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Typing of Urogenital, Maternal, and Neonatal Isolates of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in Correlation with Clinical Source of Isolation and Evidence for a Genital Specificity of *H. influenzae* Biotype IV

ROLAND QUENTIN,[1]* JAMES M. MUSSER,[2] MICHÈLE MELLOUET,[1] PIERRE-YVES SIZARET,[3] ROBERT K. SELANDER,[2] AND ALAIN GOUDEAU[1]

Laboratoire de Bactériologie et Virologie-URA CNRS 1334[1] and Laboratoire de Microscopie Électronique-Département de Biologie Humaine,[3] CHRU Bretonneau, 37044 Tours Cedex, France, and Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802[2]

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Over a period of 6 years, 114 strains of *Haemophilus influenzae* and *Haemophilus parainfluenzae* were isolated from genital, mother-infant, or neonatal infections. Their serotypes, biotypes, antibiotic resistance phenotypes, and outer membrane protein (OMP) electrophoretic patterns were characterized and correlated with the various clinical outcomes. Genital *H. influenzae* and *H. parainfluenzae* appeared to behave mostly as opportunistic pathogens; for instance, 62% of the cases of endometritis or pelvic inflammatory disease were related to the presence of an intrauterine device. However, as seen clearly in one case, the strains may be sexually transmitted. The analysis of OMP patterns proved to be a very convenient method to seek evidence for the sexual origin of the infection. *H. influenzae* was more often involved in complicated genital infections than was *H. parainfluenzae*. Nontypeable and biotype II *H. influenzae* strains were the more frequent isolates, except in pelvic inflammatory diseases, in which biotype I prevailed, and in mother-infant infections, in which one-fourth of the cases were due to biotype IV. Characterization of *H. influenzae* isolates did not support a general concept of specific genital strains. However, strains of biotype IV clearly stood out with two characteristics: (i) a peritrichous fimbriation and (ii) a very peculiar homogeneous OMP pattern comprising an OMP of molecular weight ≈18,000 unique to this biotype. These characteristics were also found in *H. influenzae* biotype IV strains isolated from genital infections in the United States and used as controls. *H. influenzae* biotype IV strains may thus correspond to a group somewhat adapted to the genital tract.

The first neonatal, perinatal, and genital infections due to *Haemophilus* strains were described in the early 1900s (22, 40, 66), but the number of cases, while remaining low, has increased only in the last 15 years (3, 10, 16, 20, 37, 58, 72). A specific tropism of some *Haemophilus influenzae* strains to the genital tract has been suggested from biotyping and serotyping studies (2, 58, 72); this is not unanimously admitted (41). *Haemophilus parainfluenzae* has also been shown to be involved in venereal diseases (27).

Over a continuous period of 90 months, we have collected a series of 114 *H. influenzae* and *H. parainfluenzae* isolates from various genital, mother-infant, and neonatal infections. Strains were characterized according to biotype and serotype, capacity to produce a β-lactamase, phenotype of resistance to antibiotics, and outer membrane protein (OMP) patterns. Some of them were studied for the presence of fimbriae by negative-staining electron microscopy.

Aims of the study were (i) to estimate the incidence of genital and neonatal *Haemophilus* infections; (ii) to better understand the pathogenesis of genital infection; (iii) to correlate strain characteristics with clinical manifestations, especially in regard to the involvement of the upper genital tract or infections occurring during pregnancy; (iv) to discuss the interest of OMP electrophoretic pattern determination in epidemiological studies; and (v) to dispute the concept of specific genital strains developed by some authors.

H. influenzae biotype IV has been reported to be isolated mostly from neonatal or mother-infant infections in the United States and France (58, 72). However, clinical outcome has been shown to be more severe in Houston, Tex. (72), than in Tours, France (58). To account for this difference, we have compared our own *H. influenzae* biotype IV isolates to nine strains originating in the United States and kindly provided by R. J. Wallace.

MATERIALS AND METHODS

Patients. *Haemophilus* spp. were isolated from 1 October 1979 through 31 March 1987 in patients from the Centre Hospitalier Régional et Universitaire Bretonneau in Tours, France. Patients belonged to two groups: women consulting for genital or urinary tract infections and newborn infants suspected to be at risk of infection on the basis of clinical signs in the mother (fever, genital or urinary tract infections, premature rupture of membranes, or premature onset of labor) or overt clinical signs in the child (fever, respiratory distress syndrome, shock, jaundice, or leukopenia). In these cases, bacterial investigations included two placental smears corresponding to both sides of the placenta, an aspiration of the gastric fluid, and swabs of ears, mouth, anus, and nose. In addition, urine analysis, blood cultures, and lumbar punctures were carried out in three circumstances: when the neonate had clinical or biological signs of infection, when direct examination of the gastric fluid and/or placental smears showed an abundant and monomorphic population of bacteria, or when pure cultures were obtained. In these three

* Corresponding author.

circumstances, newborn infants immediately received an appropriate antibiotic treatment.

Isolation of bacteria. Cultures of genital samples, urine samples, placenta, and gastric fluid were plated on tryptic soy agar with 5% horse blood incubated aerobically, on chocolate agar supplemented with Polyvitex incubated in 8 to 10% CO_2, and on Colombia agar with 5% sheep blood incubated anaerobically (bioMérieux, Charbonnières les Bains, France). Blood cultures were performed utilizing aerobic Castañeda bottles and anaerobic Schaedler broth (bioMérieux). Cerebrospinal fluid cultures were carried out on chocolate agar with Polyvitex and in beef liver glucose broth (Diagnostics-Pasteur, Marnes-la-Coquette, France). The presence of *Neisseria gonorrhoeae* was determined by culture on chocolate agar-Polyvitex with vancomycin-colistin-nystatine as the inhibitor. The presence of *Chlamydia trachomatis* was determined by direct immunofluorescence assays on urethral, cervical, or peritoneal swabs by using reagents from Syva bioMérieux.

Identification and biotyping of *Haemophilus* sp. For primary identification of *Haemophilus* strains, requirements for hemin and nicotinamide adenine dinucleotide were determined on Mueller-Hinton agar (Diagnostics-Pasteur) with Rosco tablets (Rosco, Taastrup, Denmark) and Oxoid disks (Oxoid Ltd., Basingstoke, England). Further identification was carried out by using the API 20E identification system (API Systems, Montalieu Vercieu, France) that includes testing for 11 enzymatic activities and nine carbohydrate fermentations. A full differentiation between *H. influenzae* and *H. parainfluenzae* can be achieved with API 20E as well as the determination of Kilian's biotypes (12, 34, 38). For some dubious strains, additional controls of urease, indol, and porphyrin tests were performed. *H. influenzae* originating from the United States was characterized and described previously (9, 72).

Antigenic serotyping. Capsular serotypes were determined upon isolation with coagglutination reagents (Phadebact-Haemophilus test; Pharmacia Diagnostics, Uppsala, Sweden) and controlled by slide agglutination and/or countercurrent immunoelectrophoresis by using specific antisera (Difco Laboratories, Detroit, Mich.).

β-Lactamase activity. β-Lactamase activity was screened with a chromogenic cephalosporin (Cefinase; bioMérieux).

Antibiotic resistance phenotypes. The MICs of ampicillin, kanamycin (Bristol, Paris, France), chloramphenicol (Merck Sharp & Dohme-Chibret, Clermont-Ferrand, France), tetracycline, and cefotaxime (Roussel Uclaf, Compiègne, France) were determined by the agar dilution method (67). Constant volumes of increasing concentrations from 0.016 to 64 μg/ml for ampicillin, 0.063 to 32 μg/ml for kanamycin, 0.016 to 16 μg/ml for chloramphenicol, 0.032 to 128 μg/ml for tetracycline, 0.004 to 1 μg/ml for cefotaxime were incorporated in Mueller-Hinton agar (bioMérieux) to which 5% Fildes enrichment medium (Difco) was added. Approximately 10^4 bacteria were applied to the agar surface of the plates with an inoculum-replicating apparatus (multipoint inoculator A400; Denley, Sussex, England). The last concentration inhibiting the culture was determined after 24 to 48 h of incubation at 37°C in conventional atmosphere. *Staphylococcus aureus* NCTC 6571 was used as a technical control.

Electron microscopy studies. *H. influenzae* and *H. parainfluenzae* fimbriae were studied by electron microscopy after negative staining (5, 35, 69). A 15-mm-thick carbon film was floated onto the surface of the bacterial suspension for 30 s and then transferred to the surface of a solution of 1.5% uranyl acetate in distilled water. After a few seconds, the carbon film was collected on a 400-mesh copper grid, washed with distilled water, and air dried. Observation was carried out with a JEOL 1200 EX microscope at 80 kV.

OMP electrophoretic patterns. The method for determining OMP electrophoretic pattern was derived from that described by Barenkamp et al. (8) and by Murphy et al. (49). *Haemophilus* isolates were grown on chocolate agar in 7% CO_2 for 18 h. Bacteria were suspended in 10 ml of 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.4, at a concentration of 2×10^9 cells per ml. They were sonicated four times for 30 s with 30-s cooling intervals in a sonifier (MSE, Crawley, United Kingdom) set at an amplitude of 6 μm. Large cellular debris and intact bacteria were removed by centrifugation at 1,600 × *g* for 15 min. Cell envelopes were collected by centrifugation at 50,000 × *g* for 60 min at 4°C. Pellets were suspended in 2 ml of 0.01 M HEPES buffer, pH 7.4, containing 1% sarcosyl (*N*-lauroyl sarcosine; Serva, N.Y.) and incubated at room temperature for 30 min to solubilize inner membrane proteins. Insoluble fractions containing outer membranes were collected by centrifugation at 50,000 × *g* for 60 min at 4°C, suspended in 100 μl of distilled water, and stored at −20°C. Protein concentration was determined by the method of Bradford (13). Outer membrane preparations were diluted 1:2 in Tris-sodium dodecyl sulfate buffer (0.0625 M Tris hydrochloride, 2% sodium dodecyl sulfate, 5% dithiothreitol, 3% glycerol, 0.005% bromophenol blue) and heated at 100°C for 10 min. The stacking gel contained 4% polyacrylamide, and the resolving gel contained 11% (43, 45). Protein (15 to 18 μg) was applied to each slot of the gel. Electrophoresis was performed at a constant 60 V until the dye left the stacking gel and at 200 V thereafter. Molecular weight protein standards (Bio-Rad Laboratories, Richmond, Calif.) were run in each gel. When electrophoresis was completed, gels were washed and fixed in 25% aqueous isopropanol for 2 h and then stained overnight in a solution containing 0.1% Coomassie blue (brilliant blue G; Sigma Chemical Co., St. Louis, Mo.), 25% methanol, and 10% acetic acid. Destaining was carried out by immersion in the same solution with gentle agitation.

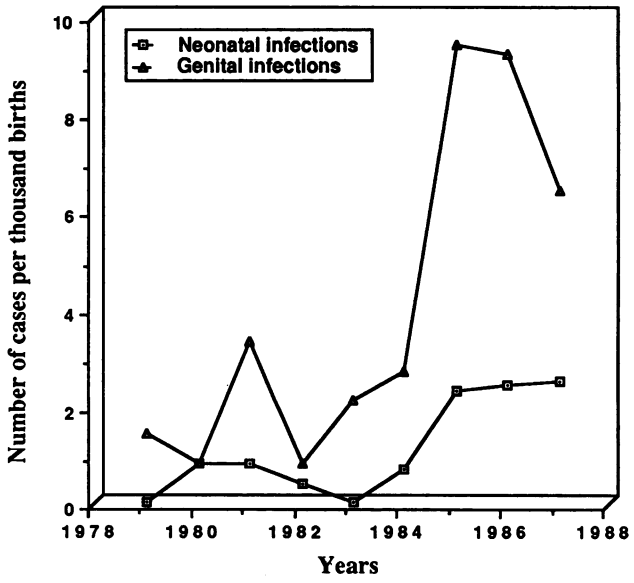

FIG. 1. Incidence of genital and neonatal infections due to *Haemophilus* sp. in Tours, France.

TABLE 1. Typing of 84 *H. influenzae* strains isolated from genitourinary and neonatal infections

Source of isolate	No. of strains	No. of strains with serotype:		No. of strains with β-lactamase activity	No. of strains with antibiotic resistance phenotype[a]	No. of strains with biotype:					
		Nontypeable	Type			I	II	III	IV	V	VI
Cervicovaginitis	17	16	1b	3	1 Ap; 2 Ap Km; 1 Cm	3	7	5	1	1	0
Urethritis	3 (M[b])	3	0	0	0	0	1	0	2	0	0
Bartholin's gland abscess	6	6	0	0	0	0	4	1	0	1	0
Sperm culture	3	3	0	1	1 Ap	1	0	2	0	0	0
Endometritis	25	25	0	0	1 Tc	3	10	7	1	2	2
Salpingitis	5	3	1a 1b	0	0	3	2	0	0	0	0
Orchiepididymitis	1	1	0	0	0	0	0	1	0	0	0
Spontaneous abortion	4	3	1a	0	0	0	4	0	0	0	0
Mother-infant infections	20	17	3b	3	1 Ap Cm Km Tc; 1 Ap Km Tc; 1 Ap	3	8	2	5	1	1
Total (%)	84	77 (91.6)	7 (8.3)	7 (8.3)	9 (10.7)	13 (15.4)	36 (42.8)	18 (21.4)	9 (10.7)	5 (5.9)	3 (3.5)

[a] Ap, Ampicillin; Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline.
[b] M, Male patient.

RESULTS

During the study period, 114 *Haemophilus* strains were isolated from 112 patients. In two cases, a mixed etiology, *H. influenzae* and *H. parainfluenzae*, was diagnosed. Two other coinfections were found involving *H. influenzae* with *Streptococcus pneumoniae* in one case and *H. influenzae* with *Streptococcus agalactiae* in the other. In no instance, were *C. trachomatis* and *N. gonorrhoeae* isolated along with *Haemophilus* sp. A significant increase in the incidence of genital and neonatal infection caused by *Haemophilus* sp. was observed during the study period (Fig. 1). The mean age of patients with *Haemophilus* genital infections was 29 years (range, 18 to 56 years excluding three prepuberal girls of 3, 4, and 12 years of age). *H. influenzae* was more often isolated than *H. parainfluenzae* in infections occurring during pregnancy (24 of 29 patients) and in female genital infections (53 of 68 patients); in contrast, *H. parainfluenzae* prevailed in male genital infections (10 of 17 patients).

Clinical circumstances of strain isolation. (i) Urinary and lower genital tract infections (48 isolates). *Haemophilus* sp., especially *H. parainfluenzae*, can be rather frequently isolated from the genital tract. Therefore, strains considered to be causative agents of lower genital tract infection were those isolated in the following circumstances: patient with overt clinical signs of cervicovaginitis or urethritis and leukocytes in genital discharges and bacteria in pure and abundant culture.

By these conditions, *H. influenzae* or *H. parainfluenzae* were involved in 22 cases of cervicovaginitis (the three young girls were in this group) and 13 cases of urethritis (Tables 1 and 2).

Seven *Haemophilus* strains were isolated from six patients with a Bartholin's gland abscess; a mixed infection with *H. influenzae* and *H. parainfluenzae* was noted in one patient. Two *Haemophilus* strains were isolated from urinary tract infections, and four were collected from spermocultures.

(ii) Upper genital tract infections (37 isolates). A total of thirty *Haemophilus* strains were isolated from 29 cases of endometritis (Tables 1 and 2). Eighteen patients (62%) had intrauterine devices (IUD); two patients had delivered healthy babies 2 and 3 weeks prior to the infection; two patients had complicated leiomyomas (one case with bleeding and one case with necrosis). No infectious risk factor could be found in seven cases. Strains were isolated from culture of the IUD in IUD-related endometritis, from intracervical aspiration in postpartum endometritis, and from

TABLE 2. Typing of 30 *H. parainfluenzae* strains isolated from genitourinary and neonatal infections

Source of isolate	No. of strains[a]	No. of strains with β-lactamase activity	No. of strains with antibiotic resistance phenotype[b]	No. of strains with biotype:		
				I	II	III
Cervicovaginitis	5	0	1 Cm 1 Tc	0	5	0
Urethritis	10 (8M, 2F)	1	1 Ap Cm Km Tc	1	8	1
Bartholin's gland abscess	1	1	1 Ap Km Tc	0	1	0
Urinary tract infection	2 (1M, 1F)	0	1 Tc	0	2	0
Sperm culture	1	0	1 Cm Tc	0	1	0
Endometritis	5	0	0	0	5	0
Salpingitis	1	0	0	0	1	0
Spontaneous abortion	1	0	0	0	1	0
Mother-infant infection	4	0	0	2	2	0
Total (%)	30	2 (6.6)	6 (20)	3 (10)	26 (86.7)	1 (3.3)

[a] Numbers and letters in parentheses indicate the number of strains isolated from males (M) or females (F).
[b] Ap, Ampicillin; Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline.

TABLE 3. Maternal circumstances of delivery and initial neonatal syndrome caused by *H. influenzae* and *H. parainfluenzae*[a]

Patient no.	Maternal disease	Term of pregnancy (wk)	Birth wt (g)	Sex of newborn	Neonatal clinical syndrome
1	Fever, RTI	37	3,000	M	Shock followed by lethargy, RDS, phlyctena, hypocalcemia, hypoglycemia
2	PRM	39	2,700	M	No disease
3	Fever, RTI	38	2,900	M	Shock followed by lethargy, purpura
4	Amnionitis, UTI	39.5	2,920	F	Hypocalcemia
5	PBR at 32 wk	32	1,700	M	Jaundice
5A			1,400	M	RDS, hypoglycemia
6	Amnionitis, PRM	40	3,100	M	Fever, tachycardia
7	RTI, PBR at 32 wk	35.5	2,400	F	RDS, jaundice
8		32	1,670	F	Leukopenia, hypoglycemia
9	Amnionitis, PRM	33	1,830	M	Apnea at 5 min
9A			1,400	M	Shock, malformation
10	UTI	39	3,370	F	Leukopenia, jaundice
11	Amnionitis, PRM	31	1,590	F	Meconial amniotic fluid, RDS, jaundice
12	Amnionitis, PRM	33	2,500	F	No disease
13	Amnionitis, PRM PBR at 33 wk	39	3,400	F	No disease
14	Amnionitis	41	3,120	F	Bradycardia during delivery
15	GTI	36	2,400	F	Jaundice followed by conjunctivitis
16	Amnionitis, PRM	41	3,620	M	Leukocytosis
17		41	3,600	F	Meconial amniotic fluid
18	PBR at 31 wk, PRM	38	2,900	F	Lethargy during 48 h, poor feeding
19	PRM	40	2,600	M	Tachycardia during delivery Shock followed by lethargy
20	Amnionitis	35	2,600	M	RDS, irritability, jaundice
21		34	2,400	F	Jaundice, irritability CSF leukocytes, 12/mm^3; glucose, 2.2 mmol/liter; proteins, 0.89 g/liter; *H. influenzae* type b
22	UTI, GTI	33	2,350	F	Lethargy
23	PRM	41	3,250	M	No disease
24	PRM	38.5	3,270	M	Leukopenia, jaundice, hypocalcemia

[a] RTI, Respiratory tract infection; PRM, premature rupture of membranes; UTI, urinary tract infection; PBR, risk of premature birth; GTI, lower genital tract infection; RDS, respiratory distress syndrome; M, male; F, female; CSF, cerebrospinal fluid.

biopsies of the endometrium in the other cases. In two cases, there was a mixed etiology: *H. influenzae* plus *H. parainfluenzae* and *H. influenzae* plus *S. agalactiae*.

Six *Haemophilus* strains were isolated in female patients with pelvic inflammatory disease, from direct cultures of peritoneum fluid collected during celioscopy. Four of these women had IUDs; cultures of the device were positive in all cases. One patient was the sexual partner of one of our patients with urethritis. No favorable circumstances could be demonstrated in this case. Only one case of upper genital tract infection was observed among male patients: an orchiepidydimitis caused by *H. parainfluenzae*.

(iii) Infections during pregnancy (29 isolates). Five *Haemophilus* strains were isolated from curettage product of spontaneous septic miscarriages (Tables 1 and 2). These miscarriages occurred between 8 and 11 weeks of amenorrhea in four cases; the fifth case occurred during the week 24 and had a mixed etiology (*H. influenzae* and *S. pneumoniae*).

Clinical circumstances and initial clinical signs observed in 24 mother-infant infections are reported in Table 3. Small gram-negative bacilli were observed in placental and gastric fluid smears in 18 cases. Twenty strains of *H. influenzae* and four strains of *H. parainfluenzae* were isolated from gastric fluid and placenta in pure culture. There were two pairs of twins infected with identical strains; only one strain for each pair is shown in Tables 1 and 2. All infants received either ampicillin or cefotaxime starting at birth for 7 to 21 days, 14 infants because of clinical signs combined with a positive direct examination of gastric fluid smears, four infants because of clinical signs, and four infants because of positive examination alone. Blood culture remained negative for all neonates. Cerebrospinal fluid culture was positive in one case (case 21). Only one child died early after birth (case 9A); he was a premature twin born with several severe malformations.

Typing of isolates. (i) Antibiotic resistance. All ampicillin-resistant strains produced a β-lactamase (Tables 1 and 2). There were no cefotaxime-resistant isolates. Other resistances belonged to various phenotypes.

(ii) Serotype. Of 84 *H. influenzae* strains, 77 were nontypeable (Tables 1 and 2). Three strains reacted with all coagglutination reagents; they were found negative by counterimmunoelectrophoresis. Of 20 neonatal *H. influenzae* infections, 3 were due to serotype b strains, one with a neonatal meningitis.

(iii) Biotype. *H. influenzae* biotype II was the more frequent (42.8% of the cases) (Tables 1 and 2). However, biotype I prevailed in pelvic inflammatory diseases, and biotype IV was observed in 25% of neonatal infections and in two of three cases of urethritis.

(iv) Fimbriation. Strains belonging to each *Haemophilus* biotype were studied. All strains had been frozen, thawed, and plated three to six times before examination. Under these conditions, *H. parainfluenzae* and *H. influenzae* biotype IV retained a fimbriation that was clearly peritrichous (Fig. 2).

(v) OMP electrophoretic patterns. *H. influenzae* OMP patterns were heterogeneous for biotypes I, II, III, V, and

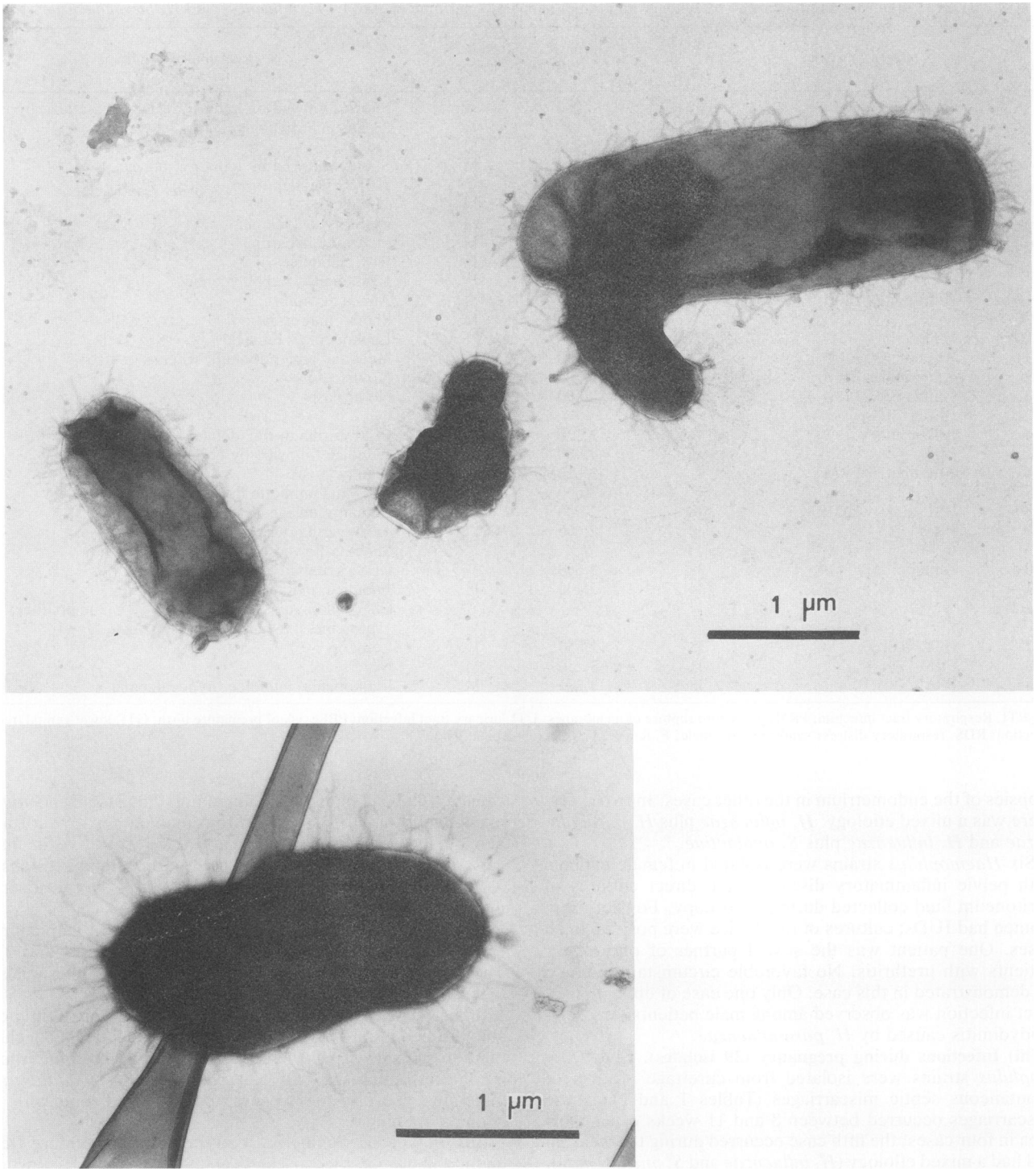

FIG. 2. Electron micrograph after staining with uranyl acetate showing peritrichous fimbriae in *H. influenzae* biotype IV (top) and *H. parainfluenzae* (bottom).

VI. Pattern variability involved primarily two constant groups of major proteins. The first group consisted usually of two highly variable proteins in the 32,000- to 43,000-molecular-weight range. The second group was composed of one or two proteins in the 27,000- to 32,000-molecular-weight range. This group was less variable and included a casi-constant protein with a molecular weight of ≈31,000. A protein of molecular weight ≈16,000 was common to all strains.

A comparative analysis of the nine *H. influenzae* biotype IV strains of our series and nine American biotype IV strains showed that this biotype formed a singular subset characterized by its extreme homogeneity: 16 isolates of 18 had strictly identical electrophoretic patterns. The major OMPs

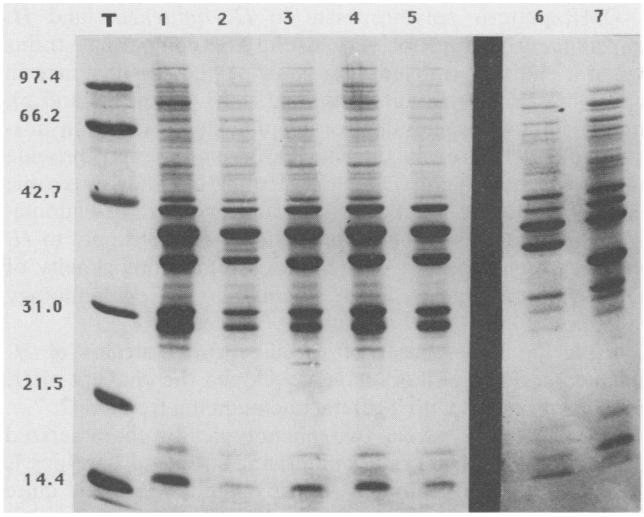

FIG. 3. Homogeneity of OMP electrophoretic patterns of *H. influenzae* biotype IV strains isolated from mother-infant and genital infections in France (n = 9) and the United States (n = 9). Of 18 strains, 16 had the same patterns (lanes 1 to 5). Two French strains had a different pattern; one was isolated from a case of endometritis (lane 6), and the other was from a male patient with urethritis (lane 7). T, Molecular size markers (in kilodaltons).

described above were found in all biotype IV strains, but in addition, a protein with a molecular weight of ≈18,000 was present in 17 of 18 biotype IV strains (Fig. 3).

H. parainfluenzae OMP electrophoretic patterns were highly variable (Fig. 4). This species showed a variability group composed of two major proteins located in the 32,000- to 43,000-molecular-weight range and one or two proteins in the 27,000- to 32,000-molecular-weight range, including an almost constant protein of ≈32,000. A protein of molecular weight ≈22,000 was present in 25 isolates, and two proteins of molecular weight 17,000 and 18,000 were present in 27 isolates. Because of this extreme heterogeneity within the species, identical patterns found in isolates of two sexual

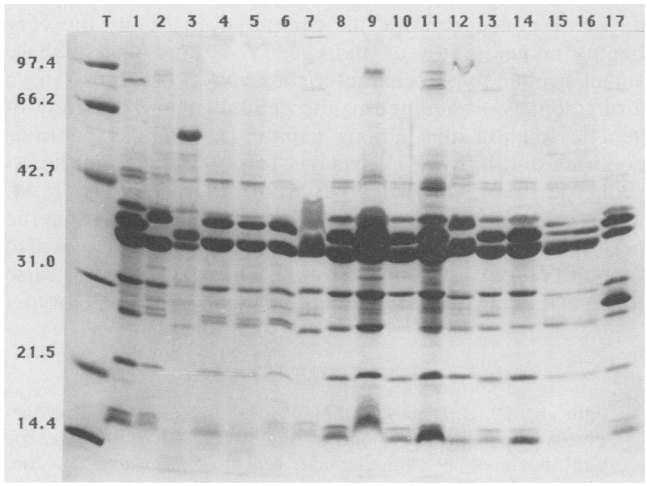

FIG. 4. Heterogeneity of OMP electrophoretic patterns of *H. parainfluenzae* isolated from genital and mother-infant infections. Lanes 4 and 5 show two strains originating from a woman suffering from pelvic inflammatory disease and her sexual partner suffering from urethritis. T, Molecular size markers (in kilodaltons).

partners argued strongly for a sexual transmission (Fig. 4, lanes 4 and 5).

DISCUSSION

Incidence of *Haemophilus* sp. in genital infections and mother-infant infections. Our results point out that *Haemophilus* is not an exceptional cause of genital infections and mother-infant infections. During a period of 90 months, we have diagnosed genital infections due to *H. influenzae* or *H. parainfluenzae* in 83 patients of which 42 suffered from severe or complicated infections (29 had endometritis, 6 had salpingitis, 6 had Bartholin's gland abscesses, and 1 had orchiepididymitis). This relatively high incidence contradicts previously reported data that comprise less than 100 well-documented cases of genital infections since the turn of the century (1, 3, 6, 11, 14, 15, 17–19, 23–25, 26, 30–33, 36, 38, 40, 42, 46, 51, 52, 55, 57, 60–64, 71, 72, 75). It is unclear whether this discrepancy is related to a recent increase of *Haemophilus* genital infection incidence, especially in France, or to the fact that a fair number of *Haemophilus* infections may go unrecognized because of inadequate laboratory techniques. We are more in favor of the first assumption since, without changing our bacteriology routine, we observed an increased incidence of *Haemophilus* genital infections, from 22 cases between 1 October 1979 to 31 December 1984 to 61 cases between 1 January 1985 and 31 March 1987. Colonization of the genital tract by *Haemophilus* sp. may occur via fecal carriage (56). The exact role of sexual practices, e.g., oral-genital contact (27), in the increasing frequency of *Haemophilus* genital infections remains to be assessed.

Neonatal infections and infection during pregnancy due to *H. influenzae* have been described regularly since 1975 (3, 4, 10, 16, 20, 37, 47, 53, 58, 68, 72, 74). Authors reporting the largest series (16, 58, 72) point to an increasing frequency, *H. influenzae* accounting for 1.4% of positive blood cultures in mothers and neonates in Houston, Tex., in 1975 and up to 5.7% in 1981 (72). Reported rates of neonatal bacteriemia due to *H. influenzae* ranged from 0.14/1,000 births in Houston (1976 to 1981) to 0.49/1,000 births in Providence, R.I. (1980 to 1984) (16, 72). In our continuous series of 21,034 births, the rates of *Haemophilus* sp. isolated from mother-infant infections were 0.64/1,000 births in the period from 1979 to 1984 and 2.83/1,000 births in the period from 1985 to 1987.

Route of infection. Presence of an IUD appeared to be a predominant factor in the spread of infection and accounted for 62% of our endometritis cases and four of six pelvic inflammatory disease cases. Other preexisting lesions such as uterine leiomyomas may have played a role. The fact that *Haemophilus* sp.-infected patients appeared to be slightly older than the usual patient with a sexually transmitted disease can be taken as a further proof that *Haemophilus* sp. behave mostly as opportunistic infectious agents. During pregnancy, premature birth (10 cases) and premature rupture of membranes (11 cases) were leading causes of amniotic contamination and neonatal infection. These data indicate that most of the complicated genital infections, such as mother-infant infections, originate from the commensal flora of the lower genital tract (2, 28, 37, 51, 54, 65). Lack of favoring circumstances in two acute salpingitis cases and in 6 endometritis cases may suggest the existence of more virulent and sexually transmitted strains. Isolation of two strictly identical strains in a woman with salpingitis and her

one-time partner is a strong argument in favor of this hypothesis.

Strain typing in relation to clinical episode. Strains responsible for upper or complicated genital infections (endometritis, pelvic inflammatory diseases, and Bartholin's gland abscesses) were no more often capsulated (2 of 36) than those causing lower genital tract infections (1 of 24), indicating that capsulation was not a factor for invasion of the genital tract; one exception was in cases of salpingitis, in which 2 of 5 *H. influenzae* strains were capsulated. Accordingly, we did not observe more resistance to antibiotics among *H. influenzae* and *H. parainfluenzae* strains isolated from pelvic inflammatory diseases than among strains collected from uncomplicated urogenital infections. This is different from what has been reported for *N. gonorrhoeae*, for which isolates from upper genital tract infections are more resistant (59).

As usual, *H. influenzae* biotype II was the more frequent. However, biotype I prevailed in acute salpingitis. Such a predominance can be compared with the frequency of biotype I in pneumonia (48, 50, 73) and raises the question of a special affinity of this biotype for ciliated cell epithelium.

Neonatal infections with nontypeable *H. influenzae* resulted in a number of septicemia and meningitis cases in series reported in the United States (16, 72). These invasive diseases in newborns were absent from our study. We have tried to determine the reason for this difference. Clinical circumstances in the mothers and initial clinical manifestations in the neonates—especially signs of pulmonary tract infection—were similar in Tours (Table 3) to those reported in Houston and Providence. Our isolates were similar to U.S. strains in terms of serotype, biotype, and antibiotic resistance. A high frequency of *H. influenzae* biotype IV has been reported in mother-infant infections, suggesting that biotype IV may represent a specific neonatal biotype pathogen (9, 72). In our series, this biotype accounted for 5 of 20 neonatal *H. influenzae* infections. Our biotype IV strains were compared with nine other strains isolated in the United States. It was found that they shared a striking homogeneity of OMP electrophoretic patterns. It is thus quite unlikely that a major phenotypic divergence explains clinical differences observed between biotype IV infections in the United States and in France. More favorable outcomes in our series may be due to two factors: (i) a smaller number of highly premature newborn infants and (ii) a rapid antibiotic treatment of infants who were not clinically septic but were heavily colonized with *Haemophilus* sp. as assessed by positive direct examination of gastric fluid and placental smears. This corroborates the view of Khuri-Bulos and McIntosh (37), who suggested that early treatment of newborns who are heavily colonized may abort the infection and prevent invasive disease.

***Haemophilus* OMP typing as an epidemiological tool.** Analysis of the *H. influenzae* type b OMP electrophoretic patterns is a widely used epidemiological tool (7, 8, 70), especially for typing strains isolated from meningitis. OMP typing systems have also been proposed for nonserotypeable strains of *H. influenzae* (44, 49). Because of the extreme heterogeneity of the OMP patterns, our clinical isolates could not be classified according to these subtyping schemes. Densitometer scanning of gels and computerized analysis of whole OMP patterns might help to establish a more precise degree of similarity among strains. In addition, to validate a subtyping system, it will be important to assess whether phenotypic grouping based on OMP patterns accurately reflects a genotypic homogeneity.

OMP pattern polymorphism in *H. influenzae* and *H. parainfluenzae* appears very useful for comparing strains when a chain of transmission is suspected, for instance in cases of sexually acquired disease (Fig. 4, lanes 4 and 5), mother-infant transmission, or hospital staff-infant transmission. Furthermore, an unusual OMP pattern may provide useful information in *Haemophilus* mixed infections (one case of Bartholin's gland abscess and one case of endometritis in our series). These conclusions will not apply to *H. influenzae* biotype IV, where the extreme homogeneity of OMP patterns makes them unsuitable for epidemiology studies.

Exploring the concept of specific genital strains of *H. influenzae*. Some authors have developed the concept of *H. influenzae* strains with a genital and neonatal tropism (2, 72). Our data clearly reveal two phenotypically characterized populations. The first one, comprising strains of biotypes I, II, III, V, and VI, mostly uncapsulated, appeared quite similar in terms of biotype frequency, phenotype of antibiotic resistances, and OMP electrophoretic pattern to populations that have been reported in otitis, conjunctivitis, and upper respiratory tract infections (2, 9, 19, 21, 29, 39, 44, 49, 50, 73).

The second one, composed of 16 *H. influenzae* biotype IV strains, stands out as a peculiar group characterized by its homogeneous OMP pattern, including a unique protein of molecular weight ≈18,000, and by its peritrichous fimbriation. It is likely that these characteristics found for biotype IV involved in mother-infant infections both in France and in the United States reflect a selection of these strains from the very heterogeneous mainstream of the species.

Our data show that the incidence of complicated genital infections as well as mother-infant and neonatal infections due to *H. influenzae* and *H. parainfluenzae* has been increasing in the past few years. These bacteria mostly behave as opportunistic pathogens, but they may be sexually transmitted. Analysis of OMP patterns proves to be a convenient method to seek evidence for the sexual origin of an infection. However, more strains ought to be analyzed in order to estimate the probability of two strains being identical by chance. The study of *H. influenzae* isolates does not support a general concept of specific genital strains. Only the very homogeneous group of biotype IV strains which share singular phenotypic characteristics may correspond to a group somewhat adapted to the genital tract. The study of genetic relationships among genital *H. influenzae* strains confirms that biotype IV isolates form a unique multilocus genotype that greatly diverges from other biotypes (J. M. Musser, manuscript in preparation). It is noteworthy that the peritrichous fimbriation of *H. influenzae* biotype IV was also found in *H. parainfluenzae* strains. These singularities raise the question of the real intrageneric position of this biotype.

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