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Susceptibility of *Mobiluncus* Species to 23 Antimicrobial Agents and 15 Other Compounds

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The susceptibility of 12 strains of *Mobiluncus curtisii* and 10 strains of *M. mulieris* to 23 antimicrobial agents and 15 other compounds was determined. All strains were susceptible to chloramphenicol, clindamycin, rifampin, tobramycin, vancomycin, virginiamycin, and all beta-lactam antibiotics tested, including imipenem. One strain of *M. mulieris* was resistant to erythromycin and josamycin. All were resistant to colistin, cycloserine, nalidixic acid, and neomycin. Tetracycline had variable activity. All *M. curtisii* strains were resistant to metronidazole and its hydroxy metabolite. Of 10 *M. mulieris* strains, 5 were resistant to metronidazole and 2 were resistant to its hydroxy metabolite. All 12 *M. curtisii* and 1 of 10 *M. mulieris* strains were resistant to tinidazole. *M. curtisii* and *M. mulieris* produced two mutually exclusive clusters of MICs when tested against ampicillin, cefoxitin, cephalothin, moxalactam, alizarin red, Evans blue, and sodium fluoride. *Gardnerella vaginalis* was more susceptible to Nile blue A than was either *M. curtisii* or *M. mulieris*. Clindamycin and imipenem may be useful agents in the therapy of metronidazole-resistant bacterial vaginosis. Metronidazole, tinidazole, and Nile blue A may be of value in the development of a selective agar for *Mobiluncus* species.

Mobiluncus curtisii and *M. mulieris* (11), curved, motile, anaerobic rods, are clearly associated with bacterial vaginosis (BV) (10, 12, 13). Although their role in BV is not yet understood, reports of their presence in up to 50% of women with (10) and only 0 to 5% without (10, 12) BV have provoked a great deal of interest in these organisms. Several researchers have also reported that most or all strains of short curved rods (*M. curtisii*) (2, 4, 9, 10, 12) and some strains of long curved rods (*M. mulieris*) (2, 4, 10, 12) are resistant to metronidazole, the drug of choice for treating BV. One could speculate that the presence of metronidazole-resistant *Mobiluncus* spp. plays a significant role in recurrent BV, which occurs in 10 to 12% of women treated with metronidazole (1). In that case, it will be important to know what other antimicrobial agents are active against them. Several reseachers have published susceptibility patterns, but recommended methods have rarely been used (5). When methods have been modified, quality control has only rarely been included (2).

The purpose of these studies was to use standardized methods to determine the susceptibility of 12 strains of *M. curtisii* and 10 strains of *M. mulieris* to a wide variety of antimicrobial agents and to use similar methods to determine their susceptibility to a selection of stains, dyes, and metabolic inhibitors as part of their phenotypic characterization. I also hoped that these data would guide me toward development of a selective medium for *Mobiluncus* species.

MATERIALS AND METHODS

Source of strains. Susceptibilities were determined by using 22 well-characterized strains of *Mobiluncus* spp., including the type strains. The methods used for isolation, identification, and storage of these organisms have been published elsewhere (10, 11). The activity of five antimicrobial agents against 10 of the *M. curtisii* and 3 of the *M. mulieris* strains was previously determined by using a different method (10).

Activity of selected agents was also determined for six clinical isolates of *Gardnerella vaginalis* recovered from frozen stock. These isolates had been identified based on the presence of pleomorphic gram-variable rods which produced beta hemolysis on HBT agar (Regional Media Laboratories, Lenexa, Kans.) incubated in 5 to 10% carbon dioxide (7, 14). One isolate was provided by L. Barth Reller, University of Colorado, Denver.

In vitro susceptibility testing. Susceptibilities were determined within five passages of recovery from frozen stock. MICs were determined either by microbroth dilution (antimicrobial agents) or agar dilution (other). Tests were performed in Wilkins-Chalgren broth (Anaerobe Broth, Experimental; Difco Laboratories, Detroit, Mich.) supplemented with 1% soluble starch (Difco), 2% rabbit serum (GIBCO Diagnostics, Madison, Wis.) and, for agar dilution tests only, 1.5% agar (Difco). Media were prepared on the bench top and reduced overnight in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) in an atmosphere of 10% hydrogen–10% carbon dioxide–80% nitrogen before inoculation.

The 23 antimicrobial agents tested were ampicillin (Sigma Chemical Co., St. Louis, Mo.), cefazolin (Smith Kline & French, Philadelphia, Pa.), cefoxitin (Merck Sharp & Dohme, Rahway, N.J.), cephalothin, chloramphenicol (Sigma), clindamycin (The Upjohn Co., Kalamazoo, Mich.), colistin methanesulfonate, cycloserine (Sigma), erythromycin (Upjohn), hydroxymetronidazole (G. D. Searle, Skokie, Ill.), imipenem (Merck Sharp & Dohme), josamycin (S. M. Finegold, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.), metronidazole (Searle), moxalactam (Eli Lilly & Co., Indianapolis, Ind.), nalidixic acid, neomycin, penicillin G, rifampin, tetracycline (Sigma), tinidazole (Ortho Diagnostics, Raritan, N.J.), tobramycin, vancomycin (Eli Lilly), and virginiamycin (Upjohn). The 15 other compounds tested were alizarin red S, azure II

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TABLE 1. MICs (μg/ml) of 23 antimicrobial agents against 12 strains of *M. curtisii* and 10 strains of *M. mulieris*

Agent	MIC range tested	*M. curtisii*			*M. mulieris*		
		MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Ampicillin	0.015–16	0.125–0.25	0.125	0.25	≤0.015–0.062	0.031	0.062
Cefazolin	0.2–200	≤0.2–1.56	0.78	1.56	≤0.2–0.78	0.2	0.4
Cefoxitin	0.062–64	1–4	2	4	0.25–0.5	0.25	0.5
Cephalothin	0.2–200	0.78–1.56	1.56	1.56	≤0.2–0.4	0.4	0.4
Chloramphenicol	0.062–64	4–8	8	8	2–4	4	4
Clindamycin	0.015–16	0.062–0.125	0.125	0.125	≤0.015–4	0.031	0.062
Colistin	4–4,096	32–64	64	64	64–256	128	256
Cycloserine	0.2–200	200–>200	200	>200	100–>200	100	>200
Erythromycin	0.2–200	≤0.2	≤0.2	≤0.2	≤0.2–>200	≤0.2	≤0.2
Hydroxymetronidazole	0.5–512	128–512	256	512	4–>512	8	128
Imipenem	0.004–4	0.031–0.125	0.062	0.125	0.031–0.062	0.062	0.062
Josamycin	0.004–4	0.008–0.015	0.015	0.015	≤0.004–>4	0.004	0.004
Metronidazole	0.5–512	32–512	128	256	2–>512	4	256
Moxalactam	0.062–64	1–4	4	4	≤0.062–0.5	0.25	0.5
Nalidixic acid	0.3–200	200–>200	>200	>200	100–200	200	200
Neomycin	0.062–64	2–8	4	8	4–8	4	8
Penicillin G	0.004–4	0.015–0.125	0.062	0.062	0.008–0.015	0.015	0.015
Rifampin	0.004–4	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004
Tetracycline	0.2–200	0.39–12.5	6.25	12.5	≤0.2–6.25	≤0.2	6.25
Tinidazole	0.5–256	>256	>256	>256	4–>256	4	8
Tobramycin	0.031–64	0.25–1	1	1	0.25–1	0.5	1
Vancomycin[a]	0.001–1	0.5	0.5	0.5	0.5–1	0.5	0.5
Virginiamycin	0.2–200	≤0.2	≤0.2	≤0.2	≤0.2–0.39	≤0.2	≤0.2

[a] Concentrations from 2 to 1,024 μg/ml were included for testing of the quality control organism only.

(Sigma), basic fuchsin (Harleco, Philadelphia, Pa.), brilliant green (Difco), deoxycholate, Evans blue (Sigma), gentian violet (Difco), janus green (Sigma), malachite green (Difco), methyl orange (Sigma), Nile blue A (75% pure; Eastman Kodak Co., Rochester, N.Y.), oxgall, resazurin (Difco), safranine, and sodium fluoride (Sigma).

Stationary-phase growth was used to prepare the inoculum. With 48-h-old growth on agar, a suspension was made to match a McFarland 0.5 standard in Wilkins-Chalgren broth. This suspension was appropriately diluted to produce an inoculum of approximately 10^6 CFU/ml or spot. MICs were determined after 48 h of anaerobic incubation at 35°C. The MIC in broth was the minimum concentration at which there was no visible turbidity. The MIC on agar was the minimum concentration at which there was no more than a slight haze of growth or no more than two distinct colonies.

Quality control. Quality control of the antimicrobial agents was performed by using *Bacteroides fragilis* ATCC 25285 for cefoxitin, chloramphenicol, clindamycin, imipenem, metronidazole, moxalactam, and tetracycline; *Clostridium perfringens* ATCC 13124 for penicillin G, *Escherichia coli* ATCC 25922 for cefazolin and tobramycin; and *Enterococcus (Streptococcus) faecalis* ATCC 29212 for ampicillin, cephalothin, erythromycin, and vancomycin.

RESULTS

Antimicrobial susceptibility. No quality control values were known for colistin, cycloserine, hydroxymetronidazole, josamycin, nalidixic acid, neomycin, rifampin, tinidazole, or virginiamycin. The tobramycin MIC was 32 times higher than expected, and the erythromycin MIC was four times greater than expected. The remaining 12 drugs were in control.

The results for the 22 *Mobiluncus* strains are in Table 1. All strains were susceptible to ampicillin, cefazolin, cefoxitin, cephalothin, chloramphenicol, clindamycin, imipenem, moxalactam, penicillin G, rifampin, tobramycin, vancomycin, and virginiamycin. All strains but one were susceptible to erythromycin and josamycin. All strains were resistant to colistin, cycloserine, nalidixic acid, and neomycin. All *M. curtisii* strains were resistant to metronidazole and hydroxymetronidazole. Whereas the MICs for 50 and 90% of the strains tested (MIC_{50} and MIC_{90}, respectively) were similar for metronidazole and its hydroxy metabolite against *M. mulieris*, the distribution of individual MICs was not. Whereas only 5 (50%) of 10 *M. mulieris* strains were susceptible to ≤8 μg of metronidazole per ml, 8 (80%) of 10 were susceptible to ≤8 μg of the hydroxy metabolite per ml (data not shown). All 12 *M. curtisii* and 1 of 10 *M. mulieris* strains were resistant to tinidazole. There was a bimodal distribution of tetracycline MICs; 10 of 12 *M. curtisii* and 2 of 10 *M. mulieris* strains were resistant.

MICs could not be used to differentiate *M. curtisii* subsp. *curtisii* from *M. curtisii* subsp. *holmesii* (data not shown). However, *M. curtisii* and *M. mulieris* formed two distinct clusters of MICs when tested against ampicillin, cefoxitin, cephalothin, and moxalactam. In all of these cases, *M. mulieris* was more susceptible than *M. curtisii*. The only antimicrobial agent that was noticeably more active against *M. curtisii* than *M. mulieris* was colistin. One strain of *G. vaginalis* had a tinidazole MIC of 16 μg/ml, and that of the remaining five was 64 μg/ml.

Stains, dyes, and metabolic inhibitors. No quality control values were available for these compounds. The MICs of these agents against *Mobiluncus* spp. and *G. vaginalis* are shown in Table 2; interpretations of susceptibility or resistance could not be made.

MICs could not be used to differentiate *M. curtisii* subsp. *curtisii* from *M. curtisii* subsp. *holmesii* (data not shown). However, *M. curtisii* and *M. mulieris* formed two distinct clusters of MICs when tested against alizarin red, Evans blue, and sodium fluoride. *M. mulieris* was more susceptible to each of these agents. *G. vaginalis* was more susceptible to Nile blue A than was either *M. curtisii* or *M. mulieris*.

TABLE 2. MICs of 15 stains, dyes, and metabolic inhibitors against 12 *M. curtisii*, 10 *M. mulieris*, and 6 *G. vaginalis* strains

Agent	Range tested[a]	*M. curtisii*		*M. mulieris*		*G. vaginalis* MIC_{90}
		MIC range	MIC_{90}	MIC range	MIC_{90}	
Alizarin red	0.03–0.2	0.05–0.1	0.1	≤0.03	≤0.03	≤0.03
Azure II	1×10^{-4}–0.015	0.005–0.015	0.01	0.005–0.01	0.005	0.005
Basic fuchsin	5×10^{-5}–0.015	5×10^{-4}–0.005	0.005	0.005	0.005	$>5 \times 10^{-4}$[b]
Brilliant green	5×10^{-7}–0.01	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-5}
Deoxycholate	0.01–1	0.1	0.1	0.1	0.1	NT[c]
Evans blue	0.02–1	0.1–1	0.1	≤0.3	≤0.3	≤0.03
Gentian violet	5×10^{-6}–0.0015	5×10^{-5}	5×10^{-5}	5×10^{-5}	5×10^{-5}	$\leq 5 \times 10^{-4}$
Janus green	5×10^{-5}–0.015	5×10^{-4}–0.005	0.005	5×10^{-4}–0.005	0.005	$>5 \times 10^{-4}$[b]
Malachite green	2–200	20	20	20	20	>2[b]
Methyl orange	0.001–1	0.1	0.1	0.1	0.1	0.05
Nile blue A	0.005–1	0.1–0.5	0.5	0.05–0.1	0.1	0.01
Oxgall	0.01–1	≤0.01–1	1	1	1	>0.01[b]
Resazurin	1×10^{-4}–0.1	0.01	0.01	0.01	0.01	0.01
Safranine	1×10^{-5}–0.005	5×10^{-4}	5×10^{-4}	5×10^{-4}–0.005	0.005	$>10^{-5}$[b]
Sodium fluoride	0.025–0.25	0.1–0.25	0.25	≤0.025–0.05	0.05	0.1

[a] Units are in grams per 100 ml, except for malachite green, which is in micrograms per 100 ml.
[b] Single concentration tested.
[c] NT, Not tested.

DISCUSSION

Standard microtiter methods could not be used to determine the susceptibility of *Mobiluncus* spp. to antimicrobial agents because Wilkins-Chalgren broth does not adequately support their growth. To enhance growth, soluble starch (1%) and rabbit serum (2%) were added. Quality control organisms were used to show that these supplements did not alter the activity of the drugs tested. Of those agents whose values were known, only tobramycin and erythromycin were less active than expected. The anoxic capneic environment rather than the medium supplements may have decreased the activity of tobramycin and erythromycin (3). In cases in which quality control values are lacking, it would not be prudent to use the data published herein to choose an agent for human use. The information may be valuable in choosing selective agents for in vitro use.

We are aware of six prior reports containing *Mobiluncus* susceptibility patterns (2, 4, 5, 9, 10, 12). It should be noted that both strains of *M. curtisii* and one of the two strains of *M. mulieris* tested by Carlone et al. (2) were tested by Spiegel et al. (10) and that all four strains were tested in the present study. Only one of six studies that tested beta-lactam antibiotics found them inactive against *Mobiluncus* spp. (5). With the exception of a single strain described in the present study, Hammann et al. (5) were the only researchers to report erythromycin resistance to *Mobiluncus* spp. (17 of 17 strains).

The activity of tetracycline against *Mobiluncus* spp. appears to be heterogeneous. Results may vary with the method used since the same two strains of *M. mulieris* tested by Carlone et al. (2), which had MICS of 16 and 32 μg/ml, had MICs in the range ≤0.2 to 6.25 μg/ml in the present study.

Results of testing with the remaining agents was relatively consistent. We found higher MICs of chloramphenicol than had previously been reported. The resistance of these organisms to colistin and nalidixic acid has already been noted (4), and selective media containing these two antimicrobial agents have been used for primary isolation of *Mobiluncus* species.

Even though the quality control value was high, for reasons discussed above, tobramycin was very active against *Mobiluncus* spp. Aminoglycoside activity was reported in all four published works in which they were tested (2, 5, 9, 12). As has been noted previously (9, 12), it is highly unusual to detect aminoglycoside susceptibility in an anaerobic organism. The significance of this observation is unknown.

Whereas we found that MICs of ampicillin, cefoxitin, cephalothin, and moxalactam could apparently differentiate between *M. curtisii* and *M. mulieris*, Sprott et al. (12) found that erythromycin, metronidazole, and hydroxymetronidazole could serve that function.

All studies that differentiated *M. curtisii* (short curved rods) from *M. mulieris* (long curved rods) found that most or all strains of the former were resistant to metronidazole (2, 4, 9, 10, 12). There is no such consensus regarding *M. mulieris*. The British and European literature reports them to be susceptible (4, 9, 12), whereas we and others (S. L. Hillier and F. D. Schoenknecht, Abstr. Int. Conj. S. T. D. Meet. 1984, 86, p. 134) found some susceptible and some resistant strains. Skarin et al. (9) found that metronidazole was more active against *Mobiluncus* spp. than was tinidazole and that both drugs showed mutually exclusive clusters of MICs for the two species. In our hands, the range of MICs of the two drugs was almost the same when all members of the genus were considered, but *M. mulieris* had MICs from 2 to >512 μg/ml for metronidazole and 4 to 8 μg/ml for tinidazole. The relative resistance of *Mobiluncus* spp. to metronidazole and tinidazole may be advantageous in the development of a selective medium for their isolation from vaginal fluid.

The activity of the hydroxy metabolite of metronidazole was determined because of a previous report that it is more active against *G. vaginalis* than is the parent compound (8). The greater activity of the metabolite against *M. mulieris* reported here may help explain why some patients with metronidazole-resistant strains respond to therapy with this agent. Sprott et al. (12), however, did not find increased activity of hydroxymetronidazole against their strains.

Although *Mobiluncus* strains are susceptible to beta-lactam antibiotics, the presence of beta-lactamase-producing organisms in the vagina precludes their effective use in treating BV. Based on our data, oral or topical forms of clindamycin and imipenem may represent potential alternate modes of therapy when metronidazole fails. Both of these

antimicrobial agents have broad anaerobic coverage, and neither would be susceptible to the beta-lactamases of vaginal *Bacteroides* sp. Both, however, are active against vaginal lactobacilli and so may inhibit their regrowth during therapy. There is evidence that this contributes to treatment failures seen after treatment with ampicillin or amoxicillin (1). Resistance to these alternate agents may also develop. Our single strain, which had a clindamycin MIC of 4 μg/ml, was isolated from a woman with BV that had been refractory to therapy with metronidazole and clindamycin. Hers was the strain resistant to erythromycin and josamycin, and it had the highest metronidazole MIC. Hammann et al. (5) reported clindamycin MICs as high as 8 μg/ml, which is considered resistant.

Caution should be exercised in interpreting these data because susceptibility was determind based on achievable levels in serum. The levels in vaginal fluid may be lower, or drugs which do achieve adequate levels may not be active in that environment (3).

Many of the discrepancies between our data and those of others may be method dependent. Because there are no universally accepted methods for susceptibility testing, interpretation of results from laboratories in distant geographic locations can be difficult if not impossible. Modifications in media and incubation conditions which are made to support the growth of fastidious organisms may affect the results as seen in this study. Controls must be included to monitor the effects of these changes on the results.

The MICs of the stains, dyes, and sodium fluoride pointed out several compounds which might be useful in differentiating *M. curtisii* from *M. mulieris*, i.e., alizarin red, Evans blue, and sodium fluoride. Because sodium fluoride is a poison and cannot be placed in glass, its use will be limited. However, the use of alizarin red or Evans blue to differentiate the two species may be much faster than the currently recommended procedures (10). This awaits further study.

Of all the antimicrobial agents (6) and other compounds tested, only Nile blue A has shown greater activity against *G. vaginalis* than *Mobiluncus* spp. The relative resistance of *Mobiluncus* spp. to metronidazole and tinidazole and the selective inhibition of *G. vaginalis* by low concentrations of Nile blue A will be used to pursue development of a selective agar for *Mobiluncus* species.

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LITERATURE CITED

1. **Amsel, R., C. W. Critchlow, C. A. Spiegel, K. C. S. Chen, D. Eschenbach, K. Smith, and K. K. Holmes.** 1982. Comparison of metronidazole, ampicillin, and amoxicillin for treatment of bacterial vaginosis (nonspecific vaginitis): possible explanation for the greater efficacy of metronidazole, p. 225–237. *In* S. M. Finegold (ed.), U.S. Metronidazole Conference, Proceedings from a Symposium. Biomedical Information Corp., New York.
2. **Carlone, G. M., M. L. Thomas, R. J. Arko, G. O. Guerrant, C. W. Moss, J. M. Swenson, and S. A. Morse.** 1986. Cell wall characteristics of *Mobiluncus* species. Int. J. Syst. Bacteriol. **36**:288–296.
3. **Durfee, M. A., P. S. Forsyth, J. A. Hale, and K. K. Holmes.** 1979. Ineffectiveness of erythromycin for treatment of *Haemophilus vaginalis*-associated vaginitis: possible relationship to acidity of vaginal secretions. Antimicrob. Agents Chemother. **16**:635–637.
4. **Durieux, R., and A. Dublanchet.** 1980. Les "*Vibrions*" anaérobies des leucorrhées. I. Technique d'isolement et sensibilité aux antibiotiques. Med. Mal. Infect. **10**:109–115.
5. **Hammann, R., A. Kronibus, A. Viebahn, and H. Brandis.** 1984. *Falcivibrio grandis* gen. nov. sp. nov. and *Falcivibrio vaginalis* gen. nov. sp. nov., a new genus and species to accommodate anaerobic motile curved rods formerly described as "*Vibrio mulieris*" (Prevot 1940) Breed et al. 1948. Syst. Appl. Microbiol. **5**:81–96.
6. **McCarthy, L. R., P. A. Mickelsen, and E. G. Smith.** 1979. Antibiotic susceptibility of *Haemophilus vaginalis* (*Corynebacterium vaginale*) to 21 antibiotics. Antimicrob. Agents Chemother. **16**:186–189.
7. **Piot, P., E. van Dyck, P. A. Totten, and K. K. Holmes.** 1982. Identification of *Gardnerella* (*Haemophilus*) *vaginalis*. J. Clin. Microbiol. **15**:19–24.
8. **Ralph, E. D., and Y. E. Amatnieks.** 1980. Relative susceptibilities of *Gardnerella vaginalis* (*Haemophilus vaginalis*), *Neisseria gonorrhoeae*, and *Bacteroides fragilis* to metronidazole and its two major metabolites. Sex. Transm. Dis. **7**:157–160.
9. **Skarin, A., E. Holst, and P.-A. Mårdh.** 1983. Antimicrobial susceptibility of comma-shaped bacteria isolated from the vagina. Scand. J. Infect. Dis. Suppl. **40**:81–84.
10. **Spiegel, C. A., D. A. Eschenbach, R. Amsel, and K. K. Holmes.** 1983. Curved anaerobic bacteria in bacterial (nonspecific) vaginosis and their response to antimicrobial therapy. J. Infect. Dis. **148**:817–822.
11. **Spiegel, C. A., and M. Roberts.** 1984. *Mobiluncus* gen. nov., *Mobiluncus curtisii* subspecies *curtisii* sp. nov., *Mobiluncus curtisii* subspecies *holmesii* subsp. nov., and *Mobiluncus mulieris* sp. nov., curved rods from the human vagina. Int. J. System. Bacteriol. **34**:177–184.
12. **Sprott, M. S., H. R. Ingham, R. S. Pattman, R. L. Eisenstadt, G. R. Short, H. K. Narang, P. R. Sisson, and J. B. Selkon.** 1983. Characteristics of motile curved rods in vaginal secretions. J. Med. Microbiol. **16**:175–182.
13. **Thomason, J. L., P. C. Schreckenberger, W. N. Spellacy, L. J. Riff, and L. J. LeBeau.** 1984. Clinical and microbiological characterization of patients with nonspecific vaginosis associated with motile, curved anaerobic rods. J. Infect. Dis. **149**:801–808.
14. **Totten, P. A., R. Amsel, J. Hale, P. Piot, and K. K. Holmes.** 1982. Selective differential human blood bilayer media for isolation of *Gardnerella* (*Haemophilus*) *vaginalis*. J. Clin. Microbiol. **15**:141–147.