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Isolation and Molecular Characterization of β-Lactamase-Producing *Haemophilus parainfluenzae* from the Genital Tract

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Three *Haemophilus parainfluenzae* strains isolated from the urogenital tract harbored a β-lactamase-coding 3.2-megadalton plasmid identical, by restriction endonuclease digestion and hybridization with radioactive and biotin-labeled probes specific for the TEM-1 β-lactamase and Tn*A* sequences, to the 3.2-megadalton "African-type" plasmid found in *Neisseria gonorrhoeae*.

Since its first description in 1976 (6), β-lactamase-producing *Haemophilus parainfluenzae* has been isolated more frequently from the respiratory tract than β-lactamase-producing *Haemophilus influenzae* (16). This high frequency of recovery stimulated the interest of investigators (4, 17) to define the basis of resistance in this species. Usually considered a component of the normal flora of the upper respiratory tract, *H. parainfluenzae* is less commonly encountered as a urogenital commensal but has nevertheless been considered to be a causative agent of urethritis in humans (3, 5, 19). Rarely, β-lactamase-producing *H. parainfluenzae* has been isolated from genital specimens (19, 20). In the course of a survey of respiratory and anogenital tract colonization by *Haemophilus* spp., 362 patients attending the Dekalb County Sexually Transmitted Diseases Clinic, Decatur, Ga., were screened for the presence of *Haemophilus* spp. (A. Y. Martel, F. O. Sottnek, W. E. DeWitt, S. J. Kraus, and W. L. Albritton, 5th Int. Meet. Int. Soc. STD Res., Seattle, Wash., 1 to 3 August 1983). Seventy patients (19.3%) harbored *Haemophilus* spp. in the anogenital area, and three strains, 11, 259, and 361, were found, by hydrolysis of the chromogenic cephalosporin nitrocefin (Oxoid Ltd., Basingstoke, England), to produce β-lactamase. The three strains required V factor for growth and were identified as biotype II *H. parainfluenzae* by the methods of Kilian (7, 8).

Molecular characterization. The plasmid content of the three β-lactamase-producing *H. parainfluenzae* strains was analyzed by the alkaline lysis method (1). All β-lactamase-producing strains harbored a plasmid (pLQ920) of 3.7 megadaltons (MDa), along with smaller plasmids of molecular sizes between 0.75 and 2.2 MDa. The presence of cryptic plasmids of less than 2.0 MDa was previously observed in *H. parainfluenzae* (2, 11, 17, 20). Plasmid DNA purification by cesium chloride-ethidium bromide gradients (10) yielded the same patterns of bands (Fig. 1). The purified plasmid DNA was used to transform *Escherichia coli* HB101 (10). All ampicillin-resistant clones harbored the pLQ920 plasmid with or without the smaller plasmid bands (results not shown), indicating that the β-lactamase was encoded by plasmid pLQ920. The plasmid DNA of one of these transformants was compared with pLQ920 from strains 11 and 361 isolated by electroelution (10); after digestion with restriction enzymes *Hae*III, *Rsa*I, *Acc*I, and *Alu*I, all showed identical digestion patterns. pLQ920 was also compared with p88557, a 3.2-MDa plasmid isolated from an "African-type" penicillinase-producing *Neisseria gonorrhoeae* strain (12) by using digestion with restriction enzymes *Bam*HI, *Pst*I, and *Pvu*II. The two plasmids gave identical digestions with these enzymes (results not shown).

β-Lactamase characterization. The β-lactamases of the three *H. parainfluenzae* strains were compared by isoelectric focusing as previously described (15), and all had

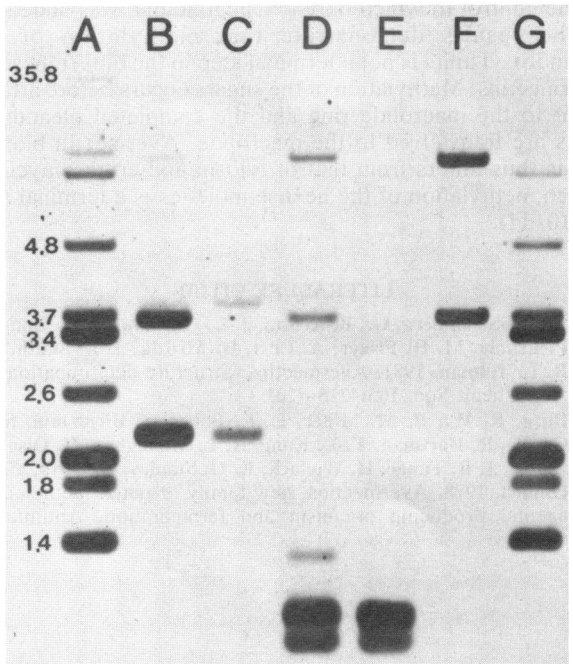

FIG. 1. Ethidium bromide-stained agarose gel electrophoresis of plasmids prepared by cesium chloride-ethidium bromide gradients from *H. parainfluenzae* 11, 259, and 361 (lanes B, C, and D, respectively). Lane E, Strain 363, a non-β-lactamase-producing biotype II *H. parainfluenzae* strain isolated simultaneously with strain 361 from the same patient and used as a negative control. Lanes A and G, *E. coli* V517 (molecular size marker) (9). Lane F, p88557 isolated from penicillinase-producing *N. gonorrhoeae*.

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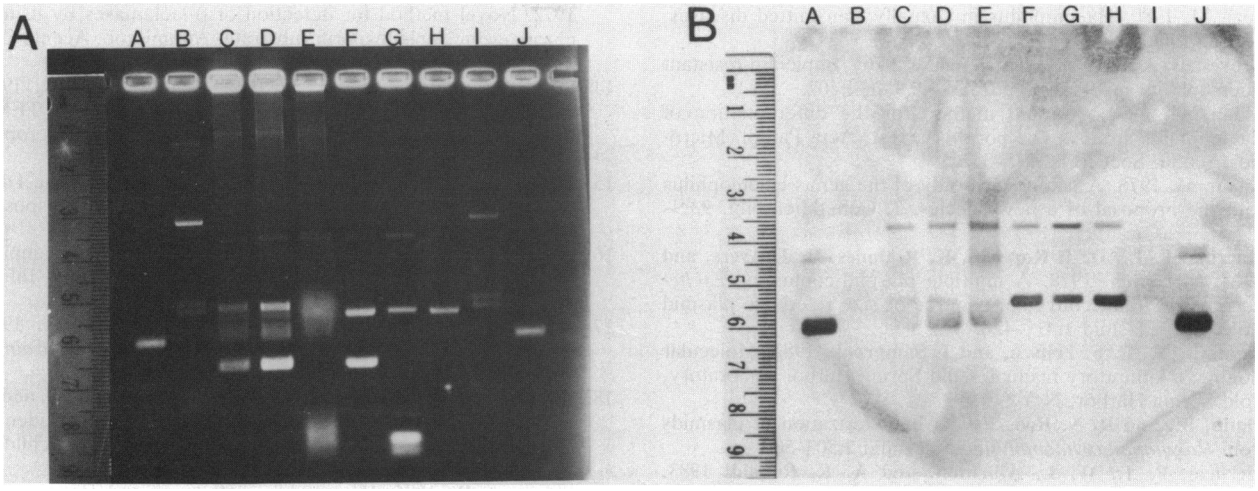

FIG. 2. Southern transfer and hybridization with a nonradioactive labeled *Hin*fI-*Taq*I probe. (A) Ethidium bromide-stained agarose gel electrophoresis and (B) its transfer onto a nylon membrane after colorimetric development using a commercial streptavidin-alkaline phosphatase kit (Bethesda Research Laboratories). Lanes A and J correspond to pBR322 (positive control), and lanes B and I correspond to pTY27 (a hybrid plasmid consisting of a 2.2-kilobase *Bam*HI-*Hin*dIII fragment, containing the OXA-1 β-lactamase gene, cloned into pACYC184); lanes C, D, and E, minipreparations (1) of *H. parainfluenzae* 11, 259, and 361, respectively; lanes F and G, CsCl gradient preparations of *H. parainfluenzae* 11 and 361, respectively; lane H, electroeluted pLQ920 plasmid of *H. parainfluenzae* 361.

a pI of 5.4 characteristic of TEM-1 (data not shown). The β-lactamase production was further characterized with a series of DNA molecular probes. Three radiolabeled probes, a 656-base-pair *Hin*fI-*Taq*I restriction fragment known to be specific for the TEM family and two oligonucleotides, TEM-1, specific for the TEM-1 β-lactamase gene, and OIR1, corresponding to the 5′ first 15 nucleotides of each inverted repeat of Tn*3*, were used as described elsewhere (14a). In colony hybridization experiments, the three probes hybridized only with the β-lactamase-producing strains and the positive controls, showing that the β-lactamases of the three clinical isolates were of the TEM-1 type (results not shown). Hybridization with the OIR1 probe showed that all β-lactamase-producing *H. parainfluenzae* strains had sequences in common with the inverted repeats of Tn*3*. A Southern transfer of an *H. parainfluenzae* DNA preparation was hybridized with the *Hin*fI-*Taq*I fragment probe nick translated with biotinylated dUTP (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Colorimetric development as recommended by the supplier (Bethesda Research Laboratories) revealed pLQ920 and its dimer but neither the chromosomal DNA nor other plasmids of the strains (Fig. 2). This demonstrated unambiguously that plasmid pLQ920 encodes the β-lactamase gene.

Discussion. Several reports describe the presence of *Haemophilus* spp. other than *H. influenzae* in the anogenital area (2, 13, 19). To our knowledge, only two other investigators have described the presence of β-lactamase-producing *H. parainfluenzae* in the genital tract (19, 20). In one report (20), two biotype II *H. parainfluenzae* strains had two plasmid species of 3.3 and 2.0 MDa. The cleavage pattern of the 3.3-MDa plasmids was identical to that of the African-type plasmid of penicillinase-producing *N. gonorrhoeae*. The transfer of these penicillinase-producing plasmids by conjugation was unsuccessful. We have shown that the penicillinase-producing plasmid pLQ920 can be transformed into *E. coli* HB101. The identity of pLQ920 and p88557, as determined by restriction enzyme digestion, suggests that only 40% of Tn*A* sequences is present in pLQ920 and that the hybridization with the OIR1 probe is due to the remaining right inverted repeat of Tn*3* (12). The OIR1 probe hybridized also with p88557 (data not shown).

Recent reports of nongonococcal urethritis (3, 19, 20) raised the potential role of *H. parainfluenzae* as a urogenital pathogen. We think that *Haemophilus* spp. other than *H. ducreyi* could be considered at least as commensals if not pathogens in the urogenital tract but their lack of intrinsic pathogenicity and fastidious growth make them rarely encountered clinically. Moreover, their presence might well serve as a reservoir of antibiotic resistance plasmids that may be acquired by the pathogenic species *N. gonorrhoeae* in the urogenital tract and *H. influenzae* in the respiratory tract (2). Nonradioactive DNA probes such as described in this report offer a potential tool to characterize the spread of ampicillin resistance among *Haemophilus* spp. They have the advantages of longer shelf life and greater availability and do not carry the hazard associated with their radioactive counterparts. A nonradioactive DNA probe has already served in the identification of enteroinvasive *E. coli* in clinical material (18).

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