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Comparison of Isolation of *Haemophilus vaginalis* (*Corynebacterium vaginale*) from Peptone-Starch-Dextrose Agar and Columbia Colistin-Nalidixic Acid Agar

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A total of 447 cervical or vaginal specimens were inoculated in parallel onto peptone-starch-dextrose (PSD) and Columbia colistin (10 mg/ml)-nalidixic acid (15 μg/ml) (CNA) agar and were incubated for 48 h at 35°C in an atmosphere with 2 to 10% CO_2. One hundred (22.4%) of the cultures were positive for *Haemophilus vaginalis*. Forty-eight of the isolates were recovered from both PSD and Columbia CNA agar, five from PSD only, and 47 from Columbia CNA agar only ($P < 0.001$). On Columbia CNA agar, 76 of the isolates were detected after 24 h of incubation, and the remainder were detected within 4 days of incubation.

Since its original description by Gardner and Dukes in 1955 (10) as an etiological agent in nonspecific vaginitis, both the taxonomic position and the clinical significance of *Haemophilus vaginalis* have been questioned. Zinnemann and Turner (20) suggested in 1963 that the species belongs to *Corynebacterium*; however, ultrastructural and biochemical studies by Criswell et al. (3) have demonstrated characteristics more commonly associated with those of gram-negative rather than of gram-positive organisms. While most workers agree that the organism does not belong in the genus *Haemophilus*, it remains listed as *H. vaginalis* (species *incertae sedis*) in the latest edition of *Bergey's Manual* until such a time as its taxonomic position is settled (12).

In their original article (10) and in a later one with their co-workers (2), Gardner and Dukes have presented a considerable amount of data regarding this organism's pathogenicity, including its ability to infect normal volunteers. Lewis et al. (13, 14) in a study of 1,008 pregnant and nonpregnant women found that the incidence of cultures positive for *H. vaginalis* was significantly greater ($P < 0.001$) in patients with vaginitis than in those without vaginitis. In their entire study population, 88.6% of patients with vaginitis and 10.4% of patients without vaginitis had positive cultures. Studies by Frampton and Lee (9) and by Mendel and Haberman (15), however, have resulted in substantially higher rates of isolation of *H. vaginalis* from vaginal cultures of asymptomatic women and have failed to demonstrate a statistically significant difference between controls and patients with vulvovaginitis. In a study of the vaginal flora in 100 asymptomatic women by Tashjian et al. (18) at the Mayo Clinic, *H. vaginalis* was isolated from 21. Recently, McCormack et al. (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 229, 1975) reported that *H. vaginalis* was more prevalent in women with documented vaginal discharge and that its presence in vaginal cultures was also related to sexual experience.

Reported differences in recovery rates of *H. vaginalis* from vaginal cultures and in its prevalence in the presence or absence of vulvovaginitis may be related to such variables as the populations studied, criteria used for the diagnosis of vulvovaginitis, specimen collection and transport, the media used for cultures, and the methods and criteria used for identification of the organism. The present study was prompted by our observation that organisms resembling *H. vaginalis* were more frequently isolated from Columbia CNA agar than from chocolate blood agar or from soybean-casein digest agar with 5% sheep blood.

MATERIALS AND METHODS

Swabs (Culturette, Marion Scientific Corp., Rockford, Ill.) of material from the cervix and vagina submitted for bacteriological culture were inoculated in parallel onto soybean-casein digest agar (tryptic soy, Difco Laboratories, Detroit, Mich.) containing 5% sheep blood, Columbia CNA (colistin, 10 μg/ml; nalidixic acid, 15 μg/ml) agar (BioQuest, Cockeysville, Md.), and proteose peptone-starch-dextrose agar (PSD). Plates were incubated at 35°C in an atmosphere with 2 to 10% CO_2. PSD agar

plates were prepared and were examined with a dissecting microscope after 48 h of incubation, as recommended by Dunkelberg et al. (6). The other plates were examined macroscopically both after 24 and 48 h of incubation.

Colonies suspected of representing *H. vaginalis* on the basis of characteristic Gram stain morphology and negative catalase reaction were identified as follows. After their isolation on CNA, several colonies were inoculated into carbohydrate fermentation media (19) with Andrade indicator, supplemented with 10% sterile horse serum and containing dextrose, maltose, levulose, and sucrose. These tests were incubated at 35°C in room air for a maximum of 7 days. Other tests included those for nitrate reduction, indole production, and oxidase (19).

Reference strains A2508 and A7057 of *H. vaginalis* were obtained from R. E. Weaver, Center for Disease Control, Atlanta, Ga., and were used for purposes of quality control of the media and their reactions.

Additional studies with *H. vaginalis* CDC-A2508 were carried out to determine relative growth rates on 5% sheep blood in Columbia agar base, on 5% sheep blood in soybean-casein digest agar base, and on CNA (BioQuest, Cockeysville, Md.). A broth suspension of the organism was prepared so as to contain 10^4 colony-forming units/ml; 0.1/ml was then spread with a sterile glass rod over the agar surface. Plates were incubated for 48 h at 35°C in 2 to 10% CO_2 and then examined macroscopically.

RESULTS

All catalase-negative and oxidase-negative, gram-negative bacilli which exhibited colonial and microscopic morphology consistent with that of *H. vaginalis* and which fermented dextrose (100%), maltose (100%), and levulose (92%) but not sucrose (3%) were considered to be *H. vaginalis*, according to criteria published by King (11). Other characteristics included their failure to reduce nitrates or to produce indole. Early in the study several isolates resembling *H. vaginalis* were submitted to the Center for Disease Control, Atlanta, Ga., and their identity was confirmed by R. E. Weaver. All subsequent strains' reactions were identical to those of both the confirmed and the reference strains.

Of 447 cultures of cervical and vaginal material, *H. vaginalis* was recovered from 100. Forty-seven isolates were recovered from CNA only, 5 from PSD only, and 48 from both CNA and PSD. The greater recovery of *H. vaginalis* on CNA than on PSD was statistically significant ($P < 0.001$). Colonies were initially detected on CNA after 1 day of incubation in 76 instances, and the remainder were detected within 4 days of incubation. Colonies were indistinct and often overgrown by other bacteria on sheep blood agar.

Examination of the agar plates inoculated with the suspension of *H. vaginalis* CDC-A2508 demonstrated colonies on 5% sheep blood in Columbia agar base and on CNA which were at least twice the diameter of those on 5% sheep blood in soybean-casein digest agar base.

DISCUSSION

A variety of media have been utilized for the cultivation of *H. vaginalis* including proteose no. 3 agar (Difco) containing 10% sheep blood (10), Casman agar with rabbit blood (1), blood agar with 1% maltose (7), peptone-maltose-dextrose agar (4), dextrose-starch agar (16), proteose PSD agar (6), and starch-dextrose agar with purple broth base (17). Whereas proteose peptone no. 3 (Difco) has been frequently used in media for the isolation of *H. vaginalis*, the other components included in such media have varied. Moreover, there have been few studies comparing isolation rates of the organisms in different media. Although Smith recovered more strains of *H. vaginalis* on chocolate blood agar with GC medium base (Difco) and on 10% sheep blood agar (tryptose base) than on PSD and more strains on starch agar than on chocolate blood agar or 10% sheep blood agar, he never compared isolation rates on PSD and starch agar concurrently (17). Nonetheless, it would appear from his data that *H. vaginalis* was more readily and frequently isolated from starch agar than from PSD. Smith speculated that the absence of dextrose in starch agar may have reduced overgrowth of *H. vaginalis* by other bacteria and, therefore, facilitated the recognition of this organism (17).

In our study CNA yielded more positive cultures with *H. vaginalis* than did PSD. The reasons for our findings are not completely evident; however, the composition of the Columbia agar base (8) used in Columbia CNA agar generally resembles that of Casman agar (1) and appears to contain all of the growth requirements for *H. vaginalis* described by Dunkelberg and McVeigh (5). Moreover, Columbia CNA agar does not contain dextrose but does contain antimicrobial agents to which *H. vaginalis* is resistant (16) but which do suppress overgrowth of other bacteria, the presence of which on noninhibitory media interferes with the recognition of colonies of *H. vaginalis*.

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