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Methods for Quantitative and Qualitative Evaluation of Vaginal Microflora during Menstruation

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The quantitative and qualitative changes in the bacterial flora of the vagina during menstruation have received inadequate study. Similarly, the effect of vaginal tampons on the microbial flora as well as the relationship between the microbial flora of the vagina and that of the tampon has not been adequately evaluated. The purposes of the present study were (i) to develop quantitative methods for studying the vaginal flora and the flora of tampons obtained during menstruation and (ii) to determine whether there were differences between the microflora of the tampon and that of the vaginal vault. Tampon and swab samples were obtained at various times from eight young healthy volunteers for 8 to 10 menstrual cycles. Samples consisted of swabs from women wearing menstrual pads compared with swab and tampon samples taken at various times during the menstrual cycle. Samples were analyzed for total facultative and anaerobic bacterial counts, and the six dominant bacterial species in each culture were identified. Statistical evaluation of the results indicates that total bacterial counts decreased during menstruation and that swab and tampon samples yielded similar total counts per unit weight of sample. The numbers of bacteria in tampons tended to be lower than in swabs taken at the same time. Overall, during menstruation, the concentrations of lactobacilli declined, but otherwise there was little difference among the species found during menstruation compared with those found in intermenstrual samples. Cotton tampons had little discernible effect on the microbial flora.

Only recently have methods for the study of anaerobic bacteria permitted a more complete analysis of the vaginal bacterial flora (3–5, 7, 9–13, 16). Most published reports describing the human vaginal bacterial flora do not describe changes occurring during menstruation. Bartlett et al. (3) did use more modern methods for anaerobic study and measured changes in the bacterial flora in two groups of young healthy volunteers. Their first group consisted of 17 women sampled with a single culture each taken whenever in the course of the menstrual cycle the volunteer appeared for a vaginal examination, which was given for a variety of reasons. There were no menstrual samples. The second group consisted of five healthy volunteers who obtained from themselves duplicate vaginal swabs during a single menstrual cycle, including the menstrual period. The combined data from the two groups indicated a 100-fold decrease in the mean number of aerobes during the last week of the menstrual cycle, compared with numbers during the first week of menstrual flow. The number of anaerobes remained relatively constant throughout the cycle, although there was considerable variation in the individual species recovered. Sautter and Brown (13) sampled seven volunteers by taking 2 to 3 weekly samples during 1 month. They found that the various species of bacteria isolated from a given individual remained relatively constant, but that the numbers of each species varied at different times in the cycle. The effect of tampons was not evaluated in any of the previous studies.

Attempts have been made to relate the use of tampons to changes in the vaginal microflora during menstruation. In a 6-month study that excluded menstruating women, Morris and Morris (10) found that the microflora of women using tampons was not significantly different from that of women using other forms of catamenial protection. Smith et al. (14) found a significant association between menstruation and the frequency of isolation of *Staphylococcus aureus*, but no differences in the rate of colonization with this organism in users of tampons compared with the rate in users of menstrual pads. However, the reported data dealt only with the frequency of recovery of bacterial species and did not include quantitative data.

Thus, it has not been possible from the available data to determine whether the presence of a tampon alters significantly the microbial flora of the genital tract during menstruation. Therefore, a systematic study of the effect of a commonly used cotton tampon on the bacterial flora of menstruating healthy young women has been undertaken.

MATERIALS AND METHODS

Experimental design. In the following studies, young women were asked to volunteer to be monitored through successive menstrual cycles, as described below. During each menstrual period and at appropriate intermenstrual times, tampons or vaginal swabs or both were used to obtain microbiologic samples. These studies were designed to address the following questions: (i) do quantitative and qualitative bacterial analyses of tampons differ from data obtained by using a vaginal swab for sampling, and (ii) does the presence of a tampon alter the vaginal microflora compared with the microflora in women using external catamenial pads?

Volunteers were followed for an average of nine complete menstrual cycles. Swab and tampon samples were obtained on days 2, 4, and 21 after the start of menstrual flow. Only all-cotton tampons (Tampax regular) were used. During the initial phase of the study, in which the primary objective was to validate the methods of analysis used for tampons, the tampons were worn for 2 hours prior to sampling on each of the sample days. This sampling schedule was used for the

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TABLE 1. Sampling schedule[a] and protocol for evaluation of vaginal microflora during menstruation

Cycle no.	Catamenial protection used	Tampon retention time (h)[b]	Source of sample[c]
1, 2, 3	Tampon	2	Tampon
4	Tampon	2	Tampon, vaginal swab
5, 6	Pad	NA[d]	Vaginal swab
7, 8, 9	Tampon	6	Tampon, vaginal swab

[a] Samples were taken on days 2, 4, and 21 of each cycle.
[b] Time tampon was worn by subject before samples were taken. NA, Not applicable.
[c] During tampon cycles, swab was taken immediately after tampon was removed.
[d] Pad samples were not associated with retention time.

first three menstrual cycles. During the fourth menstrual cycle, vaginal swab samples obtained immediately after removal of the tampons were included. The second phase of the study allowed comparison of vaginal swabs obtained from the same women wearing catamenial pads (Maxi-thins) with the initial phase of tampon use. The final phase of the study allowed comparison of a longer tampon retention time prior to sampling with the previous data on pad and shorter tampon retention time. Each participant changed pads or tampons according to her usual pattern throughout menstruation, except on the days on which samples were obtained. On sampling days, each participant inserted an all-cotton tampon for the time specified by the protocol (Table 1).

Volunteers. Samples were obtained from eight healthy female volunteers between the ages of 20 and 25 years for 8 to 10 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 from a given cycle. Criteria for exclusion from the study included pregnancy, genital abnormalities, vaginal infections and antibiotic therapy, and douching 1 month prior to the start of sampling. Each woman provided information concerning height, weight, contraceptive practices, prior catamenial-product use, regularity and duration of menstrual flow, and pregnancies and outcome, as well as other clinical information pertinent to the study. Pelvic examinations were performed on each woman prior to the start of the protocol, and cultures for *Chlamydia trachomatis* and *Trichomonas vaginalis* were obtained. No attempt to culture women specifically for *Neisseria gonorrhoeae* was made, since the culture methods used (see below) were adequate to detect this organism.

Sample processing. At the various sample times described above, vaginal swabs or tampons or both were obtained for microbiologic processing. The tampon samples were removed at the clinic by the volunteers and placed into a sterile specimen cup. This sample was immediately given to a research assistant who aseptically removed the withdrawal strings and placed the tampon into a sterile preweighed blender jar containing 100 ml of sterile phosphate-buffered saline (PBS; pH 7.2). The jar was reweighed, and the sample was reduced to a slurry by 30 s of mixing. The tampon sample weight was determined by subtracting the combined weight of the jar, buffer, and average weight of a regular tampon (2.24 g; $s = 0.12$; standard error = 0.001; $n = 120$) from the combined weight of the jar, buffer, and sample tampon. The blender jar and sample were placed into an anaerobic container, the anaerobic jar 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 study participants by the double-swab technique described previously (12). Briefly, volunteers were given a package containing a sterile wrapped swab and a sterile swab in a sterile tube which had been preweighed with an analytical balance. Both swabs were inserted simultaneously into the vagina as far as possible, with care being taken to avoid contact with the external labial surfaces. The swabs were rotated several times to saturate the cotton tip, and both swabs were removed carefully, with the preweighed swab being replaced into the sterile tube and the other swab being placed into prereduced Cary-Blair transport medium (GIBCO Diagnostics, Lawrence, Mass.). The preweighed swab and tube were reweighed, with the difference being 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.

Preliminary studies compared PBS and a prereduced Cary-Blair medium for recovery of obligate anaerobes from the tampons. No significant difference between the two transport media was found (Onderdonk, unpublished data), and PBS was therefore used in these studies.

Bacteriological analysis. Quantitative bacteriological analysis of processed tampons or swabs took place within 3 h of collection. Specimens were passed into an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.). The tampon in PBS was agitated to suspend 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.

Five serial dilutions made with separate pipettes were made of both the tampon and the swab specimens in PBS to achieve decimal dilutions through 10^{-5}. Samples (0.1 ml) of the undiluted specimen and of the five dilutions were plated onto several selective and nonselective media with sterile plastic spreaders. The culture media for recovering anaerobic bacteria were (i) prereduced brucella-base agar with 5% sheep blood, containing 0.01 g of both hemin and vitamin K_1 per liter (BMB); (ii) BMB with 150 μg of neomycin sulfate per ml; and (iii) prereduced Brucella-base agar with 5% laked sheep blood, 100 μg of kanamycin per ml, 7.5 μg of vancomycin per ml, and hemin and vitamin K_1 as described above. Media for recovery of facultative anaerobes were (i) 5% sheep blood in tryptic soy agar, (ii) mannitol salts agar, and (iii) MacConkey agar. Chocolate agar was used for the recovery of fastidious organisms (Scott Laboratories, Inc., Fiskeville, R.I.).

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

Facultative gram-positive cocci were identified by established criteria (8). Members of the family *Enterobacteriaceae* and gram-negative bacilli were identified by either the API 20E System (Analytab Products, Plainview, N.Y.) or the AMS Vitek System (Vitek Systems, Inc., Hazelwood, Mo.). Gram-positive, catalase-negative aerobic or microaerophilic bacilli that produced large amounts of lactic acid, as determined by gas-liquid chromatography, were

classified as lactobacilli without being further identified by species. Aerobic gram-positive, sporeforming, catalase-positive rods were classified as *Bacillus* sp. Catalase-positive, gram-positive pleomorphic bacilli were classified as *Corynebacterium* sp. No further classification was performed for facultative *Lactobacillus*, *Corynebacterium*, or *Bacillus* species. No special efforts were made to identify *Gardnerella vaginalis* in these samples. Obligate anaerobes were classified by gas-liquid chromatography analysis of glucose fermentation products and antibiotic susceptibility patterns as performed by standard procedures (6, 15). Final identification included the use of the Anastat II system of biochemical tests and a computer database (Scott). Concentrations of organisms recovered from the tampon and swab samples were expressed in 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 analysis of quantitative data. Evaluations of the total anaerobic and the total facultative populations were performed by a mixed three-way analysis of variance (1). This technique can be visualized as a three-dimensional cube, with the first two dimensions being day and sample type (e.g., 2-h swab or 6-h tampon) and the third dimension being 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 due to deviations between cycles and within cycles to be evaluated for each volunteer alone and for the group as a whole. 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 $1 \leq i \leq 72$; $j = 2, 4$, or 21; and k = SP, ST_2, ST_6, T_2, T_6 (SP, ST_2, and ST_6 refer to the vaginal swabs from women using pads or using tampons for 2 and 6 h prior to sampling, respectively; T_2 and T_6 refer to tampons from women after 2 and 6 h of use, respectively), then 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 due 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 20 to 25 years, height ranged from 5'2" to 5'11" (ca. 157 to 180 cm), weight ranged from 106 to 160 lb (ca. 47 to 72 kg), and all eight volunteers reported having regular menstrual periods of consistent duration. The average length of cycles ranged from 21 to 35 days, and the duration of menstrual flow was between 4 and 8 days. Contraceptive protection was used by four of eight volunteers, with one woman using a diaphragm, one woman using both condom and diaphragm protection, and two women using both diaphragms and contraceptive jelly.

Quantitative bacteriological analysis. (i) Effect of menstruation on total counts. Since little information was available on the changes in microflora during menstruation, the first analysis dealt with the changes in total counts in two successive cycles during which women used only catamenial pads. Only vaginal swabs were analyzed for this purpose. The mean aerobic counts (i.e., facultative anaerobes) in log CFU per gram of sample weight from the swabs of women wearing menstrual pads ranged from 7.41 (standard deviation [SD], 1.26) on day 2 to 8.14 (SD, 1.04) on day 4 and 8.47 (SD, 1.41) on day 21 after the start of menstrual flow. Total counts for obligate anaerobes ranged from 7.33 (SD, 1.1) on day 2 to 7.07 (SD, 1.43) on day 4 and 7.68 (SD, 1.17) on day 21. These differences in aerobic and anaerobic vaginal swab counts were not statistically significant with the statistical model described previously ($P > 0.05$). The differences might have been more significant if the number of observations had been larger.

Since menstruation alone did not appear to result in dramatic shifts in total counts (although significant fluctuations in population levels for the various species occurred, as reported below), the data from all of the various samples and sample types were evaluated to determine whether other factors might result in statistically significant shifts in total microbial populations. The facultative and aerobic counts for all samples, regardless of type, were significantly lower on day 2 compared with counts on either day 4 or 21 ($P < 0.01$). The obligately anaerobic population on day 2 was significantly less than that on day 4 ($P = 0.038$), and the population on day 4 was significantly less than that on day 21 ($P = 0.008$). When swab samples obtained from women during catamenial pad use were compared with vaginal swab samples obtained from women using tampons for either 2 or 6 h, it was found that the total facultative counts after 2 h of tampon use were significantly lower than for the same women during pad use ($P = 0.007$). On the other hand, the samples obtained after 6 h of tampon use yielded counts which were significantly higher than for the same women wearing pads ($P < 0.001$). No significant differences in the total anaerobic counts were associated with pad versus tampon use.

A major part of the analysis was to determine, if possible, whether tampons served as a focus for microbial growth during use. This analysis was performed by comparing total counts from tampon samples with total counts from vaginal swab samples obtained from the same volunteers at the same sample times. The data indicate that there were no significant differences in total counts between tampon and swab samples when the 2-h tampon samples were compared with the vaginal vault swab samples, although the tampon counts from the menstrual samples were consistently lower than the swab samples. Total facultative and obligately anaerobic population counts for the swab samples obtained after 6 h of tampon use were significantly higher than for the tampons themselves ($P < 0.001$).

A visual comparison of the total counts for aerobic and facultative populations and for the obligately anaerobic microflora at the various sample times is given in Fig. 1. As can be seen, for the facultative bacterial populations, the swabs obtained from the women when they were using pads and when they were using tampons for 6 h showed no significant changes, although there was a trend towards a gradual increase in populations from day 2 to 21. For the swab samples from women using tampons for 2 h, there were also no significant changes from day 2 to day 21, but a tendency for bacterial counts to decrease on day 21 was noted (Fig. 1A). A significant increase in total anaerobic counts was noted between days 2 and 21 for vaginal swab samples (Fig. 1B). A comparison of tampon and swab counts (Fig. 1C and D) shows trends similar to those noted for swab samples, with the tampon counts being consistently lower than the corresponding vaginal swab counts for both aerobes and anaerobes for most sampling conditions. The exception is a nonsignificant reversal of this trend on day 21 for the 2-h aerobic counts. Finally, a comparison of the tampon counts with the vaginal vault counts during pad use (Fig. 1E and F) reveals similar trends, with the exception again being the

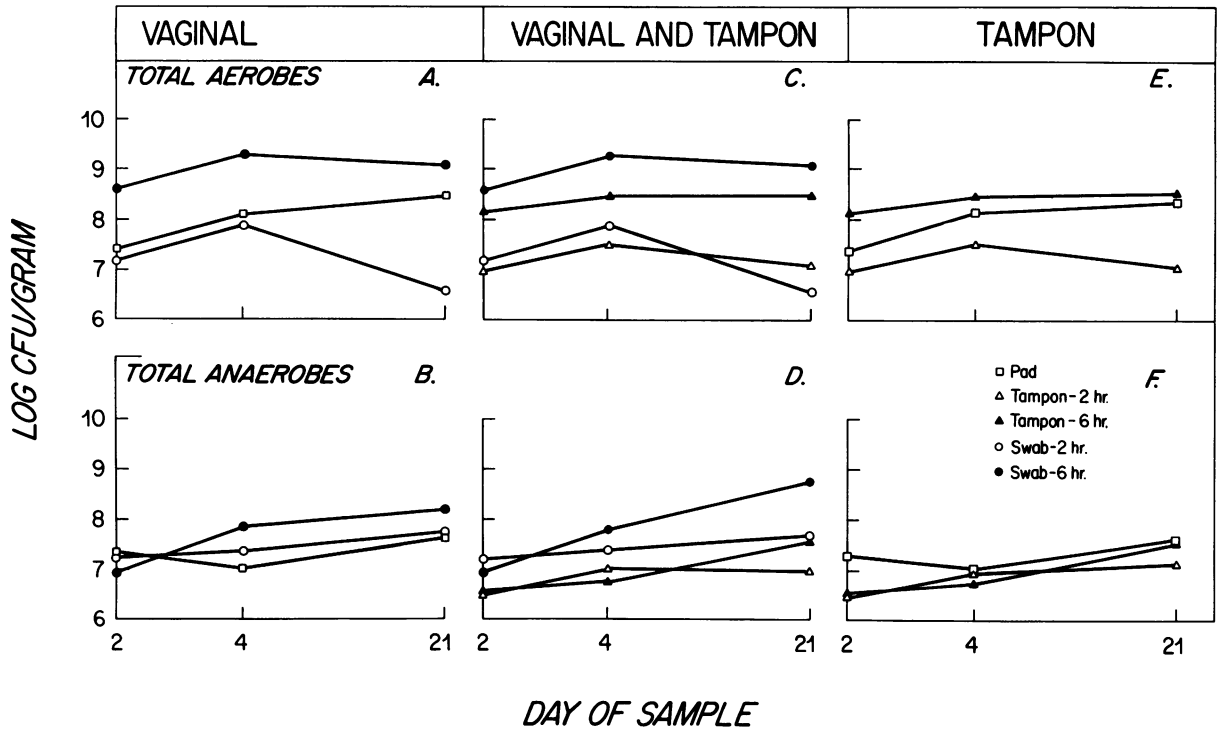

FIG. 1. (A) Comparison of total aerobic and facultative bacterial populations in vaginal swab samples from women using catamenial pads, tampons for 2 h, or tampons for 6 h. (B) Comparison of total anaerobic bacterial populations in vaginal swab samples from women using catamenial pads, tampons for 2 h, or tampons for 6 h. (C) Comparison of total aerobic and facultative bacterial populations in tampon and concomitant swab samples at 2 and 6 h of tampon use. (D) Comparison of total anaerobic bacterial populations in tampon and concomitant swab samples at 2 and 6 h of tampon use. (E) Comparison of total aerobic and facultative bacterial populations in 2- and 6-h tampon samples with the population in swab samples taken during catamenial pad use. (F) Comparison of total anaerobic bacterial populations in 2- and 6-h tampon samples with the population in swab samples taken during catamenial pad use.

decline in counts on day 21 for the 2-h aerobic tampon samples.

(ii) Qualitative evaluation of the vaginal microflora. The six dominant species present in each specimen are shown by rank order according to frequency of isolation (Table 2). This ordering was expanded to include *S. aureus* and facultative gram-negative rods when present, regardless of counts. The rank order of the most frequently isolated species changed little when categorized by day, sample type (i.e., swab or tampon), or catamenial product. There were, however, large fluctuations in the percentage of total flora represented by the various species relative to time of sample. Members of the genus *Lactobacillus* were the major microflora component on day 21, ranging from 52 to 99% of the total microflora, but members of this genus were found less frequently and in lower relative concentrations on day 2 regardless of sample type or catamenial product use. The most frequently isolated species was *Staphylococcus epidermidis*, yet this species accounted for less than 21% of total counts on day 2, less than 30% on day 4, and less than 1% on day 21.

Several additional observations with regard to the qualitative makeup of the microflora in this study deserve mention. Although the genus *Lactobacillus* was the numerically dominant genus isolated from 21-day 2-h tampon samples, this genus was not represented among the dominant microflora in the concomitant vaginal swab samples. Despite the absence of this genus in the swab samples, it was isolated among the top six isolates in 10 of 30 tampon samples from the same individuals obtained during the study. Although certain genera and species such as *Peptococcus* spp., *S. epidermidis*, *Lactobacillus* spp., and *Bacteroides* spp. were the most frequent isolates in significant numbers, many different species were isolated on one or more occasions in the 289 samples as part of the numerically dominant microflora. An alphabetical listing of the various species isolated and the frequency of isolation is presented in Table 3

A total of 1,615 different isolates were characterized during these studies, of which 111 did not fit an existing genus or species designation. Of the 111 unidentified isolates, 58 were obligate anaerobes, 23 were facultative organisms, and 30 were thought to represent one of three undescribed gram-positive rod phenotypes. *N. gonorrhoeae* was not isolated in any of the samples, and *S. aureus* and *Escherichia coli*, when present, represented only a small percentage of the total microflora.

DISCUSSION

The purpose of these studies was to determine whether the methods employed were capable of detecting quantitative or qualitative changes, or both, in vaginal microflora during the menstrual cycle, including changes occurring during menstrual flow. In addition, the study was designed to determine whether tampons were capable of provoking microbial changes either directly through microbial growth within the tampon or indirectly by affecting the microbial ecology in the vagina. Although the number of volunteers was small, the total microbial counts for women using catamenial pads were consistent with previously published findings (2, 12)

from a similar sampling procedure. The methods used in this study both for sampling and for statistical analysis appear to be able to detect changes in vaginal microflora associated with a variety of fixed and random effects.

The effect of day of sample when evaluating changes in microflora associated with menstrual flow was examined in detail. The total aerobic counts on day 2 of menstrual flow were generally lower than those for the intermenstrual sample obtained on day 21. The exception to this observation was for samples obtained after 2 h of tampon use on day 21, when there were no menses. Both the swab and the tampon samples after 2 h of tampon use revealed aerobic and facultative counts which were lower than the counts obtained on day 2 of menstrual flow. Although the low aerobic counts on day 2 of menstrual flow may be explained by a "washout" effect, the low counts on day 21 suggest an inhibitory effect due to the use of a tampon for a short time. This inhibitory effect does not appear to be sampling error, since the total anaerobic counts do not show a similar pattern. Results of the use of a cotton tampon for 6 h prior to sampling were compared with either the tampon samples after 2 h of tampon use or the swab samples from the same women when they were using pads. One can argue that the higher counts for facultative species on day 2 after 6 h of tampon use are due to retention of the menstrual fluids for a longer time, resulting in increased bacterial counts. However, the same explanation does not necessarily apply to the 21-day samples when fluid volume is minimal. Therefore the reason for the higher menstrual counts after 6 h of tampon use remains unclear. In all events, the significance of these changes in bacterial counts, which rarely changed by more than 1 log, is uncertain.

The finding that the total anaerobic populations show only modest changes during the menstrual cycle regardless of sample type and catamenial product was unexpected. The data suggest that the anaerobic microflora are more resistant to environmentally induced changes than previously thought. Despite the relatively aerobic environment created by tampon insertion on day 21, the obligately anaerobic counts both on the tampon and within the vaginal vault tended to be consistent, with no apparent decline in numbers associated with tampon use. The protective effect of a mucosal surface may explain the persistence of these microbes at the vaginal vault surface. However, the survival of these organisms within or on the tampon suggests that the local environment is less inhibitory than expected.

A comparison of the tampon microflora with that of the vaginal vault suggests that tampons per se do not serve as a focus for microbial multiplication within the vaginal vault. If tampons were a focus for microbial multiplication, the total counts per gram of sample would be higher in the tampon samples than in the concomitant vaginal swab samples. In fact, the counts of both aerobic and obligately anaerobic microorganisms were consistently lower in tampons than in samples obtained by the vaginal swab technique.

The data from this study indicate that the tampon samples reflect the total counts observed within the vaginal vault. Although tampons may induce environmental changes which alter the growth of microorganisms at the vaginal surface, such growth appears to occur at the level of the mucosal surface itself and not within the tampon. This observation is clearly at variance with the hypothesis that this form of catamenial protection provides a focus for uncontrolled microbial growth. It should also be pointed out that the sensitivity of the techniques used in this study results in statistical significance with less than a 10-fold change in total

TABLE 2. Rank order of dominant species present in each specimen

Sample[a]	Bacterial genus or species isolated	Results on day[b]					
		2		4		21	
		F	%	F	%	F	%
T2	*Bacteroides*	12	2.07	20	0.73	13	0.05
	Clostridium	ND	ND	ND	ND	10	2.49
	Corynebacterium	17	9.04	14	2.20	16	1.00
	E. coli	8	1.93	9	5.16	ND	ND
	Lactobacillus	10	42.30	10	35.70	25	96.00
	Peptococcus	17	2.07	12	2.00	10	0.49
	Peptostreptococcus	ND	ND	7	1.52	ND	ND
	Staphylococcus sp.	30	11.40	32	28.34	29	0.72
	S. aureus	8	1.02	7	0.94	3	0.01
ST2	*Bacillus*	4	11.60	4	9.70	5	1.07
	Bacteroides	3	0.57	5	0.97	ND	ND
	Clostridium	ND	ND	3	6.27	2	0.72
	Corynebacterium	ND	ND	3	1.19	3	0.02
	E. coli	4	0.40	2	1.34	ND	ND
	Lactobacillus	ND	ND	ND	ND	4	96.00
	Peptococcus	4	6.51	6	3.36	5	0.28
	Staphylococcus sp.	8	10.30	8	18.07	7	0.13
	S. aureus	2	2.42	2	0.93	ND	ND
SP	Aerobic gram-negative rod	5	23.30	ND	ND	ND	ND
	Bacillus	14	6.12	14	19.71	13	0.05
	Bacteroides	4	0.75	ND	ND	ND	ND
	Clostridium	4	3.07	4	0.86	ND	ND
	Corynebacterium	18	0.41	6	6.53	5	2.81
	E. coli	ND	ND	6	0.70	5	0.05
	Lactobacillus	10	33.60	8	35.87	12	90.96
	Peptococcus	14	5.98	9	1.16	5	0.08
	Peptostreptococcus	ND	ND	3	0.50	ND	ND
	Staphylococcus sp.	17	1.19	16	1.53	15	0.17
	S. aureus	ND	ND	1	0.10	2	0.5
	Streptococcus (group D)	ND	ND	ND	ND	8	0.63
	Streptococcus sp.	ND	ND	3	0.10	ND	ND
T6	*Bacillus*	7	0.02	5	0.71	12	0.01
	Bacteroides	7	0.04	5	0.04	3	0.01
	Corynebacterium	9	0.62	5	3.38	14	0.02
	E. coli	9	0.03	8	0.24	ND	ND
	Lactobacillus	14	28.80	14	48.92	21	52.10
	Peptococcus	18	0.36	13	0.09	20	0.02
	Staphylococcus sp.	20	7.08	17	14.10	20	0.01
	S. aureus	5	2.57	5	3.02	3	0.01
	Streptococcus (group D)	ND	ND	6	5.86	ND	ND
	Streptococcus sp.	ND	ND	ND	ND	7	0.31
ST6	*Bacillus*	9	0.76	6	0.09	6	0.02
	Bacteroides	ND	ND	10	0.02	10	0.02
	Corynebacterium	13	0.78	11	0.36	15	0.05
	E. coli	8	0.05	5	0.75	4	0.01
	Lactobacillus	13	45.80	15	27.90	21	61.00
	Peptococcus	14	0.32	12	0.09	20	0.01
	Staphylococcus sp.	20	2.24	16	3.94	20	0.03
	S. aureus	5	0.06	5	2.43	4	0.01
	Streptococcus (Group D)	7	0.48	4	21.16	ND	ND

[a] T2, 2-h tampon sample; ST2, swab concomitant with 2-h tampon sample; SP, swab from women wearing catamenial pad; T6, 6-h tampon sample; ST6, swab concomitant with 6-h tampon sample.

[b] Day during menstrual cycle after day 1 of period. F, Frequency at which strains of species indicated were isolated; %, percentage of the total population present; ND, not among six predominant isolates.

TABLE 3. Frequency distribution by genus and species

Organism	Total no. of observations
Actinomyces spp.	5
Bacillus spp.	122
Bacteroides spp.	29
B. asaccharolyticus	4
B. bivius	10
B. disiens	2
B. fragilis	12
B. furcosus	2
B. melaninogenicus	25
B. uniformis	3
B. vulgatus	7
Bifidobacterium breve	2
Bordetella bronchiseptica	3
Clostridium spp.	15
C. beijerinckii	2
C. bifermentans	11
C. clostridiiforme	2
C. glycolicum	2
C. putrefaciens	2
C. ramosum	2
C. sordellii	2
C. symbiosum	2
Corynebacterium spp.	142
E. coli	76
Eubacterium spp.	12
E. combesii	2
E. cylindroides	2
E. saburreum	3
Fusobacterium spp.	2
F. nucleatum	4
Gaffkya anaerobia	9
Klebsiella pneumoniae	31
Lactobacillus spp.	161
L. brevis	2
L. casei	2
L. fermentum	7
L. leichmannii	3
L. plantarum	2
Peptococcus spp.	24
P. asaccharolyticus	31
P. indolicus	3
P. magnus	84
P. prevotii	37
Peptostreptococcus spp.	9
P. anaerobius	6
P. micros	5
P. productus	7
Propionibacterium acnes	2
P. avidum	2
Pseudomonas spp.	3
P. maltophilia	2
P. putida	3
Staphylococcus spp.	277
S. aureus	51
Streptococcus spp.	35
Group A	3
Group B	17
Group D	52
Anaerobic *Streptococcus* spp.	9
S. intermedius	7
Sarcina ventriculi	3
Yeasts	
Unidentified	3
Candida spp.	4
C. albicans	9
Unidentified anaerobes[a]	
Gram− rods	7
Gram+ rods	22
Gram− coccobacilli	3
Gram+ coccobacilli	8
Gram+ cocci	16
Unidentified aerobes and facultatives	
Gram− rods	15
Gram− coccobacilli	3
Gram+ rods	3
Unidentified Gram+ nonsporeforming anaerobic rods	
Type I	7
Type II	16
Type III	7
Organisms isolated only once	
Aerobes (facultative)	11
Anaerobes	28

[a] Gram−, Gram negative; gram+, gram positive.

counts. The clinical importance of such modest changes in microbial numbers has never been documented. No clinical problems relative to the tampons arose in any of the volunteers during the study regardless of sample type, sample time, or catamenial product used.

The results of the qualitative analyses of the predominant species isolated during the course of this study were consistent with previous studies for intermenstrual samples. During menstrual flow, the genus *Lactobacillus* was replaced by a variety of other gram-positive organisms including facultative and anaerobic cocci of various genera. Regardless of time or sample type, the vaginal and tampon microfloras were overwhelmingly gram positive. Although members of the genus *Bacteroides* were present with regularity, they were present in only a small part of the total microflora. When present in any volunteer, gram-negative rods such as *Bacteroides melaninogenicus* or *E. coli* tended to persist for several cycles. Other organisms were sporadic in occurrence, suggesting either that they were present at detectable levels only rarely or that their presence represented a random attempt at colonization with little ability to persist as an integral part of the vaginal microflora. In three of the women, *S. aureus* was classified as one of these sporadic isolates. In two other volunteers, *S. aureus* was recovered continually from menstrual samples but sporadically from intermenstrual samples. The remaining three women failed to yield *S. aureus* under any of the test conditions.

These studies have provided data which document a

sampling method and statistical design which allow changes in the vaginal microflora associated with a variety of factors to be studied. Additional clinical studies are in progress using these techniques to determine whether the composition of different tampons contributes to changes in the vaginal microflora during menstruation.

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