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Identification of a Complete *dfrA14* Gene Cassette Integrated at a Secondary Site in a Resistance Plasmid of Uropathogenic *Escherichia coli* from Nigeria

In a study of transferable antimicrobial resistance in uropathogenic *Escherichia coli* from humans in Nigeria, a small plasmid of 6.8 kb, designated pSTOJO1, was identified by transformation into *E. coli* JM107 to mediate resistance to sulfamethoxazole (Smz) and trimethoprim (Tmp). The MICs of Smz and the combination Tmp/Smz (4) were >1,024 μg/ml and >16/304 μg/ml, respectively, for both the original *E. coli* strain and *E. coli* JM107(pSTOJO1). Plasmid pSTOJO1 was mapped (Fig. 1a), and the two *Hin*dIII-*Kpn*I fragments of 3.9 and 2.9 kb were cloned into pBluescript II SK$^+$ (Stratagene, Amsterdam, The Netherlands). The sequence of a 3,839-bp segment of pSTOJO1 including the resistance genes was determined on both strands by primer walking starting at the *Hin*dIII cloning site of both fragments. It has been deposited with the EMBL database under accession no. AJ313522. Within this segment, a disrupted streptomycin resistance gene, *strA*, and three intact reading frames corresponding to the sulfonamide resistance gene *sul2*, the trimethoprim resistance

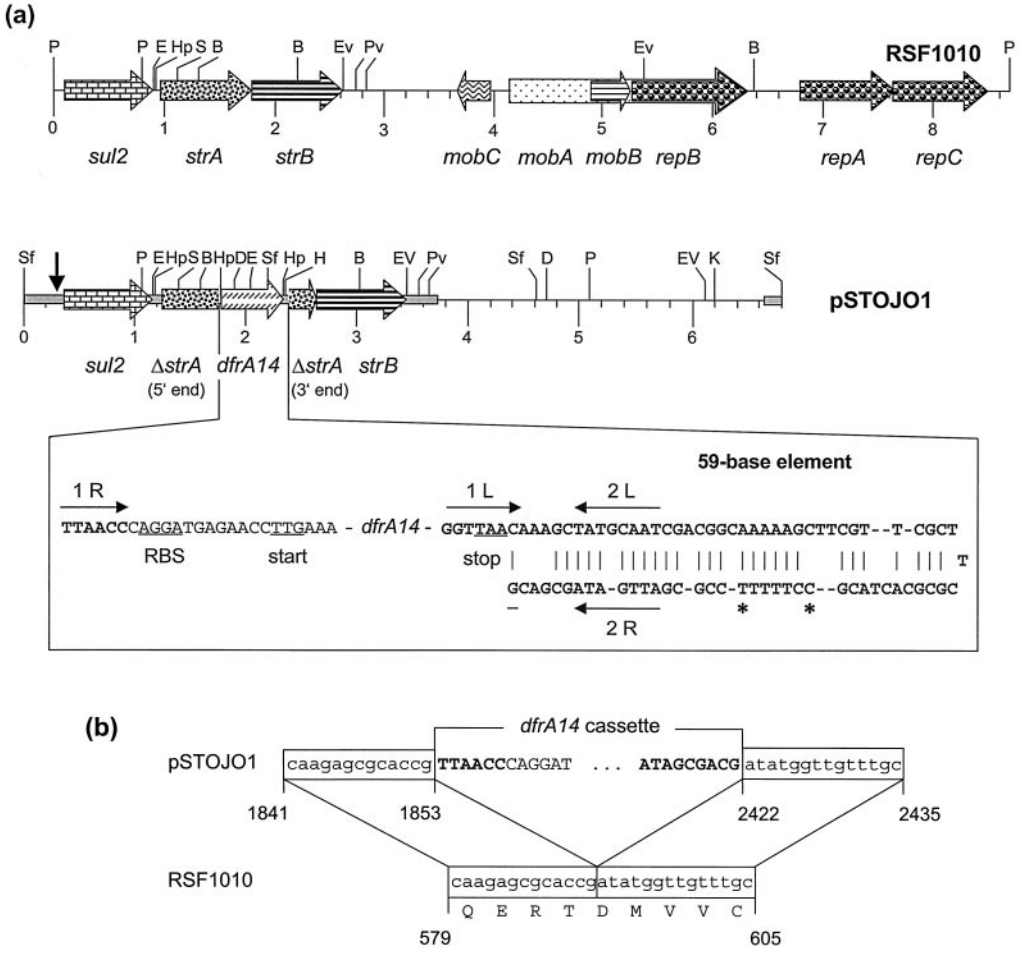

FIG. 1. (a) Restriction map and structural organization of plasmids RSF1010 (8) and pSTOJO1. Restriction endonucleases: B, *Bcl*I; D, *Dra*I; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; and Sf, *Sfu*I. A distance scale in kilobases is presented below the map. The reading frames for the genes *sul2*, Δ*strA*, *strA*, *dfrA14*, *strB*, *mobA-C*, and *repA-C* are shown as arrows, with the direction of transcription indicated by the arrowhead. The gray bar in pSTOJO1 indicates the sequenced part. The vertical arrow at the left end of the map of pSTOJO indicates the beginning of the RSF1010-related part of the pSTOJO1 sequence. Essential parts of the *dfrA14* cassette are shown in more detail below the map of pSTOJO1. The ribosome binding site (RBS) and translational start and stop codons are underlined. In the 59-be, the putative IntI1 integrase binding domains 1L, 2L, 2R, and 1R (11) are indicated by arrows. The entire 59-be of the cassette is shown in boldface. The C marked with an asterisk is missing in the 59-be of the *dfrA14* cassette of plasmid pUK1329 from *E. coli* (Z50805), and the T marked with an asterisk is missing in that of plasmid pHCM1 from *S. enterica* serovar Typhi (AL513383). (b) Comparison of the integration site of the *dfrA14* cassette within *strA* in pSTOJO1 and the corresponding *strA* sequence of RSF1010 (8). The numbering refers to the positions in the database entries for pSTOJO1 (AJ313522) and RSF1010 (M28829).

gene *dfrA14* (formerly known as *dhfrIb*), and the gene *strB* were detected (Fig. 1a).

The arrangement of the genes *sul2-strA-strB* closely resembled that known from plasmid RSF1010 (8), and sequence analysis confirmed 99% identity of the pSTOJO1 sequence from positions 353 to 1853 as well as 2422 to 3839 to the corresponding RSF1010 sequence. Except for these regions covering the genes *sul2*, *strA*, and *strB*, plasmids pSTOJO1 and RSF1010 appeared to be unrelated (Fig. 1a). The *strA* gene was disrupted by the insertion of a 568-bp element that carried a *dfrA14* gene coding for a dihydrofolate reductase (2, 12). The *dfrA14* gene was identical to the corresponding gene recently detected on a plasmid from *Salmonella enterica* serovar Typhimurium DT104 (AF393510) and exhibited 1-, 2-, and 5-bp differences from the sequences of the *dfrA14* genes found in *S. enterica* serovar Typhi (AL513383) or *E. coli* (Z50805 and Z50804).

The gene *dfrA14* has been reported to be part of a gene cassette, the size of which and the structure and length of the 59-base element (59-be) of which have been unknown (5). Analysis of the pSTOJO1 sequence identified the *dfrA14* cassette to be 568 bp. The 59-be of the *dfrA14* cassette consists of 87 bp and shows a central axis of symmetry (Fig. 1a). Since the submission of the pSTOJO1 sequence to the EMBL database, another two database entries for complete *dfrA14* cassettes have become available (AL513383 and Z50805). Both 59-be sequences comprised 86 bp; the differences from the 59-be of the *dfrA14* cassette from pSTOJO1 are indicated in Fig. 1a. In pSTOJO1, the *dfrA14* cassette was found to be integrated at a secondary site within the *strA* gene. A similar situation was also seen in the *E. coli* plasmid pUK1329 (Z50805). No base pairs were lost or gained at the integration site (Fig. 1b), suggesting precise integration of the cassette. As a result, the *strA* gene was inactivated. Integration of a gene cassette at a secondary site was assumed to be an IntI-catalyzed recombination event, which involves a secondary recombination site (5, 6). The *strA* sequence at the integration site, GATAT, corresponded to the consensus sequences for secondary sites: Gt/aT (7) or Ga/tTa/ca/t (1). Precise integration of a complete *aadB* cassette at a secondary site between the genes *repB* and *repA* of RSF1010 (6), as well as in plasmid pRAY of a clinical isolate of *Acinetobacter* (9), has previously been reported. Moreover, truncation of an RSF1010-like *strA* gene by the insertion of the non-cassette-borne Tmp resistance gene, *dfrA9*, has also been reported (10) and is believed to have occurred as a consequence of the high selective pressure imposed by the frequent use of Tmp (3). A similar condition can be assumed for the development of the Smz/Tmp resistance plasmid pSTOJO1, since sulfonamides and Tmp are among the most frequently used antimicrobial drugs in Nigeria. Since integrase genes as well as plasmids carrying *sul2-strA-strB* genes are widespread among gram-negative bacteria, it is impossible to determine in retrospect where or when the recombination event between the *dfrA14* cassette and the *strA* gene occurred.

REFERENCES

1. **Francia, M. V., F. de la Cruz, and J. M. Garcia Lobo.** 1993. Secondary sites for integration mediated by the Tn*21* integrase. Mol. Microbiol. **10:**823–828.
2. **Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld.** 1995. Trimethoprim and sulfonamide resistance. Antimicrob. Agents Chemother. **39:**279–289.
3. **Jansson, C., A. Franklin, and O. Sköld.** 1992. Spread of a newly found trimethoprim resistance gene, *dhfrIX*, among porcine isolates and human pathogens. Antimicrob. Agents Chemother. **36:**2704–2708.
4. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard, 5th ed. NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
5. **Recchia, G. D., and R. M. Hall.** 1995. Gene cassettes: a new class of mobile element. Microbiology **141:**3015–3027.
6. **Recchia, G. D., and R. M. Hall.** 1995. Plasmid evolution by acquisition of mobile gene cassettes: plasmid pIE723 contains the *aadB* gene cassette precisely inserted at a secondary site in the IncQ plasmid RSF1010. Mol. Microbiol. **15:**179–187.
7. **Recchia, G. D., H. W. Stokes, and R. M. Hall.** 1994. Characterisation of specific and secondary recombination sites recognised by the integron DNA integrase. Nucleic Acids Res. **22:**2071–2078.
8. **Scholz, P., V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian, and E. Scherzinger.** 1989. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. Gene **75:**271–288.
9. **Segal, H., and B. G. Elisha.** 1997. Identification and characterization of an *aadB* gene cassette at a secondary site in a plasmid from *Acinetobacter*. FEMS Microbiol. Lett. **153:**321–326.
10. **Sköld, O.** 2001. Resistance to trimethoprim and sulfonamides. Vet. Res. **32:**261–273.
11. **Stokes, H. W., D. B. O'Gorman, G. D. Recchia, M. Parsekhian, and R. M. Hall.** 1997. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. Mol. Microbiol. **26:**731–745.
12. **Young, H. K., M. J. Qumsieh, and M. L. McIntosh.** 1994. Nucleotide sequence and genetic analysis of the type Ib trimethoprim resistant, Tn*4132*-encoded dihydrofolate reductase. J. Antimicrob. Chemother. **34:**715–725.

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