APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1987, p. 2779–2784
0099-2240/87/122779-06$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 53, No. 12

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

A. B. ONDERDONK,[1]* G. R. ZAMARCHI,[1] M. L. RODRIGUEZ,[1] M. L. HIRSCH,[2] A. MUÑOZ,[2] AND E. H. KASS[2]

Infectious Disease Research Laboratory, Department of Pathology, Tufts University School of Veterinary Medicine,[1] and Channing Laboratory and Department of Medicine, Harvard Medical School and Brigham and Women's Hospital,[2] Boston, Massachusetts 02130

Received 1 December 1986/Accepted 21 August 1987

The effect of vaginal tampons on the microbial flora during menstruation has recently been studied by several investigators. However, little information regarding the qualitative effects attributable to particular tampon fibers is available. The purpose of the present study was to compare the effects of polyacrylate rayon tampons and cotton-viscose rayon blend tampons on the qualitative bacterial counts obtained from tampons and concomitant vaginal swabs and to determine whether either of these tampon types alters the qualitative makeup of 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 as described previously (A. B. Onderdonk, G. R. Zamarchi, M. L. Rodriguez, M. L. Hirsch, A. Muñoz, and E. H. Kass, Appl. Environ. Microbiol. 53:2774–2778). The genus and species of the six dominant bacterial species in each sample were identified, if possible. A statistical evaluation of the qualitative makeup of the microflora revealed that the same numerically dominant phenotypes were present regardless of sample type, sample time, or catamenial product. Predictable changes in total numbers among the dominant species were also noted when the data were evaluated by day of menstrual cycle. The correlation between the total numbers of each dominant species present was evaluated by day of cycle, and the findings are discussed.

Previously published reports describing normal human vaginal microflora have not adequately described either the changes occurring during menstruation or the qualitative composition of the microflora. Bartlett et al. (1) evaluated both areas by studying a small group of volunteers. These volunteers submitted self-obtained swabs during a single menstrual cycle, including the menstrual period. The most noteworthy finding was that 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.

Sautter and Brown (10) sampled a small number of volunteers several times throughout 1 month. They found that the variety of bacteria isolated from any individual remained relatively constant but that the numbers of each microbial species varied at different times in the cycle.

A more extensive study was conducted by Johnson et al. (3) in which they sampled 34 women during both the menstrual and intermenstrual phases of the cycle by a vaginal wash method. Qualitative differences in the microflora during the menstrual cycle were evaluated during this study, and it was noted that a greater variety of organisms were isolated from the menstrual samples than from the intermenstrual samples.

The effect of tampon use was not evaluated in any of these studies, although 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. Smith et al. (11) did find a significant association between menstruation and the recovery of *Staphylococcus aureus*, but found no difference in the rate of colonization with this organism attributable to the use of a specific catamenial product. The results, however, were reported in terms of frequency of isolation and did not include any quantitative data.

The most extensive published report to date on this question has been the work done in our laboratory (8). The qualitative results indicate that the concentrations of lactobacilli declined during menstruation but that otherwise there was little qualitative difference among the various species found during menstruation compared with those isolated from samples obtained intermenstrually.

Our previous studies evaluated only all-cotton tampons. The present study assessed the qualitative microflora during the use of cotton-viscose rayon blend 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 composition are as follows: Regular, 100% bleached cotton; Super, 70% cotton and 30% viscose rayon; Super Plus, 100% polyacrylate rayon.

Experimental design. Women were asked to volunteer to be monitored through successive menstrual cycles. Samples were obtained on days 2, 4, and 21 after the start of menstrual flow in the form of tampons and concomitant self-obtained vaginal swabs, as described previously (7). Each participant changed pads or tampons according to her usual pattern except on days when samples were obtained.

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 as described previously (7).

* Corresponding author.

TABLE 1. Average isolation frequency and counts by day and sample type

Sample and organism	Day 2		Day 4		Day 21	
	%[a]	Count[b]	%	Count	%	Count
Tampon						
Staphylococcus spp.	96.62	6.49	97.80	6.70	95.16	4.80
Lactobacillus sp.	84.99	6.45	84.49	6.43	87.59	7.33
Bacteroides spp.	82.91	5.19	83.81	5.07	73.45	4.01
Corynebacterium sp.	58.05	3.76	56.53	3.72	61.69	3.38
Peptococcus spp.	56.82	3.88	51.28	3.45	53.21	3.19
Streptococcus spp.	54.62	3.87	58.76	4.19	39.51	2.01
E. coli	45.55	2.55	49.46	2.67	26.99	1.58
Anaerobic *Streptococcus* spp.	32.08	2.17	30.51	2.03	35.67	2.02
G. vaginalis	21.01	1.71	19.16	1.58	13.42	1.18
Vaginal swab						
Staphylococcus spp.	95.98	6.73	98.75	7.17	96.17	5.66
Bacteroides spp.	89.23	5.88	88.17	6.02	73.63	4.60
Lactobacillus sp.	78.49	6.21	83.56	7.08	90.92	7.91
Peptococcus spp.	63.54	4.43	66.94	4.79	57.23	3.65
Corynebacterium sp.	59.31	4.15	62.75	4.54	58.60	3.84
Streptococcus spp.	57.17	3.91	58.32	4.30	45.29	2.71
Anaerobic *Streptococcus* spp.	41.12	2.79	37.44	2.70	42.79	2.74
E. coli	40.98	2.35	57.63	3.27	36.46	2.12
G. vaginalis	16.37	1.06	21.24	1.86	13.31	1.50

[a] Percentage of samples from which organism was recovered.
[b] Log_{10} CFU per gram.

Sample processing. At the various sample times explained above, vaginal swabs and tampons were obtained for processing as described previously (8). Briefly, 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 difference by using the average weight of the particular tampon type (Super = 2.88 ± 0.12 g, $n = 50$; Super Plus = 3.56 ± 0.15 g, $n = 50$). 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).

Bacteriological analysis. 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 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 in either 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.

Facultative gram-positive cocci were identified by established criteria (4). 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 further species identification. Aerobic, gram-positive, sporeforming catalase-positive rods were classified as *Bacillus* spp. Catalase-positive, gram-positive pleomorphic rods were classified as *Corynebacterium* spp. No further classification was performed for facultative *Lactobacillus*, *Corynebacterium*, or *Bacillus* species. Gram-positive or gram-variable, catalase-negative pleomorphic rods showing beta-hemolysis on HBT agar were presumptively identified as *G. vaginalis*. This preliminary identification was confirmed by using a modification of the criteria established by Piot et al. (9).

Obligate anaerobes were classified by gas-liquid chromatographic analysis of glucose fermentation products and antibiotic susceptibility patterns as performed by standard procedures (2, 12). Final identification included the use of the Anastat II system of biochemical tests and a computer data base (Scott). The concentrations of organisms recovered from the tampon and swab samples were expressed as

TABLE 2. Average frequency of isolation and counts by product and sample type

Sample and organism	% of samples yielding organism (log_{10} CFU/g)[a]				
	Regular	S2	S6	SP2	SP6
Tampon					
Staphylococcus spp.	98.04 (5.69)	96.20 (5.97)	96.24 (5.98)	93.05 (5.78)	97.22 (6.17)
Lactobacillus sp.	83.58 (6.53)	91.16 (7.04)	84.96 (6.62)	85.00 (6.55)	85.13 (6.56)
Bacteroides spp.	73.41 (4.47)	80.59 (4.66)	83.24 (4.82)	78.11 (4.28)	86.45 (5.02)
Peptococcus spp.	63.11 (4.29)	63.21 (3.86)	55.11 (3.58)	43.89 (2.66)	49.26 (3.15)
Corynebacterium sp.	56.98 (3.38)	60.74 (3.55)	55.91 (3.63)	57.61 (3.43)	61.22 (3.19)
Streptococcus spp.	42.89 (2.45)	46.47 (3.03)	54.08 (3.58)	53.28 (3.49)	50.93 (3.49)
E. coli	30.68 (1.75)	43.60 (2.01)	52.32 (2.79)	36.77 (2.02)	21.73 (2.34)
Anaerobic *Streptococcus* spp.	22.06 (1.39)	32.79 (2.09)	44.24 (2.81)	31.61 (1.91)	38.63 (2.48)
G. vaginalis	18.26 (1.52)	17.58 (1.42)	23.04 (1.83)	8.16 (0.71)	16.50 (1.40)
Vaginal swab					
Staphylococcus spp.	91.91 (5.94)	100.00 (6.77)	100.00 (6.81)	95.83 (6.45)	97.09 (6.63)
Lactobacillus sp.	77.69 (6.58)	91.26 (7.72)	85.26 (7.08)	82.22 (6.93)	85.19 (7.04)
Bacteroides spp.	77.23 (5.26)	84.39 (5.37)	88.07 (6.05)	83.44 (5.25)	85.13 (5.58)
Peptococcus spp.	71.20 (4.82)	63.20 (4.30)	56.49 (4.09)	57.50 (3.85)	64.46 (4.40)
Corynebacterium sp.	62.99 (4.27)	58.17 (3.97)	64.93 (4.78)	56.11 (3.87)	58.90 (4.01)
Streptococcus spp.	57.10 (3.79)	51.80 (3.46)	55.27 (3.77)	54.72 (3.69)	49.07 (3.48)
E. coli	35.05 (2.09)	48.00 (2.54)	55.11 (3.11)	40.78 (2.50)	46.19 (2.66)
Anaerobic *Streptococcus* spp.	30.64 (1.99)	43.16 (2.78)	49.67 (3.54)	39.83 (2.65)	38.96 (2.76)
G. vaginalis	18.38 (1.11)	20.14 (1.71)	21.85 (1.85)	9.50 (1.39)	14.99 (1.33)

[a] Regular, cotton; S2 and S6, viscose rayon at 2 and 6 h, respectively; SP2 and SP6, polyacrylate rayon at 2 and 6 h, respectively.

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.

RESULTS

Comparison of qualitative data by frequency of isolation. A total of 805 tampon and swab samples were processed during this study. A mean of 8.72 facultative isolates and an equal number of obligately anaerobic isolates were characterized per sample for a total of 15,225 separate isolates. The distribution of isolates and frequency of isolation were similar to the results published previously, with over 100 separate phenotypes being represented among the 15,225 isolates (8). The most frequent isolates were categorized by groups and were remarkably similar regardless of day, sample type, or product. The percentage of samples from which each phenotypic group was isolated and the mean counts by day and sample type are shown in Table 1. For tampon samples obtained on day 2, the most frequent isolates were *Staphylococcus* spp. (96.62% of samples), *Lactobacillus* sp. (84.99%), *Bacteroides* spp. (82.91%), *Corynebacterium* sp. (58.05%), *Peptococcus* spp. (56.82%), facultative *Streptococcus* spp. (54.62%), and *Escherichia coli* (45.55%). On all sample days, *Staphylococcus* spp. and *Lactobacillus* sp. were the first and second most frequent isolates from tampon samples, respectively.

Vaginal swab samples obtained from the same subjects concomitant with tampon samples and swab samples from subjects using catamenial pads revealed a somewhat different order of frequency of isolation when compared with tampon samples as described above. The most frequent isolate for all sample days was *Staphylococcus* spp. On days 2 and 4, *Bacteroides* spp. was more frequently isolated than *Lactobacillius* sp. from these samples (89.23 and 88.17%, respectively). *Corynebacterium* sp. and *Peptococcus* spp. were isolated with almost equal frequency (57.23 to 66.94%) on the various sample days. *E. coli* was most frequently isolated on day 4 (57.63%). Facultative *Streptococcus* spp. were most frequently isolated on day 4 (58.32%) and least frequently on day 21 (45.29%).

An analysis of frequency of isolation by product and sample type is shown in Table 2. When evaluating frequency of isolation by product irrespective of day, *Staphylococcus* spp. were the most common isolates for all tampons. *Lactobacillus* sp. were isolated with similar frequency from all products, as were *Corynebacterium*, *Peptococcus*, and

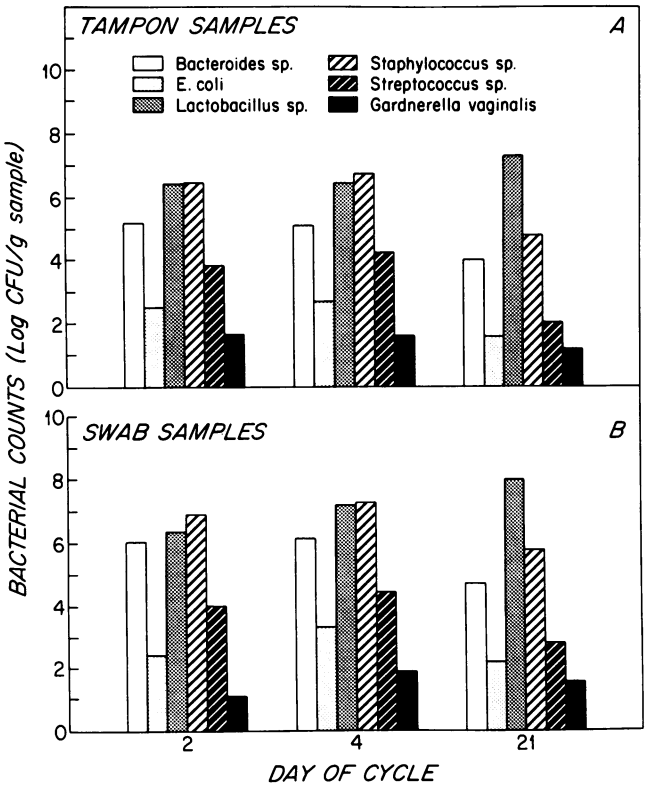

FIG. 1. Total counts for most frequently isolated organisms by day.

TABLE 3. Direction of correlation between organisms on days 2, 4, and 21

Day and organism	*Bacillus* sp.	*Bacteroides* spp.	*Corynebacterium* sp.	*E. coli*	*G. anaerobia*	*Klebsiella* spp.	*Lactobacillus* sp.	*Peptococcus* spp.	*Peptostreptococcus* spp.	*Propionibacterium* spp.	*Staphylococcus* spp.	*Streptococcus* spp.	*Streptococcus* spp. (ANO_2)[a]	*Candida* spp.	*G. vaginalis*
Day 2															
Bacillus sp.			+												
Bacteroides spp.						+	−	+	+					−	+
Corynebacterium sp.				−				+			−	−		−	
E. coli						−		−			+	+			
G. anaerobia										+					
Klebsiella spp.									+						
Lactobacillus sp.											−	+			
Peptococcus spp.									+			+		−	+
Peptostreptococcus spp.															
Propionibacterium spp.												−	−		+
Staphylococcus spp.												+	−		+
Streptococcus spp.															
Streptococcus spp. (ANO_2)															
Candida spp.															
G. vaginalis															
Day 4															
Bacillus spp.													+		
Bacteroides spp.					+	+		+	+		+			−	−
Corynebacterium sp.						−		+							
E. coli									−		+	+			−
G. anaerobia															
Klebsiella spp.									+		+				
Lactobacillus sp.									−	+		+			
Peptococcus spp.									+			+	+	−	
Peptostreptococcus spp.															
Propionibacterium spp.															+
Staphylococcus spp.												+			
Streptococcus spp.													−		−
Streptococcus spp. (ANO_2)														−	
Candida spp.															+
G. vaginalis															
Day 21															
Bacillus spp.															
Bacteroides spp.			+	+			−	+	+		+	+	+		+
Corynebacterium sp.				−	+	−	+	+							
E. coli							−				+	+	+		
G. anaerobia								+							
Klebsiella spp.												+			+
Lactobacillus sp.											−	−			
Peptococcus spp.															
Peptostreptococcus spp.															
Propionibacterium spp.															
Staphylococcus spp.													+		
Streptococcus spp.															
Streptococcus spp. (ANO_2)															
Candida spp.															
G. vaginalis															

[a] ANO_2, Anaerobic.

Bacteroides spp. Few differences could be seen when comparing frequency of isolation by product type. *E. coli* was isolated more often from vaginal swabs and tampons from women using Super tampons for 2 or 6 h, and anaerobic *Streptococcus* spp. were also more often isolated from these samples. A more detailed evaluation of *E. coli* isolation rates revealed that the frequency of isolation from vaginal swabs and tampon samples of women using Super tampons for 2 or 6 h was greatest on days 2 and 4 (data not shown). The frequency of isolation of *G. vaginalis* irrespective of product ranged from 13.31% of observations on day 21 to 21.24% on day 4. *G. vaginalis* was most commonly observed in women

using Super tampons for 6 h and women using catamenial pads.

Comparison of qualitative data by counts. Although the frequency of isolation changes little regardless of sample day, sample time, or product, the total counts for the most frequently isolated organisms changed considerably when compared by day (Fig. 1). For tampon samples, the counts for *Lactobacillus* sp. made this the numerically dominant isolate on day 21 ($10^{7.33}$). *Staphylococcus* spp. counts were equal to *Lactobacillus* sp. counts on day 2 ($10^{6.5}$), slightly higher on day 4 ($10^{6.70}$ versus $10^{6.43}$), and significantly lower on day 21 ($10^{4.80}$ versus $10^{7.33}$). Interestingly, the counts for *Bacteroides* spp. declined from day 2 to day 21 ($10^{5.19}$ versus $10^{4.01}$). A similar decline was noted for *Peptococcus*, *Streptococcus*, and *Corynebacterium* spp. and *E. coli*.

Trends similar to those described for tampon samples were noted for the corresponding vaginal swab samples. Swab samples tended to yield somewhat higher counts than the same sample groups and times described for the various tampon products (Fig. 1).

The correlation between the counts for the most frequently isolated organisms was performed by day, irrespective of sample type or product. Positive and negative correlations were listed if $P \leq 0.001$. These data, shown in Table 3, indicate that a negative correlation exists for counts between many of the anaerobic species and *Lactobacillus* sp. regardless of day. A similar relationship appears to be present between *E. coli* and *Lactobacillus* sp. and between *Lactobacillus* sp. and *Staphylococcus* spp. There also appears to be a positive correlation between *Candida* spp. and *G. vaginalis* on day 4, but a negative correlation between *Candida* and *Peptococcus* spp. for days 2 and 4. Not surprisingly, there appears to be a positive correlation between counts for *Bacteroides* spp. and most of the other anaerobic species, regardless of day.

S. aureus was isolated from 15 of 805 samples and at some point from 7 of the 18 subjects. One volunteer yielded *S. aureus* on five occasions, one volunteer yielded it on two occasions, and the rest were from single samples. No particular sample time, product, or sample type predominated.

DISCUSSION

Qualitative changes in the vaginal microflora were evaluated in the present study and were tabulated by day, product, and sample type. The most frequently isolated species were similar for all sample days, regardless of sample type and product. The numerically dominant species were members of the genera *Lactobacillus* and *Staphylococcus*, with members of the genera *Bacteroides*, *Peptococcus*, *Streptococcus*, and *Peptostreptococcus* serving as major sources for frequent isolation. Several interesting observations resulted from the qualitative assessment of the microflora. It was noted that *Bacteroides* spp. were isolated in higher numbers on days 2 and 4 than on day 21. A similar trend was noted for many of the other anaerobic species as well. Although the frequency of isolation varied little by day, the decline in total populations of certain species suggests that major shifts in environmental factors occur during menstruation. Clearly, factors such as E_h, pH, and undissociated short-chain fatty acid concentrations can serve as potent control mechanisms for microbial populations in vivo. Since the E_h is thought to be lowest for intermenstrual samples, anaerobiosis per se does not appear to be a factor in these observed differences. It is of interest to note that anaerobic populations for species other than *Lactobacillus* were highest during the early stages of menstrual flow, a time at which tampons are most likely to be changed with greatest frequency (with the possible introduction of oxygen into the vaginal vault). The apparent resilience of the anaerobic microflora under these conditions suggests that pH and undissociated short-chain fatty acid concentrations are more important control mechanisms for obligate anaerobes than previously thought.

Although well over 100 phenotypes were characterized from vaginal swabs and tampon samples during these studies, the same phenotype appeared with apparent consistency in virtually all samples, regardless of subject. The exceptions to this were *E. coli* and other facultative gram-negative rods, which were often isolated for several consecutive cycles and then were not detected again. The increased frequency of isolation of *E. coli* during use of Super tampons and at higher counts than for other products warrants further study.

S. aureus was isolated infrequently, although it was isolated from 7 of 18 subjects during this study. *S. aureus* followed a pattern similar to that noted for *E. coli* in one subject and was a sporadic isolate in the other six volunteers. None of the strains of *S. aureus* isolated during these studies produced toxic shock syndrome toxin-1 when tested.

G. vaginalis was detected by the use of selective media and identified as described previously. This organism was present in 8.16 to 23.04% of samples, depending on day and product. The suppressive effect of the Super Plus tampon on *G. vaginalis* suggests that the lower frequency of isolation during use of this product was related to the extremely low counts normally present in the vaginal vault of healthy women. The positive correlation between the counts of *G. vaginalis* and *Candida* spp. suggests that these two organisms proliferate under similar environmental conditions.

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 the data provided by this study. Similarly, many of the relationships shown by the correlation matrix are the subject of ongoing research to determine the nature of the various relationships and the control mechanisms which govern the complex ecosystem present within the vaginal vault. At this time, it does not appear that tampon use significantly alters the normal qualitative 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.

LITERATURE CITED

1. **Bartlett, J. G., A. B. Onderdonk, E. Drude, C. Goldstein, M. Anderka, S. Alpert, and W. M. McCormack.** 1977. Quantitative bacteriology of the vaginal flora. J. Infect. Dis. **136**:271–277.
2. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
3. **Johnson, S. R., C. R. Petzold, and R. P. Galask.** 1985. Qualitative and quantitative changes of the vaginal microbial flora during the menstrual cycle. Am. J. Reprod. Immunol. **9**:1–5.
4. **Lennette, E. M., A. Balows, W. J. Hausler, Jr., and J. P. Truant.** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
5. **Morris, C. A., and D. F. Morris.** 1967. "Normal" vaginal microbiology of women of childbearing age in relation to the use of oral contraceptives and vaginal tampons. J. Clin. Pathol.

20:636–640.

6. **Onderdonk, A. B., B. F. Polk, N. E. Moon, B. Goren, and J. G. Bartlett.** 1977. Methods for quantitative vaginal flora studies. Am. J. Obstet. Gynecol. **122**:777–781.
7. **Onderdonk, A. B., G. R. Zamarchi, M. L. Hirsch, A. Muñoz, and E. H. Kass.** 1987. Quantitative assessment of vaginal microflora during the use of tampons of various compositions. Appl. Environ. Microbiol. **53**:2774–2778.
8. **Onderdonk, A. B., G. R. Zamarchi, J. A. Walsh, R. D. Mellor, A. Muñoz, and E. H. Kass.** 1986. Methods for quantitative and qualitative evaluation of vaginal microflora during menstruation. Appl. Environ. Microbiol. **51**:333–339.
9. **Piot, P., E. Van Dyck, P. A. Totten, and K. K. Holmes.** 1982. Identification of *Gardnerella* (*Haemophilus*) *vaginalis*. J. Clin. Microbiol. **15**:19–24.
10. **Sautter, R. L., and W. J. Brown.** 1980. Sequential vaginal cultures from normal young women. J. Clin. Microbiol. **11**:479–484.
11. **Smith, C. B., V. Noble, R. Bensch, P. A. Ahlin, J. A. Jacobsen, and R. H. Lathon.** 1982. Bacterial flora of the vagina during the menstrual cycle. Ann. Intern. Med. **96**:948–951.
12. **Sutter, V. L., V. L. Vargo, and S. M. Finegold.** 1975. Wadsworth anaerobic bacteriology manual, 2nd ed. University of California Press, Los Angeles.