Tampon Sampling for Diagnosis of Bacterial Vaginosis: a Potentially Useful Way To Detect Genital Infections?

DAVID WILKINSON,1,2,3 NOMFANELO NDOVELA,4 AYESHA KHARSANY,4 CATHERINE CONNOLLY,1 AND A. WILLEM STURM4

Centre for Epidemiological Research in South Africa, Medical Research Council,1 and Hlabisa Hospital,2 Hlabisa, and Department of Medical Microbiology, University of Natal, Durban,3 South Africa, and Liverpool School of Tropical Medicine, Liverpool, United Kingdom4

Received 3 March 1997/Returned for modification 17 April 1997/Accepted 10 June 1997

Genital tract infections are important causes of ill health in developing countries, but diagnosis is difficult. Bacterial vaginosis (BV) was correctly diagnosed by using a vaginal specimen obtained by tampon sampling in 22 of 24 women (91.6%) for whom BV was diagnosed by Gram staining. The yield for other vaginal infections was higher (28% for Trichomonas vaginalis and 32.7% for Candida albicans) than it was for cervical infections (0% for Neisseria gonorrhoeae and 30% for Chlamydia trachomatis). Tampon sampling was acceptable to patients and may facilitate diagnosis of genital infections in developing countries.

Bacterial vaginosis (BV) is an important cause of premature labor and low birth weight (2–4), and its treatment has been shown to improve pregnancy outcome (6, 7). In developing countries, where prematurity and low birth weight are major causes of perinatal death and the prevalence of BV is high, diagnosis is difficult. Facilities for performing a speculum examination rarely exist in primary care clinics, and access to microscopy is limited. The aim of this study was to determine if BV can be diagnosed by microscopy of a vaginal specimen obtained by inserting a tampon.

A cross-sectional study to determine the prevalence of bacterial vaginosis as well as infection with Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, and Candida albicans among consecutive consenting women attending the family planning clinic of a rural district hospital in South Africa was done. Demographic and clinical data were collected for all consenting women, and a speculum and gynecological examination was conducted by a female doctor. A swab inserted high into the vagina was used to make a smear for Gram staining and was then placed in Amies transport medium for culture of T. vaginalis. A cervical swab was used to make a smear for detection of C. trachomatis by direct immunofluorescence (DIF) (Microtrak slide; Syva) and was placed in Amies transport medium for culture for N. gonorrhoeae. Immediately following the clinical examination, a tampon was inserted into the vagina for 1 h. On removal, it was placed into 5 ml of brain heart infusion broth (BHI; Difco, Detroit, Mich.) to prevent it from drying out. Specimens were transported at 4°C to the Department of Medical Microbiology, University of Natal, Durban, for further processing within 10 h of sampling.

On removal from the container, the tampon was inspected for the presence of discharge. If present, this was used to make smears for Gram staining and DIF. If no discharge was visible, the tampon tip was used. Then, another 5 ml of BHI was poured on the tampon and squeezed out by using forceps. This fluid was aspirated in a pipette and used for further processing. Specimens obtained by conventional sampling and tampon sampling were analyzed in the same way. BV was diagnosed by microscopy of the Gram-stained smear of the high-vaginal swab using Nugent's criteria, a semiquantitative scoring system for the presence of lactobacillus, anaerobic gram-negative rod, and curved-rod morphotypes (8). The presence of yeast cells or pseudohyphae on this Gram stain was considered diagnostic of candidiasis. Diamond's medium, incubated at 37°C for a maximum of 7 days, was used to grow T. vaginalis. Growth was observed by wet-mount microscopy done every day from day 2 on. The C. trachomatis DIF slide was prepared according to the manufacturer's instructions and viewed by means of fluorescence microscopy. A smear was considered positive if 3 or more typically fluorescing inclusion bodies were seen. Culture for N. gonorrhoeae was performed by using New York City medium (Oxoid) with incubation for 48 h at 37°C in a CO2 incubator. Positivity was confirmed by the ability of isolated colonies to ferment glucose and not maltose, and by their being oxidase positive.

All women were treated for identified infections, and their partners were referred for treatment. Proportions were compared by the Chi-square test by using EpiInfo 6 software. Ethical approval for the study was granted by the ethics committee of the University of Natal Medical School.

A total of 175 consecutive women were sampled by both techniques. As shown in Table 1, the sensitivity and specificity of tampon sampling for diagnosis of BV were high. All women with BV had abnormal vaginal discharge. The two barriers to diagnosing BV in resource-poor settings are obtaining the specimen and doing microscopy. If pregnant women were to insert a tampon prior to an antenatal clinic visit, an appropriate sample could be obtained. Although most women studied (152, 87%) do not use tampons when menstruating, most (86%) said they would be prepared to repeat the test. Most women (95%) in our setting receive antenatal care (9), and this typically occurs at remote primary care clinics. A blood sample taken at the antenatal care facility for estimating hemoglobin concentration and for syphilis serology is sent to the district hospital laboratory, and it would also be possible to send a tampon for microscopic analysis for diagnosis of BV.

Tampon sampling was less sensitive for detection of other organisms. The material with which tampons are made may have interfered with the viability of these fastidious organisms. This possibility is supported by the higher number of positive results for those conditions diagnosed by microscopy (BV, candidiasis, and chlamydial infection) than for those diagnosed...
by culture (gonococcal infection and trichomoniasis). The sensitivity of detection of microscopy-diagnosed infections from samples from tampons was 60.2%, compared with 21.2% for culture-diagnosed infections ($P < 0.0001$). The interval between sampling and processing has been shown to determine the yield of *N. gonorrhoea* when sampling is carried out in this way (5).

The higher level of sensitivity for diagnosing BV compared to yeast infection might reflect inherent differences in each disease: while BV typically presents as a sticky discharge affecting the whole vagina, candidiasis is characterized by a patchy distribution over the vaginal walls, often located mainly at the vaginal orifice and the vulva. Due to its sticky nature, the discharge of bacterial vaginosis may have covered most of the tampon, while yeasts may have been present on limited parts.

Rates of recovery of cervical pathogens from vaginal specimens are by nature lower than rates for endocervical specimens. Although the tampon comes into contact with the cervix, the amount of endocervical material absorbed by it depends on the amount produced. Also, it is not possible to be sure which part of the tampon touched the cervix. It is therefore not surprising that the overall yield of cervical pathogens from the tampon (28.6%) was significantly lower than the yield of vaginal pathogens (54.3%; $P = 0.02$).

Bowden et al. (1) have reported that PCR for *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis* with tampon specimens is highly sensitive and specific. However, such techniques do not allow for susceptibility testing and are, because of the absence of an identifiable causative agent, not suitable for diagnosis of BV: conventional microbiology is still needed.

The need to improve pregnancy outcome by reducing the incidence of prematurity and low birth weight in resource-poor settings is considerable. The low-birth-weight rate in Hlabisa is 8%. Of 134 babies admitted to the neonatal nursery over a 6-month period, 114 (85%) had a primary diagnosis of either prematurity or low birth weight. One-third of the babies born at gestational ages of <34 weeks died, as did 17% of those with birth weights of <2,500 g (unpublished data). Operational research is now needed to determine the feasibility and cost-effectiveness of tampon sampling for pregnant women to prevent the complications associated with bacterial vaginosis.

In conclusion, tampon sampling of vaginal contents enables an accurate diagnosis of BV and therefore offers the opportunity of timely intervention to limit the incidence of adverse pregnancy outcome. Further work to increase the sensitivity of tampon sampling for detection of sexually transmitted organisms is warranted, and this is important as it could provide a useful tool for community-based surveys and diagnosis of genital tract infections.

### TABLE 1. Diagnosis of genital tract infections by tampon sampling and conventional sampling in 175 women attending a family planning clinic

<table>
<thead>
<tr>
<th>Genital tract infection</th>
<th>No. (% of women positive)</th>
<th>$P$ value</th>
<th>Sensitivity (%)$^a$</th>
<th>Specificity (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional sampling</td>
<td>Tampon sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>24 (13.7)</td>
<td>22 (12.6)</td>
<td>0.75</td>
<td>88.3</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>7 (4.0)</td>
<td>0 (0)</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>14 (8.0)</td>
<td>6 (3.4)</td>
<td>0.06</td>
<td>30</td>
</tr>
<tr>
<td><em>T. vaginalis</em></td>
<td>26 (14.3)</td>
<td>7 (4.0)</td>
<td>0.0008</td>
<td>28</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>55 (31.4)</td>
<td>28 (16.0)</td>
<td>0.0006</td>
<td>32.7</td>
</tr>
</tbody>
</table>

$^a$ Tampon sampling compared with conventional sampling.

### REFERENCES