

THE VAGINAL BACTERIA FLORA IN PREGNANT AND NON-PREGNANT WOMEN

By

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ABSTRACT

The bacterial flora of the vagina was assessed in healthy Nigerian women (twenty pregnant and twenty-four non-pregnant women). They all harboured many types of microorganisms in their vagina. The mean number of microbial types isolated was five for the pregnant and three for the non-pregnant women. *Staphylococcus* was detected in 18 of the pregnant women and 16 of the non-pregnant women. Amongst the anaerobes *Peptostreptococcus* was isolated in 14 of the pregnant women and 6 of the non-pregnant women. Lactobacilli in 7 and 12 respectively. *Bacteroides* was not isolated from the vagina of pregnant women but from 50% of the vaginal swabs obtained from the non-pregnant women.

Statistically, the difference between the bacterial flora of the pregnant and non-pregnant women investigated is not significant.

INTRODUCTION

Several laboratories have studied the microbial flora of the female genital tract. Gorbach, Menda, Thadepalli and Keith (1973) found the most common anaerobes to be bacteroides followed by peptostreptococci. Corbishley (1977) found that both vagina and cervix harboured many types of microorganisms in common but that the concentrations of the organisms differed between the sites. The purpose of this report is to compare the bacterial flora of pregnant women with that of non-pregnant women.

MATERIALS AND METHODS

Cultures were obtained from the vagina of 20 non-pregnant women attending the Gynecology Out-Patient Clinic at the University of Nigeria Teaching Hospital, Enugu; 20 pregnant women attending prenatal clinic at the University of Nigeria Teaching Hospital Enugu (Department of Gynecology and Obstetrics) and four non-pregnant students of the University of Nigeria Nsukka. Five of the pregnant women were in their first trimester of pregnancy and the rest were in their mid or last trimester of pregnancy. The women were all healthy and were between 18 and 40 years old and the average age was 28. Women receiving birth control pills or antibiotics and women with signs of genital infections were excluded from this study.

SAMPLING TECHNIQUE

Vaginal secretions were obtained during pelvic examination. Cotton-tipped applicators were inserted and then rotated in the posterior fornix. Care was taken to avoid any contact of the swab with the external genitals, or other sources of contamination. Similar swabs were also obtained from the four students with the help of a medical doctor at the University of Nigeria Medical Centre.

The swabs were dipped into Stuart Transport Medium (Oxoid) and delivered to the Microbiology Laboratory of the University of Nigeria Nsukka, within four hours of collection, for the processing. Each of the swabs was inoculated onto five primary culture media. The media used were:

(1) blood agar (oxid) (2) MacConkey agar (oxid) (3) blood agar with neomycin (4) tomato juice agar (oxid) and (5) chocolate agar. Plates (1) and (2) were incubated aerobically, plates (3) and (4) anaerobically and plate (5) was incubated in 10% carbon dioxide for the isolation of *Neisseria gonorrhoea*.

The anaerobic system was obtained by using Fildes and McCintosh jars from Messrs Baird and Tatlock. The jars were evacuated and replaced with oxygen-free CO₂ (10%) and hydrogen (90%). The anaerobic jars were incubated for 72 hours at 37°C. To check the system, viable *Pseudomonas aeruginosa*, an obligate aerobe was included. This will fail to grow if true anaerobiosis was established. An environment of 10% CO₂ was achieved by burning a candle stick in a closed container in which plate (5) was incubated. Aerobic Culture were examined after 24 hours and again at 48 hours incubation.

All colonial types on each medium were investigated further by microscopy, biochemical and physiological tests, and divided into genera or species by routine laboratory methods, (Cruickshank, Duguid, Marmion, and Swain 1975), to give the categories listed in Table III.

RESULT

Most specimens yielded polymicrobial growth. Total isolates were 99 from 20 pregnant women and 77 from 24 non-pregnant women. The mean number of isolates per specimen being five for the pregnant women and three for the non-pregnant women. (See Table I).

Seventy five percent of the pregnant women showed positive cultures for both aerobes and anaerobes. Twenty five percent showed positive cultures for aerobes only while none showed positive cultures for anaerobes only. For the non-pregnant women 58.3% showed positive cultures for both aerobes and anaerobes, 41.7% showed positive cultures for aerobes only and none showed positive cultures for anaerobes only. (See Table II).

Statistical analysis of the data showed no significant difference in the vaginal flora of pregnant and non-pregnant women at 5% error.

(No. of Microbial
Isolates per
specimen.

0
1
2
3
4
5
6
7
8

Total 99

No. of positive
cultures

Aerobes &
anaerobes

Aerobes only

Anaerobes only

Total

TABLE I

FREQUENCY OF MULTIPLE ISOLATES OF MICROBIAL SPECIES FROM THE VAGINA OF PREGNANT AND NON-PREGNANT WOMEN.

| PREGNANT | | NON-PREGNANT | |
|---|-------------------|---|-------------------|
| No. of Microbial Isolates per specimen. | No. of specimens. | No. of Microbial Isolates per specimen. | No. of specimens. |
| 0 | 0 | 0 | 0 |
| 1 | 0 | 1 | 2 |
| 2 | 1 | 2 | 5 |
| 3 | 3 | 3 | 10 |
| 4 | 6 | 4 | 3 |
| 5 | 5 | 5 | 2 |
| 6 | 4 | 6 | 1 |
| 7 | 1 | 7 | 1 |
| 8 | 1 | — | — |
| Total 99 | 20 | 77 | 24 |

TABLE II

INCIDENCE OF POSITIVE CULTURES FROM THE VAGINA OF PREGNANT AND NON-PREGNANT WOMEN.

| PREGNANT | | NON-PREGNANT | |
|---------------------------|------------------|---------------------------|----------------------|
| No. of positive cultures. | Percent of total | No. of Positive cultures. | Percentages of total |
| Aerobes & anaerobes 15 | 75% | 14 | 58.3 |
| Aerobes only 5 | 25% | 10 | 41.7 |
| Anaerobes only 0 | 0% | 0 | 0% |
| Total 20 | 100% | 24 | 100% |

TABLE III
FREQUENCY OF ISOLATES

| Organisms | Pregnant Women (20) | Non-Pregnant Women (24) | Total (44) | Percentage Occurrence |
|-------------------------------|------------------------|----------------------------|---------------|-----------------------|
| <i>Aerobes:</i> | | | | |
| <i>Staphylococcus</i> sp. | 18 | 16 | 34 | 77.4 |
| <i>Strep. faecalis</i> | 4 | 6 | 10 | 25.0 |
| <i>Micrococcus</i> sp. | 4 | 2 | 6 | 17.7 |
| <i>Diphtheroids</i> | 12 | 2 | 14 | 31.9 |
| <i>E. coli</i> | 8 | 6 | 14 | 31.9 |
| <i>Klebsiella pneumoniae</i> | 6 | 2 | 8 | 18.2 |
| <i>Proteus</i> sp | 2 | 0 | 2 | 4.6 |
| <i>Lactobacillus</i> sp | 4 | 8 | 12 | 27.3 |
| <i>Bacillus</i> sp | 4 | 4 | 8 | 18.2 |
| <i>Anaerobes:</i> | | | | |
| <i>Peptococcus</i> sp | 8 | 12 | 20 | 45.5 |
| <i>Peptostreptococcus</i> sp. | 14 | 6 | 20 | 45.5 |
| <i>Clostridium</i> sp | 2 | 0 | 2 | 4.6 |
| <i>Lactobacillus</i> sp | 3 | 4 | 7 | 15.9% |
| <i>Bacteroides</i> sp | 0 | 12 | 12 | 27.3% |

The aerobes isolated were *Staphylococcus* sp (*S. epidermidis* and *S. Aureus*). *Streptococcus* (*S. faecalis* only) *Micrococcus* Sp. *Diphtheroids*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* and *Bacillus*. The anaerobic isolates were *Peptococcus* *Peptostreptococcus*, *Bacteroides*, *Clostridium* and *Lactobacillus*. The genus *Lactobacillus* covers both anaerobic and facultative isolates. *Neisseria gonorrhoea* was not isolated in any of the samples.

Table III shows the frequencies of the isolates. (See Table III).

DISCUSSION

The study shows no statistical significant difference between pregnant and non-pregnant vaginal flora. This could be due to the smallness of the sample size. But there is a difference both in types and relative concentrations of the bacteria isolated. The vaginal flora may vary depending on factors such as age, hormonal influence, pregnancy, use of contraceptives, use of antibiotics and state of health of the individual (Hurley, *et al* (1974). Bartlett, *et al* (1977)

have pointed out constant fluctuations in types and relative concentrations of the bacteria isolated.

Staphylococcus aureus was the most frequent isolate in non-pregnant women. Corynebacterium and Streptococcus faecalis were the most frequent isolates from the vaginal site. deLouvois (1977) reported that the incidence of Staphylococcus aureus in pregnant women was 77.4%.

Lactobacillus was the most frequent isolate in pregnant women. Lactobacillus was found in 27.3% of the samples. Hurley (1974) reported that Lactobacillus was the dominating bacterium in the vaginal flora of women at puberty. The incidence of Lactobacillus was 27.3%; deLouvois (1977) reported that the incidence of Lactobacillus was 0.0%. Swenson (1977) reported that the incidence of Neisseria gonorrhoea increased in non-pregnant women.

The absence of Neisseria gonorrhoea was confined to the vaginal flora. Neisseria gonorrhoea was not isolated amongst a wide range of socio-economic groups.

Of the anaerobes, Peptococcus and Peptostreptococcus were the most frequent isolates in non-pregnant women. Peptococcus was isolated 45.5% for the non-pregnant women and Peptostreptococcus was isolated 45.5% for the non-pregnant women. Clostridium was isolated 4.6% for the non-pregnant women. Lactobacillus was isolated 15.9% for the non-pregnant women. Bacteroides was isolated 27.3% for the non-pregnant women.

The isolation of Bacteroides was noteworthy. In this study, Bacteroides was isolated 27.3%; Corbinaux *et al* (1973), reported that Bacteroides was isolated 15% in the vaginal flora. An explanation of the difference in the incidence of Bacteroides in the vaginal flora of both genera has been given by deLouvois *et al* (1973). Swenson *et al* (1977) reported that the incidence of Clostridium in the vaginal flora was 4.6%.

The difference in the incidence of Bacteroides is significant. 60% of the Bacteroides cover organisms were isolated in both pregnant and non-pregnant women.

have pointed out that the vagina harbours an unstable bacterial population that is subjected to constant fluctuations based on well defined hormonal influences and ill-defined extraneous factors. The results of this study have confirmed these views. Here, there are fluctuations both in types and relative concentrations of the bacteria isolated from non-pregnant and pregnant women.

Staphylococcus possessed the highest percent incidence 90% and 66.6% in pregnant and non-pregnant females respectively, 73% of these Staphylococci were *S. epidermidis* and 27% *S. aureus*. Corbishley (1977) observed 89% and 73% coagulase negative staphylococci in vaginal and cervical swabs respectively. 17% coagulase positive staphylococci were observed in each site. deLouvois, *et al* (1975) observed 66.1% and 4.6% *S. epidermidis* and *S. aureus* respectively in pregnant women.

Lactobacilli have been thought to be preponderant in the vagina. In this investigation they were found in 35% and 50% of the vaginal swabs of pregnant and non-pregnant women respectively. Hurley *et al* (1974) pointed out that in children and women of menopausal age the predominating bacteria are the cocci, while lactobacilli predominated during pregnancy and at puberty. The incidence in both groups was 43.2%. Other surveys show incidences of lactobacilli varying between 0% and 82%: Morris and Morris (1967) 49.1%; Gorbach *et al* (1973) 73%; deLouvois, *et al* (1975) 81.8%; Ohm and Galask, (1975) 75%; Swenson, *et al.* (1973) 0.0%. Swenson *et al.* (1973) studied the anaerobic bacterial infection of the female genital tract, and recovered all the anaerobes found in the study except *Lactobacillus*. Saigh, *et al.* (1977) observed the inhibitory effect of endocervical flora mainly lactobacilli on the growth of *N. gonorrhoea*. They found that when the endocervical flora is low or absent the pathogen increased in number.

The absence of *N. gonorrhoea* in this study is explained by the fact that this study was confined to mainly married middle class women. In a separate study the incidence of *N. gonorrhoea* amongst Nigerian women will be reported. The study will cover more samples and a wider range of socio-economic groups in Nigeria.

Of the anaerobes isolated *Peptostreptococcus* occurred more in pregnant women than in non-pregnant women, 70% and 25% respectively. *Peptococcus* was 40% for the pregnant and 50% for the non-pregnant. In other studies, (Gorbach *et al* 1973) isolated 33% and 10% *Peptostreptococcus* and *Peptococcus* respectively. Swenson *et al* (1973) isolated *Peptostreptococcus* sp. 19 times and *Peptococcus* sp. 21 times in 91 infections of the genital tract.

The isolation of bacteroides 50% in non-pregnant women and none in pregnant women is noteworthy. In other studies on genital tracts, bacteroides were isolated. Swenson *et al.* (1973), 66.6%; Corbishley (1977), 24% from vaginal swabs and 20% from cervical swabs. Gorbach *et al* (1973), 57%. The women in these studies were not pregnant. DeLouvois *et al* (1975) isolated 15 bacteroides spp. from 280 unselected pregnant women (5.4%), the most reasonable explanation of the absence of bacteroides is probably the small number sampled, but the incidence of bacteroides is definitely less in pregnant women. *Proteus* and *Clostridium* were isolated (10% each) in pregnant women but none was isolated in non-pregnant females. In other studies both genera have been isolated both in healthy and in infected female genital tracts. Gorbach *et al.* (1973), 10% and 17%; Corbishley (1977), 3% and 7% for vagina and 1% and 7% for cervix, deLouvois *et al.* (1975), 6.1% and 0.0% *Proteus* and *Clostridium* respectively.

Swenson *et al.* (1973) studying the anaerobic bacterial infections of the female genital tract isolated *Clostridium* six times from 91 infections of the female genital tract. On the whole, the incidence of the two genera in vagina is low.

The difference in the incidence of diphtheroids in pregnant and non-pregnant women is significant. 60% for pregnant and 8.3% for non-pregnant women. Diphtheroid bacterial here, cover organisms suspected but not confirmed as *Corynebacterium*. Percentage occurrence in both pregnant and non-pregnant women was 31.9%. In other works, deLouvois *et al.* (1975)

observed 83.4% incidence of Corynebacteria in pregnant women. Corbishley (1977) observed 69% and 64% diphtheroids from vaginal and cervical swabs respectively.

Most of the anaerobes and some of the aerobes isolated are frequently involved in genital tract infections suggesting that the normal flora of vagina is potentially pathogenic.

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