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# Global Molecular Epidemiology of IMP-Producing *Enterobacteriaceae*

**Yasufumi Matsumura,[a,b] Gisele Peirano,[c,d] Mary R. Motyl,[e] Mark D. Adams,[f*] Liang Chen,[g] Barry Kreiswirth,[g] Rebekah DeVinney,[a] Johann D. D. Pitout[a,c,d,h]**

Departments of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada[a]; Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan[b]; Departments of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada[c]; Division of Microbiology, Calgary Laboratory Services, Calgary, Alberta, Canada[d]; Merck & Co., Inc., Rahway, New Jersey, USA[e]; J. Craig Venter Institute, La Jolla, California, USA[f]; Public Research Institute TB Center, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA[g]; Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa[h]

**ABSTRACT** International data on the molecular epidemiology of *Enterobacteriaceae* with IMP carbapenemases are lacking. We performed short-read (Illumina) whole-genome sequencing on a global collection of 38 IMP-producing clinical *Enterobacteriaceae* (2008 to 2014). IMP-producing *Enterobacteriaceae* (7 varieties within 11 class 1 integrons) were mainly present in the South Pacific and Asia. Specific $bla_{IMP}$-containing integrons (In809 with $bla_{IMP-4}$, In722 with $bla_{IMP-6}$, and In687 with $bla_{IMP-14}$) were circulating among different bacteria in countries such as Australia, Japan, and Thailand. In1312 with $bla_{IMP-1}$ was present in *Klebsiella pneumoniae* from Japan and *Citrobacter freundii* from Brazil. *Klebsiella pneumoniae* ($n = 22$) was the most common species; clonal complex 14 (CC14) from Philippines and Japan was the most common clone and contained In1310 with $bla_{IMP-26}$ and In1321 with $bla_{IMP-6}$. The *Enterobacter cloacae* complex ($n = 9$) consisted of *Enterobacter hormaechei* and *E. cloacae* cluster III. CC78 (from Taiwan) containing In73 with $bla_{IMP-8}$ was the most common clone among the *E. cloacae* complex. This study highlights the importance of surveillance programs using the latest molecular techniques for providing insight into the characteristics and global distribution of *Enterobacteriaceae* with $bla_{IMP}$ genes.

**KEYWORDS** *Enterobacteriaceae*, IMP, metallo-β-lactamases, molecular epidemiology

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Address correspondence to Johann D. D. Pitout, johann.pitout@cls.ab.ca.

* Present address: Mark D. Adams, The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA.

B.K., R.D., and J.D.D.P. are co-senior authors.

Carbapenems are often the last line of effective therapy available for the treatment of serious infections due to multidrug-resistant bacteria. The rapid evolution of carbapenem resistance in *Enterobacteriaceae* during the last decade is an emerging global threat (1, 2). Enzymes that hydrolyze the carbapenems, known as carbapenemases, are the most important causes of carbapenem resistance. Carbapenemase-producing *Enterobacteriaceae* (CPE) have acquired multiple resistance genes, making treatment of infections due to these bacteria challenging (1, 2).

The most common carbapenemases among CPE are *Klebsiella pneumoniae* carbapenemases (KPCs) (Amber class A), IMPs, VIMs, NDMs (class B or metallo-β-lactamases [MBLs]), and OXA-48-like (class D) enzymes (1). Metallo-β-lactamases hydrolyze all β-lactams except aztreonam, although resistance levels may vary according to different subtypes. After the initial discovery of IMP-1 in Japan in 1991, bacteria with IMP enzymes have been detected worldwide (1). IMPs are common among MBL-producing *Pseudomonas aeruginosa* but remain relatively rare among members of the *Enterobacteriaceae* (3). IMP-producing *Enterobacteriaceae* are mainly identified in Asia-Pacific (i.e., China, Japan, Taiwan, and Australia) (1, 4). The most common species associated with IMPs among the *Enterobacteriaceae* include *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter* spp. (2, 3). IMP genes are often situated within class 1 integrons harbored

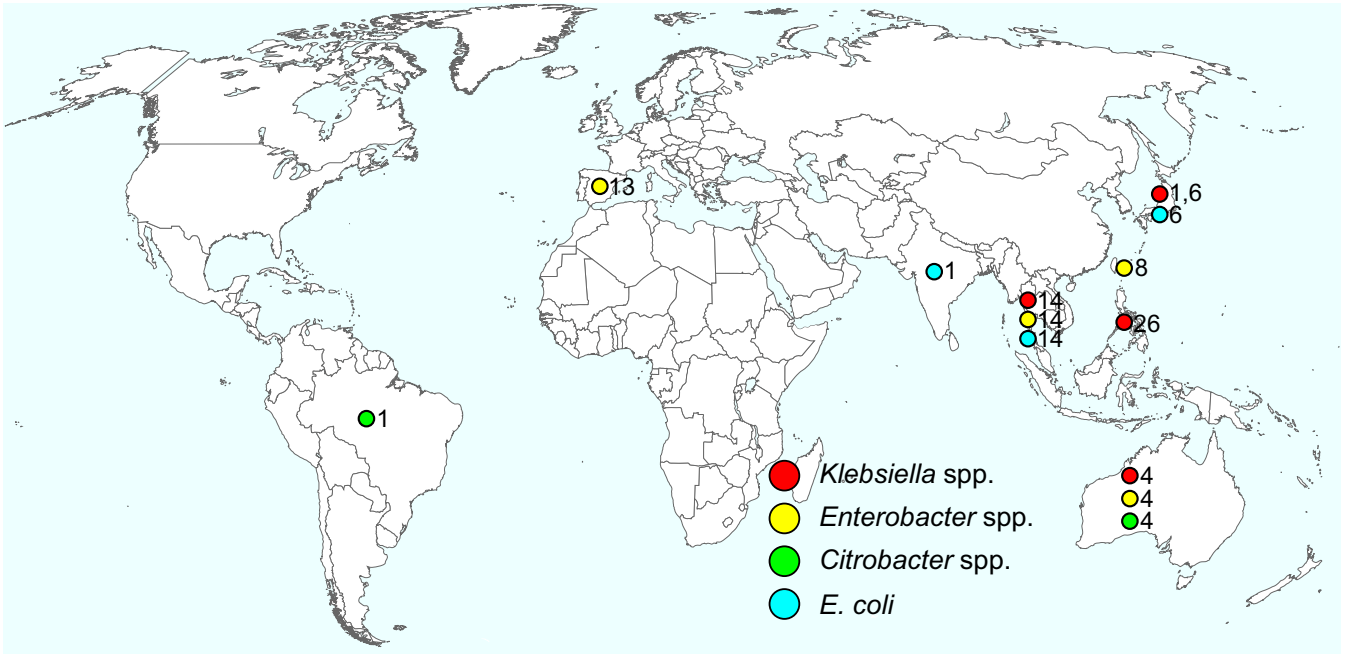


**FIG 1** Global distribution of IMP-producing *Enterobacteriaceae* isolates in this study. Numbers beside circles indicate IMP subtypes.

on broad-host-range plasmids, with the exception of some $bla_{IMP}$-encoding class 2 and 3 integrons (2, 3). These mobile genetic elements play an important role in the interspecies distribution of IMP types of carbapenemases (5).

Comprehensive global data regarding the molecular epidemiology of CPE with $bla_{IMP}$ are currently lacking. We designed a study that utilized short-read whole-genome sequencing to describe the molecular characteristics and international distribution of $bla_{IMP}$ among *Enterobacteriaceae* obtained from the Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance system.

## RESULTS AND DISCUSSION

**Geographic distribution showed IMP-producing *Enterobacteriaceae* mainly in Asia.** The 38 IMP-producing *Enterobacteriaceae* isolates were obtained from eight countries, mainly from the South Pacific ($n = 21$), Asia ($n = 15$), and 1 each from Brazil and Spain (Fig. 1; see also Data Set S1 in the supplemental material). Common sources were intra-abdominal specimens and urine samples ($n = 27$ and 11, respectively). The isolates included the following species: *K. pneumoniae* ($n = 22$), *Enterobacter cloacae* complex ($n = 9$), *Citrobacter* spp. ($n = 4$), and *E. coli* ($n = 3$) (Fig. 1 and Table 1).

**TABLE 1** IMP subtypes and integrons of the *Enterobacteriaceae* isolates

| Carbapenemase (no.) | No. of each species (country) | | | | Defined integron (species, no.)[a] |
|---|---|---|---|---|---|
| | *Klebsiella* spp. | *E. cloacae* complex | *Citrobacter* spp. | *E. coli* | |
| IMP-1 (5) | 3 (Japan) | | 1 (Brazil) | 1 (India) | In1312 (*Klebsiella* spp., 2; *Citrobacter* spp., 1), In1311 (*Klebsiella* spp., 1), In1313 (*E. coli*, 1) |
| IMP-4 (8) | 2 (Australia) | 3 (Australia) | 3 (Australia) | | In809 (*Citrobacter* spp., 3; *E. cloacae* complex, 1) |
| IMP-6 (4) | 3 (Japan) | | | 1 (Japan) | In722 (*Klebsiella* spp., 1; *E. coli*, 1), In1321 (*Klebsiella* spp., 1) |
| IMP-8 (4) | | 4 (Taiwan) | | | In73 (*E. cloacae* complex, 3) |
| IMP-13 (1) | | 1 (Spain) | | | In1319 (*E. cloacae* complex, 1) |
| IMP-14 (3) | 1 (Thailand) | 1 (Thailand) | | 1 (Thailand) | In687 (*Klebsiella* spp., 1; *E. coli*, 1), In1314 (*E. cloacae* complex, 1) |
| IMP-26 (13) | 13 (Philippines) | | | | In1310 (*Klebsiella* spp., 6) |

[a]In1310 to In1314, In1319, and In1321 were novel integrons found in this study.

**TABLE 2** Details of class 1 integrons with $bla_{IMP}$

| Integron no. (major type) | Integron no. (variant) | No. | Gene cassette | Promoter type (no.) | Downstream of gene cassettes (no.) | Accession no. of the integron |
|---|---|---|---|---|---|---|
| In73 | | 3 | $bla_{IMP-8}$-*aacA4-catB4* | PcH1 (1), UD[a] (2) | *qacEΔ1-sul1*-IS*6100*[b] (1), UD (2) | AF322577 |
| In687 | | 2 | $bla_{IMP-14}$-*aacA34* | UD (2) | UD (2) | LC169587 |
| In722 | In722 | 2 | *aacA4*-$bla_{IMP-6}$-*aadA2-tnpA*[c] | PcH1 (2) | *qacEΔ1-sul1-orf5-orf6*-IS*6100* (1), *qacEΔ1-sul1*-Δ*orf5-chrA-padR*-IS*6100* (1) | AB616660 |
| | In1311 | 1 | *aacA4*-$bla_{IMP-1}$-*aadA2-tnpA* | PcH1 (1) | *qacEΔ1-sul1-orf5-orf6*-IS*6100* (1) | LC169566 |
| | In1321 | 1 | *aacA4*-$bla_{IMP-6}$-*aadA2-tnpA*[c] | PcH1 (1) | *qacEΔ1-sul1-orf5-orf6*-IS*6100* (1) | LC169589 |
| In809 | | 4 | $bla_{IMP-4}$-*qacG-aacA4-catB4* | $PcW_{TGN-10}$ (2), $PcH2_{TGN-10}$ (1), UD (1) | *qacEΔ1-sul1*-Δ*orf5*-IS*CR1-sapA-orf2-qnrB2-pspF*-Δ*qacEΔ1-sul1-orf5*-IS*5075-chrA-padR*-IRt[d] (1), *qacEΔ1-sul1*-Δ*orf5*-IS*4321R*-like-*chrA-padR*-IRt (1), UD (2) | JX101693 |
| In1310 | | 6 | $bla_{IMP-26}$-*qacG-aacA4* | UD (6) | *qacEΔ1-sul1*-IS*CR1* (5), *qacEΔ1-sul1-orf5*-Δ*tniB-tniA*-IRt (1) | LC169564, LC169565 |
| In1312 | | 3 | $bla_{IMP-1}$-*aacA31*-$bla_{OXA-142}$ | PcH1 (2), $PcS_{TGN-10}$ (1) | *qacEΔ1-sul1-orf5-orf6*-IS*6100* (3) | LC169567 |
| In1313 | | 1 | $bla_{OXA-10}$, *aacA4*-$bla_{IMP-1}$-*qacG* | $PcW_{TGN-10}$ (1) | *tniR-tniQ-tniB-tniA*-IRt (1) | LC169568 |
| In1314 | | 1 | $bla_{IMP-14}$-$bla_{OXA-10}$, *aacA4* | $PcW_{TGN-10}$ (1) | UD (1) | LC169569 |
| In1319 | | 1 | $bla_{IMP-13}$-*aacA4*-$bla_{OXA-2}$ | PcH1 (1) | *qacEΔ1-sul1-orf5*-Δ*orf6*-IS*6100* (1) | LC169585 |

[a]UD, undetermined due to a contig break in the 5′-conserved segment (CS) or 3′-CS.
[b]Ninety-seven base pairs of the 5′ side of 3′-CS was absent.
[c]In722 and In1321 have a different *aacA4* allele (*aacA4′-3* and *aacA4′-36*, respectively).
[d]IRt, inverted repeat of Tn*402*-like transposon.

The 38 genomes were sequenced at an average depth of 120 (standard deviation [SD], 56.3) (Data Set S1). Assembled genomes had an average number of contigs of 104 (SD, 41.5) and an $N_{50}$ value of 283,649 bp (SD, 107,564 bp). We confirmed the presence of $bla_{IMP}$ in the draft genomes of all of the isolates.

The presence of resistance genes, antibiotic resistance profiles, plasmid replicons, and plasmid addiction systems is shown in Fig. S1 in the supplemental material. Table 1 shows the geographical distribution of the different species, types of carbapenemases, and integrons. We identified 7 $bla_{IMP}$ variants, namely $bla_{IMP-1}$ ($n = 5$), $bla_{IMP-4}$ ($n = 8$), $bla_{IMP-6}$ ($n = 4$), $bla_{IMP-8}$ ($n = 4$), $bla_{IMP-13}$ ($n = 1$), $bla_{IMP-14}$ ($n = 3$), and $bla_{IMP-26}$ ($n = 13$). The following genes were present in more than 1 species: $bla_{IMP-1}$, $bla_{IMP-4}$, $bla_{IMP-6}$, and $bla_{IMP-14}$ (Table 1). *Enterobacteriaceae* with $bla_{IMP-14}$ (i.e., *K. pneumoniae*, *Enterobacter* spp., and *E. coli*) and $bla_{IMP-13}$ (*Enterobacter* spp.) were obtained from urine samples in Thailand and Spain; the remainder of the $bla_{IMP}$ genes were present in both urine samples and intra-abdominal specimens. The distribution of the different $bla_{IMP}$ subtypes was similar to previously published data (i.e., $bla_{IMP-1}$ and $bla_{IMP-6}$ were present in Japan, $bla_{IMP-4}$ in Australia, $bla_{IMP-8}$ in Taiwan, $bla_{IMP-14}$ in Thailand, and $bla_{IMP-26}$ in Philippines (Table 1) (2, 6–8).

**Characterization of class 1 integrons identified 11 different integron types, including 7 novel cassette combinations.** All of the $bla_{IMP}$ genes were situated within class 1 integrons. We were unable to sequence the complete integron-associated gene cassettes in 13 isolates due to the limitations associated with short-read sequencing.

We identified 11 different integron types containing $bla_{IMP}$, including 7 novel cassette combinations (Table 2). The novel cassette combinations included the following: $bla_{IMP-26}$-*qacG-aacA4* (In1310), *aacA4*-$bla_{IMP-1}$-*aadA2-tnpA* (In1311), $bla_{IMP-1}$-*aacA31*-$bla_{OXA-142}$ (In1312), $bla_{OXA-10}$, *aacA4*-$bla_{IMP-1}$-*qacG* (In1313), $bla_{IMP-14}$-$bla_{OXA-10}$, *aacA4* (In1314), $bla_{IMP-13}$-*aacA4*-$bla_{OXA-2}$ (In1319), and *aacA4*-$bla_{IMP-6}$-*aadA2-tnpA* (In1321). In1310 containing $bla_{IMP-26}$-*qacG-aacA4* was the most common cassette. The novel integron In1312 with $bla_{IMP-1}$-*aacA31*-$bla_{OXA-142}$ had international, intercontinental, and intergenus distribution (present in *K. pneumoniae* from Japan and *Citrobacter freundii* from Brazil). Country-specific $bla_{IMP}$ subtypes corresponded to the specific integron types previously characterized in that country, i.e., $bla_{IMP-4}$, In809 from Aus-

tralia (9); $bla_{IMP-6}$, In722 from Japan (10); $bla_{IMP-8}$, In73 from Philippines (11); and $bla_{IMP-14}$, In687 from Thailand (7).

The *aacA* variant (especially *aacA4*) aminoglycoside acetyltransferase genes were the most prevalent gene cassettes in the different integron cassette combinations. They were present in all 11 $bla_{IMP}$-containing integrons. The second most common cassette was the aminoglycoside adenylyltransferase gene (*aad* variant), which was present in 3 $bla_{IMP}$-containing integrons.

The $bla_{OXA-142}$ cassette had previously been identified in class 1 integrons among *P. aeruginosa* from Bulgaria (without $bla_{IMP}$) (12) and from Taiwan (with $bla_{IMP}$) (13). This suggests that appropriate surveillance and control methods may need to extend beyond the *Enterobacteriaceae*.

Integrons with weak promoters (i.e., PcW and PcH1) were common, whereas strong promoters (i.e., PcS and PcH2) were rare (Table 2; see also Table S1 in the supplemental material). There was no correlation between the type of promoter (weak versus strong) and MICs to ertapenem and imipenem. It seems that carbapenem MICs are influenced by various factors, such as type of IMP, porin deficiency, presence of other extended-spectrum β-lactamases (ESBLs), and efflux pumps. We were able to characterize the downstream structures in 9 $bla_{IMP}$-containing integrons (Table 2; see also Table S2 in the supplemental material). All integrons contained 3′-conserved segment (CS) structures immediately downstream of the gene cassettes except for $bla_{IMP-1}$-containing In1313 with a Tn*402*-like *tniRQBA* structure (14) (Table 2). The majority contained In4-like structures consisting of 3′-CS-IS*6100* (with or without partial deletion of 3′-CS and *chrA-padR* insertion).

The integron diversity observed in this study most likely represents the following: (i) the sequential evolution over time of structurally similar cassettes (i.e., $bla_{IMP-1}$, $bla_{IMP-6}$ with only one single nucleotide variant [SNV] difference) situated within homogenous integrons (i.e., variants of In722), and (ii) the multiple acquisition of the same IMP variant within more genetically divergent integron structures (e.g., $bla_{IMP-14}$ situated within In687 and In1314).

***K. pneumoniae* subsp. *pneumoniae* with two dominant clonal complexes.** The phylogenetic relationships in Fig. 1 identified all of the 22 *K. pneumoniae* isolates as *K. pneumoniae* subsp. *pneumoniae*. *K. pneumoniae* isolates consisted of 6 clonal complexes (CCs) and 2 sequence types (STs) (Fig. 2). The most prevalent CCs (with ≥4 isolates) included CC14 ($n = 11$; from Japan and Philippines) and CC37 ($n = 4$; from Japan). In1310 with $bla_{IMP-26}$ from Philippines and In1321 with $bla_{IMP-6}$ from Japan were present in CC14. CC14 is the only clone with international distribution. ST14 and ST37 are global multidrug-resistant clones and have been associated with the production of AmpC β-lactamases, extended-spectrum β-lactamases (ESBLs), and carbapenemases (15, 16).

OmpK35 and OmpK36 deficiencies and variants are responsible for alterations in porins that contribute to increased MICs to carbapenems (15). The majority of the study isolates had OmpK35 deficiency due to premature stop codons and wild-type OmpK36 (Fig. 2).

K1, K2, K5, K20, K54, and K57 capsular types are associated with community-acquired invasive infections due to *K. pneumoniae* (17). CC14 isolates from this study were positive for K2 and present in Japan and Philippines (Fig. 2). Brisse et al. reported that CC14-K2 was not associated with *rmpA* (i.e., regulator of mucoid phenotype) and mouse lethality compared to CC65-K2 (18).

Hypervirulent *K. pneumoniae* strains often possess siderophore clusters (i.e., yersiniabactin, aerobactin, colibactin, and salmochelin) as well as *rmpA* or *rmpA2* (16). Yersiniabactin, which is encoded by a pathogenicity island that includes *ybt*, *irp12*, and *fyuA* genes (16), was present in isolates from this study belonging to CC14 and ST626 (Fig. 2).

***E. cloacae* complex consisted of *Enterobacter hormaechei* subsp. *steigerwaltii* and subsp. *oharae* and *E. cloacae* cluster III.** The *E. cloacae* complex is made up of 13

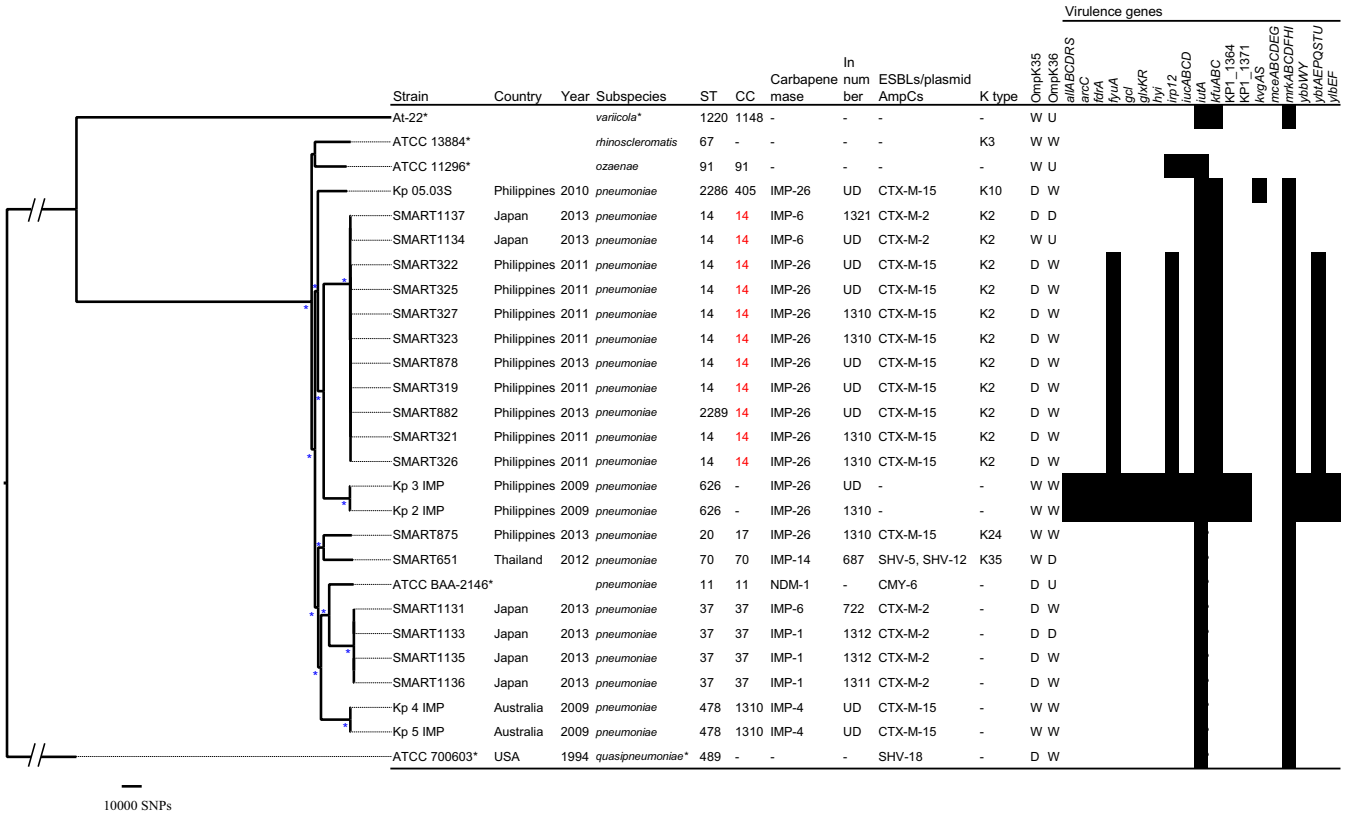


**FIG 2** Phylogenetic tree of IMP-producing *K. pneumoniae*. This maximum-likelihood phylogram is based on a 4,437,167-bp core genome and a total of 417,610 SNPs. The core genome was identified using *K. pneumoniae* subsp. *pneumoniae* ATCC BAA-2146 as a reference genome. The tree included 22 study isolates and 5 reference strains (marked with asterisks). The tree is rooted by using the outgroup of *Klebsiella quasipneumoniae* ATCC 700603, and asterisks indicate bootstrap support of >90% from 100 replicates. In the subspecies column, *Klebsiella variicola* and *K. quasipneumoniae* (marked with asterisks) are not subspecies of *K. pneumoniae* but distinct species. ST2286 and ST2289 were novel types found in this study. A CC marked with red distributed internationally. OmpK35 and OmpK36 columns indicate predicted mutation of porins: W, wild; D, deficient (due to a premature stop codon); V, variant associated with increased MIC of carbapenems; U, variant with unknown significance. Virulence genes of *clbA-R* (collibactin), *iroBCDN* (salmochelin), and *rmpA* and/or *rmpA2* were sought but not found.

groups that are difficult to distinguish using phenotypic methods (19). Recent studies showed that *E. hormaechei* and *E. cloacae* cluster III are the most prevalent clinical species among the *E. cloacae* complex (20, 21). *E. hormaechei* subsp. *steigerwaltii* is the most prevalent subspecies, followed by *E. hormaechei* subsp. *oharae*, while *E. hormaechei* subsp. *hormaechei* is generally rare (20, 21).

The *E. cloacae* complex ($n = 9$) was the second most common species in our study and consisted of *E. hormaechei* subsp. *steigerwaltii*, *E. hormaechei* subsp. *oharae*, and *E. cloacae* cluster III (Fig. 3). *In silico* multilocus sequence type (MLST) analysis identified 7 CCs among the *E. cloacae* complex (Fig. 3). *E. cloacae* cluster III CC78 (with $bla_{IMP-8}$ from Taiwan) was the most common CC among the *E. cloacae* complex. Previous molecular epidemiology studies have shown that CC78 includes global clones associated with $bla_{CTX-M-15}$ or $bla_{VIM-1}$, especially among European countries (22).

***Citrobacter* spp. and *Escherichia coli*.** The *Citrobacter* species isolates ($n = 4$) included in our study belonged to STs 97 to 100 (Fig. 4). Two isolates (SMART316 and SMART314) were classified as *Citrobacter* spp. based on the phylogenetic tree constructed with type strains (Fig. 4) (23). The average nucleotide identity (ANI) value between these 2 isolates and the 5 most closely related *Citrobacter* species (i.e., *C. freundii*, *Citrobacter braakii*, *Citrobacter werkmanii*, *Citrobacter youngae*, and *Citrobacter pasteurii*) was <95% (i.e., the cutoff value of species definition) (see Table S3 in the supplemental material). ANI is a promising method of defining species using whole-genome sequencing replacing DNA-DNA hybridization (24).

The phylogenetic relationship of *E. coli* isolates ($n = 3$) is shown in Fig. S2 in the supplemental material. Two of the *E. coli* isolates belonged to the multidrug-resistant

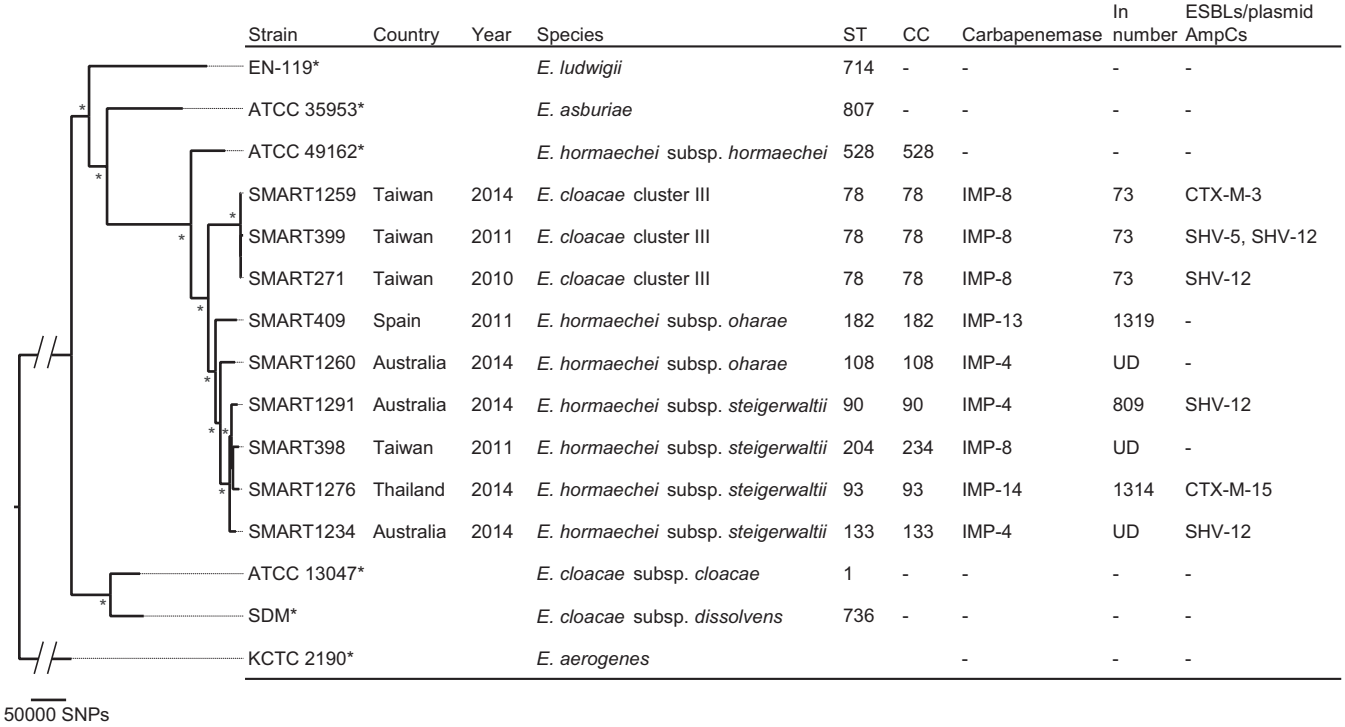


**FIG 3** Phylogenetic tree of IMP-producing *Enterobacter* spp. This maximum-likelihood phylogram is based on a 1,803,217-bp core genome and a total of 478,902 SNPs. The core genome was identified using *E. cloacae* subsp. *cloacae* ATCC 13047 as a reference genome. The tree included 9 study isolates and 6 reference strains (marked with asterisks). The tree is rooted by using the outgroup of *Enterobacter aerogenes* KCTC 2190, and asterisks indicate bootstrap support of >90% from 100 replicates. ST512, ST514, and ST520 were novel types found in this study.

ST131 pandemic clone, which has been associated with fluoroquinolone resistance and ESBLs, including the recent acquisition of carbapenemases (25). The $bla_{IMP-14}$ in one of the ST131 isolates (SMART640) was nested within a 54-kb multidrug resistance region located on an epidemic IncA/C2 plasmid (26).

This study has several limitations. Our collection may not represent the global prevalence of IMP and integron subtypes. We were unable to determine all of the

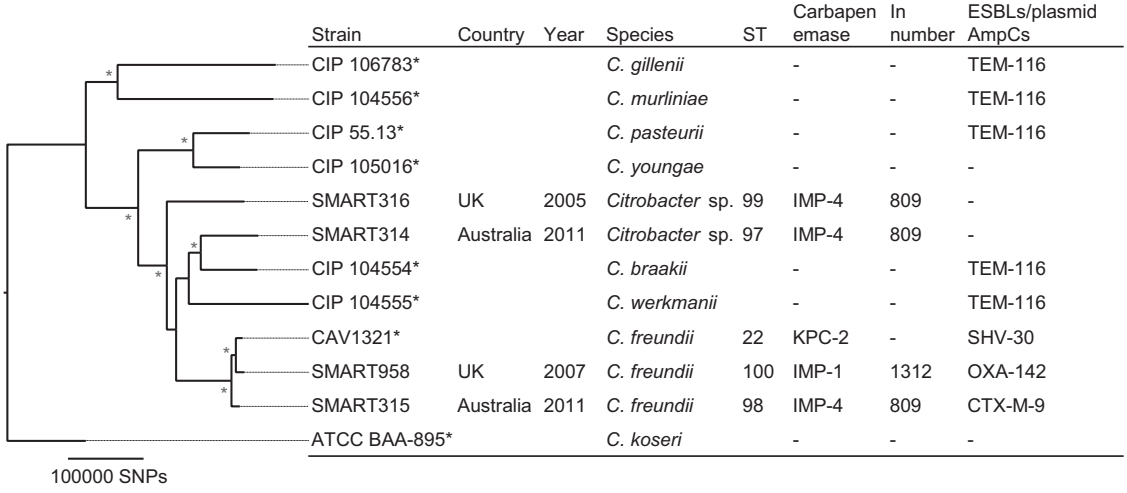


**FIG 4** Phylogenetic tree of IMP-producing *Citrobacter* spp. This maximum-likelihood phylogram is based on a 2,410,533-bp core genome and a total of 609,249 SNPs. The core genome was identified using *C. freundii* CAV1321 as a reference genome. The tree included 4 study isolates and 8 reference strains (marked with asterisks). The tree is rooted by using the outgroup of *Citrobacter koseri* ATCC BAA-895, and asterisks indicate bootstrap support of >90% from 100 replicates. STs 97 to 100 were novel types found in this study.

integron structures due to the limitation of short-read sequencing. Long-read sequencing techniques, including the detailed analysis of plasmids, would provide more knowledge on the location, mobile elements, and plasmid backbones of these carbapenemases.

**Summary.** We used whole-genome sequencing with comprehensive molecular analysis to elucidate the global epidemiology at a large scale of $bla_{IMP}$-containing *Enterobacteriaceae* and showed that some $bla_{IMP}$ subtypes with associated integrons were present in certain countries within multiple species. Examples include the following: (i) In809 was present in *E. hormaechei* subsp. *steigerwaltii*, *C. freundii*, and a *Citrobacter* sp. from Australia; (ii) In722 was present in *K. pneumoniae* and *E. coli* from Japan; (iii) In687 was identified in *K. pneumoniae* and *E. coli* from Thailand; and (iv) In1312 was present in *K. pneumoniae* from Japan and *C. freundii* from Brazil. This study identified certain high-risk global clones with specific IMP integrons (i.e., *K. pneumoniae* ST14 from Philippines contained In1310, *K. pneumoniae* ST37 from Japan contained In722, In1311, and In1312, and *E. cloacae* ST78 from Taiwan contained In73).

This study highlights the importance of surveillance programs using the latest molecular techniques for providing insight into the characteristics and the global distribution of CCs and their association with integrons on containing $bla_{IMP}$ genes. Our results suggest that specific $bla_{IMP}$-containing integrons are circulating locally among different bacteria in countries such as Australia, Japan, and Thailand, while the identification of high-risk clones has the potential to expand the global distribution of CPE. This emphasizes the importance of identifying global types of IMPs among different *Enterobacteriaceae* species and clones.

## MATERIALS AND METHODS

**Bacterial isolates.** We included 38 IMP-producing clinical, nonrepeat *Enterobacteriaceae* collected from a global surveillance program, namely the Merck Study for Monitoring Antimicrobial Resistance Trends (SMART) (2008 to 2014) (see Data Set S1 in the supplemental material). The SMART program included isolates from intra-abdominal and urinary tract infections. The program collected consecutive clinically relevant Gram-negative aerobes at each institution. These isolates initially underwent microdilution panel susceptibility testing and molecular screening for $bla_{IMP}$ as described previously (25). Overall, 107,366 isolates were obtained from 2008 to 2014; of these, 755 were positive for $bla_{KPC}$, 281 for $bla_{OXA-48}$-like, 271 for $bla_{NDM}$, 89 for $bla_{VIM}$, and 38 for $bla_{IMP}$.

**Whole-genome sequencing.** We used the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA) to prepare libraries for sequencing. Samples were multiplexed and sequenced on an Illumina NextSeq 500 for 300 cycles (151 bp paired end).

**Genomic analysis.** Draft genomes were obtained using SPAdes version 3.8.1 (27). Species identification was performed using SILVA 16S rRNA gene database release 123 (28). In addition, we used the *hsp60* gene for identification of *Enterobacter* spp. (19) and a whole-genome-based phylogenetic tree, including type strains for identification of *Klebsiella* spp. and *Citrobacter* spp. Average nucleotide identity (ANI) was calculated using JSpecies (24).

To define the presence of resistance genes other than $\beta$-lactamases, plasmid replicons, and virulence genes of *Klebsiella* spp. and *E. coli* (detailed below), we used raw sequence data and SRST2 (29) (default settings: thresholds for detection of 90% identity and 90% coverage) in combination with the ARG-ANNOT (30) and PlasmidFinder (31) databases. To perform MLST and to define the presence of $\beta$-lactamases and other genes of interest, we used draft genomes and BLAST+ (32) in combination with the following databases or typing schemes: NCBI BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), NCBI Beta-Lactamase Data Resources (http://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/), plasmid addiction systems (33), and MLST (http://bigsdb.pasteur.fr/klebsiella/; http://pubmlst.org/ecloacae/; http://pubmlst.org/cfreundii/; http://mlst.ucc.ie/mlst/dbs/Ecoli/). We used BLAST+ thresholds of 90% nucleotide identity and 90% coverage, except for the detection of gene alleles in which we used 100% identity ($\beta$-lactamases and *ompK35* and *ompK36* at protein level and the others at nucleotide level).

The goeBURST algorithm implemented in PHYLOViZ software (34) was used to demonstrate relationships between STs and to define the founder of a clonal complex (CC). We defined CCs at the single-locus variant level. We used the assembled contigs with $bla_{IMP}$ to determine their genetic environments. Integrons were classified according to INTEGRALL (http://integrall.bio.ua.pt/), and promoters of gene cassettes were characterized according to a previous study (35). For *Klebsiella* isolates, we performed *in silico* detection of the K capsular type based on *wzi* alleles (17), virulence genes (http://bigsdb.pasteur.fr/klebsiella), and promoters and coding sequences of *ompK35* and *ompK36* (36, 37). For *E. coli* isolates, we performed *in silico* phylogenetic grouping (38), virulence genotyping (39), O:H typing (40), *fimH* typing (41), and detection of *H*30Rx-status (42).

**Phylogenetic analysis.** We used a core genome single nucleotide polymorphism (SNP)-based approach to create a phylogenetic tree for each *Enterobacteriaceae* genus. First, we made the reference

genome-like pseudochromosomes that contained only SNPs. For study isolates and 6 reference strains downloaded from the NCBI database (see Data Set S2 in the supplemental material) for which complete genomes were not available, SNPs were identified using trimmed reads mapping to a genus-specific reference genome (Data Set S2) followed by GATK Best Practices workflow (43) and SAMtools (44) (depth of sequencing, >10; Phred score, >20). Complete genomes and draft genomes for which raw reads were not available on the NCBI database (Data Set S2) were aligned against the reference genome of the genus using progressiveMauve to obtain pseudochromosomes that contained only SNPs (45). The SNP-only core genome was identified as the common block of >500 bp to all of the study isolates. A maximum-likelihood tree was built using these core genomes and RAxML (46) and visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

**Accession number(s).** We deposited the sequencing data in the DDBJ and NCBI databases under accession numbers DRA004879 and SRP046977. The sequences of new integrons described in this study ranged from accession numbers LC169564 to LC169589.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02729-16.

**SUPPLEMENTAL FILE 1,** XLSX file, 0.1 MB.

**SUPPLEMENTAL FILE 2,** PDF file, 0.3 MB.

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