Putative Integrative Mobile Elements That Exploit the Xer Recombination Machinery Carrying *bla*_{IMI}-Type Carbapenemase Genes in *Enterobacter cloacae* Complex Isolates in Singapore

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ABSTRACT Whole-genome sequencing was performed on 16 isolates of the carbapenemase-producing *Enterobacter cloacae* complex to determine the flanking regions of *bla*_{IMI}-type genes. Phylogenetic analysis of multilocus sequence typing (MLST) targets separated the isolates into 4 clusters. The *bla*_{IMI}-type genes were all found on Xer-dependent integrative mobile elements (IMEX). The IMEX elements of 5 isolates were similar to those described in Canada, while the remainder were novel. Five isolates had IMEX elements lacking a resolvase and recombinase.

KEYWORDS antimicrobial resistance, carbapenemase, mobile genetic elements

Enterobacter cloacae complex (ECC) isolates producing the carbapenemase IMI-1 were isolated in the United States as early as 1984. However, the flanking regions of the *bla*_{IMI-1} gene, and the mechanism of its mobilization into this species, have remained elusive until only recently (1).

First, Antonelli et al. characterized the Xer-dependent integrative mobile elements (IMEX) responsible for inserting the gene for the closely related carbapenemase NMC-A in members of the ECC (2). Then, Boyd et al., studying isolates in Canada, found that similar IMEX were responsible for mobilizing *bla*_{IMI}-type genes into ECC (3).

As a result of an extensive screening program for carbapenemase-producing *Enterobacteriaceae*, 16 *bla*_{IMI}-type-positive ECC were isolated from patients in a large teaching hospital from April 2013 to March 2015. All were isolated from stool or rectal swabs except sample DU20756, which was isolated from urine. MICs to a range of antimicrobials were determined using Etest (bioMérieux, Marcy l'Etoile, France).

DNA was extracted and whole-genome sequencing was performed to determine the flanking regions of the *bla*_{IMI}-type genes. The sequencing libraries were prepared using the NEBNext Ultra DNA library prep kit for Illumina (NEB E7370L; NEB Inc., Singapore) and custom primers with an 8-bp barcode index and sequenced on an Illumina HiSeq 4000 (Illumina Inc., San Diego, CA). *De novo* genome assemblies were created using the Velvet assembler (version 1.2.10) with parameters optimized by Velvet Optimizer (k-mer length ranging from 81 to 127), scaffolded with Opera (version 1.4.1), and finished with FinIS (version 0.3) (4, 5, 6). Genomes were initially annotated with rapid annotation of microbial genomes using subsystems technology (RAST) with default parameters (7). An additional assembly was done using SPAdes (version 3.11.0) using the “-cov-cutoff auto” and “-careful” parameters to validate the junction of two scaffolds for strain DS36577 (8). The Velvet assembly genome sequences (annotated by the GenBank

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FIG 1 Phylogenetic analysis of the *E. cloacae* complex isolates in this study with selected reference strains based on concatenated partial *hsp60* sequences. Scale bars are nucleotide substitutions per site.

Prokaryotic Annotation Pipeline) and raw sequencing reads have been deposited at GenBank under BioProject [PRJNA393769](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA393769).

Fifteen isolates had *bla*_{IMI-1} and one isolate (DS2686) had *bla*_{IMI-4}. The *rpoB* and *hsp60* genes of all sequenced isolates had $\geq 96\%$ nucleotide identity to the respective ECC genes (2). Detection of antimicrobial resistance genes and multilocus sequence typing (MLST) based on the ECC database (www.pubmlst.org) (9) was performed by SRST2 analysis of the raw sequencing reads (10). Cluster analysis using partial *hsp60* sequences (Fig. 1) of the ECC in this study (and selected strains from GenBank) was performed as described by Boyd et al. (3).

Phylogenetic analysis of the MLST profiles divided the isolates into four groups, A, B, C, and D (Fig. 2). The MICs of antimicrobials tested were consistent with the resistance gene profiles.

The *bla*_{IMI}-type genes of isolates in this study were all found within IMEX elements with conserved features similar to those previously described (Fig. 3) (3). The initial

FIG 2 Neighbor-joining tree of isolates with *bla*_{IMI-like} genes based on phylogenetic analysis of MLST profiles showing resistance gene profiles and MICs to a range of antimicrobials. AMK, amikacin; TET, tetracycline; CIP, ciprofloxacin; GEN, gentamicin; SXT, sulfamethoxazole-trimethoprim; CRO, ceftriaxone; IPM, imipenem.

Velvet-based assembly of DS36577 placed the IMEX sequence on two different contigs. We mapped the raw reads back to this assembly and found 60 reads that supported joining these two contigs; furthermore, a SPAdes-based assembly resulted in the IMEX element from DS36577 on a single contig that was consistent with the joining of the two Velvet-based contigs. Finally, SPAdes-based assemblies were concordant with the Velvet-based assemblies for all the other strains. Therefore, diagrams in Fig. 4 are drawn based on the SPAdes-based assembly for consistency. The IMEX sequences of strains DS36577, DS15987, DS2686, and DS18454 from cluster D and strain DS05262 from cluster C were more than 94% similar to that of *EcloIMEX-2* (3). The IMEX elements of the remaining Singapore isolates were more divergent from those found in Canadian and Japanese isolates, with higher levels of sequence variation found between the resolvase and elongation factor P-like protein (*yeiP*) genes.

FIG 3 The *xerC/xerD* recombination sites defining the left (*dif-L*) and right (*dif-R*) chromosomal/IMEX junctions and the internal *dif-Lrec* site, with nucleotides identical to those for *EcloIMEX-1* indicated as dashes.

FIG 4 Comparison of the genomic region of isolates carrying the *bla*_{IMI-like} gene in this study and the corresponding regions of *Enterobacter ludwigii* AOU-8/14 EludIMEX-1 (KR919803) and *E. cloacae* EcloIMEX-2 (KR057494). Homologous regions to EcloIMEX-2 are indicated by gray shading, with the percent identity in the gray box on the right.

Notably, five isolates (all three from cluster A, one from cluster B, and one from cluster C) had IMEX elements that lacked a resolvase and recombinae. Importantly, the entire IMEX element in these strains was contained within a single assembled contig, suggesting that the lack of these genes was not due to breaks in the assembly.

In conclusion, we have shown that bla_{IMI}-type genes in ECC in Singapore are also found within IMEX elements, and we believe this is only the second publication to demonstrate this association. Together with the results of the Canadian study, the chromosomal diversity of the isolates and the heterogeneity of their IMEX elements hint at a potentially large and/or widely distributed environmental source of bla_{IMI}-type genes.

Accession number(s). Raw sequencing reads have been deposited at GenBank under Bioproject [PRJNA393769](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA393769).

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