IS26-Associated In4-Type Integrons Forming Multiresistance Loci in Enterobacterial Plasmids

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Three distinct multiresistant loci from enterobacterial plasmids each comprised an integron and an IS26-associated sequence. Sequence comparison suggested a common ancestral structure that derived from an IS26 insertion into the 5′ conserved segment of an In4-type integron and evolved through acquisition of gene cassettes and IS26-mediated recruitment of additional resistance genes of diverse origin.

Resistance to multiple antibiotics in enterobacteria is largely attributed to acquisition of multiresistance plasmids (MRPs) (20). Sequencing data from MRPs have shown that resistance genes often occur in clusters carried by class 1 integrons. The latter, though not mobile themselves, are commonly associated with various transposons, such as Tn21, Tn1696, and Tn1412 of the Tn3 family (1, 13, 17).

We have previously reported on three MRPs: the SHV-5-encoding pSEM from Salmonella enterica serovar Typhimurium that belonged to IncL/M and carried In-t3 (18), the IncN plasmid from to the IncL/M incompatibility group (data not shown), carrying integron the first 113 bp of the common 5′/H11032 end of integrons.

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These experiments showed that the three integrons were associated with IS6100 and, therefore, belonged to a lineage of class 1 integrons related to In4 from Tn1696 (14).

**Sequences flanking the 5′ end of integrons.** To determine the sequences flanking the 3′ end of In-t3, In-111, and In-e541, the respective plasmids were partially digested with various endonucleases and the fragments were ligated into the chloramphenicol-resistant phagemid PBC-SK (+) (Stratagene, La Jolla, Calif.). E. coli DH5α was used as a host of recombinant plasmids. Recombinant clones were screened by PCR assays and cloning procedures as above.

**Sequences upstream the extreme left IS26.** The IS26-bounded sequence (7,996 bp) comprised eight open reading frames: truncated putative endonuclease (ygbM), fuculose-1-phosphate aldolase (fucA), putative tRNA synthase (ygbK), putative oxidoreductase (ygbL), blaSHV-5, recF, and a truncated lactose transport gene (ΔlacY) (Fig. 1). It exhibited >90% homology with a chromosomal segment of K pneumoniae including the intrinsic blaSHV-gene (www.genome.wustl.edu/projects/bacterial/kpneumoniae/).

Sequences upstream the extreme left IS26 (at least 60 bp in each locus), compared with the relevant flanks of the other

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IS26 elements, did not include duplications characteristic of IS26-mediated transposition.

A GenBank search revealed two more MRPs resembling pSEM, the IncFII p1658/97 plasmid from E. coli (AF550679; unpublished data) and the IncL/M pACM1 from Klebsiella oxytoca (U90945, AY081221, AY309067, AY309066) (15, 16). They both carried In4-type integrons similar to In-t3 in which the 5′CS was truncated by an IS26 at the same position as in the integrons discussed here. Additionally, these integrons were associated with SHV-5-encoding sequences flanked by IS26 that, however, were differently oriented than those in pSEM.

**Formation and spread of IS26/Δ5′CS-containing multiresistant loci.** It is likely that the mobile elements of the IS6 family, including IS26, do not exhibit any marked target site specificity (8). Therefore, the hypothesis of independent IS26 insertions into the same “hot-spot” of different class 1 integrons that, additionally, all belonged to the In4 family was discarded. The key features of the loci described here were the following: (i) the occurrence of the IS26-In4 structure in distinct replicons and (ii) the presence of multiple copies of IS26 in the sequences adjoining the 5′CS that were, most probably, inserted independently as indicated from the absence of target site duplications. Based also on the properties of IS26 (2, 4, 5, 8), it can be proposed that the IS26/Δ5′CS-containing multiresistant loci derived from a common structure. The initial step would be the insertion in the 5′CS of a plasmid-borne In4-type integron of an IS26-1 derived either by intramolecular transposition or by transposition of an element located on a different replicon. IS26-1 probably facilitated recruitment of diverse IS26-flanked sequences, such as aphA and the β-lactamase-encoding chromosomal fragments observed here, by homologous recombination as suggested by the lack of target site duplications. Also, IS26-mediated cointegration of different replicons, subsequently resolved by RecA-dependent homologous recombination, can explain mobilization of IS26-In4 among distinct MRPs.

IS26 is widely spread among plasmids (2, 6, 12, 21) and implicated in the dissemination of resistance genes in several ways. Compound IS26 transposons carrying from one to nine resistance genes have been described previously (5, 12). Also, IS26 elements seem to facilitate mobilization of chromosomal sequences containing resistance genes (3, 6). The findings of this study suggest that association of IS26 with a class 1 integron of the In4 lineage was probably a critical step in the evolution of diverse multiresistance plasmids found in clinical enterobacteria.

**Nucleotide sequence accession numbers.** The GenBank accession numbers of the sequences presented here are AJ245670 and AJ009829 (pSEM), AY260546 (pAK33), and AY339625 and AY340637 (p541).

**REFERENCES**


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