An increasing number of antibiotic resistance genes found in the mobile gene pool of Acinetobacter species are part of transposons that are mobilized by the insertion sequence ISAba1. ISAba1 includes a strong, outward-facing promoter, originally identified by Segal et al. and later re-positioned, and overexpression from this promoter can convert intrinsic genes into resistance genes. For example, the widespread oxa23 carbapenem resistance gene is known to originate from an intrinsic gene encoding a class D β-lactamase that is found in the chromosome of Acinetobacter radioresistens. For clarity and simplicity, the A. radioresistens gene is referred to using the designation oxaAr; a term that encompasses all chromosomal alleles. An oxaAr variant has been mobilized twice from the A. radioresistens chromosome by an ISAba1 inserted upstream to create Tn6208A and Tn6208B (see Nigro and Hall). Subsequently, larger compound transposons bounded by two copies of ISAba1 in inverse (Tn6206) or direct (Tn6209) orientation have arisen from Tn6208B. Though oxaAr is not known to confer resistance to carbapenem antibiotics, oxa23 in its new context is expressed from the strong outward-facing ISAba1 promoter and confers resistance. ISAba1 has also mobilized the intrinsic ampC gene from an Acinetobacter baumannii to form Tn6168, which confers resistance to third-generation cephalosporins due to increased expression driven by the promoter in ISAba1. Tn6168 has been found, in addition to the intrinsic ampC gene, in the chromosome of a group of A. baumannii ST11 isolates and in a plasmid in an A. baumannii ST49 outbreak.

Tn6252, which includes the oxa235 gene bound by inversely oriented copies of ISAba1, was first reported in the chromosome of ST10 isolate LAC-4. The upstream ISAba1 is oriented such that the strong promoter internal to ISAba1 drives expression of the oxa235 gene (Figure 1a). The cloned oxa235 gene (originally called blaOXA-235) had been shown to confer modest levels of resistance to carbapenem antibiotics. Later, Tn6252 was also found in the potentially conjugative plasmid pRCH51-3 (GenBank accession number KY216144) and was responsible for the reduced susceptibility to carbapenems of a sporadic A. baumannii isolate RCH51. Tn6252 was also found in a GC2 outbreak. In each case, the 3267 bp Tn6252 is surrounded by a 9 bp target site duplication (TSD), as is typical of ISAba1 transposition, indicating that it is an active transposon.

Here, we have examined the distribution of Tn6252 and the origin of the oxa235 gene. Though Tn6252 is not often encountered (21 entries were found in the GenBank nucleotide database as of August 2021), examination of the locations of Tn6252 in those sequences revealed 12 positions in the chromosome and 5 in plasmids, usually flanked by a 9 bp duplication indicative of movement.

The similarity (85% identity) of OXA-235 (and two minor variants OXA-236 and OXA-237) with a single amino acid difference) to OXA-134, which was encoded by an intrinsic gene in the chromosome of an Acinetobacter lwoffi isolate, suggesting a possible chromosomal source, had been noted. Here, the closest match of 98.92% identity was to an intrinsic gene encoding a class D β-lactamase (KX360744) reported to be from the chromosome of an Acinetobacter schindleri isolate. The segment of DNA that includes the oxa235 gene found between the ISAba1 copies was found to share 92%–96% identity to the corresponding region in most of the A. schindleri chromosomes for which complete or draft sequences are available and was more distantly related to the corresponding region in A. lwoffi genomes (Figure 1b).

For simplicity, the A. schindleri gene, covering all alleles, is referred to here as oxaAsc. A phylogeny of the OXA variants encoded by the oxa235 gene (OXA-235, OXA-236 and OXA-237) and those currently assigned to A. lwoffi or A. schindleri in ResSeq (Figure 1c) also revealed a clear separation of the alleles currently designated as ‘OXA-134 family’ into two groups corresponding to those encoded by A. lwoffi and A. schindleri chromosomes and the oxa235 alleles clearly group with those derived from A. schindleri. The species assignment of the A. schindleri genomes was confirmed using ribosomal RNA gene sequences (Figure S1, available as Supplementary data at JAC Online). The first recorded allele of the oxaAsc gene in ResSeq (https://www.ncbi.nlm.nih.gov/resseq/) is designated blaOXA-276 and we recommend that the two groups currently designated ‘OXA-134 family’, but corresponding to different species origins, should be separated into OXA-134 and OXA-276 groups.

We also recommend that context should be considered in order to distinguish the intrinsic chromosomally located genes that are not known to confer resistance to carbapenems from the mobilized oxa23 and oxa235 genes that have spread into other
Figure 1. Origin of oxa235. (a) Comparison of Tn6252 with A. schindleri ACE chromosomal sequence. The extent and orientation of genes are indicated by arrows with the gene names below. The chromosomal oxa gene and oxa235 are shown in red, while other genes and open reading frames are shown in white. ISAba1 is shown as an orange box with arrows inside to indicate the transposase genes. Grey shading indicates regions shared between the two sequences. Drawn to scale from GenBank accession numbers CP015615 (A. schindleri ACE) and KY216144 (Tn6252). (b) Comparison of A. schindleri and A. lwofii chromosomal sequences with oxa235 and the central segment of Tn6252. (c) Maximum likelihood tree of oxa genes from the oxaAlw, oxaAsc and oxa235 groups. In Geneious Prime, nucleotide sequences were aligned using Clustal Omega with default settings and the tree was constructed using PhyML with the GTR substitution model optimized for topology/length/rate, and confidence was assessed by performing 100 bootstraps. Percentage support from bootstrapping is shown on the branches. Where oxa allele numbers have been assigned by the NCBI Bacterial Antimicrobial Resistance Reference Gene Database, these are given in parentheses. Where no allele number has been assigned, the name of the strain that the gene derived from has been given. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
species and now because of their context confer resistance to carbapenem antibiotics.

Recently, we reported mobilization of the chromosomal folA gene of an A. schindleri isolate by ISAba60 to generate the dfrA44 trimethoprim resistance gene.13 However, the dfrA44 gene and surrounds match a region in the chromosomes of the completely sequenced A. schindleri isolates with >98.3% identity. Hence, closer matches for the oxa235 gene may be found in the future.

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**Transparency declarations**
None to declare.

**Supplementary data**
Figure S1 is available as Supplementary data at Supplementary data at JAC Online.

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**Characterization of VIM-1-, NDM-1- and OXA-48-producing Citrobacter freundii in France**

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During recent decades, carbapenem resistance in Gram negatives has become a worldwide threat leading to more restricted antimicrobial treatment options.1,2 In Enterobacteriales, carbapenem resistance is linked to the emergence and dissemination of carbapenemase producers.1,2 In Enterobacteriales, the most prevalent carbapenemases belong to the Ambler class A with mainly KPC1,2 and few other rare enzymes (GES, IMI, SME etc.).6 The NDM-, VIM- and IMP-type metallo β-lactamases (Ambler class B)5,6 and the carbapenem-hydrolysing Ambler class D oxacillinase of OXA-48-type.7

Usually, metallo β-lactamase-encoding genes are localized on diverse plasmids that also carry several genetic resistance determinants to other antimicrobials.6 In contrast, the blaOXA-48 gene is nearly always localized on an archetypal IncL plasmid of approximately 62 kb that does not contain any other resistance genes.8 Most often, carbapenemase-producing Enterobacteriales produce only one carbapenemase. However, the number of isolates that produce two carbapenemases has increased during the last

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