RESEARCH

Molecular characteristics of clinical IMP-producing *Klebsiella pneumoniae* isolates: novel IMP-90 and integron In*2147*

Liuyang Yang[1†], Guangcun Zhang[1†], Qiang Zhao[1], Ling Guo[1] and Jiyong Yang[1*]

Abstract

Background Since the first report of carbapenem-resistant *Klebsiella pneumoniae* isolates in China in 2007, the prevalence of CRKP and CRE has increased significantly. However, the molecular characteristics of IMP-producing *Klebsiella pneumoniae* (IMPKp) are rarely reported.

Methods A total of 29 IMPKp isolates were collected from a Chinese tertiary hospital from 2011 to 2017. Clinical IMPKp were identified by VITEK®MS, and further analyzed by whole-genome DNA sequencing with HiSeq and PacBio RSII sequencer. Sequencing data were analyzed using CSI Phylogeny 1.4, Resfinder, PlasmidFinder and the MLST tool provided by the Centre for Genomic Epidemiology. The analysis results were visualized using iTOL editor v1_1. The open reading frames and pseudogenes were predicted using RAST 2.0 combined with BLASTP/BLASTN searches against the RefSeq database. The databases CARD, ResFinder, ISfinder, and INTEGRALL were performed for annotation of the resistance genes, mobile elements, and other features. The types of bla_{IMP} in clinical isolates were determined by BIGSdb-Pasteur. Integrons were drawn by Snapgene, and the gene organization diagrams were drawn by Inkscape 0.48.1.

Results Four novel ST type, including ST5422, ST5423, ST5426 and ST5427 were identified. The IMP-4 and IMP-1 were the dominant IMP type. The majority of bla_{IMP}-carrying plasmids belonged to IncN and IncHI5. Two novel bla_{IMP}-carrying integrons (In*2146* and In*2147*) were uncovered. A novel variant bla_{IMP-90} presented in novel integron In*2147* has been identified.

Conclusions IMPKp showed low prevalence in China. Novel molecular characteristics of IMPKp have been identified. Continuous monitoring of IMPKp shall also be carried out in the future.

Keywords Carbapenemase, Metallo-β-lactamases, IMP, *Klebsiella pneumoniae*, Integron

Introduction

The spread of carbapenem-resistant *Enterobacteriaceae* (CRE) has become a major public health problem. Carbapenems are the most effective drugs for the treatment of Gram-negative bacterial infections. In China, since the first report of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates in 2007, the prevalence of CRKP and CRE has increased significantly [1].

Resistance to carbapenems involves multiple mechanisms, and the production of various carbapenemases is the most common mechanism. Based on their

[†]Liuyang Yang and Guangcun Zhang have authors contributed equally to this work.

*Correspondence:
Jiyong Yang
yangjy301@163.com
[1] Laboratory Medicine Department, First Medical Center of Chinese PLA General Hospital, Beijing 100853, China

Yang *et al. Ann Clin Microbiol Antimicrob* (2023) 22:38

Page 2 of 8

dependency on actions for enzyme activity, carbapenemases can be divided into two different groups: serine/non-metallo- (zinc-independent; classes A, C, and D) and metallo-carbapenemases (MBLs; zinc-dependent; class B) [2]. MBLs include various clinically and epidemiologically important enzymes, such as IMP, VIM and NDM. Since the first report of IMP-1 from *Pseudomonas aeruginosa* in Japan, IMP-type enzymes have been reported globally. Compared with NDM, IMP exhibited a relatively low prevalence among clinical *K. pneumoniae* isolates [3]. The CRKP harboring the bla_{IMP-1}, bla_{IMP-4}, bla_{IMP-22} and bla_{IMP-26} have been recovered from East Asia, Europe, Australia, or North America [3]. In China, the main IMP variants include IMP-4, IMP-8, and IMP-26 [1].

The plasmid-mediated dissemination of carbapenemase-coding genes plays an important role in emergence and spread of CRKP. The most encountered bla_{IMP} genes located in plasmids with diverse incompatible groups include HI2/HI5, FI/FII, L/M, A/C, P and N [4]. Various integrons that contain bla_{IMP} genes had been characterized such as In*1763*, In*809*/In*823*, In*722*, In*73* and In*687*, and these integrons harbored bla_{IMP-1}, bla_{IMP-4}, bla_{IMP-6}, bla_{IMP-8} and bla_{IMP-14}, respectively [5, 6].

Few studies have reported the complete sequence of bla_{IMP}-harbouring plasmids, which limited our understanding of the transmission mechanism of bla_{IMP} genes. In this study, 29 clinical IMP-producing *K. pneumoniae* (IMPKp) isolates were analyzed by whole-genome sequencing and further molecular analysis. A novel IMP-type enzyme (IMP-90) and novel bla_{IMP}-harbouring integron (In*2147*) were identified.

Material and methods

Bacterial strains and antimicrobial susceptibility testing

A total of 29 IMPKp isolates were collected from July 2011 to November 2017 from our hospital (Additional file 1: Table S1). All isolates were identified by VITEK®MS (bioMérieux SA, Marcy-l'Étoile, France). At first, the MICs of antimicrobial agents [ceftazidime (CAZ), cefepime (CFP), ceftazidime-avibactam (CAZ-AVI), imipenem (IPM), meropenem (MEM), ertapenem (ETP), amikacin (AK), ciprofloxacin (CIP) and sulfamethoxazole/trimethoprim (SXT)] were measured by BioMérieux VITEK 2 AST-GN09 and AST-GN13 according to the manufacturer's instructions. Then, were measured with broth microdilution method by using the Biofosun R Gram-negative panels (Biofosun Biotechnology Corporation Ltd., Shanghai, China). If the MICs of carbapenem antibiotics exceeded the upper limit of detection [IPM (MICs > 8 mg/L), ETP (MICs > 8 mg/L) or MEM (MICs > 4 mg/L)], the dry strip method was performed to correct the results by using E-test (Autobio Diagnostics

Corporation Ltd., Zhengzhou, China). All susceptibility results were interpreted according to the 2021 CLSI guidelines [7].

Whole-genome DNA sequencing

All isolates were subjected to draft-genome sequencing using a paired-end library with an average insert size of 350 bp (ranged from 150 to 600 bp) on a HiSeq sequencer (Illumina, CA, USA). All sequences were assembled using SOAPdenovo (SOAP Version 2.21). The N_{50}, N_{90}, coverage rate and scaffold number were used to identify de novo characteristics. In addition, one or two strains from each subclade were selected for subsequent long PacBio reads sequencing using a sheared DNA library with average size of 15 kb (ranged from 10 to 20 kb) on a PacBio RSII sequencer (Pacific Biosciences, Menlo Park, CA, United States). The paired-end short Illumina reads were used to correct the long PacBio reads utilizing proofreads, and then the corrected PacBio reads were assembled de novo utilizing SMARTdenovo.

Data analysis

WGS data for all isolates were analyzed using CSI Phylogeny 1.4, Resfinder, PlasmidFinder and the MLST tool provided by the Centre for Genomic Epidemiology (https://cge.cbs.dtu.dk/services). Determination of capsular type for strains was conducted by using Kleborate (https://github.com/katholt/Kleborate). The tree file was visualized by iTOL (https://itol.embl.de), and annotated information were edited by iTOL editor v1_1.

Sequence annotation

The open reading frames and pseudogenes were predicted using RAST 2.0 combined with BLASTP/BLASTN searches against the RefSeq database as previous described [8]. Plasmid replicon types were identified using PlasmidFinder and corrected by the RAST 2.0. The databases CARD, ResFinder, ISfinder, and INTEGRALL were performed for annotation of the resistance genes, mobile elements, and other features. The types of bla_{IMP} in clinical isolates were determined by BIGSdb-Pasteur. Integrons were drawn by Snapgene, and the gene organization diagrams were drawn by Inkscape 0.48.1.

Transformation experiments

As previously described [9], we cloned the open reading frame of bla_{IMP-90} and bla_{IMP-8} into the chloramphenicol-resistant pHSG398 vector (Takara Bio, Shiga, Japan) at the *Eco*RI-*Kpn*I site and used it to transform *Escherichia coli* DH5α cells (Takara Bio).

Yang *et al. Ann Clin Microbiol Antimicrob* (2023) 22:38

Page 3 of 8

Nucleotide sequence accession numbers

All genome sequences in this study were submitted to GenBank under the accession BioProject No. PRJNA862666.
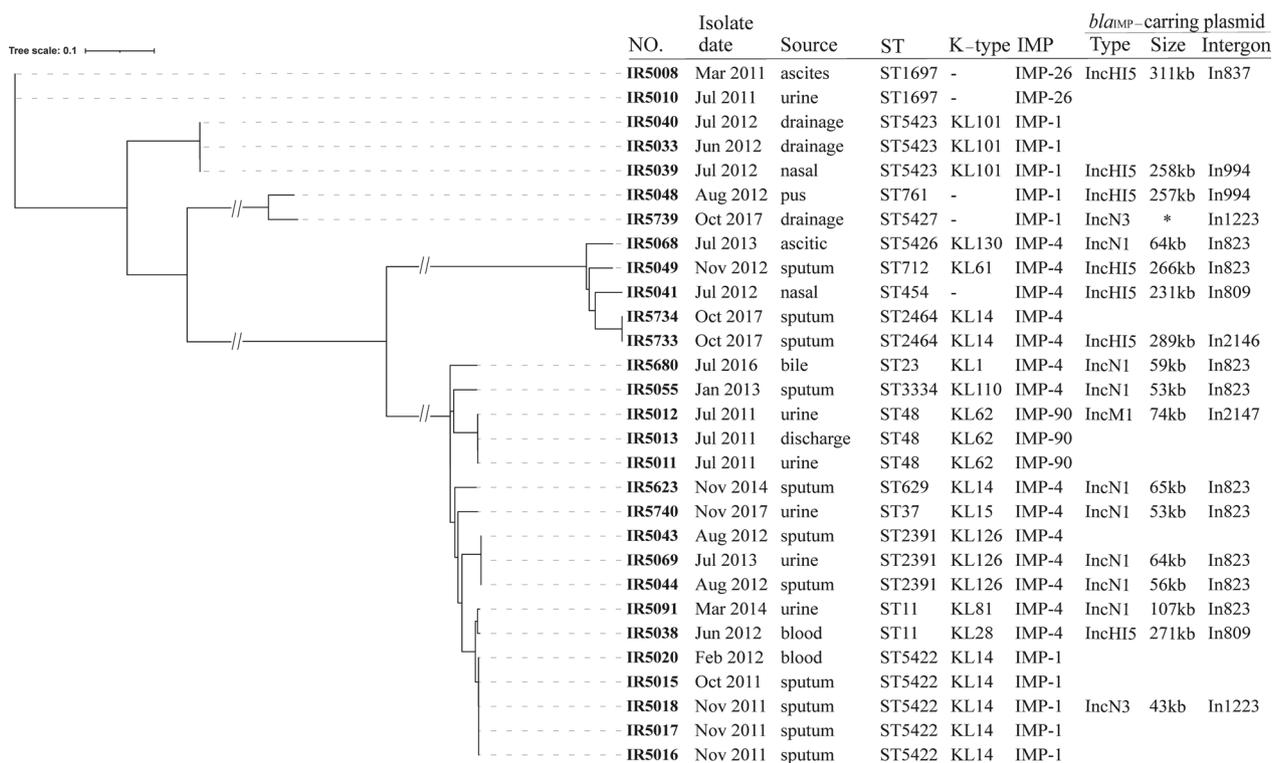
Results

Clinical characteristics of IMPKp isolates

A total of 29 IMPKp isolates were collected from July 2011 to November 2017 (Fig. 1). The sputum (n=11, 37.9%) and urine (n=6, 20.7%) were the main sources of these isolates. The strains were mainly isolated from the departments of hepatobiliary (n=9), neurology (n=6), surgical care unit (n=4) and respiratory medicine (n=4). In this study, four novel STs (ST5422, ST5423, ST5426 and ST5427) with the allelic profile (*gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*) 3-3-1-1-1-1-9, 42-22-25-96-115-20-712, 16-24-21-31-47-257-760 and 17-73-90-39-541-18-148, respectively, have been identified. The ST5422 (n=5), ST5423 (n=3), ST48 (n=3), ST2391 (n=3) outbreaks may occur in our hospital (Fig. 1, Additional file 1: Table S1). The antimicrobial susceptibility results were listed in Table S1. About 55.2% (n=16) of IMPKp isolates carried various bla_{CTX-M}, while nine isolates (24.1%) carried *armA* which was related to the amikacin resistance.

The plasmids and integrons related to known bla_{IMP}

The complete genome sequences of eighteen strains were analyzed. All bla_{IMP} gnes were located on the plasmids (Fig. 1 and Additional file 1: Table S1). In total, three known bla_{IMP} variants have been identified, including bla_{IMP-1}, bla_{IMP-4} and bla_{IMP-26}. The gene bla_{IMP-4} was distributed in 10 different ST types. Besides the ST761, other isolates harboring the bla_{IMP-1} were novel ST types, including ST5422, ST5423, and ST5427 (Fig. 1). The bla_{IMP-1}-carrying plasmids belonged to IncN3 and IncHI5 with a size ranging from 43 to 258 kb. Plasmids harboring bla_{IMP-4} were IncN1 and IncHI5 with a size ranging from 53 to 289 kb. The bla_{IMP-26} located on a 311 kb IncHI5 plasmid (Fig. 1 and Additional file 1: Table S1). All bla_{IMP}-carrying plasmids carried various resistance genes (Additional file 1: Table S1).

The bla_{IMP-1} was presented in the In*994* and In*1223*, the bla_{IMP-4} was presented in the In*809*, In*823*, and novel identified In*2146*, while the bla_{IMP-26} was presented in the In*837* (Fig. 2 and Additional file 1: Table S1). The integrons carrying the bla_{IMP-4} and bla_{IMP-26} exhibited a certain degree of similarity. Compared with the bla_{IMP-4}-carrying In*809*, the novel In*2146* (bla_{IMP-4}-*attCDΔ::Kl. pn.I3-aacA4'-attC-3'CS*) seems like inserted the *Kl. pn.I3* and lost the resistance genes *qacG2* and *catB3*.

NO.	Isolate date	Source	ST	K–type	IMP	Type	Size	Intergon
IR5008	Mar 2011	ascites	ST1697	-	IMP-26	IncHI5	311kb	In837
IR5010	Jul 2011	urine	ST1697	-	IMP-26			
IR5040	Jul 2012	drainage	ST5423	KL101	IMP-1			
IR5033	Jun 2012	drainage	ST5423	KL101	IMP-1			
IR5039	Jul 2012	nasal	ST5423	KL101	IMP-1	IncHI5	258kb	In994
IR5048	Aug 2012	pus	ST761	-	IMP-1	IncHI5	257kb	In994
IR5739	Oct 2017	drainage	ST5427	-	IMP-1	IncN3	*	In1223
IR5068	Jul 2013	ascitic	ST5426	KL130	IMP-4	IncN1	64kb	In823
IR5049	Nov 2012	sputum	ST712	KL61	IMP-4	IncHI5	266kb	In823
IR5041	Jul 2012	nasal	ST454	-	IMP-4	IncHI5	231kb	In809
IR5734	Oct 2017	sputum	ST2464	KL14	IMP-4			
IR5733	Oct 2017	sputum	ST2464	KL14	IMP-4	IncHI5	289kb	In2146
IR5680	Jul 2016	bile	ST23	KL1	IMP-4	IncN1	59kb	In823
IR5055	Jan 2013	sputum	ST3334	KL110	IMP-4	IncN1	53kb	In823
IR5012	Jul 2011	urine	ST48	KL62	IMP-90	IncM1	74kb	In2147
IR5013	Jul 2011	discharge	ST48	KL62	IMP-90			
IR5011	Jul 2011	urine	ST48	KL62	IMP-90			
IR5623	Nov 2014	sputum	ST629	KL14	IMP-4	IncN1	65kb	In823
IR5740	Nov 2017	urine	ST37	KL15	IMP-4	IncN1	53kb	In823
IR5043	Aug 2012	sputum	ST2391	KL126	IMP-4			
IR5069	Jul 2013	urine	ST2391	KL126	IMP-4	IncN1	64kb	In823
IR5044	Aug 2012	sputum	ST2391	KL126	IMP-4	IncN1	56kb	In823
IR5091	Mar 2014	urine	ST11	KL81	IMP-4	IncN1	107kb	In823
IR5038	Jun 2012	blood	ST11	KL28	IMP-4	IncHI5	271kb	In809
IR5020	Feb 2012	blood	ST5422	KL14	IMP-1			
IR5015	Oct 2011	sputum	ST5422	KL14	IMP-1			
IR5018	Nov 2011	sputum	ST5422	KL14	IMP-1	IncN3	43kb	In1223
IR5017	Nov 2011	sputum	ST5422	KL14	IMP-1			
IR5016	Nov 2011	sputum	ST5422	KL14	IMP-1			

Fig. 1 Genetic characteristics of IMP-producing *K. pneumoniae*. All the bla_{IMP}-carrying plasmid were analyzed by the HiSeq + PacBio RSII, except that in IR5739 isolates was identified by HiSeq (marked with asterisks)

Yang *et al. Ann Clin Microbiol Antimicrob*   (2023) 22:38

Page 4 of 8
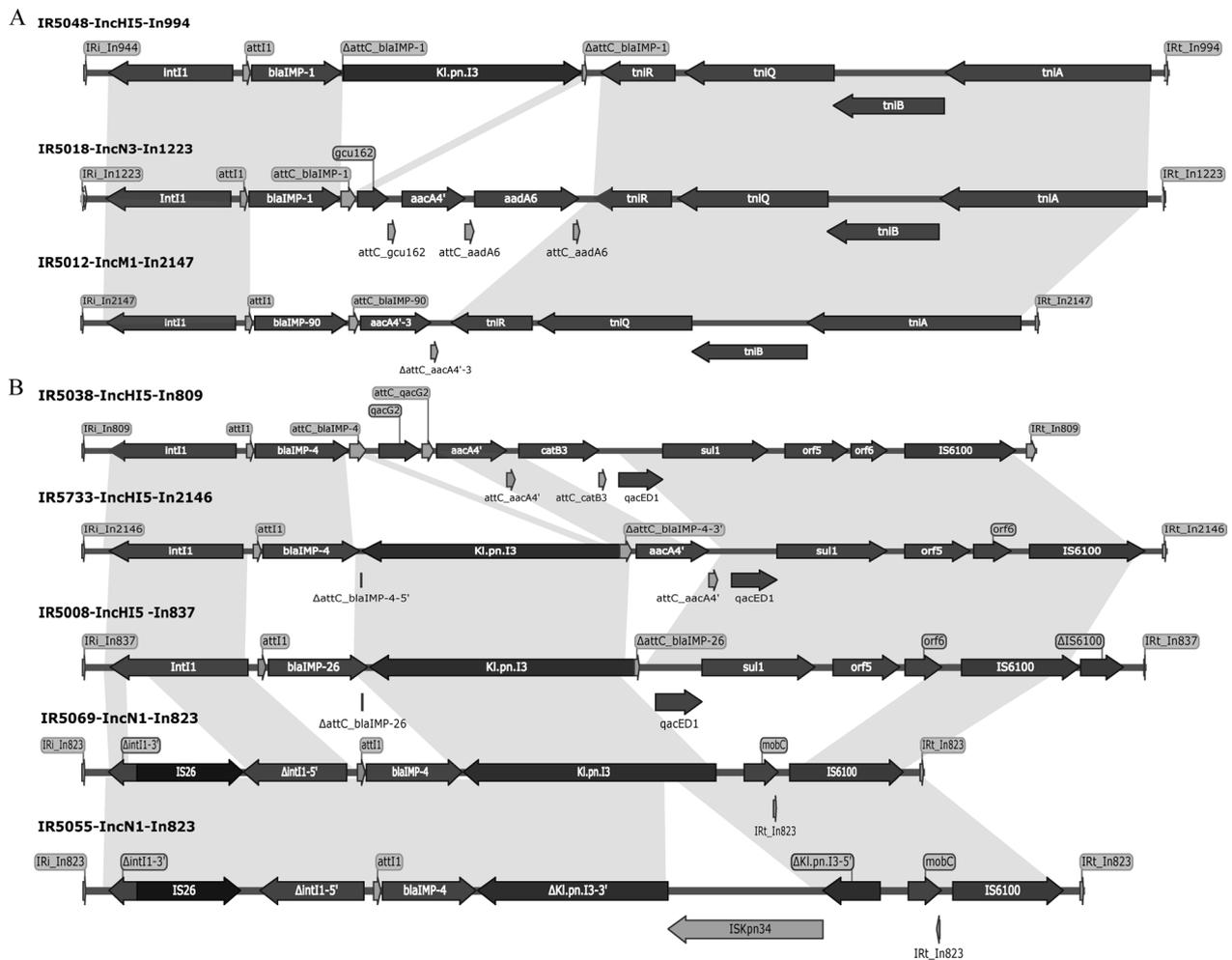
Fig. 2 Comparative analysis of the bla_{IMP}-carrying integrons. **A** The In*994*, In*1223* and In*2147* shown the similarity. **B** The In*809*, In*2146*, In*837* and In*823* shown the similarity

Meanwhile, there was a possibility that the In*2146* was derived from the In*823* by inserting the *aacA4* and bla_{IMP-4} mutation (Fig. 2). The In*809*, In*823* and In*2146* contained 3'-conserved segment (CS) structures immediately downstream of the gene cassettes. The majority contained In*4*-like structures consisting of 3'-CS-IS*6100* (with or without partial deletion of 3'-CS and *chrA-padR* insertion).

Novel metallo-β-lactamase IMP-90 and integron In2147
In this study, a novel IMP-type MBL, bla_{IMP-90}, was identified. An IMPKp ST48 emerged and caused an outbreak in 2011 (Fig. 1). Whole-genome sequencing revealed that the isolate carried a novel bla_{IMP} variant, which was designated as the bla_{IMP-90}. IMP-90 differed from IMP-8 by an amino acid substitution (Ser262Gly). The bla_{IMP-90} was located on a 72905-bp IncM1 plasmid (Fig. 3), and was presented in a novel integron In*2147*

(bla_{IMP-90}-*attC-aacA4'-3-attCD*), which showed similarity with In*994* and In*1223* (Fig. 2). The IR5012 was susceptible to imipenem (MIC=1 ug/mL), suggesting that the IMP-90 had a weaker imipenem hydrolyzing activity compared with other IMP enzymes (Additional file 1: Table S1). Meanwhile, the transformation experiments of bla_{IMP-90} and bla_{IMP-8} demonstrated that the IMP-90 exhibited similar carbapenem hydrolyzing activity with IMP-8.

Discussion
The prevalence of IMPKp varies by regions and often induces outbreaks in clinical departments. A multicenter study showed that the MBLs were not equally common, with more than 60% of MBL-positive CRE isolates carrying the bla_{NDM}, while only 9% of the isolates produced the IMP [3]. In China, the predominance of MBL among *Enterobacteriaceae* was NDMs, and the IMP has been

Yang *et al. Ann Clin Microbiol Antimicrob*   (2023) 22:38

Page 5 of 8
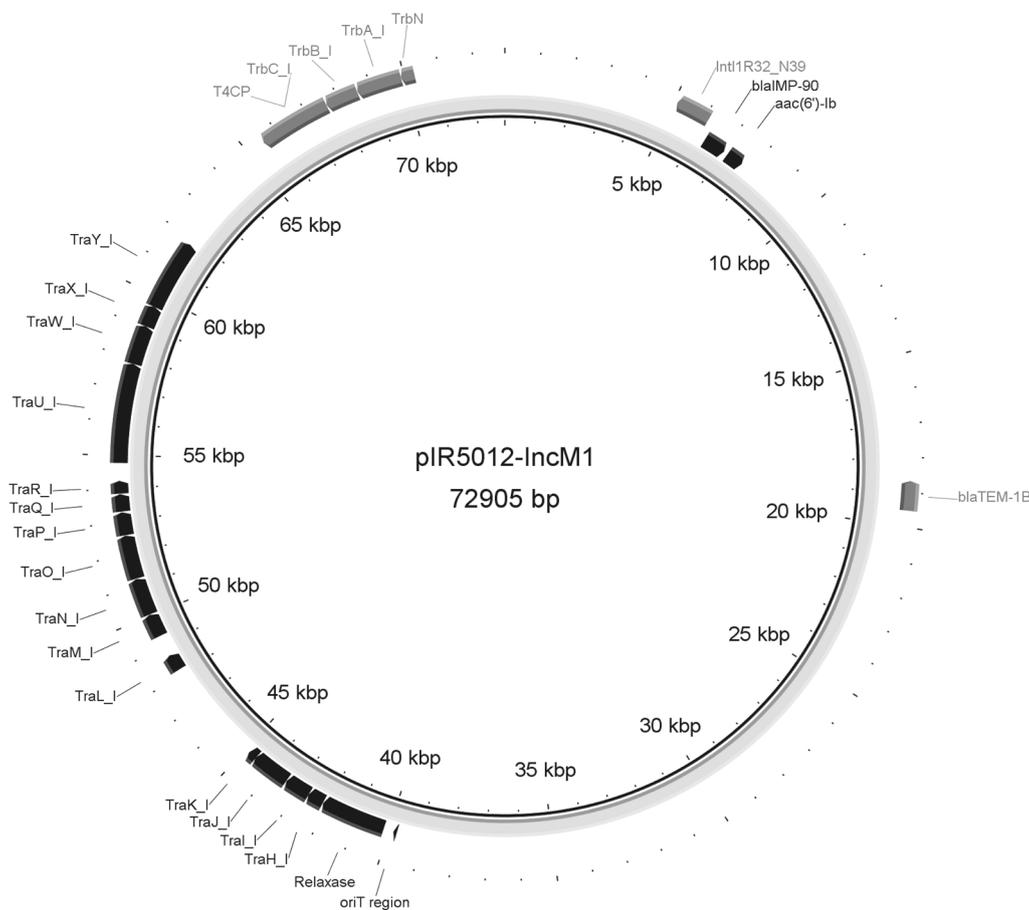
Fig. 3 Backbone structure of the bla_{IMP-90}-encoding plasmid pIMP-90-IR5012

sporadically detected [10]. We have reported the molecular characterization of some clinical IMPKp isolates [11]. In this study, a larger collection (n = 29) of IMPKp were collected and analyzed by more accurate methods such as whole-genome sequencing, and thus limited results in previous study are revised and supplemented. The high homology among some strains indicated the existence of small-scale outbreaks caused by various IMPKp clones (Fig. 1). Most IMPKp isolates exhibited low-level resistance or even susceptible to various carbapenems (Additional file 1: Table S1), indicating the low hydrolysis capacity of IMP, and may leading to a low epidemic level of IMPKp. In this study, IMP-4 (14/29, 48.28%) was the most popular enzyme type, followed by IMP-1 (10/29, 34.48%), and this was consistent with previous finding [12].

The ST type of IMPKp isolates in this study was relatively dispersed (Fig. 1). The *K. pneumoniae* CG258 are the most common carbapenem-resistant isolates reported worldwide, including the ST11 and the ST5422 in this study. The *tonB* allele of ST11 (*tonB*-4) is widely distributed in approximately 80 unrelated STs of CG258, but the *tonB*-79 allele of ST258 has only been observed among less than 10 STs (http://www.pasteur.fr/mlst). Some researchers considered that the *tonB*-79 was probably derived from *tonB*-4 by acquisition of site substitutions [13]. In this study, among the four novel STs (ST5422, ST5423, ST5426 and ST5427), only ST5422 [allelic profile (*gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*) was 3-3-1-1-1-1-9] may arise from ST11 (allelic profile: 3-3-1-1-1-1-4) by substituting *tonB*-4 with *tonB*-9. However, although it has high homology with ST11, completely different characteristics of IMP-coding genes suggested the independent evolutionary path of this novel identified clone (Fig. 1).

Consistent with previous study [14], we found that all known IMP-coding genes were located on IncN and IncHI5 plasmids in this study (Fig. 1). IncN and IncHI plasmids are both conjugative plasmids and have been reported to be part of a broad-host-range group [15]. Based on the nucleotide sequence homology over the backbones, the IncN group can be divided into three

Yang *et al. Ann Clin Microbiol Antimicrob*   (2023) 22:38

Page 6 of 8

subgroups, including IncN1, IncN2 and IncN3 [16]. In this study, the predominant bla_{IMP}-carrying plasmids belonged to N1 with a smaller size (Fig. 1). The bla_{IMP-1}-carrying IncN3 plasmid was identified in UK [5], and this was the first report to identify this plasmid in China, showing the necessity of investigating prevalence and evolutionary history of IncN3 plasmids. The IncHI plasmids are important vectors in the dissemination of heavy metal resistance genes and antimicrobial resistance genes, and usually have a size larger than 200 kb [17]. In this study, all bla_{IMP}-carrying IncHI5 plasmids carried conserved IncHI5 backbones, including *repHI5B* and a *repFIB*-like, *parABC*, and *tra1*, mediating replication, partition and conjugal transfer, respectively. Further studies are needed to continuously monitor the prevalence of bla_{IMP}-carrying IncN and IncHI5 plasmids in different sources, especially among clinical settings [11, 14].

To date, various genetic context of bla_{IMP-4} has been identified, e.g. bla_{IMP-4}-*qacG-aacA4-aphA15*, bla_{IMP-4}-*Kl.pn.I3-mobC* and bla_{IMP-4}-*Kl.pn.I3-qacEΔ-sul1*. Of note, the structure of bla_{IMP-4}-*Kl.pn.I3* seems unique in isolates from China revealed by blasting in GenBank, thus it could be used as an epidemiological marker for bla_{IMP-4} detection in China. In*823* harboring bla_{IMP-4} has been identified in IncHI5 plasmid [18]. In this study, all In*823* harboring the bla_{IMP-4} was presented in the IncN1 plasmid, and the bla_{IMP-4}-*attCDΔ::Kl.pn.I3* was the most common cassette. Meanwhile, the integrons in IncHI5 were In*809* and the novel integron In*2146* (Figs. 1 and 2). In*809* with four cassettes (bla_{IMP-4}-*qacG2-aacA4-catB3*) is widely disseminated in *Enterobacteriaceae* and *Acinetobacter* spp. in Asia−pacific region [19]. In*2146* may be derived from In*809*, and is considered as an In*809*-like integron (bla_{IMP-4}-*Kl.pn.I3-qacG2-aacA4-catB3Δ*), of which a group II intron *Kl.pn.I3* was inserted into the *attC* site of the bla_{IMP-4} cassette (Fig. 2). The bla_{IMP-1}-carrying In*994* and In*1223* has been identified in previous studies [16], and it has also been found in this study (Fig. 1). In addition, the integron In*837* (*intI1-bla_{IMP-26}-attCDΔ::Kl.pn.I3-3′CS*, Fig. 2) showed low homology with other bla_{IMP-26}-carrying integrons, and had different genetic structure such as *intI1-bla_{IMP-26}-ltrA-qacEΔ1-sul1*, *intI1-bla_{IMP-26}-qacG-aacA4-aac(6′)-orf-catB3*, and *intI1-bla_{IMP-26}-qacG-aac(6′)-Ib-aac(6′)-orf3-orf4-catB3-dfrA1-tnpA-istB-orf5*, respectively [20]. In general, country-specific bla_{IMP} subtypes corresponded to the specific integron types previously characterized in that country, i.e., bla_{IMP-4}-carrying In*809* in Australia [21]; bla_{IMP-6}-carrying In*722* in Japan [22]; bla_{IMP-8}-carrying In*73* in Philippines [23]; and bla_{IMP-14}-carrying In*687*

in Thailand [24]. In this study, we identified 7 different bla_{IMP}-carrying integron types, including 2 novel cassette combinations (In*2146* and In*2147*; Fig. 2). All the four class I integrons have a complete set of IRi/IRt, *intI1*, and *attI1*. All integrons carried the strong promoters PcS (In*2147* and In*809*) or the PcW$_{TGN-10}$ (In*837*, In*994*, In*823*, In*1223* and In*2146*) (Additional file 1: Table S1), and these promoters can drive the high-level expression of cassette-borne genes [25, 26].

IMP-90 is a novel variant with an amino acid substitution at Ser262Gly compared with IMP-8. The IMP-90-producing isolates showed a sensitive feature to imipenem (MIC = 1 mg/L), while the bla_{IMP-90}-carrying *E. coli* DH-5α (competent cell) showed a reduced resistance to imipenem, compared with the bla_{IMP-8} (data not published). Previous studies show that isolates producing the IMP-type metallo-beta-lactamases with Ser262Gly substitution all exhibited susceptibility to imipenem, including the IMP-6 and IMP-68, which were the Ser262Gly substitution variants of IMP-1 and IMP-11, respectively [9, 27]. These results indicate that the Ser262Gly substitution may affect the hydrolysis ability of IMP variants to imipenem. The bla_{IMP-90} presented in the novel integron In*2147* located on a IncM1 plasmid (Figs. 2 and 3). Plasmids belonging to the IncL/M are the important mobile genetic platforms for dissemination of clinically important resistance genes, e.g. bla_{CTX-M} and bla_{OXA-48} [28]. Although L and M plasmids showed high level of DNA homology (approximately 94% overall nucleotide identity), the ExcA, TraY, and TraX proteins exhibited evident division (35%, 59%, and 75% amino acid identity, respectively). IncM plasmids have a wide host range, while IncM1 and IncM2 showed the 99% amino acid identity of the entry exclusion ExcA and TraY proteins [29]. This was the first report about the bla_{IMP}-carrying IncM1 plasmid. Conjugation between isolates carrying various IncM plasmids is compromised by inhibitory interactions of the exclusion system, which is highly conserved across all IncM plasmids (Fig. 3) [29]. Therefore, more attention should be paid to the transmission of drug resistance mediated by the IncM-type plasmids, and continuous monitoring shall also be carried out.

Conclusions

In conclusion, we collected and analyzed 29 IMPKp isolates from a Chinese tertiary hospital during the year 2011 to 2017. In this study, we identified four novel ST type, including the ST5422, ST5423, ST5426 and ST5427. The dominant IMP type was IMP-4 and IMP-1. The majority of bla_{IMP}-carrying plasmids belonged to IncN

Yang *et al. Ann Clin Microbiol Antimicrob*   (2023) 22:38

Page 7 of 8

and IncHI5. Two novel bla_{IMP}-carrying integrons (In*2146* and In*2147*) were uncovered. A novel variant bla_{IMP-90} presented in novel integron In*2147* has been identified.

Abbreviations

IMPKp	IMP-producing *Klebsiella pneumoniae*
CRE	Carbapenem-resistant *Enterobacteriaceae*
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
Inc	Incompatibility
RAST	Rapid Annotation using Subsystem Technology
CLSI	Clinical and Laboratory Standards Institute
MLST	Multi-locus sequence typing
MDR	Multi-drug resistance
IRi	Inverted repeat at the integrase end
CS	Conserved segment
IRt	Inverted repeat at the *tni* end
E. coli	*Escherichia coli*
MIC	Minimal inhibit concentration
CAZ	Ceftazidime,
CFP	Cefepime
CAZ-AVI	Ceftazidime-avibactam
IPM	Imipenem
MEM	Meropenem
ETP	Ertapenem
AK	Amikacin
CIP	Ciprofloxacin
SXT	Sulfamethoxazole/trimethoprim

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-023-00588-w.

> **Additional file 1: Table S1.** Characteristics of 29 clinical bla_{IMP}-carrying *Klebsiella pneumonia* isolates.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest to declare.

References

1. Zhang R, Chan EW, Zhou H, Chen S. Prevalence and genetic characteristics of carbapenem-resistant *Enterobacteriaceae* strains in China. Lancet Infect Dis. 2017;17(3):256–7.
2. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007;20(3):440–58.
3. Kazmierczak KM, Karlowsky JA, de Jonge BLM, Stone GG, Sahm DF. Epidemiology of carbapenem resistance determinants identified in meropenem-nonsusceptible *Enterobacterales* collected as part of a global surveillance program, 2012 to 2017. Antimicrob Agents Chemother. 2021;65(7): e0200020.
4. Matsumura Y, Peirano G, Motyl MR, et al. Global molecular epidemiology of IMP-producing *Enterobacteriaceae*. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.02729-16.
5. Turton JF, Davies F, Taori SK, et al. IncN3 and IncHI2 plasmids with an In1763 integron carrying bla (IMP-1) in carbapenem-resistant *Enterobacterales* clinical isolates from the UK. J Med Microbiol. 2020;69(5):739–47.
6. Zhao Y, Chen X, Hu X, et al. Characterization of a carbapenem-resistant *Citrobacter* amalonaticus coharbouring bla (IMP-4) and qnrs1 genes. J Medical Microbiol. 2021. https://doi.org/10.1099/jmm.0.001364.
7. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100, 31st edition. J Clin Microbiol. 2021;59(12):e0021321.
8. Guo L, Wang L, Zhao Q, et al. Genomic analysis of KPC-2-producing Klebsiella pneumoniae ST11 isolates at the respiratory department of a tertiary care hospital in Beijing China. Front Microbiol. 2022. https://doi.org/10.3389/fmicb.2022.929826.
9. Kubota H, Suzuki Y, Okuno R, et al. IMP-68, a novel IMP-type metallo-β-lactamase in imipenem-susceptible Klebsiella pneumoniae. mSphere. 2019. https://doi.org/10.1128/mSphere.00736-19.
10. Zhang R, Liu L, Zhou H, et al. Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. EBioMedicine. 2017;19:98–106.
11. Lai K, Ma Y, Guo L, An J, Ye L, Yang J. Molecular characterization of clinical IMP-producing Klebsiella pneumoniae isolates from a Chinese tertiary hospital. Ann Clin Microbiol Antimicrob. 2017;16(1):42.
12. Wang Q, Wang X, Wang J, et al. Phenotypic and Genotypic Characterization of Carbapenem-resistant Enterobacteriaceae: Data From a Longitudinal Large-scale CRE Study in China (2012–2016). Clin Infect Dis Off Publ Infect Dis Soc America. 2018;67((suppl_2)):S196–205.
13. Breurec S, Guessennd N, Timinouni M, et al. Klebsiella pneumoniae resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. Clin Microbiol Infect Offi Publ Eur Soc Clin Microbiol Infect Dis. 2013;19(4):349–55.
14. Liu W, Dong H, Yan T, et al. Molecular characterization of bla (IMP) (-) (4)-carrying Enterobacterales in henan province of China. Front Microbiol. 2021;12:626160.
15. Yamagishi T, Matsui M, Sekizuka T, et al. A prolonged multispecies outbreak of IMP-6 carbapenemase-producing Enterobacterales due to horizontal transmission of the IncN plasmid. Sci Rep. 2020;10(1):4139.
16. Jiang X, Yin Z, Yin X, et al. Sequencing of bla(IMP)-carrying IncN2 plasmids, and comparative genomics of IncN2 plasmids harboring class 1 integrons. Front Cell Infect Microbiol. 2017;7:102.
17. Cain AK, Hall RM. Evolution of IncHI2 plasmids via acquisition of transposons carrying antibiotic resistance determinants. J Antimicrob Chemother. 2012;67(5):1121–7.
18. Liang Q, Jiang X, Hu L, et al. Sequencing and genomic diversity analysis of IncHI5 plasmids. Front Microbiol. 2018;9:3318.
19. Ho PL, Lo WU, Chan J, et al. pIMP-PH114 carrying bla IMP-4 in a Klebsiella pneumoniae strain is closely related to other multidrug-resistant IncA/C2 plasmids. Curr Microbiol. 2014;68(2):227–32.
20. Tada T, Nhung PH, Miyoshi-Akiyama T, et al. Multidrug-resistant sequence Type 235 pseudomonas aeruginosa clinical isolates producing IMP-26 with increased carbapenem-hydrolyzing activities in vietnam. Antimicrob Agents Chemother. 2016;60(11):6853–8.
21. Partridge SR, Ginn AN, Paulsen IT, Iredell JR. pEI1573 Carrying blaIMP-4, from Sydney, Australia, is closely related to other IncL/M plasmids. Antimicrob Agents Chemother. 2012;56(11):6029–32.

Yang *et al. Ann Clin Microbiol Antimicrob*   (2023) 22:38

Page 8 of 8

22. Kayama S, Shigemoto N, Kuwahara R, et al. Complete nucleotide sequence of the IncN plasmid encoding IMP-6 and CTX-M-2 from emerging carbapenem-resistant Enterobacteriaceae in Japan. Antimicrob Agents Chemother. 2015;59(2):1356–9.

23. Yan JJ, Ko WC, Wu JJ. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-beta-lactamase, IMP-8, from a clinical isolate of Klebsiella pneumoniae. Antimicrob Agents Chemother. 2001;45(8):2368–71.

24. Kansakar P, Dorji D, Chongtrakool P, Mingmongkolchai S, Mokmake B, Dubbs P. Local dissemination of multidrug-resistant Acinetobacter baumannii clones in a thai hospital. Microb Drug Resist. 2011;17(1):109–19.

25. Collis CM, Hall RM. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob Agents Chemother. 1995;39(1):155–62.

26. Nesvera J, Hochmannová J, Pátek M. An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium Corynebacterium glutamicum. FEMS Microbiol Lett. 1998;169(2):391–5.

27. Yano H, Ogawa M, Endo S, et al. High frequency of IMP-6 among clinical isolates of metallo-β-lactamase-producing Escherichia coli in Japan. Antimicrob Agents Chemother. 2012;56(8):4554–5.

28. Carattoli A. Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol. 2011;301(8):654–8.

29. Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. PLoS ONE. 2015;10(5):e0123063.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.