A conjugation system initially discovered in β-lactamase-producing gonococci mobilized small non-selftransmissible R plasmids encoding β-lactamase (penicillinase) production into other gonococci, Neisseria, and Escherichia coli. This conjugation system was mediated by a separate selftransmissible plasmid of 23.9 × 10^6 daltons, pFA2. Conjugative plasmids capable of mobilizing R plasmids were also found in nearly 8% of the non-penicillinase-producing gonococci. These were similar to pFA2 in size, buoyant density, and restriction endonuclease digest patterns but were less efficient than pFA2 in mobilization of the penicillinase plasmid pFA3. The presence of conjugative plasmids in gonococci isolated before the appearance of penicillinase-producing strains indicates that a conjugation system for plasmid transfer predated the appearance of R plasmids in gonococci.

Recently, strains of Neisseria gonorrhoeae with plasmid-mediated β-lactamase (penicillinase) production have been isolated in several nations (6, 26). Although plasmid-mediated antibiotic resistance is common in many other gram-negative bacteria (3), antibiotic resistance in gonococci has been thought to be a result of chromosomal loci (7). Chromosomal mutations result in only low-level resistance to penicillin (31). Acquisition of plasmid-mediated β-lactamase production by a penicillin-sensitive strain of gonococcus, however, results in an approximately 100- to 1,000-fold increase in resistance to penicillin, depending on inoculum size (25).

β-Lactamase-producing gonococcal clinical isolates contain plasmids of about 4.7 or 3.4 million daltons (Mdal) in addition to the 2.6-Mdal cryptic plasmids found in most gonococci (12, 28). The 4.7- and 3.4-Mdal plasmids are responsible for β-lactamase production, as evidenced by loss of these plasmids in spontaneously cured (β-lactamase-negative) derivatives, as well as by acquisition of these plasmids in β-lactamase-positive transformants and transconjugants (12, 28). Transfer of the penicillinase plasmids by conjugation to other gonococci, Neisseria flava, and Escherichia coli has been reported (12, 15, 28). Since all gonococcal conjugative donors of the β-lactamase plasmids also possess plasmids of 24 Mdal, it has been postulated that conjugation might be mediated by the 24-Mdal plasmids (12, 28).

In this communication, we show that the 24-Mdal plasmids are selftransmissible and are indeed responsible for transfer of the R plasmids. Similar conjugative plasmids were found in penicillin-sensitive strains isolated well before the appearance of penicillinase-producing gonococci.

**MATERIALS AND METHODS**

**Strains and materials.** Table 1 presents a list of relevant strains and their genotypes. Strain and plasmid nomenclatures generally adhere to the proposals of Novick et al. (23). Media and growth conditions were as described previously (31). The presence of β-lactamase production was confirmed by a chromogenic cephalosporin indicator (Glaxo) (24) or by a phenol red-penicillin G indicator solution (30). Most antibiotics were obtained from sources previously described (31). Ampicillin was from Bristol Laboratories, and nalidixic acid was from Sterling-Winthrop.

**Conjugation procedure.** Conjugation was performed by the following two methods. (i) Donor and recipient cells were cultivated together in GCB broth for 2 to 16 h at 37°C, washed in medium A of Davis and Mingioli (10), and spread on agar media containing appropriate antibiotics to select for transconjugants. (ii) Donor and recipient cells from plates inoculated 16 to 20 h previously were suspended in GCB broth medium to approximately 5 × 10^8 colony-forming units per ml. Portions (0.1 ml) of donor and recipient suspensions were placed on a sterile membrane filter resting on GCB agar (Difco Laboratories) that contained Kellogg supplements I and II. In some experiments, 0.05 ml of a solution containing 1 mg of pancreatic deoxyribonuclease ( Worthington Biochemicals Corp.) per ml was added to the filter to inhibit transformation. Filters were incubated at 37°C in the presence of 3% CO_2 for 1 to 16 h, depending on the experiment. After this incubation, filters were agitated in 1.0 ml of medium A to suspend the cells. The cells were pelleted by brief centrifugation and resuspended.
and portions were spread on selective media. Recipient Neisseria strains contained markers for nalidixic acid (Nal), fusidic acid (Fus), rifampin (Rif), or streptomycin (Str) resistance to allow selection against the donor cells. Media for selection of penicillinase-producing (Pc') gonococcal transconjugants contained 0.1 to 0.5 μg of penicillin G per ml in addition to one or more of the above antibiotics. E. coli recipients contained one of the same chromosomal antibiotic resistance markers, and the MacConkey agar (Difco) used for selection of Pc' E. coli transconjugants contained 50 μg of ampicillin per ml in addition to antibiotics to which the donor was sensitive. Background growth of recipient gonococci on original plates often necessitated replica plating for transconjugant colonies to be isolated. The filter conjugation method generally yielded higher conjugation frequencies than direct conjugation in broth culture. In the filter conjugation technique, the number of transconjugants obtained varied widely with the brand and lot of filters used. Gelman Metrical GA6 filters or Amicon Microporous filters produced the highest conjugation frequencies.

Resistance mobilization test. Strains lacking resistance (R) plasmids were scored for the presence of conjugative plasmids by a procedure similar to that described by Anderson (2). A strain lacking an R plasmid served as the potential initial donor of the conjugative plasmid. FA333, a gonococcus that contained a Pc' R plasmid but lacked a conjugative plasmid, was used as an intermediate. An Nal' derivative of E. coli C900.5 served as the final recipient. Transfer of the R plasmid into E. coli could only occur if the tested strain introduced a conjugative plasmid into strain FA333, thereby mobilizing its R plasmid for transfer. A 0.1-ml amount of a suspension containing ca. 5 x 10^8 colony-forming units of the strain being tested for conjugative ability per ml was added to an equal volume of strain FA333 (also ca. 5 x 10^8 colony-forming units per ml) on a membrane filter. After several hours of incubation, 0.1 ml of a suspension of Nal' E. coli C600.5 was added, and the conjugation was continued overnight. The cells were then resuspended and plated on media to select for Pc' E. coli transconjugants. As a control, FA333 was usually incubated with Nal' E. coli C600.5 under the same conditions.

Strain construction. A set of four homogenic derivatives of strain FA305 (Table 1) containing different conjugative plasmids was constructed. FA288 was conjugated directly with FA305 to yield FA453(pFA2)(pFA3). The derivatives of strains FA651, FA652, and FA653 were obtained by using these clinical isolates as conjugative donors and FA333(pFA3) as an intermediate in the resistance mobilization test, and scoring FA305 Pc' transconjugants for conjugative ability. This yielded FA529(pFA11)(pFA3) from strain FA651 as the donor, FA532(pFA12)(pFA3) from strain FA652 as the donor, and FA539(pFA13)(pFA3) from strain FA653 as the donor.

Since most gonococcal strains contain cryptic plasmids of around 2.6 Mdal (7, 18, 33), purification of conjugative plasmid DNA from the original host strains was difficult. This problem was circumvented by mating the homogenic derivatives of FA305 (FA453, FA529, FA532) with N. flava (ATCC 14221), which contains no plasmids. Pc' transconjugants were selected and scored for their ability to mobilize pFA3 to E. coli C600.5. Pc' (pFA3') derivatives of these conjugative donors were then isolated and used to prepare conjugative plasmid DNA for restriction enzyme studies. We were unable to obtain N. flava transconjugants when FA539(pFA13)(pFA3) was used as a donor.

Multiplicon marked donor and recipient strains were used in testing for conjugative transfer of chromosomal markers. Strain F62, a proline auxotroph, was mutagenized with ethyl methane sulfonate to yield a

### Table 1. Bacterial strains used

<table>
<thead>
<tr>
<th>Strain and plasmids*</th>
<th>Source</th>
<th>Distinguishing characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA192</td>
<td>Transformant of FA19 (31)</td>
<td>SPEC-3</td>
</tr>
<tr>
<td>FA288(pFA2)(pFA3)</td>
<td>C. Thornberry (12)</td>
<td>Like FA288 but Pc'</td>
</tr>
<tr>
<td>FA289(pFA2)(pFA3*)</td>
<td>Spontaneous Pc' cure of FA288 (12)</td>
<td>nal-1 rif-1 str-7 fus-1 met-1 pro-1</td>
</tr>
<tr>
<td>FA305</td>
<td>Construction from F62 (8, 12)</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA333(pFA3)</td>
<td>Transformant of FA19 (12)</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA453(pFA2)(pFA3)</td>
<td>Construction from FA305</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA529(pFA11)(pFA3)</td>
<td>Construction from FA305</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA532(pFA12)(pFA3)</td>
<td>Construction from FA305</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA539(pFA13)(pFA3)</td>
<td>Construction from FA305</td>
<td>Like FA305 but Pc', conjugative donor</td>
</tr>
<tr>
<td>FA651(pFA11)</td>
<td>Local isolate (1974)</td>
<td>nal-1 arg-1 met-1 pro-1</td>
</tr>
<tr>
<td>FA652(pFA12)</td>
<td>W. McCormack</td>
<td>rif-1 str-7 SPEC-3 leu-1 Pc', conjugative donor</td>
</tr>
<tr>
<td>FA653(pFA13)</td>
<td>Local isolate (1971)</td>
<td>Conjugative donor</td>
</tr>
<tr>
<td>N. flava</td>
<td>Spontaneous mutant of ATCC 14221</td>
<td>nal-2 rif-4</td>
</tr>
<tr>
<td>E. coli C600.5</td>
<td>C. A. Hutchison</td>
<td>Res&quot; Mod&quot;</td>
</tr>
</tbody>
</table>

* All gonococcal strains in this table contained cryptic plasmids of approximately 2.6 Mdal. pFA2 and pFA3 had molecular weights of 10^6 and 4.7 x 10^5, respectively, as determined by electron microscopy. pFA11, pFA12, and pFA13 migrated on agarose gels at the same rate as pFA2 and had similar EcoRI digest patterns. Their molecular weights were therefore estimated to be 24 x 10^6. pFA2 is probably identical to the recently described pLE2450 (27); pFA3 is probably identical to pMR0360.
methionine auxotroph (met-l) (8). The markers arg-l and nal-l were added to this derivative by transformation, yielding a strain (FA583) that was used as a recipient. The donor strain FA589 was a leucine auxotroph of F62 obtained by ethyl methane sulfonate mutagenesis to which the markers rif-2, str-7, spc-3, and proline prototrophy were added by transformation and the plasmids pFA2 and pFA3 were introduced by conjugation.

**Screening for plasmid content.** A loopful of cells (ca. 20 mg) scraped from an overnight plate was suspended in 200 µl of TES buffer [50 mM NaCl, 5 mM ethylenediaminetraacetate, 30 mM tris(hydroxymethyl)aminomethane (pH 8.0)]. Ten microliters of a 10-mg/ml lysozyme solution was added, and the suspension was incubated at 37°C for 30 min. A 25-µl amount of a 10% sodium dodecyl sulfate solution was then added, and the mixture was shaken gently to lyse the cells. Five microliters of a 10-mg/ml ribonuclease A (Sigma Chemical Co.) solution that had been heated to 80°C for 20 min was added, and the mixture was incubated at 37°C for 1 h. Ten microliters of a 1-mg/ml Pronase (Calbiochem) solution was then added, and the mixture was incubated at 37°C for an additional 2 h. Seventy microliters of 5 M NaCl was then added, and the lysate was kept at 4°C overnight. After centrifugation at 48,000 × g for 20 min, the supernatant was decanted, and 5- to 25-µl portions were subjected to electrophoresis on 0.8% agarose gels, using a vertical slab gel apparatus (E-C Apparatus Corp.). Electrophoresis was performed by the procedure of Meyers et al. (19). Gels were stained with a 1-µg/ml ethidium bromide solution and viewed with a long-wave UV transilluminator. Photographs were made through a Vivarat orange filter with Polaroid type 665 film. The identity of plasmid bands was determined by electron microscopy of DNA extracted from ethidium bromide-stained bands by the freeze-squeeze technique (35).

**Density gradient centrifugation.** Cesium chloride-ethidium bromide buoyant density gradients were prepared as described previously (7). Neutral cesium chloride gradients for buoyant density determinations (14) contained labeled plasmid DNA and 32P-labeled E. coli chromosomal DNA. E. coli chromosomal DNA was assumed to have a specific gravity of 1.7035 (11).

**Electron microscopy.** DNA was prepared for electron microscopy by the Kleinschmidt technique (16) and viewed with an AE1 transmission electron microscope. Open circular simian virus 40 DNA was used as an internal standard of 3.2 Mdal (36).

**Restriction endonuclease digestion.** Restriction endonucleases (EcoRI, BamHI, and HpaII) were obtained from Miles Laboratories, and digestions were performed essentially by the supplier’s instructions.

**RESULTS**

**Identification of the 24-Mdal plasmid as a conjugal plasmid.** Each of six tested clinical isolates functioning as conjugal donors of the β-lactamase plasmids contained plasmids of about 24 Mdal. This association suggested but did not prove that these plasmids were conjugal. Proof was obtained in conjugation experiments that used Pc· strain FA288 (pFA2) (pFA3) as a conjugal donor and FA305 (Pc·) as a recipient. Sixty-four Pc· transconjugants were isolated and examined for their plasmid content by agarose gel electrophoresis and for their ability to donate the Pc· plasmid pFA3 to E. coli C600.5 (Table 2). Six were found to have received pFA2 from strain FA288; only these six clones were able to mobilize pFA3 for transfer to E. coli C600.5 or to other gonococci. Thus, pFA2 was responsible for conjugal transfer of pFA3 and could mediate its own transfer between gonococci.

However, when Pc· E. coli transconjugants from a conjugation of C600.5 with FA288 were examined for plasmid content, none of 24 was found to contain pFA2. Also, resistance mobilization tests utilizing strain FA293 as a source of pFA2, a pFA3-containing derivative of strain C600.5 as an R plasmid intermediate, and an Str· Nal· derivative of strain C600.5 as a final recipient failed to yield Pc· transconjugants. Therefore, although pFA2 was able to mobilize pFA3 for transfer to C600.5, pFA2 itself was neither transferred, could not replicate, or was highly unstable in this strain of E. coli.

**R plasmid transfer was not due to stable**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Frequency of pFA3* (× 10^4)</th>
<th>pFA3 transconjugants receiving pFA2</th>
<th>Frequency of unselected recipient of pFA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5</td>
<td>13/24 (54)</td>
<td>0/64 (&lt;2)</td>
</tr>
<tr>
<td>16</td>
<td>5.0</td>
<td>21/24 (88)</td>
<td>6/64 (9)</td>
</tr>
</tbody>
</table>

* FA288(pFA2)(pFA3) (rif· nal·) served as the Pc· donor, and FA305 (rif· nal·) served as the Pc· recipient. Conjugation was performed on a membrane filter (type HA; Millipore Corp.) as described in the text; transfer frequencies in this experiment were unusually low due to the type of filter used. Pc· transconjugant colonies were selected on media containing 2.0 µg of rifampin per ml, 2.0 µg of nalidixic acid per ml, and 0.2 µg of penicillin per ml. Frequencies are expressed per input donor colony-forming units.

* Pc· transconjugants were scored for receipt of pFA2 by agarose gel electrophoresis and by the ability to mobilize pFA3 to suitable recipients. Numbers in parentheses are percentages.

* Recipient FA305 colonies were selected on media containing 2.0 µg of rifampin per ml and 2.0 µg of nalidixic acid per ml. Receipt of pFA2 was determined by the ability to function as a conjugal donor in a resistance mobilization test and by agarose gel electrophoresis. Frequencies are expressed per recipient colony-forming unit. Numbers in parentheses are percentages.

* None of the six clones receiving pFA2 also received pFA3.
**CONJUGATIVE PLASMIDS IN N. GONORRHOEAE**

Cointegrate formation. By analogy with the behavior of plasmids from *Proteus mirabilis* (22) and other organisms (4), it was possible that the Pc' plasmid (pFA3) formed a cointegrate with the conjugative plasmid (pFA2) in strain FA288. If this were the case, the conjugative plasmid from the Pc' strain would be larger, by about 4.7 Mdal (the size of pFA3), than the conjugative plasmid from the Pcs derivative FA293. Molecular weights of the conjugative plasmids from strains FA288 and FA293 were determined from electron micrographs (Fig. 1). pFA2 molecules derived from FA288 were approximately 23.9 Mdal (n, 19; standard deviation, 2.0); no measured molecule was as large as the expected size of a cointegrate (28.6 Mdal). pFA2 molecules from FA293 had a similar size, 23.8 Mdal (n, 13; standard deviation, 2.0). Thus, cointegrates of pFA2 and pFA3 do not appear to be common in FA288. The possibility remained that a rare cointegrate was responsible for transfer of the pFA3 genome during conjugation, although all transconjugants examined possessed separate pFA2 and pFA3 plasmids. The fact that many transconjugants received pFA3 without receiving pFA2 (Table 2) confirmed that pFA3 was transferred without stable linkage to pFA2. The existence of transient (unstable) cointegrates cannot be excluded without further experiments.

**Conjugative plasmids in penicillin-sensitive gonococci.** The presence of a conjugative plasmid in some $\beta$-lactamase-producing isolates and previous reports of a 24.5-Mdal plasmid in some penicillin-sensitive strains of gonococci (18, 33) indicated that conjugative plasmids might be present in non-$\beta$-lactamase-producing gonococci. Therefore, a number of isolates from different years and locales were examined for their ability to function as conjugative donors in the resistance mobilization test. Twelve of 156 strains examined gave clearly positive results as

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**Fig. 1.** Plasmids of FA288. A plasmid band from an ethidium bromide-cesium chloride gradient was prepared for electron microscopy as described in the text. The large plasmid in the center is an open circular molecule of pFA2. Open circular pFA3 is at the left side of the inset; pFA1 is at the right side of the inset. pFA3 and pFA1 are also visible in the main figure. Bar represents 1 $\mu$m.
conjugal donors. These were analyzed for plasmid content and all were found to contain plasmids of the same mobility on agarose gels as that of pFA2. Seventeen clinical isolates that failed to act as conjugal donors were screened for plasmid content, and none was found to contain a similar-sized plasmid. Three strains with conjugal plasmids were selected for further study.

Each of these conjugal plasmids, as well as pFA2, was introduced into strain FA305 (Materials and Methods), yielding a set of four homoclonic strains. Mobilization of pFA3 to other gonococci and E. coli C600.5 by this set was examined (Table 3). The plasmid derived from the naturally occurring Pc' strain FA288, pFA2, consistently mobilized pFA3 to other gonococci and C600.5 at a higher frequency than did the other three plasmids investigated. Curiously, when the conjugal plasmids pFA12 and pFA13 were introduced into FA305, they were unable to mobilize pFA3 to either E. coli or to gonococcal recipients. The original clinical isolates containing pFA12 and pFA13 were reliable (but low-frequency) donors in the resistance mobilization test.

The low frequencies of mobilization of the Pc' plasmid pFA3 by the other conjugal plasmids could be due to inherently lower frequencies of conjugation by these plasmids than by pFA2. Alternatively, all conjugal plasmids might be equally effective in establishing conjugation (as detected by selftransfer), but mobilization of pFA3 might occur more readily with pFA2 than with the other conjugal plasmids. To distinguish between these possibilities, overnight conjugations were performed by using the respective FA305 derivative as the donor and FA192, an Spc' transformant of FA19, as the recipient. Receipt of pFA2 occurred at a significantly higher frequency (23%) than did receipt of pFA13 (2%) (Table 3); pFA2 also mobilized pFA3 much more readily than did pFA13. Receipt of pFA11 by FA192 was as frequent as receipt of pFA2, but pFA11 mobilized pFA3 10- to 100-fold less frequently than did pFA2 (Table 3). Thus, the differences in selftransfer did not fully account for the large differences in mobilization of pFA3; the conjugal plasmids apparently also differed in their ability to initiate transfer of pFA3.

**Determination of buoyant density.** The buoyant density of pFA2 (Fig. 2) was 1.701 g/cm², corresponding to a guanine-plus-cytosine content of 48% (34). Chromosomal DNA of *N. gonorrhoeae* has been reported (11) to have the same buoyant density as *E. coli* chromosomal DNA; this corresponds to a guanine-plus-cytosine content of 50.4%. The lower density of pFA2 compared with that of gonococcal chromosomal DNA was confirmed by the following experiment. Whole-cell DNA was prepared by the method of Marmur (17) and centrifuged in neutral CsCl density gradients. One-drop fractions from the region of pertinent density were collected, and portions of these fractions were subjected to electrophoresis on agarose gels to determine the location of chromosomal and plasmid DNA in the gradient. The conjugal plasmids pFA2, pFA11, pFA12, and pFA13 were all found to band in similar regions that were slightly less dense than the region of chromosomal DNA.

**Restriction endonuclease analysis.** The purified conjugal plasmids pFA2, pFA11, and pFA12 were digested separately with each of the restriction endonucleases EcoRI, BamHI, and HpaII, and the digestion products were subjected to electrophoresis on agarose gels. All three plasmids were cleaved by EcoRI into four fragments, and the respective fragments from each plasmid had the same mobility (Fig. 3, tracks A to C). The BamHI digest of pFA12 (track D in Fig. 3) differed from the BamHI

### Table 3. Differences in function of conjugal plasmids in homoclonic strains

<table>
<thead>
<tr>
<th>Donor strain*</th>
<th>Frequency of unselected transfer of conjugal plasmid</th>
<th>Frequency of mobilization of pFA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F452(pFA2)(pFA3)</td>
<td>11/48 (23)</td>
<td>3.8 x 10^-4</td>
</tr>
<tr>
<td>F4529(pFA11)(pFA3)</td>
<td>5/16 (31)</td>
<td>3.7 x 10^-5</td>
</tr>
<tr>
<td>F4532(pFA12)(pFA3)</td>
<td>0/16 (&lt;6)</td>
<td>&lt;1.0 x 10^-7</td>
</tr>
<tr>
<td>F4539(pFA13)(pFA3)</td>
<td>1/48 (2)</td>
<td>&lt;3.2 x 10^-7</td>
</tr>
</tbody>
</table>

* These strains are homoclonic derivatives of FA305, constructed as described in the text.

* Overnight conjugations of homoclonic Spc' FA305 derivatives as donors and Spc' FA192 as the recipient were performed as described in the text. Recipient colonies were selected by plating dilutions of resuspended cells on media containing 200 µg of spectinomycin per ml. The presence of a conjugal plasmid was determined by agarose gel electrophoresis. Frequencies expressed per recipient colony-forming unit. Numbers in parentheses are percentages.

* Mean of three experiments. Frequencies expressed per input donor colony-forming unit.

* Mean of two experiments.
The pFA2 digest lacked the fifth and seventh bands from the top found in the other two digests; instead there was a band migrating between the fourth and fifth bands of the other two BamHI digests. In partial BamHI digests of pFA2, a band was seen at a similar position; this disappeared upon further digestion (data not shown). Addition of excess amounts of enzyme did not result in the disappearance of this band in the pFA12 digest. Apparently this fragment lacked a BamHI cleavage site present in pFA2 and pFA11. The HpaII digests of pFA2, pFA11, and pFA12 were identical for all of 17 resolvable bands (Fig. 4).

We were unable to obtain purified pFA13, the conjugative plasmid of strain FA652 (Materials and Methods). However, bands similar to those in tracks A to C of Fig. 3 were present in an EcoRI digest of the plasmids of strain FA539, which contained pFA13 obtained from strain FA652. These bands were not present in a digest of plasmids from the homogenic strain FA305, which lacked pFA13 (data not shown).

Failure to detect chromosomal transfer. The mobilization of the non-selftransmissible plasmid pFA3 by pFA2 suggested that pFA2 might also mobilize the chromosome. Experiments to detect such mobilization were performed using multiply marked conjugative donor (FA589) and recipient (FA583) strains that were not competent for genetic transformation (Materials and Methods). There was no difference in the number of apparent Arg⁺, Met⁺, Str⁺, or Spc⁺ recombinants when the recipient was mated overnight on membrane filters with donor cells compared with overnight incubation of the recipient with DNA isolated from the donor (data not shown). In both cases, recombinants for individual donor markers occurred at a frequency of less than 10⁻⁷. Recombinants from both parts of this experiment were examined for the acquisition of linked markers. Both groups showed similar low linkage frequencies (data not shown). Other experiments, not presented here, showed that low-frequency transformation occurred during prolonged matings despite initial addition of deoxyribonuclease; only by adding deoxyribonuclease hourly could transformation be prevented.

DISCUSSION

About 8% of the non-β-lactamase-producing clinical isolates of N. gonorrhoeae that we tested contained conjugative plasmids. The actual occurrence of such plasmids may be greater than 8%, since the resistance mobilization test would not detect conjugative plasmids unable to mobilize the R plasmid used (pFA3). Conjugative plasmids are known to be quite common in other gram-negative bacteria; Falkow reported that as many as 40% of E. coli isolates possessed conjugative ability (13). Previous attempts to demonstrate conjugation in gonococci (32) were unsuccessful partly because of the lack of an R plasmid whose transfer could be readily detected.

Our failure to detect mobilization of chromosomal markers is not surprising. Transfer of chromosomal markers by most conjugative plasmids is apparently quite rare (13). Also, the paucity of genetic markers presently known in gonococci allowed us to search for transfer of only a few chromosomal regions. The isolation
A B C D E F G

FIG. 3. Agarose gel (0.8%) of EcoRI and BamHI digests of conjugal plasmids. Tracks A to C, EcoRI digests of pFA11, pFA12, and pFA2, respectively; tracks D to F, BamHI digests of pFA11, pFA12, and pFA2, respectively; track G, EcoRI digest of phage lambda DNA. Arrow on left indicates position of a faint band present in tracks A, B, and C; arrow on right indicates position of a faint band in E and F.

of derepressed transfer mutants of the conjugal plasmid (20) or the use of the technique of integrative suppression (21) might result in a detectable mobilization of chromosomal markers, which would greatly facilitate future genetic studies of gonococci.

The four conjugal plasmids that we examined were similar in size, buoyant density, and restriction endonuclease digest patterns, indicating a strong degree of nucleotide homology. We found similar-sized conjugal plasmids in local strains isolated as long ago as 1971; also, these plasmids were found in a group of strains collected in Boston, Mass., in 1975. Whatever the source of the genetic material of the \( \beta \)-lactamase plasmids, a conjugal system capable of promoting the transfer of these \( R \) plasmids was already present in the gonococcus.

The data of Table 3 show that the conjugal plasmids differed widely in their ability to mobilize the \( Fc^+ \) \( R \) plasmid pFA3 from a homogenic donor. Also, there was a significant difference in the selftransfer of the conjugal plasmids pFA2 and pFA13 in overnight matings. Thus, plasmids that appear to be very similar by restriction endonuclease digest patterns can be quite different functionally. A number of explanations could account for these differences. The conjugal plasmids may differ in the amount of repression exerted on sex pilus formation or they may vary in the frequency with which plasmid DNA is activated for transfer. These differences could also be due to differences in the copy numbers of these plasmids; however, we did not notice any marked difference in the intensity of these plasmid bands on agarose gels.

FIG. 4. Agarose gel (2%) of HpaII digests of conjugal plasmids. First track, pFA11; second track, pFA12; third track, pFA2.
Although the mobilization of the Pc' plasmid pFA3 was often accompanied by transfer of the conjugative plasmid, we could find no evidence of stable cointegrate formation. Rather, the results suggested that these plasmids were transferred separately, as in the class 2 transfer system described by Anderson and Natkin (5).

The 24-Mdal plasmids described in this paper are among the smallest conjugative plasmids that have been reported (29). However, a conjugative plasmid of 17.3 Mdal has been described (9), and the portion of the E. coli F plasmid encoding transfer functions may occupy only about 15 Mdal (1). Thus, these gonococcal plasmids may be large enough to encode genetic information unrelated to transfer functions. We found that these plasmids did not confer entry exclusion (37) upon the host cells, since recipient strains differing only in the presence or absence of the conjugative plasmid served equally well as recipients of the Pc' plasmid (data not shown).

The natural selection that maintained these plasmids in gonococcal strains before the appearance of R plasmids remains obscure. However, these conjugative plasmids now provide a means of transfer of antibiotic resistance in vitro, and this transfer may occur in vivo as well.

ACKNOWLEDGMENTS

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LITERATURE CITED


