GIsul2, a genomic island carrying the sul2 sulphonamide resistance gene and the small mobile element CR2 found in the Enterobacter cloacae subspecies cloacae type strain ATCC 13047 from 1890, Shigella flexneri ATCC 700930 from 1954 and Acinetobacter baumannii ATCC 17978 from 1951

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Sir, The Acinetobacter baumannii strain ATCC 17978 for which a genome sequence is available (GenBank accession number CP001918) was isolated in France in 1951, when antibiotic therapy was in its infancy. The sul1 sulphonamide resistance determinant and dfrA10 (dhfrX) trimethoprim resistance gene were reported to be present in this isolate. However, we were unable to find either gene in this genome. In the case of sul1, the misidentified gene is likely to encode a sulphate permease that had been misannotated previously and, to avoid confusion, was later renamed sup (Figure 1d). The only dihydrofolate reductase found was the chromosomal folA gene. Nonetheless, ATCC 17978 was sulphonamide resistant. When tested using a disc diffusion assay with a disc containing 300 μg of sulfamethoxazole, the annular radius of the cleared zone was 0 mm for ATCC 17978 and a panel of 20 sulphonamide-resistant A. baumannii isolates. In contrast, 20 susceptible isolates exhibited cleared zones with annular radii of 10–14 mm, corresponding to a diameter of 26–34 mm. ATCC 17978 was not significantly more resistant to trimethoprim than the susceptible isolates. The sul2 sulphonamide resistance gene was found at 798900–799715 bp in CP000521.

The sul2 gene, when it occurs alone, is usually found located adjacent to the small mobile element CR2 with the sul2 gene facing the ori end of CR2, and this configuration was present in ATCC 17978. The insertion sequence ISAba1 was found upstream of sul2 (Figure 1c). The ISAba1-sul2 configuration has been reported previously in an A. baumannii strain, RAM, of unknown provenance (GenBank accession number AY823412) and ISAba1 supplied the promoter. The arrangement in ATCC 17978 is shown in Figure 1(c). Further bioinformatic analysis revealed that a 1080 bp segment located next to the ter end of CR2, and encoding a resolvase, is in the equivalent location in several IncA/C plasmids for which sequences are available (e.g. GenBank accession numbers AB277723 and AB305618) (Figure 1a). The resolvase gene was named resG.

In ATCC 17978, the ISAba1-sul2-CR2-resG arrangement lies within pA4, one of the alien islands identified previously. Searches for related sequences in the GenBank non-redundant DNA database revealed that the sul2 gene, CR2 and resG and a further 11 kb adjacent to resG were identical to a region from the chromosome (GenBank accession number AE014073) of Shigella flexneri 2a strain 2457T (ATCC 700930) isolated in Japan in 1954 and a region from the chromosome of the Enterobacter cloacae subspecies cloacae type strain (ATCC 13047) isolated in the USA in 1890 (GenBank accession number CP001918). The region that is identical in the Shigella and the Enterobacter genomes represents a 15456 bp genomic island (GI) that was also defined by comparison of the Shigella sequence to that of several Escherichia coli genomes that do not include it. The structure of this GI, named GIsul2, is shown in Figure 1(b). GIsul2 includes a gene encoding an integrase (a tyrosine site-specific recombinase) at one end (int in Figure 1b) and is located in the 3′ end of the guaA gene (encoding a GMP synthetase) of both the Shigella and the Enterobacter isolates. Hence GIsul2 is an integrating element. The position of a mercury ion resistance transposon closely related to Tn5393 (GenBank accession number L40585) that interrupts GIsul2 in the E. cloacae strain is indicated by an arrow in Figure 1(b). Other open reading frames (ORFs) encoding proteins related to transfer proteins (trbL and trbJ) or replication initiation proteins, repA and repC, raise the possibility that GIsul2 may be able to replicate independently as a plasmid.

The A. baumannii ATCC 17978 isolate includes all but 288 bp of GIsul2, and this appears to have been removed by ISAba1. The structure of pA4 adjacent to the integrase-encoding int end of GIsul2 is shown in Figure 1(c). It includes parts of a variant form of the transposon found in the comM gene in this strain and designated Tn6021. Tn6021 is shown for comparison in Figure 1(d). GIsul2 appears to have integrated into the trnB gene of a transposon related to Tn6021. The Tn6021-related segments are 86%–89% identical to Tn6021 and are bounded on the right (as shown in Figure 1) by a 25 bp inverted repeat (IR) identical to that found at the outer end of Tn6021. An insertion sequence that is 88% identical to
ISSBA2 is located between orf2 and orf3. However, the uspA and sup genes in Tn6021 have been replaced by a 4.2 kb segment that contains ferrous iron uptake genes (feo) and a gene for a putative efflux protein.

E. cloacae ATCC 13047 is the earliest bacterial isolate known to carry the sul2 gene and demonstrates that the association of sul2 with its companion, the small mobile element CR2, predates the era of antimicrobial therapy. The fact that GIsul2 is also in a clinical A. baumannii isolate from 1951 and a Shigella isolated 3 years later in Japan indicates that the sul2-CR2 configuration was globalized and had spread to different species early in the antibiotic era. Though sulphonamide resistance in A. baumannii has rarely been examined, sul2 has been reported in A. baumannii isolates (GenBank accession numbers FN293019 and FN293050) and we have found the sul2 gene in all of the isolates belonging to global clone 2 (formerly European clone II) that we have examined to date.10

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Transparency declarations
None to declare.

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