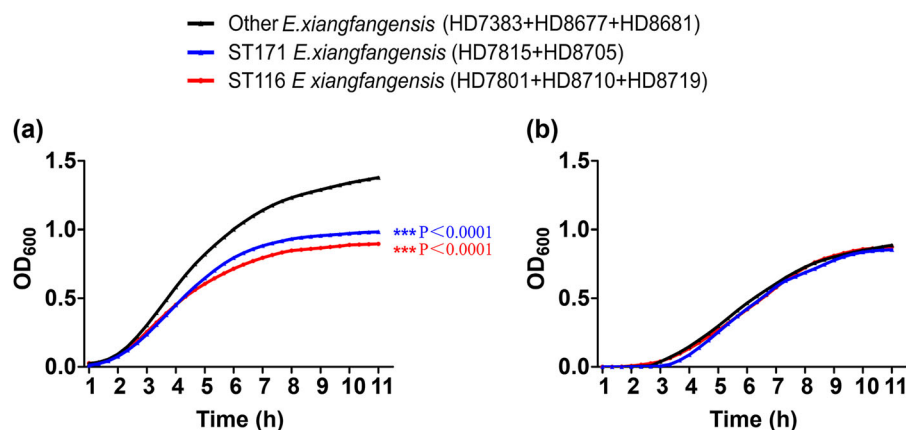
carried by IncX3-type plasmid (*n* = 12) or IncFII-type plasmid (*n* = 1) (Figure 4a). Further analysis revealed that all *bla<sub>NDM</sub>* genes were carried by Tn125-related derivatives (Figure 5). Additionally, the *bla<sub>VIM-1</sub>* gene was located within the integron In916 and carried by an unknown-type plasmid pHD2956-VIM (Figure 4b), which was identical to the reference plasmid pCY-VIM (GenBank accession number KF998104) [16]. The detailed genetic analysis of plasmids and Tn125-related derivatives is described in Supplemental Text 2.

Besides plasmids from ST171 and ST116 strains, the carbapenemase-encoding plasmids carried by other ST *Enterobacter* strains sequenced in this study were also preliminarily analysed. The result showed

that *bla<sub>NDM</sub>*-harbouring IncX3-type plasmids were the most common ones (*n* = 51) in all carbapenemase-encoding plasmids, while other plasmid types only accounted for a small proportion, such as *bla<sub>NDM</sub>*-harbouring IncC and IncFII plasmids (data not shown). Additionally, all *bla<sub>NDM</sub>* genes were carried by Tn125-related derivatives.

The IncFII-type plasmid pHD7405-NDM and IncX3-type plasmid pHD8705-NDM could be transferred into *E. coli* J53 from strain HD7405 and HD8705 through conjugal transfer experiment, respectively. However, repeated conjugation attempts failed to transfer pHD2956-VIM from strain HD2956 into *E. coli* J53, which is likely due to the lack of *tra* module in this plasmid (Figure 4b).





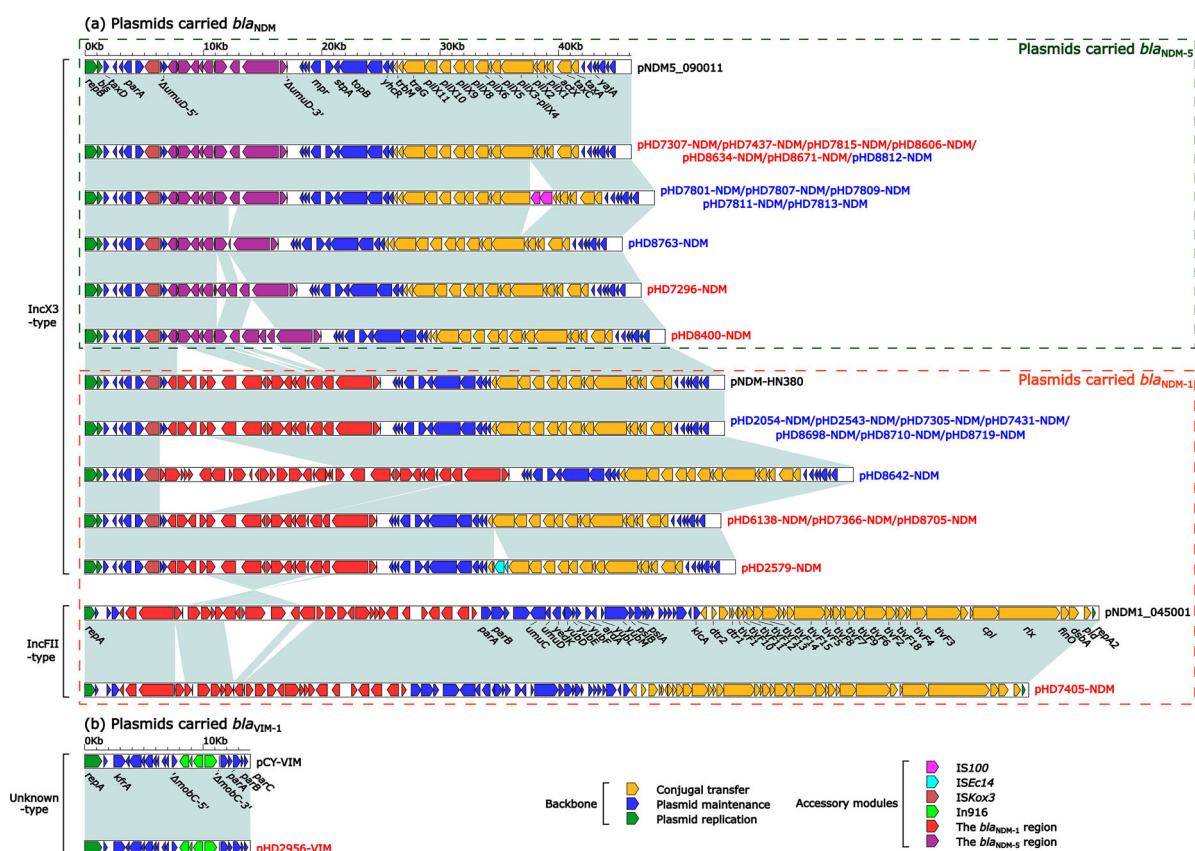
**Figure 3.** Growth curves of ST116, ST171 and other ST *E. xiangfangensis* strains. (a) Optical densities of strains in each group cultured in LB without antibiotic. (b) Optical densities of strains in each group cultured in LB with 2 µg/mL meropenem.

To assess the impact of IncFII-type and IncX3-type plasmids on the growth of strain, the growth of *E. coli* J53 transconjugants of IncFII-type plasmid pHD7405-NDM or IncX3-type plasmid pHD8705-NDM was compared. The growth curves of *E. coli* J53 and J53/pHD8705-NDM (IncX3) showed no significant difference ( $p > 0.05$ ), while the growth of J53/pHD7405-NDM (IncFII) was clearly slower ( $p < 0.05$ ) than the parental *E. coli* J53 (Figure S2). The results indicated

that the acquisition of IncX3-type plasmid had minimal impact on the growth of the parental strain, but the acquisition of IncFII-type plasmid had a high fitness cost *in vitro* growth.

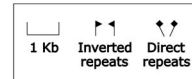
## Discussion

CR-Ent has become a new emerging threat to global public health [4]. However, poor identification of



**Figure 4.** Linear comparison of carbapenemase-encoding plasmids from ST171 and ST116 *E. xiangfangensis* strains. Genes are denoted by arrows. Genes, mobile genetic elements and other features are coloured based on function classification. Shading regions denote homology of two plasmids (light blue:  $\geq 99\%$  nucleotide identity). The names of plasmids from ST171 *E. xiangfangensis* strains were marked in red, while ST116 *E. xiangfangensis* strains in blue. The names of reference plasmids were marked in black. The accession number of plasmids pNDM5\_090011, pNDM-HN380, pNDM1\_045001 and pCY-VIM used as reference are CP036312, JX104760, CP043383 and KF998104, respectively.





*Enterobacter* species further complicates the analysis of CR-*Ent* species. This study used ANI and isDDH to examine 92 non-duplicated CR-*Ent* strains from Chinese patients. Most of these strains were initially reported as *Enterobacter cloacae* or *E. cloacae* complex in clinical laboratories. Nevertheless, the precise species identification showed that the two most common CR-*Ent* species were *E. xiangfangensis* and *E. hoffmanni*, while *E. cloacae* was not detected. Furthermore, due to the main mechanism for carbapenem

resistance in *Enterobacter* was the producing carbapenemase, we screened the carbapenemase-encoding genes in the strains from this study and publicly available genomes. The result showed that the most common CP-Ent species from human sources in the world was *E. xiangfangensis*, while the specific prevalent *Enterobacter* lineage and carbapenemase-encoding genes vary in different regions. Additionally, compared with 24% CP-Ent prevalence (46/192) in the USA [17], the proportion of CP-Ent in CR-Ent

was much higher (78.26%, 72/92) in China ( $p < 0.05$ ). It indicated distinct molecular mechanisms underlying the carbapenem resistance in *Enterobacter* between China and the USA, and the acquisition of carbapenemase genes and plasmids has been identified as the main cause of carbapenem resistance in China. Our results also highlighted the geographical impacts of rapid molecular diagnosis on CR-*Ent*. Molecular carbapenemase gene detection can likely capture ~80% CR-*Ent* in China and only some ~25% in the USA.

CP-ST171 *E. xiangfangensis* has been recognized as a high-risk lineage in the USA [6–8], which could cause infections in healthcare settings, and the clonal spread of ST171 CR-*Ent* has also been documented. Unlike the previously reported CP-ST171 *E. xiangfangensis* that mainly carried *bla*<sub>KPC</sub>, the strains in China mainly carried *bla*<sub>NDM</sub>. Indeed, the same feature was also found in CP-ST171 *E. xiangfangensis* from several other Asian countries. Phylogenetic analysis further revealed that all *bla*<sub>NDM</sub>-carrying ST171 *E. xiangfangensis* from Asia were from the same clade, suggesting that this lineage most likely originated in Asia and evolved from the same clone. Additionally, CP-ST116 *E. xiangfangensis* was also identified as an important lineage in China. CP-ST116 *E. xiangfangensis* was rarely reported in previous studies, and only two CP-ST116 *E. xiangfangensis* were deposited in the GenBank database. However, among the CP-strains sequenced in this study, ST116 *E. xiangfangensis* (20.83%,  $n = 15$ ) showed the highest prevalence, exceeding ST171 *E. xiangfangensis* (19.44%,  $n = 14$ ). These ST116 *E. xiangfangensis* carried *bla*<sub>NDM</sub>, and although belonging to two different clades, they are indicative of independent acquisition of *bla*<sub>NDM</sub> by the different ST116 strains in China. Of note, further analysis highlighted the intra-hospital clonal transmission of ST116 and ST171 *E. xiangfangensis* and the inter-hospital clonal transmission of ST171 *E. xiangfangensis*. The inter-hospital clonal transmission of ST171 *E. xiangfangensis* was also recently reported in a bloodstream infection-associated study in China [5]. Although statistical analysis of clinical data showed that ST171 and ST116 *E. xiangfangensis* were not significantly associated with poorer outcomes, ST171 *E. xiangfangensis* was more likely to infect older patients. However, the number of *Enterobacter* strains collected in this study was relatively limited; thus, more studies are needed to evaluate these findings.

Horizontal gene transfer is one of the main carbapenemase genes transfer mechanisms. In this study, *bla*<sub>NDM</sub>-harbouring IncX3-type plasmids were the most common plasmids among all carbapenemase-encoding plasmids. The IncX3-type plasmid was self-transmissible, which has been confirmed in this

study and previous reports [18,19]. Furthermore, the same IncX3-type plasmid was detected in *E. xiangfangensis* strains belonging to different clones. For example, HD7307 and HD8634 were collected from patients in different wards of the same hospital and belonged to two ST171 clones (56 SNPs between each other). The interval between the collection dates of the two strains was about one month. However, pHD7307-NDM from HD7307 was identical to pHD8634-NDM from HD8634. The self-transferability of *bla*<sub>NDM</sub>-harbouring IncX3-type plasmid in part explained the wide-ranging existence of this plasmid in *Enterobacter* strains. Additionally, all *bla*<sub>NDM</sub> genes from strains sequenced in this study were carried by Tn125-related derivatives. Tn125 was a composite transposon flanked by two copies of IS*Aba125* [20]. Tn125 is considered important in the initial dissemination of *bla*<sub>NDM</sub> to plasmid backbones [21]. In general, self-transmissible *bla*<sub>NDM</sub>-harbouring plasmids should be closely monitored in China, especially *bla*<sub>NDM</sub>-harbouring IncX3-type plasmids widely distributed in *Enterobacter* strains.

The reasons for the high prevalence of ST116 and ST171 *E. xiangfangensis* in China need to be further explored. However, the result of statistical analysis showed that ST171 and ST116 *E. xiangfangensis* were more likely to carry some important virulence genes or antimicrobial resistance genes, such as *iroDCBNE* (involving the encoding of siderophore salmochelin) in ST116 *E. xiangfangensis*, and *cgsD* (involving the encoding of curli fibres), *vipB/tssC* (involving the encoding of T6SS), and *bla*<sub>NDM</sub> in ST116 and ST171 *E. xiangfangensis*. The presence of these genes may provide certain selection advantages against host immune factors or hospital environments. IncX3-type plasmids were reported as the most common ones carrying *bla*<sub>NDM</sub>, which were mainly distributed in China and neighbouring countries [22]. Our results showed that the fitness cost of carrying an IncX3-type plasmid was minimal compared to other plasmids. Furthermore, compared with the major *bla*<sub>KPC</sub>-harbouring IncFIA-type plasmid carried by the ST171 strain in the USA [7,23], the IncX3-type plasmid was adapted to carriage by enterobacteria that colonize mammalian hosts [24]. Therefore, the ability of strains to acquire and maintain the *bla*<sub>NDM</sub>-harbouring IncX3-type plasmid might be one major driver in the prevalence of ST171 and ST116 *E. xiangfangensis* in China.

This study has some limitations. Firstly, the sample size of CR-*Ent* in this study was relatively small. The small sample size was in part because we focused on isolates associated with clinical infections and only non-duplicated isolates were included. However, this study was one of the largest studies focusing on both clinical and genomic features of CR-*Ent* in China, and the isolates were collected from different

geographical regions, and the predominant CR-*Ent* clones and plasmids were described. Secondly, although all high-quality *Enterobacter* genome sequences within GenBank were included in this study, the Genbank genomes may be biased towards submission sources with genome sequencing capacity. Nevertheless, this dataset represents the most comprehensive CR-*Ent* genomic resources currently available and the data provide important distribution information and genomic features of different global CR-*Ent* strains.

In conclusion, the most common CR-*Ent* strains recovered from humans in China were not *E. cloacae* but *E. xiangfangensis*, which is consistent with the finding that most CP-*Ent* strains recovered from human sources (including China) were *E. xiangfangensis*. The results also suggested that some species-specific factors may contribute to the predominance of *E. xiangfangensis* in CR-*Ent*, which deserves further studies. In China, ST171 and ST116 *E. xiangfangensis* were the major clinical lineages of CP-*Ent* strains and were mainly associated with *bla*<sub>NDM</sub>-harbouring IncX3-type plasmids. Further surveillance and infection control measures should be advocated to prevent the clonal dissemination of CP-ST171 and ST116 *E. xiangfangensis*, and the horizontal dissemination of *bla*<sub>NDM</sub>-harbouring IncX3-type plasmids.

### Data availability statement

The data presented in the study are included in the article/supplementary files, further inquiries can be directed to the corresponding author/s.

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### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### References

- [1] Mezzatesta ML, Gona F, Stefani S. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. *Future Microbiol.* 2012 Jul;7(7):887–902.
- [2] Davin-Regli A, Lavigne JP, Pagès JM. *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. *Clin Microbiol Rev.* 2019 Sep 18;32(4):e00002–19.
- [3] Zong Z, Feng Y, McNally A. Carbapenem and colistin resistance in *enterobacter*: determinants and clones. *Trends Microbiol.* 2021 Jun;29(6):473–476.
- [4] Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. *Clin Infect Dis.* 2019 Nov 13;69(Suppl 7):S521–S528.
- [5] Wu W, Wei L, Feng Y, et al. Precise species identification by whole-genome sequencing of *enterobacter* bloodstream infection, china. *Emerging Infect Dis.* 2021 Jan;27(1):161–169.
- [6] Hawken SE, Washer LL, Williams CL, et al. Genomic investigation of a putative endoscope-associated carbapenem-resistant *enterobacter cloacae* outbreak reveals a wide diversity of circulating strains and resistance mutations. *Clin Infect Dis.* 2018 Jan 18;66(3):460–463.
- [7] Gomez-Simmonds A, Annavaiah MK, Wang Z, et al. Genomic and geographic context for the evolution of high-risk Carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78. *mBio.* 2018 May 29;9(3):e00542–18.
- [8] Peirano G, Matsumura Y, Adams MD, et al. Genomic epidemiology of global Carbapenemase-Producing *Enterobacter* spp., 2008–2014. *Emerging Infect Dis.* 2018 Jun;24(6):1010–1019.
- [9] Brettin T, Davis JJ, Disz T, et al. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep.* 2015 Feb 10;5:8365.
- [10] Boutet E, Lieberherr D, Tognolli M, et al. Uniprotkb/Swiss-Prot, the manually annotated section of the UniProt knowledge base: how to use the entry view. *Methods Mol Biol.* 2016;1374:23–54.
- [11] O’Leary NA, Wright MW, Brister JR, et al. Reference sequence (RefSeq) database at NCBI: current status,



- taxonomic expansion, and functional annotation. *Nucleic Acids Res.* **2016 Jan 4**;44(D1):D733–D745.
- [12] Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother.* **2012 Nov**;67(11):2640–2644.
- [13] Liu B, Zheng D, Zhou S, et al. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res.* **2022 Jan 7**;50(D1):D912–D917.
- [14] Siguier P, Perochon J, Lestrade L, et al. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res.* **2006 Jan 1**;34(Database issue):D32–D36.
- [15] Tansirichaiya S, Rahman MA, Roberts AP. The transposon registry. *Mob DNA.* **2019**;10:40.
- [16] Yang L, Wu AW, Su DH, et al. Resistome analysis of *Enterobacter cloacae* CY01, an extensively drug-resistant strain producing VIM-1 metallo- $\beta$ -lactamase from China. *Antimicrob Agents Chemother.* **2014 Oct**;58(10):6328–6330.
- [17] van Duin D, Arias CA, Komarow L, et al. Molecular and clinical epidemiology of carbapenem-resistant Enterobacterales in the USA (CRACKLE-2): a prospective cohort study. *Lancet Infect Dis.* **2020 Jun**;20(6):731–741.
- [18] Xiao X, Zeng F, Li R, et al. Subinhibitory concentration of colistin promotes the conjugation frequencies of *mcr-1*- and *bla*<sub>NDM-5</sub>-positive plasmids. *Microbiol Spectr.* **2022 Mar 1**:e0216021.
- [19] Chen R, Liu Z, Xu P, et al. Deciphering the epidemiological characteristics and molecular features of *bla*<sub>KPC-2</sub>- or *bla*<sub>NDM-1</sub>-positive *Klebsiella pneumoniae* isolates in a newly established hospital. *Front Microbiol.* **2021**;12:741093.
- [20] Poirel L, Bonnin RA, Boulanger A, et al. Tn125-related acquisition of *bla*<sub>NDM</sub>-like genes in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* **2012 Feb**;56(2):1087–1089.
- [21] Acman M, Wang R, van Dorp L, et al. Role of mobile genetic elements in the global dissemination of the carbapenem resistance gene *bla*<sub>NDM</sub>. *Nat Commun.* **2022 Mar 3**;13(1):1131.
- [22] Wu W, Feng Y, Tang G, et al. Ndm metallo- $\beta$ -lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev.* **2019 Mar 20**;32(2):e00115–18.
- [23] Pereira EC, Anacker M, Houseman J, et al. A cluster of carbapenemase-producing *Enterobacter cloacae* complex ST171 at a tertiary care center demonstrating an ongoing regional threat. *Am J Infect Control.* **2019 Jul**;47(7):767–772.
- [24] Baomo L, Lili S, Moran RA, et al. Temperature-regulated IncX3 plasmid characteristics and the role of plasmid-encoded H-NS in thermoregulation. *Front Microbiol.* **2021**;12:765492.