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Construction of a Gentamicin Resistance Gene Probe for Epidemiological Studies

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A 7.7-kilobase *Bam*HI fragment was cloned from the transconjugant of a clinical isolate of *Escherichia coli* containing a 120-kilobase multiresistance IncC plasmid. The recombinant plasmid conferred resistance to kanamycin, gentamicin, tobramycin, sulfamethoxazole, and trimethoprim. This clone was used to generate a series of subclones from which a 2.0-kilobase *Bam*HI-*Hin*dIII probe containing a gentamicin 2″-*O*-adenylyltransferase [AAD(2″)] gene was obtained. This probe hybridized specifically in both colony and Southern hybridizations with the AAD(2″) gene but not with other resistance genes, including other aminoglycoside-modifying genes, or with a reference IncC plasmid lacking the AAD(2″) gene. The AAD(2″) gene may be part of a transposon, since hybridization occurred with both nonconjugative plasmids and the chromosome in some isolates.

Organisms harboring multiresistance plasmids have become a major cause of patient morbidity and mortality in hospitals since the use of antibiotics was begun (8, 25, 33). The properties of plasmids and mechanisms of transfer between organisms have been well described (6, 17). However, it has not been established whether these plasmids evolve in the hospital or are introduced, or what factors lead to the persistance of some plasmids once established in the environment.

These plasmids, which often carry genes for resistance to many unrelated antibiotics, have various structures and have arisen in response to a number of selective environmental effects, including the patient population, the procedures and instruments used in patient care, antibiotic usage patterns, the location of the hospital, cross-infection between patients and staff, and the properties of the individual plasmids and the bacteria that harbor them (33). It has been shown that conjugative plasmids bearing no resistance markers but belonging to present-day incompatibility groups existed before the advent of antibiotics (12). It therefore seems that the emergence of new resistance plasmids is the result of the insertion of new genes, probably on transposons, into existing plasmids (37).

A study of the epidemiology of resistance genes by using specific resistance gene probes could answer questions about the origins and evolution of multiresistance in the hospital environment. In this paper we describe the construction and characterization of a gentamicin resistance (Gm^r) gene probe which can be used to identify homologous genes in clinical isolates by colony and Southern hybridization techniques.

MATERIALS AND METHODS

Bacterial strains. Clinical isolates were obtained from patients at Royal North Shore Hospital of Sydney, Sydney, Australia. Susceptibilities to antibiotics were determined by agar dilution (43), and identification of the organisms was carried out by a replica plating method for biochemical analysis (28). Standard strains and plasmids are listed in Table 1. Other isolates used were from clinical specimens.

Antibiotics. Antibiotics were obtained from the following sources: amikacin, Bristol Myers; ampicillin, Commonwealth Serum Laboratories; chloramphenicol, Parke-Davis; gentamicin, Essex Laboratories; kanamycin, Bristol Myers; neomycin, Takeda Chemical Industries; rifampin, Ciba-Geigy; streptomycin, Glaxo; sulfamethoxazole, Wellcome Australia; tetracycline, Upjohn Pharmaceuticals; tobramycin, Lilly Industries Pty. Ltd.; and trimethoprim, Wellcome Australia.

Incompatibility grouping. Incompatibility grouping was carried out as described by Datta (10).

Plasmid selected for construction of the probe. A plasmid (IncC) belonging to incompatibility group C in a clinical isolate of *Escherichia coli*, VA292, was selected for the construction of the probe. IncC is common among multiresistance plasmids in clinical isolates at Royal North Shore Hospital. The MIC of gentamicin for organisms containing this group of plasmids was always greater than 5 μg/ml and in many instances was greater than 20 μg/ml. *E. coli* VA292 was resistant to ampicillin (MIC, >100 μg/ml), chloramphenicol (MIC, >8 μg/ml), gentamicin (MIC, >5 μg/ml), kanamycin (MIC, >20 μg/ml), neomycin (MIC, >20 μg/ml), sulfamethoxazole (MIC, >200 μg/ml), tobramycin (MIC, >5 μg/ml), and trimethoprim (MIC, >20 μg/ml). Many strains containing IncC plasmids are also resistant to streptomycin and tetracycline. These were not chosen for two reasons: (i) the recipient organism *E. coli* RR1 that was used in transformation experiments was chromosomally resistant to streptomycin, and (ii) the loss of tetracycline resistance from the vector plasmid pBR322 (ampicillin and tetracycline resistant [Ap^r Tc^r]) assists in identifying clones in which DNA has been inserted in the Tc^r gene.

Conjugation. Conjugation of *E. coli* VA292 and *E. coli* JP995 in broth culture was carried out by standard methods

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TABLE 1. Strains and plasmids used

Plasmid or strain	Antibiotic phenotype[a]	History or reference
Bacterial strains		
VA292	Ap Cm Gm Km Nm Su Tm Tp	Clinical isolate of *E. coli*, IncC, AAD(2″), APH(3′)
E292	Ap Cm Gm Km Nm Su Tm Tp	Transconjugant of *E. coli* JP995, IncC, AAD(2″), APH(3′)
VA272	Ap Gm Km Su Tm	Clinical isolate of *E. coli*, IncC, AAD(2″)
VA273	Ap Gm Km Sm Su Tc Tm Tp	Clinical isolate of *K. pneumoniae*, IncC, AAD(2″)
VA274	Ap Gm Km Su Tm	Clinical isolate of *E. aerogenes*, IncC, AAD(2″)
VA77	Ap Tc	Clinical isolate of *E. coli*
JP995	Rf	20
RR1	Sm	4
Plasmids		
pDGO101	Ap Gm Km Su Tm Tp	*Bam*HI[b] derived from *E. coli* E292
pDGO103	Ap Gm Km Su Tm	*Eco*RI-*Bam*HI derived from pDGO101
pDGO106	Ap Gm Km Tc Tm	*Hin*dIII derived from pDGO101
pDGO109	Ap Gm Km Tm	*Hin*dIII derived from pDGO101
pDGO112	Gm Km Tm	*Pvu*I derived from pDGO103
pDGO113	Ap Gm Km Tm	*Pvu*I derived from pDGO103
pDGO114	Gm Km Tm	*Pvu*I derived from pDGO103
pDGO116	Ap Gm Km (Su) Tm	*Pvu*I derived from pDGO101
R40a	Ap Km Su	Reference IncC, APH(3′)-1
R391	Km	Reference IncJ, APH(3′)-1
R478	Cm Km Tc	Reference IncH, APH (3′)-1

[a] Antibiotic resistance abbreviations: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Nm, neomycin; Rf, rifampin; Sm, streptomycin; Su, sulphamethoxazole; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim.
[b] Restriction endonuclease used to obtain cloned fragment.

(9). Transconjugants were selected on DST agar (Oxoid Ltd.) containing 2 μg of gentamicin and 100 μg of rifampin per ml.

Preparation of total DNA. Transconjugant cells of *E. coli* JP995 containing the clinical IncC plasmid were grown in 1 liter of Luria-Bertani (LB) broth at 35°C for 16 h. The cells were lysed by lysozyme-EDTA incubation (31), followed by treatment with 0.5% sodium dodecyl sulfate (SDS)–proteinase K (100 μg/ml) and incubation at 45°C overnight. The mixture was dialyzed for 24 h against 5 liters of 50 mM Tris-hydrochloride–50 mM EDTA (pH 8.0). Heat-inactivated RNase A (final concentration, 100 μg/ml) and RNase T1 (final concentration, 1 μg/ml) were added. After another round of SDS-proteinase K treatment, the DNA was extracted with phenol and dialyzed before restriction endonuclease digestion.

Isolation of DNA from clinical isolates. Minipreparations of DNA from clinical isolates were made by the method of Kieser (26).

Restriction digests and electrophoresis. Restriction enzymes were obtained from Boehringer Mannheim Biochemicals, Bethesda Research Laboratories, Pharmacia Fine Chemicals, or New England BioLabs and were used either as specified by the manufacturers or with general buffer systems (31). Restriction fragments were resolved in 0.5, 0.8, or 2.5% agarose run at 7 V/cm for about 1 h. In all cases the running buffer was 40 mM Tris base–2 mM EDTA adjusted to pH 7.9 with acetic acid. Gels were stained for 20 min in 1 μg of ethidium bromide per ml before visualization at 302 nm with a transilluminator (model 302, Spectroline TR). Gels were then photographed through a no. 9 Wratten filter (Eastman Kodak Co.) with a Polaroid MP-4 camera and Polaroid type 665 film. Fragments were extracted from gels by electrophoresis onto a dialysis membrane (31).

Ligation. Ligation of insert DNA to pBR322 was carried out by using T4 DNA ligase under standard conditions (31). The concentration of vector and insert DNA was determined by the criteria of Dugaiczyk et al. (15). Subclones were generated by digestion with various restriction enzymes followed by ligation in the presence of vector DNA under similar conditions.

Transformation and selection of clones. Ligated DNA was transformed into *E. coli* RR1 by standard methods (4). A 2-ml sample of transformants in recovery medium was suspended in 20 ml of 0.4% SeaPlaque agar (FMC Corp.) in LB broth kept liquid at 37°C. Transformants were selected on media containing 2 μg of gentamicin per ml, 20 μg of kanamycin per ml, 200 μg of sulfamethoxazole per ml, or 20 μg of trimethoprim per ml after overlaying the plates with 1 ml of the transformed bacterial suspension per plate, cooling the plates to solidify the agar, and then incubating them at 37°C overnight. Clones from the antibiotic plates were subcultured to microtiter walls containing 150 μl of LB broth, then replicated onto selective plates, and incubated at 37°C overnight.

Restriction mapping. Plasmid DNA from the subclones was isolated from an amplified culture (31) and precipitated with polyethylene glycol 6000 (24). The DNA was digested with a number of restriction endonucleases, and the fragments were analyzed by gel electrophoresis as described above. For multiple restriction digestions with different buffer requirements, the DNA was precipitated with ethanol and redissolved before digestion with the second enzyme.

Nick translation. Nick translation of the probe was carried out by standard methods (31). The nick-translated DNA was separated from the unreacted nucleotides by passing the reaction mixture over a Sephadex G-50 column and collecting the first radioactive peak.

Southern blot hybridization. Southern blot hybridizations were carried out on GeneScreen (New England Nuclear Corp.) as specified by the manufacturer, except for the washing procedure. For the first wash the hybridization bag was opened, the probe and the hybridization mixture were discarded, and 20 to 30 ml of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS was added. The bag was resealed and incubated at 42°C for 10 to 15 min.

The filter was then removed and washed in 2× SSC–0.1% SDS for 30 min at 42°C, followed by a 30-min wash at 60°C in 0.1× SSC–0.5% SDS.

Colony hybridization. Bacteria were transferred to Whatman no. 541 filter paper for colony hybridization (30). The hybridization procedure was done by the method of Gergen et al. (19). The filters were washed for 30 min at 37°C in 2× SSC–0.1% SDS and then twice for 30 min at 60°C in 0.1× SSC–0.5% SDS. They were then blotted and air dried at 37°C.

Autoradiography. Autoradiography was carried out by wrapping the filters in plastic wrap and exposing them to X-ray film at −70°C with two intensifying screens for 16 to 48 h.

Identification of aminoglycoside-modifying enzymes. Identification of aminoglycoside-modifying enzymes by the disk method was carried out by the method of van de Klundert et al. (42).

Preparation of crude enzyme extracts. A crude enzyme extract was made from *E. coli* RR1 carrying pDGO114 and pDGO116 (23).

Enzyme assays. The radiochemical phosphocellulose binding assay was used for the measurement of adenylyltransferase and acetyltransferase (23). All reactions were carried out in a volume of 100 μl. The adenylyltransferase assay contained 10 μl of [*U*-^{14}C]ATP (5 nmol, 40 μCi/μmol; New England Nuclear), 10 μl of antibiotic (1 mg/ml), 30 μl of buffer (100 mM Tris-hydrochloride [pH 7.8], 100 mM $MgCl_2$, 1.5 mM EDTA, 1 mM dithiothreitol), and 50 μl of crude enzyme extract. The acetyltransferase reaction contained [*U*-^{14}C]acetyl coenzyme A (10 nmol, 20 μCi/μmol), 10 μl of antibiotic (1 mg/ml), and 30 μl of buffer (100 mM Tris-maleate [pH 7.2], 30 mM $MgCl_2$, 1.5 mM dithiothreitol).

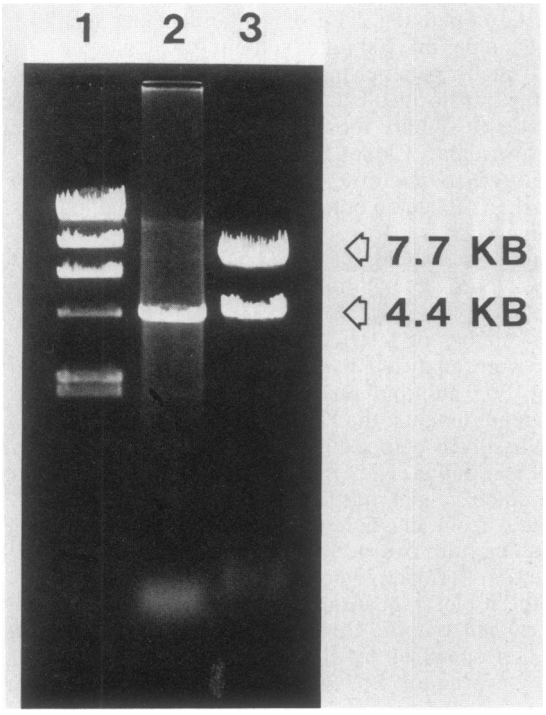

FIG. 1. Agarose gel electrophoresis of the cloned fragment from *E. coli* E292. Lanes: 1, *Hin*dIII digest of lambda; 2, *Bam*HI digest of pBR322; 3, *Bam*HI digest of pDGO101.

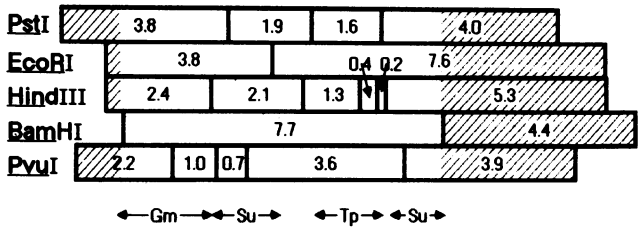

FIG. 2. Restriction endonuclease map of pDGO101. Shaded area, pBR322; unshaded area, cloned DNA. Fragment sizes are given in kilobases. Approximate locations of the genes are indicated by arrows. Antibiotic abbreviations: Gm, gentamicin; Su, sulfamethoxazole; Tp, trimethoprim.

Total aminoglycoside modification with ATP as cofactor was measured by bioassay (7).

RESULTS

Recombinant clones. A recombinant clone, pDGO101, selected on 2 μg of gentamicin per ml, contained a 7.7-kilobase (kb) *Bam*HI fragment carrying Km^r, Gm^r, Tm^r, Su^r, and Tp^r resistance genes (Fig. 1). A restriction map of the recombinant plasmid was generated by digestion of the DNA with combinations of the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, and *Pvu*I (Fig. 2). The insertion of the fragment into the *Bam*HI site inactivated the Tc^r gene of pBR322 but left the Ap^r gene functional. In subclones in which the Ap^r gene was cut, e.g., pDGO111 (which comprises the 3.6- and 3.9-kb *Pvu*I fragments of pDGO101 [Fig. 2]) and pDGO112 (which comprises the 4.6-, 1.0-, and 0.7-kb *Pvu*I fragments of pDGO103 [Fig. 3]), no ampicillin resistance was expressed. These two clones contain separate pieces of insert DNA that span the entire 7.7-kb *Bam*HI fragment. In addition to this, a Su^r gene has been mapped which spans the *Pvu*I site separating the 0.7- and 3.6-kb fragments (Fig. 2). This led us to conclude that a functional Ap^r gene from *E. coli* E292 was absent in the cloned fragment. Plasmid DNA from pDGO101 and pDGO103 was used to derive all of the subclones listed in Table 1.

The following subclones were selected for their suitability as Gm^r probes; pDGO103 (Ap^r Km^r Gm^r Tm^r Su^r) and pDGO109 (Ap^r Km^r Gm^r Tm^r), which were *Bam*HI-*Eco*RI and *Hin*dIII subclones of pDGO101, respectively; and pDGO112 (Km^r Gm^r Tm^r), pDGO113 (Ap^r Km^r Gm^r Tm^r), and pDGO114 (Km^r Gm^r Tm^r), which were *Pvu*I subclones of pDGO103. A restriction map of these selected subclones (Fig. 3) shows that pDGO112, pDGO113, and pDGO114 had a 4.6- and a 1.0-kb *Pvu*I fragment in common. The 1.0-kb *Pvu*I fragment overlaps the 2.4-kb *Hin*dIII fragment of pDGO109, which carries the Km^r, Gm^r, and Tm^r gene. This gene does not have *Bam*HI, *Eco*RI, *Hin*dIII, or *Pst*I sites but may contain a *Pvu*I site, since we have not obtained any subclones in which the 1.0-kb *Pvu*I fragment was separated from the 4.6-kb *Pvu*I fragment (Fig. 3).

Tetracycline resistance is expressed in the clone pDGO106; this may be due to the 2.4-kb *Hin*dIII fragment's carrying a promoter which allows expression of the otherwise nonfunctional Tc^r gene from pBR322 (44). The subclone pDGO109 has this same fragment in the reverse orientation, as determined by restriction mapping with *Pvu*I, but is susceptible to tetracycline.

Nature of the aminoglycoside-modifying enzymes. The aminoglycoside-modifying enzymes, adenylyltransferases,

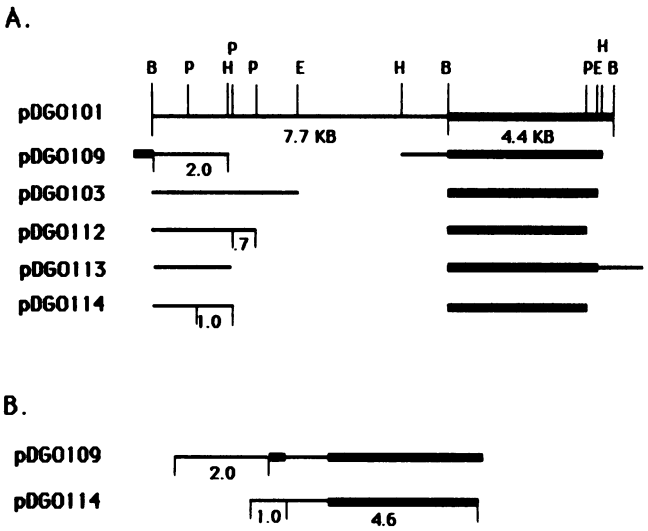

FIG. 3. Restriction map of selected subclones. Plasmids pDGO103 and pDGO109 were *Hin*dIII and *Bam*HI-*Eco*RI derivatives of pDGO101, respectively. Restriction sites are indicated above pDGO101. Abbreviations: B, *Bam*HI; P, *Pvu*I; H, *Hin*dIII; E, *Eco*RI. All other subclones were *Pvu*I derivatives of pDGO103. (A) Fragments are in the same orientation as in pDGO101. (B) Actual orientation of the fragments in the subclones.

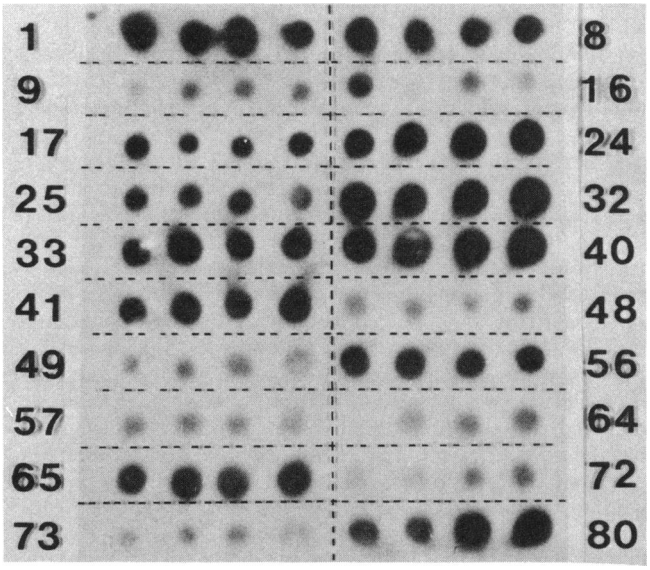

FIG. 4. Autoradiograph of the colony hybridization with a *Bam*HI-*Hin*dIII 2.0-kb fragment of pDGO103 as the probe. Wells: 1 through 4, VA292; 5 through 8, E292; 9 through 12, *E. coli* RRI; 13, E292; 14 through 16, *E. coli* JP995, 17 through 20, pDGO101; 21 through 24, pDGO103; 25 through 28, pDGO106; 29 through 32, pDGO109; 33 through 36, pDGO112; 37 through 40, pDGO113; 41 through 44, pDGO114; 45 through 48, R391; 49 through 52, R478; 53 through 56, VA272; 57 through 60, VA273; 61 through 64, VA274; 65 through 68, VA279; 69 through 72, R40a; 73 through 76, VA77; 77 through 80, pDGO116. R40a is a reference IncC plasmid. R391, R478, and R40a code for the phosphotransferase APH(3′). *E. coli* VA77, VA272, VA273, VA274, and VA279 are clinical isolates (Table 1).

acetyltransferases, and phosphotransferases, have different substrate profiles (14). All the clones that conferred resistance to gentamicin also conferred resistance to kanamycin and tobramycin but were susceptible to neomycin, indicating that they did not contain a functional phosphotransferase gene [APH(3′)]. On the basis of this resistance profile, it appeared that the enzyme was a 2″-*O*-adenylyltransferase [AAD(2″)] (14, 21). This was tested and confirmed by assaying aminoglycoside-modifying enzymes (Table 2). The substrate affinity of the enzyme for gentamicin, kanamycin, and tobramycin was approximately the same. There was no affinity for amikacin and no detectable acetyltransferase activity.

The clinical isolate *E. coli* VA292 and its transconjugant *E. coli* E292 were resistant to neomycin and were shown by bioassay (7) and the disk method (42) to contain a phosphotransferase. When total adenylyltransferase and phosphotransferase activity in pDGO114 was measured by bioassay, there was no increased inactivation of kanamycin or any inactivation of neomycin, confirming that there was no phosphotransferase activity.

Southern blot and colony hybridizations. The 2.4-kb *Hin*dIII fragment from pDGO109 and the 1.0-kb *Pvu*I fragment from pDGO114 were isolated by gel electrophoresis and nick translated for use as probes. Southern blotting hybridizations showed that both fragments hybridized successfully to the plasmid band of the Gm^r subclones (data not shown). However, the 1.0-kb fragment also hybridized with subclones that did not contain the Gm^r gene but conferred resistance to sulfamethoxazole. This indicated that the 1.0-kb fragment overlapped the Su^r gene, which maps adjacent to the Gm^r gene. The 2.4-kb *Hin*dIII fragment did not hybridize with subclones that contained Su^r genes but no Gm^r gene. Additional hybridization only occurred with the DNA from pBR322. This fragment appeared to be the most suitable for developing a probe.

The 2.4-kb fragment was further trimmed with *Bam*HI to yield a 2.0-kb fragment which did not contain any of the pBR322 DNA to test for homology with the other subclones by colony hybridization. For convenience in isolating the 2.0-kb *Bam*HI-*Hin*dIII fragment, pDGO103 was used (*Bam*HI-*Hin*dIII digestion of pDGO109 yields two similar-sized fragments). Strong hybridization was observed specifically with all Gm^r subclones.

For colony hybridization on Whatman no. 541 filter paper, the time of incubation of the plates was important for obtaining specific hybridization. Growth for 6 h at 37°C gave the best results. Longer incubation times gave increasing nonspecific hybridization due to excessive bacterial debris left on the filters, and shorter incubation times resulted in low levels of DNA on the filters and poor hybridization. Figure 4 shows the results obtained by using the 2.0-kb *Bam*HI-*Hin*dIII probe against a set of clones, clinical isolates, and strains containing reference plasmids. There was strong hybridization with clones that contained known homologous genes, including the original clinical isolate *E. coli*

TABLE 2. Aminoglycoside-modifying activity of crude enzyme extracts

Antibiotic	Adenylyltransferase activity[a]	Acetyltransferase activity
Gentamicin	14.7	0
Kanamycin	13.8	0
Tobramycin	12.7	0
Amikacin	0	0

[a] Activity is expressed as nanomoles of substrate modified per 30 min per milligram of protein.

VA292 and its transconjugant *E. coli* E292. There was no hybridization with *E. coli* RR1, the host for the clones, *E. coli* JP995, which contained no plasmid, or with organisms containing reference plasmids R391 (IncJ), R478 (IncH), and R40a (IncC), which all contained the APH(3′) gene. There was hybridization with two of the clinical isolates that were classified as IncC (*E. coli* VA272, VA279) and shown to contain AAD(2″) by the disk method. In separate experiments, clinical isolates that contained the acetyltransferase gene [AAC(3)-1] or APH(3′)-1 were shown not to hybridize.

In Southern hybridizations, the 2.0-kb fragment hybridized with the conjugative plasmid in *E. coli* VA292 and its transconjugant *E. coli* E292. There was also hybridization in *E. coli* VA292 with a band corresponding to the chromosome (Fig. 5). In other clinical isolates, hybridization commonly occurred with more than one plasmid band, including lower-molecular-weight, nonconjugative plasmids. This suggested that the ADD(2″) gene may be on a mobile genetic element.

Weak hybridization was shown to occur with the plasmid band of *Klebsiella pneumoniae* VA273, one of the IncC clinical isolates shown to contain AAD(2″) that did not hybridize on colony hybridization. No hybridizations occurred with reference plasmid R478 containing the gene for APH(3′)-I.

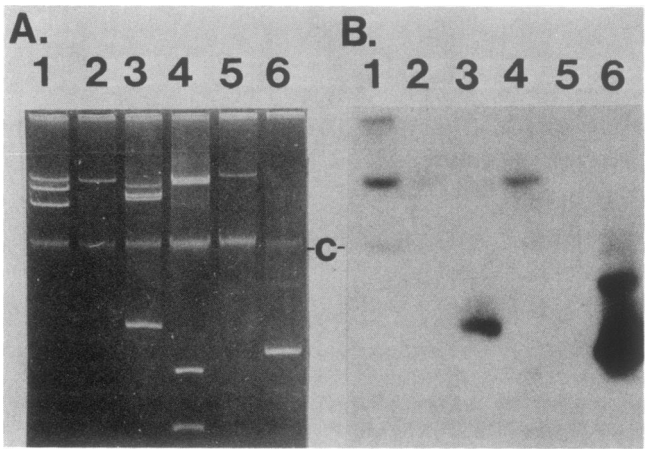

FIG. 5. Gel electrophoresis and Southern hybridization of selected isolates with the 2.0-kb *Bam*HI-*Hin*dIII probe. (A) Lanes: 1, VA292; 2, E292; 3, VA272; 4, VA273; 5, R478; 6, pDGO103. (B) Autoradiograph of the probed gel. -C-, Chromosome.

DISCUSSION

Specific DNA probes are a convenient way of detecting homologous genes and have a wide range of applications (2, 18, 27, 34, 40). In this paper we have described and characterized an AAD(2″) probe that can be used in epidemiological studies with Southern and colony hybridization techniques.

Previously, studies of plasmid epidemiology have been difficult because selective pressures in the clinical environment cause plasmids to undergo rapid structural changes (38, 39). When these changes are small, as may happen during an outbreak of disease caused by multiresistant organisms in hospitals, the endonuclease restriction profiles of isolated plasmids may be similar, showing that the same plasmid is involved in the epidemic (13, 41). However, high degrees of DNA homology, possibly corresponding to resistance determinants, have been shown on plasmids which seem to be otherwise unrelated epidemiologically (32, 39). The demonstration of different plasmid restriction endonuclease patterns does not therefore preclude homology. Whole clinical plasmids are too large and contain too much diverse genetic material to be useful as probes.

In our studies the 2-kb probe hybridized only with organisms containing the AAD(2″) gene. No hybridization occurred with organisms containing AAC(3)-1 or APH(3′)-1 genes but not AAD(2″). A colony hybridization technique was used in this study, as a large number of isolates can be processed at one time (22). The use of Whatman filter papers as the support membrane is inexpensive, and the filters can be stripped of the probe and reused many times (30). So far, no false-positives have been shown to occur and hybridization has been confirmed in Southern hybridizations. However, false-negatives occur, as shown by *K. pneumoniae* VA273 and *Enterobacter aerogenes* VA274, which were negative in colony hybridization (Fig. 4) but positive in Southern hybridizations (Fig. 5). This may be due to the low copy number of large plasmids in some clinical isolates.

There is evidence that the AAD(2″) gene probe described in this paper is part of a transposon, since hybridization of the probe was also shown to occur with nonconjugative plasmids and with the chromosome in different clinical isolates (e.g., *E. coli* VA292, Fig. 5). This has been shown to occur with other transposons (16, 29). *E. coli* VA292 was isolated during an epidemic of gentamicin-resistant gram-negative rods in this hospital, and this is consistent with the concept of transposition of an AAD(2″) to a common conjugative plasmid, in this case to an IncC plasmid. Transposon Tn*732*, which also codes for an AAD(2″), was responsible for the spread of gentamicin resistance in Toronto hospitals (35). The movement of such resistance genes can only be followed by the use of specific probes, since small changes in plasmid size cannot be detected by gel electrophoresis. A changing restriction pattern is meaningless as far as identifying specific genes is concerned.

No sequencing data are available on the AAD(2″) gene, but the genes which code for the phosphotransferase enzymes APH(3′)-I and APH(3′)-II and the acetyltransferase gene AAC(3)-IV have been sequenced, and their average size is approximately 800 bases (3, 5, 36). The AAD(2″) gene is about the same size (40). The 2-kb AAD(2″) probe described in this paper thus includes additional DNA sequences that are not part of the structural gene.

The AAD(2″) enzyme in crude extracts of the subclone pDGO114 did not modify amikacin. On the basis of this substrate profile, the enzyme should be classified as AAD(2″)-I. However, it did not completely rule out the possibility that the gene coded for AAD(2″)-II, since the substrate affinity data for AAD(2″)-II was determined with a highly purified enzyme preparation (7).

An association of the AAD(2″) gene with IncC (as we have found) and IncFII plasmids has been previously reported (11). This differs from another AAD(2″) gene probe (40), which originated from a plasmid not able to be classified by incompatibility group (F. C. Tenover, personal communication).

Earlier studies on a collection of gentamicin-resistant enterobacteria from this hospital and other hospitals in Australia have been limited to descriptions of the patterns of different resistance genes that are transferred by conjugation and have not differentiated between genes that confer resistance to the same antibiotic (1). These studies could not include resistance genes found on nonconjugative plasmids or on the chromosome. The AAD(2″) probe we have constructed will allow us to make a detailed study of the

distribution and mobility of this gene in clinical isolates regardless of the location of the gene. The construction of further probes will allow us to make a detailed assessment of the epidemiology of antibiotic resistance in hospitals.

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