JOURNAL OF BACTERIOLOGY, Feb. 1982, p. 726–732
0021-9193/82/020726-07$02.00/0

Vol. 149, No. 2

Mobilization of Nonconjugative Antibiotic Resistance Plasmids in *Haemophilus ducreyi*

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Received 10 July 1981/Accepted 1 October 1981

A clinical isolate of *Haemophilus ducreyi* was found to harbor three plasmids: a 23.5-megadalton (Mdal) phenotypically cryptic plasmid, a 7.0-Mdal ampicillin resistance plasmid, and a 4.9-Mdal sulfonamide resistance plasmid. The two smaller plasmids were transferable by conjugation to *Haemophilus* recipients, but only if the donor cell harbored the 23.5-Mdal plasmid as well, indicating that this large plasmid had mobilizing capabilities. Transfer was also possible to *Escherichia coli* recipients. *Haemophilus influenzae* transconjugants which had acquired both the 23.5-Mdal plasmid and one of the R-plasmids could subsequently retransfer the R-plasmid to other *Haemophilus* recipients at higher frequencies. A derivative of the 23.5-Mdal plasmid was isolated which was shown by restriction endonuclease analysis to contain an ampicillin resistance transposon and to have retained its conjugative ability.

Ampicillin resistance in *Haemophilus ducreyi* has been shown to be mediated by plasmids of either 5.7 or 7.0 megadaltons (Mdal) (7; J. L. Brunton et al., manuscript in preparation). Neither of these plasmids is conjugally self-transmissible to drug-sensitive strains of *H. ducreyi* or to other species of *Haemophilus*, but they may be transformed into *Escherichia coli* and into competent strains of *Haemophilus influenzae*. To date, transformation is the only well-characterized means for the transfer of small antibiotic resistance plasmids in the genus *Haemophilus*. Since it is not known if *H. ducreyi* is naturally transformable, the problem remains as to how these R-factors may be disseminated throughout this species.

Certain nonconjugative plasmids may nevertheless be conjugally transferred if a self-transmissible plasmid is also present in the cell. This ability of certain large conjugative plasmids to mobilize smaller nonconjugative plasmids is widely observed among the *Enterobacteriaceae* (23) and has been described in *Neisseria gonorrhoeae* where a 4.4-Mdal ampicillin resistance plasmid is mobilized by a coresident 24.5-Mdal phenotypically cryptic plasmid (4, 5, 19).

In this paper we report the presence and preliminary characterization of a 23.5-Mdal plasmid in a clinical isolate of *H. ducreyi* which is capable of mobilizing a small coresident 7.0-Mdal ampicillin resistance plasmid and a 4.9-Mdal sulfonamide resistance plasmid for conjugal transfer to *Haemophilus* and *E. coli* recipients. This suggests a possible means whereby small nonconjugative R-plasmids may be disseminated in *H. ducreyi* as well as to other *Haemophilus* species and could provide a link between this genus and the penicillinase-producing *N. gonorrhoeae* which possess a similar plasmid transfer system.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. All *H. ducreyi* strains were clinical isolates from Winnipeg, Canada or Nairobi, Kenya. All ampicillin-resistant strains produced a β-lactamase as detected by hydrolysis of chromogenic cephalosporin 87-312 (Glaxo Research Ltd., Greenford, England) (16). *H. influenzae* strain Rd is a widely used strain originally isolated by Alexander and Leidy (2), and HI1008 is a clinical isolate from Winnipeg. Strain 9(N3) is a lysogenic derivative of strain JC9 containing the N3 prophage and is very poorly transformable with both chromosomal (17) and plasmid (1) DNA. The *H. influenzae rec-1* strain is a recombination-deficient derivative of strain Rd and has been previously described (15, 22). The plasmids used as molecular weight standards in agarose gel electrophoresis were RP4, Sa, RSF1010, and pMB8 and have been described (13).

Media. All *H. ducreyi* and *H. influenzae* strains were routinely maintained on hemoglobin agar consisting of a GC agar base (GIBCO Diagnostics) supplemented with 1% hemoglobin and 1% CVA enrichment (GIBCO). All β-lactamase-producing strains were grown on hemoglobin agar containing 10 μg of ampicillin per ml. For mating experiments or preparation of pure plasmid DNA, *H. influenzae* strains were grown in 3.8% brain heart infusion broth supplemented with

TABLE 1. Bacterial strains and plasmids

Strain	Plasmids in Mdal	Phenotype/ genotype[a]	Source
H. ducreyi			
HD147	23.5 (pHD147), 7.0 (pHD747), 4.9 (pHD447)	Ap^r, Su^r, Tc^r	Clinical isolate, Kenya
HD9468	7.0, 4.9	Ap^r, Su^r, Tc^r	Clinical isolate, Kenya
HD9265	32 (pHD9265), 7.0	Ap^r, Tc^r	Clinical isolate, Kenya
HD35000	None	Nal^r	Clinical isolate, Winnipeg (7)
H. influenzae			
Rd	None	Nov^r	J. Setlow (1)
HI1008	None	Rif^r	Clinical isolate, Winnipeg
9(N3)	None	Sm^r	J. Bendler (1)
rec-1	None	Sm^r	J. Setlow (1)
Rd(pJB1)	5.7	Ap^r, Nov^r	Transformation of Rd with pJB1 (6, 7)
Rd(RSF0885)	4.1	Ap^r, Nov^r	Transformation of Rd with RSF0885 (9)
Rd(p22209)	4.4	Ap^r, Nov^r	Transformation of Rd with p22209
Rd(p88557)	3.2	Ap^r, Nov^r	Transformation of Rd with p88557
N. gonorrhoeae			
22209	4.4 (p22209), 2.6, 24.5	Ap^r	Clinical isolate, Winnipeg
88557	3.2 (p88557), 2.6	Ap^r	Clinical isolate, Winnipeg
E. coli C600	None	*hsdR hsdM*$^+$ *thr leu thi*	R. Gill (7)

[a] Tc^r, Tetracycline resistant; Sm^r, streptomycin resistant; Nal^r, nalidixic acid resistant; Rif^r, rifampin resistant; Nov^r, novobiocin resistant.

10 μg of hemin and 3 μg of NAD per ml (BHI broth).

Conjugation procedure. *H. ducreyi* donors were grown for 18 h on fresh hemoglobin agar containing antibiotics, scraped off, and resuspended in BHI broth to a density of approximately 10^9 colony-forming units per ml. *H. influenzae* donors and recipients were grown separately overnight without stirring in 1.0 ml of BHI broth and were then diluted with 5.0 ml of fresh BHI broth and grown with stirring to a density of 10^9 colony-forming units per ml. A 1-ml amount of donor cells was mixed with 1.0 ml of recipient cells and collected by filtration onto 0.2-μm polycarbonate filters (Bio-Rad Laboratories). The filters were then placed culture side up on the surface of a fresh hemoglobin agar plate. In all matings, 0.1 ml of a solution of DNase I (1 mg/ml; Sigma Chemical Co.) was added to both donors and recipients 10 min before mixing and flooded over the cells on the membrane filter while on the agar plate. The filters were incubated for 8 h at 35°C in 10% CO_2 after which they were placed in 2.0 ml of BHI broth and blended vigorously in a Vortex mixer to resuspend the cells. The cells were then diluted and plated on hemoglobin agar containing antibiotics selective for transconjugants alone and for transconjugants plus recipients. The frequency of conjugative transfer was expressed as the number of transconjugants formed per output recipient. Ampicillin-resistant transconjugants were checked for β-lactamase production, and their plasmid content was determined by agarose gel electrophoresis. Antibiotics were used at the following final concentrations (μg/ml): ampicillin, 10; streptomycin, 1,000; rifampin, 50; nalidixic acid, 5; novobiocin, 5; and sulfadiazine, 100.

Agarose gel electrophoresis. The complete growth of *H. ducreyi* or *H. influenzae* from five plates of hemoglobin agar was suspended in 5.0 ml of TES buffer (50 mM NaCl, 5 mM EDTA, 30 mM Tris, pH 8.0). Cells were lysed by the method of Meyers et al. (13), and the ethanol-precipitated plasmid DNA was electrophoresed through 0.7% horizontal agarose slab gels for 4 h at 80 V. Gels were stained in an ethidium bromide solution (5 μg/ml) for 0.5 h and photographed under long-wave UV light with a Polaroid MP4 camera and Kodak type 55 film.

Purification of plasmid DNA. Cleared lysates were prepared essentially as described by Clewell and Helinski (8) except that Triton X-100 (0.1%, final concentration) was substituted for Brij 58 (9). Covalently closed circular DNA was separated from bulk chromosomal DNA by cesium chloride–dye-buoyant density gradient centrifugation.

Restriction endonuclease digestion. *Hin*cII was obtained from New England Biolabs, and *Pst*I, *Eco*RI, *Hin*dIII, *Bam*HI, *Sma*I, and *Hae*II were purchased from Boehringer Mannheim Corp. Reactions were

carried out essentially as recommended by the supplier. Plasmid DNA was sometimes treated with self-digested proteinase K (2 μg/ml, final concentration; EM Laboratories) before endonuclease digestion. Fragments were separated by electrophoresis through horizontal slab gels of 1.0 to 1.5% agarose. Phage lambda DNA cleaved by *Hin*dIII was included as a molecular weight marker (14).

RESULTS

Conjugal transfer of ampicillin resistance. Figure 1 shows the plasmid complement of *H. ducreyi* strain HD147 and several representative transconjugants obtained by mating HD147 with plasmid-free strains of *H. influenzae* and selecting for transfer of ampicillin resistance. Strain HD147 (lane A) is resistant to tetracycline, ampicillin, and sulfonamide and carries three plasmids of 23.5 (pHD147), 7.0 (pHD747), and 4.9 (pHD447) Mdal. This strain is able to act as a conjugal donor of ampicillin resistance in membrane filter matings with drug-sensitive *H. influenzae* recipients. The resulting ampicillin-resistant (Ap^r) transconjugants produce β-lactamase and are seen to contain two plasmids of 23.5 and 7.0 Mdal (lane C). Neither tetracycline nor sulfonamide resistance was cotransferred when transconjugants were selected on the basis of ampicillin resistance. Strain HD9468, a clinical isolate which has the same antibiotic resistance pattern as HD147 but contains only two plasmids of 7.0 and 4.9 Mdal, could not transfer ampicillin resistance (data not shown).

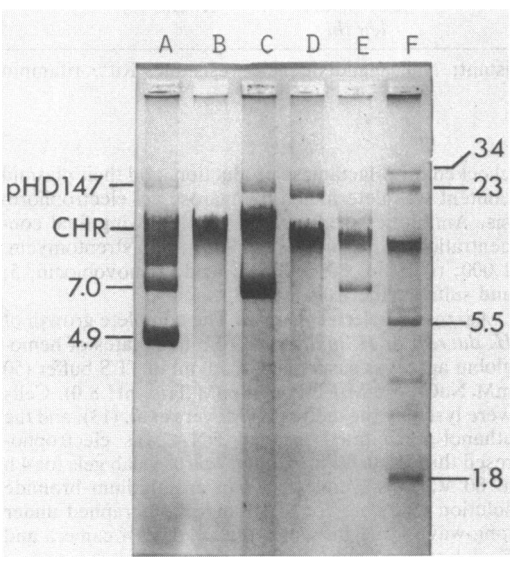

FIG. 1. Electrophoresis of plasmid DNA in 0.7% agarose gel. Lane A, strain HD147 showing three plasmids of 23.5 (pHD147), 7.0 (pHD747), and 4.9 (pHD447) Mdal; lane B, strain HI1008; lane C, Ap^r transconjugant of HD147 × HI1008; lane D, strain HI1008(pHD147), an ampicillin-sensitive derivative of the strain in lane C; lane E, strain HI1008(pHD747), Ap^r transconjugant of HD147 × HI1008 containing only the 7.0-Mdal plasmid; lane F, molecular weight standards: RP4, 34 × 10^6; Sa, 23 × 10^6; RSF1010, 5.5 × 10^6; pMB8, 1.8 × 10^6. CHR, Chromosomal DNA.

Daily subculturing of Ap^r *H. influenzae* transconjugants containing the 23.5- and the 7.0-Mdal plasmids in ampicillin-free broth over a period of 10 days yielded an ampicillin-sensitive clone which had lost the 7.0-Mdal plasmid yet still retained the larger 23.5-Mdal plasmid (lane D). This indicates that the ampicillin resistance determinant is in fact located on the 7.0-Mdal plasmid, and the 23.5-Mdal plasmid is considered to be cryptic. In one mating, an Ap^r transconjugant was isolated which was found to contain only a 7.0-Mdal plasmid (lane E); this is further evidence that ampicillin resistance is mediated only by this plasmid. This was apparently a relatively rare event since only one such clone was observed in over 50 Ap^r transconjugants screened by agarose gel electrophoresis.

These results suggest that the 23.5-Mdal cryptic plasmid of *H. ducreyi* strain HD147 does in fact have mobilizing capabilities. The 7.0-Mdal plasmid which encodes ampicillin resistance can be conjugally transferred to *H. influenzae* recipients only if the 23.5-Mdal plasmid is also present (coresident) in the cell. Other large conjugative plasmids in *H. ducreyi* do not share this mobilizing ability, however. Strain HD9265, for example, carries a 32-Mdal plasmid encoding tetracycline resistance (pHD9265) in addition to a 7.0-Mdal Ap^r plasmid (H. Deneer, M.S. thesis, University of Manitoba, Winnipeg, 1981). The plasmid pHD9265 was self-transferable to *H. ducreyi* or *H. influenzae* strains at frequencies of 10^{-5} to 10^{-3} per recipient upon selection for tetracycline resistance. In no case was cotransfer of the 7.0-Mdal plasmid observed, nor could transconjugants be isolated when just ampicillin resistance or when linked ampicillin-tetracycline resistance was selected (frequency, $<10^{-9}$).

Transformation as the mode of plasmid transfer may be ruled out for two reasons. DNase was routinely included in all matings and did not inhibit transfer, nor was the frequency of transfer appreciably altered when DNase was omitted (data not shown). Also, transfer was possible to the 9(N3) strain of *H. influenzae* (Table 2) which is poorly transformable (frequency, 10^{-9}) with both high- and low-molecular-weight plasmid DNA (1). No decrease in efficiency of ampicillin resistance transfer was noted when either the recipient or the donor strain carried the *rec-1* mutation.

Retransfer of ampicillin resistance from *H. influenzae* transconjugants. Table 2 shows the frequency of ampicillin resistance transfer from the original HD147 donor to recipient strains of

TABLE 2. Frequency of ampicillin resistance transfer in conjugative matings

Donor strain	Frequency[a] in recipient:					
	H. ducreyi HD35000	*H. influenzae* Rd	*H. influenzae* HI1008	*H. influenzae* 9(N3)	*H. influenzae rec-1*	*E. coli* C600
HD147	1.5×10^{-4}	1.6×10^{-5}	3.4×10^{-6}	2.0×10^{-6}	1.7×10^{-6}	5.3×10^{-5}
Rd(pHD147, pHD747)	4.0×10^{-5}	1.2×10^{-4}	1.1×10^{-6}	8.5×10^{-5}	6.0×10^{-4}	4.1×10^{-5}
rec-1(pHD147, pHD747)	6.3×10^{-5}	3.9×10^{-5}	1.4×10^{-5}	1.4×10^{-4}	—[b]	—
HI1008(pHD747)	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$
9(N3)(pHD147::Tn*A*)	3.4×10^{-5}	1.1×10^{-5}	3.3×10^{-6}	—	1.3×10^{-5}	—

[a] Transfer frequency is expressed as the number of Ap^r transconjugants formed per output recipient.
[b] Not determined.

H. influenzae, *H. ducreyi*, and *E. coli*. *Haemophilus* transconjugants all contained plasmids of 23.5 and 7.0 Mdal. *E. coli* transconjugants which produced β-lactamase could be isolated at frequencies similar to that seen with *H. influenzae* recipients, but the 23.5-Mdal plasmid was not detected.

When Ap^r *H. influenzae* transconjugants harboring the 23.5- and 7.0-Mdal plasmids were used as donors to sensitive *H. influenzae* recipients, transfer of ampicillin resistance was again seen but at frequencies somewhat higher than with HD147 as a donor. This was not unexpected since intraspecific *Haemophilus* matings are generally more efficient than interspecific matings (unpublished data). Transfer of ampicillin resistance could not be detected when strain HI1008(pHD747) containing just the 7.0-Mdal plasmid was used as a donor.

Transfer of sulfonamide resistance. Sulfonamide resistance in *H. ducreyi* has recently been shown to be mediated by the 4.9-Mdal plasmid present in HD147 (1a). We attempted to transfer sulfonamide resistance from HD147 in standard membrane filter matings with *E. coli* C600 and *H. influenzae* recipients. Sulfonamide-resistant (Su^r) *E. coli* transconjugants could be isolated at frequencies of 10^{-3} to 10^{-4} and, when screened by agarose gel electrophoresis, were found to contain a single plasmid of 4.9 Mdal. *H. influenzae* transconjugants arose at slightly higher frequencies of 10^{-2} to 10^{-3}, but these always contained two plasmids of 23.5 and 4.9 Mdal. Retransfer of sulfonamide resistance was not detected from *E. coli* transconjugants but was observed at frequencies of 10^{-3} when Su^r *H. influenzae* transconjugants were used as donors to other *Haemophilus* recipients. Sulfonamide resistance could not be transferred from HD9468, which contained only two plasmids of 7.0 and 4.9 Mdal, to either *E. coli* or *Haemophilus* recipients. These results demonstrate that the 23.5-Mdal plasmid (pHD147) is capable of mobilizing the 4.9-Mdal Su^r plasmid in addition to the 7.0-Mdal Ap^r plasmid.

Tetracycline resistance was never transferred in conjugative matings with HD147 and may be chromosomally mediated in this strain as previously described for other strains of *H. ducreyi* (7).

Mobilization of other nonconjugative plasmids by pHD147. It was questioned whether other small R-factors such as pJB1 (6, 7), RSF0885 (9, 18), or the two gonococcal Ap^r plasmids of 4.4 and 3.2 Mdal (18) could also be mobilized by pHD147. These plasmids were introduced individually into *H. influenzae* Rd by transformation and were tested for their ability to be mobilized by pHD147 in three-way matings conducted in a fashion similar to that described by Anderson (3). The initial donor in these matings was strain HI1008(pHD147) carrying just the 23.5-Mdal plasmid; the intermediate donor was strain Rd containing one of the small plasmids to be tested, and the final recipient was *E. coli* C600. Selection was for ampicillin- or sulfonamide-resistant *E. coli*. The results are shown in Table 3. All of the small ampicillin resistance plasmids from *Haemophilus* and *Neisseria* are mobilized by the 23.5-Mdal plasmid. Similar results are seen when *H. influenzae* recipients are used, but transfer of chromosomal markers occurs in conjugative matings between strains of *H. influenzae* (unpublished observations), making interpretation of the direction of transfer difficult in three-way-mating experiments. This problem

TABLE 3. R-plasmids mobilized by pHD147[a]

Plasmid	Size (Mdal)	Phenotype	Original source
pHD747	7.0	Ap^r	*H. ducreyi*
pJB1	5.7	Ap^r	*H. ducreyi*
RSF0885	4.1	Ap^r	*H. parainfluenzae*
p22209	4.4	Ap^r	*N. gonorrhoeae*
p88557	3.2	Ap^r	*N. gonorrhoeae*
pHD447	4.9	Su^r	*H. ducreyi*

[a] Three-way matings were modified from the method of Anderson (3) with *E. coli* C600 as the final recipient, *H. influenzae* HI1008(pHD147) as the initial donor, and *H. influenzae* Rd containing the indicated plasmid as intermediate donor. All small plasmids were mobilized.

does not occur when *E. coli* is used as the final recipient.

Restriction endonuclease analysis of pHD147. The 23.5-Mdal plasmid pHD147 was purified from HI1008(pHD147) and incubated separately with each of the restriction endonucleases *Pst*I, *Eco*RI, *Hinc*II, *Hin*dIII, *Bam*HI, *Sma*I, and *Hae*II. Agarose gel electrophoresis of the reaction mixture revealed that this plasmid DNA was not cleaved by any of the enzymes tested. In contrast, a 32-Mdal conjugative plasmid (pHD9265) introduced into, and purified from, the same background strain could be cleaved to yield the expected fragmentation pattern with these enzymes under the same reaction conditions (data not shown). Prior treatment of pHD147 DNA with proteinase K followed by incubation with restriction enzymes still failed to yield a cleavage pattern.

Isolation of pHD147::Tn*A*. From one mating of HD147 and *H. influenzae* strain 9(N3), a β-lactamase-producing transconjugant was isolated which was found to carry only a single plasmid species of 27 Mdal. This plasmid was transferable to *Haemophilus* recipients upon selection for ampicillin resistance. The frequency of this transfer was similar to that seen for the usual mobilization of the 7.0-Mdal plasmid by pHD147 (Table 2). For this reason it was suspected that the ampicillin resistance transposon Tn*A* may have transposed from the 7.0-Mdal plasmid to the cryptic mobilizing plasmid pHD147 to yield a new phenotypically marked conjugative plasmid, pHD147::Tn*A*. The plasmid pHD147::Tn*A* was purified and digested with *Pst*I, and the fragments were separated on an agarose gel (Fig. 2). It can be seen that although digestion of pHD147 with *Pst*I produces no cleavage fragments (lane A), digestion of pHD147::Tn*A* yields two small fragments of 2.0 and 0.38 Mdal plus the remainder of the mobilizing plasmid (lane B). These two small fragments correspond to *Pst*I-cleaved fragments from the 5.7-Mdal Ap^r plasmid pJB1 (lane C) and the 7.0-Mdal Ap^r plasmid pHD747 from HD147 (lane D). Both of these plasmids are known to carry the complete Tn*A* sequence (6, unpublished observations), and these fragments probably represent cleavage within Tn*A* (7).

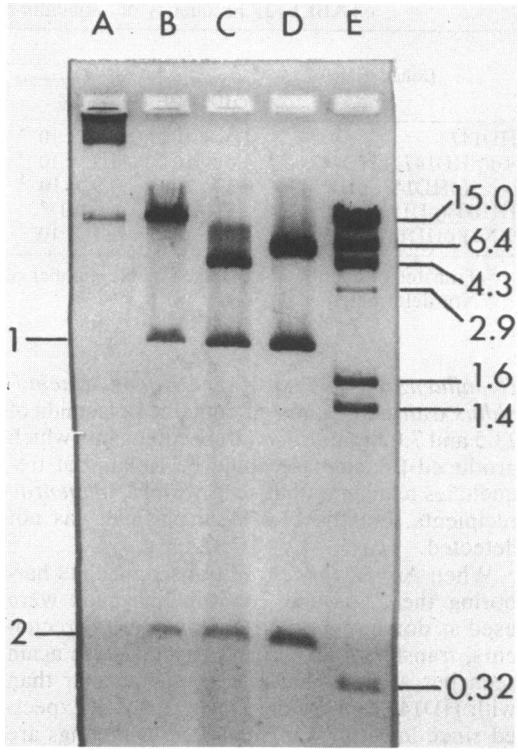

FIG. 2. Electrophoresis of *Pst*I-digested plasmid DNA in 1.2% agarose gel. Lane A, pHD147 (23.5 Mdal). The lower band in this lane is the open circular form of the plasmid. Lane B, pHD147::Tn*A* (27 Mdal). Lane C, pJB1. Lane D, pHD747 (7.0-Mdal plasmid of HD147). Lane E, *Hin*dIII-digested lambda DNA as molecular weight markers. Numbers 1 and 2 denote, respectively, the 2.0-Mdal and 0.38-Mdal internal *Pst*I-cleaved fragments of Tn*A*.

DISCUSSION

The large (>30 Mdal) R-plasmids found in *Haemophilus* species are generally self-transmissible by conjugation and, under optimal conditions, can attain transfer frequencies approaching 10^{-1} in intraspecific crosses (20; unpublished data). Small R-factors of less than 10 Mdal, however, lack the ability to mediate their own conjugal transfer. In *H. ducreyi* at least, no large conjugative plasmids mediating ampicillin resistance have yet been isolated. Nevertheless, plasmid-mediated resistance to this antibiotic is becoming increasingly common in clinical isolates of *H. ducreyi*, and recently, sulfonamide resistance encoded by a nonconjugative plasmid has been observed as well. Thus far, however, the mechanism responsible for the dissemination of these small Ap^r and Su^r plasmids has remained unclear.

The 23.5-Mdal plasmid pHD147, originally found in *H. ducreyi*, is self-transmissible but is phenotypically cryptic. It has, however, the ability to mobilize either a coresident 7.0-Mdal Ap^r plasmid or a 4.9-Mdal Su^r plasmid for transfer to sensitive *Haemophilus* or *E. coli* recipients. *Haemophilus* transconjugants acquiring ampicillin or sulfonamide resistance were found to have received both the 23.5-Mdal pHD147 plasmid and either the 7.0- or the 4.9-Mdal R-plasmid. Both the large and small plasmids were, in general, stably maintained in these *Haemophilus* transconjugants. The resulting Ap^r or Su^r transconjugants are able to retransfer

resistance to other strains including *H. ducreyi*, *H. influenzae*, and *E. coli*. In addition, pHD147 is able to mobilize other small nonconjugative plasmids, including ampicillin resistance plasmids originally isolated in *Haemophilus parainfluenzae* and *N. gonorrhoeae*. Thus, the existence of a plasmid transfer system of this type, one not strictly limited to *H. ducreyi* but able to cross species and generic lines, suggests one possible mechanism by which drug resistance genes carried on small plasmids could be disseminated throughout certain *Haemophilus* species.

A stable mobilization system in the genus *Haemophilus* has not been previously demonstrated although the apparent transfer of small Ap^r plasmids by conjugative means has been observed (20, 21, 24). However, no large conjugative plasmids could be found coresident in these strains of *Haemophilus*, and transfer ability, although not resistance, was rapidly lost upon storage, implying that a large, highly unstable sex factor was responsible for the conjugal transfer of these small R-plasmids. In contrast, pHD147 is easily detectable and appears to be quite stable.

The ability to mobilize small plasmids as seen for pHD147 does not appear to extend to other large conjugative plasmids of *H. ducreyi* or *H. influenzae*. We were unable to show that the conjugative R-plasmid pHD9265 could mobilize a coresident 7.0-Mdal Ap^r plasmid, and in addition, Stuy (25) was unable to mobilize the small Ap^r plasmid RSF0885 from an *H. influenzae* strain with a conjugative chloramphenicol-tetracycline resistance plasmid. This suggests that the mobilizing plasmid pHD147 contains unique gene sequences which are necessary to effect the mobilization of smaller R-plasmids but which are either inactive or not present on at least some large *Haemophilus* R-factors. Additional hybridization and incompatibility studies will be necessary to determine the relationship, if any, between pHD147 and other conjugative *Haemophilus* plasmids.

The mechanism of the actual mobilization process is presently unclear. There is no direct evidence for the formation of stable cointegrates between pHD147 and the 7.0-Mdal Ap^r plasmid, and indeed, one Ap^r transconjugant was isolated which carried the 7.0-Mdal plasmid alone. Also, the presence of the *rec-1* mutation in either the donor or the recipient had no effect on the frequency of mobilization. In this regard the *H. ducreyi* mobilization system resembles the *trans*-acting class-I system of Kilbane and Malamy (10). Further characterization of pHD147, both structurally and in terms of its mechanism of mobilization, may be aided by the presence of a readily identifiable and selectable phenotypic marker on the plasmid. Such a derivative of pHD147 was isolated from an Ap^r transconjugant which was found to carry only a single plasmid species of 27 Mdal. Restriction endonuclease analysis suggests that this plasmid (pHD147::Tn*A*) contains the 3.2-Mdal Tn*A* transposon encoding the β-lactamase enzyme. The cryptic 23.5-Mdal plasmid may have acquired this sequence through a transposition event from the 7.0-Mdal Ap^r plasmid, after which the remainder of the smaller plasmid was lost, or in the process of mobilization. The new pHD147::Tn*A* plasmid retains the conjugative ability of the parent pHD147, indicating that transposition of Tn*A* was onto a region of the plasmid not required for self-transferability. It is not known, however, if the mobilizing capabilities of pHD147 are affected by the insertion of Tn*A*.

Finally, it is known that the presence of a 24.5-Mdal plasmid in penicillinase-producing *N. gonorrhoeae* confers the ability to conjugally transfer a 4.4-Mdal coresident Ap^r plasmid to gonococcal recipients (4, 18, 19). Because a number of small *Haemophilus* Ap^r plasmids share considerable sequence homology with the 4.4- and 3.2-Mdal *Neisseria* R-plasmids (6, 11), it has been suggested that either the gonococcal plasmids originated in *Haemophilus* species, specifically *H. parainfluenzae* (24), or that an ampicillin resistance determinant of enteric origin simply transposed onto a small plasmid shared by members of the genera *Haemophilus* and *Neisseria* (12, 18). The finding that *H. ducreyi* possesses a means of conjugally transferring small non-self-transmissible R-factors at least between certain members of the genus *Haemophilus* in a fashion similar to that seen in *Neisseria*, suggests that a means may exist for the active transfer of such plasmids between these genera and may help establish the origins of R-plasmids in *N. gonorrhoeae* and *H. ducreyi*. At present it is not known if HD147 is capable of donating its 7.0-Mdal Ap^r plasmid to gonococcal recipients, and the extent of sequence homology between the 24.5-Mdal *Neisseria* mobilizing plasmid and pHD147 is not known. The *H. ducreyi* plasmid is unusual in that it appears to lack recognition sites for several restriction endonucleases. In contrast, Tenover et al. (26) have shown that the 24.5-Mdal gonococcal plasmid is cleaved by *Eco*RI, *Hin*cII, and *Sma*I, three enzymes which had no effect on pHD147. This suggests that these two plasmids may be structurally different, although further studies including hybridization may be necessary to verify this.

ACKNOWLEDGMENTS

We thank M. Fast, A. Ronald, and H. Nsanze for providing some of the bacterial strains.

This work was supported by the Medical Research Council of Canada (MRC-6327 and MRC-6368).

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