Penicillinase-Producing *Neisseria gonorrhoeae* in the Netherlands: Epidemiology and Genetic and Molecular Characterization of Their Plasmids

J. D. A. van Emden,* B. van Klingeren, M. Desseens-Kroon, and L. J. van Wijngaarden

*National Institute of Public Health, 3720 BA Bilthoven, The Netherlands*

Penicillinase-producing *Neisseria gonorrhoeae* strains were isolated in the Netherlands with increasing frequency during the period of 1976 to 1979. About 3% of the gonococci isolated in the first half of 1979 produced penicillinase. In contrast to the period of 1976 to 1977, most penicillinase-producing *N. gonorrhoeae* infections during the period of 1978 to 1979 were contracted in the Netherlands. The results of genetic and molecular studies on 80 penicillinase-producing *N. gonorrhoeae* strains were similar to earlier observations: resistance plasmids of only two sizes, 4.5 and 3.3 megadaltons (Md), occurred in penicillinase-producing *N. gonorrhoeae* strains, and these encoded for the TEM-1 enzyme. The 4.5-Md plasmid could be transferred to *Escherichia coli* when it coexisted with a plasmid of 24 Md. The latter plasmid was present in the vast majority of the strains carrying the 4.5-Md plasmid. One strain carried a cryptic 7.5-Md plasmid in addition to the commonly found 2.5-Md plasmid. Two penicillinase-producing strains of *Haemophilus parainfluenzae* isolated were found to carry a 3.3-Md plasmid species which was indistinguishable from the 3.3-Md gonococcal resistance plasmids. No plasmid deoxyribonucleic acid was found in two strains of penicillinase-producing *Branhamella catarrhalis*, and these strains produced a penicillinase different from the TEM-1 enzyme.

Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains were isolated for the first time in 1976 in the United Kingdom and the United States (2, 21), and since then they have been found in many other countries (4, 5, 20, 22, 35). A significant proportion of PPNG infections encountered in several Western countries seems to have been acquired from foreign sources rather than from local contacts (1). Importation has been mainly from countries in the far east and from the west coast of Africa (1, 2, 5, 18, 20, 21, 27). The origin of these PPNG strains was correlated with two different types of penicillin-resistance plasmids: a 4.4-megadalton (Md) plasmid originating in the far east countries and a 3.2-Md plasmid originating in west Africa (9, 20, 24, 25).

The emergence of PPNG strains at the end of 1976 (5) and their potential dissemination among the general population is of public health concern in the Netherlands. For this reason, the epidemiology of PPNG strains is studied centrally, and all PPNG strains isolated are asked to be sent to the National Institute of Public Health for confirmation of penicillinase production. Furthermore, the minimal inhibitory concentrations of various antibiotics are determined for all PPNG strains isolated.

To monitor changes at the genetic level, plasmids have been isolated and characterized from about 30% of all PPNG strains isolated in the period 1976 to the beginning of 1979. In this report we describe the results of the epidemiological data, sensitivity testing, and plasmid properties of PPNG strains. Furthermore, several isolates of penicillin-producing strains of *Branhamella catarrhalis* have been studied with regard to the type of penicillinase, transferability, and plasmid content.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** The gonococcal strains were obtained from clinical specimens collected from 19 different laboratories in the Netherlands. The two *Haemophilus parainfluenzae* strains were obtained from two different laboratories. Strain SC181 is a restriction-deficient derivative of *Escherichia coli* K-12 and was obtained from E. Lederberg. *E. coli* K-12 strains TEM and 1725E were provided by M. Matthew and were used as reference strains for the production of the TEM-1 and TEM-2-like penicillinases, respectively (16). *Haemophilus influenzae* HC31 is an ampicillin-resistant isolate of clinical origin. The beta-lactamase-producing *B. catarrhalis* strains were obtained from P. Piot and J. E. Brorsson. Drug resistance plasmids from PPNG strains were designated with the prefix pRIG followed by a number which is the number...
of the gonococcal isolate. In the same way the plasmids from the two *H. parainfluenzae* strains were designated with the prefix pRIH.

**Media.** Gonococci and *H. parainfluenzae* were cultivated on NVX agar (34) supplemented with 20% ascites fluid (NVXA). Incubation was at 35°C in a CO₂ incubator with an atmosphere consisting of air and CO₂ in a ratio of 19:1. Minimal inhibitory concentrations of relevant antibiotics were determined by a tube dilution method with blood agar. The tubes were inoculated with 3 drops of an inoculum containing approximately 10⁷ colony-forming units per ml. Nutrient broth and nutrient agar (94) were used for growth of *E. coli* K-12. M9 medium was prepared as described in reference 17.

**DNA isolation and characterization.** Plasmid deoxyribonucleic acid (DNA) from gonococci was obtained as follows. Two plates supplemented with penicillin G (1 μg/ml) were loop-streaked with gonococcal cultures. After 20 h of incubation at 35°C the confluent layer of gonococci was suspended in 2.4 ml of TES buffer [0.01 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane (Tris), and 0.001 M ethylenediaminetetraacetic acid (EDTA; pH 8.0)]. Lysozyme (0.125 ml, 10 mg/ml in distilled water) and 1 ml of 0.2 M EDTA (pH 8.0) were added, and the mixture was incubated for 10 min at 37°C. A solution of 20% sodium dodecyl sulfate (0.25 ml) was added; after thoroughly mixing, 1.7 g of solid CsCl was dissolved in the lysate, and the mixture was kept on ice for at least 2 h. Most of the chromosomal DNA was pelleted by centrifugation for 15 min at 30,000 rpm in a Beckman Ti50 rotor at 0°C. The supernatant was collected and subjected to equilibrium centrifugation in CsCl in the presence of ethidium bromide as described previously (33). Covalently closed circular (CCC) DNA was fractionated and purified (33).

For the isolation of plasmid DNA from *E. coli* K-12, 50-ml overnight cultures in M9 medium were subjected. Cleared lysates were prepared as described previously (7), and the plasmid DNA was collected by precipitation with polyethylene glycol 6000 (12). The DNA was dissolved in TES buffer, extracted with phenol and chloroform, and precipitated with ethanol. The precipitate was dissolved in 0.2 ml of TE buffer (0.01 M Tris and 0.001 M EDTA, pH 8.0) to convert CCC DNA into the open circular (OC) form. DNA preparations were γ-irradiated with a dose of 14,000 rad as described previously (32).

Agarose gel electrophoresis was performed on horizontal slab gels (20.5 by 16 by 0.4 cm). The gel buffer used was composed of 0.089 M boric acid, 0.089 M Tris, and 0.025 M EDTA (pH 8.2). Electrophoresis was at 40 V for 18 h. Gels were stained for 20 min in a solution of ethidium bromide (3 μg/ml) and photographed under long-wave ultraviolet irradiation with a Polaroid camera (MP-3) using Polaroid film (type 665) and a Wratten gelatin filter (Kodak, no. 9). Masses were inferred from the relative mobility of CCC DNA to standard reference CCC DNA (32). Electron microscopy of DNA was as described previously (32). OC DNA of φX174 RFII (contour length 1.70 μm) was used as internal standard for length measurements.

The restriction endonucleases *BamHI*, *AluI*, *HpaII*, and *HindII* were obtained from Boehringer; *MboII* was from Biolabs. Digestion of DNA was according to the manufacturer’s instructions. *Sau3AI* was prepared as described previously (30).

**Transfer of drug resistance.** Gonococcal resistance plasmids were transferred to *E. coli* K-12 strain SC181 by transformation as described previously (32). Conjugation was carried out by suspending a loopful of donor cells from an overnight plate in 1 ml of an overnight culture of *E. coli* K-12. The cells were centrifuged, and the pellet was spotted onto an NVX plate. This mating mixture was then incubated overnight at 37°C and suspended in nutrient broth; dilutions were spread on nutrient plates supplemented with ampicillin (100 μg/ml).

**Isoelectric focusing of beta-lactamases.** Isoelectric focusing and extraction of beta-lactamase from *E. coli* were as described previously (32). Extracts of gonococci for isoelectric focusing of beta-lactamase were prepared by treatment of a suspension of gonococcal cells in 0.01 M EDTA (pH 8.0) with lysozyme (final concentration, 0.1 mg/ml) for 10 min at 37°C followed by overnight dialysis against 0.001 M EDTA (pH 8.0).

**RESULTS**

**Epidemiology and drug sensitivity of PPNG strains.** The surveillance of PPNG strains in the Netherlands started in 1977 (34). PPNG strains isolated in regional public health laboratories and other centers are sent to the Institute usually together with basic information concerning the patients. This information is sent to the Chief Medical Officer of Health, who tries to gather more epidemiological data. However, contact tracing was usually unsuccessful since the majority of the infections were found to be acquired from untraceable prostitutes.

During the period 1976 to 1977 only 13 patients with a PPNG infection were found in this country. The incidence rose toward the end of 1978, resulting in 52 cases in that year. In 1979 a further increase was observed: the total amounted to 273 cases, 119 in the first half and 154 in the second half of the year.

In contrast to the patients in 1977, who contracted the disease in the far east or in west Africa, the majority of the patients are infected now in the Netherlands: 80% in 1978 and more than 90% in 1979. The proportion of female cases increased from 8% in 1978 to 20% in 1979. As in 1978, most PPNG infections are now acquired in Amsterdam (ca. 50%), The Hague (16%), and Rotterdam (10%). Remarkably, about half of the patients are foreign laborers.

A good estimate of the frequency of PPNGs was obtained from an inquiry among the regional laboratories. Based on the total numbers of gonococcal isolates and the numbers of penicillinase-producing isolates, it was estimated for 1978 and the first half of 1979 that about 3% of
the isolates of *N. gonorrhoeae* in this country were producing penicillinase. This figure also reflects the present incidence in Amsterdam, where about half of the gonococci in the country are isolated; in 1978 the PPNG frequency in this city was only 1%.

Susceptibility of PPNG strains to antibiotics. Previously we reported minimal inhibitory concentration values for benzylpenicillin, ampicillin (amoxycillin), spectinomycin, cefuroxime, tetracycline, and sulfamethoxazole as determined with 174 PPNG strains (35). The extended results, comprising more than 300 strains, are still consistent with the conclusion that all PPNGs tested so far are normally susceptible to the main alternatives for penicillin in the treatment of gonorrhoeae, e.g., cefuroxime and spectinomycin.

Sizes of plasmid species in the gonococcal isolates of various geographic origins. Purified plasmid DNA was extracted from 81 penicillinase-producing and 6 penicillinase-nonproducing strains. All DNA preparations were subjected to agarose gel electrophoresis, and the molecular weights of the plasmid species in each isolate were determined. The results are summarized in Table 1, and a gel loaded with representative DNA preparations is shown in Fig. 1. To distinguish CCC DNA from OC DNA, which usually contaminated the DNA preparations, the DNA samples were exposed to γ rays. By this treatment CCC DNA is converted to OC DNA. Figure 2 shows an example of an agarose gel on which irradiated and non-irradiated samples are run in juxtaposition. The OC structures of all plasmid migrations slower than the CCC structures of the corresponding DNA species in agarose under the conditions used. Seventy-one of the PPNG strains showed an identical plasmid banding pattern in agarose gels. Three different plasmid species were present in each of these isolates, having masses of 2.5 Md, 4.5 Md, and 24 Md, respectively (Fig. 1, tracks b to k; Fig. 2, tracks b and b'). The contour lengths of the different plasmid molecules in one of the preparations were determined by electron microscopy, and we measured lengths of 1.34 ± 0.05 μm, 2.45 ± 0.28 μm, and 12.6 ± 0.9 μm, respectively. These values correspond to masses of 2.6, 4.8, and 25 Md, respectively, which is in reasonable agreement with the values obtained by gel electrophoresis. Two strains carried only the 4.5-Md plus the 2.5-Md plasmid (Fig. 1, track o). In one strain, GO181, a fourth plasmid species was present in addition to those of the sizes mentioned above (Fig. 1, track p; Fig. 2, tracks f and f'). The mass of this plasmid was estimated to be 7.5 Md. Seven PPNG strains harbored a 3.3-Md plasmid in addition to the 2.5-Md plasmid, and none of these strains carried the 24-Md plasmid DNA species (Fig. 1, track n; Fig. 2, tracks e and e'). As shown in Table 1, the majority of strains carrying this plasmid combination originated in west Africa. All of the six non-PPNG isolates carried the 2.5-Md plasmid, and three of them also harbored the 24-Md species (Fig. 1, track a; Fig. 2, tracks a and a'). Penicillinase-producing strains were isolated from two male patients with clinical symptoms of gonorrhoea. These strains were at first probably erroneously identified as *N. gonorrhoeae* and freeze-dried. From the freeze-dried ampoules of both isolates, *H. parainfluenzae* was grown; both cultures had identical biotypes (II) and minimal inhibitory concentration patterns. Both of the strains, HP67 and HP70, were found to carry two plasmid species with masses of 3.2 and 2.0 Md, respectively (Fig. 1, tracks 1 and m; Fig. 2, tracks d and d').

Transfer of the resistance plasmids to *E. coli* K-12. All of the 81 plasmid DNA preparations of the PPNG strains and the two *H. para-

### Table 1. Plasmid species and origin of 81 PPNG and 6 non-PPNG strains; transferability of ampicillin resistance to *E. coli* K-12

<table>
<thead>
<tr>
<th>Masses of plasmids (Md)</th>
<th>No. of isolates and origin</th>
<th>Mass of plasmid in transformant (Md)</th>
<th>Transferability to <em>E. coli</em> K-12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The Netherlands</td>
<td>Asia</td>
<td>Africa</td>
</tr>
<tr>
<td>24 + 4.5 + 2.5</td>
<td>51&lt;sup&gt;5&lt;/sup&gt;</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>4.5 + 2.5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.2 + 2.5</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>24 + 2.5&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2.5&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> All strains were isolated from patients who were present in the Netherlands at the time of isolation. The origin points to the country where the infection was contracted.

<sup>b</sup> Numbers within parentheses indicate the number of transformants tested.

<sup>c</sup> Numbers within parentheses indicate the number of donor strains tested.

<sup>d</sup> One of these isolates, GO181, also carried a 7.5-Md plasmid.

<sup>e</sup> These strains were the only penicillinase nonproducing strains included in this study.
influenzae strains transformed E. coli K-12 strain SC181 to ampicillin resistance with frequencies of 10^4 to 10^5 transformants per μg of total plasmid DNA. This host became resistant to a level of 1 to 2 mg of ampicillin per ml. Plasmid DNA extraction from E. coli transformants showed the presence of only a single DNA species with a mass of either 4.5 or 3.3 Md, depending on which one of these plasmid species was present in donor DNA (Table 1).

Figure 3 is a photograph of a gel showing three examples of the migration of the resistance plasmids extracted from E. coli transformants in juxtaposition to the plasmid DNA isolated from the corresponding PPNG strains and strain HP67 that was used to transform E. coli K-12. Earlier studies showed that strains harboring the 24-Md plasmid can mobilize the 4.5-Md drug resistance plasmid to other gonococci and E. coli K-12, probably by a mechanism similar to conjugation (8, 25). In this study we also found a strict correlation between the ability to transfer ampicillin resistance to E. coli K-12 by mating and the presence of the 24-Md plasmid species. This is consistent with the finding that the 24-Md plasmid acts as a transfer factor and mobilizes the non-self-transferable 4.5-Md drug resistance plasmid (3, 8, 25, 28, 29). No transfer of ampicillin resistance from strains HP67 and HP70 was observed.

Stability of the resistance plasmids. Roberts et al. (24) observed that the 4.4-Md gonococcal plasmid pMR0360 in E. coli K-12 was lost from about 50% of the cells after prolonged growth in nonselective medium. In contrast, the smaller gonococcal plasmid pMR0200 (3.2 Md) was rapidly lost, so that after 120 h of growth, only 0.1% of the population was resistant. We have observed the same phenomenon with two transformants carrying the 4.5-Md plasmid and two carrying 3.3-Md plasmids. These plasmids were found to be very stable in their original gonococcal hosts: penicillinase-nonproducing gonococci were found at a frequency of less than 2% after 5 days of daily passage on nonselective medium. It should be noted that the plasmid stability in vivo might differ from that observed in vitro. In several cases (five of the six non-
PPNG strains in Table 1) we obtained PPNG and non-PPNG strains originating from the same sample of a single patient. The PPNG strains from these cases showed the same high in vitro stability as strains from patients with PPNGs only.

**Similarity between the resistance plasmids.** DNA-DNA hybridization studies have shown that the 4.4-Md and the 3.2-Md classes of gonococcal penicillinase resistance plasmids are mutually homologous (13, 14, 24). Furthermore, at least 90% of the sequences in the smaller plasmid class were found to be present in the 4.4-Md plasmid (24). We cleaved three different 4.5-Md plasmids with various restriction enzymes. The plasmid DNA was isolated from *E. coli* transformants. Cleavage with *Bam*HI and *Hpa*II resulted in identical fragmentation patterns. However, slight mutual differences in fragmentation patterns were found after digestion of the plasmids with *Hind*II (2 cleavage sites) and *Sau*3AI (>12 cleavage sites).

Digestion of two 3.2-Md gonococcal and the two 3.2-Md *H. parainfluenzae* resistance plasmids with various restriction enzymes showed no mutual differences. The fragmentation patterns of the 3.2-Md plasmids and the 4.5-Md plasmids differed only in the position of a few bands, indicating a large proportion of base sequence homology between the plasmids. As an example, Fig. 4 shows the *Alu*I and *Mbo*II fragmentation patterns of the 3.3-Md plasmids from GO263 and HP67 and of the 4.5-Md plasmid from GO181.

Sox et al. (29) found that transformation of *N. gonorrhoeae* by plasmid pFA3 resulted in the establishment of plasmids smaller than pFA3 in a minority (about 20%) of the transformants and also in the establishment of plasmids with an increased size (28 and 5.8 Md). As described above, we also found that transformation of *E. coli* usually resulted in the establishment of either the 4.5-Md or the 3.2-Md plasmid. One exception was found among transformants derived from plasmid DNA of the gonococcal strain GO222: in one transformant a plasmid of 6.0 Md was found, whereas two other transformants tested carried the 4.5-Md plasmid.

**Isoelectric focusing of gonococcal penicillinases.** The beta-lactamase produced by penicillin-resistant gonococci resembles the TEM-1 enzyme in its activity towards different penicillins and cephalosporins (4, 19, 21, 31). The isoenzyme pattern resulting from isoelectric focusing is very characteristic for the different penicillinases (16), and such patterns can reveal slight differences that are not revealed by differences in substrate activity, as is the case with

---

**Fig. 2.** Agarose gel electrophoresis of CCC DNA and change in banding pattern after conversion to OC DNA due to nicking by γ irradiation (tracks with primed letters). (a, a’) GO98; (b, b’) GO245; (c, c’) GO137; (d, d’) HP67; (e, e’) GO263; (f, f’) GO181.
TEM-1 and TEM-2. These enzymes have main enzyme components with isoelectric points of 5.4 and 5.6, respectively, but they are similar in substrate spectrum. By isoelectric focusing, Percival et al. (19) and Philips (21) found two main bands having isoelectric points of 5.4 and 5.5. This suggested that the gonococcal penicillinas might be different from the TEM-1 and TEM-2 enzymes. We performed isoelectric focusing of extracts of 18 different PPNG isolates and of strains HP67 and HP70 (Fig. 5). The main component of the beta-lactamase isolated from gonococci was found at the same position (pH 5.4) as that of the TEM-1 enzyme from E. coli (Fig. 5, tracks a to e). However, in the majority of the gonococci analyzed, a faint band at pH 5.5 was observed, whereas the faint E. coli TEM-1 band at pH 5.6 was not present.

The isoenzyme pattern of beta-lactamases extracted from E. coli transformants carrying the gonococcal resistance plasmids was identical to that of the TEM-1 enzyme extracted from E. coli (Fig. 5, tracks i to m). Therefore, it seems that the slight difference in isoenzyme patterns between N. gonorrhoeae and E. coli is due to a different expression of the plasmid DNA in the different hosts. Similar slight host dependent differences in the isoenzyme patterns have been described previously for TEM-like beta-lactamas in various Enterobacteriaceae (6). The isoenzyme patterns of strains HP67 and HP70 resemble those of TEM-1 in E. coli.

In Fig. 5 we have included an extract from an ampicillin-resistant H. influenzae strain (lane f) to show the similarity between enzymes originating from different species.

Beta-lactamase-producing B. catarrhalis. B. catarrhalis (previously named Neisseria catarrhalis) is a commonly found inhabitant of the human nasopharynx. Beta-lactamase-producing variants of this species have been reported (15). We obtained two such strains. Attempts to detect CCC DNA in these

Fig. 3. Agarose gel electrophoresis of plasmid DNA isolated from PPNG strains and in juxtaposition from E. coli K-12 transformed by corresponding gonococcal plasmid DNA preparations. a, GO181; b, SC181 (pRIG181); c, HP70; d, SC181 (pRIH70); e, GO245; f, SC181 (pRIG245); The masses indicated are those of the CCC components.

Fig. 4. Agarose gel electrophoresis of plasmid DNA isolated from E. coli transformants and cleaved by the restriction endonucleases MboII (tracks A to C) and AluI (tracks E to G). (A, E) pRIG263; (B, F) pRIH67; (C, G) pRIG181; (D) marker, bacteriophage lambda DNA cleaved with EcoRI plus HindIII.
FIG. 5. Isoelectric focusing of penicillinases isolated from PPNG strains (c to e), H. influenzae (f), H. parainfluenzae (g, h), and E. coli K-12 transformants carrying various plasmids from gonococci (i, l, m) and H. parainfluenzae (j, k). Extracts from the standard E. coli strains (a, b, n, o) producing TEM-1 and TEM-2 were included as references.

strains by ethidium bromide-cesium chloride centrifugation of lysates were unsuccessful. The beta-lactamase production in these strains was found to be an extremely stable property. No penicillinase-negative variants were found among about 1,000 colonies tested after 3 weeks of daily subculturing onto nonselective medium. Isoelectric focusing of extracts of the two strains showed two bands at pH 5.55 and pH 5.35, respectively. The isoenzyme banding pattern was identical for both strains and clearly distinct from that of the TEM-like enzymes (data not shown). Attempts to transfer the penicillin resistance to E. coli K-12 by mating were unsuccessful.

DISCUSSION

In contrast to 1977, PPNG infections from later periods were mainly contracted in the Netherlands, and PPNG infections should therefore be considered as endemic. The incidence of PPNG isolates is presently at a level of about 2 to 3% of all gonococcal isolates, mainly due to the contribution of a few “hot areas” in Amsterdam and The Hague. This incidence is significantly higher than that found in the United
States, the United Kingdom, and the Scandinavian countries (D. Lind, personal communication). The results of our experiments suggest that the successful spread of the 4.5-Md “Asia” plasmid is related to its transferability: virtually all strains carrying this plasmid harbored in addition the 24-Md plasmid that has been identified as a transfer factor (8, 28), whereas only 5 to 34% of the penicillin-sensitive isolates carry the 24-Md plasmid (26, 28). Until now, no 3.2-Md “Africa” plasmid-carrying strains have been found which also harbor the 24-Md species and transfer penicillin resistance (7, 23). The 24-Md and the cryptic 2.5-Md gonococcal plasmids constitute two remarkable homogenous groups of plasmids as based on their size, DNA-DNA homology, and their fragmentation patterns after cleavage with restriction enzymes (25, 28). Based on DNA-DNA hybridization experiments a similar high degree of relatedness has been found among the two size classes of the gonococcal resistance plasmids (14, 16). After cleavage with restriction endonucleases we observed small differences in fragmentation patterns among the 4.5-Md and the 3.2-Md plasmids. Furthermore, small differences were also found between the 4.5-Md plasmid pFA3, described by Sox et al. (29), and the 4.5-Md plasmids investigated in our studies. For instance, we found two HindII sites on these plasmids, whereas pFA3 has only one. We presume that these differences reflect small changes in the plasmid genomes that took place after their initial introduction into the species N. gonorrhoeae. Foster and Foster (10) observed similar slight changes in restriction enzyme fragment patterns of the cryptic 2.6-Md plasmid from gonococci serially passaged 300 times, compared with the plasmid cleavage patterns from nonpassaged cultures.

It is interesting that one strain, GO181, harbored a cryptic plasmid of 7.5 Md, a size which has not been described previously for a plasmid in the species N. gonorrhoeae. Sox et al. (29) found that transformation of N. gonorrhoeae by the 4.5-Md plasmid pFA3 often results in the establishment of plasmids that are smaller than pFA3, and some of them are deleted in a region such that they are undistinguishable from the 3.2-Md Africa plasmids. This led to the speculation that the 3.2-Md African variant might have arisen from a 4.5-Md Asia plasmid by a transformation-produced deletion. In relation to this it is very interesting that we found a 3.2-Md plasmid in two isolates of H. parainfluenzae. The cleavage patterns were similar to those of the African variant of the gonococcal resistance plasmids; therefore, all these plasmids carry the same sequences of DNA. Previously, natural isolates of penicillinase-producing strains of H. influenzae and H. parainfluenzae have been described which carry 4.5-Md resistance plasmids, which show significant homology with the 4.5-Md gonococcal plasmids (13, 14, 24). Because H. parainfluenzae can be transformed efficiently by plasmid DNA (11), this organism might have acquired the 3.2-Md plasmid by transformation with DNA released from penicillinase-producing strains of Neisseria or Haemophilus.

By isoelectric focusing we analyzed the type of beta-lactamase of 18 PPNG strains, and all were of the TEM-1 type. This is consistent with the previous enzymatic studies on PPNG strains and the finding that the gonococcal resistance plasmids carry about 40% of the translocatable element TnA (9).

B. catarrhalis is a common component of the human indigenous flora of the nasopharynx, and the minority of beta-lactamase-producing B. catarrhalis strains might be a potential source of beta-lactamase genes transferable to genera like Neisseria and Haemophilus (15). However, we found no indication that penicillinase production in the two B. catarrhalis strains investigated is transferable or plasmid mediated. Furthermore, the beta-lactamase in these strains was not of the TEM-like enzyme. Similar results have been obtained with other penicillinase-producing B. catarrhalis strains (18) and also with the commensal Neisseria perflava (23).

ACKNOWLEDGMENTS

We acknowledge J. Borst for the typing of cultures and M. Schouls for his technical assistance.

LITERATURE CITED


