IMI-Type Carbapenemase-Producing *Enterobacter cloacae* Complex, France and Overseas Regions, 2012–2022

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We characterized a collection of IMI-like–producing *Enterobacter* spp. isolates (n = 112) in France. The main clone corresponded to IMI-1–producing sequence type 820 *E. cloacae* subspecies *cloacae* that was involved in an outbreak. Clinicians should be aware of potential antimicrobial resistance among these bacteria.

The *Enterobacter cloacae* complex (ECC) is highly diverse; its many species and subspecies can be distinguished by using phenotypic methods or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Whole-genome sequencing enables the precise determination of the bacterial species inside this complex; 22 species, including 6 subspecies, have been assigned to the ECC. IMI and NmcA, which are Ambler class A carbapenemases conferring antimicrobial resistance, are typically associated with the ECC (*1*), but they are rarely reported in other bacterial species (*2*,*3*) despite a worldwide distribution.

A total of 24 NmcA/IMI-type variants have been identified in accordance with the Beta-Lactamase DataBase (http://www.bldb.eu) (*4*). The $bla_{\text{IMI/NmcA}}$ genes can be either chromosome or plasmid encoded; bla_{NmcA}, $bla_{\text{IMI-1}}$, $bla_{\text{IMI-4}}$ and $bla_{\text{IMI-9}}$ have been described as chromosome encoded (*5*–*7*). The chromosome-encoded $bla_{\text{IMI/NmcA}}$ genes are usually described into XerC/XerD recombinase-dependent integrative mobile elements (IMEX) called *Eclo*IMEX elements. For all IMI producers, the genetic features showed an integration of *Eclo*IMEX structures at the same position between *setB* and *yeiP* genes. For chromosomal variant, the bla_{IMI} gene were mostly identified in *E. cloacae* subsp. *cloacae* as *E. bugandensis* or *E. ludwigii* strains (*6*,*8*,*9*). In contrast, the plasmid-encoded genes (such as $bla_{\text{IMI-2}}$ or $bla_{\text{IMI-6}}$) were mostly identified on a IncFII(Yp)-type plasmid in *E. asburiae* isolates (*3*,*6*,*10*). We characterized a large collection of IMI/NmcA producers collected in France.

The Study

We included all nonduplicate IMI-producing and NmcA-producing isolates showing antimicrobial resistance received at the French National Reference Center for Antimicrobial resistance (F-NRC) during 2012–2022 (n = 112) (Appendix 1 Table 1, https://wwwnc.cdc.gov/EID/article/30/6/23-1525-App1.xlsx). Mass spectrometry showed that all strains belonged to the ECC. Since 2014, each year, 3–20 IMI/NmcA producers were identified, representing 0.03%–0.91% of all carbapenemase-producing Enterobacterales analyzed at F-NRC. No IMI/NmcA producers were found before 2014. (Appendix 2 Figure 1, https://wwwnc.cdc.gov/EID/article/30/6/23-1525-App2.pdf).

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Disc diffusion antimicrobial susceptibility testing revealed resistance to third-generation cephalosporins for 1 strain (257D9, overexpression of *ampC* confirmed with CLOXA agar) of the 112 tested. We determined MICs for last-resort antibiotics against highly resistant bacteria on 30 IMI/NmcA producers belonging to several sequence types (STs) (Appendix 1 Table 2). Relebactam restored imipenem activity for 67% of the strains and vaborbactam restored susceptibility to meropenem for all strains with lower MICs than imipenem/relebactam. Then, 37% of the tested strains were susceptible to colistin.

We performed WGS on the 112 IMI-/NmcA producers and identified 74 IMI-1 producers (Appendix 2 Figures 1, 2). Of those, 44 IMI-1-producing ECC were involved in an outbreak in Mayotte and La Réunion islands.

We confirmed ECC species identification using average nucleotide identity (ANI) calculation (Appendix 1 Table 3; Appendix 3, https://wwwnc.cdc.gov/EID/article/30/6/23-1525-App3.xlsx). *E. cloacae* subsp. cloacae was the most prevalent species (n = 56 [50.0%]) (Figure). Multilocus sequence typing (MLST) assigned 42 known unique STs for 105 strains. The 7 remaining isolates belonged to new or undetermined STs. Major STs (≥4 isolates) were ST820 (n = 45), ST250 (n = 5), ST657 (n = 5), ST1516 (n = 4), and ST1517 (n = 4) (Figure). Of note, 44 of the ST820 strains corresponded to the strain isolated in the Mayotte/La Réunion outbreak; the last IMI-1 *E. cloacae* subsp. *cloacae* of ST820, 193I8, was isolated in Paris and was not clonally related to the outbreak strains. That strain exposed >1,200 single-nucleotide polymorphisms (SNPs) corresponding with the other IMI-1 ECC ST820 isolates from Mayotte or La Réunion.

Genes encoding NmcA, IMI-1, IMI-4, IMI-12, and IMI-13 were localized on the chromosome, whereas those coding for IMI-2, IMI-6, IMI-17, IMI-19, IMI-25, IMI-26 and IMI-27 were carried on plasmids. We characterized genetic environments of $bla_{\text{IMI/NmcA}}$ genes using Illumina (https://illumina.com) and MinION long-read (Oxford Nanopore, https://nanoporetech.com) sequencing. All chromosome-encoded $bla_{\text{IMI/NmcA}}$ genes were located into a *Eclo*-IMEX-type genetic element (Appendix 2 Figure 4, panel A), except $bla_{\text{IMI-13}}$, which possessed a distinct genetic environment (Appendix 2 Figure 4, panel B). We detected already-characterized *Eclo*IMEX-type and 6 new variants, named *Eclo*IMEX-11–16 (Appendix 2 Figure 4, panel A). Those *Eclo*IMEX elements were ≈15–≈39.4-kb long, possessed a highly

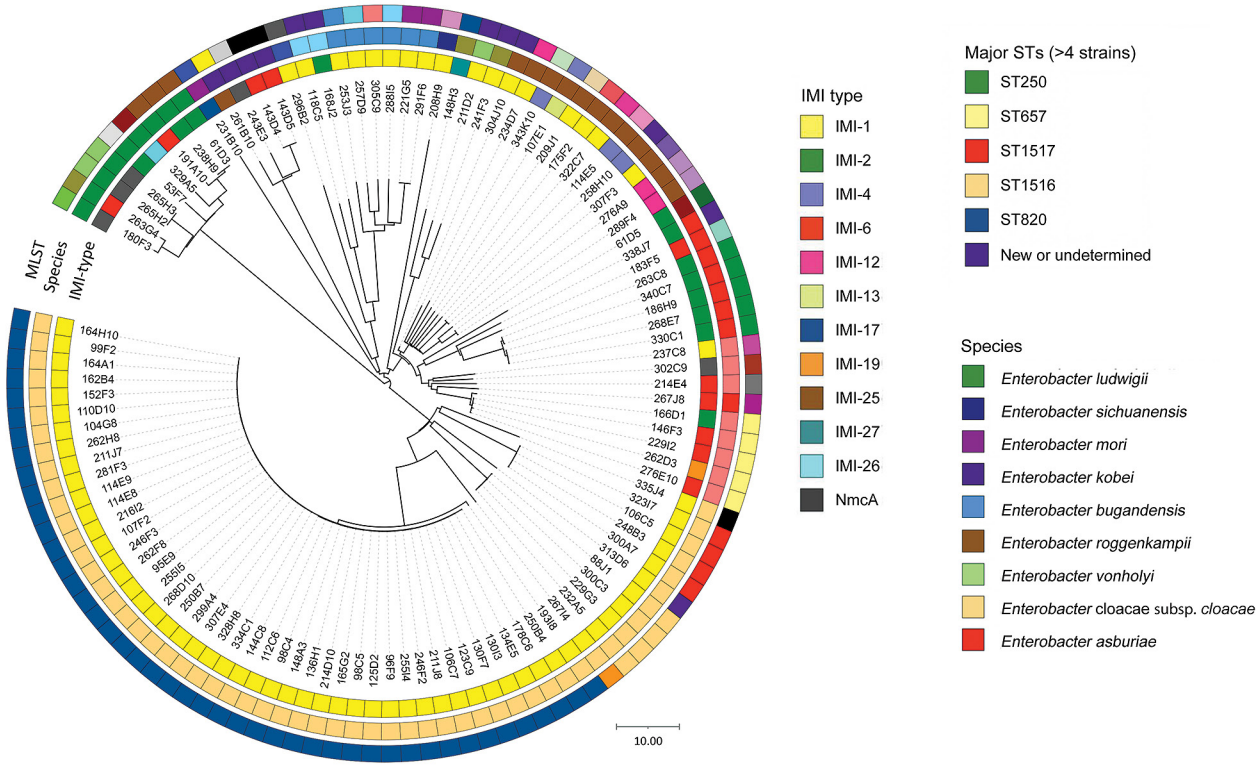

Figure. Phylogenetic relationship and global characterization of 112 IMI-producing *E. cloacae* complex received by the French National Reference Center, France, 2012–2022. The phylogenetic tree was built with a single-nucleotide polymorphism analysis approach from whole-genome sequencing data. MLST, multilocus sequence type; ST, sequence type.

conserved 5′ region, and were inserted between *setB* and *yieP* genes. We observed a strong correlation between bla_{NmcA} and *Eclo*IMEX-1. In contrast, we identified bla_{IMI-1} on 9 different *Eclo*IMEX elements. We saw no correlation between the *Enterobacter* species and the type of *Eclo*IMEX. The bla_{IMI-13} gene was inserted in the chromosome between genes encoding a hypothetical protein and an Inovirus-type Gp2 protein. We identified several complete or partially deleted insertion sequences (IS) close to bla_{IMI-13} (Appendix 2 Figure 4, panel B); however, the mechanism of bla_{IMI-13} acquisition is unclear.

All bla_{IMI-6} genes were carried on a IncFII(Yb)-type plasmid (160–200 kb) (Appendix 1 Table 4). Similarly, bla_{IMI-2} genes were carried on a IncFII(Yp)-type plasmid for 75% (8/12) of the IMI-2 producers. The plasmidic replicase was not identified in the 4 remaining IMI-2 producers. The long-read sequencing performed on strains producing new IMI variants enabled a more precise identification of plasmid type and size (Appendix 1 Table 4). The close genetic environments of the bla_{IMI} genes included several IS that differed according to the bla_{IMI} variants (Appendix 2 Figure 3). Conjugation experiments performed in *E. coli* J53 used as recipient strain confirmed those plasmids were conjugative except the 1 carrying bla_{IMI-17}.

We built an SNP matrix for the 44 IMI-1 *E. cloacae* subsp. *cloacae* ST820 isolates involved in the Mayotte/La Réunion outbreak to confirm their clonality. Those strains were closely related (1–62 SNPs between 2 isolates). We also performed a Bayesian analysis to estimate the date of the most recent ancestor and the evolutionary rate of that population. We estimated the evolutionary rate of the clone to 3.94×10^{-7} substitutions per site and per year (95% highest posterior density [HPD], $2.50–5.33 \times 10^{-7}$), corresponding to 1.63 SNPs per genome per year (95% HPD 1.04–2.21 SNPs). The common ancestor of the 44 IMI-1–producing *E. cloacae* subsp. *cloacae* ST820 isolates has an estimated date of 1994.7 (95% HPD 1990.8–2000.2) (Appendix 2 Figure 5).

Conclusions

Consistent with previous findings (*6*,*9*), our collection of IMI producers included uncommon species of ECC, such as *E. cloacae* subsp. *cloacae*, a rarely described species; IMI-1, IMI-2 and IMI-6 were the most prevalent variants. We identified no isolates of *E. hormaechei*, the most prevalent carbapenemase-producing ECC species (*11*,*12*).

Genetic environments and plasmid types of IMI-2 producers identified in this study were similar to those previously described (*2*,*3*,*13*); IncFII(Yp)-type plasmids were most common. The close genetic environment of bla_{IMI-2} observed in our isolates has been reported on a plasmid identified in *E. coli* (*2*). The genetic environment of bla_{IMI-6} was previously reported in an *E. cloacae* isolate described by Boyd et al. (*6*). Regarding the chromosome-encoded IMI and NmcA variants (n = 85), we described a variety of *Eclo*IMEX elements (n = 11) including 6 novel elements; that the same *Eclo*IMEX could be identified in different ECC species suggests that XerC/D recombinases enable the mobility of these $bla_{IMI-/NmcA}$–carrying *Eclo*IMEX structures specifically between ECC species. Finally, the evolution rate of the IMI-1–producing *E. cloacae* subsp. *cloacae* ST820 clone (1.63 SNPs/genome/year) is similar to the 0.5–3 SNPs/year for a genome reported for a population of multidrug-resistant ECC in the United Kingdom (*14*) and the 2.5–3 SNPs/year for a genome identified for ST171 and ST78 carbapenem-resistant ECC (*15*).

In conclusion, in IMI/NmcA producers in France, we observed a large diversity of ECC species, STs, genetic supports, and genetic environments. Future work should elucidate why *E. cloacae* subsp. *cloacae* is highly prevalent among IMI producers; why $bla_{IMI/NmcA}$-carrying plasmids were almost always found alone in IMI-producing isolates that always do not carry any other resistance genes; and whether *Eclo*IMEX genetic elements are mobilizable. Clinicians should remain aware of potential antimicrobial resistance among ECC species.

About the Author

Dr. Emeraud is assistant professor at the INSERM. Her main field of research interest includes epidemiology, genetics, and biochemistry of β-lactamases in Gram negatives.

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