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Detection of an In*104*-like integron carrying a bla_{IMP-34} gene in *Enterobacter cloacae* isolates co-producing IMP-34 and VIM-1

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Sir,

The complex class-1 integron In*104* is a 13 kb multi-resistance region (MRR). This integron belongs to the In4 group and is often found in a 43 kb genomic island named *Salmonella* genomic island 1 (SGI1), which confers resistance to ampicillin/amoxicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and tetracyclines (ACSSuT phenotype) in *Salmonella enterica* serovar Typhimurium.[1] While In*104* has disseminated widely among *Salmonella* spp., its distribution in other pathogens remains unclear. The aim of this study was to gain insight into the structure of an In*104*-like integron harbouring a carbapenemase gene detected in two closely related *Enterobacter cloacae* isolates.

Two carbapenemase-producing *E. cloacae* isolates (Ecl20710 and Ecl20712) were obtained during a national surveillance study for carbapenem-resistant Enterobacteriaceae (CRE) in China during 2011–12. Ecl20710 was isolated from the secretions of burn wounds of a 45-year-old patient in August 2011. This patient was admitted to the burn department with major burns in a hospital located in Shandong province, China. Ecl20712 was isolated from the sputum of a 2 month neonate in September 2011 in the same hospital. This patient was admitted to the paediatric ICU due to lung infection. Both strains were resistant to numerous drugs, including imipenem (≥16 mg/L) and meropenem (≥8 mg/L), and remained susceptible to tigecycline (1 mg/L) and colistin (0.25 mg/L) (Table S1, available as Supplementary data at *JAC* Online). The two isolates were assigned to ST200 and shared 100% sequence similarity as shown by average nucleotide identity analysis, suggesting the occurrence of clonal dissemination.

The two isolates shared an identical resistome composed of 25 genes, including: bla_{IMP-34} and bla_{VIM-1} for carbapenem resistance; *armA*, *aph(6)-Ib*, *aph(6)-Id*, *ant(3″)-Ia* (two copies), *aadA2* and *aadA5* for aminoglycoside resistance; bla_{ACT-16}, bla_{TEM-1b} and Δbla_{SED1} for β-lactam resistance; *fosA* and *fosA3* for fosfomycin resistance; *oqxAB* for quinolone resistance; *mph*(A) for macrolide resistance; *catA1* and *floR* for phenicol resistance; *sul1* (three copies) for sulphonamide resistance; *dfrA17* (two copies) for trimethoprim resistance; and *tet*(G) for tetracycline resistance. Except for bla_{ACT-16}, *fosA* and *oqxAB*, the resistance genes were carried by plasmids (Table S2). Carbapenemase genes bla_{IMP-34} and bla_{VIM-1} have been individually detected in China;[2,3] however, to the best of our knowledge, this is the first report of the coexistence of the two genes.

Completion of the Ecl20710 genome results in a chromosome of 4888062 bp and five plasmids, including pIMP-20710 (IncHI2/2A, 286652 bp), p2-20710 (IncA/C2, 170955 bp), p3-20710 (IncFIB, 108358 bp), p4-20710 (untypeable, 91816 bp) and pVIM-20710 (untypeable, 39601 bp). This is in concordance with the S1-PFGE result (Figure S1). pIMP-20710 carrying the bla_{IMP-34} gene was successfully transferred into *Escherichia coli* J53 cells. A conjugant carrying the pIMP-20710 plasmid was resistant to imipenem and showed intermediate resistance to ertapenem (Table S1). The bla_{VIM-1}-carrying plasmid pVIM-20710 failed to be transferred. IncHI2/2A-type plasmids are frequently associated with carbapenemase genes and we previously found that IncHI2/2A-type plasmids were prevalent among ESBL-producing *E. cloacae* in China,[4] suggesting the necessity of surveillance for carbapenemase-encoding IncHI2/2A-type plasmids in the future.

The bla_{IMP-34} gene was first detected in In*808* (*intI1-qacF-aacA4-*bla_{IMP-34}*-qacE2-qacEΔ1-sul1-orf5*) carried by an IncL/M plasmid, pKOI-34 (AB715422), in *Klebsiella oxytoca* isolated in Japan.[5] The bla_{IMP-34} gene was detected in an MRR in pIMP-20710. The MRR is an In*104*-like complex class-1 integron, including a truncated In*2*, an IS*CR3*-mediated co-transposition region carrying *floR* and *tetAR*(G) genes, and a partially duplicated 5′-CS (Figure 1). The 3′-CS and IS*6100* carried by In*104* were absent here and the original gene cassettes were replaced by a *dfrA12* gene cassette, an *aadA2* gene cassette and a bla_{IMP-34} gene (Figure 1). The *attC* site of the bla_{IMP-34} gene cassette was interrupted by the insertion of a gene encoding a group IIC intron, and the right half of *attC* and the 135 bp group IIC intron gene was truncated (Figure 1). It is known that group II introns have a retroelement capability and are able to function as mobile genetic elements, capable of integrating into specific chromosome sites,[6,7] as well as transposing independently to novel sites.[8,9] We previously noted that IMP genes are frequently associated with group II introns in Chinese isolates;[10] however, the biological/epidemiological significance

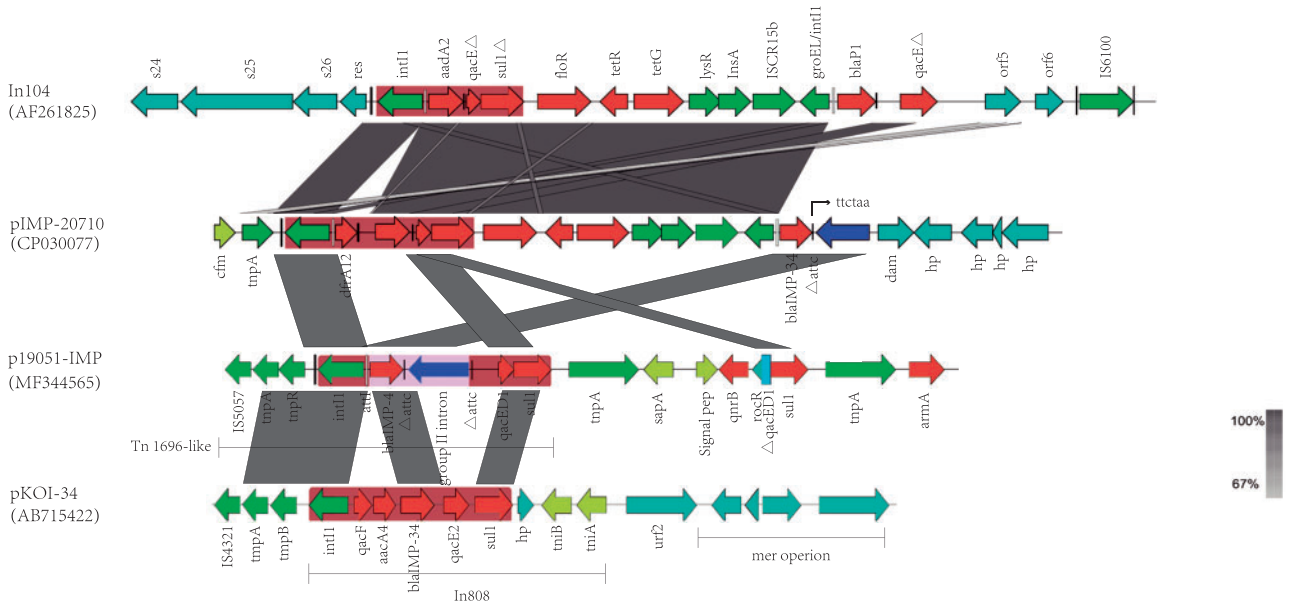

Figure 1. Genetic features of the bla_{IMP-34}-harbouring MRR detected in pIMP-20710. Gene classes are indicated by different colours and the class 1 integron is highlighted by a red box. The MRR is an In*104*-like complex integron carrying a truncated In*2*, an IS*CR3*-mediated co-transposition region and a partially duplicated 5′-CS. The *attC* site of the bla_{IMP-34} gene cassette was interrupted by an insertion of a group IIC intron and the 5′-end of the group IIC intron gene was further truncated. The last 6 bp of the remaining *attC* site is shown. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

remains unclear. We here suppose that the bla_{IMP-34} gene cassette was first incorporated into the In*104*-like integron and was later truncated, probably by recombination. While IMP-type carbapenemase genes often reside within a variety of integron structures incorporated as gene cassettes, to the best of our knowledge this is the first report of an IMP-type gene associated with an In*104*-like integron. This very first detection of the carbapenemase gene on an In*104*-like structure is alarming, since it might facilitate the widespread distribution of carbapenemases among Enterobacteriaceae. In fact, a similar structure is detected in multiple species by BLAST comparison in GenBank, including *K. pneumoniae*, *Proteus mirabilis*, *Providencia* spp., *Pseudomonas aeruginosa* and *Laribacter hongkongensis*. This suggests that In*104*-like has disseminated across species.

In summary, to the best of our knowledge we report for the first time the clonal dissemination of *E. cloacae* ST200 co-producing IMP-34 and VIM-1 in a clinical setting, which may represent a reservoir of MBLs facilitating their wide dissemination. The In*104*-like complex integron associated with IMP-type carbapenemases identified in this study raises great concern about the infection control of CRE.

Nucleotide sequence accession numbers

The complete sequences of Ecl20710 and Ecl20712 have been deposited in GenBank under the accession numbers CP030076–CP030081 (Ecl20710) and QLNR00000000 (Ecl20712).

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 and Figure S1 are available as Supplementary data at *JAC* Online.

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