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# Previously Undescribed 6.6-Kilobase R Plasmid in Penicillinase-Producing *Neisseria gonorrhoeae*

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**A penicillin-resistant *Neisseria gonorrhoeae* strain was isolated. The resistance was due to the production of TEM-1 β-lactamase encoded by a plasmid. This 6.6-kilobase plasmid was compared with the previously known 7.4- and 5.3-kilobase penicillin R plasmids of *N. gonorrhoeae*.**

Since 1976, numerous penicillin-resistant strains of *Neisseria gonorrhoeae* have appeared. Their resistance is related to the synthesis of a plasmid-encoded TEM-1 β-lactamase (3, 15, 20). Two kinds of plasmids coding for this enzyme have been described so far: a 5.3-kilobase (kb) plasmid, found in strains isolated from patients who have been in Africa, and a 7.4-kb plasmid from Asia (14, 19, 22). These two plasmids are very similar; both carry the same part of a Tn*2* transposon (5, 16), and the only difference between them is a 2.1-kb fragment inserted in the 5.3-kb plasmid to give the 7.4-kb one (1). In 1982, a penicillin-resistant *N. gonorrhoeae* strain (HN1) was isolated at Nimes Hospital from an inflamed Fallopian tube. The strain owed its resistance to the production of a β-lactamase. We have characterized the enzyme and the genetic determinant encoding its synthesis.

β-Lactamase production was detected with nitrocefin (Glaxo Pharmaceuticals, Ltd.) (13). The enzyme was purified 110-fold on a cyanogen bromide-Sepharose column, using cephalosporin C as ligand (4). The substrate profile of the β-lactamase, determined by the spectrophotometric method of Samuni (17), was similar to that of TEM-1. Like TEM-1, the HN1 enzyme was inhibited by cloxacillin and was not inhibited by 1 mM NaCl. Isoelectric focusing on a polyacrylamide gel (9) gave pIs of 5.48 for TEM-1, 5.7 for TEM-2, and 5.48 for the β-lactamase from strain HN1. The substrate profile of the enzyme, its sensitivity to inhibitors, and its pI indicate therefore that it is a TEM-1 β-lactamase, as are the β-lactamases of other penicillinase-producing *N. gonorrhoeae* strains previously described (6, 20).

Plasmid DNA was extracted from *N. gonorrhoeae* HN1 according to the method described by Elwell and Falkow (2). It was studied by agarose gel electrophoresis and compared with plasmid DNA extracted from *N. gonorrhoeae* strain 1347, containing a 4.2-kb cryptic plasmid and a 5.3-kb R plasmid (kindly provided by J. Y. Riou [7]), and from *Escherichia coli* OF37 containing the 7.4-kb gonococcus R plasmid pJ102 (given by O. Fayet [5]). *N. gonorrhoeae* strain HN1 contained two plasmids of 4.2 and 6.6 kb (Fig. 1). Plasmid DNA of strain HN1, purified by cesium chloride ultracentrifugation with ethidium bromide (2), was successfully used to transform *E. coli* HB101 (8) to ampicillin resistance according to the method of Elwell and Falkow (2). Transformed clones produced β-lactamase and contained a single plasmid termed pGF1. The size of this plasmid, 6.6 kb, was between those of the two previously described gonococcal β-lactamase plasmids (7.4 and 5.3 kb). The homology between plasmid pGF1 and the 7.4-kb plasmid pJ102 (5) was studied by the Southern blotting method (18). Whole plasmid pJ102 was used as a probe. Its hybridization with pGF1 fragments generated by cleavage with *Alu*I, *Hin*fI, and a combination of *Bam*HI plus *Hin*cII was studied. Hybridization of the same fragments with a probe consisting of the pGF1 plasmid itself was used as a reference. All fragments generated by *Bam*HI plus *Hin*cII hybridized strongly with pJ102 (data not shown). Of the fragments cut by *Hin*fI, the 0.35-kb one did not hybridize and the 0.62- and 0.385-kb fragments hybridized less strongly than the control (Fig. 2). Hydrolysis of pGF1 by restriction endonucleases gave the following results. *Hin*cII cut it once, as did *Pst*I and *Ava*I. *Bam*HI digestion generated two fragments of approximately

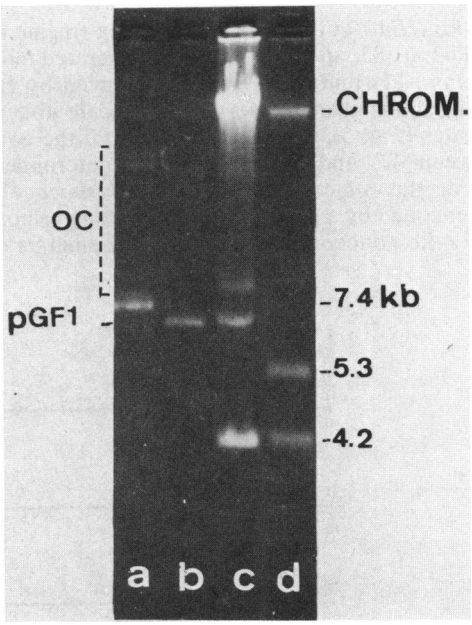


FIG. 1. Agarose gel electrophoresis of cleared lysates. Lane a, *E. coli* OF37 containing pJ102; lane b, *E. coli* HB101 containing plasmid pGF1; lane c, *N. gonorrhoeae* HN1; lane d, *N. gonorrhoeae* 1347 containing 4.2- and 5.3-kb plasmids. OC refers to the open circular forms. CHROM. refers to chromosomal DNA.

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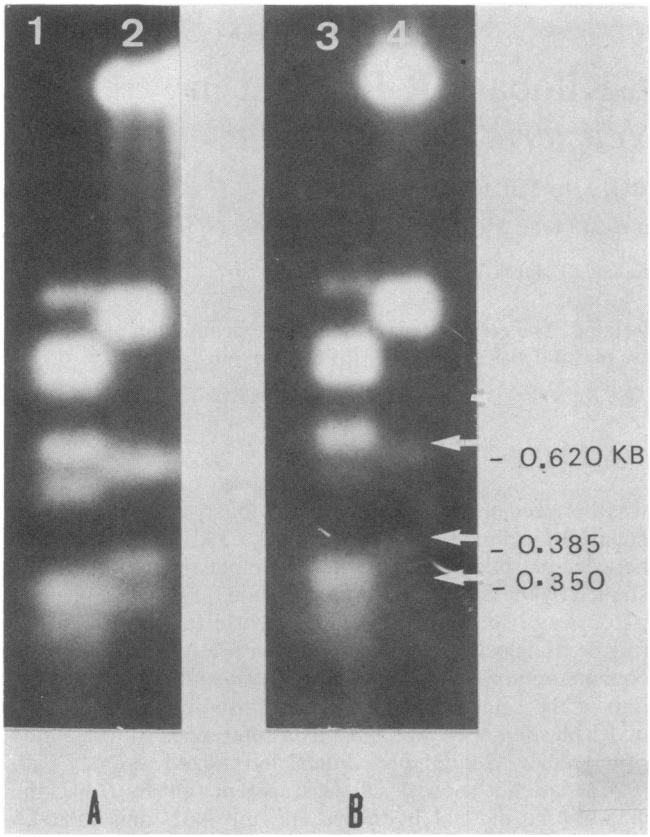


FIG. 2. Southern blotting hybridization autoradiogram. pGF1 was cleaved by *Alu*I (lanes 1 and 3) or *Hin*fI (lanes 2 and 4). The probes used were pGF1 (A) and pJ102 (B). Arrows indicate the fragments of pGF1 cut by *Hin*fI which hybridized weakly or not at all with pJ102.

3 and 3.6 kb. *Hin*fI cut the plasmid into six fragments (2.9, 1.2, 1.1, 0.62, 0.385, and 0.35 kb). *Alu*I generated more than six fragments. Plasmid pGF1 was not cleaved by *Hin*dIII. Double digestions were performed to locate the various cleavage sites (Fig. 3). The exact location of the two *Hin*fI sites between 4.7 and 5.8 kb was not determined. The synthesis of the β-lactamase of *N. gonorrhoeae* HN1 is therefore encoded by a plasmid that has extensive homology with the 7.4-kb gonococcal β-lactamase plasmid as demonstrated by the Southern blotting hybridization. Furthermore, the restriction sites corresponding to the Tn*2* transposon, which encodes the β-lactamase in the 5.3- and 7.4-kb plasmids, are in the expected locations (one *Pst*I site, one *Hin*cII site, two *Hin*fI sites, and one *Bam*HI site) (10–12). However, pGF1 is different in size (6.6 kb) from the two previously known R plasmids (5.3 and 7.4 kb), and hybridization by the Southern blotting method showed the presence of a *Hin*fI fragment that did not hybridize with the 7.4-kb pJ102. Comparison of the sites of *Bam*HI cleavage of the 7.4- and 5.3-kb plasmids (5.3-kb plasmid pNG18 [1] is cleaved in 2.4- and 2.9-kb fragments; 7.4-kb plasmid pJ102 is cleaved in 2.4- and 5-kb fragments) with those of pGF1 allows us to locate this insertion approximately: in pGF1 the *Bam*HI fragment encompassing *Pst*I and *Hin*cII sites corresponded to the 2.4-kb *Bam*HI fragment in the 5.3- and 7.4-kb plasmids and was modified to give a 3.6-kb fragment (Fig. 3).

The simplest explanation of the origin of pGF1 is therefore that it derives from the 5.3-kb plasmid by insertion of this DNA fragment. As far as we know, the 6.6-kb plasmid we report here has not been previously described (plasmid pG04717, recently described, differs from the 7.4-kb plasmid by a 2.9-kb deletion [21]).

Current research is presently in progress in our laboratory to determine the origin of the supplementary sequence.

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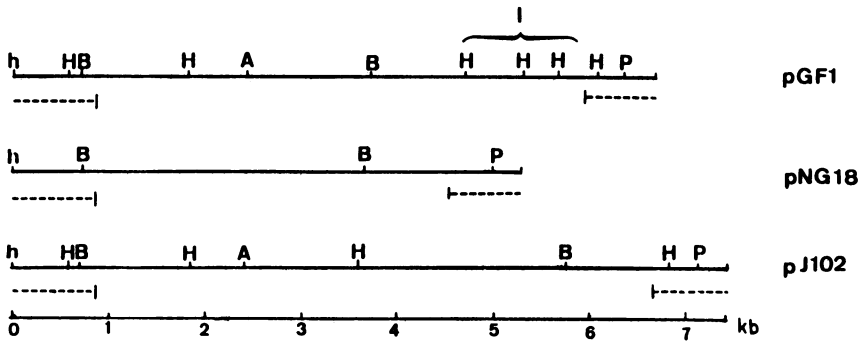


FIG. 3. Restriction endonuclease map of pGF1 compared with the maps of 7.4-kb pJ102 and 5.3-kb pNG18. I represents a DNA fragment of unknown origin. Regions underlined with dashed lines represent the ampicillin transposon. Abbreviations: h, *Hin*cII; H, *Hin*fI; B, *Bam*HI; A, *Ava*I; P, *Pst*I. The presence of *Hin*fI sites in pNG18 has not been evaluated (1).

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