PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 571-574 (1976)

The Bacterial Flora of the Female Rat Genital Tract (39261)

BRYAN LARSEN, A. J. MARKOVETZ, AND R. P. GALASK

Departments of Microbiology and Obstetrics and Gynecology, The University of Iowa, Iowa City, Iowa 52242

Vaginitis represents a complex and challenging clinical problem. While most cases encountered are due to either yeasts or trichomonads, bacterial agents, especially *Corynebacterium vaginale,* have been suggested to have an etiologic role in vaginal disease (1). Improved understanding of the pathogenesis of both vaginitis and veneral diseases depends upon a better understanding of factors which promote the colonization of the genitalia by the indigenous flora. It would therefore be useful to have an animal model for the study of the mechanisms involved in genital bacterial colonization. The rat was chosen as a possible experimental model since it would serve as an easily handled subject that has been well studied with respect to both reproductive endocrinology and vaginal cytology. Further, the laboratory rat may be obtained in an axenic state. Since no information on the indigenous microflora of female rat genitalia appears in the literature, as a primary step in understanding the mechanisms of genital tract colonization, the indigenous bacterial flora will be described in this report.

Methods. Randomly-bred virgin female rats were obtained from Small Animal Supply Company, Omaha, Nebraska, and were housed in stainless steel cages. Bedding (Sanicel, Vivarium Research, Whitehousestation, N. J.) was changed weekly and cages were washed, but no exceptional sanitation procedures were utilized. Rats used in this study were between the ages of 3 and 9 months. Vaginal and cervical tissues were obtained for culture by sacrificing the rat by etherization. A midline incision was made down to the level of the vulva, while skin, muscle layers, and pubis were retracted to prevent contamination. The vagina and cervix were removed *in toto,* placed on a sterile field, and the vagina and cervix were dissected apart. The vagina and cervix were then divided longitudinally, and one-half of each tissue specimen was placed immediately in chopped-meat medium. The chopped-meat medium was placed in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Michigan) for 4 days at 37° and was then subcultured onto 5% sheep blood agar. Anaerobic isolates thus obtained were identified using Holdeman and Moore (2) as a reference. The portions of vaginal or cervical tissues not used for anaerobic culture were gound in a sterile mortar and pestle with 1-ml sterile saline, streaked for isolation on 5% sheep blood agar, and incubated at 37° for 18 hr. Aerobic isolates were identified by standard procedures. Measurement of vaginal pH was accomplished by inserting a microcombination glass pH electrode (Microelectrodes Inc., New Hampshire) into the vagina of an etherized rat to a depth of 5 mm. Readings were taken using a standard pH meter with an expandable scale.

Results. Aerobic cultures were performed on genitalia of 25 rats, and anaerobic cultures were done on 22 of the 25 rats. The species recovered from these rats are presented in Table I, which shows that the most commonly isolated organisms were alpha and nonhemolytic streptococci, *Pasteurella pneumotropica* and diphtheroid bacilli. It was apparent that this group of rats was relatively homogenous with respect to the species comprising the genital microflora, as evidenced by the small number of species reported in Table I. Further, the frequency distribution of the number of bacterial isolates per rat (Table II) emphasizes the similarity among the rats studied. More than half of the rats had five or six species comprising their flora, and all rats had at least three species. Because of the possible differences in the vagina and cervix as separate ecologic niches, the differences in bacteria isolated from these two tissues were considered. Table III shows that more isolates were obtained from the vagina than from the cervix. More often vaginal isolates were not recovered from the cervix than were

TABLE I. BACTERIAL SPECIES ISOLATED FROM FEMALE RAT GENITALIA.

Organism	Frequency of isolation (%)
Facultative gram positive bacilli	
Diphtheroids	52.0
Lactobacillus sp.	36.0
Facultative gram positive cocci	
Alpha-hemolytic streptococci	72.0
Nonhemolytic streptococci	56.0
Staphylococcus epidermidis	48.0
Beta-hemolytic streptococcus (not group A)	8.0
Micrococcus sp.	8.0
Facultative gram negative bacilli	
Pasteurella pneumotropica	68.0
Proteus mirabilis	48.0
Escherichia coli	16.0
Proteus vulgaris	12.0
Anaerobic species[a]	
Bacteroides sp.	40.9
Fusobacterium sp.	36.4
Veillonella sp.	13.6
Gram positive cocci	13.6
Propionibacterium sp.	4.5
Eubacterium sp.	4.5

[a] Anaerobic culture was performed in 22 of the 25 rats studied.

TABLE II. FREQUENCY DISTRIBUTION OF THE NUMBER OF BACTERIAL ISOLATES FROM THE GENITAL TRACTS OF 22 VIRGIN FEMALE RATS.

Number of isolates[a]	Number of rats	Percentage of rats
0	0	0
1	0	0
2	0	0
3	2	9.1
4	2	9.1
5	7	31.8
6	7	31.8
7	2	9.1
8	1	4.5
9	1	4.5

[a] Mean number of isolates per rat, 5.55.

cervical isolates not found in the vagina. However, Table IV shows that no particular tissue specificity was found when the bacterial species isolated from either the vagina alone or the cervix alone were compared.

Because of the apparent importance of vaginal acidity as a factor predisposing the human vagina to colonization by certain bacterial species (3), the vaginal pH in 20 rats was determined. The pH values ranged from 6.12 to 7.62 with a mean value of 6.95. The standard error of the mean was 0.35 pH units. It is unclear whether or not the vaginal pH in the rat has an effect upon the bacterial flora.

TABLE III. DIFFERENCES IN SPECIES OF BACTERIA ISOLATED FROM THE VAGINA AND CERVIX IN 22 VIRGIN FEMALE RATS.

Rat	Number of vaginal isolates	Number of cervical isolates	Disparity in species isolated	
			Cervix[a]	Vagina[b]
1	4	4	1	1
2	3	4	2	1
3	5	5	1	1
4	3	4	2	1
5	3	6	4	1
6	3	1	0	2
7	5	4	1	2
8	5	5	0	0
9	4	3	2	3
10	5	5	1	1
11	4	4	1	1
12	6	0	0	6
13	5	5	0	0
14	6	3	0	3
15	7	7	2	2
16	4	5	1	0
17	5	3	0	2
18	7	6	0	1
19	6	6	2	2
20	3	2	0	1
21	5	5	0	0
22	4	4	2	2
Total	102	91	22	33

[a] The number of species found only in the cervix.
[b] The number of species found only in the vagina.

TABLE IV. FREQUENCY OF ISOLATION OF VARIOUS BACTERIAL SPECIES FROM THE VAGINA ONLY OR CERVIX ONLY.

Organism	Frequency of isolation	
	Vagina only	Cervix only
Staphylococcus epidermidis	4	4
Diphtheroids	4	3
Bacteroides sp.	4	2
Lactobacillus sp.	4	0
Nonhemolytic streptococcus	3	3
Alpha-hemolytic streptococcus	3	1
Proteus sp.	3	1
Pasteurella pneumotropica	2	3
Micrococci	1	1
Beta-hemolytic streptococci	1	0
Escherichia coli	1	0
Eubacterium sp.	1	0
Peptostreptococcus sp.	1	0
Veillonella alcalescens	0	2
Peptococcus sp.	0	1
Fusobacterium sp.	0	1

Discussion. The development of an ani-

mal model for the study of the interactions of a host and the microflora of the genital tract is important since the well being of the vaginal mucosa may in part depend upon its bacterial flora (3). The microflora of the rat genital tract has been described. Comparison of the genital flora in rats with data available regarding the flora in women shows some similarities. A comparable frequency in the isolation of diphtheroids has been observed in humans (4–7). Similar frequencies of isolation of *Staphylococcus epidermidis* were also found; this study showed a 48% frequency of isolation in rats and studies in humans showed 30 (8), 64.5 (4), 57 (9), 66.1 (7), and 41% (5). Notable differences between rats and humans included the low frequency of lactobacilli in rats, while the reported frequencies in humans approach 100% (5, 7, 8, 10, 11). Because of the association of lactobacilli with acidic pH (12, 13), the relatively high pH values observed in the rats studied may be related to the observed low frequency of lactobacilli. The high frequency of isolation of gram negative rods in rats, notably *Proteus sp.* is interesting. In the human vagina, *Proteus* is usually isolated from less than 10% of vaginal cultures (5, 7, 9, 11, 14). The reason for this difference may be due to fundamental differences in the microenvironments provided by the two host species. Alternatively, the association of rats with fecal material in their cages may contribute to the high percentage of rats carrying *Proteus sp.* An additional difference in rat vaginal flora and human vaginal flora was the finding of *Pasteurella pneumotropica* in 68% of the rats studied. This organism has not been isolated from the human vagina. The strength of the association of *Pasteurella pneumotropica* with tissues of the rat was suggested by a study in which this organism was found to be associated with cesarian-derived barrier held rats (16). The authors suggested that animals harbored this organism as fecal flora but felt that cesarian-derived offspring were infected *in utero* since this organism was isolated from uteri of 2 or 12 infected adult rats and because uteri of rats inoculated *per os* with *Pasteurella pneumotropica* harbored the organism 22 days after inoculation.

This study has demonstrated differences existing between cervical and vaginal flora. While these differences may be due in part to technical reasons, it is also likely that the differences reflect the concept that the vagina and cervix represent different microenvironments. This appears to be true with respect to pH in the human vagina and cervix since the human vagina generally has a lower pH than the cervix.

Summary. A study of the genital microflora in virgin female rats of reproductive age has demonstrated that alpha and nonhemolytic streptococci, *Pasteurella pneumotropica,* diphtheroid bacilli, *Staphylococcus epidermidis,* and *Proteus mirabilis* are the predominant genital organisms. The pH of the rat vagina was demonstrated to be near neutrality. It is suggested that the higher pH in the rat vagina as compared to the human vagina may account for some of the differences between human and rat genital microflora.

A. J. Markovetz is the recipient of a Development Award-Research Career Program (5KO4 GM14681) from the National Institute of General Medical Sciences.

The assistance of Marilyn J. Ohm in identifying anaerobic bacteria for this study is gratefully acknowledged.

1. Dunkelberg, W. E., Jr., Skaggs, R., and Kellogg, D. S., Jr., Amer. J. Clin. Pathol. **53,** 370 (1970).
2. Holdeman, L. V., and Moore, W. E. C. (eds.), *Anaerobe Laboratory Manual.* Virginia Polytechnic Institute and State University, Blacksburg, Virginia (1972).
3. Cruickshank, R., and Sharman, A., J. Obst. Gynec. Brit. Emp. **41,** 190 (1934).
4. Weinstein, L., Yale J. Biol. Med. **10,** 247 (1938).
5. Ohm, M. J., and Galask, R. P., Amer. J. Obst. Gynec. **122,** 683 (1975).
6. Keith, L., England, D., Bartizal, F., Brown, E., and Fields, C., Brit. J. Vener. Dis. **48,** 51 (1972).
7. Hurley, R., Stanley, V. C., Leask, B. G. S., and DeLouvois, J., *in* The Normal Microbial Flora of Man (F. A. Skinner and J. G. Carr, eds.) p. 164. Academic Press, New York (1974).
8. Harris, J. W., and Brown, J. H., Johns Hopkins Hospital Bull. **43,** 190 (1928).
9. Gorbach, S. L., Menda, K. B., Thadepalli, H., and Keith, L., Amer. J. Obst. Gynec. **117,** 1053 (1973).
10. Hunter, C. A., and Long, K. R., Amer. J. Obst. Gynec. **75,** 865 (1958).
11. Walsh, H., Hildebrandt, R. J., and Prystowsky, H.,

Obst. Gynec. **22,** 665 (1965).
12. Hunter, C. A., and Long, K. R., Amer. J. Obst. Gynec. **75,** 872 (1958).
13. Cruickshank, R., J. Pathol. Bacteriol. **39,** 213 (1934).
14. Bollinger, C. C., Obst. Gynec. **23,** 251 (1964).
15. Ansbacher, R., Boyson, W. A., and Morris, J. A., Amer. J. Obst. Gynec. **99,** 394 (1967).
16. Moore, T. D., Allen, P. M., and Ganaway, J. R., Lab. Animal Sci. **23,** 657 (1973).
17. Trussel, R. E., and MacDougal, R. G., Amer. J. Obst. Gynec. **39,** 77 (1940).

Received October 20, 1975. P.S.E.B.M. 1976, Vol. 151.