DYNAMICS OF VAGINAL MICROFLORA

by

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Introduction

Many reports describing the microbial flora of the female genital tract, in health and disease, have been published since Doderlien's first report in 1892. Organisms frequently isolated during infections are Bacteroids, Clostridia, Streptococci, Coliforms and Candida. The presence of these organisms have been also reported in the vagina of healthy females. However, the flora that is reported as normal depends largely upon the socio-economic status of the group under study, the area of the genital tract studied and the methods used. Besides, vaginal microbial ecosystem seems to be dynamic and is governed by various physiological factors including vaginal acidity and hormonal status. Galask *et al* (1976) have concluded, from their studies on rats, that estrogen appears to promote colonization of bacteria while progesterone has antagonistic effect. There are no such reports in humans. Thus, normal cyclic hormonal pattern may influence the colonization of bacteria in the genital tract. Hence the knowledge of the indigenous microbial flora of the vagina and its variation, if any, in different phases of the menstrual cycle is of great importance in obstetrics and gynaecology because of its possible role in endogenous infections. A thorough knowledge of the dynamics of the vaginal microbial flora could help to reduce the postoperative morbidity if elective surgery can be appropriately synchronised with reduction in the microbial flora. The aim of the present study was to determine the normal microbial flora of vagina in relation to hormonal status.

Material and Methods

Subjects selected for this study were 80 clinically asymptomatic females of the reproductive age group attending the Sterility Clinic of "Mother and Child Hospital" in Bombay. These were predominantly cases of primary sterility. In some of these cases with anovulatory cycles, effect of unopposed oestrogen throughout the cycle on the bacterial

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flora could also be studied. The subjects under study were not undergoing any antibiotic treatment and they were instructed strictly to avoid coitus and douche during the period of investigation. High vaginal swabs were collected from these females on the 6th, 10th, 14th, 18th and 22nd day of their menstrual cycle.

Collection of samples was done at the Haffkine Institute where the material was immediately processed for bacterial analysis. With sterile bivalve speculum, vaginal swabs were taken avoiding any contamination.

Swabs were streaked onto (a) Blood agar plates—for aerobes. (b) MacConkey's agar plates—for selection of gram negative aerobes. (c) Tomato juice agar plates—for selecting Lactobacilli. (d) Neomycin blood agar plates containing 100 gm/ml of neomycin—for anaerobes. (e) Sabourad's agar plates—for yeasts and fungi.

One set consisting of one blood agar plate, one tomato juice agar plate and one Neomycin blood agar plate were incubated anaerobically in the chrome acid anaerobic jar, immediately after streaking, for 72 hours at 37°C. Other set consisting of one blood agar plate, MacConkey agar plate and one Sabourad's agar plate were incubated aerobically for 48 hours at 37°C. The isolates were identified by the standard methods (Gradwohl's Clinical Laboratory Methods and Diagnosis).

Differences between bacterial studies carried out on different days in respect of isolation rates of various bacterial species were also studied. Total number of different species isolated from each individual was separately noted down from each group. Mean number of species isolated per patient was calculated. Similarly, fraction of the total number of cases examined on each of the 6, 10, 14, 18 and 22 days that yielded 0, 1, 2, 3, 4 and 5 or more different species per patient are presented in bar diagram (Fig. 1 and 2).

Results

Vaginal microflora from the 80 asymptomatic females irrespective of the phase of the cycle, are summarised in Table I.

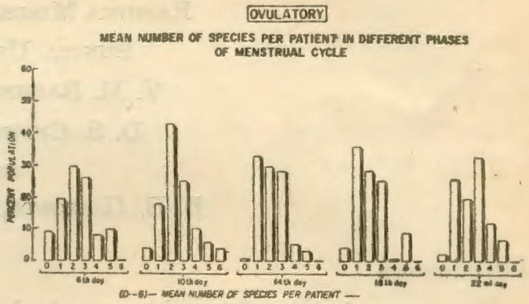

Fig. 1

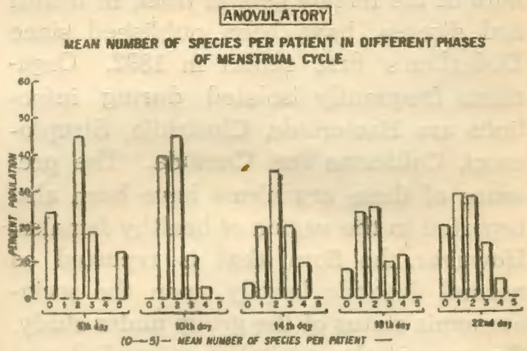

Fig. 2

It can be seen that micrococci, staphylococcus epidermidis, diptheroids, lactobacilli and candida form the predominant flora. Hemolytic and non-hemolytic streptococci, streptococcus faecalis and peptostreptococci were also noted in significant number of cases while gram negative aerobes were isolated in small numbers in the present study.

It can be seen that no correlation exists between the incidence of various organisms and the different phases of the menstrual cycle (Tables II and III). The

TABLE I
Vaginal-cervical Microflora From 80 Asymptomatic Females

Isolates		Isolated from No. of cases	Isolates		Isolated from No. of cases
Aerobes (Gram Positive)		%	*Aerabes (Gram Negative)*		%
Micrococci	64	80.00	E. Coli	5	6.25
Staph. epidermidis	49	61.25	Pseudomonas	4	5.00
Staph. citreus	16	20.00	Alcaligenes faecalis	7	8.75
Staph. aureus	5	6.25	Flavobacterium	2	2.5
Hemolytic streptococci	20	25.00	Achromobacter Lwoffi	0	0
Non-hemolytic ,,	21	26.25	,, anitratum	1	1.25
Streptococcus faecalis	18	22.50	Proteus	1	1.25
Diphtheroids	37	46.25	Citrobacter	4	5.00
Aerobic spore-bearers	42	43.5	Klebsiella	2	2.5
			Moraxella	1	1.25
			Aeromonas hydrophulia	1	1.25
			Plesiomonas	1	1.25
			Edwardsiella	1	1.25
			Haemophillus spp.	2	2.50
Anaerobes (Gram Positive)			*Anaerobes (Gram negative)*		
Clostridium	4	5.00	Bacteriodes	2	2.50
Lactobacilli	45	56.25	Veillonella	1	1.25
Peptostreptococci	21	26.25			
Peptococci	3	4.75			
Yeasts spp.	31	38.75			

data is not very much different for anovulatory cycles (Table IV and V).

It was not possible to carry out a quantitative assessment of colonisation for each organism. Similarly, since so many different organisms were encountered in the culture, it was not possible to get the estimate of total number of organisms colonising the vaginal mucous membrane.

However, one could get a semiquantitative estimate of the degree of colonisation by two indirect approaches: (1) Comparison of the number of cases (for different phases of menstrual cycle) where swabs did not yield any growth at all. (2) Comparison of the mean number of species isolated per patient for different phases of menstrual cycle, shown in Fig. 1. Studies on patients with ovulatory cycles revealed that the maximum number of cases which yielded no growth were encountered on the sixth day. As can be seen from (Table VI) as many as 45.17% of cases of normal ovulatory cycles did not yield any growth when swabs were cultured for aerobes and facultative anaerobes. However, when the same cases were examined on days 10, 14. 18 and 22, the average number of cases that did not yield any growth were 24% (± 10.8 S.E.). This difference is quite significant (P = .99). Similar figures for anaerobes is 22.59 and 12.01 respectively. This also implies that some of the cases that did not yield any aerobic growth, did show colonisation when the swabs were cultured for anaerobes. Reference to Fig.

TABLE II

Dynamics of Vaginal Microflora of 48 Cases with Normal Ovulatory Cycle (Aerobic and Facultative)

Isolates	Site	Day of the menstrual cycle										
			6th		10th		14th		18th		22nd	
		Total cases	31	%	47	%	48	%	48	%	45	%
Micrococci	Vg		9	29.00	22	47.00	19	39.59	14	29.17	17	37.78
Staphylococcus	Vg		10	32.25	17	36.16	18	37.5	18	37.5	16	35.55
Streptococci	Vg		5	16.13	11	23.41	11	22.92	16	31.84	8	16.666
Diphtheroids	Vg		5	16.13	10	21.27	9	18.75	11	22.92	9	18.75
Yeast	Vg		1	3.23	8	17.02	9	18.75	5	10.42	10	22.22
Coliforms	Vg		—	0	—	0	—	0	—	0	—	0
Non-coliforms	Vg		4	13.00	1	2.13	—	0	—	0	1	2.22
Spore-bearers	Vg		3	9.67	5	10.64	8	16.66	9	18.73	9	18.75
No Growth	Vg		14	45.17	14	29.78	6	12.5	14	29.12	11	24.44

TABLE III

Dynamics of Vaginal Microflora in 48 Cases with Normal Ovulatory Cycle (Anaerobic)

Isolates	Site	Day of the menstrual cycle										
			6th		10th		14th		18th		22nd	
		Total cases	31	%	47	%	48	%	48	%	45	%
Peptostreptococcus	Vg		5	16.13	10	21.27	13	27.08	6	12.5	7	14.58
Peptococcus	Vg		—	0	—	0	2	4.178	2	4.16	2	4.16
Lactobacilli	Vg		10	32.25	16	24.04	13	27.08	13	27.08	13	27.08
Bacteroides	Vg		—	0	1	2.127	—	0	1	2.08	—	0
Clostridium spp.	Vg		—	0	2	4.25	1	2.084	4	8.32	3	6.38
Veillonella	Vg		—	0	—	0	—	0	—	0	—	0
No Growth	Vg		7	22.59	7	14.89	4	8.34	7	14.58	4	8.88

TABLE IV

Dynamics of Vaginal Microflora of 15 Cases with Anovulatory Cycle (Aerobic and Facultative)

Isolates	Site	Day of the menstrual cycle										
		Total cases	6th 14	%	10th 14	%	14th 15	%	18th 15	%	22nd 15	%
Micrococci	Vg		2	14.29	9	64.28	7	46.67	2	13.33	3	20.00
Staphylococci	Vg		—	—	8	57.15	3	20.00	4	26.66	3	20.00
Streptococci	Vg		1	7.14	5	35.72	4	26.66	4	26.66	4	26.66
Diphtheroids	Vg		1	7.14	1	7.14	2	13.33	1	6.67	3	20.00
Yeast	Vg		1	7.14	4	28.58	5	33.34	4	26.66	3	20.00
Coliforms	Vg		—	—	—	—	—	—	1	6.67	—	—
Non-coliforms	Vg		1	7.14	—	—	—	—	—	—	—	—
Sporebearers	Vg		2	14.29	1	7.14	3	20.00	3	20.00	2	13.33
No Growth	Vg		4	28.58	—	—	1	6.67	4	26.66	4	26.66

TABLE V

Dynamics of Vaginal Microflora of 15 Cases with Anovulatory Cycle (Anaerobic)

Isolates	Site	Day of the menstrual cycle										
		Total cases	6th 14	%	10th 14	%	14th 15	%	18th 15	%	22nd 15	%
Peptostreptococcus	Vg		1	7.145	1	7.145	—	—	3	20.00	2	13.33
Peptococcus	Vg		1	7.145	—	—	—	—	—	—	—	—
Lactobacilli	Vg		3	21.43	4	28.58	5	33.34	5	33.34	2	13.33
Bacteroides	Vg		—	—	—	—	—	—	—	—	—	—
Clostridium spp	Vg		—	—	—	—	—	—	—	—	—	—
Veillonella	Vg		—	—	—	—	—	—	—	—	—	—
No Growth	Vg		11	78.53	7	50.00	9	65.79	8	57.15	11	78.53

1 will show that there is essentially no difference in the number of cases that yielded 0, 1, 2 or 3 species per patient when cases were examined during different phases of menstrual cycle. Studies on cases of anovulatory cycles (Table VI)

TABLE VI
Comparison of Culture Negative Cases Between Day 6 and Rest of the Cycle

	% of of cases showing no growth on 6th day	% mean number of cases showing no growth during the rest of the cycle
Ovulatory Cycle		
Aerobic	45.17	24 ± 10.8
Anaerobic	22.59	12.01 ± 11.67
Anovulatory Cycle		
Aerobic	28.58	15 ± 20.68
Anaerobic	78.53	63 ± 22.9

99% Confidence Limits

revealed some interesting features. Unlike ovulatory cases number of cases of anovulatory cycles that did not yield any growth on days 6, 10, 14, 18 and 22 did not vary significantly.

Comparison of day 6 culture negative swabs between ovulatory and anovulatory cases show some interesting features. Much larger number of ovulatory cases were culture negative on day 6 for aerobes, when compared with anovulatory cases (45.17 vs. 28.58). The results of anaerobic cultures were exactly opposite. As many as 78.53% of anovulatory cases were culture negative on day 6 as compared to 22.59% of ovulatory cases.

Discussion

The population selected mainly belonged to low socio-economic group which forms the majority in our country. Bulk of the cases selected were that of primary sterility. This group was readily available with us and it was also intended to see the effect of unopposed oestrogen on the microbial flora of vagina in the cases with anovulatory cycle. As regarding the microbial flora, 80 female subjects were studied and the flora which have been reported here is not very different from that reported by several other workers. However, the incidence of bacteroides was found to be very low in the present study. Similarly Gupta *et al* (1973) have reported very low incidence of bacteroides (4.8%) in asymptomatic females in New Delhi. Several recent reports in the West, quote a proportionately higher incidence of bacteroides in the vagina. But this may be on account of the type of diet consumed by the Western population as suggested by Hill *et al* (1971). They reported that vegetarians had fewer bacteroides in their faeces than the non-vegetarian population.

As regarding the dynamics of microbial ecosystem we were greatly impressed by the laboratory experiments on rats of Ohm and Galask (1976). Their studies indicated that oestrogen favoured the colonization and progesterone had antagonistic effect. There were no reports in the literature about such studies in humans. Neary *et al* (1973) studied the microbial flora in 246 cases before and after vaginal hysterectomy. They found that the rate of isolation of bacteria was higher in cases falling in the first half of the cycle than those in the later half of the cycle. They also noted that incidence of bacteroides was higher in the first week. However, the conclusions were based on single swab collected from different subjects in various menstrual phases. In our studies, samples were collected at different intervals throughout the cycle from each case.

Quantitative studies were not practical. However, the incidence of culture negative cases does give some indication of dynamics of the vaginal flora. In ovulatory cycles, maximum number of culture negative cases were encountered on day 6. For aerobes and facultative anaerobes 45% of day 6 swabs were culture negative as against 24% for the rest of the cycle. This is not in agreement with the conclusion of Bartlett *et al* (1977) who reported minimum colonisation in the 4th week of the cycle.

The corresponding figures for anaerobic flora, i.e. 22.59% v/s 12.01% also indicate that the least anaerobic growth is encountered on day 6 in ovulatory cycle. However, it is important to point out that even on the day when maximum number of culture negative cases were encountered, i.e. day 6 as many as 78% of them yielded anaerobes. As many as 45% of these were culture negative for aerobes. Therefore, it should not be assumed that on day 6 the vagina is comparatively bacteria free for surgical purposes, because even if the aerobes are not isolated, the same cases may have anaerobes colonising the vaginal mucuous membrane. This point is further stressed by our analysis of data as shown in the histogram of Fig. 1. In this analysis, mean number of species per patient has been calculated for both aerobes and anaerobes. One can easily see that when aerobes and anaerobes are considered together there is no difference of culture negative cases (i.e. O species/patient) when studied on different days of menstrual cycles. Even the fraction of cases that yield 1, 2 and 3 different species per patient also does not seem to differ in different phases of menstrual cycle. Of course it is needless to stress again that no menstrual phase related differences in culture negative cases of anovulatory cycles were encountered.

It also appears that ovulatory cycles favour anaerobic growth and anovulatory cycles aerobic growth. Culture negative ovulatory cases for aerobes and anaerobes can be compared from (Table VI). One can notice that in all phases of the ovulatory cycles one encountered more culture negative swabs for aerobes than anaerobes. Table VI also reveals similar figures in anovulatory cycles. Number of culture negative cases for aerobes are 25% or less on different days of the anovulatory cycle. As against this 60-78% of the same cases were culture negative for anaerobes when investigated on the same days. Hormonal and other microbiological factors leading to these striking differences, need to be investigated further.

Conclusion

In conclusion, the vaginal microbial flora seem to be very much dynamic and the factors which control it remained unidentified to a great extent. None of the phases of ovulatory cycles could be deemed comparatively bacteria free and thus safe for surgical purposes.

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