Semiquantitative Culture of *Gardnerella vaginalis* in Laboratory Determination of Nonspecific Vaginitis

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To evaluate the usefulness of quantitative cultures of *Gardnerella vaginalis* in the laboratory determination of nonspecific vaginitis, the actual and relative numbers of *G. vaginalis* in genital cultures of a general patient population were assessed semiquantitatively, and the laboratory results were then correlated with the clinical findings. Of the 1,585 women studied, 417 (26.3%) yielded *G. vaginalis* in culture. Of these, only 113 (27.1%) were found to have symptoms and signs consistent with nonspecific vaginitis. *G. vaginalis* was obtained in pure or predominant growth from 87 of 100 consecutive cases with nonspecific vaginitis and 32 of 100 consecutive cases without the symptoms or signs of vaginitis (*P* < 0.001). Hence, the positive predictive value of isolation of *G. vaginalis* in pure and predominant growths was determined to be 73% (87 of 119). Conversely, *G. vaginalis* was isolated in mixed or light growth significantly more often from asymptomatic women than from symptomatic patients, i.e., 68 versus 13 cases. Therefore, the negative predictive value of isolation of *G. vaginalis* in mixed and light growths was found to be 84% (68 of 81). Quantitation of the relative amount of *G. vaginalis* growth had higher predictive values as compared with the assessment of *G. vaginalis* growth alone. We conclude that quantitative culture of *G. vaginalis* is essential to obtain maximum reliability of culture results in the laboratory determination of nonspecific vaginitis. Although quantitated cultures of *G. vaginalis* have high predictive values, laboratory results must be interpreted in conjunction with the clinical findings.

*Gardnerella vaginalis* has been implicated as the etiological agent of nonspecific vaginitis (NSV) in numerous studies (1, 11, 13, 15, 19, 20) following the initial report by Gardner and Dukes (6). However, the significance of the isolation of this organism from the lower genital tract has been controversial because asymptomatic women are frequently found to have positive cultures for *G. vaginalis* (5, 12, 14). In asymptomatic women *G. vaginalis* occurs only in small numbers, whereas in symptomatic women *G. vaginalis* is the predominant organism recovered (9, 15, 18, 19). Therefore, to determine the clinical significance of *G. vaginalis*, semiquantitative culture of this organism in genital specimens has been suggested analogous to urine culture in the diagnosis of bacteriuria (9). A survey of the literature indicates that several studies incriminating *G. vaginalis*, or disputing its role in vaginitis, failed to include quantitative culture and to consider its significance in the laboratory determination of NSV (1, 2, 4, 13, 14). These factors have contributed to the contradictory conclusions reached in earlier studies. This study was undertaken to determine the predictive value of quantitative cultures of *G. vaginalis* in the laboratory determination of NSV. The actual and relative numbers of *G. vaginalis* in routinely processed genital specimens of women with and without symptoms of NSV were assessed semiquantitatively, and the laboratory results were correlated with the clinical findings.

MATERIALS AND METHODS

Study population. The study population consisted of women seen in general practice, gynecological outpatient clinics, family planning clinics, and venereal disease clinics. Their age range was 14 to 79 years, with a mean age of 28 years, and most were sexually active. Their medical history was taken, and they were clinically examined for symptoms and signs of NSV. NSV was defined as a condition characterized by persistent vaginal discharge that was often malodorous with or without pruritus, which was not attributable to uterine infection, trichomoniasis, candidiasis, or gonorrhea. Women with candida, trichomonas, or gonococcal infection and those who had received antibiotic treatment within the previous month were excluded from the study population.

Of the 1,585 patients qualified as test subjects, 417 (26.3%) yielded *G. vaginalis* in culture. Of these 417
women harboring *G. vaginalis*, 113 (27.1%) were considered to have NSV. Presence of vaginal discharge was confirmed in all 113 cases; offensive odor was noted in 91 of them, pruritus in 52, and dysuria in 21. These 113 were considered to be the *G. vaginalis*-positive asymptomatic study population. The remaining 304 (72.9%) women who harbored *G. vaginalis* but showed no symptoms or signs of vaginitis were designated as the *G. vaginalis*-positive asymptomatic study population. One hundred consecutive cases from each of the culture-positive symptomatic and asymptomatic populations were chosen to assess correlation between the semi-quantitated growth of *G. vaginalis* and the clinical manifestations of vaginitis.

In addition, a study population consisting of 83 asymptomatic student nurse volunteers was also included in the survey. Their age range was 16 to 30 years, with a mean age of 21 years.

**Isolation and identification of *G. vaginalis***. Vaginal fluid was obtained with swabs and submitted to the laboratory in Amies charcoal transport medium (Difco Laboratories). V agar (9) was used as the primary isolation medium for *G. vaginalis*. This was prepared with 5% whole human blood but without the addition of Proteose Peptone no. 3 (Difco). One quadrant of the plate was inoculated with the swab, and, using a loop, the inoculum was cross-streaked from the primary to secondary and tertiary streak areas for isolation of colonies. The inoculated plates were incubated for up to 48 h at 37°C in 5 to 8% CO₂ and were examined for colonies showing diffuse beta-hemolysis. These colonies were tested for the following characteristics of *G. vaginalis*: Gram stain morphology showing gram-negative to gram-variable coryneform coccobacillary to bacillary forms, absence of hemolysis on sheep blood agar, negative catalase and oxidase reactions, and a positive hippurate hydrolysis reaction. When all of the above criteria were met, the isolate was identified as *G. vaginalis*. When one or more of the above tests were not in agreement, additional confirmatory tests comprising fermentation of dextrin, lactose, maltose, salicin, and starch and sensitivity to bacitracin were carried out. All of the above tests were performed according to the methods described elsewhere (8).

**Semi-quantitative culture of *G. vaginalis***. Two methods were used to quantitate the growth of *G. vaginalis* on the primary isolation medium.

(i) **Method I**. By method I, the amount of *G. vaginalis* growth was assessed semiquantitatively in terms of its ratio to the total aerobic genital flora. For this, an objective grading system comprising four categories, pure, predominant, mixed, and light growth, was used. Pure growth was defined as the growth of *G. vaginalis* composed of a minimum of 50 colonies with other bacterial species constituting no more than 10% of the total number of colonies. When colonies of *G. vaginalis* clearly outnumbered those of other organisms representing normal genital flora, the predominant growth category was assigned. When colonies of *G. vaginalis* were grown in approximately equal numbers as those of the normal genital flora, the growth was designated as mixed. Light growth was defined as growth of *G. vaginalis* comprised of colonies considerably lesser in number than those of the genital flora.

(ii) **Method II**. By method II, the actual numbers of *G. vaginalis* rather than the ratio of its growth to the genital flora were taken into account. This quantitation scheme has been used by others (21) and is as follows: 1+, <10 colonies in the primary streak area; 2+, >10 colonies in the primary and <10 colonies in the secondary streak areas; 3+, >10 colonies in the secondary and <10 colonies in the tertiary streak areas; 4+, >10 colonies in the tertiary streak area.

**RESULTS**

When growth of *G. vaginalis* was quantitated by method I, of the 200 culture-positive symptomatic and asymptomatic cases chosen, 18 (9%) had pure; 101 (50.5%), predominant; 66 (33%), mixed; and 15 (7.5%), light growths of *G. vaginalis* (Table 1). A greater number of women with NSV yielded *G. vaginalis* in pure or predominant growth compared with those without symptoms or signs of NSV (87 versus 32; *P* < 0.001). On the other hand, *G. vaginalis* was isolated in mixed or light growth from a larger number of asymptomatic women than from those with NSV (68 versus 13). In patients with NSV, correlation of clinical symptoms to culture results was found to be closely related to the amount of *G. vaginalis* isolated. Isolation of *G. vaginalis* in pure culture correlated with the disease in 88.9%, predominant growth in 70.3%, mixed growth in 16.7%, and light growth in 13.3%. A reverse order was observed in asymptomatic women.

There was less correlation between the clinical symptoms and the semiquantitated culture of *G. vaginalis* when the growth was assessed by method II (Tables 1 and 2).

**Occurrence of *G. vaginalis*** in 1,472 asymptomatic women representing the general patient population who were attending clinics for various reasons was compared with a group of 83 asymptomatic student nurse volunteers (Table 3). The rate of vaginal colonization with *G. vaginalis* was found to be approximately 21% in both groups, but minor differences occurred in

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**TABLE 1.** Semi-quantitated culture of *G. vaginalis* in asymptomatic and symptomatic women by method I

<table>
<thead>
<tr>
<th>Patients</th>
<th>No. of cases</th>
<th>Quantity (%) of <em>G. vaginalis</em> growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>100</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>100</td>
<td>16 (88.9)</td>
</tr>
</tbody>
</table>

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TABLE 2. Semiquantitated culture of *G. vaginalis* in asymptomatic and symptomatic women by method II

<table>
<thead>
<tr>
<th>Patients</th>
<th>No. of cases</th>
<th>4+ (n = 107)</th>
<th>3+ (n = 57)</th>
<th>2+ (n = 29)</th>
<th>1+ (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>100</td>
<td>40 (37.4)</td>
<td>33 (57.9)</td>
<td>20 (69.0)</td>
<td>7 (100.0)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>100</td>
<td>67 (62.6)</td>
<td>24 (42.1)</td>
<td>9 (31.0)</td>
<td>0</td>
</tr>
</tbody>
</table>

Semiquantitative categorization of *G. vaginalis* growth between these groups. Nevertheless, the majority (67 to 76%) of asymptomatic women colonized with *G. vaginalis* yielded this organism as a mixed or light growth. In the remaining cases (24 to 33%), it was isolated in pure or predominant growth.

**DISCUSSION**

Our study showed that *G. vaginalis* may occur in as many as 21% of women who have no symptoms or signs of vaginitis. However, in most of these cases, *G. vaginalis* was found in relatively small numbers. In contrast, in the majority of women with NSV, *G. vaginalis* constituted a greater percentage of the total aerobic vaginal flora. These findings are similar to those of others (7, 9, 21) and indicate a close correlation between relative numbers of *G. vaginalis* present in the vaginal canal and the clinical manifestations of NSV. Our data establish that quantitative culture of *G. vaginalis* significantly contributes to the interpretation of laboratory findings in the diagnosis of *G. vaginalis*-associated vaginitis.

When growth of *G. vaginalis* was quantitated in relation to the genital flora, the positive predictive value of pure and predominant growths was 73% (87 of 119 cases), and the negative predictive value of mixed and light growth was 84% (68 of 81 cases; Table 1). In comparison, when the actual numbers rather than the relative numbers of *G. vaginalis* were assessed, the positive predictive value of 3+ and 4+ growths was 55% (91 of 164 cases), and the negative predictive value of 1+ and 2+ growths was 75% (27 of 36 cases; Table 2). Thus, the determination of the relative numbers of *G. vaginalis* in cultures of genital specimens provides results with higher predictive values than the assessment of *G. vaginalis* growth alone for the diagnosis of NSV. Therefore, we feel that growth of *G. vaginalis* should be assessed in relation to the total aerobic genital flora and that pure growth of *G. vaginalis*, or its clear predominance, be heavily weighed as clinically significant. However, it should be remembered that up to one-third of cases yielding *G. vaginalis* in such proportions are likely to be asymptomatic (Table 3). Conversely, isolation of *G. vaginalis* in mixed or light growth may not exonerate this organism in a small percentage of cases with *G. vaginalis*-associated vaginitis. It is necessary, therefore, to interpret quantitative cultural findings in conjunction with the clinical picture when determining the clinical significance of *G. vaginalis* isolation.

Media to permit the selective growth of *G. vaginalis* have been developed to isolate this organism from genital specimens (10, 16, 21). Such media, designed to yield a higher isolation rate of *G. vaginalis*, fail to indicate the relative numbers of this organism in mixed genital flora. Use of nonselective media, on the other hand, permits relative quantitation of *G. vaginalis* growth. With a nonselective medium, we observed a higher degree of correlation between clinical symptoms and specimens yielding a pure growth of *G. vaginalis* than specimens yielding predominant, mixed, or light growths.

It appears that *G. vaginalis* can occur as part of the normal vaginal microflora and is a low-grade opportunistic pathogen producing symptoms when present in increased numbers, and only under certain conditions. Local environmental factors may play a part in determining the outcome of vaginal colonization with *G. vaginalis* (7). Recently, several studies have indicated that NSV is a synergistic infection requiring participation of other organisms, particularly anaerobes, in addition to *G. vaginalis* (3, 4, 15, 17, 19). This may help to explain why a

TABLE 3. Occurrence of *G. vaginalis* in asymptomatic general patient population and a group of student nurse volunteers

<table>
<thead>
<tr>
<th>Population</th>
<th>No. tested</th>
<th>No. (% yielding <em>G. vaginalis</em>)</th>
<th>Distribution (% of <em>G. vaginalis</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pure</td>
</tr>
<tr>
<td>Patient</td>
<td>1,472</td>
<td>304 (20.7)</td>
<td>7 (2.3)</td>
</tr>
<tr>
<td>Volunteer</td>
<td>83</td>
<td>17 (20.5)</td>
<td>0</td>
</tr>
</tbody>
</table>
large number of women who are colonized with \textit{G. vaginalis} remain symptomless. They may lack the anaerobic synergent and thus remain free of the symptoms. As is recognized that a large number of women who are colonized with \textit{G. vaginalis} do not have vaginitis, it has also been known that certain percentage of patients with symptoms and signs consistent with NSV do not yield \textit{G. vaginalis} in culture (10, 13, 19, 20). As studies continue to elucidate the etiology and pathogenesis of NSV which now appears to be a polymicrobial infection, \textit{G. vaginalis} may best serve as an indicator organism for the laboratory determination of NSV in the majority of women with this syndrome. Semiquantitation of its relative numbers in the microflora of vaginal cultures will considerably enhance the predictive value of the laboratory report.

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LITERATURE CITED