AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EPIDEMIOLOGY AND SURVEILLANCE

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

Sophie Octavia,[a,c] Tse Hsien Koh,[b] Oon Tek Ng,[c,d,e] Kalisvar Marimuthu,[c,e,f] Indumathi Venkatachalam,[g,i] Raymond T. P. Lin,[a,c,h] Jeanette W. P. Teo[h]

[a]National Public Health Laboratory, National Center for Infectious Diseases, Singapore
[b]Singapore General Hospital, Department of Microbiology, Singapore
[c]National Centre for Infectious Diseases, Singapore
[d]Tan Tock Seng Hospital, Department of Infectious Diseases, Singapore
[e]Nanyang Technological University, Lee Kong Chian School of Medicine, Singapore
[f]National University of Singapore, Yong Loo Lin School of Medicine, Singapore
[g]Singapore General Hospital, Department of Infectious Diseases, Singapore
[h]National University Hospital, Department of Laboratory Medicine, Singapore
[i]Singapore General Hospital, Department of Infection Prevention & Epidemiology, Singapore

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 $bla_{IMI\text{-}type}$ in the *Enterobacter* (8). It is hypothesized that the EcloIMEX 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

Citation Octavia S, Koh TH, Ng OT, Marimuthu K, Venkatachalam I, Lin RTP, Teo JWP. 2020. Genomic study of bla_{IMI}-positive *Enterobacter cloacae* complex in Singapore over a five-year study period. Antimicrob Agents Chemother 64:e00510-20. https://doi.org/10.1128/AAC.00510-20.

Address correspondence to Jeanette W. P. Teo, Jeanette_Teo@nuhs.edu.sg.

Received 17 March 2020
Returned for modification 21 April 2020
Accepted 23 May 2020
Accepted manuscript posted online 1 June 2020
Published 22 July 2020

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. 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.*

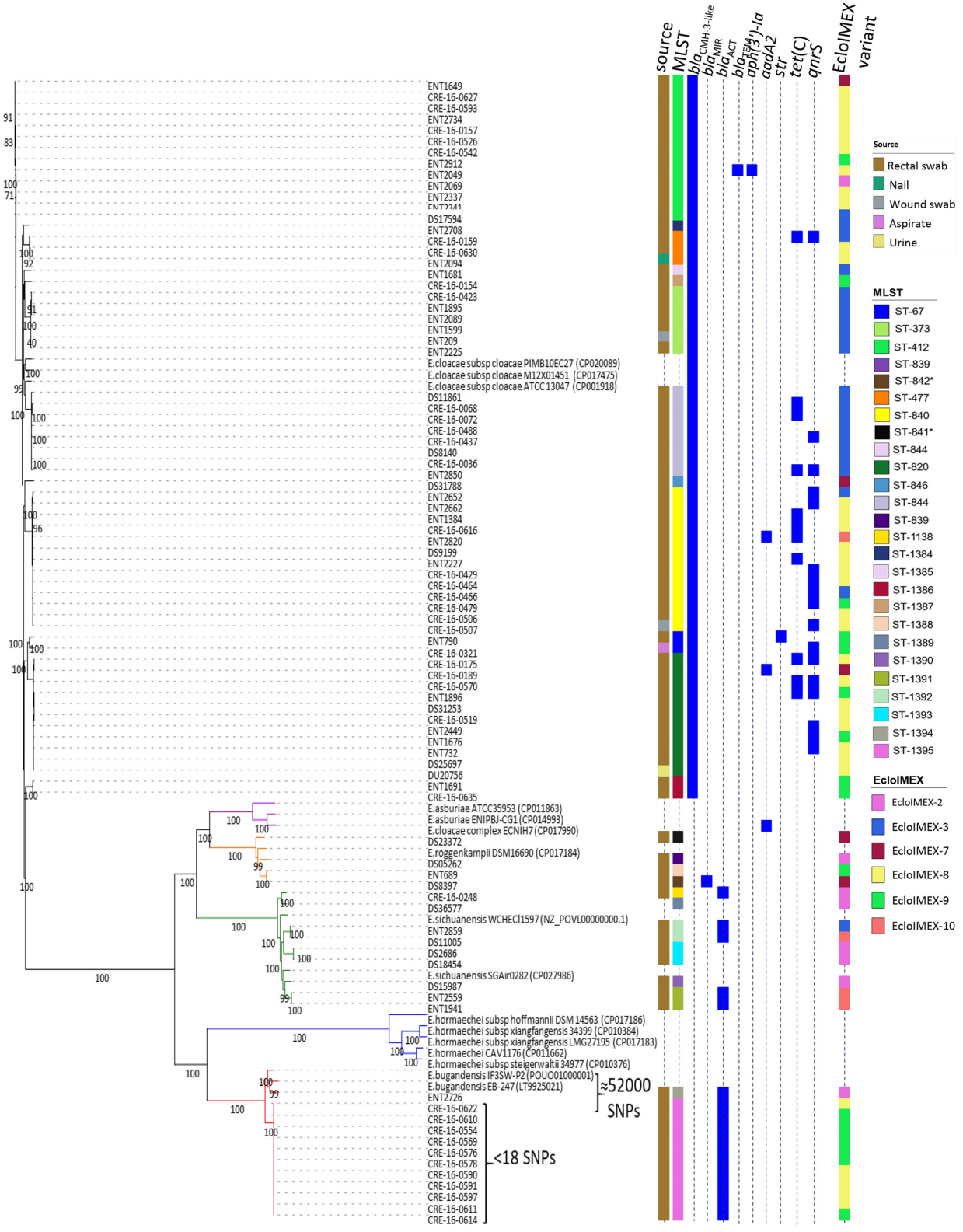

FIG 1 Core SNP phylogenetic tree of 87 bla$_{IMI\text{-}type}$ *Enterobacter* spp. The metadata includes specimen source, sequence types (ST), and the presence of resistance genes. Genomes with black branch labels belong to *E. cloacae*, orange branch labels to *E. roggenkampii*, green branch labels 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.

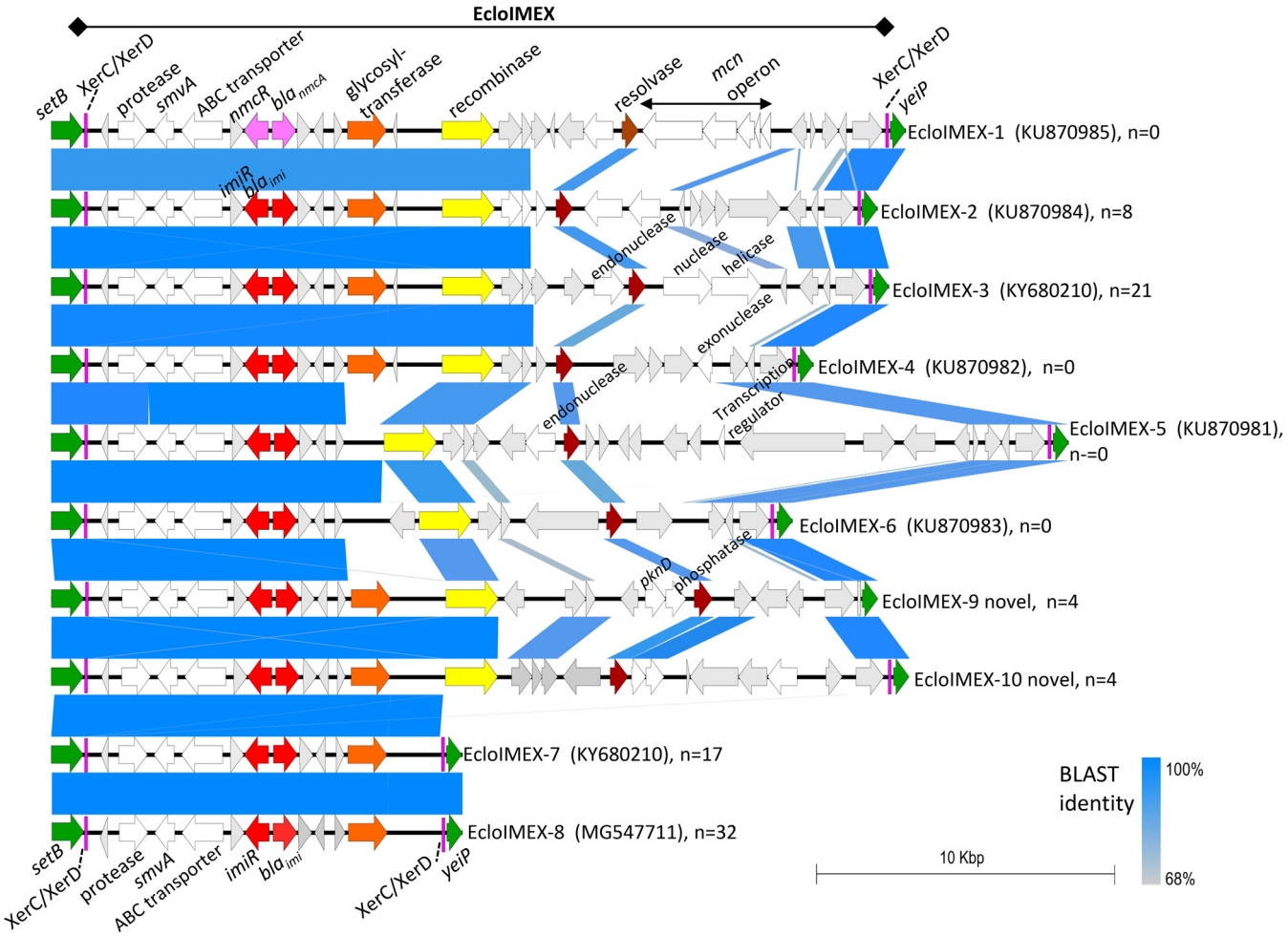

FIG 2 Comparison of bla_{nmcA} (EcloIMEX-1) and bla_{IMI}-harboring genetic elements from reference types (EcloIMEX-2 to -8) and sequenced isolates ($n = 87$). Short vertical pink bars represent XerC/XerD recombination sites. The red arrows represent bla_{IMI} and its repressor. White arrows represent 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. roggenkampii* 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 EcloIMEX-1 as more IMEX elements from *E. cloacae* complex were characterized (8) (Fig. 2). Currently there are eight different EcoIMEX elements with relatively heterogenous organization between them (4) (Fig. 2). Diverse EcloIMEX variants types were detected in our study, namely, EcloIMEX-2, -3, -7, and -8, as well as two new variants which we designated EcloIMEX-9 and -10 (Fig. 2). The distribution of EcloIMEX variants showed that EcloIMEX-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 EcloIMEX 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.

ACKNOWLEDGMENTS

Grant support was provided by the NMRC Clinician-Scientist Individual Research Grant (NMRC/CIRG/1463/2016), Singapore Ministry of Education Academic Research Fund Tier 2 grant: New Delhi Metallo-Beta-Lactamase: A global multicenter, whole-genome study (MOE2015-T2-2-096), NMRC Collaborative Grant: Collaborative Solutions Targeting Antimicrobial Resistance Threats in Health Systems (CoSTAR-HS) (NMRC CGAug16C005), and NMRC Clinician Scientist Award (NMRC/CSA-INV/0002/2016). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We have no conflicts of interest to declare.

REFERENCES

1. Walther-Rasmussen J, Høiby N. 2007. Class A carbapenemases. J Antimicrob Chemother 60:470–482. https://doi.org/10.1093/jac/dkm226.
2. Rojo-Bezares B, Martín C, López M, Torres C, Sáenz Y. 2012. First detection of blaIMI-2 gene in a clinical Escherichia coli strain. Antimicrob Agents Chemother 56:1146–1147. https://doi.org/10.1128/AAC.05478-11.
3. Hopkins KL, Findlay J, Doumith M, Mather B, Meunier D, D'Arcy S, Pike R, Mustafa N, Howe R, Wootton M, Woodford N. 2017. IMI-2 carbapenemase in a clinical Klebsiella variicola isolated in the UK. J Antimicrob Chemother 72:2129–2131. https://doi.org/10.1093/jac/dkx103.
4. Koh TH, Rahman NBA, Teo JWP, La M-V, Periaswamy B, Chen SL. 2017. Putative integrative mobile elements that exploit the xer recombination machinery carrying bla_{IMI}-type carbapenemase genes in Enterobacter cloacae complex isolates in Singapore. Antimicrob Agents Chemother 62:e01542-17. https://doi.org/10.1128/AAC.01542-17.
5. Di Luca MC, Skaare D, Aasnaes B, Sundsfjord A, Samuelsen O. 2016. Identification of a novel IMI carbapenemase variant (IMI-9) in Enterobacter cloacae complex. Int J Antimicrob Agents 48:764–765. https://doi.org/10.1016/j.ijantimicag.2016.09.004.
6. Aubron C, Poirel L, Ash RJ, Nordmann P. 2005. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. Emerg Infect Dis 11:260–264. https://doi.org/10.3201/eid1102.030684.
7. Chu Y-W, Tung VWN, Cheung TKM, Chu M-Y, Cheng N, Lai C, Tsang DNC, Lo J. 2011. Carbapenemases in enterobacteria, Hong Kong, China, 2009. Emerg Infect Dis 17:130–132. https://doi.org/10.3201/eid1701.101443.
8. Boyd DA, Mataseje LF, Davidson R, Delport JA, Fuller J, Hoang L, Lefebvre B, Levett PN, Roscoe DL, Willey BM, Mulvey MR. 2017. Enterobacter cloacae complex isolates harboring bla_{NMC-A} or bla_{IMI}-type class A carbapenemase genes on novel chromosomal integrative elements and plasmids. Antimicrob Agents Chemother 61:e02578-16. https://doi.org/10.1128/AAC.02578-16.

9. Antonelli A, D'Andrea MM, Di Pilato V, Viaggi B, Torricelli F, Rossolini GM. 2015. Characterization of a novel putative Xer-dependent integrative mobile element carrying the bla(NMC-A) carbapenemase gene, inserted into the chromosome of members of the Enterobacter cloacae complex. Antimicrob Agents Chemother 59:6620–6624. https://doi.org/10.1128/AAC.01452-15.
10. Annavajhala MK, Gomez-Simmonds A, Uhlemann A-C. 2019. Multidrug-resistant Enterobacter cloacae complex emerging as a global, diversifying threat. Front Microbiol 10:44. https://doi.org/10.3389/fmicb.2019.00044.
11. Chavda KD, Chen L, Fouts DE, Sutton G, Brinkac L, Jenkins SG, Bonomo RA, Adams MD, Kreiswirth BN. 2016. Comprehensive genome analysis of carbapenemase-producing Enterobacter spp.: new insights into phylogeny, population structure, and resistance mechanisms. mBio 7:e0209316. https://doi.org/10.1128/mBio.02093-16.
12. Pati NB, Doijad SP, Schultze T, Mannala GK, Yao Y, Jaiswal S, Ryan D, Suar M, Gwozdzinski K, Bunk B, Mraheil MA, Marahiel MA, Hegemann JD, Spröer C, Goesmann A, Falgenhauer L, Hain T, Imirzalioglu C, Mshana SE, Overmann J, Chakraborty T. 2018. Enterobacter bugandensis: a novel enterobacterial species associated with severe clinical infection. Sci Rep 8:5392. https://doi.org/10.1038/s41598-018-23069-z.
13. Singh NK, Bezdan D, Checinska Sielaff A, Wheeler K, Mason CE, Venkateswaran K. 2018. Multidrug resistant Enterobacter bugandensis species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains. BMC Microbiol 18:175. https://doi.org/10.1186/s12866-018-1325-2.
14. Sutton GG, Brinkac LM, Clarke TH, Fouts DE. 2018. Enterobacter hormaechei subsp. hoffmannii subsp. nov., Enterobacter hormaechei subsp. xiangfangensis comb. nov., Enterobacter roggenkampii sp. nov., and Enterobacter muelleri is a later heterotypic synonym of Enterobacter asburiae based on computational analysis of sequenced *Enterobacter* genomes. F1000Res 7:521. https://doi.org/10.12688/f1000research.14566.2.
15. Wu W, Feng Y, Zong Z. 2018. Enterobacter sichuanensis sp. nov., recovered from human urine. Int J Syst Evol Microbiol 68:3922–3927. https://doi.org/10.1099/ijsem.0.003089.
16. Norgan AP, Freese JM, Tuin PM, Cunningham SA, Jeraldo PR, Patel R. 2016. Carbapenem- and colistin-resistant Enterobacter cloacae from Delta, Colorado, in 2015. Antimicrob Agents Chemother 60:3141–3144. https://doi.org/10.1128/AAC.03055-15.
17. Matteoli FP, Passarelli-Araujo H, Pedrosa-Silva F, Olivares FL, Venancio TM. 2019. Population structure and pangenome analysis of Enterobacter bugandensis uncover the presence of bla(CTX-M-55), bla(NDM-5) and bla(IMI-1), along with sophisticated iron acquisition strategies. Genomics 112:1182–1191.
18. Teixeira P, Tacão M, Pureza L, Gonçalves J, Silva A, Cruz-Schneider MP, Henriques I. 2020. Occurrence of carbapenemase-producing Enterobacteriaceae in a Portuguese river: bla(NDM), bla(KPC) and bla(GES) among the detected genes. Environ Pollut 260:113913. https://doi.org/10.1016/j.envpol.2020.113913.
19. López-Hernández I, García Barrionuevo A, Díaz de Alba P, Clavijo E, Pascual A. 2019. Characterization of NDM-1- and CMH-3-producing Enterobacter cloacae complex ST932 in a patient transferred from Ukraine to Spain. Enferm Infecc Microbiol Clin S0213-005X:30297-6. https://doi.org/10.1016/j.eimc.2019.10.007.
20. Weigel LM, Steward CD, Tenover FC. 1998. gyrA mutations associated with fluoroquinolone resistance in eight species of Enterobacteriaceae. Antimicrob Agents Chemother 42:2661–2667. https://doi.org/10.1128/AAC.42.10.2661.
21. Miltgen G, Bonnin RA, Avril C, Benoit-Cattin T, Martak D, Leclaire A, Traversier N, Roquebert B, Jaffar-Bandjee M-C, Lugagne N, Filleul L, Subiros M, de Montera A-M, Cholley P, Thouverez M, Dortet L, Bertrand X, Naas T, Hocquet D, Belmonte O. 2018. Outbreak of IMI-1 carbapenemase-producing colistin-resistant Enterobacter cloacae on the French island of Mayotte (Indian Ocean). Int J Antimicrob Agents 52:416–420. https://doi.org/10.1016/j.ijantimicag.2018.05.015.
22. Brouwer MSM, Rapallini M, Geurts Y, Harders F, Bossers A, Mevius DJ, Wit B, Veldman KT. 2018. Enterobacter cloacae complex isolated from shrimps from Vietnam carrying bla(IMI-1) resistant to carbapenems but not cephalosporins. Antimicrob Agents Chemother 62:e00398-18. https://doi.org/10.1128/AAC.00398-18.
23. Köck R, Daniels-Haardt I, Becker K, Mellmann A, Friedrich AW, Mevius D, Schwarz S, Jurke A. 2018. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. Clin Microbiol Infect 24:1241–1250. https://doi.org/10.1016/j.cmi.2018.04.004.