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Isolation and Biochemical Characterization of *Haemophilus* Species Isolated Simultaneously from the Oropharyngeal and Anogenital Areas

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Several reports have described the high frequency of pharyngeal isolation of *Haemophilus* species. Few studies have compared the simultaneous isolation rate of this species in the oropharyngeal and anogenital areas. Using two selective media, heart infusion agar (HIA) supplemented with 5% defibrinated rabbit blood, 1% IsoVitaleX, and either bacitracin alone (100 μg/ml) or bacitracin (5 μg/ml), vancomycin (3 μg/ml), and polymyxin B (1 μg/ml), we isolated *Haemophilus* species in both areas in 89 of 399 (22.2%) patients consulting a sexually transmitted diseases clinic. Of those, 56 were males and 33 were females. We recovered *Haemophilus* species in the oropharyngeal area in 384 patients (96%), while rectal and genital areas were colonized in 48 (12.0%) and 55 (13.8%) patients, respectively (both areas were colonized in 14 patients). *Haemophilus parainfluenzae* was isolated almost twice as often in the anogenital area as was *H. influenzae*. *H. influenzae* biotypes II and III and *H. haemolyticus* were the more prevalent XV-requiring haemophili isolated from the oropharynx, while *H. influenzae* biotype IV was more prevalent in the anogenital area. *H. parainfluenzae* biotypes I, II, and III were more prevalent in the oropharynx, while biotypes I and II were more prevalent in the anogenital area.

Despite an increasing number of reports of serious genital infections caused by *Haemophilus* species other than *Haemophilus ducreyi* (2–4, 18; M. Fuzi, Letter, Lancet ii:476, 1980), much remains to be learned about the epidemiology and the natural urogenital ecology of these organisms. Recently, several authors (16, 21) suggested that *H. influenzae* biotype IV should be considered a genital and neonatal pathogen. However, the isolation of other biotypes of these species from the genital area has been reported (1, 2, 13, 14). Partly due to the routine use of media that do not support the growth of *Haemophilus* species (7) or to overgrowth by commensal microbial flora, reports of anogenital infections by *H. influenzae* (2, 14) and by *H. parainfluenzae* (3, 4, 18, 20; M. Fuzi, Letter, 1985) are sparse. The development of selective media inhibiting the less fastidious microbial flora while permitting the growth of *Haemophilus* species allowed a recovery rate varying between 0.2 and 5.3% (1, 13, 19) from colonized areas.

We conducted a survey comparing the simultaneous colonization rates of the oropharyngeal and anogenital areas by *Haemophilus* species.

MATERIALS AND METHODS

The first 10 patients presenting to the clinic on Monday and Tuesday of each week were studied. The majority of the population studied included homosexual males and heterosexual females who consulted for sexually transmitted disease (STD) symptoms and the sexual contacts of these patients. These were complemented by asymptomatic patients at high risk for STD and patients with urogenital complaints not necessarily related to STD (prostatitis, vulvar itching, increased vaginal discharge, etc.), either heterosexuals or homosexuals. Pregnant patients were excluded, as were those who had taken antibiotics in the 2 weeks prior to the study and those who did not give oral consent.

Pharyngeal specimens were obtained by touching a cotton swab to both tonsils and the oropharynx. A cotton swab was touched to the posterior fornix to collect vaginal specimens. Saline was used to moisten a cotton swab before its insertion 2 to 3 cm into the rectum. Male urethral specimens were collected on a Calgiswab inserted 2 cm into the urethral meatus. Specimens were plated directly on two selective media: heart infusion agar supplemented with 5% defibrinated rabbit blood, 1% IsoVitaleX, and either bacitracin alone (100 μg/ml) or bacitracin (5 μg/ml), vancomycin (3 μg/ml), and polymyxin B (1 μg/ml). All plates were inoculated in one quadrant and then cross-streaked with a wire loop for colony isolation. After 48 to 72 h of incubation at 35°C in a candle jar or a 5% CO_2 incubator, the plates were inspected for the presence of suspected colonies. A few representatives of morphologically different colonies suspected of being *Haemophilus* sp. were plated on nonselective enriched chocolate agar and reincubated for 24 h in the same conditions. Organisms were identified as *Haemophilus* sp. if they had typical colony morphology and cellular morphology as determined by Gram staining and if they required V or XV factors for growth on heart infusion agar. Biotyping was done by the method of Kilian as previously described (10) and interpreted as shown in Table 1. Serotyping by using the Centers for Disease Control *H. influenzae* polyvalent antisera or a commercial coagglutination kit (Difco Laboratories, types a through f), determination of the production of porphyrin from δ-aminolevulinic acid (9), and acid formation from glucose, lactose, and xylose completed the identification of the strains. The presence of beta-hemolysis was recorded on the initial selective plates.

Statistical analysis. The statistical analyses of the data in the different tables were done by using the likelihood ratio

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TABLE 1. Differential characteristics of *Haemophilus* species in this study[a]

Species and biotype	Characteristic[b]							
	ALA conversion to porphyrin	V-factor requirement	Indole	Urease	ODC	Hemolysis	Acid formation from:	
							Glucose	Lactose
H. influenzae								
I	−	+	+	+	+	−	+	−
II	−	+	+	+	−	−	+	−
III	−	+	−	+	−	−	+	−
IV	−	+	−	+	+	−	+	−
V	−	+	+	−	+	−	+	−
VI	−	+	−	−	+	−	+	−
VII	−	+	+	−	−	−	+	−
VIII	−	+	−	−	−	−	+	−
H. haemolyticus	−	+	+/−	+	−	+	+	−
H. parainfluenzae								
I	+	+	−	−	+	−	+	−
II	+	+	−	+	+	−	+	−
III	+	+	−	+	−	−	+	−
IV	+	+	+	+	+	−	+	−
V	+	+	−	−	−	−	+	−
VI	+	+	+	−	+	−	+	−
VII	+	+	+	+	−	−	+	−
H. paraphrophilus	+	+	−	−	−	−	+	+
H. segnis	+	+	−	−	−	−	W	−
H. parahaemolyticus	+	+	−	+	+/−	+	+	−

[a] Characteristics of various species and biotypes were obtained from references 11, 12, 17, and 18.
[b] +, Positive reaction; −, negative reaction; +/−, variable; W, weak reaction; ALA, δ-aminolevulinic acid; V, NAD; ODC, ornithine decarboxylase.

chi-square test for independence between the rows and the columns (6).

RESULTS

Of the 399 patients (179 males and 220 females) screened for *Haemophilus* species, 384 patients (96%) harbored a species in the oropharynx, while 89 patients (22.2%; 56 males and 33 females) had *Haemophilus* species isolated from the anogenital area. A total of 37 males were simultaneously colonized in the oropharyngeal and the genital areas, and 31 males were simultaneously colonized in the oropharyngeal and the rectal areas. A total of 18 females were simultaneously colonized in the oropharyngeal and the genital areas, and 17 were simultaneously colonized in the oropharyngeal and rectal areas. A total of 14 patients (3.5%; 12 males and 2 females) were simultaneously colonized in all three areas. More than twice as many males were colonized in the genital area as were females ($P < 0.001$), while the rectal area was colonized almost twice as often in males as in females. From the 89 patients colonized in the anogenital area, V-dependent *Haemophilus* species, including *H. parainfluenzae*, *H. segnis*, *H. paraphrophilus*, and *H. parahaemolyticus*, were more than twice as prevalent ($P < 0.008$) as the XV-dependent *Haemophilus* species (Table 2), while these two types were equally prevalent in the oropharynx (P was nonsignificant). XV- and V-dependent *Haemophilus* species were also unevenly distributed in males and females. While both XV- and V-dependent *Haemophilus* species were equally prevalent in the pharynxes of males (28 of 56) and females (18 of 33), very few patients of either sex harbored them simultaneously in the anogenital area (3 of 56 males and 4 of 33 females; $P < 0.001$). Exclusive colonization of the pharynx by either XV- or V-dependent *Haemophilus* species was the same in males (14 versus 14) while the distribution was different in females (3 versus 12, respectively). The exclusive colonization of the anogenital area by XV- or V-dependent *Haemophilus* species was different. While XV-dependent *Haemophilus* species were about half as prevalent as V-dependent *Haemophilus* species in males (18 versus 34, respectively), the difference was more striking in females (4 versus 27, respectively). The biotype distribution of XV-dependent *Haemophilus* species according to anatomic site is summarized in Table 3. Biotypes II and III *H. influenzae* along with *H. haemolyticus* were more prevalent in the oropharynx, while biotype IV *H. influenzae* was more likely to be present in the anogenital area ($P < 0.001$). The biotype distribution of V-dependent *Haemophilus* species according to anatomic site is summarized in Table 4. Oropharyngeal *H. parainfluenzae* was usually biotype I, II, or III. Biotype II was the most common genital *H. parainfluenzae* isolate, and biotypes I and II were the most

TABLE 2. Distribution of XV- and V-requiring *Haemophilus* species according to anatomic site

Anatomic site	No. of patients (total no. of strains isolated) colonized by:	
	XV-requiring *Haemophilus* species	V-requiring *Haemophilus* species
Oropharynx	62 (105)	71 (152)
Vagina or urethra[a]	18 (26)	40 (95)
Rectum	13 (25)	39 (96)

[a] Vaginal and urethral specimens were obtained from females and males, respectively, as described in Materials and Methods.

TABLE 3. Biotype and species distribution of XV-requiring *Haemophilus* species according to anatomic site in 89 patients colonized by at least one *Haemophilus* strain in the anogenital area

Anatomic site	No. of patients colonized by *H. influenzae* biotype:								No. of patients colonized by *H. haemolyticus*
	I	II	III	IV	V	VI	VII	VIII	
Oropharynx	1	20	29	5	0	0	12	8	30
Vagina or urethra[a]	0	5	4	12	0	0	1	0	4
Rectum	0	2	5	18	0	0	0	0	0

[a] Vaginal and urethral specimens were obtained from females and males, respectively, as described in Materials and Methods.

common rectal *H. parainfluenzae* isolates ($P < 0.001$). *H. parahaemolyticus* was also more prevalent in the oropharyngeal area. Among other V-dependent *Haemophilus* species, we found beta-hemolytic *H. parainfluenzae* biotypes I, IV, and VII, beta-hemolytic *H. segnis*, and beta-hemolytic *H. paraphrophilus* other than *H. paraphrohaemolyticus*. None of these strains could conform to the differential characteristics of Table 1.

DISCUSSION

The method used in this study would likely not detect mixed flora of the same species but different biotypes unless they were of different colonial morphologies. This weakness is acknowledged, but the selection of two to three colonies with the same morphology did not help to differentiate more *Haemophilus* species. Different patient selection or isolation methods could explain the higher *Haemophilus* species recovery rate from the anogenital area in this study (22.2%) compared with that reported in the literature (1, 13, 19). The low *Haemophilus* isolation reported by Sturm (19) and Messing et al. (13) may be due to their use of different selective media and may reflect the better selective properties of the two media used in our study. We had a much lower recovery rate with the selective media containing vancomycin compared with the alternative media containing bacitracin. This contrasts with data from other investigators (1, 19), who have reported a low *Haemophilus* isolation rate with medium containing bacitracin as the only selective agent. We cannot exclude the possibility that patients seen in the STD clinic may represent a more sexually active population than those observed by other investigators and, for this reason, have a higher colonization rate than the rest of the population. This, however, was not addressed previously (13). For the oropharynx, our colonization rate (96%) was identical to those reported by others, suggesting that the difference in media may not be the sole explanation for our observations (5, 8).

Sturm (19) compared the genital tract isolates with those isolated from the upper airways and observed that males harbored twice as many *Haemophilus* species in their genital secretions as did females. This is comparable with our results and might be explained by differences in anatomical environment, such as pH, moisture, or inhibition by normal commensal flora. Whether one factor prevails over the others remains to be determined. We found an anogenital colonization rate twice as frequent for V-requiring *Haemophilus* species as that for XV-requiring *Haemophilus* species, while the oropharyngeal colonization rate for the two types of species was about the same (Table 2). Like others (1, 19, 21), we noted a difference in the oropharyngeal and anogenital biotype distribution of *H. influenzae*. A significant proportion of anogenital *H. influenzae* are biotype IV, while the oropharyngeal isolates are mainly biotype II and/or III (Table 3). The explanation of this biotype distribution difference is unresolved but might be due to a more permissive ecological environment in the different anatomical sites or to a specific colonization tropism for this particular biotype. While biotypes I and II *H. parainfluenzae* were more prevalent in the anogenital area, biotypes I, II, and III *H. parainfluenzae* were equally prevalent in the oropharynx. These results agree with those of other investigators (13, 19), and the reasons for this difference might well be the same as those for the biotype distribution pattern of *H. influenzae*.

The presence of *Haemophilus* species in the genital area could be explained by a natural ecological colonization by particular *Haemophilus* species either by the spread of the strains from the oropharynx through orogenital contact or by a contiguous genital extension from the gastrointestinal tract. This study does not support the hypothesis of orogenital transmission of *Haemophilus* species as presented by Fuzi (Letter, 1985), since the *Haemophilus* biotype distribution was different in the oropharynx and the anogenital area (Table 3 and 4). Genital *Haemophilus* species and biotypes were similar to those we isolated from the rectum, suggesting an interplay of *Haemophilus* colonization in these two anatomic sites. However, we recovered a few biotype IV *H. influenzae* in the oropharynx and a few biotype II and III *H. influenzae* in the anogenital area, suggesting that orogenital transmission remains a possibility in some patients. A study on the oropharyngeal colonization rate among sexual partners of patients harboring *Haemophilus* species in their genital tracts might give a clue to confirm this hypothesis. A contiguous genital spread of *Haemophilus* species from the lower gastrointestinal tract, as suggested by Palmer (15), appears to be the most attractive explanation for our observation. XV-requiring *Haemophilus* species are as prevalent

TABLE 4. Biotype and species distribution of V-requiring *Haemophilus* species according to anatomic site in 89 patients colonized by at least one *Haemophilus* strain in the anogenital area

Anatomic site	No. of patients colonized by *H. parainfluenzae* biotype:							No. of patients colonized by:			
	I	II	III	IV	V	VI	VII	*H. segnis*	*H. paraphrophilus*	*H. parahaemolyticus*	Other *Haemophilus* species[a]
Oropharynx	23	29	29	0	9	0	1	6	9	35	11
Vagina or urethra[b]	6	61	14	0	0	0	0	5	2	2	5
Rectum	32	28	6	0	9	1	0	0	5	6	9

[a] Beta-hemolytic *H. parainfluenzae* biotypes I, IV, and VII, beta-hemolytic *H. segnis*, and beta-hemolytic *H. paraphrophilus* other than *H. paraphrohaemolyticus*.

[b] Vaginal and urethral specimens were obtained from females and males, respectively, as described in Materials and Methods.

as V-requiring *Haemophilus* species in the oropharynx but are only half as prevalent in the anogenital area, showing the existence of colonization selection throughout the gastrointestinal tract. Considering the anatomical proximity of genital and anal areas in women, the contiguous colonization rate of the genital tract by *Haemophilus* species would appear to be more striking in women than in men. This appeared to be more evident with V-requiring *Haemophilus* species. Their presence in urogenital areas was significantly more frequent ($P < 0.001$) in females than in males, supporting this hypothesis. However, the anal area did not appear to be more frequently colonized than the genital area in either sex. Finally, of the 14 patients (12 males and 2 females) simultaneously colonized in all three sites, only three patients harbored the same biotype in all sites, while 10 patients had the same biotype in both genital and anal areas but not in the oropharynx. Which of the two sites (genital and anal) became colonized first remains to be determined. Multiple identification methods are thus needed to better define the ecology of this microorganism in different anatomical sites. The use of more sensitive markers, such as isoenzyme polymorphism, restriction fragment length polymorphism using specific DNA probes, or plasmid DNA analysis, might give a clue to *Haemophilus* species natural genital ecology.

With the recent emergence of reports on urogenital infections caused by *Haemophilus* species, a better definition of the natural ecology of the genital tract may be of clinical importance. We demonstrated a 22.2% colonization rate in a population attending an STD clinic. This high colonization rate might reflect the high sexual activity of this population but, in some instances, might well be due to disease directly caused by the presence of *Haemophilus* species, as speculated by Wallace et al. (21). On the other hand, a diseased genital tract may favor colonization by this potential pathogen. Because of their lack of intrinsic pathogenicity, *Haemophilus* species other than *H. influenzae* type b or *H. ducreyi* could naturally colonize the anogenital area and, if particular factors are present, ultimately cause disease. The potentiating factors that could explain the appearance of disease by *Haemophilus* species remain to be defined.

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