Molecular Characterization of \textit{bla}_{IMP}^-4-Carrying Enterobacterales in Henan Province of China

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Carbapenem-resistant Enterobacterales (CRE) pose a serious threat to clinical management and public health. We investigated the molecular characteristics of 12 \textit{IMP}-4 metallo-\(\beta\)-lactamase-producing strains, namely, 5 \textit{Enterobacter cloacae}, 3 \textit{Escherichia coli}, 2 \textit{Klebsiella pneumoniae}, and 2 \textit{Citrobacter freundii}. These strains were collected from a tertiary teaching hospital in Zhengzhou from 2013 to 2015. The minimum inhibitory concentration (MIC) results showed that each \textit{bla}_{IMP}^-4-positive isolate was multidrug-resistant (MDR) but susceptible to colistin. All of the \textit{E. coli} belonged to ST167, two \textit{C. freundii} isolates belonged to ST396, and diverse ST types were identified in \textit{E. cloacae} and \textit{K. pneumoniae}. S1-PFGE, Southern blotting, and PCR-based replicon typing assays showed that the \textit{bla}_{IMP}^-4-carrying plasmids ranged from \(\sim 52\) to \(\sim 360\) kb and belonged to FI1, FIB, HI2/HI2A, and N types. N plasmids were the predominant type (8/12, 66.7%). Plasmid stability testing indicated that the \textit{bla}_{IMP}^-4-carrying N-type plasmid is more stable than the other types of plasmids. Conjugative assays revealed that three of the \textit{bla}_{IMP}^-4-carrying N plasmids were transferrable. Complete sequence analysis of a representative N type (plIMP-ECL14–57) revealed that it was nearly identical to plIMP-FJ1503 (KU051710) (99% nucleotide identity and query coverage), an N-type \textit{bla}_{IMP}^-4-carrying epidemic plasmid in a \textit{C. freundii} strain. PCR mapping indicated that a transposon-like structure [IS6100-mobC-intron (K1.pn.I3)-\textit{IS}26] was highly conserved in all of the N plasmids. IS26 involved recombination events that resulted in variable structures of this transposon-like module in FI1 and FIB plasmids. The \textit{bla}_{IMP}^-4 gene was captured by a \textit{sul1}-type integron In1589 on HI2/HI2A plasmid plIMP-ECL-13–46.

**Keywords:** \textit{bla}_{IMP}^-4, transposon-like structure, class 1 integron, carbapenem-resistant Enterobacterales, N plasmid

**INTRODUCTION**

The Zn(II)-containing metallo-\(\beta\)-lactamases (MBLs) comprise Imipenemase (IMP), New Delhi metallo-\(\beta\)-lactamase (NDM), and Verona Integron-encoded Metallo-\(\beta\)-lactamase (VIM) types that belong to class B \(\beta\)-lactamase according to the Ambler classification. MBLs can hydrolyze nearly all \(\beta\)-lactams, including carbapenems, which are important antibiotics in clinical practice and the “last line” drugs for treating infections.
caused by multiple drug-resistant (MDR) Gram-negative bacteria (Boyd et al., 2020). The rapid spread of MBLS among Enterobacterales has led to the increased prevalence of carbapenem-resistant Enterobacterales (CRE), and this presents a challenge for infection treatment worldwide (Nordmann and Poirel, 2019). Unlike NDMs, IMP-type β-lactamases are not often detected in CRE from China (Zhang et al., 2017; Wang et al., 2018). The most commonly encountered blaIMP−4 gene has been found captured by class 1 integrons and carried by plasmids belonging to multiple replicon types including HI2, L/M, A/C, and N for dissemination (Lai et al., 2017; Matsumura et al., 2017). An epidemic N plasmid in Enterobacterales isolates was recently recovered from Shanghai, Guangdong, and Fujian provinces of China and was responsible for the dissemination of blaIMP−4 gene (Wang et al., 2017). It is not known if this type of plasmid is prevalent in other regions of China and if it is involved in the spread of blaIMP genes. We conducted a retrospective study to investigate the prevalence and molecular characterization of IMP-positive Enterobacterales isolates in Henan Province within the north central region of China.

MATERIALS AND METHODS

Bacterial Isolates and Antimicrobial Susceptibility Testing

From January 2013 to December 2015, a retrospective survey for MBLS in CRE isolated from a tertiary teaching hospital of Zhengzhou University identified 12 blaIMP−4 positive isolates, which were recovered from different types of clinical specimens (Table 1). The study and consent procedure was approved by the Ethical Committee of Zhengzhou University. PCR and sequencing were used to identify MBL encoding genes, including blaIMP, blaNDM, and blaVIM, as described previously (Doyle et al., 2012). Antimicrobial susceptibility of the 12 blaIMP−4-positive isolates and their transconjugants was determined using microbroth and agar dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLS1, 2019). Escherichia coli ATCC25922 was used as the quality control.

Bacterial Genotyping

Multilocus sequence typing (MLST) for Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter freundii, and E. coli isolates were performed using previously described methods (Qin et al., 2014; Liu et al., 2015). The PCR products were purified and sequenced, and the allelic profiles and sequence types (STs) were assigned using online databases (https://pubmlst.org/ for K. pneumoniae, E. cloacae, and C. freundii, http://mlst.warwick.ac.uk/mlst/dbs/ Ecoli for E. coli).

Conjugation Assay, S1-PFGE, and Southern Blotting

Conjugation experiments were conducted using methods described previously at 25, 30, and 37°C. Briefly, the blaIMP−4-positive isolates served as the donor, while E. coli EC600 (rifampin resistant) was used as the recipient strain. Transconjugants were selected on Mueller–Hinton (MH) agar supplemented with sodium rifampin (200 μg/ml) and meropenem (2 μg/ml). The presence of the blaIMP−4 gene and other resistance genes in transconjugants was confirmed by PCR, DNA sequencing, and antimicrobial susceptibility. S1-PFGE and Southern blotting were conducted, according to published methods, to estimate sizes of blaIMP−4 plasmids (Qin et al., 2014).

Plasmid Sequencing and Genetic Environments of blaIMP−4 Analysis

The plasmids of the blaIMP−4-positive strains were extracted using the Qiagen Midi kit (Qiagen, Hilden, Germany) and transformed into E. coli DH5α by electroporation. Transformants were selected on Luria–Bertani (LB) agar plates containing meropenem (2 μg/ml), and we confirmed the presence of the blaIMP−4 gene by using PCR and sequencing. Plasmid replicons were determined using the PCR-based replicon typing method (Carattoli et al., 2005). Plasmids were sequenced based on the Illumina HiSeq2000 platform with 2 × 100 bp paired-end reads (Majorbio Company, Shanghai, China) and the Nanopore MinION (long-read) sequencing platform. The sequencing reads were assembled de novo using SOAPdenovo v2.04. Open reading frame prediction and annotation were done with Glimmer 3.021 and BLAST at NCBI2. Plasmid comparisons were performed using BRIG3 (Alikhan et al., 2011) and Easyfig4 tools (Sullivan et al., 2011). The complete sequence of the plasmids pIMP-ECL14-57, pIMP-KP-13-9, pIMP-CF-15-127, and pIMP-CF-15-288 and the ~46 kb fragment from pIMP-ECL-13-46 were deposited in GenBank with accession nos. MH727565 (pIMP-ECL14-57), CP068028 (pIMP-KP-13-9), CP068026 (pIMP-CF-15-127), CP068027 (pIMP-CF-15-288), and (CP068240) (pIMP-ECL-13-46, partial sequence). The final dataset of pIMP-KP-13-9, pIMP-CF-15-127, and pIMP-CF-15-288 and the ~46 kb fragment from pIMP-ECL-13-46 is available as a fasta file from Figshare; doi: 10.6084/m9.figshare.13515482. The genetic environments surrounding the blaIMP−4 gene on the other seven N plasmids were investigated by PCR mapping and sequencing, and the plasmid pIMP-ECL14-57 and an H2 plasmid pIMP4-SEM1 (KX810825) were used as references. PCR primers were designed from the reference sequences and are listed in Supplementary Table 1. The locations of the primers are shown in Figure 1D.

Plasmid Stability

Plasmid stability tests for plasmids were conducted as described previously (Wang et al., 2017). Briefly, the blaIMP−4-harboring transformants from ECL14-57, CF-15-127, and ECL-13-46, which were representative of blaIMP−4-carrying N, F, and H2 plasmids characterized in this study, respectively, were used as

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1http://cbcb.umd.edu/software/glimmer/
2https://blast.ncbi.nlm.nih.gov/Blast.cgi
3http://brig.sourceforge.net/
4http://mjsull.github.io/Easyfig/
5https://figshare.com/s/a1dc76cc2fa6d00d1b4
the test strains. The overnight growths of the bacteria in LB broth were inoculated into 2 ml of a fresh LB broth and incubated for 12 h at 37°C (time zero). The above process was repeated every 12 h (equivalent to 10 generations each). At time zero and after passage without antibiotic for 50, 100, 150, and 200 generations, a sample of the culture was diluted and spread onto a LB plate. One hundred colonies were picked and replica plated onto a pair of plain and antibiotic-containing (0.5 µg/ml meropenem) LB plates. Plasmid stability was determined by the percentage of colonies growing on the antibiotic-containing plates.

RESULTS

Overview of the $\text{bla}_{\text{IMP-4}}$-Positive Isolates

A total of 12 (12/317, 3.79%) $\text{bla}_{\text{IMP-4}}$ positive isolates, namely, 5 E. cloacae, 3 E. coli, 2 K. pneumoniae, and 2 C. freundii strains, were obtained from 317 CRE. These strains were recovered from different sample types including urine, blood, wound, abdominal drainage, sputum, and cerebrospinal fluid (Table 1). Over half of the $\text{bla}_{\text{IMP-4}}$-carrying isolates (7/12, 58.33%) were collected from the ICU department, and the mortality among the patients infected with a $\text{bla}_{\text{IMP-4}}$-positive isolate was 25% (3/12) (Table 1). These patients were diagnosed with different clinical diseases and none of them had a history of foreign travel.

For the antimicrobial susceptibility profiles, all the $\text{bla}_{\text{IMP-4}}$-positive isolates were susceptible to colistin (minimum inhibitory concentrations (MICs) of $\leq 2 \mu g/ml$); tigecycline also had high activity against these isolates (MIC$_{50} = 0.5 \mu g/ml$) (Table 2). Our observation is consistent with previous data from both China and other countries which showed that colistin and tigecycline are effective for the treatment of infections caused by CRE (Wang et al., 2018).

### Bacterial Genotyping, Conjugation, and Plasmid Analysis

MLST was performed for all the IMP-4-positive E. cloacae, E. coli, C. freundii, and K. pneumoniae isolates. Based on the MLST results, five E. cloacae isolates were distributed to four ST types, namely, ST133 ($n = 2$), ST231 ($n = 1$), ST754 ($n = 1$), and ST97 ($n = 1$). All of the three E. coli isolates belonged to ST167, which is regarded as the most common clone of E. coli in China (Zhang et al., 2017; Wang et al., 2018). Two C. freundii isolates belonging to ST396. ST14- and ST17-type K. pneumoniae carried $\text{bla}_{\text{IMP-4}}$ in this study (Table 1). Overall, the observation of diversity in the isolates of E. cloacae for carrying $\text{bla}_{\text{IMP-4}}$ indicated that the mobile genetic elements, such as conjugative plasmids and...

### Table 1 | Characteristics of $\text{bla}_{\text{IMP-4}}$-positive CRE isolates.

| Isolate | Age/sex | Clinical features | MLST | Plasmid name | Type and size (kb) |
|---------|---------|------------------|------|--------------|-------------------|
| ECL-13–46 | 25 years/female | Urine | Multiple injury and lung infection/neurosurgery | ST231 | pIMP-ECL-13–46 | HI2/HI2A/360 |
| ECL-14–57 | 72 years/female | Blood | Viral encephalitis and lung infection/EICU | ST754 | pIMP-ECL-14–57 | N/52 |
| ECL-15–65 | 54 years/male | Wound | Arterial ischemia and thrombosis of right lower/vascular surgery | ST97 | pIMP-ECL-15–65 | N/52 |
| ECL-15–101 | 60 years/male | Urine | Prostatic hyperplasia with urinary retention/urology | ST133 | pIMP-ECL-15–101 | N/52 |
| ECL-15–284 | 45 years/male | Abdominal drainage | Severe acute pancreatitis/ICU | ST133 | pIMP-ECL-15–284 | N/52 |
| KP-13–9 | 6 months/male | Sputum | Lung infections and asphyxia/PICU | ST14 | pIMP-KP-13–9 | Fil/110 |
| KP-15–285 | 6 months/male | Sputum | Severe pneumonia/PICU | ST17 | pIMP-KP-15–285 | N/52 |
| EC-13–25 | 78 years/female | Urine | Bronchiectasis/respiratory and sleep department | ST167 | pIMP-EC-13–25 | N/52 |
| EC-13–28 | 72 years/male | Sputum | ACVD/NSICU | ST167 | pIMP-EC-13–28 | N/52 |
| EC-14–52 | 58 years/female | Urine | Renal calculi/urology | ST167 | pIMP-EC-14–52 | N/52 |
| CF-15–288 | 19 years/female | CSF | Cerebral hemorrhage/NSICU | ST396 | pIMP-CF-15–288 | FIB/130 |
| CF-15–127 | 26 years/male | CSF | Headache and dizziness/NSICU | ST396 | pIMP-CF-15–127 | FIB/130 |

*ECL, E. cloacae strains; EC, E. coli strains; KP, K. pneumoniae strains; CF, C. freundii strains. *aMLST, multilocus sequence typing; -, not detected. 
*ICU, intensive care unit; NSICU, neuroscience ICU; EICU, emergency ICU; PICU, pediatric ICU; ACVD, acute cardiovascular disease. 
*CSF, cerebrospinal fluid.
transposons, might be responsible for the horizontal transfer of \( bla_{IMP-4} \) among different clones.

The \( bla_{IMP-4} \) gene was always carried by a plasmid, so S1-PFGE and Southern blotting were performed to identify \( bla_{IMP-4} \) harboring plasmids. The \( bla_{IMP-4} \) genes in all 12 CRE isolates were located on plasmids with sizes ranging from \( \sim 52 \) to \( \sim 360 \) kb. The \( \sim 52 \) kb plasmids were predominant among those carrying \( bla_{IMP-4} \) (8/12, 66.7%). Conjugative assays revealed that only three \( \sim 52 \) kb \( bla_{IMP-4} \)-carrying plasmids were successfully transferred to \( E. coli \) EC600 from the donors by conjugation at frequencies of \( 3.2 \times 10^{-14} \times 10^{-5} \) per donor cell. The other nine IMP-4-encoding plasmids which failed to transfer to the recipient strain by conjugation were electrotransformed into \( E. coli \) DH5\( \alpha \). PCR-based replicon typing analysis for both transconjugants and transformants showed that all the \( \sim 52 \) kb \( bla_{IMP-4} \)-carrying plasmids were distributed in four \( E. cloacae \), three \( E. coli \), and one \( K. pneumoniae \) isolates belonging to plasmid replicon type N (Table 1 and Figure 1A). The details concerning plasmid name, size, and replicon type are summarized in Table 1.

**Sequence Analysis of \( bla_{IMP-4} \)-Carrying Plasmids and Genetic Environments of \( bla_{IMP-4} \)**

A representative N-type \( bla_{IMP-4} \)-carrying plasmid named pIMP-ECL14-57, which came from \( E. cloacae \) strain ECL14-57, had 51,795 bp, with an average GC content of 50.52%, encoding 54 predicted open reading frames (ORFs). It shared extensive similarity with pIMP-FJ1503 (99% nucleotide identity and query coverage) (KU051710), an N-type \( bla_{IMP-4} \)-carrying plasmid in a carbapenem-resistant \( C. freundii \) strain CRE1503 isolated from Hong Kong (Figure 1A). Comparative genomic analysis between these two plasmids revealed only two differences: (1) the intact \( Iskpn19 \) element downstream of \( qnrS1 \) that was carried by pIMP-FJ1503 is inserted by an IS26 element in pIMP-ECL14-57 and (2) the \( Int1 \) gene immediately upstream of \( bla_{IMP-4} \) was complete in pIMP-ECL14-57 but was truncated in pIMP-FJ1503 (Figure 1A). Only two resistance genes, namely \( bla_{IMP-4} \) and \( qnrS1 \), conferring resistance to carbapenems and quinolones, respectively, were identified in each plasmid. The \( bla_{IMP-4} \) gene-associated class 1 integron In823 was carried by a transposon-like structure [IS\( 6100-mobC \)-intron (K1.pn.13)-\( bla_{IMP-4} \)-Int11-IS\( 626 \)] bracketed by two 5 bp direct repeats (DR: AACAG) inserted between the \( EcorII \) and \( wpu1 \) genes. In addition, this \( bla_{IMP-4} \)-carrying transposon-like structure was also identified in the other seven N plasmids by using PCR mapping and sequencing (Figure 1D).

The FII plasmid pIMP-KP-13-9 was 112,209 bp long with an average GC content of 51.19% and encoding 138 predicted ORFs (Figure 1B). This plasmid showed 98.94% nucleotide identity and 84% query coverage with pIMP1572 (MH464586), a plasmid carrying both \( bla_{IMP-26} \) and \( tet(A) \) variants (Yao et al., 2020). Different from the plasmid pIMP1572, a Tn1721-like transposon structure carrying the \( tet(A) \) variant which is responsible for tigecycline was absent in pIMP-KP-13-9. Interestingly, a 3,447 bp region comprising an IS\( 26 \), \( int1 \), the \( bla_{IMP-4} \) gene, and \( \Delta \)intron (K1.pn.13) in the \( bla_{IMP-4} \)-carrying transposon-like structure

| Isolate | IPM (\( \mu \)g/mL) | MEM | ATM | CAZ | LVX | GEN | AMK | CHL | TET | TGC | CST | FOF | AMP | CFZ | CFX | TZP |
|----------|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ECL-13-46 | \(<64\) | >64 | >64 | >64 | 64 | >64 | >64 | >64 | >64 | >64 | 1 | 2 | \(<1,024\) | ND | ND | ND | 256 |
| ECL-14-57 | 32 | 64 | 64 | 64 | 64 | 16 | >64 | >64 | 4 | 1 | 64 | ND | ND | ND | 256 |
| ECL-15-65 | >64 | >64 | >64 | >64 | 2 | 8 | 2 | >64 | 8 | 1 | 0.5 | \(<1\) | ND | ND | ND | \(<512\) |
| ECL-15-101 | >64 | >64 | >64 | >64 | 64 | 8 | 4 | >64 | 8 | 4 | 1 | \(<1\) | ND | ND | ND | \(<512\) |
| ECL-15-284 | 64 | >64 | >64 | >64 | >64 | 16 | >64 | 8 | 0.25 | 1 | 64 | ND | ND | 64 |
| KP-13-9 | 64 | 64 | 64 | >64 | 8 | 1 | 4 | 4 | 8 | 0.25 | 1 | 16 | \(<256\) | \(<256\) | \(<256\) | 128 |
| KP-15-285 | >64 | >64 | >64 | >64 | 4 | 1 | 2 | 4 | \(\geq64\) | 0.5 | 0.5 | 16 | \(<256\) | \(<256\) | \(<256\) | 64 |
| EC-13-25 | 8 | 16 | >64 | >64 | 64 | >64 | 8 | >64 | 0.25 | 0.5 | 2 | \(<256\) | \(<256\) | \(<256\) | 256 |
| EC-13-26 | 4 | 4 | 64 | 64 | 16 | >64 | 8 | 64 | 0.5 | 0.125 | \(<1\) | \(<256\) | \(<256\) | \(<256\) | 512 |
| EC-14-52 | 4 | 16 | 64 | 64 | 0.5 | >64 | 64 | 4 | 64 | 0.25 | 0.5 | 16 | \(<256\) | \(<256\) | \(<256\) | 256 |
| CF-15-288 | 64 | >64 | >64 | >64 | 4 | 8 | 1 | 4 | \(\geq8\) | 2 | 1 | 4 | ND | ND | ND | 512 |
| CF-15-127 | 64 | 32 | 64 | >64 | >64 | 64 | 8 | 8 | 8 | 1 | 2 | 4 | ND | ND | ND | 256 |

Isolate \( a \) Antibiotic susceptibilities of \( bla_{IMP-4} \)-positive CRE and their transconjugants.

1. M. freundii isolates are intrinsically resistant to AMP, CFZ, and CFX.
in N plasmids was reversed in pIMP-KP-13-9 due to IS26-mediated recombination indicated by the presence of target site duplications (TSD) of 8 bp (CCTGCAGAG).

The two FIB plasmids pIMP-CF-15-127 and pIMP-CF-15-288 obtained from different ST396 _C. freundii_ strains were nearly identical (96.85% nucleotide identity and 100% query coverage) (Figure 1C), both of which harbored 268 predicted ORFs. The backbone region of pIMP-CF-15-127/pIMP-CF-15-288 (∼57.6 kb) containing _repA_ (replication), _umuCD_ (SOS mutagenesis), _sopAB_ (plasmid-partition), and partial type IV
secretion system (T4SS) encoding gene cluster shared 51% query coverage and 99.06% nucleotide identity with PCN061p6 (CP006642) from an O9 E. coli strain. A partial, but not an intact, T4SS encoding region in the two plasmids could explain the lack of conjugation of FIB plasmids. A similar blaIMP−4−carrying transposon-like structure in N plasmids was also found in pIMP-CF-15-127/pIMP-CF-15-288, while the IS26 element located immediately downstream of the int1 gene was disrupted by a 6,689 bp pIMP-HZ1 (KU886034)-derived segment encompassing IS26, qnrS1, and multiple functional genes (Figure 1D).

Overall, the blaIMP−4−associated In823 flanked by IS6100 and IS26 in N plasmids was conserved, and IS26 involved recombination events that resulted in variable structures of this transposon-like module in the FII and FIB plasmids. Analysis of a ~46 kb blaIMP−4−carrying segment from the H12/H12A plasmid pIMP-ECL-13-46 (failure to obtain complete sequence by WGS) revealed that the blaIMP−4 gene was present in the sul1-type integron In1589, which was first identified in an H12 plasmid pIMP-4-EC62 obtained from E. cloacae EC62 of swine origin (Zhu et al., 2019).

### Stability of blaIMP−4-Carrying Plasmids

Plasmid stability analysis revealed that the N-type plasmid pIMP-ECL14-57 in transformants from ECL14-57 could be maintained at 100% over 200 generations of multiplication in the absence of antibiotics. However, drastic loss of the F-type plasmid pIMP-CF-15-127 and H12-type plasmid pIMP-ECL-13-46 in transformants from CF-15-127 and ECL-13-46 was observed after 50 generations of multiplication, with 35 and 3%, respectively, retaining the blaIMP−4−harboring plasmid after 150 generations. These results revealed that, among the plasmids carrying blaIMP−4, the N type is more stable than the F type and H12 type.

### DISCUSSION

The IMP-4-type MBL, first identified in clinical Acinetobacter spp. from Hong Kong (Chu et al., 2001), has spread to Australia but has not been frequently detected as KPC-2 and NDM among CRE in mainland China (Xiong et al., 2016). The incidence (3.79%) of IMP-4-producing Enterobacterales observed in the CRE of this study was comparable to that found in a recent nationwide survey of CRE (3.6%) (Wang et al., 2018). The blaIMP−4 gene was found in four species, namely, E. cloacae, E. coli, K. pneumoniae, and C. freundii, which are the most common species carrying blaIMP genes (Wang et al., 2018). A report from Australia indicated that IMP-4 was the predominant MBL type among CRE, particularly in carbapenem-resistant E. cloacae (CRECL) (Sidjabat et al., 2015). Our previous study together with recent findings from China revealed the dominance of NDM-type MBL among CRECL; whether IMP-4 is the second most common MBL in CRECL needs further study (Liu et al., 2015; Jin et al., 2018).

All of the blaIMP−4 genes in this study were carried by plasmids with diverse replicons. These included H12, N, F, and especially the predominant N plasmids. The N type is a broad host range plasmid that carries a variety of resistance determinants and shows resistance to extended-spectrum-β-lactams, sulfonamides, quinolones, aminoglycosides, tetracyclines, and streptomycin (Eikmeyer et al., 2012). N plasmids are also associated with the spread of carbapenem-resistant determinants, such as blanDM and blakPC (Poirel et al., 2011; Partridge et al., 2012; Eilertson et al., 2017; Jiang et al., 2017; Partridge et al., 2018; Schweizer et al., 2019). This type of plasmid was recently identified as an epidemic plasmid for carrying blaIMP−4 among Enterobacterial species in China (Lai et al., 2017; Wang et al., 2017), and it was responsible for horizontal transmission of blaIMP−6 among Enterobacterales from Japan (Yamagishi et al., 2020). Our findings are consistent with these studies and indicate the prevalence of N blaIMP−4−carrying epidemic plasmids among CRE in other regions of China. Additionally, FII plasmids, which are carriers of the blakPC gene in K. pneumoniae (Partridge et al., 2018; Yang et al., 2020), were found to carry the blaIMP gene in this study. Association with these widespread types of plasmids may accelerate dissemination of blaIMP genes among K. pneumoniae.

Class 1 integrons are common vehicles for carrying the blaIMP genes. Multiple blaIMP−harboring class 1 integrons with considerable cassette array diversity, such as In992, 1312 (blaIMP−1). In809, 823, 1456, 1460, 1589 (blaIMP−4), In722, 1321 (blaIMP−6), In73 (blaIMP−8), In687 (blaIMP−14), In1310, 1386 (blaIMP−26), and 1385 (blaIMP−38), were identified in Enterobacterales and non-fermenting Gram-negative bacilli including Pseudomonas aeruginosa and Acinetobacter spp. (Lee et al., 2017; Matsumura et al., 2017; Papagiannitsis et al., 2017; Wang et al., 2017; Dolejska et al., 2018; Zhan et al., 2018; Zhu et al., 2019). Among these, the blaIMP−4−carrying In823 integron was the most frequently detected structure on N-type plasmids in isolates recovered from different regions of China including Henan Province (Feng et al., 2016; Wang et al., 2017).

### CONCLUSION

In conclusion, we determined the prevalence and molecular characterization of blaIMP−4−positive Enterobacterales in clinical specimens collected at a teaching hospital in Henan Province. Previously reported epidemic N-type plasmids exhibited superior stability compared with F- and H12-type plasmids. N-type plasmids were the predominant plasmids carrying blaIMP−4 among the collected Enterobacterales. Associated with self-transmissible N plasmids, widespread FII plasmids and a successful epidemic E. coli ST167 clone might facilitate further dissemination of blaIMP−4 among the Enterobacterales. Surveillance is needed to monitor the spread of blaIMP−4−harboring Enterobacterales.

### DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, MH727565, CP068028, CP068026, CP068027, and CP068240.
ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Zhengzhou University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SQ and ZW designed the study. WL, TY, CL, and SZ performed the experiments. HD, JC, LL, and XF analyzed the bioinformatics data. SQ and JC wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.626160/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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