Quantitative bacteriology of the vaginal flora during the menstrual cycle

M. WILKS and SOAD TABAQCHALI

Department of Medical Microbiology, St Bartholomew's Hospital Medical College, West Smithfield, London EC1A 7BE

Summary. The vaginal bacteriology of 10 healthy asymptomatic women was assessed during the menstrual cycle. Samples were taken from the posterior vaginal fornix for quantitative analysis. There were no significant alterations in the total vaginal flora at different stages of the menstrual cycle. The mean number of species isolated per specimen declined from 4·6 in week 1 to 2·9 in week 4. This decline was not caused by a decrease in the occurrence or concentration of any one organism or group of organisms. The vaginal pH decreased from a mean of 6·6 in week 1 to 4·3 in week 4, this increased acidity could not be attributed to the action of lactobacilli as their total incidence or concentration did not change during the menstrual cycle.

Introduction

There have been two important changes in studies of the vaginal bacterial flora in recent years. First, methods more suitable for the recovery of anaerobic organisms were introduced (Gorbach *et al.*, 1973); and second, several methods of quantitative sampling were developed (Levison *et al.*, 1977, Onderdonk *et al.*, 1977; Linder *et al.*, 1978). Most studies, whether quantitative or qualitative, have been based on the analysis of a single sample at an arbitrary point in the menstrual cycle, although hormonal factors have long been considered to be an important influence on the vaginal flora (Cruickshank and Sharman, 1934).

If there were significant differences in the bacterial flora at different stages in the menstrual cycle, then vaginal flora studies would have to be standardised. Furthermore, this could have important implications for gynaecological surgery which could be timed to minimise the risks of endogenous infection.

To assess the occurrence of any variations in the vaginal flora, a group of asymptomatic women with a regular menstrual cycle was studied.

Patients and methods

Patients

Ten asymptomatic women volunteers who had a regular menstrual cycle of about 28 days and who were

not using oral contraception were studied. None had taken antimicrobial agents in the preceding month.

Sampling

The method of sampling was as described by Wilks *et al.* (1982). Briefly, samples were collected at weekly intervals from the posterior vaginal fornix, using a sterile metal loop, into preweighed tubes of a modified pre-reduced Cary-Blair medium.

Processing of specimens

Specimens, after reweighing, were placed in an anaerobic chamber and serially diluted in VPI dilution blanks (Holdeman *et al.*, 1977). For anaerobic incubation, samples (100 µl) were spread on to the surface of the following media which had been poured in the chamber: Brucella blood agar, neomycin (100 µg/ml)-blood agar and kanamycin (50 µg/ml)-vancomycin (5 µg/ml)-lysed blood agar, prepared according to the methods of Sutter *et al.* (1980) except that horse blood 10% v/v was substituted for sheep blood 5% v/v; Rogosa Agar (Difco) with a final pH of 5·4, as selective medium for lactobacilli. The dilution tubes were then removed from the chamber and 100-µl samples were spread on plates of the following media for aerobic cultivation: blood agar (Tissue Culture Services, 10 Henry Road, Slough, Berks), MacConkey Agar (Oxoid) and modified Thayer Martin Medium (Riddell and Buck, 1970). Anaerobic culture plates were incubated in the chamber for up to 7 days at 37°C and the aerobic plates for 2–3 days in an atmosphere of air + CO₂ 5%.

Different colony types were enumerated and subcultured on to fresh media for identification. Anaerobic organisms were identified by the methods of Holdeman *et al.* (1977). Biochemical tests were performed with Scott

prerduced sugars (Scott Laboratories, Rhode Island, USA). Aerobic and facultatively anaerobic organisms (henceforth referred to collectively as aerobes) were identified by standard microbiological techniques (Cowan, 1974). The pH of the vaginal secretions was measured with Merck narrow range pH papers (BDH, Poole, Dorset).

Results

The organisms isolated and the concentration at which they occurred are shown in the table. Fluctuations in the total bacterial counts and the counts of aerobes and anaerobes from week to week are shown in fig. 1.

The number of different isolates per specimen decreased significantly from a mean of 4.6 in week 1 to 4.0 in week 2, 4.1 in week 3 and 2.9 in week 4 (Kruskal-Wallis, $p < 0.05$). This decrease was accounted for by a general decline in the incidence of most types of bacteria and was not due to a particular decrease in one particular organism or group of organisms. The concentration of organisms isolated did not decrease. The incidence and concentration of lactobacilli are shown in fig. 2, together with the mean vaginal pH, which fell from a mean of 6.5 in week 1 to 4.6 in week 2 and 4.3 in weeks 3 and 4. The pH in week 1 was significantly higher than that recorded in the other weeks (Kruskal-Wallis; $p < 0.05$).

Discussion

The vaginal bacterial flora has long been considered to be indirectly controlled by oestrogens. The

Fig. 1. Mean bacterial counts during the menstrual cycle of 10 healthy women: ●, mean total bacterial count; ○, mean total anaerobic count; ■, mean total aerobic count.

Fig. 2. Occurrence and isolation of lactobacilli during the menstrual cycle: ●, mean total lactobacilli count; ■, mean aerobic lactobacilli count; ○, mean anaerobic lactobacilli count; ▲, pH; () number of specimens positive for lactobacilli.

production of oestrogen increases glycogen in the vaginal epithelium which is metabolised to glucose and then to lactic acid by the microbial flora consisting mainly of lactobacilli (Cruickshank and Sharman, 1934). The resultant acidity in the vagina selectively favours its colonisation by acidotolerant organisms such as lactobacilli. In the rat, there is evidence to show that oestrogen does control the number of bacteria present. Oestrogen treatment of ovariectomised rats results in an increase in bacterial counts of 10^3 – 10^4 (Galask *et al.*, 1976). However no correlation has been shown in man between hormones and the vaginal flora. The vagina does not provide a constant acidic environment, as is often stated. In this study there was a rapid decline in vaginal pH after the onset of menstruation from a mean value of 6.5 to 4.6. This could be due to the action of lactobacilli on the vaginal epithelium which is being shed, but there was no increase in the concentration of lactobacilli at this time. Furthermore, it has not been shown unequivocally that lactobacilli can produce acid from glycogen *in vitro* (Brown, 1982). The increase in the concentration of aerobic lactobacilli from week 1 to week 2 and a decrease in the concentration of anaerobic strains of lactobacilli may be correlated with the fall in pH. An acidic environment is considered to be especially unfavourable for anaerobes because an increase in hydrogen ion concentration is accompanied by an uptake of electrons and by the development of a more positive oxidation-reduction potential (Hentges and Maier, 1972). However no other organisms were affected.

Although hormone levels were not measured in this study, the subjects were chosen because they

Table. Bacteria isolated from the vagina of 10 healthy women during one menstrual cycle

Subject no.	Organism	Viable count (log ₁₀ cfu/ml) during week of menstrual cycle			
		1	2	3	4
1	<i>Lactobacillus</i> spp.	6.7	7.6	4.3	0
	Coryneforms	4.9	5.1	0	0
	<i>Staphylococcus epidermidis</i>	7.1	0	6.4	7.5
	* <i>Bifidobacterium</i> spp.	6.9	6.4	5.7	0
	* <i>Propionibacterium</i> spp.	7.3	0	0	0
	* <i>Peptostreptococcus anaerobius</i>	8.4	0	7.2	0
	* <i>Lactobacillus</i> spp.	0	5.7	5.5	6.7
	* <i>Bacteroides bivius</i>	0	0	0	8.2
	* <i>Clostridium perfringens</i>	0	0	6.8	0
2	<i>Lactobacillus</i> spp.	5.6	7.2	8.2	7.6
	<i>Gardnerella vaginalis</i>	6.9	0	0	8.1
	<i>Staphylococcus epidermidis</i>	4.7	0	6.8	7.2
	* <i>Lactobacillus</i> spp.	8.4	6.9	7.8	6.1
	* <i>Bacteroides melaninogenicus</i>	6.9	7.4	0	0
	* <i>Bifidobacterium</i> spp.	8.2	7.5	8.0	7.0
3	<i>Staphylococcus epidermidis</i>	8.5	6.6	5.8	6.2
	<i>Enterococcus faecalis</i>	6.4	0	0	0
	<i>Lactobacillus</i> spp.	0	5.7	7.2	0
	* <i>Lactobacillus</i> spp.	8.3	7.9	6.8	0
	* <i>Peptococcus prevotti</i>	6.4	7.3	8.4	8.2
	* <i>Bacteroides bivius</i>	0	6.9	6.9	5.8
4	<i>Lactobacillus</i> spp.	6.7	7.8	7.1	4.9
	<i>Gardnerella vaginalis</i>	7.2	5.3	0	0
	<i>Staphylococcus epidermidis</i>	6.9	7.2	7.5	7.6
	Coryneforms	8.2	6.5	0	0
	<i>Enterococcus faecalis</i>	6.4	0	0	0
	* <i>Lactobacillus</i> spp.	8.3	7.9	6.8	0
	* <i>Peptococcus prevotti</i>	6.4	7.3	8.4	8.2
5	Coryneforms	8.4	6.9	8.2	7.2
	<i>Staphylococcus epidermidis</i>	0	0	6.1	6.8
	* <i>Lactobacillus</i> spp.	8.1	8.2	8.4	8.9
	* <i>Eubacterium lentum</i>	5.9	0	0	0
	* <i>Bifidobacterium</i> spp.	4.7	6.9	7.2	7.8
	* <i>Peptostreptococcus productus</i>	8.6	8.7	6.8	0
	* <i>Peptostreptococcus</i> spp.	0	5.6	0	0
6	<i>Gardnerella vaginalis</i>	0	0	7.8	0
	<i>Lactobacillus</i> spp.	7.9	0	0	0
	* <i>Peptococcus asaccharolyticus</i>	8.4	8.9	8.2	6.1
	* <i>Propionibacterium</i> spp.	0	0	6.7	0
	* <i>Fusobacterium nucleatum</i>	0	6.1	0	0
	* <i>Clostridium</i> spp.	0	0	5.3	0
7	Coryneforms	6.9	7.6	5.9	7.3
	<i>Lactobacillus</i> spp.	4.9	7.3	7.9	7.1
	<i>Streptococcus</i> (group B)	0	0	5.3	0
	* <i>Lactobacillus</i> spp.	7.3	4.6	5.5	0
8	Coryneforms	7.8	6.5	5.6	6.3
	<i>Lactobacillus</i> spp.	8.2	7.8	0	0
	* <i>Bacteroides fragilis</i>	6.7	0	0	0
	* <i>Lactobacillus</i> spp.	7.3	0	8.6	6.9
	* <i>Clostridium difficile</i>	0	0	0	4.9
9	<i>Lactobacillus</i> spp.	8.5	8.3	8.2	8.9
	<i>Staphylococcus epidermidis</i>	6.2	5.9	7.8	6.7
	Coryneforms	6.5	6.1	0	7.2
	* <i>Bacteroides disiens</i>	7.2	7.6	8.3	9.2

Continued overleaf

Table. Bacteria isolated from the vagina of 10 healthy women during one menstrual cycle

Subject no.	Organism	Viable count (log ₁₀ cfu/ml) during week of menstrual cycle			
		1	2	3	4
10	Coryneforms	6.1	5.9	0	7.2
	<i>Enterococcus faecalis</i>	0	4.1	5.6	0
	* <i>Peptococcus asaccharolyticus</i>	7.6	6.3	6.8	0
	* <i>Peptostreptococcus magnus</i>	4.9	0	0	0
	* <i>Bacteroides disiens</i>	8.4	8.6	6.8	0
	* <i>Bacteroides melaninogenicus</i>	8.2	8.4	7.3	0

* Anaerobic isolates.

had a regular menstrual cycle and were not taking oral contraceptives; therefore any effect due to changes in hormone levels during the menstrual cycle should be readily apparent. Hormone fluctuations during the menstrual cycle may be too rapid for any effects on the bacterial flora to become apparent. However, recent qualitative studies of the vaginal flora, in which samples were taken from pre- and post-menopausal women showed no correlation between hormone status and bacterial growth (Osborne *et al.*, 1979; Mehta, 1982).

Lactobacilli, *Staphylococcus epidermidis*, *Bacteroides* spp, coryneforms bifidobacteria and anaerobic cocci were the most common organisms found in this study. The isolation of other organisms such as streptococci and *Gardnerella vaginalis* was unusual. Furthermore, these organisms did not appear to persist and they were rarely isolated from more than one sample per patient. The prevalence and concentration of organisms observed in this study is similar to that obtained in an earlier study based on single samples from 102 women (Wilks *et al.*, 1984). The apparent simplicity of the flora compared to that of the gastro-intestinal tract may be more apparent than real. Methods used in vaginal

flora studies are based on those devised for intestinal studies, and may not provide the optimum conditions for the isolation of vaginal organisms.

There has been only one other published quantitative study of the vaginal flora during the menstrual cycle. Bartlett *et al.* (1977) detected relatively consistent mean levels of anaerobes and a significant decrease in concentrations of aerobes in premenstrual specimens from five women compared with those in the specimens collected in the week following onset of menstruation. However the converse effect was noted in a recent semi-quantitative study of 15 volunteers in whom it was found that aerobic bacteria remained relatively consistent during the sampling times but there was marked variation in the concentration of anaerobic gram-positive bacilli, mainly lactobacilli. These decreased from a mean count of 9.4 (log 10) cfu/g before menstruation to 6.7 (log 10) cfu/g during menstruation (Sautter and Brown, 1980). The volunteers in this study were chosen because they had a regular menstrual cycle and were asymptomatic. Although the number of subjects is small, the absence of any major cyclical changes in the vaginal bacterial flora is clear.

REFERENCES

- Bartlett J G, Onderdonk A B, Drude E, Goldstein C, Anderka M, McCormack W M 1977 Quantitative bacteriology of the vaginal flora. *Journal of Infectious Diseases* **136**: 271-277.
- Brown W J 1982 Variations in the vaginal bacterial flora: a preliminary report. *Annals of Internal Medicine* **96**: 931-934.
- Cowan S T 1974 Cowan and Steele's Manual for the identification of medical bacteria, 2nd edn. Cambridge University Press, Cambridge.
- Cruikshank R, Sharman A 1934 The biology of the vagina in the human subject; glycogen in the vaginal epithelium and its relation to ovarian activity. *Journal of Obstetrics and Gynaecology* **41**: 190-207.
- Galask R P, Larsen B, Ohm M J 1976 Vaginal flora and its role in disease entities. *Clinical Obstetrics and Gynaecology* **19**: 61-81.
- Gorbach S L, Menda K B, Thadepalli H, Keith L 1973 Anaerobic microflora of the cervix in healthy women. *American Journal of Obstetrics and Gynaecology* **117**: 1053-1055.
- Hentges D J, Maier B R 1972 Theoretical basis for anaerobic methodology. *American Journal of Clinical Nutrition* **25**: 1299-1305.
- Holdeman L C, Cato E P, Moore W E C 1977 Anaerobe laboratory manual, 4th edn. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
- Levison M E, Corman L C, Carrington E R, Kay C D 1977 Quantitative microflora of the vagina. *American Journal of Obstetrics and Gynaecology* **127**: 80-85.
- Lindner J G E M, Plantena F H F, Hoogkamp-Korstanje J A A

- 1978 Quantitative studies of the vaginal flora of healthy women and of obstetric and gynaecological patients. *Journal of Medical Microbiology* **11**: 233–241.
- Mehta P V 1982 Vaginal flora: a dynamic ecosystem. *Journal of Reproductive Medicine* **27**: 455–458.
- Onderdonk A B, Polk B F, Moon N E, Goren B, Bartlett J G 1977 Methods for quantitative vaginal flora studies. *American Journal of Obstetrics and Gynecology* **128**: 777–781.
- Osborne N G, Wright R C, Grubin L 1979 Genital bacteriology: a comparative study of premenopausal women with postmenopausal women. *American Journal of Obstetrics and Gynecology* **135**: 195–198.
- Riddell R H, Buck A C 1970. Trimethoprim as an additional selective agent in media for the isolation of *N. gonorrhoeae*. *Journal of Clinical Pathology* **23**: 481–483.
- Sautter R L, Brown W J 1980 Sequential vaginal cultures from normal young women. *Journal of Clinical Microbiology* **11**: 479–484.
- Sutter V L, Citron D M, Finegold S 1980 Wadsworth Anaerobic Bacteriology Manual, 3rd edn. C V. Mosby, St. Louis, MO, USA.
- Wilks M, Thin R N, Tabaqchali S 1982 Quantitative methods for studies on vaginal flora. *Journal of Medical Microbiology* **15**: 141–147.
- Wilks M, Thin R N, Tabaqchali S 1984 Quantitative studies of the vaginal flora in genital disease. *Journal of Medical Microbiology* **18**: 217–231.