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Quantitative Vaginal Microflora in Women Convalescent from Toxic Shock Syndrome and in Healthy Controls

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We performed sequential and quantitative vaginal cultures obtained from 8 women within 4 days to 3 years (average, 15.8 months) after their recovery from classic menstrual toxic shock syndrome (TSS) and from 11 healthy women who served as age-matched controls. Apart from tampon use, which was significantly less frequent in TSS women after their acute illness, no demographic differences were observed in the two groups. Significantly lower total aerobic and anaerobic bacterial counts were found in TSS women than in healthy controls ($P < 0.05$, Mann-Whitney test). These differences were most profound during the menstrual (aerobes) and premenstrual (aerobes and anaerobes) sample times, whereas no difference in bacterial counts was observed in the mid-cycle samples. Although the less frequent usage of tampons among TSS women after their acute illness might explain the lower aerobic counts in menstrual specimens, this is unlikely to explain the significantly lower aerobic and anaerobic counts observed in premenstrual samples when tampons were not used in either group. It is possible that these differences in the quantitative vaginal microflora were a direct result of recent TSS in these women. Alternatively, disruption of the normal indigenous microflora could have predisposed these women to acute TSS by alteration of the resistance of vaginal colonization to pathogenic microorganisms.

Although toxinogenic *Staphylococcus aureus* is almost invariably isolated from vaginal cultures of women with menstrual toxic shock syndrome (TSS), the nature and quantitation of other components of the vaginal microflora are seldom characterized. Sanders et al. (3) recently postulated that ecological imbalances within the genital microflora might favor colonization by *S. aureus* and predispose women to TSS. We report here our findings of the quantitative vaginal microflora in 8 women who recovered from definite menstrual TSS and 11 healthy university women who served as age-matched controls. These data support the hypothesis that the normal indigenous microflora of the genital tract may be disrupted in women who have had TSS thus increasing their susceptibility to vaginal colonization by pathogenic microorganisms.

MATERIALS AND METHODS

All eight fulfilled the case definition of menstrual TSS (2), and vaginal cultures were obtained within 4 days to 3 years (average, 15.8 months) after recovery from acute illness during 1980 to 1983. Control women were recruited from the University of British Columbia Student Health Service, and cultures were obtained from them during the same study period (April through September 1983). Except for tampon use, which was significantly less frequent among TSS women after their acute illness, there were no other differences in the demographic characteristics of these two groups (Table 1). One patient had received antibiotics during an acute episode of TSS. None of the others had received antibiotics within 2 weeks before or throughout the duration of the study. Each woman was studied for at least one menstrual cycle on specified cycle days (days 5 ± 2, 15 ± 2, and 21 ± 2).

Vaginal cultures were obtained with prereduced, anaerobically sterilized (PRAS) swabs from the lateral walls and posterior vaginal fornix and processed immediately in an anaerobic chamber. After elution and vortexing in 1 ml of PRAS VPI diluent salt solution (6), three 100-fold serial dilutions were prepared and 0.1-ml samples were plated in PRAS medium and in routine medium. These media included MacConkey agar, mannitol salt agar, PRAS brain heart infusion agar supplemented with 10% defibrinated sheep blood and vitamin K-hemin (0.01%, vol/vol), and brain heart infusion agar containing vancomycin and kanamycin (7.5 and 75 μg/ml, respectively). Anaerobic plates were retained within the anaerobic chamber and incubated for 7 days. Aerobic plates were taken out of the chamber and incubated for 48 h in 5% CO_2 or air. Colony types were enumerated and identified. This quantitative culture technique was highly reproducible, as was demonstrated by duplicate sampling in three subjects (correlation coefficient, 0.994). Isolates from menstrual (day 5 ± 2) specimens were further identified to species level, and obligate anaerobes were identified with PRAS differential medium and by gas-liquid chromatographic analysis of fatty acid fermentation products (6). Identification of obligate anaerobes to species level was done by the method of Holdeman and Moore (6), and that of aerobes was done by standard procedures. Bacterial counts from TSS and control women (expressed as $\log_{10}$ CFU per milliliter of diluent) were compared at various phases of the menstrual cycle by the nonparametric Mann-Whitney rank sum test (5). Menstrual cultures were also compared with paired mid-cycle and premenstrual cultures in TSS and control women by the Wilcoxon paired rank sign test (5).

RESULTS

The mean $\log_{10}$ CFU of aerobic and anaerobic vaginal isolates from the two groups are shown in Fig. 1. The total aerobic counts were significantly lower in TSS women in both menstrual (day 5 ± 2) cultures ($P < 0.01$, Mann-

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TABLE 1. Demographic characteristic of TSS and control women

Characteristic	TSS women (n = 8)	Controls (n = 11)	P
Mean age ± SD (yr)	27.7 ± 6.8	23.1 ± 2.5	NS
Contraceptive method (no.)			NS
Pill	3	6	
Intrauterine device	2	2	
Other	2	1	
None	1	2	
Catemenial product (no.)			<0.05
Tampon	3	10	
Napkin	5	1	
Sexual activity			NS
Mean no. of partners past mo	0.75	0.91	
Mean no. of partners past yr	1.0	1.2	

[a] NS, Not statistically significant.

Whitney test) and in premenstrual (day 21 ± 2) cultures ($P < 0.05$). Similarly, total anaerobic counts were significantly lower in TSS women, but only for premenstrual cultures ($P < 0.025$). No significant differences were noted for the mid-cycle (day 15 ± 2) samples.

For both TSS and control groups, the quantitative vaginal flora in menstrual cultures were further compared with those in paired mid-cycle and premenstrual cultures, respectively. No significant differences were observed.

The specific species and genera of quantitative vaginal microflora obtained from menstrual cultures for either group was summarized in Table 2. Complete data were available from 6 of 8 TSS women and from 7 of 11 control women. Apart from the total absence of *Gardnerella vaginalis* in the TSS group ($P < 0.01$, Fisher exact test), no other significant differences were noted.

TABLE 2. Quantitative vaginal microflora from menstrual (day 5 ± 2) cultures of TSS and control women

Organism	Log_{10} CFU in[a]: TSS women (n = 6)	Controls (n = 7)
Aerobes		
Diphtheroids	4 (4.7)	5 (6.0)
Lactobacilli	3 (4.3)	3 (6.3)
Gardnerella vaginalis	0	5 (5.6)
Escherichia coli	2 (5.5)	4 (4.5)
Other *Enterobacteriaceae*	1 (5.0)	2 (1.5)
Streptococci	2 (4.0)	5 (5.4)
Staphylococcus aureus	3 (4.0)	2 (4.0)
S. epidermidis	5 (3.8)	7 (4.1)
Anaerobes		
Peptostreptococcus intermedius	1 (4.0)	2 (6.0)
P. anaerobius	0	2 (6.0)
P. micros	2 (4.5)	2 (3.5)
P. prevotii	1 (4.0)	1 (4.0)
Peptococcus magnus	0	2 (5.0)
P. asaccharolyticus	0	1 (5.0)
Gaffkya anaerobia	0	1 (5.0)
Veillonella parvula	2 (4.5)	2 (7.5)
Propionibacterium acnes	0	1 (7.0)
Eubacterium lentum	0	1 (7.0)
Lactobacillus casei	1 (6.0)	0
L. fermentum	1 (6.0)	0
Clostridium ramosum	2 (6.5)	0
Bifidobacterium adolescentis	1 (6.0)	0
Bacteroides capillosus	1 (7.0)	1 (8.0)
B. asaccharolyticus	1 (5.0)	1 (6.0)
B. melaninogenicus	0	1 (4.0)
B. bivius	2 (5.5)	0
Unidentified gram-negative bacilli	1 (6.0)	3 (5.3)

[a] Numbers of women from whom isolates were recovered are shown in parentheses. The mean concentration is expressed as log_{10} CFU per milliliter of vaginal diluent.

DISCUSSION

Our sequential studies of quantitative vaginal microflora indicated significant differences in both total aerobic and anaerobic bacterial counts between TSS and control women at various phases of the menstrual cycle. These differences were most profound for menstrual (aerobes) and premenstrual (both aerobic and anaerobic) cultures, whereas no differences were noted for mid-cycle specimens. Apart from the absence of *G. vaginalis* in the TSS group, no other differences among individual genera or species could be detected, owing to the small number of patients studied. The reason for lower total aerobic or anaerobic bacterial counts in TSS women is not known. These differences remained significant even when the one TSS patient who had received antibiotics during acute illness was excluded from analysis. Although the less-frequent usage of tampons among TSS

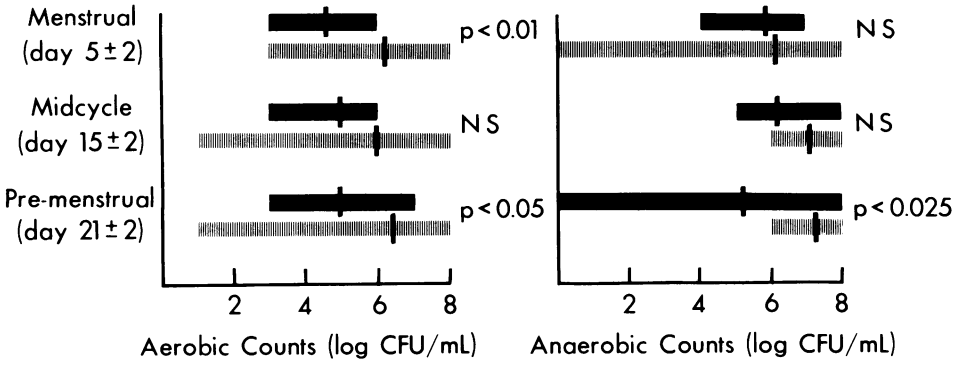

FIG. 1. Total aerobic and anaerobic counts (mean and range log_{10} CFU per milliliter) in vaginal microflora of 8 TSS (solid bars) and 11 control (hatched bars) women at different days of menstrual cycle. Comparisons of results between TSS and control women were done by the nonparametric Mann-Whitney rank sum test. NS, Not statistically significant.

women might explain the lower aerobic counts in menstrual specimens, this is unlikely to account for the significantly lower aerobic and anaerobic counts observed in premenstrual samples when tampons were not used in either group.

Sequential quanitative cultures of the vaginal microflora during the menstrual cycle have been infrequently studied. Both Bartlett et al. (1) and Sautter and Brown (4) have noted that, despite great variations of specific bacteria in multiple samples of a given individual, there is remarkable consistency in recovery of major categories of organisms from a particular subject. We noted similar findings in both TSS and control women. It is of interest that Sautter and Brown (4) also observed the high prevalence and predominance of *G. vaginalis* in sequential quantitative vaginal cultures from seven normal young women. These studies raise the question of a protective role of *G. vaginalis* as normal vaginal flora. Except for the single TSS patient during the acute episode, none of the TSS or control women were symptomatic throughout the duration of the study. Unlike Bartlett et al. (1), we did not find a significant difference in total aerobic counts between premenstrual and menstrual samples in either TSS or control women.

Since the indigenous microflora appears to be a major defense mechanism against colonization by pathogenic organisms, it is possible that these differences in quantitative vaginal microflora were a direct result of recent TSS in these women. Alternatively, disruption of the normal indigenous microflora could have predisposed these women to acute TSS by alteration of the resistance of vaginal colonization to pathogenic microorganisms. Further efforts to characterize quantitatively and sequentially the complete vaginal flora in both healthy and TSS women appear to be warranted, particularly if the effect of tampon use can be better delineated by prospective studies in which tampon or napkin usage is randomly assigned.

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