Detection of Trichomonosis in Vaginal and Urine Specimens from Women by Culture and PCR

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Vaginal trichomonosis is a highly prevalent infection which has been associated with human immunodeficiency virus acquisition and preterm birth. Culture is the current “gold standard” for diagnosis. As urine-based testing using DNA amplification techniques becomes more widely used for other sexually transmitted diseases (STDs) such as gonorrhea and chlamydia, a similar technique for trichomonosis would be highly desirable. Women attending an STD clinic for a new complaint were screened for trichomonads had microscopic and/or culture evidence of the organism. Two women were positive for trichomonads by wet prep or culture only in the urine. The sensitivity and specificity of PCR using urine specimens were 64 and 100%, respectively. These results indicate that the exclusive use of urine-based detection of T. vaginalis is not appropriate in women. PCR-based detection of T. vaginalis using vaginal specimens may provide an alternative to culture.

Although bacterial sexually transmitted diseases such as syphilis, gonorrhea, and chlamydia are declining in the United States, the rate of infections caused by Trichomonas vaginalis remains constant. Vaginal trichomonosis has been linked to preterm birth and acquisition of human immunodeficiency virus (5, 20); however, increased screening efforts have not materialized. Despite its limited sensitivity (19), direct microscopic examination of the vaginal fluid remains the most widely utilized diagnostic test for this infection. Culture of the organism using vaginal specimens is the current “gold standard” (4); however, PCR techniques are currently being designed. As urine-based testing using DNA amplification techniques becomes more widely used for gonorrhea and chlamydia (22), a similar technique for trichomonosis would be highly desirable.

In order to evaluate the possible use of urine for the diagnosis of trichomonosis in women, we tested urine and vaginal fluids for the presence of T. vaginalis using direct microscopy, culture, and PCR and compared the relative sensitivities of these methods.

MATERIALS AND METHODS

Women attending the Jefferson County Department of Health sexually transmitted disease clinic for either screening or a new complaint were eligible for entry into the study. The study was approved by the Institutional Review Boards of the University of Alabama at Birmingham and the Jefferson County Department of Health. During the routine pelvic examination, additional swab specimens for trichomonads had microscopic and/or culture evidence of the organism. The sensitivity and specificity of PCR using urine specimens were 64 and 100%, respectively. These results indicate that the exclusive use of urine-based detection of T. vaginalis is not appropriate in women. PCR-based detection of T. vaginalis using vaginal specimens may provide an alternative to culture.
PCR was confirmed by sequence analysis of the 300-bp PCR product from random samples as previously described (34). Inhibition assays were performed on discrepant samples. The reaction mix used was as previously described with the exception that DNA from a clinical isolate of T. vaginalis (8 ng of DNA in a 50-μl volume) was added with 10 μl of sample. Serial dilutions were made of a live culture of a clinical isolate of Trichomonas and tested to detect the lower limit of sensitivity for the PCR assay. Dilutions were made in deionized water, and then DNA was extracted and processed as described above.

Statistical methods. Statistical comparisons were made using the EpiInfo software program, version 6 (A. Dean, J. Dean, and D. Columbeer, Centers for Disease Control and Prevention, Atlanta, Ga.). Fisher’s exact test was used to compare categorical variables. Ninety-five percent confidence intervals were calculated to evaluate statistically significant differences between collection methods (6). Trichomoniasis was defined as the detection of motile trichomonads by either direct microscopy or culture in either vaginal or urine specimens. This was the comparator for all other methods.

RESULTS

Samples were obtained from 190 women. The overall prevalence of trichomoniasis was 28% (53 of 190). The sensitivities and specificities of direct microscopy, culture, and PCR of both urine and vaginal swab samples compared to the previously defined gold standard are shown in Table 1. The most sensitive method for the detection of T. vaginalis was culture of the vaginal fluid, with a sensitivity of 94.3%. Direct microscopy of either urine or vaginal fluid was the least sensitive, at only 58.5%.

PCR-based detection of T. vaginalis from vaginal swabs was equivalent to culture with a sensitivity and specificity of 88.7 and 97.1%, respectively. Four vaginal swab samples were positive by PCR but negative by wet prep and culture. DNA sequence analysis of the PCR products from all four women was performed. A BLAST search of the National Institutes of Health GenBank confirmed that the 300-bp PCR product corresponded to T. vaginalis DNA. Sufficient DNA was available for only one of these four specimens to be tested with alternative T. vaginalis-specific PCR primers (TVA5 and -6) (27), and this was also positive for T. vaginalis DNA. The lower limit of T. vaginalis detection by the PCR assay was found to be one organism (Fig. 1). Motile trichomonads were detected in the urine by direct microscopy or culture from 74.5% (38 of 51) of women with vaginal trichomoniasis detected by similar means. Two women were positive for trichomonads in the urine but not in vaginal specimens. The sensitivity and specificity of PCR for urine specimens were 64.2 and 100%, respectively, compared to the previously defined gold standard (Table 1). The sensitivity of PCR for urine specimens from only those women with motile trichomonads detected in the urine by wet mount or culture was still only 68.4% (26 of 38).

Inhibition assays were performed for 32 urine samples from women with either a culture or a vaginal PCR sample positive for T. vaginalis. Inhibitors for PCR were present in 3 of 32 (9%) samples. PCR inhibitor was eliminated in these three specimens by use of a threefold increase of Taq, confirming that these samples were false negatives.

Seventy-five percent (40 of 53) of women with trichomonads complained of vaginal symptoms such as discharge, odor, and itching. Among women with trichomonads, the presence of symptoms was not associated with a positive vaginal fluid wet prep for T. vaginalis (24 of 40, 62.5%; versus 6 of 13, 46.2%; P = 0.47). Review of the medical records for the four women who had positive PCRs but negative culture and wet preps was unrevealing.

DISCUSSION

Infection with T. vaginalis remains highly prevalent worldwide despite the availability of an inexpensive, single-dose, curative antibiotic regimen (10). To date, little emphasis has been placed on the importance of decreasing rates of this infection even though it has been associated with human immunodeficiency virus acquisition and increased risk of preterm birth (5, 20). One strategy for increasing diagnosis and treatment of trichomoniasis is the use of a screening test with increased sensitivity compared to the traditional wet prep of vaginal fluid. Culture methods are currently the gold standard and should be considered for widespread clinical use (2, 4).

PCR techniques have proven superior to culture for other infections such as gonorrhea and chlamydia, and moreover urine has been found to be a suitable testing substrate for these techniques in men and women (7, 32). A similar approach would further facilitate screening for trichomonads. Although urine specimens are suitable for the culture of T. vaginalis in males (18), there are limited published data on rates of urethral colonization in women. In one study of the incidence of urinary tract trichomoniasis, 18 of 25 (72%) women with vaginal trichomoniasis had a positive urine culture for T. vaginalis obtained from a catheterized specimen (J. Finley, P. Breeden, W. Lushbaugh, and J. Cleary, Abstr. Int. Congr. Sex. Transm. Dis., abstr. 679, p. 175, 1997). Another reference states that urethral colonization occurs in up to 90% of women, but data

TABLE 1. Comparison of diagnostic tests for T. vaginalis in females

<table>
<thead>
<tr>
<th>Diagnostic method</th>
<th>No. true positive</th>
<th>No. false positive</th>
<th>No. false negative</th>
<th>No. true negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95% CI</td>
<td>95% CI</td>
<td>Positive</td>
</tr>
<tr>
<td>Vaginal swab culture</td>
<td>50</td>
<td>0</td>
<td>3</td>
<td>137</td>
<td>94.3</td>
<td>83.4–98.5</td>
<td>100</td>
</tr>
<tr>
<td>Vaginal PCR</td>
<td>47</td>
<td>4</td>
<td>6</td>
<td>133</td>
<td>88.7</td>
<td>76.3–95.3</td>
<td>100</td>
</tr>
<tr>
<td>Urine culture</td>
<td>32</td>
<td>0</td>
<td>22</td>
<td>137</td>
<td>58.5</td>
<td>44.2–71.6</td>
<td>100</td>
</tr>
<tr>
<td>Urine PCR</td>
<td>34</td>
<td>0</td>
<td>19</td>
<td>137</td>
<td>64.2</td>
<td>49.7–76.5</td>
<td>100</td>
</tr>
<tr>
<td>Urine wet prep</td>
<td>31</td>
<td>0</td>
<td>22</td>
<td>137</td>
<td>58.5</td>
<td>44.2–71.6</td>
<td>100</td>
</tr>
</tbody>
</table>

* n = 190. The gold standard is trichomonads visualized from any wet prep or culture (n = 53). CI, confidence interval.
are not presented (17). Among adolescent women, the sensitivity of detection of trichomonads by direct microscopy of centrifuged urine specimens was 64%, and use of this technique improved the level of detection achieved by using direct microscopic examination of vaginal fluid alone by 12% (3). Our data are comparable to those of the first study in that the percentage of women with trichomoniasis who had evidence of motile trichomonads in the urine was approximately 75%. This rate of urethral colonization and/or infection with T. vaginalis is comparable to that previously reported for gonorrhea and chlamydia (23, 32). For gonorrhea, detection of urethral colonization in women by culture was not found to be necessary for diagnosis, based on only six patients with wet-mount- or culture-proven gonorrhea (23, 32). For reasons discussed later, we compared PCR results from urine specimens to vaginal cultures and wet prep and found that the sensitivity of PCR for urine specimens was 100% (33). However, this comparison was based on only six patients with wet-mount- or culture-proven trichomoniasis.

Several groups of investigators have reported their findings on the development of a PCR technique for trichomonads. In 1992, Riley et al. published a report of primers (TVA5 and TVA6) for the detection of T. vaginalis. Subsequently, many additional primer sets have been described. The sensitivity and specificity of these primers in clinical studies using vaginal swab specimens have varied, with sensitivities of 85 to 100% being reported (Table 2). The sensitivity and specificity of our PCR method using vaginal swab specimens were comparable to those of other published studies. Unlike PCR for infections such as gonorrhea and chlamydia, which appears to have greater sensitivity than culture methods (7, 32), PCR for trichomonads does not appear to offer a diagnostic advantage. This may be due to the fact that T. vaginalis is much less fastidious for culture than is Neisseria gonorrhoeae or Chlamydia trachomatis. Successful culture of T. vaginalis requires only the multiplication of a single organism, the same as that needed for PCR. PCR of vaginal swabs may be advantageous in settings where incubation of cultures is not possible and shipping of specimens to a reference laboratory is required. Self-obtained vaginal swab specimens, which have been shown...

### Table 2. Summary of PCR primers for T. vaginalis and results of clinical trials using PCR in females

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Reference no.</th>
<th>Principal primers</th>
<th>Type of specimen</th>
<th>Sensitivity/specificity of PCR (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riley et al. (1992)</td>
<td>27</td>
<td>TR 7 and 8, TV A5 and 6, TR 5 and 6, FE 1 and 2</td>
<td>Laboratory isolates</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Pâces et al. (1992)</td>
<td>24</td>
<td>TV-E650-1c</td>
<td>Laboratory isolates</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kengne et al. (1994)</td>
<td>15</td>
<td>TVK 3, 4 and 7</td>
<td>Laboratory isolates</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Katiyar and Edlind (1994)</td>
<td>14</td>
<td>BTUB 9 and 2 (beta-tubulin genes)</td>
<td>Laboratory isolates</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Alderete et al. (1995)</td>
<td>1</td>
<td>AP651 and -2c</td>
<td>Laboratory isolates</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Jeremias et al. (1994)</td>
<td>13</td>
<td>TVA5 and -6</td>
<td>Vaginal swab</td>
<td>100/97.8</td>
<td>Prevalence, 11.5% (6 of 52); asymptomatic</td>
</tr>
<tr>
<td>Heine et al. (1997)</td>
<td>8</td>
<td>TVA5 and -6</td>
<td>Vaginal swab</td>
<td>91.8/95.2</td>
<td>Prevalence, 14.6% (44 of 300); symptomatic and asymptomatic</td>
</tr>
<tr>
<td>Shaio et al. (1997)</td>
<td>30</td>
<td>TV-E650-1</td>
<td>Vaginal swab</td>
<td>100/100</td>
<td>Prevalence, 8.9% (40 of 451); symptomatic and asymptomatic</td>
</tr>
<tr>
<td>Madico et al. (1998)</td>
<td>21</td>
<td>BTUB 9 and 2</td>
<td>Vaginal swab</td>
<td>96/98</td>
<td>Prevalence, 6.6% (23 of 350)</td>
</tr>
<tr>
<td>Paterson et al. (1998)</td>
<td>26</td>
<td>TVA5 and -6</td>
<td>Tampon specimen</td>
<td>92.7/92.1</td>
<td>Prevalence, 8.6% (51 of 590); delayed (up to 5 days) inoculation of culture medium may have affected results</td>
</tr>
<tr>
<td>van der Schee et al. (1999)</td>
<td>33</td>
<td>TVK3 and -7</td>
<td>Vaginal swab</td>
<td>95.8/98.1</td>
<td>Prevalence, 5.7% (48 of 846)</td>
</tr>
<tr>
<td>Ryu et al. (1999)</td>
<td>28</td>
<td>TV-E650-1</td>
<td>Vaginal swab</td>
<td>100/96.8</td>
<td>Prevalence, 2.4% (10 of 426); symptomatic and asymptomatic</td>
</tr>
</tbody>
</table>

*a* Positive culture and/or wet prep was used as the gold standard. NA, not available.

*b* Prevalence is based on wet prep and culture.

*c* Original cloning sequence.
to be appropriate specimens for PCR testing of gonorrhea and chlamydia (11, 12) as well as for culture of T. vaginalis (29), may also be useful for the PCR technique. In addition, PCR may be superior to culture for the diagnosis of T. vaginalis in males. Although Hobbs et al. in their study of T. vaginalis in males found the sensitivity of PCR using urethral swabs to be only 82%, the authors suggest that technical factors may have played a role (9). There are no published studies on the use of PCR for detection of trichomonads in male urine specimens.

Of note also are the many different primers which have been used for the detection of T. vaginalis by PCR (Table 2). Direct comparisons of these primers, and perhaps the development of new primers, could prove useful with regards to refining the technique and improving sensitivity.

Evaluation of the four false-positive specimens in our study suggested that these may represent true infection. All specimens were processed in a biological hood which would greatly limit the possibility of contamination. All PCR products were consistent with T. vaginalis sequences. Although only one specimen had sufficient quantity available to test with additional primers, the result was positive. However, even if these are regarded as true-positive results, the sensitivity of PCR did not exceed that of culture.

In summary, T. vaginalis was detected in the urine of 75% of women with trichomoniasis using standard methods. PCR of urine for T. vaginalis had a sensitivity less than that of microscopy and culture. PCR for T. vaginalis using vaginal swab specimens was equivalent to culture. PCR of vaginal swab specimens may be considered in settings where incubation of cultures is not feasible or in settings where self-collection techniques are utilized. Important areas for future investigations include further studies of rates of urethral colonization with T. vaginalis in females, enhancement of the sensitivity of PCR assays including comparison and refinement of T. vaginalis-specific primers, and suitability of PCR for detection of trichomonads in male urine specimens.

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REFERENCES