APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1987, p. 2774–2778
0099-2240/87/122774-05$02.00/0

Vol. 53, No. 12

Quantitative Assessment of Vaginal Microflora during Use of Tampons of Various Compositions

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Received 1 December 1986/Accepted 21 August 1987

Although the effect of vaginal tampons on the microbial flora during menstruation has recently been studied by several investigators, quantitative effects attributable to particular tampon fibers have received less attention. The purposes of the present study were (i) to determine and then to compare the effects of polyacrylate rayon tampons and viscose rayon tampons on the normal vaginal flora, (ii) to compare quantitative bacterial counts obtained from these tampons with those obtained from concomitant vaginal swabs, and (iii) to determine whether either of these tampon types alters the vaginal microflora when compared with the microflora in the same women using all-cotton tampons or external catamenial pads. Tampon and swab samples were obtained at predetermined times from 18 women for an average of seven menstrual cycles. Samples consisting of swabs from women wearing menstrual pads were compared with swab and tampon samples taken at predetermined times during the menstrual cycle from women using cotton, polyacrylate rayon, or viscose rayon tampons. Samples were analyzed for total aerobic, facultative, and anaerobic bacterial counts. Statistical evaluation of the results indicated that, on the whole, total bacterial counts decreased during menstruation and that the numbers of bacteria in tampons tended to be lower than those in swab samples taken at the same time. The tampon type had little effect on the vaginal microflora.

The majority of published reports describing normal human vaginal microflora have not adequately described the changes occurring during menstruation. Bartlett et al. (2) addressed these changes by studying two groups of healthy young women volunteers. Cultures were obtained from the first group by vaginal swabs whenever in the course of the menstrual cycle the woman appeared for a vaginal examination. No menstrual samples were included in this group. To compensate for the latter, they studied a second group of women. These women submitted self-obtained swabs during a single menstrual cycle, including the menstrual period. When pooled with the results from the first group, a 100-fold decrease in the number of aerobes was seen in the last week of the menstrual cycle as compared with numbers obtained during menstrual flow. The number of anaerobes remained relatively constant during the entire cycle, although there was considerable variation in the species recovered at different times in the cycle.

A more extensive study was conducted by Johnson et al. (3). They sampled 34 women by a vaginal wash method during both the menstrual and intermenstrual phases of the cycle. Quantitative assessment of the microflora during the menstrual cycle revealed that the total number of bacteria did not change significantly from one sample period to another.

Although the effect of tampon use was not evaluated in any of the above studies, other investigators have attempted to relate the use of tampons to changes in the vaginal microflora during menstruation. Morris and Morris (5) conducted a 6-month study to assess this effect but included no samples taken during menstruation. They found that the microflora of women using tampons did not differ significantly from that of women using other forms of catamenial protection.

The most extensive published study addressing this question was performed in our laboratory (8). To assess the effect of cotton tampons and tampon use on the normal vaginal microflora, we studied eight healthy young women for 4 to 10 consecutive menstrual cycles. Samples consisting of vaginal swabs from women wearing catamenial pads were compared with vaginal swab and tampon samples obtained at various times during the menstrual cycle. The results indicate that total bacterial counts decreased during menstruation and that concomitant swab and tampon samples yielded similar total counts per unit weight of sample. The vaginal vault total counts, as reflected by the swab samples, tended to be higher than the counts obtained from tampons taken at the same time.

Although the work described above showed that cotton tampons had little discernible effect on the microbial flora, there is no information regarding other tampon fibers. Therefore, we studied, by methods similar to those used previously (8), the quantitative and qualitative (7) effects of two additional commonly used tampons with different fiber components. This report assesses the quantitative microflora during the use of cotton-viscose rayon and polyacrylate rayon tampons.

MATERIALS AND METHODS

Tampons. All tampons were commercially available products of Tampax brand (Tambrands, Inc., Palmer, Mass.). The specific brands used and their compositions are as follows: Regular, 100% bleached cotton; Super, 70% cotton

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TABLE 1. Experimental design

Cycle no.	Product and retention time (h)	Sample type
1	Pad[a]	Vaginal swab only
2	Regular[b] (2)	Tampon and swab
3 and 4	Super[c] (2)	Tampon and swab
5 and 6	Super (6)	Tampon and swab
7 and 8	Super Plus[d] (2)	Tampon and swab
9 and 10	Super Plus (6)	Tampon and swab

[a] No retention time associated with pads.
[b] All cotton.
[c] Viscose rayon.
[d] Polyacrylate rayon.

and 30% viscose rayon; Super Plus, 100% polyacrylate rayon.

Experimental design. Women were asked to volunteer to be monitored through successive menstrual cycles as described below. During each menstrual cycle, one intermenstrual and two menstrual samples were submitted in the form of tampons and concomitant self-obtained vaginal swabs. The study was designed to answer the following questions. (i) Do quantitative vaginal microbial populations from women using polyacrylate rayon tampons differ from those obtained from the same women using cotton-viscose rayon tampons? (ii) Do the quantitative microbiologic findings accompanying the use of either of these tampon types differ from the cultural data obtained from concomitant vaginal swabs? (iii) Does the use of either of these tampon types alter the vaginal microflora when compared with the microflora when the same women used all-cotton tampons or external catamenial pads?

Volunteers were followed for an average of seven complete menstrual cycles, with samples being obtained on days 2, 4, and 21 after the start of menstrual flow. The initial phase consisted of two menstrual cycles. The primary objective of this phase was to determine whether the microflora of these women was similar to that of the previously sampled group (8). The women used catamenial pads and submitted swab samples for one complete cycle. The following cycle the women submitted all-cotton tampons (Tampax Regular) worn for 2 h before sampling on each of the sample days along with concomitant vaginal swab samples. The objective of the second phase of the study was to assess any changes in the microflora attributable to tampon composition or retention time or both, the women used tampons composed of either cotton-viscose rayon (Tampax Super) or polyacrylate rayon (Tampax Super Plus) and participated for an average of four cycles per product. Samples were obtained for two cycles during which the tampon was worn for 2 h before sampling. For the remaining two cycles, samples were obtained after the tampons had been worn for 6 h before sampling. Each participant changed pads or tampons according to her usual pattern except on days when samples were obtained (Table 1).

Volunteers. Samples were obtained from 18 healthy female volunteers between the ages of 18 and 24 years for an average of 7 (± 3.35) menstrual cycles. The variable length of time for volunteers in this study was due to exclusion of cycles from data analysis if all the samples required by the experimental design were not obtained during a given cycle. Criteria for exclusion from the study included pregnancy, genital abnormalities, vaginal infections, antibiotic therapy, and douching 1 month before the start of sampling. Each woman provided information concerning height, weight, contraceptive practices, prior catamenial product use, regularity and duration of menstrual flow, pregnancies and outcome, as well as other clinical information pertinent to the study. Pelvic examinations were performed on each woman before the start of the study, and cultures for *Chlamydia trachomatis* and *Trichomonas vaginalis* were obtained. No attempt was made to test women specifically for *Neisseria gonorrheae* since the culture methods used in this study (see below) were adequate to detect this organism.

Sample processing. At the various sample times described above, vaginal swabs and tampons were obtained for processing as described previously (8). The tampon samples were removed and placed in a preweighed blender jar containing 100 ml of sterile phosphate-buffered saline (pH 7.2). The jar was reweighed, and the tampon was reduced to a slurry by 30 s of mixing. The sample weight, which ranged from 0.12 to 21.34 g depending on day and sample time, was determined by subtracting the average weight of the particular tampon type (Super = 2.88 ± 0.12 g, $n = 50$; Super Plus = 3.56 ± 0.15 g, $n = 50$) from the total weight. The blender jar and sample were placed into an anaerobic jar which was evacuated, and the atmosphere was replaced with oxygen-free nitrogen (99.99%). The entire process required 3 to 5 min after removal of the tampon.

Swab samples were obtained by the double-swab technique described previously (6). Volunteers were given a sterile wrapped swab and a sterile swab in a sterile tube in a preweighed assembly. Both swabs were inserted simultaneously into the vagina as far as possible with care being taken to avoid contact with the exterior labial surfaces. The swabs were rotated to achieve saturation and carefully removed, and the preweighed swab was returned to the sterile tube. The other swab was placed into prereduced Cary-Blair transport medium (GIBCO Diagnostics, Lawrence, Mass.). The swab and tube were reweighed, with the difference providing an estimate of the sample weight. All swab weights were measured with an accuracy of 0.1 mg. All samples were then transported to the microbiology laboratory for processing within 3 h.

Bacteriological analysis. The quantitative bacteriological analysis is described in detail elsewhere (8). Briefly, upon arrival at the laboratory, the samples were passed into an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.). The tampon in phosphate-buffered saline was agitated to resuspend the slurry, while the swab specimen was agitated on a Vortex mixer for 2 to 3 min until the sample was completely dispersed into the Cary-Blair medium.

Samples (0.1 ml) of the undiluted specimen and five serial dilutions in phosphate-buffered saline were spread onto several selective and nonselective media for the recovery and enumeration of aerobes (facultative anaerobes) and obligate anaerobes. The culture media for recovering anaerobes were (i) prereduced brucella-base agar with 5% sheep blood, enriched with hemin and vitamin K_1 (BMB); (ii) BMB with 100 μg of neomycin sulfate per ml; and (iii) prereduced brucella-base agar with 5% laked sheep blood, 100 μg of kanamycin and 7.5 μg of vancomycin per ml, and supplemented with hemin and vitamin K_1. Media for recovery of facultative anaerobes were (i) 5% sheep blood in tryptic soy agar (TSA), (ii) mannitol salts agar, and (iii) MacConkey agar. Chocolate agar was used for the recovery of fastidious organisms and human blood bilayer medium with Tween (HBT) was used for the isolation of *Gardnerella vaginalis*. All of the above media were obtained from Scott Laboratories, Inc. (Fiskeville, R.I.).

Plates inoculated for the recovery of obligate anaerobes

TABLE 2. Effect of sample day[a] and product type on bacterial counts

Product type	Counts (P)[b]					
	Aerobic			Anaerobic		
All	$2 \ll 21$ (0.0001)	$4 \cong 21$ (0.31)	$2 \ll 4$ (0.0001)	$2 \ll 21$ (0.0001)	$4 \ll 21$ (0.003)	$2 \ll 4$ (0.0001)
Regular (cotton)	$2 \cong 21$ (0.15)	$4 \cong 21$ (0.44)	$2 < 4$ (0.02)	$2 < 21$ (0.03)	$4 \cong 21$ (0.18)	$2 \cong 4$ (0.46)
Super (cotton-rayon)	$2 \ll 21$ (0.001)	$4 \cong 21$ (0.12)	$2 \cong 4$ (0.09)	$2 \ll 21$ (0.001)	$4 < 21$ (0.02)	$2 \ll 4$ (0.009)
Super Plus (rayon-polyacrylate)	$2 \ll 21$ (0.009)	$4 \cong 21$ (0.87)	$2 \ll 4$ (0.0001)	$2 \ll 21$ (0.0004)	$4 \cong 21$ (0.19)	$2 < 4$ (0.03)

[a] Sampling days = 2, 4, or 21.
[b] Results are shown with double-sided P values: $\cong$, $P > 0.05$; $<$, $0.05 > P > 0.01$; $\ll$, $P < 0.01$.

were incubated in an anaerobic chamber for a minimum of 60 h at 35°C before enumeration. Media used for the isolation of facultative species were removed from the chamber and incubated for 48 h either in air (TSA, mannitol salts agar, MacConkey agar) or 5% carbon dioxide (chocolate agar, HBT) at 35°C. After incubation, the various colony types were described, enumerated, isolated, and identified.

The concentrations of organisms recovered from the tampon and swab samples were expressed as CFU per gram of vaginal secretions, so that all counts were based on a consistent denominator unaffected by the amount of fluid absorbed by the tampons.

Statistical evaluation of quantitative data. The quantitative data for total anaerobic and total aerobic-facultative bacterial populations were evaluated by previously described procedures (1). The method employed used a mixed three-way analysis of variance and can be visualized as a cube with the three dimensions being day, retention time, and cycle. The interactions among all three dimensions were then tested for differences in both total anaerobic and total aerobic counts. This technique allows the random effects owing to deviations between cycles and within cycles to be evaluated for each subject alone and for the group as a whole. The various tampon types were evaluated separately by these methods and then were compared with each other for the same tampon use times and sampling days. Owing to the less absorbent nature of the all-cotton tampon, this fiber could be compared with the other tampon fibers only at the 2-h sample time. Specifically, if Y_{ijk} denotes the log of the anaerobic or facultative count at the jth day of the ith woman-cycle under kth treatment where $1 \leq i \leq 148$; $j = 2$, 4, or 21; and k = pad, cotton at 2 h, Super at 2 or 6 h, and Super Plus at 2 or 6 h from women using pads or tampons for 2 or 6 h, the model takes the following form: $Y_{ijk} = \mu + \beta_j + \gamma_k + a_i + e_{ijk}$, where β and γ represent the fixed effects of day and treatment, respectively; a_i is the random effect owing to deviations (with variance σ_a^2 between cycles); and e_{ijk} is the within-cycle deviations (with variance σ_e^2).

RESULTS

Demographic characteristics. The age of volunteers ranged from 18 to 25 years, height ranged from 4′11″ (150 cm) to 5′9″ (175 cm), weight ranged from 88 to 160 lb, (40 to 73 kg), and all 18 volunteers reported having regular menstrual periods of consistent duration. The average length of each woman's cycles ranged from 20 to 35 days, and the duration of menstrual flow was from 3 to 7 days. Contraceptive protection was used by eight of the women, with three using diaphragms, two condoms, two oral contraceptives, and one contraceptive sponges.

Quantitative bacteriological analysis. (i) Effect of sample day and product type on total bacterial counts. The first part of this analysis was directed toward an evaluation of the changes which occur in the total bacterial counts of both obligate and facultatively anaerobic populations during the menstrual cycle. The statistical comparison of sample day for all products combined and for each product type alone is summarized in Table 2. A statistical difference in total counts was considered to be significant if $P \leq 0.05$.

For the aerobic counts for all products combined, the counts on day 2 were less than those for day 21. When the individual product types were considered, Regular tampons yielded aerobic counts which were similar between day 2 and 21, while Super and Super Plus yielded lower counts on day 2 than on day 21. Aerobic counts on day 2 ranged from $10^{7.21}$ CFU/g of sample for the 2-h Super Plus tampon cultures to $10^{8.41}$ for the catamenial pad swab cultures. The aerobic counts for day 4 were the same as for day 21 for each product alone and for all products combined. Counts for day 4 ranged from $10^{7.94}$ for the 2-h Super Plus tampon cultures to $10^{8.59}$ for the 6-h Super Plus swab cultures and for day 21 ranged from $10^{7.96}$ for the 2-h Super Plus tampon cultures to $10^{8.80}$ for the 6-h Super Plus swab cultures. The combined results for all products yielded aerobic counts for day 2 which were significantly lower than those for day 4. A similar trend was noted for the individual product types with the exception of the Super tampons, which yielded aerobic counts on day 2 that were not significantly different from those for day 4.

The anaerobic counts for day 2 were less than those for day 21 for all products combined and for the individual product types. Counts for day 2 ranged from $10^{7.14}$ for the 2-h Super tampon cultures to $10^{8.40}$ for the 6-h Super swab cultures. Combined results for all products on day 4 were less than those on day 21, although both the Regular and Super Plus products yielded anaerobic counts which were not significantly different for these two times. Anaerobic counts for day 4 ranged from $10^{7.29}$ for the 2-h Super Plus tampon cultures to $10^{8.87}$ for the 6-h Super swab cultures and for day 21 ranged from $10^{7.98}$ for the 2-h Super Plus swab cultures to $10^{8.69}$ for the 6-h Super swab cultures. The day 2 anaerobic counts were less than the day 4 counts when all products were evaluated. However, when analyzed separately, the Regular tampon yielded anaerobic counts which were similar to those for these two sample days.

(ii) Comparison of sample types by product. Vaginal swabs from women using Regular tampons revealed no differences in counts for either the aerobic or anaerobic microflora when compared with vaginal swabs obtained during the use of catamenial pads. A comparison of the vaginal swab and tampon cultures showed no significant difference in aerobic counts and a marginal significance level for the anaerobic counts, with tampon counts being slightly lower than vaginal swab counts.

A comparison of vaginal swab counts from women using

TABLE 3. Comparison of tampon products by sample type[a]

Product	Counts (P)	
	Aerobic	Anaerobic
Tampon	Regular = Super 2 hr (0.18) Regular > Super Plus 2 h (0.04) Super 2 h = Super Plus 2 h (0.40) Super 6 h = Super Plus 6 h (0.37)	Regular = Super 2 h (0.35) Regular ≤ Super Plus 2 h (0.07) Super 2 h = Super Plus 2 h (0.17) Super 6 h = Super Plus 6 h (0.24)
Tampon-associated swab	Regular = Super 2 h (0.80) Regular = Super Plus 2 h (0.26) Super 2 h = Super Plus 2 h (0.11) Super 6 hr = Super Plus 6 h (0.39)	Regular = Super 2 h (0.49) Regular = Super Plus 2 h (0.73) Super 2 h = Super Plus 2 h (0.25) Super 6 h = Super Plus 6 h (0.23)

[a] Results are shown with double-sided *P* values.

Super tampons for 2 and 6 h with counts from women using catamenial pads showed no significant differences between these groups for either aerobic or anaerobic counts. For both the 2 and 6 h sample times, the anaerobic and aerobic counts from tampons were significantly lower than the corresponding vaginal swab counts. Samples obtained during the use of Super Plus tampons yielded results statistically identical to those described for Super tampons, with no differences detected between vaginal swab cultures during pad or tampon use and tampon counts being consistently lower than vaginal swab counts obtained at the same sample times.

(iii) Comparison of tampon products by sample type. A comparison of tampon products by sample type is shown in Table 3. For aerobic counts obtained from tampon samples, the only difference noted was a marginal one between Regular and Super Plus tampons at the 2-h sample time. Regular tampons yielded higher counts than the Super Plus tampon samples. No difference was noted between any of the product types for vaginal swab samples.

The comparison of anaerobic counts by product and sample type yielded results similar to those of the aerobic count comparisons. Regular tampon counts were slightly higher than the tampon counts from Super Plus, and none of the counts obtained from vaginal swabs were significantly different, regardless of product and sample time.

DISCUSSION

Previous studies in this and other laboratories have detailed the changes in the vaginal microflora associated with menstruation. These quantitative changes appear to occur regardless of the catamenial product used (tampon versus pad) or the fiber composition of the product (cotton, cotton-viscose rayon blend, polyacrylate rayon). As in our previous studies, the total counts for both obligate anaerobes and facultative populations were lowest at day 2 and highest at day 21. A significant difference in total counts between tampon products was detected for Super Plus tampons used for 2 h versus all-cotton tampons used for the same time period. The lower counts associated with the Super Plus tampons indicate that the same inhibitory effect for certain bacterial populations noted in vitro may occur during in vivo use as well (4, 9). When comparing vaginal swab samples from women using pads with samples from the same women using the various tampon products, no significant differences were detected. In fact, the total counts for the anaerobic and facultative microflora from vaginal swabs from women wearing catamenial pads were higher on day 2 than those from women wearing the Super Plus tampon after 2 h of use. In our previous study, it was noted that the total counts for both obligate anaerobes and the facultative microbial populations were lower for tampon samples than for the corresponding vaginal swab samples. These previous studies were performed only with all-cotton tampons. The present study indicates that the same relationship between tampon and vaginal swab counts exists for other fibers as well. This is of particular importance when considering the hypothesis that tampons may serve as a nidus for unrestricted microbial growth within the vaginal vault. Clearly, there are no data from the present study to support this hypothesis for any of the tampon products evaluated to date. Based on the information presented here and on previous observations (8), it does not appear that the various tampon products tested alter the quantitative vaginal microflora to a significant degree.

Comparisons of vaginal microflora by birth control method and other subject-related factors were not assessed in the present study, but are the subject of continued evaluation of these data. This study represents one of the most detailed microflora studies ever undertaken, and it will take several years to evaluate fully all the parameters which may be associated with changes in the vaginal microflora. From these results, however, it does not appear that tampon use significantly alters the normal changes which occur in the vaginal microflora during menses.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Marilee Burrell, Stephen Notarnicola, Charles Ford, and Vincent Carey.

This research was funded by a grant from Tambrands, Inc.

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