Effect of Menstrual Cycle and Different Age on Vaginal Microflora of Healthy Women

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Abstract The composition of vaginal microflora of forty healthy women whose ages ranged from 20 to 50 years (menstrual cycle: estrogenic phase, 15 persons; luteal phase, 25 persons) was anaerobically and aerobically determined. The total numbers in the vaginal specimens obtained from all the subjects were approximately 10⁹ CFU/g of vaginal secretions. Numbers of anaerobic bacteria in the vaginal specimens were higher than those of aerobic bacteria. Lactobacilli were the predominant microorganisms in the vaginal specimens. The numbers of bacteroides, streptococci, and *Gardnerella vaginalis*, and incidences of staphylococci and peptostreptococci were increased with aging whereas the numbers of lactobacilli decreased with aging.

Key words: aging; menstrual cycle; vaginal microflora

The vaginal microflora has interested many investigators (1, 3, 7, 10) to recognizing of its significant association with vaginal tract infection. Early studies on the vaginal microflora utilized the stained smears of vaginal secretions, because the taxonomic guidelines were limited and cultural methods were incomplete. In the late 1800s, some workers thought that "Doderlein's bacilli" were the main microorganisms in the vagina. With subsequent studies and improved methods for culture and differentiation of bacteria, numerous bacterial species were detected as common or infrequent members of the vaginal microflora (16, 20).

Yeasts or *Trichomonas vaginalis* as pathogens of the vagina and cervix vaginalis have been commonly recognized. Recently, *Chlamydia trachomatis* has been established as one of the causes of vaginal discharge (21). On the other hand, disease state belonging to the category commonly known as nonspecific vaginitis (NSV) also exists (6). The term "NSV" is used to describe the vaginitis of which specific etiological agents (the genera of *Candida*, *Trichomonas*, and *Chlamydia*) cannot be demonstrated. Although many investigators have studied the cause of NSV, this problem has not yet been solved. To understand the pathogenesis of NSV, there-

fore, a closer look at the vaginal microflora in healthy women is required.

The present study was undertaken to analyze the quantitative and qualitative compositions of vaginal microflora in healthy women and to determine the effects of menstrual cycle and age on the human vaginal microflora.

MATERIALS AND METHODS

Volunteers. Vaginal secretions were collected with a sterile sponge from 40 healthy women aged 20 to 50 years. Of forty vaginal specimens, 15 samples were collected on the estrogenic phases from the end of menstruation to the 10th day after the end of menstruation and the remaining 25 samples were collected on the luteal phase from the 11th day after the end of menstruation to the beginning of menstruation.

Collection of vaginal specimens. The sterile sponge was weighed before use. After the sample processing, the sponges absorbing vaginal fluid were placed into a sterile transport medium (14) filled with 100% CO₂ gas atmosphere. Samples sealed into the medium were kept at 4°C until processed. Sample weight was determined by subtracting the combined weight of the tubes, buffer, and sterile sponge.

Culture media and methods. The method for bacterial analysis of the vaginal microflora in this study was essentially the same as that of Mitsuoka et al (13). After thorough mixing, a series of 10-fold dilutions (10^{-1} to 10^{-7}) was made in anaerobic diluents (12). From appropriate dilutions, 0.05 ml aliquots were spread onto three non-selective agars: modified Eggerth-Gagnon (EG) agar for anaerobes (13); glucose liver blood (BL) agar for anaerobes (13); trypticase soy blood (TS) agar (BBL Microbiology Systems, Cockeysville, Md.) for aerobes (13), as well as eleven selective agars: bifidobacteria selective (BS) agar for bifidobacteria (13); eubacteria selective (ES) agar for eubacteria (12); neomycin-brilliant green-taurocholate-blood (NBGT) agar for bacteroides (13); neomycin-Nagler (NN) agar for lecithinase-positive clostridia (13); modified veillonella selective (VS) agar for veillonellae and megasphaerae (13); modified lactobacilli selective (LBS) agar for lactobacilli (13); peptone-starch-glucose (PSG) agar for *Gardnerella vaginalis* (4); triphenyltetrazolium chloride-acridine orange-thallosulfate-aesculin-crystal violet (TATAC) agar for enterococci and streptococci (13); phenylethyl alcohol egg yolk suspension (PEES) agar for staphylococci and micrococci (13); potato dextrose (PD) agar (Difco, Detroit) for yeasts and molds (13); and deoxycholate hydrogen sulfide lactose (DHL) agar (Eiken, Tokyo) for enterobacteria (13). Nine agars (EG, BL, BS, NBGT, ES, NN, VS, LBS, and PSG) were incubated at 37°C for 2 days in an anaerobic steel wool jar (19) filled with an atmosphere of oxygen-free CO₂. Two agars (TATAC and PEES) were incubated aerobically at 37°C for 48 hr; TS and DHL agars were incubated at 37°C for 24 hr.

The isolates from vaginal specimens were identified by Gram stain, colony and cell morphology, aerobic growth, spore formation, and catalase test. For the bacterial species identified, the bacterial count per g of vaginal contents was

calculated and converted into a logarithmic equivalent. The total viable count was calculated from the sum of the counts of each bacterial species.

RESULTS

The composition of vaginal microflora of the 40 healthy women employed is shown in Table 1. The total bacterial counts were 10^9 CFU/g of vaginal secretions. Lactobacilli were isolated predominantly except for one specimen. Eubacteria and bifidobacteria were also isolated in higher number, but less commonly found than lactobacilli.

The changes of the vaginal microflora during the menstrual cycle are also given in Table 1. Lactobacilli were isolated predominantly throughout both estrogenic and luteal phases. The number of eubacteria was also high during the menstrual cycle. The incidences and the numbers of bacteroides and peptostreptococci were at moderate levels throughout the phases. However, bifidobacteria was found at the luteal phase only.

A relationship between the age and the composition of vaginal microflora is shown in Table 2. The kinds of microorganisms isolated from younger women were less than those of the older ones. Three bacterial groups—bifidobacteria, veillonellae, and enterobacteria—were not isolated from healthy women aged 20 to 29 years. The numbers of bacteroides, eubacteria, staphylococci, streptococci, and *G. vaginalis*, and frequencies of occurrences of staphylococci and peptostrepto-

Table 1. Effect of menstrual cycle on the vaginal microflora of healthy women

Microflora	Total (n=40)	Menstrual cycle	
		Estrogenic phase (n=15)	Luteal phase (n=25)
Anaerobic bacteria			
Bacteroides	6.5 ± 2.6^a (58) ^b	6.4 ± 3.1 (47)	6.5 ± 2.6 (64)
Eubacteria	8.6 ± 2.4 (15)	9.5 ± 1.2 (13)	8.1 ± 2.8 (16)
Peptostreptococci	6.4 ± 2.3 (55)	7.0 ± 2.3 (60)	5.4 ± 2.2 (52)
Bifidobacteria	7.4 ± 2.6 (13)	≤ 2.0 (0)	4.4 ± 2.6 (20)
Veillonellae	3.5 ± 0.3 (8)	3.3 ± 0.2 (13)	3.8 (4)
Lecithinase-negative clostridia	3.0 (3)	≤ 2.0 (0)	3.0 (4)
Lactobacilli	9.0 ± 1.1 (70)	8.5 ± 1.4 (67)	9.2 ± 1.1 (72)
Aerobic bacteria			
Enterobacteria	4.4 ± 1.4 (23)	4.3 ± 1.5 (33)	5.3 ± 1.9 (16)
Streptococci	5.6 ± 2.1 (60)	6.2 ± 2.3 (53)	5.3 ± 2.0 (64)
Staphylococci	4.2 ± 1.1 (55)	4.1 ± 1.1 (53)	4.2 ± 1.2 (56)
Corynebacteria	5.6 ± 1.9 (45)	5.3 ± 1.5 (47)	5.8 ± 2.2 (44)
<i>Gardnerella vaginalis</i>	6.7 ± 1.9 (33)	6.8 ± 1.9 (27)	6.7 ± 2.1 (36)
Yeasts	6.5 ± 2.1 (23)	5.0 ± 0.3 (13)	7.0 ± 2.2 (28)
Total bacteria ^c	9.4 ± 0.9	8.9 ± 1.2	9.5 ± 0.8

^a Mean $\pm$ SD of log bacterial counts/g of vaginal specimens.

^b Frequencies of occurrences (%).

^c Megasphaerae, lecithinase-positive clostridia, and bacilli were detected (≤ 2.0).

Table 2. Effect of aging on the vaginal microflora of healthy women

Microflora	Age		
	20-29 years old (n=7)	30-39 years old (n=18)	40-50 years old (n=15)
Anaerobic bacteria			
Bacteroides	6.0±2.8 ^a (57) ^b	4.8±2.2 (50)	8.1±2.1 (67)
Eubacteria	3.9 (14)	9.0±0.5 (11)	9.9±0.4 (20)
Peptostreptococci	6.8±3.5 (29)	5.2±2.1 (67)	7.2±2.1 (53)
Bifidobacteria	≤2.6 (0)	7.1±2.9 (22)	8.7 (7)
Veillonellae	≤2.0 (0)	≤2.0 (0)	3.5±0.3 (20)
Lecithinase-negative clostridia	≤2.0 (0)	3.0 (6)	≤2.0 (0)
Lactobacilli	9.8±1.1 (86)	8.8±1.3 (67)	8.6±1.0 (67)
Aerobic bacteria			
Enterobacteria	≤2.0 (0)	4.3±1.5 (33)	4.1±1.7 (20)
Streptococci	3.5±0.3 (71)	5.8±2.0 (78)	6.8±2.4 (33)
Staphylococci	3.9±0.3 (43)	4.4±1.5 (44)	4.1±0.9 (73)
Corynebacteria	3.9 (14)	5.9±1.9 (50)	5.4±2.0 (53)
<i>Gardnerella vaginalis</i>	6.7±1.9 (33)	6.4±1.9 (39)	7.8±2.2 (27)
Yeasts	6.6±0.9 (43)	5.8±2.2 (28)	4.0 (7)
Total bacteria ^c	9.7±1.0	9.1±1.0	9.4±0.9

^a Mean±SD of log bacterial counts/g of vaginal specimens.

^b Frequencies of occurrences (%).

^c Megasphaerae, lecithinase-positive clostridia, and bacilli were detected (≤2.0).

cocci were increased with age, whereas the numbers and incidences of lactobacilli were not increased with age.

DISCUSSION

Although quantitative studies on the vaginal microflora of healthy women have been reported by several workers (1, 2, 9, 10), the small amount of vaginal secretion raises the question of sampling error. Bartlett et al (1) reported that the discrepancy of the composition of vaginal microflora was responsible for the difference of the recovery of total bacteria. Onderdonk et al (17) indicated that 10⁹–10¹⁰ bacteria per ml of vaginal specimen may be expected in the vaginal specimens. The results in the present study indicate that vaginal microflora of healthy women with a normal menstrual cycle tended to be constant and total bacterial counts averaged 10⁹ per g of vaginal secretions. Lactobacilli was isolated from the vaginal specimens as the predominant microorganism in the present study. If the number of lactobacilli decreased in the vagina, other microorganisms, such as bacteroides and/or eubacteria occurred in large numbers (10⁸–10⁹). Thus, lactobacilli in the vagina may be related to the stability of resident vaginal microflora.

Bartlett et al (2) showed the changes in the vaginal microflora during the menstrual cycle and showed that aerobic bacteria increased during menstruation. Some workers (2, 8, 18) have proposed that changes in pH, cellular content, hormonal and biochemical conditions of the vagina may be related to the changes of

the vaginal microflora during the menstrual cycle. Advanced age may also influence the change of hormonal secretions. Therefore, further studies are needed to confirm the relationship among the changes of the vaginal microflora at the species level, the menstrual cycle, and aging.

Gardner et al (5) reported that *G. (Haemophilus) vaginalis* is closely associated with bacterial vaginosis, which occasionally causes invasive diseases such as endometritis, amnionitis, and bacteremia (11, 15). It has been reported that this organism is often present in the vaginal flora as a potential pathogen (20). In the present study, *G. vaginalis* was recovered from 13 of 40 healthy women (33%).

The relationships between age and the composition of the vaginal microflora of healthy women have not been reported earlier. The findings in this study indicated that the incidence and number of lactobacilli in younger women were higher than that of older women. On the contrary, the number of *G. vaginalis* in younger women was lower than that of older women.

If the vaginal specimens are cultured anaerobically as a routine, the role of anaerobic bacteria in the vaginal microflora will be elucidated. It has been reported that the increasing prevalence of bacteroides and peptostreptococci indicates a disturbance of the normal flora (10). A difference on the numbers of bacteroides between younger women aged 20–29 years and older women aged 40–50 years was noted. We consider that these findings may be useful for the study of relationships between incidences of NSV and aging.

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