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Resistance Plasmids of *Pseudomonas aeruginosa*: Change from Conjugative to Nonconjugative in a Hospital Population

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Properties of a population of carbenicillin- and gentamicin-resistant, tobramycin-susceptible *Pseudomonas aeruginosa* at Veteran's Administration Hospital, Cincinnati, Ohio, have been followed during a 16-month period. As originally described, the strains were isolated from patients with urinary tract colonizations and were predominantly Parke-Davis immunotype 7. For the majority of these organisms, antibiotic resistance was correlated with the presence of a self-conjugative plasmid of incompatibility group P-2. The source and relative incidence of multiply resistant isolates have remained constant during the current study, but the immunotype has shifted form type 7 to type 2. Concomitantly, the population has lost the property of conjugative transfer of resistance, and resistant strains are now compatible with P-2 plasmids. A group P-2 R plasmid, pMG5, will mobilize resistance markers, demonstrating that the multiple resistance of the nonconjugative strains is mediated by R plasmids. Additionally, gentamicin resistance due to either conjugative or nonconjugative plasmids is correlated with the presence of similar gentamicin acetyltransferase activity. pMG5-mobilized plasmids are shown to be incompatible with pMG5. pMG5 is also shown to mobilize resistance markers from nontransferring antibiotic-resistant strains representing populations from Parkland Memorial Hospital, Dallas, Texas, and Cleveland Clinic Foundation, Cleveland, Ohio.

Clinical isolates of *Pseudomonas aeruginosa* are inherently resistant to a number of antibiotics but typically have been susceptible to gentamicin and carbenicillin. Although this remains generally true, several reports of resistance to these antibiotics have appeared in recent years, including reports of R plasmid-mediated resistance (3, 7, 9, 12, 15-17). We have described a hospital population of *P. aeruginosa* containing a high incidence of strains resistant to several antibiotics, including gentamicin and carbenicillin. This population was identified primarily from patients with urinary tract complications (11). Antibiotic resistance was shown to be due to the presence of a conjugative R plasmid of P-2 incompatibility type (10).

Properties of this hospital pseudomonas population have been monitored over ensuing months. Despite continued change in actual patients involved, the incidence and source of strains of the same pattern of multiple resistance have remained essentially constant. However, the conjugative properties of resistant strains shifted; originally 80% readily transferred R plasmids, and currently none of them do so. This shift occurred concurrently with a change in immunotype of the multiply resistant organisms from predominantly Parke-Davis immunotype 7 to exclusively immunotype 2. Based upon plasmid mobilization and enzyme activity studies reported here, we suggest that the R plasmid population has evolved in the clinical environment to become deficient in its properties of incompatibility and conjugation. Genetic and epidemiological aspects of such plasmid populations are discussed.

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MATERIALS AND METHODS

Chemicals, media, buffers, and antibiotics. [1-^{14}C]acetyl coenzyme A, 50 mCi/mmol, was purchased from New England Nuclear Corp. T-Y broth consists of 10 g of tryptone (Difco) and 5 g of yeast extract (Difco) per liter in water. Buffer A is tris(hydroxymethyl)aminomethane (10 mM) and NH_4Cl (50 mM) in water at pH 7.8. Buffer B is tris(hydroxymethyl)aminomethane-succinate (0.3 M), $MgCl_2$ (30 mM), and dithioerythritol (Pierce Chemical Co., 3 mM) in water at pH 7.8. The following antibiotics were received as gifts: gentamicin sulfate complex and gentamicin components, Scher-

ing Corp.; kanamycin sulfate complex and kanamycin components, Bristol Laboratories; tobramycin sulfate, Eli Lilly and Co.; neomycin sulfate, Upjohn Co.; derivative kanamycin B (DKB), J. Y. Homma, The Institute of Medical Sciences, University of Tokyo, Tokyo, Japan. All other antibiotics, media, and chemicals have been described (11).

Bacterial strains and nomenclature. Local clinical strains were isolated, identified, and characterized as to antibiotic resistance pattern at Veteran's Administration Hospital, Cincinnati. Resistance patterns were determined by use of a modified disk method. These and other locally derived strains are designated by the prefix PL. Origins and pertinent characteristics of the major strains are summarized in Table 1. Antibiotics are abbreviated as follows: gentamicin, GM; carbenicillin, CB; kanamycin, KM; streptomycin, SM; sulfa, SU; tetracycline, TC; tobramycin, TB. Corresponding antibiotic resistance traits are indicated by Gm, Cb, Km, Sm, Su, Tc, and Tb. Immunotypes were determined by the method of Fisher et al. (5) using sera and control strains supplied by Parke-Davis (see reference 11).

Identification of conjugal and nonconjugal strains. Procedures for bacterial mating and for selection of tranconjugants were essentially as described (11). Generally 4-h matings of exponential cells were used. Under these conditions, the frequency of R plasmid transfer to PL1 using typical conjugative (Tra^+) strains is nearly 100%. Depending upon the number of cells used, nonconjugative (Tra^-) strains are defined as those showing no evidence of transfer of resistance trait to PL1, in experiments that would detect transfer at frequencies $<10^{-7}$ to $<10^{-8}$ based on donor titer at the end of the mating period (11).

Compatibility testing and mobilization of resistance markers. Multiply resistant strains were mated with PU21(pMG5), and transconjugants were selected by plating mating mixtures on minimal agar media supplemented with TB at 30 μg/ml (TB medium). Transconjugant clones were routinely purified by two successive single-colony isolations on TB medium. When using Tra^- strains harboring pMG5 as donors in matings with PL1, selection was on complex medium agar plates supplemented singly with antibiotics as follows: GM, 1 to 4 μg/ml; CB, 50 μg/ml; SM 100 μg/ml; TC 100 μg/ml; TB, 1 to 4 μg/ml. Counterselection was with rifampin at 100 μg/ml.

TABLE 1. *P. aeruginosa* strains

Strain	Pertinent characteristics	Source
PL1	*met, leu, rif;* R^-	Previously described (11)
PL2	prototroph, *rif;* R^-	Spontaneous revertant of 280 *met rif* (11)
PU21(pMG5)	*ilvB112, leu-1, str-1, rif;* pMG5: Tb, Su	G. A. Jacoby (8)
PL98	Prototroph, Gm, Cb, Sm, Tc	Clinical isolate
PL107	Prototroph, Gm, Cb, Sm, Tc	Clinical isolate
PL183	Prototroph, Gm, Cb, Sm, Tc	Clinical isolate
PL187	Prototroph, Gm, Cb, Sm, Tc	Clinical isolate
B15166	Prototroph	B. Minshew (6)
P77	Prototroph	D. Butler, Cleveland Clinic Foundation

Assay of gentamicin acetyltransferase. Cells were grown in 1 liter of T-Y broth, supplemented with GM at 4 μg/ml, in a 2-liter Erlenmeyer flask, with 1 ml of an aerated overnight culture as inoculum. Flasks were shaken at 37 C until the cultures had reached the end of exponential growth (about 6 h). Cells were then pelleted by centrifugation, and the pellet was rinsed with 200 ml of buffer A; the cells were then suspended at 1 g (wet weight)/5 ml of buffer A. The suspension was maintained on ice and was sonicated with a Branson microtip sonifier at 75 W for 3 min in 30-s bursts. Particulates were removed by centrifugation of the mixture for 3 h at 105,000 × *g*. The supernatant fluid was aspirated off and filtered in buffer A on a column of Sephadex G-50. The enzyme fraction was then frozen and stored at −20 C until use. (Enzyme activities of such preparations proved stable to storage for several months.)

GM acetyltransferase of thawed preparations was assayed by a modification of the method of Benveniste and Davies (2). All assays were done at 30 C in duplicate. Assay mixtures combined 10 μl of buffer B, 5 μl of enzyme extract, and 5×10^{-3} μmol of substrate antibiotic in 5 μl of a water solution; reactions were started by the addition of 5×10^{-3} μmol of [1-^{14}C]acetyl coenzyme A at 4 μCi/μmol in 10 μl of a water solution. For each time period, a 10-μl aliquot of the reaction mixture was spotted onto a 1-cm^2 piece of Whatman P-81 phosphocellulose paper. After 15 s at room temperature, such papers were submerged in water at 80 C for 3 min. They were then transferred to a sintered-glass filter, rinsed four times with 200-ml volumes of water, and dried at 60 C for 20 min. Radioactivity of dried samples was determined using a toluene-based scintillation fluid and a Packard Tri-Carb counter. Control values were obtained from reaction mixtures lacking either substrate or enzyme. Enzyme assays were linear for 20 min. One enzyme unit is defined as that amount of enzyme producing 10^{-9} mol of acetylated antibiotic per min at 30 C. Specific activity values are expressed as units of enzyme per milligram of soluble protein; protein determinations were by the method of Lowry et al. (13).

RESULTS

Sustained incidence of multiply resistant organisms among urinary tract isolates. Our initial description of this pseudomonas population was based upon strains isolated in December 1973–January 1974. Examination of hospital records during the period February 1974 through March 1975 indicates a fairly constant incidence of resistant strains in the population. Data for a representative period, July through October 1974, appear in Table 2. Whereas clini-

TABLE 2. *P. aeruginosa isolated from 166 patients during July 1974 through October 1974*[a]

Month	No. of isolates	Source and antibiotic resistance pattern[b]					
		Gm^S Cb^S			Gm^R Cb^R		
		Urine[c]	Sputum	Other[d]	Urine	Sputum	Other
July	64	20	17	6	10	1	0
August	74	20	22	11	8	1	2
September	52	22	11	5	6	3	0
October	55	25	10	5	8	2	0

[a] Not included are repeated isolates of the same resistance pattern from a single source from each patient per month. Isolates from a separate source from a patient are included.

[b] Superscript S and R refer to susceptible or resistant with respect to the gentamicin (Gm) or carbenicillin (Cb) resistance trait. Strains of two minor classifications, Gm^R Cb^S and Gm^S and Cb^R, were obtained from among all sources, to comprise the additional monthly isolates.

[c] Includes isolates from urine, surface or wounds of the urinary tract, or from Foley catheters of patients.

[d] Includes isolates from other wounds, skin surfaces, and body fluids.

cal isolates of *P. aeruginosa* have been numerous from such sources as sputum, wounds, and skin surfaces, urine and urinary tract sources have consistently yielded nearly all of the multiply resistant strains. The characteristic resistance pattern has also remained stable, with resistance by disk method to CB, GM, SM, TC, SU, and chloramphenicol; none of the strains is TB resistant.

Changes in immunotype and in conjugative properties of the hospital population of antibiotic-resistant pseudomonas. In our earlier study this resistance pattern was correlated with a homogeneous population of readily conjugative R plasmids. Nearly all (ca. 80%) of the antibiotic-resistant isolates tested showed resistance transfer; with the representative plasmid studied, RPL11, donor cells transferred resistance to recipient *P. aeruginosa* strain PL1 at a frequency of nearly 100%. Clinical isolates were also characterized as to immunotype using the Parke-Davis system. The majority of strains donating the RPL11 resistance pattern were immunotype 7 (11). These properties have now been determined for the resistant strains indicated in Table 2. Results appear in Fig. 1. The strains consist of 41 isolates from 32 patients, with only one isolate per source for any patient per month. Their properties indicate a change in the hospital pseudomonas population to a form unable to transfer resistance to PL1, indicated in Fig. 1 as Tra^-. Concomitantly, the antibiotic-resistant population, which was heterogeneous in immunotype and included type 7, changed to become exclusively immunotype 2. Less complete data for the period of November 1974 through March 1975 have shown a preponderance of immunotype 2 throughout, with occasional detection of resistant strains of various other immunotypes. The resistant population has remained Tra^- since September 1974.

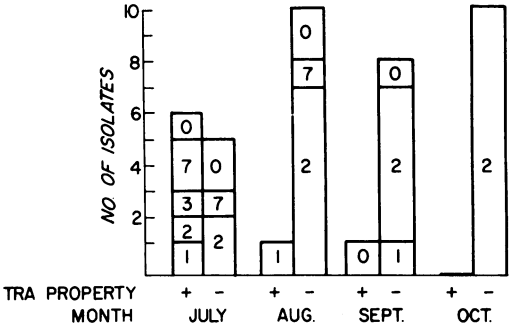

FIG. 1. *Immunotype and conjugal properties of Gm^R Cb^R P. aeruginosa isolates as described in Table 2. Numbers within histograph segments indicate Parke-Davis immunotype 1, 2, 3, or 7; 0 signifies nontypable or multitype strains. Length of each segment shows the number of patients with isolates of that immunotype. Tra + or − indicates demonstration, or failure to demonstrate, conjugal self-transfer by procedures detailed in Materials and Methods. Results are for 41 strains isolated from a total of 32 patients.*

Mobilization of resistance plasmids from the Tra^- strains. Since the Tra^- pseudomonas retained the antibiotic resistance pattern characteristic of Tra^+ strains isolated earlier, it seemed likely that Tra^- strains contain similar but now nonconjugative R plasmids. RPL11, our prototype plasmid of the Tra^+ population, is a member of incompatibility group P-2 (10). Therefore, if Tra^- strains contain plasmids similar to RPL11, their resistance should be eliminated by another P-2 plasmid. Alternatively, a P-2 plasmid might mobilize resistance. P-2 plasmid pMG5, a conjugative plasmid described by Jacoby (8), was selected since it mediates the Tb and Km resistance traits, but not Cb, Gm, Sm, or Tc, which are markers of RPL11. Representative Tra^+ and Tra^- strains were mated as

recipients with donor strain PU21(pMG5). TB-resistant transconjugants were obtained readily and were purified by single-streak reisolation on the same TB selection medium. Maintenance of the recipient resistance pattern was determined by testing clones for growth on GM, CB, SM, or TC. Data for representative strains are presented in Table 3. Strain PL11 did not retain recipient resistance markers in the presence of the Tb trait of pMG5. All of six additional Tra^+ strains tested showed essentially the same property, demonstrating the expected incompatibility of these plasmids with the P-2 plasmid. Of 10 Tra^- strains tested, eight maintained all four recipient markers in the presence of the pMG5 Tb trait (data of PL187). The other two Tra^- strains, PL98 and PL107, showed partial retention of recipient markers under the same conditions.

To test mobilization, Tra^-(pMG5) strains showing retention of the recipient resistance markers were used as donors to PL1. One or more isolates of such variants of 8 of the 10 Tra^- strains were tested. All of these transferred Tb at high frequency. Four of the eight, including variants of PL183 and PL187, showed a low level of transfer of each of the four recipient resistant markers; PL98 and PL107 variants showed a low level of transfer of Sm; two of the donors transferred only Tb. Actual efficiencies of mobilization were determined for PL187(pMG5). Based upon the titer of donor cells at the end of mating, values were: Tb, 9×10^{-1}; Gm, 4×10^{-5}; Cb, 5×10^{-5}; Sm, 5×10^{-5}; Tc, 2×10^{-5}.

When such PL1 transconjugant clones were scored for acquiring additional markers in the cross, several antibiotic-resistant patterns were observed. Representative data for transconjugants from a mating with PL183(pMG5) are shown in Table 4. Whether initial selection had been with GM, CB, or SM, isolates frequently had acquired the four resistant markers typical of RPL11 and were KM and TB sensitive. Less frequently, selection on either GM or CB yielded transconjugants with the total pattern, including TB and KM. In addition, the subpatterns TC, SM, GM, TB, KM and SM, GM, TB, KM were obtained among clones selected initially on GM; CB only was seen with CB selection; and SM, TB, KM was the predominant pattern among clones selected on SM. These results demonstrate that hospital strains do contain nonconjugative R plasmids, compatible with pMG5 and mobilized by that P-2 plasmid.

pMG5-mobilized plasmids are conjugative and are incompatible with pMG5. For subsequent serial transfer experiments, two PL1 transconjugants whose antibiotic resistance patterns, Gm, Cb, Sm, Tc, were acquired from PL183(pMG5) and PL187(pMG5) were termed strains PL1(RPL183-pMG5) and PL1(RPL187-pMG5), respectively. They were used as donors in matings with strain PL2. Transfer of their resistance patterns was observed at a frequency of about 10^{-4}, typical for PL1 donor strains (10).

These same PL1 transconjugant strains were then tested for their efficiency as recipients in crosses using PU21(pMG5) as donor of Tb. Their frequency of conversion to TB resistance was lower by 70-fold relative to a control using PL1 as recipient. Twenty clones each of PL1(RPL183-pMG5) and PL1(RPL187-pMG5), which had acquired Tb from PU21(pMG5) in these tests, were then examined for maintenance of the Gm, Cb, Sm, and Tc markers. Procedures were as described in Table 3 using

TABLE 3. *Compatibility of resistance markers in pMG5-containing transconjugants of multiply resistant clinical isolates*[a]

Clinical isolates	Tra[b]	No. of clones retaining recipient markers + Tb/no. retaining Tb			
		Gm	Cb	Sm	Tc
PL11	+	0/20	0/20	0/20	0/20
PL187	−	20/20	20/20	20/20	19/20
PL98	−	12/16	16/16	16/16	8/16
PL107	−	3/16	0/16	16/16	0/16

[a] Multiply resistant clinical isolates were mated as recipients with a donor of pMG5. Selection and isolation of transconjugant clones was on minimal agar supplemented with TB (30 μg/ml). Clones were scored on similar media and on complex agar supplemented singly with GM (30 μg/ml), CB (750 μg/ml), SM (2,000 μg/ml), and TC (2,000 μg/ml).

[b] Tra indicates presence (+) or absence (−) of conjugal self-transfer (see Fig. 1, legend).

TABLE 4. *Resistance patterns in PL1 transconjugants containing plasmids mobilized from PL183(pMG5)*

Selection antibiotic	Clones tested[a]	Pattern					Distribution
		CB	TC	SM	GM	TB	
GM	16	R	R	R	R	R	2
		R	R	R	R	S	9
		S	R	R	R	R	4
		S	S	R	R	R	1
CB	20	R	R	R	R	R	6
		R	R	R	R	S	11
		R	S	S	S	S	3
SM	20	R	R	R	R	S	5
		S	S	R	S	R	15

[a] Single-colony reisolates from selection plates were scored for growth on each of the other antibiotics as described in Materials and Methods. Plates were scored after being incubated overnight at 37 C.

the levels of antibiotics for PL1 transconjugants as indicated in Materials and Methods. As with the original Tra$^+$ strains described in Table 3, none of these clones retained recipient markers. Thus these non-self-conjugative R plasmids, having been established as Tra$^+$ after mobilization with pMG5, are now incompatible with that P-2 plasmid.

Multiply resistant clinical isolates code for similar gentamicin acetyltransferase activity. As an additional study of possible genetic similarity of Tra$^+$ and Tra$^-$ strains, we have examined their enzymatic inactivation of GM. GM acetyltransferase activities were assayed, since the antibiotic pattern, GM-resistant, TB-susceptible, is consistent with the properties of GM acetyltransferase-1. The Gm traits of RPL11 and of the Tra$^-$ plasmids of PL183 and PL187 were chosen for study. All were prepared in a common host to yield strains PL1(RPL11), PL1(RPL183-pMG5), and PL1(RPL187-pMG5).

Enzyme preparations from these strains contained similar levels of GM acetylating enzyme; the range of specific activity values is 2.3 to 3.7. Based upon the GM acetyltransferase values of respective strains as equaling 100, relative activity profiles for nine additional substrates were obtained and are shown in Table 5. All three plasmids code for the same substrate specificity for acetylation.

Detection of Tra$^-$ R plasmids among other populations of antibiotic-resistant pseudomonas—examples from Dallas and Cleveland. Holmes et al. recently described a population of GM-resistant strains among *P. aeruginosa* isolated at the Parkland Memorial Hospital, Dallas, Texas (6). None of their 35 different attempts at bacterial mating demonstrated any R plasmids in these strains. Using one of their isolates, B15166, our application of the mating conditions described above with PL1 as recipient was also negative.

TABLE 5. *Substrate specificity of acetyltransferase activity of three R plasmids of different origin*

Substrate	Relative reaction rate for enzymes from cells of PL1 containing plasmid:[a]		
	RPL11	RPL183-pMG5	RPL187-pMG5
GM	100	100	100
GM A	34	36	28
GM Cl	166	129	134
GM Cla	101	91	88
Sisomicin	104	101	93
TB	95	86	81
KM A	2.4	1.2	1.1
KM B	98	86	88
DKB[b]	72	72	74

[a] Composite data from two assay experiments. GM, TB, and KM B were tested in each series of assays; paired values agreed within ±5%. Rates with different substrates in a series are expressed relative to an assigned value of 100 for the acetylation of GM in that series.

[b] DKB is 3′,4′-dideoxykanamicin.

A similar situation exists for GM- and CB-resistant strains of *P. aeruginosa* characterized at the Cleveland Clinic Foundation, Cleveland, Ohio (D. A. Butler, L. L. Cerat, J. M. Serkey, and T. L. Gavan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C84, p. 41). In this case, our tests of 12 resistant strains provided by that clinic failed to show evidence of conjugative transfer.

Both B15166 and a representative strain from Cleveland Clinic, P77, were then examined for plasmid mobilization as described above. pMG5 was transferred to each strain. P77(pMG5) was then shown to transfer Gm, Cb, and Sm traits to PL1; B15166(pMG5) transferred Gm and Sm traits. Serial transfer of markers from PL1 transconjugants to PL2 was also observed. These derived conjugative plasmids now are apparently members of incompatibility group P-2 inasmuch as PL1 strains bearing those plasmids now exlude pMG5.

DISCUSSION

Multiple antibiotic-resistant *P. aeruginosa* are stably established in this hospital. During the period in which we first obtained strains, December 1973–January 1974, one or more *P. aeruginosa* isolates were recorded from each of 87 patients. Twenty-six percent of these patients harbored multiply resistant strains. For the 4-month period represented in Table 2 and Fig. 1 of this report, 32 of the 166 patients yielding clinical isolates of *P. aeruginosa* had strains with the same pattern of antibiotic resistance, an incidence of 19%.

In our previous studies, 80% of the resistant strains were shown to contain conjugative R plasmids (11). Plasmid RPL11 was described as a prototype for the great majority of these (10). RPL11 mediates transfer of resistance to GM, CB, SM, TC, SU, chloramphenicol, and mercury ion. Transfer is restricted to *Pseudomonas* species and the plasmid belongs to the P-2 incompatibility group (10). A few other conjugative R plasmids in this population were shown to mediate transfer of subsets of the same resistance markers carried by RPL11; no pseudomonas plasmids were detected that mediated markers other than those of RPL11 (Km or Tb, for example). Conjugative self-transfer could not be demonstrated for approximately 20% of

the strains possessing the major antibiotic resistance pattern.

During the intervening months from January to July, and progressively through September 1974, there was a decrease and finally a loss of the conjugative property from the resistant population (Fig. 1)

We began a study of plasmid relationships among these strains. Availability of pMG5 as a P-2 plasmid that mediates Tb but not Gm made possible the mobilization experiments. In general, our results show that strains possessing the resistance pattern of RPL11, but lacking conjugative transfer of that resistance, will transfer the RPL11 pattern after being infected with pMG5. Many of the resultant transconjugants can then serially donate the entire RPL11 pattern.

Apparently the nonconjugating strains contain a form of R plasmids incapable of self-transfer. These plasmids replicate stably in their host strains and are compatible with the P-2 incompatibility group (data of Table 3). Upon mobilization of these R plasmids by pMG5, they can become converted into conjugative R plasmids of incompatibility group P-2.

The resistance patterns presented in Table 4 indicate that pMG5 and resident plasmids can interact to form various patterns. A major type is that of the resident, but now conjugative, plasmid. This type might have arisen through dissociation of pMG5 into R determinants, plus a resistance transfer factor which then became stably associated with the resident plasmid. This model is consistent with well-documented cases of dissociable plasmid cointegrates in *Enterobacteriaceae* (1). Among *Pseudomonas* species, the K factor is characterized as a factor capable of mobilizing a variety of nonconjugative degradative plasmids in *P. putida* (4). The capacity of K factor to mobilize the antibiotic-resistant plasmids of this population of *P. aeruginosa* remains to be tested.

The frequency of recombinant resistance patterns documented in Table 4 shows that pMG5 need not mobilize according to this model. Mobilization may occur by recombinant interaction betweeen pMG5 and the resistant plasmid, with or without subsequent loss of specific resistance traits.

From our data a high incidence of conjugative R plasmids is unusual among *P. aeruginosa*. The 80% incidence of R transfer ability seen in January 1974 has not been typical of the long-term population. For this hospital the data in Table 2 and Fig. 1 show that the incidence of antibiotic resistance remained high during and after the loss of capacity for self-transfer. Incidence of multiply resistant strains remained comparably high at least through March 1975, although we have detected only nonconjugative plasmids in strains isolated since September 1974.

Nonconjugative R plasmids were apparently the basis of recent multiply resistant *P. aeruginosa* populations in Parkland Memorial Hospital in Dallas, Texas, and in the Cleveland Clinic in Ohio. Holmes et al. reported no evidence of conjugative transfer among 35 different matings using GM-resistant Parkland Memorial Hospital strains. A strain representative of the Parkland Memorial Hospital population, B15166, and 12 GM-resistant *P. aeruginosa* isolates obtained from the Cleveland Clinic failed to donate resistance to PL1 in our tests. However, both Parkland Memorial Hospital strain B15166 and the one Cleveland Clinic isolate tested, P77, did transfer their respective resistance traits upon mobilization with pMG5.

Concomitant with the change in the plasmid from conjugative to nonconjugative, the immunotype of plasmid-bearing strains in the local hospital changed from predominantly type 7 to exclusively type 2. It is possible that a *P. aeruginosa* type 2 population containing an unrelated nonconjugative plasmid replaced the type 7 population due to some selective pressure in the environment. The extensive pattern of multiple resistance has remained constant throughout this period, however, and we have shown that several of the plasmids code for GM acetyltransferase of the same substrate specificity. It thus seems likely that the changes in conjugability and incompatibility have evolved within the same plasmid population. Studies to determine the molecular deoxyribonucleic acid relationships of the two major plasmids are in progress.

From the continued incidence of type 2 strains bearing nonconjugative resistance plasmids, it seems clear that the spread of multiple resistant pseudomonas among patients in this hospital is due to the dissemination of a plasmid-containing strain and is not dependent upon the infectivity of a plasmid among a variety of strains (11).

Several of these patients would be expected to be prone to pseudomonas infections due to problems of therapy for neoplasia, renal insufficiency, etc. Despite the high incidence of resistant pseudomonas in the urinary tracts of these patients, it appears that these strains are relatively low in virulence. Slepack et al. have shown that RPL11 has no antivirulent effects in a test system using rats (14). The clinical signif-

icance of these resistant organisms is being investigated by local infectious disease personnel.

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