

Biotypes of *Gardnerella vaginalis*

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A simple and reproducible scheme for identifying biotypes of *Gardnerella vaginalis* has been developed, based on reactions for lipase, hippurate hydrolysis, and beta-galactosidase. Among a total of 359 strains tested, eight biotypes were observed, the most common ones being types 1 (beta-galactosidase positive, lipase positive, hippurate positive), 2 (beta-galactosidase negative, lipase positive, hippurate positive), and 5 (beta-galactosidase negative, lipase negative, hippurate positive). The distribution in biotypes was similar among isolates from Antwerp, Seattle, and Nairobi. There were no differences in biotypes between strains isolated from patients with and without bacterial vaginosis (nonspecific vaginitis). Up to 14% of women with bacterial vaginosis harbored at least two different biotypes of *G. vaginalis* in the vagina. *G. vaginalis* strains isolated before and after treatment for bacterial vaginosis belonged to identical biotypes when the time interval between two specimens was less than 1 week. Similarly, *G. vaginalis* isolates from the vaginas of women with bacterial vaginosis and from the urethras of their male sex partners belonged to identical biotypes when strains were isolated within the same 24-h period from both partners ($P < 0.005$).

Gardnerella vaginalis is the predominant organism in vaginal fluid from women with bacterial vaginosis (BV) (nonspecific vaginitis), a condition in which there is also an increase in various anaerobic bacteria (12). Cure of BV correlates with a disappearance or a significant reduction of *G. vaginalis* and anaerobes, whereas the recurrence of the condition is linked with a reappearance or increase of *G. vaginalis* (8, 11). In some studies, there was a correlation between the recurrence of successfully treated BV and sexual reexposure, and the majority of male sex partners of women with BV harbored *G. vaginalis* in their urethra (1, 7).

A better understanding of the natural history and epidemiology of BV may be facilitated by the development of a typing system for *G. vaginalis*. Strains of *G. vaginalis* have been subdivided into seven serological groups by precipitin tests, but this system has not been used in clinically defined conditions. A simple and reproducible system for biotyping *G. vaginalis* is presented here.

MATERIALS AND METHODS

Strains. All *G. vaginalis* strains were isolated on human blood-bilayer Tween 80 (HBT) agar (11) and fully identified as described previously (9).

Of the 359 *G. vaginalis* strains tested to study the distribution of biotypes, 268 were fresh clinical isolates from the microbiology laboratory, Institute of Tropical Medicine, Antwerp, Belgium; 67 strains were clinical isolates from the Division of Infectious Diseases, Seattle Public Health Hospital, Seattle, Washington; and 24 strains were vaginal discharge cultures of women at the Special Treatment Clinic, Nairobi, Kenya.

A total of 48 pairs of *G. vaginalis* strains were isolated from the vaginas of women with BV before treatment and after treatment at a follow-up visit with or without a relapse of the disease. Twenty-four pairs of strains were isolated from the vaginas of women with BV and from the urethras of their male sex partners in Seattle and Antwerp.

In 76 cases of BV, four colonies typical of *G. vaginalis* were separately subcultured from the primary isolation medium, identified, and subsequently tested for determination of biotypes.

Biochemical tests. Hippurate hydrolysis was determined in a rapid test modified from Harvey (2) as described previously (9).

Lipase (3) was tested on a medium consisting of 4 g of Proteose Peptone no. 3 (Difco Laboratories, Detroit, Mich.), 0.5 g of Na₂HPO₄, 0.2 g of NaCl, 0.2 g of glucose, 0.02 ml of a 5% solution of MgSO₄, 2.5 g of Bacto-Agar (Difco), and 85 ml of distilled water. After autoclaving, 10 ml of egg yolk emulsion (Oxoid Ltd.) and 5 ml of horse serum were added. The inoculated plates were incubated in an anaerobe jar (Gaspak; BBL Microbiology Systems, Cockeysville, Md.) at 36°C for 3 days, after which they were observed closely under oblique light for the appearance of an oily iridescent sheen over and immediately around growth.

Beta-galactosidase activity was determined with a substrate solution containing 0.4% 2-nitrophenyl-β-D-galactopyranoside (Merck & Co., Inc., Rahway, N.J.), 75 ml of distilled water, 25 ml of buffer solution (5), NaH₂PO₄ · 1H₂O, and 40 ml of distilled water; the pH was adjusted to 7.0 with NaOH (5 N), and distilled water was added to a final volume of 50 ml.

Tubes containing 0.5 ml of the test medium were inoculated with a loopful (2-mm diameter) of bacteria from an overnight culture on HBT and incubated at 37°C (water bath). The results were read after 4 h and finally 18 h for the appearance of a yellow color.

RESULTS

All combinations of the three biochemical tests were observed in the *G. vaginalis* isolates typed. These biotypes have been given numbers (Table 1). Reactions for lipase, hippurate hydrolysis, and beta-galactosidase were highly reproducible and stable. Seven isolates were tested at least five times before and after 5 to 20 consecutive subcultures, before and after lyophilization, and before and after freezing at -70°C. Identical reactions were observed for each of the

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TABLE 1. Biotypes of *G. vaginalis*

Feature	Biotype no.							
	1	2	3	4	5	6	7	8
Beta-galactosidase	+	-	-	+	-	+	-	+
Lipase	+	+	+	+	-	-	-	-
Hippurate hydrolysis	+	+	-	-	+	+	-	-

respective tests. Passage on HBT medium containing 1% lactose did not induce a positive beta-galactosidase reaction among 10 beta-galactosidase-negative *G. vaginalis* strains.

The distribution of the eight biotypes among *G. vaginalis* isolates from Belgium, the United States, and Kenya are shown in Table 2. There was no major difference in occurrence of the different biotypes between the Antwerp and the Seattle isolates. Types 1, 2, and 5 were most common, the hippurate-negative types being fairly rare. There was no significant difference in the distribution of biotypes among 55 strains of *G. vaginalis* from consecutive patients with BV compared with 48 isolates from women without signs or symptoms of BV (data not shown).

Biotyping of 48 pairs of strains isolated before and after treatment from women with BV showed that strains from the same women belonged to the same biotype when samples were collected less than 7 days apart (7 patients). However, pairs of strains obtained from the same patient yielded different biotypes in 6 of 16 (38%) and 12 of 25 (48%) of the cases for intervals of 1 week and of 2 or more weeks, respectively. Paired isolates from women who had normal examinations at the time of the second sampling were not more often identical than pairs from women who had persisting or recurring BV.

To determine whether different biotypes were present simultaneously in the same vaginal sample, four colonies of *G. vaginalis* were cloned from the primary isolation plate and subsequently biotyped. Of the 76 samples treated in this way, two different biotypes were detected in 11 (14%) cases, and three different types were detected in 1 case.

To determine whether the same *G. vaginalis* biotypes were shared by women with BV and their sex partners, single clones were selected and typed from each of 24 pairs of women with BV and their male sex partners.

All but 1 of 12 isolates from the vaginas of women with BV belonged to the same biotype as strains isolated on the same or the subsequent day from the urethras of their male sex partners ($P < 0.005$; χ^2 with 9 degrees of freedom). Of 12 pairs of isolates collected from sex partners after an interval of more than 1 day, only 5 yielded an identical biotype. Of the identical pairs, six belonged to biotype 1, five belonged to biotype 2, four belonged to biotype 5, and one belonged to biotype 6, which is similar to the distribution of biotypes in all the strains tested.

DISCUSSION

The proposed typing scheme for *G. vaginalis* provides a stable and simple marker system which may be useful for the

TABLE 2. Biotypes among *G. vaginalis* strains of different geographic origins

Place of isolation	No. of strains	% of isolates of the following biotypes:							
		1	2	3	4	5	6	7	8
Antwerp	268	26	24	5	8	30	4	2	1
Seattle	67	38	18	4		31	9		
Nairobi	60	60	8	4		16	4	8	

study of the epidemiology of *G. vaginalis* infections. It is cheap and within the technical skills of the average medical microbiology laboratory. The distribution of the different biotypes among the strains was not equal. The predominant types were 1, 2, and 5, which included over 80% of the *G. vaginalis* isolates tested. Biotypes among strains from Antwerp and Seattle were similarly distributed. Although in this study no particular biotype or test result was associated with BV, this does not exclude the possibility that some *G. vaginalis* strains are more associated with BV. The biochemical markers we selected may not have been associated with virulence, and there is a need for additional markers, such as serotypes. In addition, the multiple biotypes found in a single vaginal sample made this association hard to analyze. We are planning to type isolates from deep-seated infections, such as bacteremia, endometritis, salpingitis, and abscesses, to study any correlation between biotypes and virulence factors.

The change in biotype observed in specimens from the same woman may be due to reinfection in between two samplings. However, of the 12 women in Antwerp from whom *G. vaginalis* was isolated twice within 7 days, only 2 had had sexual intercourse during the interim, and the biotypes of the paired strains in both women were identical. Among the strains isolated at least 14 days apart from the same patient, it was not possible to differentiate between reinfection and persistence. The occurrence of more than one type of *G. vaginalis* in the vagina of each woman may also explain the variation in biotype in the same woman. To test this hypothesis, four colonies from the same primary isolation plate were typed, and we found that 11 (14%) of 76 women harbored at least two biotypes in the vagina. Failure to detect additional mixed cultures may have been due to the limited number of colonies cloned for further testing. Variation in serotypes or phage types and mixed infections in the same individual have also been observed for other bacteria colonizing the genital tract, including group B streptococci (4) and *Ureaplasma urealyticum* (6, 12).

The isolation of identical biotypes from almost all sex partners when both partners were sampled within 24 h suggests that *G. vaginalis* is sexually transmitted. Overall, one-third of the paired *G. vaginalis* isolates from couples belonged to different biotypes. However, since 14% of the women were found to harbor two biotypes and we only treated one biotype from each sex partner, it is possible that the divergent results represent a sampling error. Similarly, Sandström and Moberg have found that *Neisseria gonorrhoeae* strains from sex partners may be of different auxotypes (10). It should be stressed that sexual transmission of *G. vaginalis* does not necessarily imply that BV itself is a sexually transmitted disease. The presence of other bacterial flora and of as yet unknown host factors may be equally important.

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LITERATURE CITED

- Gardner, L. H., and C. D. Dukes. 1955. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified "nonspecific vaginitis." *Am. J. Obstet. Gynecol.* **69**:962-976.
- Harvey, S. M. 1980. Hippurate hydrolysis by *Campylobacter fetus*. *J. Clin. Microbiol.* **11**:435-437.
- Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.). 1977. *Anaerobe laboratory manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.

4. Jackson, D. H., S. M. Hinder, J. Stringer, and C. S. F. Easmon. 1982. Carriage and transmission of group B streptococci among STD clinic patients. *Br. J. Vener. Dis.* **58**:334-337.
5. Le Minor, L., and F. Ben Hamida. 1962. Avantages de la recherche de la beta-glactosidase sur celle de la fermentation de lactose en milieu complexe dans le diagnostic bactériologique, en particulier des Entérobacteriaceae. *Ann. Inst. Pasteur (Paris)* **102**:267-277.
6. Lin, J.-S., and E. H. Kass. 1973. Serotypic heterogenicity in isolates of human genital T-mycoplasmas. *Infect. Immun.* **7**:499-500.
7. Pfeifer, T. A., P. S. Forsyth, M. A. Durfee, H. M. Pollock, and K. K. Holmes. 1978. Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N. Engl. J. Med.* **298**:1429-1434.
8. Piot, P., E. Van Dyck, P. Godts, and J. Vanderheyden. 1983. A placebo-controlled double blind comparison of tinidazole and triple sulfonamide cream for the treatment of non-specific vaginitis. *Am. J. Obstet. Gynecol.* **147**:85-89.
9. Piot, P., E. Van Dyck, P. A. Totten, and K. K. Holmes. 1982. Identification of *Gardnerella (Haemophilus) vaginalis*. *J. Clin. Microbiol.* **15**:19-24.
10. Sandström, E., and I. Moberg. 1980. Mixed infection as a model for gonococcal adaptation in vivo, p. 282-288. In D. Danielson and S. Normark (ed.), *Genetics and immunobiology of pathogenic Neisseria*. University of Umea, Umea, Sweden.
11. Spiegel, C. A., R. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes. 1980. Anaerobic bacteria in non-specific vaginitis. *N. Engl. J. Med.* **303**:601-607.
12. Viarengo, J., F. Hebrant, and P. Piot. 1980. *Ureaplasma urealyticum* in the urethra of healthy men. *Br. J. Vener. Dis.* **56**:169-172.