## STUDY OF VAGINAL MICROBIAL FLORA IN CONTRACEPTIVE USERS

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### ABSTRACT

### BACKGROUND

The present study was carried out at Government Medical College, Nagpur, to study the vaginal microbial flora in women using contraceptive measures. The lactobacillus dominant flora is a protective barrier against development of various microbial infections of vagina. The alteration of this protective mechanism is associated with an increased risk of bacterial vaginosis. The vaginal flora can be disrupted by a variety of factors like use of contraceptive measures, sexual intercourse, antimicrobial use and douching. The effects of specific contraceptive methods on the vaginal flora are not clear. The present study is therefore undertaken to study the vaginal microbial flora in contraceptive users.

### MATERIALS AND METHODS

The vaginal swab was taken in all cases for detection of microbial flora in women who were using various contraceptive measures and nonusers. Isolation of organisms was done by using various bacteriological tests. The microbial data obtained in the two groups was compared. Student's T-test was used for statistical analysis.

### RESULTS

Gardnerella vaginalis, E. coli, Enterococci, group B streptococci and Candida spp were grown in the patients using oral contraceptives as well as copper-T. However, the growth of Gardnerella vaginalis (69.12%), group B streptococci (60%) and Candida spp (58%) was more in patients using copper-T while, growth of E. coli (51.72%) and Enterococci (72.22%) was more in patients using oral contraceptives. This difference in the growth of various organisms, however, was not significant.

### CONCLUSION

It is concluded that the vaginal swab for microbial flora should be studied in all women using contraceptive measures. Presence of any abnormal microorganisms should alarm for immediate measures to control the infection and thus prevent establishment of vaginitis.

### **KEYWORDS**

Microbial Flora, Contraceptive Measures, Gardnerella Vaginalis, Vaginitis.

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### BACKGROUND

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Anaerobic bacterial vaginosis and invasion of vagina with other microbial flora is very common in women in reproductive age. It is more common in patients who use various contraceptive measures.1 Such patients usually present with discharge which is putrid and of fishy odour. The normal vaginal ecosystem is an important host defence mechanism against exogenous urogenital infections. The vaginal secretions normally show various organisms and taken as normal microbial flora. The commonest organisms which dwell normally in the vaginal flora include lactobacilli, micrococci, diphtheroids and coagulase-negative staphylococci. The lactobacillus dominant flora is a

Financial or Other, Competing Interest: None. Submission 18-08-2017, Peer Review 23-08-2017, Acceptance 13-09-2017, Published 20-09-2017. Corresponding Author: Dr. Neelima Suresh Tankhiwale, Flat No. 203, Himalaya Prestige, Gopal Nagar, South Ambazari Road, Nagpur, Maharashtra-440022. E-mail: nstankhiwale@gmail.com DOI: 10.18410/jebmh/2017/892 protective barrier against development of various microbial infections of vagina. The alteration of this protective mechanism is associated with an increased risk of Bacterial vaginosis, Escherichia coli colonisation and many other infections.<sup>2</sup> In a study by Ekanem et al (2012).<sup>3</sup> Out 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 organisms isolated included Escherichia coli, Staphylococcus aureus, Candida albicans, Clostridium species and betahaemolytic Streptococci.

The vaginal flora can be disrupted by a variety of factors, like use of contraceptive measures, sexual intercourse, antimicrobial use and douching.<sup>4</sup> The effects of specific contraceptive methods on the vaginal flora, particularly that involve spermicide use, are not clear. Whereas, some studies have shown a significant decrease in the prevalence of vaginal lactobacilli among diaphragm- spermicide users.<sup>5,6</sup> the effects of other birth control methods, such as Oral Contraceptive Pills (OCPs) and the IUD, on the vaginal microbial ecology are even less well delineated.

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Bruna et al (2016)<sup>7</sup> stated that Vulvovaginal Candidiasis (VVC) is an infection caused by Candida species that affects millions of women every year. Behavioural risk factors such as use of oral contraceptives, intrauterine device, spermicides and condoms are some of the factors, which increase the risk of vulvovaginal candidiasis.

Ceruti et al (1994)<sup>8</sup> in their study evaluated the various methods of contraception with regards to prevalence of Bacterial Vaginosis, (BV), Trichomoniasis (TV), candidiasis and vulvovaginitis. The authors noticed that the IUD users showed a significant increase of BV, TV and other bacteria. The oral contraceptive users had a significant (p < 0.05) increase in candidiasis and BV. The present study is therefore undertaken to study the vaginal microbial flora in contraceptive users.

**Aim**- The present study was aimed to study the vaginal microbial flora in contraceptive users.

### Objectives

- 1. To study the microbial flora in women using contraceptive measures and nonusers.
- 2. To correlate the microbial flora with method of contraception.

## MATERIALS AND METHODS

The present work was carried out at Department of Obstetrics and Gynaecology and Department of Microbiology, Government Medical College, Nagpur. The study was carried out during the period of April 2004 to March 2008 on women attending Obstetrics and Gynaecology OPD during this period. The work included study group (group A) comprising of 355 women who were using various contraceptive measures The study also included 300 women (group B) who did not use any contraceptive measures. The microbial data obtained in the two groups was compared.

Type of Study- This was prospective cross-sectional study.

Study Setting- Government Medical College, Nagpur.

## **Inclusion Criteria**

The study included all women who attended gynaecology OPD. These patients were divided in two groups. Group A included all women who were using any of the contraceptive measures and group B included nonusers.

## **Exclusion Criteria**

- 1. Pregnant ladies.
- 2. Women with menstrual problems like DUB, menorrhagia, etc.
- 3. Patients who were not willing to participate in the study.

The participants were divided in two groups. Group A (study group) included 355 women who were using various contraceptive measures. Group B (control group) comprised

of 300 women who were nonusers of contraceptive measures.

The preliminary clinical examination including the pervaginal digital and per speculum examination was carried out in all cases immediately after their inclusion in the study.

The high vaginal secretions from posterior and lateral fornices of vagina were collected in all the participants from group A as well as group B with sterile cotton swab by per speculum examination.

In group B (nonusers), the vaginal swab was subjected for Gram stain and culture of the organisms.

In group A (study group), the swab was subjected for Gram stain, culture of the sample on blood agar with haemin and menadione supplement, blood agar with gentamycin and Robertson cooked meat broth, pH estimation of the fluid, microscopy, presence of clue cells and amine test. Gardnerella vaginalis was identified by its characteristic morphology in Gram stain, suitable biochemical tests and use of Amsel's criteria (Amsel et al, 1983).<sup>9</sup> These criteria included-

- Increased homogeneous thin vaginal discharge.
- pH of the secretion greater than 4.5.
- Amine odour when potassium hydroxide 10% solution is added to a drop of vaginal secretions.
- Presence of clue cells in wet preparations and Gram stain.

The presence of any three out of four criteria was considered as suggestive of bacterial vaginosis.

The presence of microbial flora in women from group A and group B were studied. The correlation of microbial flora with method of contraception was evaluated.

Data was analysed using Student's t-test.

### RESULTS

The study included 355 cases in study group (group A) and 300 women (group B) who were nonusers of any contraceptive measures. The age range was between 18 to 23 years with the mean age of 21.62 years.

### Nature of Contraception Used in Group A

Amongst 355 cases from group A, oral contraceptive as measure of contraception was used in 36.62% women while Copper-T was used in 63.38% women.

Nature of Contraception	Number of Patients	Percentage			
Oral contraceptives	130	36.62%			
Copper-T	225	63.38%			
Total	355	100%			
Table 1. Nature of Contraceptive Measures Used					

## The Microbial Organisms Isolated

The microorganisms isolated in group A were Gardnerella vaginalis, E. coli, enterococci, group B streptococci and Candida species. The growth of these organisms was statistically significant in group A compared with study group.

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Microbial Flora Isolated	Group A (n=355)	%	Group B (n=300)	%	Statistical Significance	
Lactobacilli	270	76.05%	240	80%		
Anaerobic peptostreptococci	155	43.66%	128	42.67%		
Coagulase-negative staphylococci	128	36.05%	105	35%		
Micrococci	100	28.17%	90	30%		
Gardnerella vaginalis	136	38.30%	0	0	HS for gp A	
E. coli	145	40.84%	15	5%	S (p=<0.001) for gp A	
Enterococci	90	25.35%	15	5%	S (p=<0.001) for gp A	
Group B streptococci	85	23.94%	21	7%	S=<0.002) for gp A	
Candida spp.	100	28.16%	18	6%	S=<0.001) for gp A	
Table 2. Microbial Organisms Isolated in Two Groups (Group n=355 and Group B n=300)						



Graph Showing Significant Microorganisms Isolated in Two Groups

	Total	Type of Contraceptive Measure				
Organisms	Number	Oral Contraceptives (n=130)	Copper-T (n=225)			
Gardnerella vaginalis	136	42 (30.88%)	94 (69.12%)			
E. coli	145	75 (51.72%)	70 (48.28%)			
Enterococci	90	65 (72.22%)	25 (27.78%)			
Group B streptococci	85	34 (40%)	51 (60%)			
Candida spp.	100	42 (42%)	58 (58%)			
Table 3. Correlation of Microbial Flora with   Type of Contraceptive Measures (n=355)						

Gardnerella vaginalis, E. coli, Enterococci, group B streptococci and Candida spp were grown in the patients using oral contraceptives as well as copper-T. However, the growth of Gardnerella vaginalis (69.12%), group B streptococci (60%) and Candida spp (58%) was more in patients using copper-T, while growth of E. coli (51.72%) and Enterococci (72.22%) was more in patients taking oral contraceptives. This difference in the growth of various organisms, however, was not significant.

## DISCUSSION

The female genital tract allows the entrance of pathogenic microorganisms in the vaginal cavity. However, the normal vaginal microflora acts as important defence mechanism to maintain the environment healthy and prevents proliferation of microorganisms stranger to the vagina.<sup>10</sup> Lactobacilli are important constituent of normal bacterial flora, which helps to prevent the development of vaginal infection by external agents. Apart from normal vaginal microbial flora specially lactobacilli, other factors, which make the vagina susceptible

for infection include fluctuation in bacterial flora, biofilms, race/ethnicity, genetic polymorphism, vaginal immunity, organism to vaginal mucosa adhesion of and instrumentation or implantation of Intrauterine Devices (IUD) such as contraceptive method.<sup>11</sup> Persistent alteration in the hormonal balance as in those women who consume contraceptive pills is yet another factor, which makes the vagina susceptible for infections.<sup>12,13</sup> Sonia E Fosch et al (2013)<sup>14</sup> observed that there is a positive association between oral contraceptives and normal microbiota, the use of an intrauterine device generates a higher risk for bacterial vaginosis, whereas the use of oral contraceptives reduces it, condom use of periodic manner increases the risk of vaginal inflammatory states, but with normal microbiota and in patients without contraception an increase in the risk of microbial vaginitis is detected.

The identification of vaginal microbial flora in women who use contraceptive measures is an important aspect. The studies on this aspect of device-related vaginal infections are scanty, hence the present work is undertaken to find out the microbial flora in women using contraceptive measures.

In the present study, amongst 335 women who were using oral contraceptives or copper-T as contraceptive method, Gardnerella vaginalis, E. coli, Enterococci, group B streptococci and Candida spp were commonly grown microorganisms. The growth of Gardnerella vaginalis (69.12%), group B streptococci (60%) and Candida spp (58%) was more in patients using copper-T, while growth of E. coli (51.72%) and Enterococci (72.22%) was more in patients taking oral contraceptives. This difference in the growth of various organisms, however, was not significant.

In the study carried out by Kalpana Gupta et al (2000)<sup>15</sup> on 331 working women, the microbial flora in women using contraceptive measures included lactobacilli 80%, G. vaginalis 40%, group B streptococci 20%, Candida 18% and coagulase-negative staphylococci, E. coli and enterococci in the range of 15% to 20%. Similarly, in women using cervical cap as contraceptive measure, lactobacilli were 88%. G. vaginalis isolation was similar to those using oral contraceptives, but E. coli was isolated in higher percentage (40%) in women using cervical cap. Thus, the findings in the present study were similar to those of Kalpana et al (2000).<sup>15</sup>

The isolation of candida in the vaginal secretion needs attention. Adhesion of Candida to host surfaces is required for initial colonisation of human tissues, contributes to persistence of the microorganism within the host and is essential in the establishment of infection. Various studies

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have confirmed that Candida species have the ability to adhere to this type of cells. In addition, Candida species can adhere to the surface of medical devices, through a protein called adhesion and often promoting device-related infections in women using Intrauterine Device (IUD) as contraceptive method.<sup>16</sup>

It is therefore concluded from the present work that the vaginal swabs in women using contraceptive measures should be evaluated periodically to find out the microbial flora other than normal vaginal dwellers. Presence of any abnormal microorganisms should alarm for immediate measures to control the infection and thus prevent establishment of vaginitis.

### CONCLUSION

It is concluded from the present study that the vaginal swab for presence of microorganisms should be evaluated in all women using various contraceptive measures. The isolation of Gardnerella vaginalis, E. coli, Enterococci, group B streptococci and Candida spp. should alarm the possibility of development of symptomatic vaginitis.

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