Genomic Study of *bla*_{IMI}-Positive *Enterobacter cloacae* Complex in Singapore over a Five-Year Study Period

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ABSTRACT The *bla*_{IMI} gene is rarely detected outside the *Enterobacter* genus. Genomic characterization of 87 *bla*_{IMI}-positive *Enterobacter cloacae* complex members revealed that the largest phylogenomic clade was made up of *E. cloacae* subsp. *cloacae* (71.3%), followed by the newly described species *E. bugandensis* (13.8%), *E. sichuanensis* (10.3%), and *E. roggenkampii* (4.6%). IMI-1 was the predominant carbapenemase variant (86/87, 98.9%). All the *bla*_{IMI} genes were associated with chromosomally integrated Xer-dependent integrative mobile elements (IMEXs), with two new variants detected.

KEYWORDS *Enterobacter*, *Enterobacteriaceae*, antimicrobial resistance, carbapenemase, imipenemase, phylogenomics, whole genome

The imipenemases (IMI) are a relatively uncommon group of Ambler class A carbapenemases (1). Although typically associated with the *Enterobacter cloacae* complex (ECC), IMI-type genes have on rare occasion been found outside the *Enterobacter* spp. For example, plasmid-mediated IMI-2 has been found in *Escherichia coli* (2) and in *Klebsiella variicola* (3). *bla*_{IMI} can be both chromosomally and plasmid encoded. IMI-1, IMI-4 (4), IMI-7 (4), and IMI-9 (5) variants are chromosomally located, whereas IMI-2 (6), IMI-3 (7), IMI-5, and IMI-6 (8) are plasmid encoded.

Xer recombinase-dependent integrative mobile elements (IMEXs) are presumably responsible for the mobility of *bla*_{IMI-type} in the *Enterobacter* (8). It is hypothesized that the EcolIMEX elements exit the chromosome through recombination mediated by the XerD/C recombinases (9). This form of site-specific and Xer recombination-dependent mechanism of mobility appears to limit the diffusion of this group of carbapenemases, in comparison to other types of more “freely” mobile genetic elements such as transposons and genetic islands. However, some *bla*_{IMI} types may be acquired by IncFII plasmids and associated with insertion sequences (2, 3).

A local study had previously investigated the genomic features of 16 *bla*_{IMI}-positive *Enterobacter* isolates obtained from a single hospital (4). Here, we undertook the effort to describe the population structure, antimicrobial resistance genes, and genetic context of *bla*_{IMI}-positive isolates from a larger collection ($n = 87$) which had been collected via a national surveillance program for non-carbapenem-susceptible *Enterobacteriales*. The genomic data from the initial 16 isolates were also incorporated into

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this study. A total of 87 nonduplicate *bla*_{IMI}-positive isolates were collected over 2011 to 2015. These isolates came from a retrospective collection of phenotypically carbapenem-resistant *Enterobacteriaceae* deposited at the reference National Public Health Laboratory, Singapore (see the Material and Methods in the supplemental materials). PCR screening was used to determine the carbapenemase genotype. Of the 87 *bla*_{IMI}-positive isolates, 5 were clinical isolates and 82 were from screening rectal swabs (see the Material and Methods in the supplemental materials). The *bla*_{IMI}-positive isolates made up 3.1% (87/2815) of all carbapenemase genotypes screened at the reference laboratory for the duration of the study period. Illumina MiSeq sequencing (Illumina Inc., San Diego, CA, USA) was used to generate 300-bp paired-end reads which were then assembled. An average sequencing depth of 60× was achieved for the genomes. Raw reads for all isolates have been deposited in NCBI under the BioProject ID [PRJNA632459](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA632459). Detailed microbiological methods and genomics analysis are presented in the supplemental materials.

ECC is polyphyletic, with current literature indicating that *Enterobacter* spp. genomic structure is diverse (10). For example, in a comparative genomics study investigating 97 clinical carbapenemase-producing *Enterobacter* spp. (none encoding *bla*_{IMI}), 18 phylogenomic groups were deduced (11). A similarly varied population structure was reflected in Boyd's study (8), where 9 *bla*_{IMI}-bearing genomes generated four different phylogenomic groups. In contrast, our population structure for *Enterobacter* bearing *bla*_{IMI} appears to be rather restricted, with 87 genomes classified into only 4 clades (Fig. 1). Our largest clade was made up of *E. cloacae* subsp. *cloacae* (62/87, 71.3%), followed by *E. bugandensis* (12/87, 13.8%), *E. sichuanensis* (9/87, 10.3%), and *E. roggenkampii* (4/87, 4.6%) (Fig. 1). The average nucleotide identity (ANI) values for within-clade isolates was >98%, and <91% ANI compared to other nonclade genomes, hence lending support for the phylogenetic clustering.

The ECC bacteria are diverse and associated with a wide range of serious nosocomial infections, such as pneumonia, urinary tract infections, and septicemia, and the emergence of carbapenem resistance in the ECC severely limits the treatment options. *E. bugandensis* is a recently described species associated with neonatal sepsis (12). It is considered to be an emerging pathogen with a multidrug resistant (MDR) phenotype (13). Contemporary genomic investigations have reclassified *E. roggenkampii* as a distinct novel species rather than a subspecies of *E. hormaechei* (14). *E. sichuanensis* is a newly described species first recovered from a urine specimen in Sichuan, China (15). Carbapenemase carriage has been reported in *E. bugandensis* (*bla*_{NDM-5} and *bla*_{IMI-1}) (16, 17) and *E. roggenkampii* (*bla*_{NDM-1}) (18), as well as in *E. sichuanensis* (*bla*_{KPC}) (11). Interestingly, our *E. sichuanensis* isolates clustered with a locally collected environmental air sample, *E. sichuanensis* SGAir0282 (Fig. 1), which was a noncarbapenemase producer. Single-nucleotide polymorphism (SNP) analysis of IMI-bearing *E. sichuanensis* revealed nucleotide differences of more than 40,000 compared to *E. sichuanensis* SGAir0282, suggesting that the study isolates were not clonally related to the environmental sample.

IMI-1 was the predominant carbapenemase variant (86/87, 98.9%) with only one other isolate carrying IMI-4. No other carbapenemases or plasmid-mediated *mcr* colistin resistance were found. Also detected was *bla*_{CMH-3-like}, a recently described AmpC type β-lactamase of the *E. cloacae* complex that is closely related to *bla*_{MIR} and *bla*_{ACT} (19). The presence of chromosomally mediated AmpC β-lactamase was detected in all the genomes. Acquired aminoglycoside (*aadA2*, *aph*(3')-Ia, *strA*, and *strB*) and quinolone (*qnrS*) resistance determinants were also sporadically detected (Fig. 1). The quinolone-resistance determining regions (QRDR) of *gyrA*, *gyrB*, and *parC* did not reveal the presence of substitutions commonly associated with quinolone resistance, such as Ser83 in *GyrA*, Ser463 in *GyrB*, and Ser80 in *ParC* (20) (Table S2 in the supplemental material).

Epidemic high-risk carbapenem-resistant *Enterobacter* lineages belonging to clonal complexes (CC) CC74 (including sequence type [ST] 78), CC114 (including ST66), and CC171 (10, 11) were not observed in our study. Overall, the sequence types for *E.*

FIG 1 Core SNP phylogenetic tree of 87 bla_{IMI}-type *Enterobacter* spp. The metadata includes specimen source, sequence types (ST), and the presence of resistance genes. Genomes with black branch labels belong to *E. cloacae*, orange branch labels to *E. roggenkampii*, green branch labels represent *E. sichuanensis*, and red branch labels belong to *E. bugandensis*. The presence of resistance determinants is represented by blue squares. The bootstrap values are indicated on the nodes. NCBI genome accession numbers are provided in brackets after the species name. The tree was illustrated using iTOL v4 (<https://itol.embl.de/>). Scale bars represent nucleotide substitutions per site. The symbol * indicates the sequence type was assigned based on the next closest sequence type.

FIG 2 Comparison of *bla*_{nmcA} (EcolIMEX-1) and *bla*_{IMI}-harboring genetic elements from reference types (EcolIMEX-2 to -8) and sequenced isolates ($n = 87$). Short vertical pink bars represent XerC/XerD recombination sites. The red arrows represent *bla*_{IMI} and its repressor. White arrows represent genes that encode metabolic functions. Hypothetical proteins are indicated by light grey arrows. Easyfig (<https://mjsull.github.io/Easyfig/>) was used to produce the linear BLAST comparisons for the *bla*_{IMI} genetic structures.

cloacae subsp. *cloacae* were diverse, with 24 different STs (Fig. 1). The majority (22/25, 88%) of the non-*E. cloacae* subsp. *cloacae*, i.e., *E. bugandensis*, *E. sichuanensis*, and *E. roggenskampii* had previously unassigned STs (Fig. 1) (Table S2), which was not unexpected as these were newly described species. We noted a cluster of 11 clonal *E. bugandensis* sequence types possessing the same MLST, with <18 single nucleotide polymorphisms (SNPs). By comparison, ~52,000 SNPs were observed between this cluster and the *E. bugandensis* reference genomes (Fig. 1). The 11 isolates originated from different patients, which hinted at the possibility of hospital spread. However, based on deeper epidemiological investigation, we were not able to attribute association at ward, procedure, or department level.

The initial description of an *Enterobacter* Xer-dependent integrative mobile element, EludIMEX-1, was from *E. ludwigii* (9), which harbored NMC (a close variant of IMI). The EludIMEX-1 was later redesignated EcolIMEX-1 as more IMEX elements from *E. cloacae* complex were characterized (8) (Fig. 2). Currently there are eight different EcolIMEX elements with relatively heterogeneous organization between them (4) (Fig. 2). Diverse EcolIMEX variants types were detected in our study, namely, EcolIMEX-2, -3, -7, and -8, as well as two new variants which we designated EcolIMEX-9 and -10 (Fig. 2). The distribution of EcolIMEX variants showed that EcolIMEX-8 was the most predominant type (32/87, 36.8%) (Fig. 1). There appeared to be no association between the *Enterobacter* species and the type of EcolIMEX variant (Fig. 1).

In all the isolates, the EcloIMEX was chromosomally integrated at the same position, i.e., the intergenic region between the sugar efflux transporter B, *setB*, and an elongation factor P-like protein, *yeiP* (Fig. 2). This feature was also observed in previous work (4, 8). The sequences flanking bla_{IMI-1}, spanning *setB* to the recombinase gene, are typically homologous/conserved regions of ~18 kb exhibiting 90% to 99% sequence identity (8) (Fig. 2). Genes in this region include a protease, ABC transporter, methyl viologen resistance protein SmvA (major facilitator superfamily efflux pump), and a glycosyltransferase. The same gene conservation was also observed in the new variants EcloIMEX-9 and -10 (Fig. 2). The exceptions are EcloIMEX-7 (4) and EcloIMEX-8 (21), which have lost the recombinase gene.

The structure of EcloIMEX-8 (GenBank accession no. [MG547711](#)) is very close to that of EcloIMEX-7 (GenBank accession no. [KY680208.1](#)), with 99% nucleotide sequence homology. *E. cloacae* complex isolates bearing bla_{IMI} in EcloIMEX-8 elements were responsible for a clonal outbreak in the French overseas department of Mayotte (21). It was interesting to note that EcloIMEX-7 was the first instance of an EcloIMEX isolated from a nonclinical *E. cloacae* complex obtained from farmed shrimp (22). Unfortunately, the occurrence of carbapenemase-producing microorganisms in food-producing animals is not unusual (23).

In this study, we did not detect epidemic high-risk carbapenem-resistant *Enterobacter* lineages. Instead, diverse STs of IMI-producing ECC were observed in our collection of isolates. Additionally, non-*E. cloacae* subsp. *cloacae* have been isolated from screening rectal swabs with a possible hospital cluster due to *E. bugandensis*, suggesting the widespread dissemination of IMI across ECC. IMI-1 remained the most predominant variant. The bla_{IMI} genetic regions were not plasmid borne but were carried on different *Enterobacter* Xer-dependent integrative mobile elements, including the two newly designated variants. Our findings provide further insights into the diversity of IMI-producing ECC in nosocomial settings.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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We have no conflicts of interest to declare.

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