

**TO ASSESS THE BACTERIAL MICROFLORA OF THE VAGINA AND CERVIX IN
WOMEN OF CHILDBEARING AGE**¹Dr. Syeda Maham Zainab, ²Dr. Abdul Hakim and ³Dr. Uzair Hassan¹PMDC # 84483-P.²PMDC #: 89230-P.³PMDC # 87933-P.

*Corresponding Author: Dr. Syeda Maham Zainab

PMDC # 84483-P.

Article Received on 15/01/2019

Article Revised on 05/02/2019

Article Accepted on 26/02/2019

ABSTRACT

Background: The female lower genital is habited by various microorganisms as normal resident which help to protect the woman against various form of infections. **Aims and objectives:** This is to assess the bacterial microflora of vagina and cervix in women of rural community in a developing country. **Subjects and Methods:** A qualitative comparative study of the bacterial flora of the vagina and cervix of 220 nonpregnant women of childbearing age (18- 40 years) in Lahore, Pakistan was undertaken. **Results:** The study revealed that both aerobic and microaerophilic organisms as well as the strictly anaerobic bacteria constituted the microflora of the lower genital of this group of women. Of the 220 women sampled, Lactobacilli were the most frequently isolated organism in both the cervix and vagina, occurring in 62.2% and 75.6% samples respectively. Proteus species were the least in incidence, occurring in only 4.1% and 5.3% in the vaginal and cervical specimens respectively. The following pathogenic organisms were isolated in both the vagina and cervix: Escherichia coli, Staphylococcus aureus, Candida albicans, Clostridium species and Beta-haemolytic Steptococci. In general, the same types of organisms were isolated in both the cervical and vaginal samples, although the incidence in the two sources varied. **Conclusion:** the organism from the cervix and vagina were essentially the same despite the anatomical and physiological differences of these structures.

KEYWORDS: Candida albicans, Clostridium species and Beta-haemolytic Steptococci.**INTRODUCTION**

The microbiological flora of the lower female genital tract provides a dynamic, complex example of microbial colonization, the regulation of which is not fully understood.^[1,2] When an exogenous bacterial species, with its array of virulence factors, are introduced into the host infective process may be prevented by these endogenous microorganisms.^[1,3] The vagina and the cervix are said to be physiologically and anatomically different. The vagina of women in the reproductive age group, with level of oestrogens and progesterones, is highly acidic ($p^H = 3.8-4.2$). This acidity is thought to be as a result of breakdown of glycogen present in the vaginal mucosa to lactic acid by Lactobacillus species.^[1,3,4]

Conversely, the environmental condition of the cervix is quite distinct form that of the vagina. The endocervix is more alkaline ($P^H = 7.8$) compare to the situation in the vaginal vault. ($p^H 3.8 - 4.2$), hence the microflora of the cervix would be expected to contain less number of acidogenic and acidoophilic organisms than the vagina.^[3,5,6]

It is also, suggested that, the presence of the columnar epithelium at the endocervical region of the cervix as compared to the squamous epithelium on the rest of the cervix and vagina could have an important effect on bacterial adherence these may also have effect on the quantitative and qualitative colonization of the two environments and subsequent protection against disease process.^[2,4,7] Some worker have now suggested the influence of race, ethnicity and other demographic factors as playing modulating Influence in the final endogenous microbiological flora of these women.⁸⁻¹⁰ For example, it has been shown that The occurrence of hydrogen-peroxide-producing lactobacilli, purportedly active in antimicrobial defense, is lower in black women and that It has been reported that the vaginal pH of Black women is higher than that of white women.^[4,9-11] It would therefore, be necessary to study the bacterial flora of women different geographical locations with diverse ethno-social and demographic background.

The study was, therefore, carried out among the people of Lahore, Pakistan with aim of assessing the bacterial

flora of the vagina and the cervix. It is hoped that the findings of this study would help to establish the differences between the flora of the vagina and the cervix and to compare the findings with that of others from different geographical settings. This would then help the clinicians and the laboratory scientists in differentiating pathological organisms for better diagnosis and treatments particularly in this resource constrained environments like ours where isolation of certain organisms appears to be routine and unreliable.

SUBJECTS AND METHODS

Location

This prospective comparative study of bacterial flora of nonpregnant women of reproductive age group was carried in the gynecological outpatient clinic of General Hospital Lahore over one year period (1st January to 31st December, 2017)

The hospital is the only secondary health care facility in the Lahore local government area of the state serving a population 1.5 million people. The area is inhabited mainly by people of annang tribe whose occupation include subsistent farming, public service, and petty trading. Female literacy level is about 37.4% and practice in polygamous marriage.

Ethical consideration/ consent

Relevant written approval was obtained from the hospital ethical committee. Oral informed consent was then received from all the women who agree to participate in this study. The aims and objectives as well as the process of the study were then carefully described by one of the authors to the participants. Those who refuse to participate were excluded but were assured of confidentiality and normal care in the hospital without prejudice to their decisions.

Recruitments

Patients attending routine gynecological clinic which is conducted twice a week were recruited for the study. Patients between the ages of 18 and 40 years were included. Those excluded from the study were: women with clinical or laboratory evidence of genital infections, immune suppressive conditions, hormonal contraceptive methods, recent antibiotics within one month, recent pregnancy, vaginal bleeding and whose samples could not be adequately taken or processed.

Evaluation of the subjects

After the initial discussions and explanation all consenting participant were then attended to by the authors. Relevant clinical history, examinations and other laboratory tests were carried out to evaluate the patients for exclusion criteria and appropriate care was then given to the patient according to her need.

Collection of samples

After explaining the procedures the patients, samples were then collected with sterile cotton tipped swabs on

wooden applicator sticks encased in plastic tubes using aseptic technique, a sterile speculum was inserted into the vagina and while the cervix was in view, swabs were taken from the cervical canal and the posterior vaginal fornix, care being taken to avoid contacts with surfaces other than those designated for sampling. The swabs were immediately placed in Stuart's transport media contained in Bijou bottles, which were freshly boiled to expel. Each swab stick was broken off in the medium and the bottles screwed down firmly.^[3]

Processing of Samples

The specimens were returned to the laboratory and each inoculated onto sterile plates of blood agar, chocolate agar and MacConkey (bile salt) agar within 2 hours of collections. The plates were incubated aerobically, microaerophilically in candle extinction jar and anaerobically in Gas-Pak jar at 37°C for 48 hours. Anaerobic plates with no growth after the initial 48 hours incubation were re-incubated for another 24 hours. At the end of incubation, the cultures were read and appropriate colonies subcultured for purity. The routine laboratory methods involving microscopical, biochemical and physiological tests were used to identify the different organisms.^[12] The collection and processing of samples were undertaken in the laboratory was undertaken by the same set of staff to minimize observer errors.

Data processing

The data was process using SPSS version 11 statistical package. Significant value between variables were calculated using chi-square with values put $p = < 0.05$. It was then presented on a frequency table and percentages.

RESULTS

A total of 225 subjects were recruited for the study and 5 samples from the vaginal specimen were discarded due to poor sampling.

Both the vagina and the cervix contained the same group of organisms. They comprised the aerobic bacteria such as *Escherichia coli*, *Proteus* species and *Staphylococci* as well as the microaerophilic bacteria, *Gardnerellavaginalis*. Strict anaerobic bacteria isolated included: *Bacteroides*, *Peptococcus*, *Peptostreptococcus*, *Lactobacilli* and *Clostridia* species.

Table 1 shows the distribution of the various isolates from the vagina and cervix of the 220 women sampled. *Lactobacilli* constituted the highest number of isolates, occurring in 75.6% of the vaginal samples and 62.2% of the cervical specimens. *Proteus* species were the least frequently isolated bacteria and constituted only 4.4% of either the vaginal or cervical samples. The incidence and type of bacterial species isolated from the vagina and cervix of non-pregnant women of childbearing age in Lahore were about the same.

DISCUSSION

This study seems to suggest no significant quantitative and qualitative difference between the microflora of vagina and the cervix despite marked embryological origin, epithelial lining, secretions and biochemical differences of these two environments. These have been the findings of other studies.^[1,3,13,14] The main reason for this is not clear but may be associated with several factor such as inappropriate or suboptimal methods of sample collection, failure to use appropriate transport systems or enriched media or a lack of stringent anaerobic technique in the processing and culture of specimens.^[2,15-17] It however, suggests that any of these pathogens isolated from these sites may be regarded as normal flora. This study also, shows that the microflora of the female lower genital tract is considerably complex and inhabited by different micro-organisms.^[18,19]

Table 1: Bacterial Isolates from the Vagina and Cervix of Non-Pregnant Women in Lahore.

Organism	Vaginal Swab N = 220 (%)	Cervical Swab N = 225(%)
<i>Lactobacillus sp.</i>	166 (75.5)	140 (62.2)
<i>Diphtheroids</i>	147 (66.8)	120 (53.3)
<i>Staphylococcus epidermidis</i>	129 (58.6)	103 (45.8)
<i>Staphylococcus aureus</i>	117 (53.1)	95 (42.2)
<i>Staphylococcus faecalis</i>	88 (40.0)	75 (33.3)
<i>Bacteroides sp.</i>	73 (33.2)	66 (29.3)
<i>Peptostreptococcus.p</i>	66 (30.0)	50 (22.2)
<i>Escherichia coli</i>	49 (22.2)	40 (17.8)
<i>Candida albicans</i>	39 (17.8)	30 (13.3)
<i>Gardnerellavaginalis</i>	34 (15.5)	25 (11.1)
<i>Streptococcus agalactiae</i>	27 (12.2)	13 (5.6)
<i>Peptococcus sp</i>	24 (11.1)	35 (15.6)
<i>Clostridium sp.</i>	17 (7.7)	13 (5.8)
<i>Proteus sp.</i>	9 (4.1)	12 (5.3)
Total Isolates	985	817
Mean No. of isolates/subject	4.5	3.6

The organisms isolated in this study are not significantly different from other studies in different settings.^[1-5] The composition of the vaginal ecosystem is not static but changes over time and in response to endogenous and exogenous influences.^[2,3] Variables include stage of the menstrual cycle, pregnancy, use of contraceptive agents, frequency of sexual intercourse, specific sexual partners, vaginal douching, use of panty liners or vaginal deodorants, and utilization of antibiotics or other medications with immune or endocrine activities.^[4,15,19,21] Exposure to an altered milieu will cause a fluctuation in the local environment and heighten or diminish the selective advantage of specific vaginal microbes.^[1,2,16] Some of these factors were difficult to control in this study, However, this study carried out in this rural community the influence of various exogenous factors appears to suggest that they have minimal effects in determining the microflora of female lower genital tract. But the effect of ethnic and genetic composition of our women require further independently verification in this community as suggested by other workers.^[8,10,11]

The most frequently isolated microbes include Lactobacilli species which occurred in 75.6% of the vaginal and 62.2% of the cervical cultures. The prevalence, of Lactobacilli in the vaginal isolates of other workers vary between 49% and 82%.^[3,7,18] The high frequency of diphtheroids in vaginal and cervical samples (66.8% and 53.3% respectively) is suggestive of the fact that they are constantly exposed to the external surfaces. *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* occurred within the range of 17% - 59% in the vagina and cervix. This is not very surprising because of the proximity of the genital tract to the anal orifice. This is important in cases of genital infections where most of pathogens would be from the gut as secondary invaders.^[1,3]

The isolates that occurred less frequently were *Proteus* species (4.1% and 5.3%) and *Clostridium* species (7.8% and 5.6%) in the vaginal and cervical specimens

respectively. All the organisms isolated from the vagina were also isolated from the cervix in proportions, which did not differ significantly. This is consistent with the findings by previous investigators.^[1,4,7,13,21] This is surprising as the vagina and cervix are physiologically different but could be explained by the fact that the female genital tract is canal which effectively communicate each other.

Though the present study and the ones cited above have shown that the vagina and cervix are inhabited by similar multiple organisms, however, some differences have been noticed when paired specimens from individual patients were analyzed.^[4,15] It is also interesting to note in this study that there were no differences in the incidence or type of bacterial species isolated from the vagina and cervix of women of childbearing age in Lahore. The mean number of bacterial isolates per specimen was 4.5 for the vagina and 3.6 for the cervix. These results are similar to those of studies,^[1,4] who recorded the means of 5 and 4 for the vagina and cervix respectively.

It has been stated that there is local secretory Immune System in the endocervix producing IgA, which destroys bacteria in the presence of complement and lysosome, blocks bacterial adhesion to mucosal cells and promotes agglutination and phagocytosis.^[1-5] These reasons may explain why the incidence figures for bacterial isolates from the cervix tends to be less than those from the vagina.

Care was taken to exclude women on or with recent history of antibiotics therapy from the study because it is known that such therapy may not only kill the causative agent of infection, but also the normal flora may be eliminated and thereby create a suitable environment for opportunistic organisms to flourish.

Most bacterial isolated from the female lower genital tract are potential pathogens. Thus the normal flora of human vagina and cervix should be seen as a complex flora of interacting and competing micro-organisms. The conditions of low pH and oxygen tensions, the production of inhibitory substances by other organisms and by the tract itself, and the competition or nutrients will all affect the female genital tract. Besides, it must be remembered always that when the conditions are right, these organisms could also be the agents of infection in the female genital tract and beyond.^[1,4]

CONCLUSION

The microflora of the cervix and vagina of women in Lahore Pakistan Nigeria is not significantly different from each other and those from other geographical locations. Independent study is required to assess the effect of genetic and ethnic composition of individual on the micro flora.

REFERENCES

1. Larsen B, Monif GR understanding the bacteria flora of female genital tract. *Clin Infect Dis*, 2001; 32: e69-e77.
2. Bartlett JG, Moon NE, Goldstein PR, Goren B, Onderdonk AB, et al. Cervical and vaginal bacterial flora: ecologic niches in the female lower genital tract. *Am J ObstetGynecol*, 1978; 130: 658-661.
3. Archibong E I, Itam IH, Okpara AA, Ikefem CA A prospective comparative study of bacteria flora of vagina and cervix in non-pregnant women of child bearing age in Calabar, Nigeria. *Mary Slessor J Med*, 2003; 3: 1-4.
4. Witkin SS, Linhares IM, Giraldo P Bacterial flora of female genital tract: Function and immune regulation. *Best practice and research clinical obstet and gynaecol*, 2007; 21: 347-354.
5. Morris CA, Morris DF Normal vaginal microbiology for women of childbearing age in relation to the use of contraceptives and vaginal tampons. *J Clin.Pathol*, 1967; 20: 636-640.
6. Larsen B, Galask RP Vaginal microbial flora: composition and influence of host physiology. *Ann Intern Med*, 1982; 96: 926-930.
7. Priestlley CFJ, Jones BM, Dhar J & Goodwin L What is normal vaginal flora? *Genitourin Med*, 1997; 73: 23-28.
8. Pavlova SI, Kilic AO, Kilic SS, So JS, Nader-Macias ME, et al. Genetic diversity of vaginal *lactobacilli* from women in different countries based on 16S rRNA gene sequences. *J Appl Microbiol*, 2002; 92: 451-459.
9. Antonio MA, Hawes SE & Hillier SL The identification of vaginal *Lactobacillus species* and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis*, 1999; 180: 1950-1956.
10. Stevens-Simon C, Jamison J, McGregor JA, Douglas JM Racial variation in vaginal pH among healthy sexually active adolescents. *Sex Transm Dis*, 1994; 21: 168-172.
11. Fiscella K, Klebanoff MA Are racial differences in vaginal pH explained by vaginal flora? *Am J Obstet Gynecol*, 2004; 191: 747-750.
12. Isenberg HD Essential procedure for clinical microbiology. ASM press, Washington DC, 1988.
13. Ohm MJ, Galask RP Bacterial flora of the cervix from 100 pre-hysterectomy patients. *Am J Obstet Gynecol*, 1975; 122: 683-687.
14. Monif GR, Thompson JL, Stephens HD, Baer H Quantitative and qualitative effects of Betadine liquid on the aerobic and anaerobic flora of the female genital tract. *Am J Obstet Gynecol*, 1980; 137: 432-438.
15. Eschenbach DA, Patton DL, Hooton TM, Meier AS, Stapleton A, et al. Effects of vaginal intercourse with and without a condom on vaginal flora and vaginal epithelium. *J Infect Dis*, 2001; 183: 913-918.

16. Schwebke JR, Richey CM & Weiss HL Correlation of behaviors with microbiologic changes in vaginal flora. *J Infect Dis*, 1999; 180: 1632-1636.
17. Zheng HY, Alcorn TM, Cohen MS Effects of H₂O₂ producing *lactobacilli* on *Neisseria gonorrhoeae* growth and catalase activity. *J Infect Dis.*, 1994; 170: 1209-1215.
18. Tashjian JH, Coulam CB, Washington JA Vaginal flora in asymptomatic women. *Mayo Clin Pract.*, 1976; 51: 557-561.
19. Bartlett JG, Onderdonk AB, Drude E, Goldstein C, Anderka M, et al. Quantitative bacteriology of the vaginal flora. *J Infect Dis.*, 1977; 136: 271-277.
20. Roy S, Sharma M, Ayyagari A, Malhotra S A quantitative study of *Bacterial vaginosis*. *Indian J Med Res.*, 1994; 100: 172-176.
21. Hill GB Anaerobic flora of the female genital tract. Anaerobic bacteria, selected topics. Plenum Press, New York, 1980.