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Factors Affecting Isolation and Identification of *Haemophilus vaginalis* (*Corynebacterium vaginale*)

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The rate of isolation of organisms resembling *Haemophilus vaginalis* (*Corynebacterium vaginale*) from vaginal specimens was not significantly affected by anaerobic versus carbon dioxide incubation atmospheres or whether specimens were inoculated on isolation media immediately after collection or after a delay of 6 h. Forty-one clinically isolated strains were provisionally divided into 30 *H. vaginalis* strains and 11 *H. vaginalis*-like (HVL) strains based on morphological and growth characteristics. The *H. vaginalis* strains were less reactive in API-20A identification test strips, (Analytab Products, Inc.) using Lombard-Dowell broth, than in a modified basal medium that contained proteose peptone no. 3 (Difco). The numbers and kinds of substrates fermented by 30 clinical and 2 reference strains of *H. vaginalis* varied among conventional, API, Minitek (Baltimore Biological Laboratory), and rapid buffered substrate fermentation systems. A greater number and variety of carbohydrates were fermented by the 11 HVL strains more consistently in all four test systems. Analysis of volatile and nonvolatile fermentation end products by gas-liquid chromatography did not reveal significant differences between the *H. vaginalis* and HVL strains. However, the latter group grew in peptone-yeast extract-glucose broth, whereas the *H. vaginalis* strains did not grow without the addition of starch to peptone-yeast extract-glucose. All of the reference and clinical strains were similar in their susceptibilities to a variety of antimicrobial compounds except sulfonamides, which inhibited the HVL strains and bifidobacteria but not the *H. vaginalis* strains. Sulfonamide susceptibility or resistance corresponded in part to the *H. vaginalis* and HVL-bifidobacteria strain reactions on selected conventional fermentation substrates. Susceptibility or resistance to sulfonamides and metronidazole in conjunction with fermentation tests is described to aid in the separation of *H. vaginalis* from other possibly unrecognized biotypes of *H. vaginalis* or other vaginal bacteria that presumptively resemble the organism. A human blood medium known as V agar was also of considerable value in distinguishing *H. vaginalis* from HVL strains, because only the *H. vaginalis* strains produced diffuse beta-hemolysis on V agar.

Various methods have been devised for the isolation and presumptive and definitive identification of *Haemophilus vaginalis* (3-5, 10, 17). The organism may be successfully isolated from vaginal specimens by using swabs or transport media (3, 5), which in some cases may involve several hours of delay before media can be planted. Malone et al. (9) also described obligately anaerobic strains of *H. vaginalis*. There do not appear to have been any studies reported that compare recovery of *H. vaginalis* from vaginal specimens isolated on media incubated anaerobically versus a mixture of carbon dioxide and air or comparisons between isolation from specimens planted on media immediately after collection versus specimens held for longer time periods.

This paper describes the results of such evaluations together with an evaluation of methods for the identification of *H. vaginalis*, using several presumptive tests and carbohydrate reactions obtained by conventional methods, the API-20A strips (Analytab Products, Inc.) and, in part, the rapid buffered substrate fermentation test (RFT) and the Minitek system (Baltimore Biological Laboratory [BBL]).

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MATERIALS AND METHODS

Specimen processing. Calcium alginate swabs were used to collect cervical and vaginal specimens (11). One swab was rolled across the center of starch isolation medium (13), and the inoculum was further streaked with a loop. One starch plate was incubated at 37°C in 5 to 10% carbon dioxide, and the other plate was placed in a Brewer Anaerobic Jar with GasPaks (BBL) immediately after planting. A second swab was used to place discharge material into a vial containing 3 ml of heart infusion broth (Difco) supplemented with 1% proteose peptone no. 3 (Difco). The vials were left at room temperature for 6 h. Each vial was mixed on a Vortex tube mixer for 30 s, and a loopful of contents was streaked on a starch plate incubated in CO_2 and another plate was incubated in the Brewer Anaerobic Jar. The inoculation of media immediately after collection or from the vial for delayed planting was randomized in terms of which swab was collected from the discharge first. All plates were examined between 24 and 72 h for the presence of *H. vaginalis* (13).

Test strains. Thirty strains of *H. vaginalis* and 11 possible *H. vaginalis* strains were isolated from patients with vaginal discharges. Reference strains of *H. vaginalis* and *Bifidobacterium* species listed in the text were obtained from the American Type Culture Collection, Rockville, Md. Other named species were part of the laboratory stock collection derived from the California State Health Department Culture Study Program.

Other media. Tomato juice agar (Difco) and mitis-salivarius agar (BBL) were also used to aid in isolation procedures to recognize lactobacilli and streptococci. Dunkelberg's proteose peptone-starch-dextrose agar (PSD) was used to cultivate strains and to perform certain screening tests. Vaginal (V) agar of Greenwood et al. (5) was used to determine whether or not strains were hemolytic. *H. vaginalis* was reported to produce a diffuse beta-hemolysis on V agar (5). V agar is composed of Columbia agar base (BBL), 1% proteose peptone no. 3 (Difco), and 5% whole human blood.

Fermentation tests. Conventional methods used 1% substrates sterilized by filtration in Dunkelberg's semisolid fermentation medium base (4). This medium contained, per 100 ml, 2.0 g of proteose peptone no. 3 (Difco), 0.2 ml of 10% Tween 80, 0.2 ml of phenol red (1.6% stock solution), and 0.5 g of agar, with a final pH of 7.4. Tubes were stab inoculated with a needle, using growth of strains on PSD agar. The semisolid media were incubated aerobically at 37°C for 5 days. The Minitek system (BBL) was used unmodified with sterile mineral oil overlying each disk and broth inoculum. Preliminary studies indicated that the regular API-20A diluent left some strains of *H. vaginalis* inert. Therefore, the regular diluent (Lombard-Dowell broth) was compared to a second diluent which consisted of 1% proteose peptone no. 3, 0.5% yeast extract, and 0.5% sodium chloride with a final pH of 7.3. The indole, urease, gelatinase, and hydrogen sulfide tests of the API system were not evaluated. The Minitek and API systems were incubated in 5% CO_2 at 37°C and observed at 24 and 48 h. For the RFT method, heavy growth of strains from PSD agar was suspended in 5 ml of balanced salts solution, centrifuged, and resuspended in balanced salts solution to a turbidity of no. 8 McFarland nephelometer. A 0.1-ml sample of cells was added to tubes (12 by 75 mm) to which was added 0.25 ml of balanced salts solution containing phenol red and 0.05 ml of 20% filter-sterilized carbohydrate. The tubes were incubated at 37°C in a water bath and examined after 4 and 10 h.

Miscellaneous methods. Volatile and nonvolatile fermentation end products were detected with the AnaBac gas-liquid chromatograph. Commercial volatile and nonvolatile reagent standards were used to calibrate the gas-liquid chromatograph equipment at each time of use, and uninoculated broth media were tested for additional quality control. Strains were grown in prereduced peptone-yeast extract-glucose (PYG; Scott Labs) broth, but *H. vaginalis* isolates only grew in PYG broth when the medium was supplemented with 0.5% soluble starch. Gram stains and colonial morphology were based on growth on PSD agar, which was also used for "spot tests" and inhibition studies. These tests consisted of viridans streptococci (10) and hydrogen peroxide inhibition (7), inhibition by various compounds in disk or tablet form, the catalase test, and reduction of 1% tellurite (15). Esculin hydrolysis was determined on starch agar supplemented with 1 g of esculin and 0.5 g of ferric ammonium citrate per liter.

RESULTS

A total of 50 strains of vaginal bacteria resembling *H. vaginalis* were isolated by direct and delayed planting of 105 vaginal specimens on starch agar (Table 1). Forty-five strains, which included most of the first 50 isolates, were isolated from 122 specimens incubated in CO_2 and anaerobic jars. Differences between the isolation rates of the strains in the direct versus delayed method or the CO_2 versus anaerobic incubation method were not significant at the 0.05 probability level. There was no significant correlation found among any of the four parameters studied with respect to isolation rates, and none of the strains was found to be obligately anaerobic.

TABLE 1. *Effects of time delay and atmosphere of incubation on the isolation of H. vaginalis*

Conditions	No. of strains isolated	Specimens negative
Time of planting (105 specimens)[a]		
Immediate only	11	55
Delayed only	15	
Immediate + delayed	24	
Incubation atmosphere (122 specimens)[b]		
CO_2 only	10	77
Anaerobic only	14	
CO_2 + anaerobic	21	

[a] Immediate and delayed isolation.
[b] Carbon dioxide and anaerobic jar.

Forty-one isolated strains resembling *H. vaginalis* were maintained for additional studies. All strains fermented starch, were catalase negative, did not reduce 1% tellurite, and were inhibited by the growth of viridans streptococci and 1 drop of 3% hydrogen peroxide placed on PSD agar. The group of strains also produced colonies with button-like centers ("bull's-eye" colonies) on PSD agar. Thirty strains, tentatively classified as *H. vaginalis*, tended on either starch agar or PSD agar to form dry rough colonies that formed clumps in saline. Eleven other strains produced a smooth creamy texture on the same media and formed uniform suspensions in saline, but also contained gram-positive-staining beads within the gram-negative cells. These latter strains also formed coiled coma-like cell shapes and were designated *H. vaginalis*-like strains.

Initially, two strains of *H. vaginalis* were inert in the API-20A system when cell inoculum was prepared with Lombard-Dowell basal medium. Therefore, 13 randomly selected *H. vaginalis* and the 11 *H. vaginalis*-like strains were compared for fermentation reactions in the API strip, using Lombard-Dowell broth and a modified basal medium containing proteose peptone no. 3 (Table 2). The fermentation of substrates in the API strip by the *H. vaginalis*-like strains was nearly identical with either basal medium.

TABLE 2. *Comparative carbohydrate fermentation reactions in the API-20A test system by H. vaginalis and H. vaginalis-like organisms, using conventional and modified basal media*[a]

Acid produced from:	No. of strains positive of total tested			
	H. vaginalis (13)[b]		*H. vaginalis*-like (11)[b]	
	Regular	Modified	Regular	Modified
Dextrose	2	5	9	9
Maltose	6	12	9	9
Sucrose	2	3	9	9
Lactose	1	1	7	7
Arabinose	1	4	2	3
Xylose	0	3	5	6
Mannose	0	1	8	8
Raffinose	0	0	9	10
Mannitol	1	2	7	7
Salicin	0	0	9	9
Glycerol	1	0	1	3
Sorbitol	2	0	2	3
Rhamnose	0	0	0	1
Trehalose	2	1	6	5
Cellobiose	0	0	6	5
Melezitose	0	0	5	5

[a] Basal media: Regular—Lombard-Dowell medium (Trypticase peptone); modified—1% proteose peptone no. 3–0.5% yeast extract–0.5% sodium chloride, pH 7.2.
[b] Number of strains.

However, the *H. vaginalis* strains fermented dextrose and maltose more often with the modified basal medium, so this was used to test all additional *H. vaginalis* strains in the API strip.

The numbers of substrates fermented by 32 *H. vaginalis* strains in four test systems (conventional, RFT, Minitek, and API) varied with the system used (Table 3). Starch and maltose were fermented by all strains only in the conventional system. Among substrates considered to be variable fermented substrates, more positive reactions occurred in Minitek and RFT systems than with conventional media.

Twelve of the 32 *H. vaginalis* strains fermented lactose in at least one system, whereas the *H. vaginalis*-like organisms fermented lactose more consistently in all test systems (Table 4). The *H. vaginalis*-like strains also fermented a greater variety of substrates in the conventional media (Table 5) and the other fermentation systems than did *H. vaginalis*.

Analysis of volatile and nonvolatile fermentation end products of the two groups of bacteria were not significantly different (Table 6). However, the *H. vaginalis* strains did not grow in PYG without the addition of 1% soluble starch. The growth of the *H. vaginalis*-like strains in PYG was stimulated by the addition of starch but was not required for growth. One strain each

TABLE 3. *Comparative fermentation reactions of 32 strains of H. vaginalis in four test systems*

Reported reaction of organism[a]	Substrate	No. of strains positive with test system:[b]			
		Conventional	Minitek	RFT	API-20A
Positive	Starch	32	30	30	—
	Maltose	32	30	28	20,5w
	Glucose	21,1w	21,1w	23,4w	12,3w
Variable	Fructose	13,5w	—	23,2w	—
	Galactose	9,1w	—	8,4w	—
	Xylose	5,2w	26,1w	7	5,2w
	Mannose	4,2w	27	17,3w	7,1w
	Lactose	5,1w	9,1w	6	9
	Sucrose	5,1w	20	7	13,1w
	Arabinose	3,2w	10,1w	8	2
	Trehalose	4	23	5,1w	3,1w
	Rhamnose	1	0	0	0
Negative	Raffinose	2	6	—	5
	Cellobiose	1,1w	5	—	2
	Mannitol	0	3	—	2
	Salicin	0	3	—	4
	Glycerol	0	0	—	0,2w
	Sorbitol	0	8,4w	—	1
	Melezitose	0	—	—	0,1w
	Esculin	0	7	—	3

[a] References 2, 3, 8, and 9.
[b] Includes *H. vaginalis* ATCC 14018 and 14019. w, Weak positive; —, not done or test not available

TABLE 4. *Comparative lactose fermentation by H. vaginalis and H. vaginalis-like organisms*

Strains	No.	Lactose fermentation			
		Conventional	API-20A	Minitek	RFT
H. vaginalis	2	−	−	w[a]	−
	4	+	−	−	−
	24	+	−	−	−
	45	−	−	w	−
	34	−	+	−	−
	37	w	−	−	−
	50	+	+	+	+
	57	−	+	+	−
	67	+	+	+	+
	77	−	−	+	−
	83	−	+	−	−
	87	−	+	+	+
H. vaginalis-like organisms	27	+	−	+	+
	29	+	+	+	+
	30	+	+	+	−
	42	+	+	+	+
	43	+	+	+	+
	48	+	+	+	+
	52	+	+	+	+
	55	+	+	+	+
	69	+	+	+	+
	88	+	+	+	+
	46	−	−	+	−

[a] w, Weak positive.

TABLE 5. *Conventional fermentation reactions of vaginal bacteria presumptively identified as H. vaginalis-like*[a]

Acid produced from:	Strain and reaction										
	52	55	27	29	30	42	43	46	69	48	88
Starch	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	−	+	+	+	+	+	+	+	−
Galactose	+	+	+	+	+	+	+	+	+	−	−
Xylose	−	−	−	−	−	−	−	+	+	−	−
Mannose	+	+	−	+	+	+	+	−	−	+	+
Lactose	+	+	+	+	+	+	+	−	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	−	−	−	−	−	−	−	+	−	−	−
Trehalose	−	−	−	−	+	+	−	−	+	−	−
Rhamnose	−	−	−	+	−	−	−	−	−	−	−
Raffinose	+	+	−	+	+	+	+	+	−	+	+
Cellobiose	−	−	−	+	−	+	+	−	+	−	−
Mannitol	−	−	−	+	+	+	+	+	+	+	+
Salacin	+	+	−	+	−	+	+	+	+	+	+
Glycerol	−	−	+	+	−	+	+	+	+	+	−
Sorbitol	+	+	−	+	−	+	+	+	−	+	+
Melezitose	−	+	−	+	−	+	−	−	−	−	−
Esculin	−	−	−	+	−	+	−	+	−	−	−

[a] Corresponding reactions on substrates for these organisms with the API, Minitek, and RFT systems were essentially identical to conventional results.

TABLE 6. *Volatile and nonvolatile fermentation end products of vaginal strains in PYG or PYG-starch medium*

End product detected[a]	*H. vaginalis* (32 strains)	*H. vaginalis*-like (9 strains)[b]
ALf	9	2
AL	12	1
Al	4	0
ALf	1	0
aL	1	0
a	3	1
A	1	0
Af	1	0
La	0	1
ALf py	0	4

[a] A, Acetic acid; L, lactic acid; f, formic acid; py, pyruvic acid. Capital letters indicate major amounts of acid (>1 meq/100 ml).

[b] Two strains not tested.

of *Bifidobacterium adolescentis* ATCC 15703, *B. breve* ATCC 15700, and *B. infantis* ATCC 15697, which grew in unsupplemented PYG, each produced major acetic and lactic acid products and minor amounts of formic acid. Succinic acid was not detected from any of the organisms tested.

The bifidobacteria, *H. vaginalis* ATCC 14018 and 14019, and the 41 clinical strains were uniformly susceptible to: furadantin, 300 μg; metronidazole, 50 μg; ampicillin, 10 μg; thionin, 1:8,000; pyronin, 1:1,600; and Taxo A-bacitracin and sulfamethoxazole-trimethoprim, 25 μg. Variable results were obtained with 10 μg of tobramycin. The *H. vaginalis* strains were uniformly resistant to: sulfisoxazole, 2 mg; sulfathiazole, 1 mg; and sulfachloropyridazine, 1 mg. The bifidobacteria and *H. vaginalis*-like strains were susceptible to the sulfonamides. Zones of inhibition were greater than 20 mm in diameter and were produced on chocolate, blood, starch, and PSD agars. Vaginal streptococci and lactobacilli were resistant to most of the compounds tested above.

Twenty-five additional vaginal strains resembling *H. vaginalis* and five reference strains of *H. vaginalis* and bifidobacteria were tested for susceptibility to sulfonamides and for acid production from starch, maltose, dextrose, lactose, melibiose, and ribose in conventional media (Table 7). Seventeen sulfa-resistant *H. vaginalis* strains did not ferment lactose, melibiose, or ribose. Combinations of these latter substrates were fermented by 12 of 13 sulfonamide-susceptible strains.

Virtually all of the isolated and reference strains of *H. vaginalis*, including strains that were sulfonamide resistant, produced diffuse beta-hemolysis on V agar. Lactobacilli, bifido-

TABLE 7. *Relationship between sulfonamide susceptibility and conventional screening methods for identification of starch-fermenting H. vaginalis-like bacteria*

Group	No. of strains	Patterns of substrates fermented				
		Maltose	Dextrose	Lactose	Melibiose	Ribose
Sulfa resistant (17 strains)[a]	8	+	+	−	−	−
	6	+	−	−	−	−
	3	−	−	−	−	−
Sulfa susceptible (13 strains)[b]	7	+	+	+	+	+
	1	+	+	−	−	+
	1	−	+	−	+	+
	1	+	+	+	−	−
	1	−	+	−	−	−
	2	+	−	−	+	+

[a] Includes *H. vaginalis* ATCC 14018 (type strain Gardner and Dukes 594) and *H. vaginalis* ATCC 14019 (Gardner and Dukes strain 317).

[b] Includes *B. breve* ATCC 15700, *B. infantis* ATCC 15697, and *B. aldolescentis* ATCC 15703.

TABLE 8. *Rapid presumptive spot tests that are not specific for identification of H. vaginalis*

Test	Reaction of *H. vaginalis*
Viridans streptococci	Inhibited
Hydrogen peroxide, 3%	Inhibited
Tellurite reduction	Tellurite not reduced
Starch fermentation	Acid produced
Catalase	Negative
Colonial morphology on PSD	Not specific
Gram stain morphology	Variable
Volatile and nonvolatile end products	Variable
Inhibition by	
Pyronin (1:1,600)	Inhibited
Thionin (1:8,000)	Inhibited
Taxo A	Inhibited
Metronidazole (50 μg)	Inhibited

bacteria, the *H. vaginalis*-like strains, and the additional isolates that were susceptible to sulfonamides either produced alpha-hemolysis or were not hemolytic on V agar.

DISCUSSION

The isolation rates of organisms resembling *H. vaginalis* were not significantly affected by whether or not specimens were inoculated on media immediately or after a time delay of 6 h and whether or not the isolation medium was incubated in CO_2 or in an anaerobic jar. However, the largest number of strains was isolated when both of the comparison systems were used. A possible explanation for this may be that the two sets of systems allowed the use of a larger total amount of inoculum inoculated on more plates when using both systems. This could be expected to increase the isolation rates of any organism.

The presumptive identification of *H. vaginalis* based on any one of a variety of single or combined screening tests (Table 8) does not distinguish the *H. vaginalis* from other starch-fermenting bacteria of the vaginal tract that in some ways closely resemble species of the genus *Bifidobacterium*. Species of the latter genus are indigenous to the vaginal tract and may occur as aerotolerant organisms (6, 12). The incidence of these and other *H. vaginalis*-like organisms in vaginal discharges is not known. However, since the clinically isolated strains were taken at random, it appears that these organisms occur commonly. Josey and Lambe (7) described *H. vaginalis* as being small, pleomorphic, and gram variable but predominantly gram negative, with some strains having gram-positive beads. Josey and Lambe further defined the fermentation properties of the organism as producing acid from dextrose, maltose, and starch, usually from fructose and trehalose, rarely from xylose, and not from mannitol or lactose. This agrees with descriptions given for the organism by Dunkelberg (2, 3), Lapage (8), and Malone et al. (9). We did not detect dextrose fermentation in all 32 *H. vaginalis* strains by any of the four methods used. Dunkelberg (2) stated that autoclaved dextrose was weakly fermented, but none of the substrates used in our study was sterilized by autoclaving. Dunkelberg (3) also reported that heavy inocula must be used for conventional fermentation media. Inoculation of conventional media in this study was heavy but not standardized, which could account for some results obtained with dextrose. Malone et al. (9) found that 2 of 24 *H. vaginalis* strains fermented lactose, and some strains in this study were positive. The large number of substrates used in this study is not practical for routine diagnostic methods. The variability of substrates fermented by *H. vaginalis* other than starch and maltose is well known. However, careful definitive diagnostic methods are required to distinguish *H. vaginalis* from other species found in the vaginal tract or undefined variants and biotypes of *H. vaginalis*. The Minitek and API-20A systems were not reliable for identification of *H. vaginalis*. Incubation of the API strip in 5% CO_2 instead of the normal, anaerobic environment did not increase the types or numbers of substrates fermented by the strains. The Minitek system produced more positive reactions in some substrates than the other systems, but this included several sugars not considered to be fermented by *H. vaginalis* (3, 8, 9). There were

more fructose- and mannose-fermenting strains detected by the RFT method than by the other three systems, which is not inconsistent with possible reactions of *H. vaginalis*. Greenwood et al. (5), using starch, dextrose, and maltose, found the RFT and conventional methods to be identical except for 2 of 63 *H. vaginalis* strains. Additional evaluation of the RFT method should include a greater variety of substrates. The use of starch, dextrose, and maltose alone would not detect *H. vaginalis*-like organisms or other starch-fermenting species such as lactobacilli, streptococci, or bifidobacteria from the vaginal tract. Other modifications of the Minitek or API systems might be useful for *H. vaginalis* identification, but modifications might be impractical unless large numbers of isolates are being routinely identified.

Despite heavy growth in PYG or PYG-starch broth, none of the three reference strains of *Bifidobacterium* species, two reference strains of *H. vaginalis*, or the clinical isolates formed detectable levels of succinic acid in PYG or PYG-starch broth. However, since *H. vaginalis* may produce minor levels of succinate (9), like the bifidobacteria (6), analysis of volatile and nonvolatile end products would not necessarily separate *H. vaginalis* from bifidobacteria or possibly other species we have designated *H. vaginalis*-like.

The ability of the *H. vaginalis*-like strains to ferment more carbohydrates than *H. vaginalis*, their tendency to form smooth colonies and to grow promptly on media that does not contain proteose peptone no. 3, their susceptibility to sulfonamides, and their failure to produce diffuse beta-hemolysis on V agar are all significant differences separating them from *H. vaginalis*.

Susceptibility to sulfonamides or metronidazole disks (Table 9), together with selected fermentation tests, can be used as simple means to presumptively separate four major groups of vaginal bacteria that may ferment starch and be confused morphologically with *H. vaginalis*. *H. vaginalis* susceptibility to metronidazole (14) is unusual for a facultative organism, but *Bifidobacterium* species, which may be obligate anaerobes or aerotolerant (12), show considerable variation in susceptibility to metronidazole (1, 16). Therefore, additional evaluation of antimicrobial susceptibility methods for presumptive identification of vaginal bacteria is required. Melibiose was not fermented by the *H. vaginalis* strains but is commonly fermented by bifidobacteria (12) and was fermented by some of the sulfa-sensitive *H. vaginalis*-like organisms.

In our hands, a wide selection of conventional carbohydrates should be used, at least in a public health reference laboratory, to identify *H. vaginalis*. Greenwood and Pickett (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C77, p. 48) determined that *H. vaginalis* was *o*-nitrophenyl-β-D-galactopyranoside positive and hydrolyzed hippurate. These data may be useful in identification of *H. vaginalis* since bifidobacteria are hippurate negative (6). V agar of Greenwood et al. (5) also appears to have considerable value as an isolation and identification medium for *H. vaginalis*. Other starch-fermenting bacteria from the vaginal tract that may be confused with *H. vaginalis* do not appear to produce a diffuse beta-hemolysis on V agar. We are in the process of comparing V agar and starch agar for *H. vaginalis* isolation.

TABLE 9. *Simple inhibition tests for the presumptive identification of H. vaginalis from among other catalase-negative, starch-fermenting vaginal bacteria*[a]

Bacterial group or species	Susceptibility to disk of:	
	Sulfonamide (1-2 mg)	Metronidazole (50 μg)
H. vaginalis	R	S
Bifidobacteria and *H. vaginalis*-like organisms	S	S
Streptococci	R	R
Lactobacilli	R	R

[a] Agar disk diffusion method, using PSD agar incubated in 5 to 10% CO_2 for 24 h, together with demonstration of starch fermentation, lack of catalase, and cell morphology based on the Gram stain.

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