Distribution of the bla\textsubscript{OXA-23}-containing transposons Tn2006 and Tn2008 in Australian carbapenem-resistant Acinetobacter baumannii isolates

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Sir,

Worldwide, resistance to carbapenems, imipenems and meropenem is widespread among Acinetobacter causing infections and is most resistant isolates are also resistant to many other antibiotic classes. Several class D \beta-lactamas can hydrolyse carbapenems, conferring resistance. The bla\textsubscript{OXA-23} gene was the first to be found and was originally identified in a conjugative plasmid from a clinical Acinetobacter baumannii isolate recovered in the UK in 1985 and the plasmid was shown to be transferable to other Acinetobacter species, but not to Escherichia coli. The bla\textsubscript{OXA-23} gene is now predominant among A. baumannii isolates in many countries.

The bla\textsubscript{OXA-23} gene originated in Acinetobacter radioresistens and has been mobilized into other species by Iss. However, resistance is only observed if expression is elevated by the presence of a strong promoter, typically an appropriately oriented repAci6 upstream of a strong promoter, typically an appropriately oriented

To determine whether Tn2008 is present in Australian carbapenem-resistant A. baumannii isolates, the genome sequences of >100 isolates were examined for the presence of Tn2008. All of the global clone 2 (GC2) isolates described previously carried Tn2006 in AbGR1-2 (Tn617), as seen for A91. Among additional isolates, three 2008 GC2 isolates from Flinders Medical Centre (08325850, 08317005 and 08315009) carried Tn2008. In addition to the resistances conferred by bla\textsubscript{OXA-23}, these isolates were resistant to third-generation cephalosporins, tetracycline, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and various aminoglycosides. Tn2008 was in a chromosomal gene encoding a short-chain dehydrogenase and surrounded by a different 9 bp duplication (AAGCGACTC) (Figure 1b). The sequence has been deposited in GenBank under accession number KP780408.

BLAST searches were used to determine whether Tn2008 is a discrete entity that is found in further locations. A third location in the draft genomes of GC1 isolates AB5075 (GenBank accession number JHU101000012), IS-15-8 (GenBank accession number AMGH101001001) and ANC 4097 (GenBank accession number APRF01000011) was identified. In this case, the interrupted gene is in the chromosome and annotated as a ‘phage-related’ membrane protein. The duplicated bases are CAATTCACC (Figure 1b). After this work was completed, Tn2008 was found in a fourth location inside ISAba125, again surrounded by a 9 bp duplication.

A further location for Tn2008 was in plasmid pNB09A30 from Acinetobacter baylyi (GenBank accession number JF731029), but in this case an additional two bases have been carried with it (Figure 1c). Tn2008 in this location interrupted by a copy of ISAba29 is found in the draft genome of the GC1 isolate Naval-B3 (GenBank accession number AMFK0000000). The sequence reported as Tn2008 by Mugnier et al. appears to represent an incompletely sequenced version of Tn2008::ISAba29 (Figure 1c).

The fact that Tn2008 has been found in four (potentially five) different locations, each of which is uninterrupted in other available sequences, is consistent with repeated transpositional movement of the DNA segment originally named as Tn2008, even though it includes only one ISAba1. Examination of the outer end of the bla\textsubscript{OXA-23}-containing segment revealed a match with 10 of 11 bases at the inner end of the 16 bp inverted repeats (IRs) of ISAba1, with the right-hand end of Tn2008 located 5 bp away (Figure 1d). The fortuitous presence of this potential transposase binding site in the A. radioresistens-derived segment appears to allow Tn2008 to move.

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Figure 1. Transposons carrying blaOXA-23. (a) Comparison of Tn2006 and Tn2008. (b) and (c) Locations of Tn2008. (d) Comparison of the outer end of Tn2008 with the IRs of ISAba1. In (a), (b) and (c), the blaOXA-23 gene is shown as a black arrow and other genes derived from A. radioresistens are in grey (AAA ATPase, DEAD helicase and yeeA). ISs are represented as white boxes that contain arrows showing the position and orientation of their transposase genes. In (a), the positions of single nucleotide differences between Tn2006 and Tn2008 are indicated above the genes, while the additional bases in Tn2006 are indicated below. In (b) and (c), various broken lines either side of Tn2008 represent the different flanking sequences and the target site duplications are shown above. In (c), the additional two bases carried with Tn2008 are shown below. In (d), the sequence of the right-hand end of Tn2008 is aligned with the two IRs of ISAba1. IR1 is upstream of the internal ORFs and IRr is downstream. The sequence of IRr has been reversed and complemented. The bases in lower case represent internal bases adjacent to each IR.
A closely related transposon, here designated Tn2008B, that appears to be derived from Tn2006 is prevalent in China and has also been found in three independent locations. Though movement has not been observed experimentally, these findings support the conclusion that Tn2008 and Tn2008B are discrete transposons, i.e. the same unit can move repeatedly. This is in contrast to the case of IS6cp1 that can mobilize adjacent DNA segments but the length of the segment is not discrete.13

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References

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Detailed characterization of the first high-level azithromycin-resistant Neisseria gonorrhoeae cases in Ireland

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Sir,
Neisseria gonorrhoeae has developed resistance to all antibiotics used as first-line empirical monotherapy for gonococcal infections, including the last option, ceftriaxone. Consequently, in Europe, the USA and several other countries, dual antimicrobial therapy consisting of ceftriaxone (250 or 500 mg intramuscularly) combined with azithromycin (1 or 2 g orally) has been introduced recently.1,2 However, this combination is compromised by the emergence of resistance to ceftriaxone and azithromycin. More worryingly, gonococcal strains with high-level resistance to azithromycin (MIC ≥ 256 mg/L) have been described in the UK, Italy, Sweden, the USA, Argentina and Australia.2,5 Here we report the detailed characterization of the first two high-level azithromycin-resistant N. gonorrhoeae cases in Ireland.

Azithromycin susceptibility by Etest methodology (bioMérieux, Marcy-l’Étoile, France) was retrospectively determined for all available urethral isolates of N. gonorrhoeae (n = 300) collected at St James’s Hospital, Dublin, from 2008 to 2014. Fourteen (4.7%) isolates were resistant to azithromycin according to breakpoints of EUCAST (Version 5.0)10 and two of the isolates showed high-level resistance to azithromycin (MIC ≥ 256 mg/L).

The first N. gonorrhoeae isolate (NGSJH7) was cultured in April 2008 from a urethral swab of a 22-year-old heterosexual male. He was presented to his general practitioner with mild urethritis. He was Chlamydia trachomatis and HIV negative, and had not been diagnosed previously with any sexually transmitted infection. The patient was treated empirically with 500 mg of ciprofloxacin orally (recommended in 2008) and was negative in test of cure. One sexual contact was traced and samples collected, but these tested negative for N. gonorrhoeae both by molecular and culture methods.