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Site-specific prevalence and cell densities of selected microbes in the lower reproductive tract of menstruating tampon users

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Objective: To assess differences in prevalence and cell densities of enterococci, Gram negative enterics (GNEs), yeast and *Staphylococcus aureus* among four genital sites and to examine whether the presence of organisms at one site affected the presence of organisms at other sites.

Methods: Swab samples from the perineum, below and above the hymen, and the posterior fornix obtained from 52 tampon users on menstrual cycle day 3 were analyzed for site-specific prevalence and cell densities of microorganisms.

Results: Enterococci and GNEs were the most prevalent study organisms at all sites and decreased in prevalence from the perineum to the posterior fornix. Cell densities similarly decreased from below the hymen to the posterior fornix. Yeast were detected at the hymen only; *S. aureus* frequency was similarly low at all sites. Yeast and *S. aureus* site-specific cell densities were similar. The above- and below-hymen sites were similar in prevalence and cell density of organisms. An above-chance association existed between the presence of any study organism below the hymen and above the hymen and was strongest for GNEs.

Conclusions: The pattern of genital colonization with enterococci and GNEs reflects their likely gastrointestinal source. The absence of significant differences in the prevalence and cell densities of study microflora above and below the hymen combined with an above-chance association of the presence of microorganisms at these regions suggests that the regions above and below the hymen are not different with respect to the presence of the organisms evaluated in this study.

Key words: *STAPHYLOCOCCUS AUREUS*; ENTEROCOCCI; GRAM NEGATIVE ENTERICS; YEAST; HYMEN

The female genital microflora represents a dynamic ecosystem that fluctuates in response to a variety of influences[1–11]. Host factors, such as age, hormonal status, sexual behavior and parity all play a role. Bacterial vaginosis (BV) is associated with pronounced shifts in the endogenous flora, and agents such as systemic antibiotics, topical antifungal preparations, spermicides and douches may profoundly or selectively alter the balance of organisms present. Menstrual products do not cause broadscale qualitative or quantitative changes in the composition of vaginal flora[11–14] but effects on selected organisms have been observed[15,16].

Studies that examined the prevalence and relative proportions of microorganisms in the lower genital tract of women have produced disparate results[4,15,17,18]. Variability in the methods of specimen collection and culture techniques, changes in vaginal microbiology over the course of the menstrual cycle, the absence of controls for conditions that alter the endogenous flora and non-uniformity in site of sampling are likely contributing factors.

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Niche specialization may exist along the lower genital tract[5,19]. Colonization may be influenced by anatomical and epithelial-receptor differences, redox potential, pH, and proximity to the external genitalia and perineum. If so, specimens collected at a single genital site may not be representative of the entire microbial community.

Few studies have examined more than one site in the same individual and of these, several focused on a single organism[17–23]. Studies that have delineated microbial changes over the menstrual cycle[1,7,17,18,24–26] or the influence of menstrual products on genital microflora[27] have typically focused on a single vaginal site, self-obtained swabs, or vaginal washes.

This study was an investigation of the prevalence and cell densities of selected microorganisms at multiple sites in the lower genital tract. We examined a single time point during menstruation in tampon users while excluding potential confounding factors such as BV, medications, and use of topical or personal cleansing products that may affect the genital microflora. The objective was to assess differences in isolation frequency and density of selected microbes between different genital sites and to examine the possibility that colonization with any particular organism at an individual site may have affected the presence of microorganisms at the other sites examined.

SUBJECTS AND METHODS

This was a single-center study to characterize selected microbes of the lower genital tract during menses in tampon users aged 18–45 from the population of Dallas, Texas. An Institutional Review Board reviewed the protocol and all subjects signed an informed consent statement. Seventy-nine subjects were screened for eligibility, 55 enrolled and completed the study, and 52 subjects were included in the dataset.

Ten days prior to the anticipated start of the menstrual period, subjects were screened for eligibility by means of medical history and habits and practices questionnaires. They were instructed to use their own tampons exclusively for menstrual protection and to wear a tampon on day 3 of the cycle for at least 2 hours but not more than 8 hours prior to their scheduled examination. On the day of their study examination (day 3 of the menstrual cycle), additional screening for BV and chlamydia, gonorrhea, and trichomoniasis was performed.

Eligible subjects were in good health (as evidenced by their medical and gynecologic history), had a gynecological exam with negative pap smear within the past 2 years, had regular (± 3 days) menstrual cycles of 21–35 days in length, typically used ten or more tampons per menstrual period, and had menstrual flow on day 3 of the cycle that allowed for the use of a tampon for at least 2 and not more than 8 hours. Women were excluded if they were pregnant or trying to conceive; had an active medical condition such as diabetes, hepatitis, AIDS or HIV positive status; were currently using, intended to use or had used immunosuppressive drugs, chemotherapeutic agents or antibiotics within the past 6 weeks; had a history of endometrial disease, fibroids, genital herpes or toxic shock syndrome (TSS); had abnormalities of the vulva or had genital warts or lesions; had used antifungal suppositories within the prior 6 weeks or vaginal spermicides within a week of study commencement; were unwilling to refrain from douching or the use of topical products (powders, perfumes, deodorants) in the genital area from time of enrollment through to the end of the study; were unwilling to refrain from sexual intercourse for 24 hours prior to their study examination; or were found to have BV, presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* or a clinically-diagnosed active yeast infection at study commencement.

Microbiological sampling for identification of *Staphylococcus aureus*, enterococci, Gram negative enterics (GNEs) and yeast (speciated to *Candida albicans*, *C. glabrata*, *C. tropicalis* or other, if present) was performed on day 3 of a single menstrual cycle. After removal of the tampon, subjects were placed in a lithotomy position. Sterile, polyester applicator swabs (FALCON #220690 Becton Dickinson, Franklin Lakes, NJ) were used to obtain samples in sequence from four sites: a 2 cm^2 area of the perineum close to the anal area (not including the anus); the right side of the introitus (just below the hymen where the labia minora meet the vaginal opening); just above the hymeneal ring (approximately 2–3 cm past the introitus); and at the posterior fornix. Dry swabs were used to obtain the

hymen and fornix samples; moistened swabs (sterile phosphate buffered saline, PBS) were used to obtain the perineum sample. Hymen and fornix swabs were preweighed (± 0.001 g) before samples were obtained. Each site was swabbed for approximately 10 seconds by rotating the swab so that the entire swab surface made contact with the site of sampling. Care was taken to avoid contamination from the external genitalia and cross-contamination from other sampling sites. The vaginal surface above the hymen was accessed by gentle, manual manipulation of the vaginal opening, avoiding contact of the sampling site by the fingers. A speculum was then inserted midway into the vagina for visualization and sampling of the posterior fornix, beyond the top of the speculum.

After sampling the four test sites, the pH was measured (Baxter pHIX, range 3.6–6.4, JT Baker Company, Phillipsburg, NJ) at the mid-right lateral wall. Individual swab samples from the right wall of the vagina were then obtained for assessment of BV, *T. vaginalis*, *N. gonorrhoeae* and *C. trachomatis*, respectively.

All samples were processed within 30 minutes of collection. Swabs from below the hymen, above the hymen and posterior fornix were re-weighed before processing. All swabs were diluted in 5 ml of PBS, vortexed for 10 seconds and plated on selective agar. Colonies growing on Mannitol Salt agar (BBL 4321173) were presumptively identified by typical colonial morphology and Gram stain as staphylococci; subsequently *S. aureus* was identified using the Remel BactiStaph System (Remel, Lenexa, KS). Colonies growing on Mycosel Agar (BBL 4321847) were identified as *C. albicans* if germ-tube positive. Germ-tube negative organisms growing on Mycosel agar were identified as yeast, not *C. albicans*. Non-albicans yeast, when present, were identified as *C. glabrata*, *C. tropicalis* or 'other', using API20C (bioMerieux Vitek, Hazlewood, MO). Colonies isolated on Enterococcosel agar (BBL 212205) were presumptively identified as enterococci by typical colony morphology and Gram stain, and biochemically confirmed with the Remel Bacticard Strep system (Remel, Lenexa, KS). GNEs were isolated on MacConkey II (BBL 212306) agar and identified by typical colony morphology, lactose fermentation and Gram stain. Microbial cell densities were determined using an automated dilution/spiral plating methodology (Autoplater 4000, Spiral Biotech, Norwood, MA), and based on the number of colonies on each plate whose identity was confirmed and reported as colony forming units (CFU) per cm^2 (perineum) or gram (all other sites).

Because the presence of menses precluded the use of pH and discharge as diagnostic criteria, the diagnosis of BV was made based on the presence of fishy amine odor upon addition of potassium hydroxide to the vaginal sample and the presence of clue cells. Trichomoniasis was diagnosed using the wet-mount diagnostic method; gonorrhea and chlamydia were diagnosed using the GEN-PROBE® PACE® 2 system (Gen-Probe Incorporated, San Diego, Ca.).

The Wilcoxon signed-rank test and the stratified pairwise Cochran–Mantel–Haenszel (CMH) test were used for comparative analysis of the data obtained. Significance was assigned at the two-sided $p \leq 0.05$ level. The potential relationship between the presence of an organism at one site and its presence at another site was assessed using the kappa statistic. A kappa value of 0.4–0.75 indicated moderate agreement and a kappa value > 0.75 indicated strong agreement.

RESULTS

Population demographics

Of the 79 subjects screened, none had BV, trichomoniasis, chlamydia, or gonorrhea. Fifty-five met eligibility criteria and enrolled in and completed the study. Two were removed from the set of evaluable subjects because of unexplained discrepancies in the weight of samples for microbial analysis. A third subject was dropped for failing to comply with the protocol (wore a tampon for less than 2 hours prior to examination).

Demographic data are summarized in Table 1. Seventy-one percent of the subjects ($n = 37$) were Caucasian, 25% ($n = 13$) were African-American and 4% ($n = 2$) were Hispanic. Subjects ranged in age from 19–44 years with a mean of 33.2 ± 7.3 years. Mean weight was 75.15 ± 17.7 kg with a weight range of 49.5–113.6 kg. Mean height was 1.65 ± 0.07 meters, with a height range of 1.5–1.8 meters.

Table 1 Demographic characteristics in 52 evaluable study subjects

Characteristic	
Age	Years
Mean ± SD	33.2 ± 7.3
Range	19–44
Race	*n* (%)
Caucasian	37 (71)
Black	13 (25)
Hispanic	2 (4)
Height	Meters
Mean ± SD	1.65 ± 0.07
Range	1.5–1.8
Weight	Kilograms
Mean ± SD	75.2 ± 17.7
Range	49.5–113.6
Contraceptive methods	*n* (%)
None	4 (8)
Oral contraceptive	13 (25)
Spermicide and condoms	2 (4)
Condoms	9 (17)
Condoms and rhythm	1 (2)
Rhythm	1 (2)
Tubal ligation	9 (17)
Tubal ligation and abstinence	1 (2)
Vasectomy	3 (6)
Abstinence or withdrawal	9 (17)

SD, standard deviation

Oral contraceptives (OC) were used by 25% of subjects (see Table 1). Condoms, tubal ligation, and abstinence or withdrawal were the next most frequently used methods, each reported at 17% prevalence. The most common choices of tampon absorbency were Regular (6–9 g) and Super (9–12 g).

Vaginal pH and prevalence and quantitative assessment of microorganisms

Mean vaginal pH was 5.6 ± 0.08 (standard error of the mean, SEM) and ranged from 4.1–6.1. The prevalence of genital microflora detected at each anatomic site is summarized in Table 2. The enterococci and GNEs were the most prevalent organisms at all sites, but their frequency decreased along the genital tract from the perineum, across the hymen, to the posterior fornix of the vagina. Colonization with yeast species was detected below the hymen in five subjects (two with *C. albicans* and three with other species); *C. albicans* was found at the posterior fornix in another subject. The frequency of *S. aureus*, when found, was similar at all sites. Two subjects colonized with this organism were colonized at more than one genital site: one subject was colonized at all four sites; the other was colonized above and below the hymen. The cell densities of genital microflora detected at each anatomic site are also summarized in Table 2. Quantitative differences in the cell densities of flora between sites (other than the perineum) were 1.3 $\log_{10}$ units/g or less for all organisms studied.

Pairwise comparisons of the prevalence of microorganisms at each site among all study subjects (Table 3) revealed a statistically higher prevalence of enterococci and GNEs at the perineum relative to all other sites ($p \leq 0.018$, stratified CMH test). The prevalence of enterococci and GNEs was lower at the posterior fornix relative to the two hymeneal sites: statistical significance was reached when comparing the sampling sites below the hymen and at the fornix ($p = 0.018$ and $p = 0.025$ for enterococci and GNEs, respectively) and approached when comparing above the hymen to the posterior fornix ($p = 0.052$ and $p = 0.059$ for enterococci and GNEs, respectively). There were no significant differences in isolation frequency of any organism above or below the hymen and no significant differences in the prevalence of yeast and *S. aureus* among any of the evaluated sites. The study had at least 80% power to detect a minimum difference in prevalence of 15% between sites at the 0.05 level of statistical significance.

Pairwise comparisons of the cell density of microorganisms below the hymen, above the hymen, and the posterior fornix (mean of all subjects including those for whom the organism was absent, Table 4), revealed significantly higher cell densities of enterococci below the hymen compared to the posterior fornix ($p = 0.005$, Wilcoxon Signed Rank test) and significantly higher cell densities of GNEs below and above the hymen relative to the fornix ($p = 0.001$ and $p = 0.007$, respectively). Cell density of enterococci was not statistically significantly different above the hymen compared to the posterior fornix ($p = 0.055$). No significant differences in bacterial cell densities

Table 2 Prevalence and $\log_{10}$ counts of microbes at selected genital sites

		Prevalence	*Cell density in positive cases*	
Microorganism	*Site*	n *(%)*	*Mean **	*SEM*
Enterococci	Perineum	28 (53.8)	3.1	0.16
	Below hymen	19 (36.5)	4.8	0.19
	Above hymen	16 (30.8)	4.2	0.16
	Posterior fornix	8 (15.4)	4.3	0.47
GNE	Perineum	25 (48.1)	2.6	0.16
	Below hymen	14 (26.9)	4.5	0.32
	Above hymen	14 (26.9)	4	0.21
	Posterior fornix	9 (17.3)	3.2	0.21
Staphylococcus aureus	Perineum	4 (7.7)	4	0.33
	Below hymen	3 (5.7)	4.5	0.46
	Above hymen	2 (3.8)	3	0.02
	Posterior fornix	2 (3.8)	4.6	0.04
Candida albicans	Perineum	0 (0)	—	—
	Below hymen	2 (3.8)	3.4	0.58
	Above hymen	0 (0)	—	—
	Posterior fornix	1 (1.9)	3	—
Other yeast	Perineum	0 (0)		
	Below hymen	3 (5.7)	3.3	0.36
	Above hymen	0 (0)	—	—
	Posterior fornix	0 (0)	—	—

*Mean cell densities expressed as $\log_{10}$ cfu/cm^2 (perineum) and $\log_{10}$ cfu/g (other sites); SEM, standard error of the mean; GNE, Gram negative enterics

between locations below and above the hymen were observed for any organism evaluated. Also, no significant differences in bacterial cell densities between hymeneal sites and the fornix were observed for yeast and *S. aureus*.

The potential relationship between the presence of an organism at one site and its presence at any other site was assessed using the kappa statistic (Table 5). There was strong above-chance association (i.e., a predictive agreement) between the presence of GNEs below and above the hymen (weighted kappa = 0.805) and a moderate above-chance association in the presence of any individual organism at these two sites (weighted kappa = 0.574). There was no predictive agreement of the presence (or absence) of an organism among other sites.

DISCUSSION

This study examined the isolation frequency and cell density of selected microorganisms in the lower genital tract of menstruating tampon-users at progressively distal locations from the perianal area. The enterococci and GNEs were chosen as indicators of potential colonization derived from the intestinal tract; yeast species and *S. aureus* represent endogenous flora of potential pathological significance. We controlled for several variables known to influence the microbiology of the genital tract: a single time point during menstruation[9] (day 3 of the cycle) was examined in healthy women who had no current or recent history of medication or spermicide use[8], who refrained from using douches[28] or other personal products in the

Table 3 Pairwise comparison of microorganism prevalence at selected genital sites (all study subjects, $n = 52$)

Microorganism	*Sites compared (site 1 vs. site 2)*	*Site 1* n (%)	*Site 2* n (%)	p-*value* *
Enterococci	Perineum vs. below hymen**	28 (54.9)	19 (36.5)	**0.018**
	Perineum vs. above hymen**	28 (54.9)	16 (30.8)	**0.003**
	Perineum vs. posterior fornix**	28 (54.9)	8 (15.7)	**0**
	Below hymen vs. above hymen	19 (36.5)	16 (30.8)	0.467
	Below hymen vs. posterior fornix**	19 (36.5)	8 (15.7)	**0.018**
	Above hymen vs. posterior fornix**	16 (30.8)	8 (15.7)	0.052
GNE	Perineum vs. below hymen	25 (48.1)	14 (26.9)	**0.008**
	Perineum vs. above hymen	25 (48.1)	14 (26.9)	**0.008**
	Perineum vs. posterior fornix	25 (48.1)	9 (17.3)	**0.001**
	Below hymen vs. above hymen	14 (26.9)	14 (26.9)	1
	Below hymen vs. posterior fornix	14 (26.9)	9 (17.3)	**0.025**
	Above hymen vs. posterior fornix	14 (26.9)	9 (17.3)	0.059
Staphylococcus aureus	Perineum vs. below hymen	4 (7.7)	3 (5.8)	0.705
	Perineum vs. above hymen	4 (7.7)	2 (3.8)	0.414
	Perineum vs. posterior fornix	4 (7.7)	2 (3.8)	0.414
	Below hymen vs. above hymen	3 (5.8)	2 (3.8)	0.564
	Below hymen vs. posterior fornix	3 (5.8)	2 (3.8)	0.655
	Above hymen vs. posterior fornix	2 (3.8)	2 (3.8)	1
Candida albicans	Perineum vs. below hymen	0 (0)	2 (3.8)	0.157
	Perineum vs. above hymen	0 (0)	0 (0)	—
	Perineum vs. posterior fornix	0 (0)	1 (1.9)	0.317
	Below hymen vs. above hymen	2 (3.8)	0 (0)	0.157
	Below hymen vs. posterior fornix	2 (3.8)	1 (1.9)	0.564
	Above hymen vs. posterior fornix	0 (0)	1 (1.9)	0.317
Other yeast	Perineum vs. below hymen	0 (0)	3 (5.8)	0.083
	Perineum vs. above hymen	0 (0)	0 (0)	—
	Perineum vs. posterior fornix	0 (0)	0 (0)	—
	Below hymen vs. above hymen	3 (5.8)	0 (0)	0.083
	Below hymen vs. posterior fornix	3 (5.8)	0 (0)	0.083
	Above hymen vs. posterior fornix	0 (0)	0 (0)	—
Any organism	Perineum vs. below hymen	43 (82.7)	30 (57.7)	**0.003**
	Perineum vs. above hymen	43 (82.7)	27 (51.9)	**0**
	Perineum vs. posterior fornix	43 (82.7)	18 (34.6)	**0**
	Below hymen vs. above hymen	30 (57.7)	27 (51.9)	0.366
	Below hymen vs. posterior fornix	30 (57.7)	18 (34.6)	**0.007**
	Above hymen vs. posterior fornix	27 (51.9)	18 (34.6)	**0.02**

*p-values for site comparisons were based on Stratified Cochran–Mantel–Haenszel test;
**$n = 51$; GNE, Gram negative enterics

genital area, and who were willing to refrain from intercourse[8] for 24 hours prior to evaluation. Subjects with chlamydia, gonorrhea, trichomoniasis, clinically diagnosed active yeast infection, or BV were excluded to increase the likelihood that any site-to-site variability occurred within a presumptively 'normal' endogenous microbiological environment[9,11].

The mean vaginal pH of 5.6 in this study was consistent with that found by other investigators during the first few days of the menstrual cycle[29]. The menstrual period is a time of increased

Table 4 Pairwise comparisons of $\log_{10}$ counts at selected genital sites (mean of all study subjects, $n = 52$)

Microorganism	*Sites compared (site 1 vs. site 2)*	*Site 1 mean (SEM)*	*Site 2 mean (SEM)*	p-*value* *
Enterococci	Below vs. above hymen	1.8 (0.33)	1.3 (0.28)	0.192
	Below vs. posterior fornix**	1.7 (0.33)	0.7 (0.23)	**0.005**
	Above vs. posterior fornix**	1.2 (0.27)	0.7 (0.23)	0.055
GNE	Below vs. above hymen	1.2 (0.29)	1.1 (0.25)	0.144
	Below vs. posterior fornix	1.2 (0.29)	0.6 (0.17)	**0.001**
	Above vs. posterior fornix	1.1 (0.25)	0.6 (0.17)	**0.007**
Staphylococcus aureus	Below vs. above hymen	0.3 (0.15)	0.1 (0.08)	0.370
	Below vs. posterior fornix	0.3 (0.15)	0.2 (0.12)	1
	Above vs. posterior fornix	0.1 (0.08)	0.2 (0.12)	0.625
Candida albicans	Below vs. above hymen	0.1 (0.09)	0 (0)	0.5
	Below vs. posterior fornix	0.1 (0.09)	0.1 (0.06)	0.75
	Above vs. posterior fornix	0 (0)	0.1 (0.06)	1
Other yeast	Below vs. above hymen	0.2 (0.11)	0 (0)	0.25
	Below vs. posterior fornix	0.2 (0.11)	0 (0)	0.25
	Above vs. posterior fornix	0 (0)	0 (0)	—

*p-values for site comparisons were based on Wlicoxon Signed-Rank test; **$n = 51$; GNE Gram negative enterics; SEM, standard error of the mean

microbiological variability in the lower genital tract[3,8,9,27]. A greater variety of organisms are recovered at this time[1,7,12,17], irrespective of the type of menstrual product used[12,13,26].

The enterococci and GNEs were the most frequently isolated organisms in the present study and the pattern of genital colonization was consistent with the intestinal tract as a source. Specifically, the isolation frequency of enterococci and GNEs was inversely related to the distance from the anus: frequencies were significantly higher at the perineum than all other sites, and lower at the posterior fornix than the hymenal sites.

Enterococci are recovered more often during menses than nonmenstrually[3,7,26]. In the present study, the 15% isolation frequency of enterococci at the posterior fornix contrasts with a reported rate of 38% in vaginal samples from 242 menstruating pad users evaluated on days 2–3 of the cycle, using the same sampling method[26]. Wide variability of enterococci prevalence has been found in studies with less well-characterized populations, menstrual product usage, and sampling times. For example, enterococci prevalence rates of 1.4% were found in the vaginal fornix of 145 women with normal discharge[15], compared with 15% in swab samples of the posterior vagina taken from 22 women at any time during the menstrual cycle[24].

Like the enterococci, GNEs (and *Escherichia coli* in particular) have been isolated more frequently during menses or in the first half of the menstrual cycle[3,6,14,15,26,30]. Colonization with GNEs was significantly more common in pad users than tampon users[14,15,30]; because pads cover the perineal area, the higher frequency of recovery of vaginal enterococci and GNEs in pad users is consistent with the gastrointestinal tract as the source of colonization.

The cell densities of enterococci and GNEs isolated from the posterior fornix and above the hymen sites ($\log_{10}$ 3.2–4.3/g) were comparable to those found vaginally during menstruation in pad users[26] but were 2–3 $\log_{10}$ units per gram lower than reported in earlier vaginal flora studies[18,24]. Direct comparison of cell density with historical data is complicated by the fact that previous studies did not always specify sampling times or sampling methodologies, and host-related variables were not as rigorously defined.

Yeast species were found in six of the subjects (11%). Although up to 20% of women may be asymptomatically colonized with *Candida* at any

Table 5 Kappa analysis ($n = 52$)

Microorganism	*Comparison*	*Weighted Kappa (values based on Kappa analysis)*
Enterococci	Perineum vs. below hymen	0.29
	Perineum vs. above hymen	0.262
	Perineum vs. posterior fornix	0.217
	Below hymen vs. above hymen	0.271
	Below hymen vs. posterior fornix	0.122
	Above hymen vs. posterior fornix	0.278
GNE	Perineum vs. below hymen	0.334
	Perineum vs. above hymen	0.334
	Perineum vs. posterior fornix	0.132
	Below hymen vs. above hymen	**0.805**
	Below hymen vs. posterior fornix	0.725
	Above hymen vs. posterior fornix	0.614
Staphylococcus aureus	Perineum vs. below hymen	–0.071
	Perineum vs. above hymen	–0.054
	Perineum vs. posterior fornix	–0.054
	Below hymen vs. above hymen	0.371
	Below hymen vs. posterior fornix	–0.048
	Above hymen vs. posterior fornix	–0.04
Candida albicans	Perineum vs. below hymen	0
	Perineum vs. above hymen	—
	Perineum vs. posterior fornix	0
	Below hymen vs. above hymen	0
	Below hymen vs. posterior fornix	–0.025
	Above hymen vs. posterior fornix	0
Other yeast	Perineum vs. below hymen	0
	Perineum vs. above hymen	—
	Perineum vs. posterior fornix	—
	Below hymen vs. above hymen	0
	Below hymen vs. posterior fornix	0
	Above hymen vs. posterior fornix	—
Any organism	Perineum vs. below hymen	0.188
	Perineum vs. above hymen	0.211
	Perineum vs. posterior fornix	0.135
	Below hymen vs. above hymen	0.574
	Below hymen vs. posterior fornix	0.266
	Above hymen vs. posterior fornix	0.430

GNE, Gram negative enterics

time[3,7,31], factors that increase vulvovaginal colonization or infection with yeast (such as pregnancy, antibiotic treatment, diabetes or compromised immune status) were not present in the study population. Vaginal colonization with *Candida* during day 2–3 of menstruation was reported at up to 5% in a population of pad users in which the aforementioned host variables were controlled[26]. It is unlikely that contraceptive choices affected the prevalence rate of yeast in the present study. Of the six subjects colonized with yeast, one used OCs, two used condoms, and three had undergone tubal

ligation. Hormonally mediated increases in yeast are most frequently associated with high-estrogen-content OCs that are no longer commonly prescribed and recent studies indicate OC use has a minimal effect on the endogenous flora[10]. Cell densities of *C. albicans* in the present study were lower than has been reported previously[32].

The vulva is the preferred genital carriage site of *S. aureus*[33,34], with reported prevalence rates as high as 67%[33]. There is a statistical association between vulvar and vaginal carriage of this organism[7,21] but, using traditional culturing techniques, *S. aureus* is recovered only sporadically from the vagina and is more consistently found there during menstruation[16,17,26,27,35]. Using current culturing techniques, vaginal colonization rates at any time during the cycle reportedly range between 2 and 30%[7,16,21,22,26,27,35,36]. Our results are consistent: detection of vaginal *S. aureus* during menses was approximately 4% and two of four subjects were colonized with *S. aureus* at more than one genital site. Molecular techniques that classify via genotype may reveal higher prevalence rates[37]. The cell densities of vaginal *S. aureus* were 2–3 $\log_{10}$ units lower than has been previously reported[18,24].

The microbiology of the female genital tract is a dynamic ecosystem. By analogy to environmental ecosystems, local anatomical and physiological variables, as well as competition between microorganisms, may alter the qualitative or quantitative colonization of various regions of the genital tract. However, we found only two studies that examined more than one organism at multiple genital sites in the same subjects[18,19]. These studies suggested that the cervix might be a distinct ecological niche: substantial differences in cervical and vaginal colonization were seen in the same individuals[18,19]. Anatomical differences, pH, the presence of columnar epithelium on the ectocervix, the influence of age and oral contraceptives on cervical ectopy and changes induced by parturition may selectively influence microbial colonization of this site. For example, *C. trachomatis* and *N. gonorrhoeae* preferentially infect the cervix, while the group B streptococci[20] and *Mycoplasma hominis*[38] are isolated less frequently from the cervix than the vagina.

Despite the common assumption of niche specialization, few studies have systematically attempted to correlate the presence or absence of particular organisms at a single site with colonization of another genital site, and only single species have been investigated[20–23]. The present study examined enterococci, GNEs, yeast and *S. aureus* at multiple sites. We found the agreement between the presence or absence of selected microorganisms among the sites did not exceed chance alone, with one notable exception: when locations just below and just above the hymen were considered, there was a moderate above-chance association between the presence of any of the study organisms just below the hymen and the presence of any study organism directly above the hymen. This relationship (evaluated via the kappa analysis) was strongest for the GNEs. Furthermore, the isolation frequency of evaluated organisms at these two sites did not differ and there were no quantitative differences in the cell densities of any individual species between them. Taken together, these observations suggest that with respect to these organisms, the regions of the vagina just below and just above the hymen are not microbiologically distinct.

This investigation was conducted exclusively among tampon users. Studies indicate that tampon use has little effect on the overall qualitative or quantitative composition of the vaginal flora[11–14,27]. It might be speculated that the tampon removal cord that extends from the vagina could be a conduit for organisms from the external genitalia, but there is no evidence that this is the case. On the contrary, results from this and other studies suggest that vaginal colonization with externally derived enterococci and GNEs is more common when pads are used[14,15,30].

In summary, there was a decreasing prevalence of enterococci and GNEs from the perineum to the posterior fornix of the vagina, with similar prevalence of these organisms across the hymen sites. Prevalence of yeast was restricted to the above- or below-hymen sites; *S. aureus* was present at similarly low prevalence rates at all four sites. Prevalence and cell density above the hymen was similar to prevalence and cell density below the hymen for enterococci, GNEs, yeast, and *S. aureus*. The results suggest that the regions just above and below the hymen are not different with respect to the presence of these organisms. Our results are

consistent with previous studies showing that the enterococci, GNEs, yeast and *S. aureus* are part of the endogenous genital flora in a well-defined population of healthy women. The examined flora was recovered at lower levels in this defined population than reported previously among women drawn from the population at large.

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