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# Characterization of Transposon Tn*1528*, Which Confers Amikacin Resistance by Synthesis of Aminoglycoside 3′-*O*-Phosphotransferase Type VI

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***Providencia stuartii* BM2667, which was isolated from an abdominal abscess, was resistant to amikacin by synthesis of aminoglycoside 3′-*O*-phosphotransferase type VI. The corresponding gene, *aph(3′)-VIa*, was carried by a 30-kb self-transferable plasmid of incompatibility group IncN. The resistance gene was cloned into pUC18, and the recombinant plasmid, pAT246, was transformed into *Escherichia coli* DH1 (*recA*) harboring pOX38Gm. The resulting clones were mixed with *E. coli* HB101 (*recA*), and transconjugants were used to transfer pAT246 by plasmid conduction to *E. coli* K802N (*rec*$^+$). Analysis of plasmid DNAs from the transconjugants of K802N by agarose gel electrophoresis and Southern hybridization indicated the presence of a transposon, designated Tn*1528*, in various sites of pOX38Gm. This 5.2-kb composite element consisted of *aph(3′)-VIa* flanked by two direct copies of IS*15*-Δ and transposed at a frequency of $4 \times 10^{-5}$. It therefore appears that IS*15*-Δ, an insertion sequence widely spread in gram-negative bacteria, is likely responsible for dissemination to members of the family *Enterobacteriaceae* of *aph(3′)-VIa*, a gene previously confined to *Acinetobacter* spp.**

Bacterial resistance to aminoglycosides is mainly due to the synthesis of enzymes that adenylylate, phosphorylate, or acetylate these antibiotics (8). In gram-negative bacteria, amikacin is modified by 6′-*N*-acetyltransferase type I [AAC(6′)-I] enzymes. The nucleotide sequences of six genes encoding related AAC(6′)-I enzymes, designated AAC(6′)-Ia, -Ib, -Ic, -Id, -If, and -Ig, have been determined previously (30). AAC(6′)-Ib is the enzyme with activity responsible for amikacin resistance in the majority of clinical isolates (29). Two other amikacin-inactivating enzymes have been detected in gram-negative bacteria: the 3′-*O*-phosphotransferase type VI [APH(3′)-VI], which is rare in members of the families *Enterobacteriaceae* and *Pseudomonadaceae* but is common in *Acinetobacter* spp. (17), and the 4′-*O*-nucleotidyltransferase type II [ANT(4′)-II], which is extremely rare (30).

The APH(3′)-VI enzyme encoded by the *aph(3′)-VIa* gene (formerly designated *aphA-6*) was detected in *Acinetobacter baumannii* BM2580, which was isolated in 1984 (18). This enzyme inactivates amikacin, butirosin, kanamycin, and neomycin but not lividomycin. An epidemiological survey in France indicated that amikacin resistance in *Acinetobacter* spp. is mainly due to dissemination of the *aph(3′)-VIa* gene (17). In the present study we showed that the *aph(3′)-VIa* gene of *Providencia stuartii* BM2667 is flanked by direct repeats of IS*15*-Δ and transposes in *Escherichia coli*. Characterization of the *aph(3′)-VIa* gene and of its flanking regions in strains of *E. coli*, *Proteus mirabilis*, *Morganella morganii*, *P. stuartii*, and *Pseudomonas aeruginosa* suggested that IS*15*-Δ-mediated transposition plays a role in the dissemination of the *aph(3′)-VIa* gene from *Acinetobacter* spp. to members of the families *Enterobacteriaceae* and *Pseudomonadaceae*.

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## MATERIALS AND METHODS

**Bacterial strains, plasmids, and culture conditions.** The strains and plasmids used in the study are listed in Table 1. *P. stuartii* BM2667 was isolated in 1989 from the abdominal abscess of an adult in an intensive care unit at the Hôpital Saint-Michel, Paris, France. *E. coli*, *M. morganii*, and *P. mirabilis* strains resistant to amikacin and isepamicin and susceptible to habekacin, a phenotype compatible with the production of an APH(3′)-VI enzyme (18), were isolated from the feces of the same patient. *Enterobacter cloacae* 880516188 and 880516264 and *E. coli* 880516301, from the strain collection of Schering-Plough Research Institute (33), and *Pseudomonas aeruginosa* BM2683, from Hôpital Necker, Paris, France, which had similar resistance phenotypes, were included in the study. Bacteria were grown in brain heart infusion broth and agar (Difco Laboratories, Detroit, Mich.) at 37°C.

**DNA techniques.** Preparation of total DNA (27) and of small-scale (2) or large-scale (28) plasmid DNA was done as described previously. DNA was amplified with *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) and two oligonucleotides (5′-CCCCGAAGAACGTTTTC and 5′-ATCAGCAATAAACCAGC) as described previously (22). For Southern hybridization, DNA was transferred onto Nytran membranes (Schleicher & Schuell, Dassel, Germany). Prehybridization and hybridization were carried out for 5 and 15 h, respectively, at 65°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.5% sodium dodecyl sulfate and 0.05% nonfat dry milk (13). The following DNA fragments were used as probes (Fig. 1): A probe, a 312-bp *Eco*RI-*Eco*RV fragment of pAT240; B probe, a 653-bp *Sal*I fragment of pIP1089; and C probe, a 355-bp *Dde*I-*Sal*I fragment of pIP1089. DNA fragments were digested with endonucleases, purified by electrophoresis in low-temperature-gelling agarose type VII (Sigma Chemical Co., St. Louis, Mo.), extracted (28), and radiolabeled by nick translation (28).

TABLE 1. Bacterial strains and plasmids used in the study

| Strain or plasmid | Relevant properties[a] | Reference or source |
|---|---|---|
| *A. baumannii* | | |
| BM2580 | Ak Km (pIP1841) | 18 |
| BM2582 | Nal Rif | 18 |
| *A. calcoaceticus* BD413 | *trpE27* | 14 |
| *E. coli* | | |
| DH1 | *recA1 endA1 thi-1 hsdR17 supE44 gyrA96* ($Nal^r$) | 21 |
| HB101 | $F^-$ *hsdS20* ($r_B^-$ $m_B^-$) *proA2 lacY1 recA13 rpsL20* ($Str^r$) *ara-14 galK2 xyl-5 mtl-1 supE44* $\lambda^-$ | 3 |
| K802N | *hsdR hsdM*$^+$ *gal met supE rpoB nalA* | 34 |
| *P. aeruginosa* BM2683 | Ak Km | Wild strain |
| *P. stuartii* BM2667 | Ak Ap Km (pIP1848) | Wild strain |
| Plasmids | | |
| pOX38Gm | $Tra^+$ Gm 58.2 kb; F derivative; in vitro construction | 23 |
| pIP1089 | $Tra^-$ $Mob^-$ Ap Tc 6 kb (pBR322::IS*15*) | 15 |
| pIP1841 | $Tra^+$ Ak Km 63 kb | 18 |
| pIP1848 | $Tra^+$ IncN Ap Ak Km 30 kb | This study |
| pAT240 | $Tra^-$ $Mob^-$ Ap [pUC18Ω365-bp *Eco*RI-*Bgl*II from *aph(3')-VIa*] | 17 |
| pAT246 | $Tra^-$ $Mob^-$ Ak Ap Km [pUC18Ω15-kb *Hin*dIII from pIP 1848] | This study |
| pAT247 | $Tra^-$ $Mob^-$ Ak Ap Km [pUC19Ω4.4 kb *Sal*I from pAT246] | This study |
| pAT474 | $Tra^-$ $Mob^-$ Ak Ap Km Nt Tm [pUC19Ω554 bp *FokI* from *A. haemolyticus* BM2685] | 19 |
| RP4 | $Tra^+$ IncP Ap Km Tc | 7 |

[a] Abbreviations: $Tra^+$, self-transferable; $Tra^-$, non-self-transferable; $Mob^-$, nonmobilizable; Inc, incompatibility group; Ak, amikacin resistance; Ap, ampicillin resistance; Gm, gentamicin resistance; Km, kanamycin resistance; Nal, nalidixic acid resistance; Nt, netilmicin resistance; Rif, rifampin resistance; Str, streptomycin resistance; Tc, tetracycline resistance; Tm, tobramycin resistance.

**Assay for aminoglycoside-modifying enzymes.** Bacterial extracts were assayed by the phosphocellulose paper-binding technique by using [$\gamma$-$^{32}$P]ATP (30 Ci/mmol) to detect enzyme activity (11). The final concentration of aminoglycoside in the assay mixture was 66.7 μg/ml, and the reaction was allowed to proceed for 30 min at 30°C.

**Genetic techniques.** Conjugation (28) into *E. coli* HB101 and K802N and into *A. baumannii* BM2582, transformation of *E. coli* DH1 (28) and *A. calcoaceticus* BD413 (14), and determination of plasmid incompatibility (6) were performed as described previously. Transfer frequencies were expressed as the number of transconjugants per donor. Antibiotic concentrations for the selection of trancipients were as follows: amikacin, 10 μg/ml; ampicillin, 100 μg/ml; gentamicin, 5 μg/ml; nalidixic acid, 50 μg/ml; and streptomycin, 100 μg/ml. Transposition experiments were performed by plasmid conduction (32) with the conjugative plasmid pOX38Gm (23).

The frequency of transfer of cointegrates relative to that of pOX38Gm alone was used as an estimate of cointegration in the donor. The stabilities of the cointegrates were analyzed by growing randomly selected transconjugants for approximately 100 generations in broth without antibiotic (9). Colonies were screened by replica plating on medium containing ampicillin and amikacin or amikacin alone and were tested for their capacities to transfer antibiotic resistance to *E. coli* K802N.

**Enzymes and chemicals.** T4 DNA ligase and restriction endonucleases (Amersham, Buckinghamshire, England) were used according to the recommendations of the manufacturer. Lysozyme was from Sigma Chemical Co., and RNaseA (bovine pancreas) was from Calbiochem-Behring (La Jolla, Calif.). Nick-translation kits were from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.); [$\alpha$-$^{32}$P]dCTP and [$\gamma$-$^{32}$P]ATP (triethylammonium salt) were obtained from the Amersham Radiochemical Centre, Buckinghamshire, England. The following antibiotics were provided by the indicated laboratories: amikacin and ampicillin, Bristol-Myers Squibb (Princeton, N.J.); habekacin, Rhône-Poulenc Rorer Bellon (Neuilly sur Seine, France); gentamicin and isepamicin, Schering-Plough Research Institute (Kenilworth, N.J.); nalidixic acid, Sterling Winthrop (New York, N.Y.); and streptomycin, Pfizer, Inc. (Groton, Conn.).

## RESULTS

**Transfer of the amikacin resistance of *P. stuartii* BM2667.** The amikacin and ampicillin resistances of BM2667 were transferable en bloc to *E. coli* K802N by conjugation at a frequency of ca. $10^{-4}$. PCR analysis of total DNA isolated from the transconjugants (22) indicated that resistance to ampicillin was due to the presence of a TEM-type β-lactamase (data not shown). All attempts to transfer amikacin resistance from BM2667 to *A. baumannii* BM2582 by conjugation or to *A. calcoaceticus* BD413 by transformation with isolated plasmid DNA were unsuccessful.

**Plasmid contents of BM2667 and transconjugants.** Analysis by agarose gel electrophoresis of plasmid DNA from BM2667 and from the transconjugants indicated that resistances to ampicillin and amikacin were borne by a 30-kb plasmid designated pIP1848 (data not shown). The plasmid belonged to incompatibility group IncN.

**Characterization of aminoglycoside-modifying activity.** Aminoglycoside phosphotransferase activity was detected in extracts of BM2667 and K802N(pIP1848) by the phosphocellulose paper-binding assay. The fact that kanamycin B was modified whereas tobramycin (3′-deoxykanamycin B) was not indicated that the 3′-hydroxyl group was the site of phosphorylation [APH(3′)]. Amikacin, butirosin, and neomycin were substrates for phosphorylation, but lividomycin A was not, indicating that the enzyme was of type VI (18) (data not shown). The A probe, which is specific for the *aph(3′)-VIa*

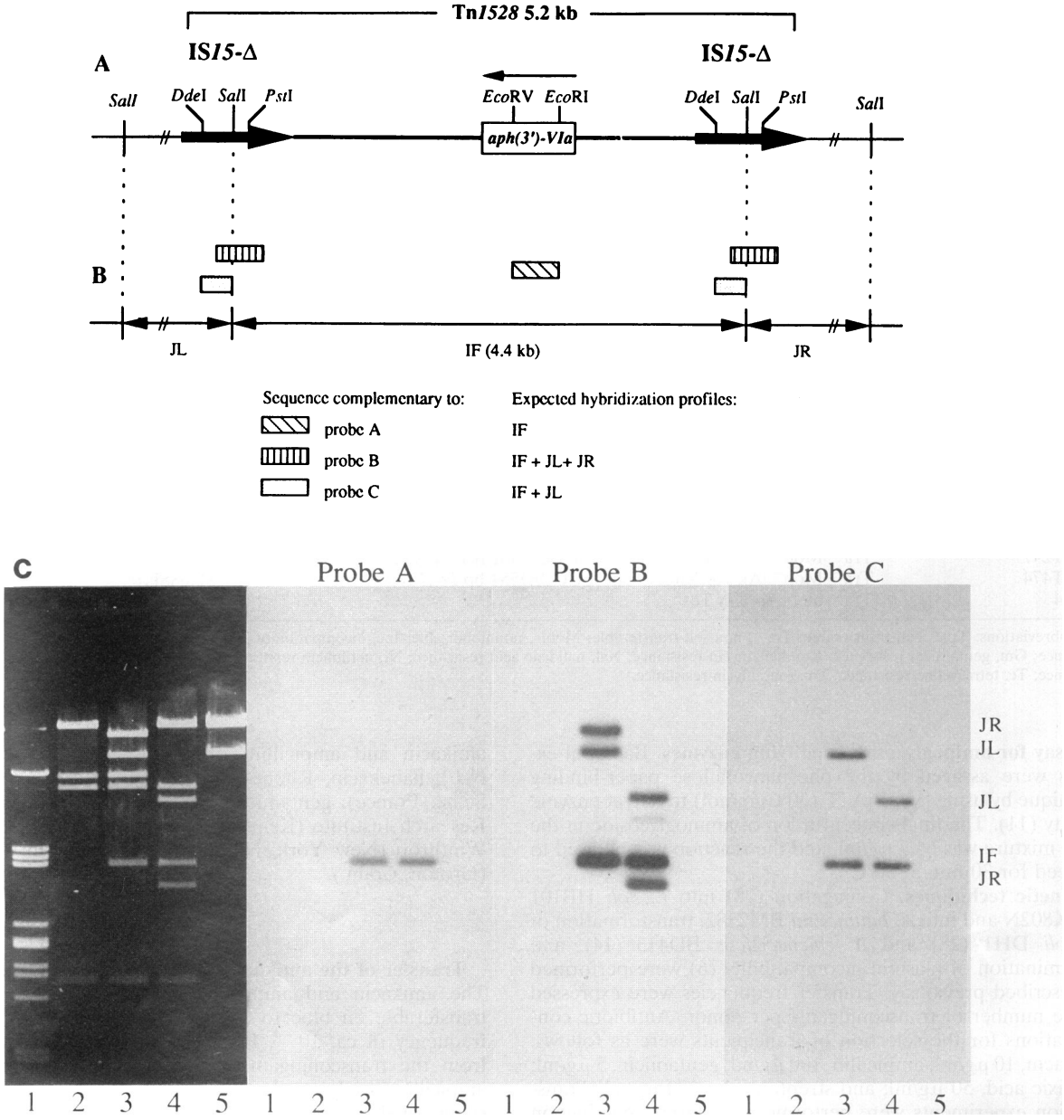


FIG. 1. Analysis of the structure of Tn*1528*. (A) Map of Tn*1528*. The thin arrow indicates the orientation of transcription of *aph(3')-VIa*; the thick arrows indicate the orientation of transcription of IS*15*-Δ. Only relevant restriction sites are shown. (B) Schematic representation of Southern blot analysis. IF, *Sal*I internal fragment of Tn*1528*; JL and JR, left and right *Sal*I junction fragments of Tn*1528*, respectively. (C) Analysis of plasmid DNA by agarose gel electrophoresis (left) and hybridization (right). Plasmid DNA was digested with *Sal*I, and the resulting fragments were separated by electrophoresis in a 0.8% agarose gel, transferred to a Nytran filter, and hybridized to the in vitro $^{32}$P-labeled probes as indicated at the top. Lanes 1 and 5, bacteriophage λ DNA digested with *Pst*I and *Sal*I, respectively; lanes 2, pOX38Gm DNA; lanes 3, pOX38Gm::Tn*1528*-1 DNA; lanes 4, pOX38Gm::Tn*1528*-2 DNA.

gene (24), hybridized to a 15-kb *Hin*dIII fragment of pIP1848 (data not shown). This fragment was also detected by the B probe, which is specific for IS*15*-Δ (15). The 15-kb fragment was purified and ligated to pUC18 linearized with *Hin*dIII, generating plasmid pAT246, which conferred resistance to amikacin (Table 1).

**Transposition of *aph(3')-VIa*.** Transposition of the *aph(3')-VIa* gene was studied by plasmid conduction (32) in *E. coli* by using pOX38Gm. Plasmid pOX38 is a conjugative F plasmid derivative which does not carry any known insertion sequence except a small region of IS*3*. pOX38Gm was constructed by cloning the *aac(3)-Ia* gene, which encodes gentamicin resistance, into pOX38 (23). Plasmid pAT246 (Tra$^-$ Mob$^-$ Ak$^r$ Ap$^r$) was introduced by transformation into *E. coli* DH1 (*recA* Nal$^r$) harboring pOX38Gm (Tra$^+$ Gm$^r$). Transfer by conjugation of amikacin resistance from *E. coli* DH1 (pOX38Gm pAT246) to *E. coli* HB101 (*recA* Str$^r$) was obtained. In three independent mating experiments, the antibiotic resistances of pAT246 and pOX38Gm were cotransferred, suggesting cointegrate formation. This observation is consistent with the fact that cointegration is an end product of the transposition process mediated by IS*15*-Δ (31). The frequency of cointegra-

tion, estimated as the frequency of transfer of pOX 38Gm::pAT246 relative to that of pOX38Gm, was $4 \times 10^{-5}$. Similar frequencies were previously reported for IS*15*-Δ (32). Under our experimental conditions, the insertion sequence-free pUC derivative pAT474 (Table 1) that was used as a negative control was not mobilized (frequency of less than $10^{-8}$).

**Stabilities of the cointegrates.** We compared the stabilities of four independent cointegrates in *recA* and *rec*$^+$ hosts. The cointegrates were introduced by conjugation into the *rec*$^+$ host *E. coli* K802N. Cells were selected on plates containing amikacin plus nalidixic acid. The *E. coli* HB101(pOX38Gm::pAT246) donors and the corresponding *E. coli* K802N (pOX38Gm::pAT246) recipients were grown for approximately 100 generations in the absence of antibiotic and were plated onto brain heart infusion agar, and the colonies were replica plated onto selective media containing amikacin plus gentamicin with and without ampicillin. All HB101 donors (252 colonies) grew on the two media, whereas approximately 20% of the 326 K802N recipients did not grow on plates containing ampicillin. This observation indicates that pOX 38Gm::pAT246 cointegrates are stable in a *recA* genetic background, whereas they are resolved in *rec*$^+$ bacteria. Cointegrates generated by IS*15*-Δ are stable in *recA* hosts since this element does not encode a site-specific resolvase (32). However, in the *rec*$^+$ background, homologous recombination occurs between the two copies of IS*15*-Δ which are present in direct orientation in cointegrates generated by replicative transposition (5, 32).

**Structure of the transposable element.** Two derivatives of pOX38Gm resulting from cointegrate resolution were compared with pOX38Gm (Fig. 1). Digestion of pOX38Gm DNA with *Sal*I generated five fragments of 36.3, 10.4, 8.8, 1.75, and 0.95 kb. One of the two plasmids lacked the 10.4-kb fragment and had three additional fragments with a total size of 15.6 kb. Thus, the plasmid differed from pOX38Gm by the insertion of a 5.2-kb element containing two *Sal*I sites. The 5.2-kb element, designated Tn*1528* (20), was inserted in a different locus of the second plasmid that lacked the 36.3-kb *Sal*I fragment. Hybridization with the A probe specific for *aph(3')-VIa* indicated that the resistance gene was located in a 4.4-kb *Sal*I fragment internal to Tn*1528* since this fragment was present in both plasmids. The IS*15*-Δ B probe hybridized to a total of three fragments including the internal one. Thus, the *aph(3')-VIa* gene was flanked by two copies of IS*15*-Δ. The 355-bp *Dde*I-*Sal*I fragment of IS*15*-Δ corresponding to the C probe was used to determine the orientation of IS*15*-Δ. If the two copies of IS*15*-Δ were present in opposite orientations, the C probe should hybridize to the 4.4-kb internal fragment (converging copies of IS*15*-Δ) or to the two-junction fragments (diverging copies of IS*15*-Δ). The probe hybridized to the internal fragment and to a single-junction fragment of each plasmid, indicating that the two copies of IS*15*-Δ are in the same orientation.

**Location of *aph(3')-VIa* on Tn*1528*.** The 4.4-kb *Sal*I fragment of Tn*1528* was cloned into pUC19, generating pAT247. This plasmid had unique sites for the restriction enzymes *Eco*RV and *Sph*I (data not shown). An *Eco*RV site is located 558 bp downstream from the start codon of the *aph(3')-VIa* gene (24), and the *Sph*I site is part of the polylinker in pUC19. Plasmid pAT247 DNA digested with *Eco*RV and *Sph*I generated two fragments of 5.1 and 2.0 kb. The A probe specific for *aph(3')-VIa* and the C probe internal to IS*15*-Δ hybridized with the 2-kb fragment. Taken together, these data indicate that *aph(3')-VIa* and the two copies of IS*15*-Δ are present in opposite orientations (Fig. 1).

**Dissemination of the *aph(3')-VIa* gene.** Plasmid DNAs from the strains of *E. coli*, *M. morganii*, and *P. mirabilis* isolated from the feces of the patient infected with *P. stuartii* BM2667 were digested with *Sal*I and analyzed by agarose gel electrophoresis and by Southern hybridization by using the *aph(3')-VIa* and IS*15*-Δ probes. The restriction and hybridization profiles observed were indistinguishable from those of BM2667, indicating the presence of Tn*1528* in these strains.

We studied strains two strains of *E. cloacae*, one strain of *E. coli*, and *P. aeruginosa* BM2683; these strains were resistant to amikacin and isepamicin, were susceptible to habekacin, and were presumed to encode an APH(3')-VI enzyme. The *aph(3')-VIa* gene was detected by dot blot hybridization in each strain. Transfer of amikacin resistance was obtained by conjugation from the *E. coli* strain and from one of the two *E. cloacae* strains and by mobilization with plasmid RP4 from *P. aeruginosa* BM2683. The total and plasmid DNAs of the four clinical isolates were digested with *Sal*I and were analyzed by Southern hybridization with the A and B probes. The *aph(3')-VIa* gene was present on plasmids in the strains that were able to transfer amikacin resistance and was found only in the total DNAs of the other remaining strains. In the four strains, the IS*15*-Δ B probe hybridized to the *Sal*I fragment that also contained *aph(3')-VIa*. A 4.4-kb *Sal*I fragment hybridizing to the *aph(3')-VIa* and IS*15*-Δ probes that should correspond to the central portion of Tn*1528* was detected only in *P. aeruginosa* BM2683. This fragment was part of a 15-kb Tra$^-$ Mob$^+$ plasmid in BM2683 (data not shown).

In *A. baumannii* BM2580, *aph(3')-VIa* is part of plasmid pIP1841, which is self-transferable to other *A. baumannii* strains (18). Following subculture in the absence of antibiotic for approximately 100 generations, we obtained clones that were no longer able to transfer amikacin resistance. Southern analysis of *Hin*dIII or *Sal*I digests of the total and plasmid DNAs of the parental strain and of three such derivatives indicated that *aph(3')-VIa* is located on differently sized fragments in BM2580 and in the three derivatives that also contained IS*15*-Δ. Although none of these fragments comigrate with the 4.4-kb *Sal*I fragment internal to Tn*1528*, these data indicate that in *A. baumannii* BM2580 the *aph(3')-VIa* gene can translocate from pIP1841 to the host chromosome and that IS*15*-Δ may be involved in the transposition process.

## DISCUSSION

We showed that the amikacin resistance determinant in plasmid pIP1848 is located on a 5.2-kb transposable element designated Tn*1528*. This transposon is a typical composite element (for a recent review, see reference 10) in which the amikacin resistance gene is flanked by two directly repeated copies of IS*15*-Δ (Fig. 1). Insertion sequence IS*15*-Δ (15, 16) is widely spread in gram-negative bacteria including *Acinetobacter* spp. (16). This ubiquitous element is closely related to IS*6*, (1), IS*26* (25), IS*46* (5), IS*140* (4), IS*160* (26), and IS*176* (35). Failure to detect, in plasmid conduction experiments, the translocation of amikacin resistance alone confirms that the 820-bp insertion sequence (31) essentially mediates the formation of cointegrates and does not encode a site-specific resolvase (32). The instability of the cointegrates, when present in a *rec*$^+$ host, is due to intramolecular recombination between the two copies of IS*15*-Δ (32). Transposon Tn*1528* confers resistance to amikacin and structurally related aminoglycosides, including isepamicin, by synthesis of aminoglycoside 3′-*O*-phosphotransferase type VI, an enzyme which was confined so far to *Acinetobacter* spp. Because of the transposition event that it mediates, it is likely that IS*15*-Δ played a role in the mobilization of the corresponding gene, *aph(3')-VIa*, from

this bacterial genus to members of the families *Enterobacteriaceae* and *Pseudomonadaceae*. The facts that the structural genes for the enzyme and IS*15*-Δ were found to coreside in *E. coli* and *E. cloacae* and were physically linked in a Tn*1528*-like structure in *P. aeruginosa* are further support for this notion. Moreover, transposition of IS*15*-Δ could account for the presence, in certain strains of *A. baumannii*, of two nontandemly repeated copies of *aph(3')-VIa* (17) and for the migration of the resistance determinant from a plasmid to the chromosome in strain *A. baumannii* BM2580.

Members of the IS*15*-Δ family have been shown to be responsible for dissemination of the kanamycin resistance gene *aph(3')-Ia* (12, 15) and the *aac(3)-IIa* gene, which confers resistance to gentamicin, netilmicin, and tobramycin (4). They were also found to be associated with the genes *hphB*, which specifies a hygromycin B phosphotransferase, and *aac(3)-IVa*, which mediates resistance to apramycin, gentamicin, netilmicin, and tobramycin (4). Results of the present study indicate that this transposable module is, in addition, involved in the spread of amikacin and isepamicin resistance in strains of the families *Enterobacteriaceae* and *Pseudomonadaceae* and in *Acinetobacter* spp.

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## REFERENCES

1. **Berg, D. E., J. Davies, B. Allet, and J. D. Rochaix.** 1975. Transposition of R factor genes to bacteriophage lambda. Proc. Natl. Acad. Sci. USA **72**:3628–3632.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
4. **Bräu, B., and W. Piepersberg.** 1983. Cointegrational transduction and mobilization of gentamicin resistance plasmid pWP14a is mediated by IS*140*. Mol. Gen. Genet. **189**:298–303.
5. **Brown, A. M., G. M. Coupland, and N. S. Willets.** 1984. Characterization of IS*46*, an insertion sequence found on two IncN plasmids. J. Bacteriol. **159**:472–481.
6. **Chabbert, Y. A., M. R. Scavizzi, J. L. Witchitz, G. R. Gerbaud, and D. H. Bouanchaud.** 1972. Incompatibility groups and classification of fi⁻ resistance factors. J. Bacteriol. **122**:666–675.
7. **Datta, N., R. W. Hedges, E. J. Shaw, R. B. Sykes, and M. H. Richmond.** 1971. Properties of an R factor from *Pseudomonas aeruginosa*. J. Bacteriol. **108**:1244–1249.
8. **Davies, J.** 1991. Aminoglycoside-aminocyclitol antibiotics and their modifying enzymes, p. 691–713. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
9. **Galas, D. J., and M. Chandler.** 1982. Structure and stability of Tn*9*-mediated cointegrates, evidence for two pathways of transposition. J. Mol. Biol. **154**:245–272.
10. **Galas, D. J., and M. Chandler.** 1989. Bacterial insertion sequences, p. 109–162. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
11. **Haas, M. J., and J. E. Dowding.** 1975. Aminoglycoside-modifying enzymes. Methods Enzymol. **43**:611–628.
12. **Iida, S., J. Meyer, P. Linder, N. Goto, R. Nakaya, H. J. Reif, and W. Arber.** 1982. The kanamycin resistance transposon Tn*2680* derived from the R plasmid Rts1 and carried by phage pIKm has flanking 0.8-kb-long direct repeats. Plasmid **8**:187–198.
13. **Johnson, D. A., J. Sportsman, and J. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Technol. **1**:3–8.
14. **Juni, E.** 1972. Interspecies transformation of *Acinetobacter*: genetic evidence for a ubiquitous genus. J. Bacteriol. **112**:917–931.
15. **Labigne-Roussel, A., and P. Courvalin.** 1983. IS*15*, a new insertion sequence widely spread in R plasmids of gram-negative bacteria. Mol. Gen. Genet. **189**:102–112.
16. **Labigne-Roussel, A., G. Gerbaud, and P. Courvalin.** 1981. Translocation of sequences encoding antibiotic resistance from the chromosome to a receptor plasmid in *Salmonella ordonez*. Mol. Gen. Genet. **182**:390–408.
17. **Lambert, T., G. Gerbaud, P. Bouvet, J. F. Vieu, and P. Courvalin.** 1990. Dissemination of amikacin resistance gene *aphA6* in *Acinetobacter* spp. Antimicrob. Agents Chemother. **34**:1244–1248.
18. **Lambert, T., G. Gerbaud, and P. Courvalin.** 1988. Transferable amikacin resistance in *Acinetobacter* spp. due to a new type of 3'-aminoglycoside phosphotransferase. Antimicrob. Agents Chemother. **32**:15–19.
19. **Lambert, T., G. Gerbaud, and P. Courvalin.** 1993. Characterization of *Acinetobacter haemolyticus aac(6')-Ig* gene encoding an aminoglycoside 6'-*N*-acetyltransferase which modifies amikacin. Antimicrob. Agents Chemother. **32**:2093–2100.
20. **Lederberg, E. M.** 1981. Plasmid reference center registry of transposon (Tn) allocations through July 1981. Gene **16**:59–61.
21. **Low, B.** 1968. Formation of merodiploids in matings with a class of Rec⁻ recipient strains of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **60**:160–167.
22. **Mabilat, C., and P. Courvalin.** 1990. Development of "oligotyping" for characterization and molecular epidemiology of TEM β-lactamases in members of the family *Enterobacteriaceae*. Antimicrob. Agents Chemother. **34**:2210–2216.
23. **Makris, J. C., P. L. Nordmann, and W. S. Reznikoff.** 1988. Mutational analysis of insertion sequence *50* (IS*50*) and transposon 5 (Tn*5*) ends. Proc. Natl. Acad. Sci. USA **85**:2224–2228.
24. **Martin, P., E. Jullien, and P. Courvalin.** 1988. Nucleotide sequence of *Acinetobacter baumannii aphA-6* gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. Mol. Microbiol. **2**:615–625.
25. **Mollet, B., S. Iida, J. Shepherd, and W. Arber.** 1983. Nucleotide sequence of IS*26*, a new prokaryotic mobile genetic element. Nucleic Acids Res. **11**:6319–6330.
26. **Nies, B. A., J. F. Meyer, and B. Wiedemann.** 1985. Tn*2440*, a composite tetracycline resistance transposon with direct copies of IS*160* at its flanks. J. Gen. Microbiol. **131**:2443–2447.
27. **Roussel, A. F., and Y. A. Chabbert.** 1978. Taxonomy and epidemiology of gram-negative bacterial plasmids studied by DNA-DNA filter hybridization in formamide. J. Gen. Microbiol. **104**:269–276.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Shaw, K. J., R. S. Hare, F. J. Sabatelli, M. Rizzo, C. A. Cramer, L. Naples, S. Kocsi, H. Munayyer, P. Mann, G. H. Miller, L. Verbist, H. Van Landuyt, Y. Glupczynski, M. Catalano, and M. Woloj.** 1991. Correlation between aminoglycoside resistance profiles and DNA hybridization of clinical isolates. Antimicrob. Agents Chemother. **35**:2253–2261.
30. **Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller.** 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol. Rev. **57**:138–163.
31. **Trieu-Cuot, P., and P. Courvalin.** 1984. Nucleotide sequence of the transposable element IS*15*. Gene **30**:113–120.
32. **Trieu-Cuot, P., and P. Courvalin.** 1985. Transposition behavior of IS*15* and its progenitor IS*15*-Δ: cointegrates are exclusive end products. Plasmid **14**:80–89.
33. **Woloj, M., M. Catalano, K. J. Shaw, F. Sabatelli, R. S. Hare, and G. H. Miller.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 676.
34. **Wood, W. B.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118–133.
35. **Wrighton, C. J., and P. Strike.** 1987. A pathway for the evolution of the plasmid NTP16 involving the novel kanamycin resistance transposon Tn*4352*. Plasmid **17**:37–45.
36. **Yanish-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.