# Study of Aerobic Isolates from Vaginal Swabs in Pregnant Women in a Stand-alone Diagnostic Laboratory of Central Madhya Pradesh

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## Abstract

Introduction: Colonisation of vagina and cervical epithelium by a variety of micro organisms can cause pelvic infection in pregnant women. The pH and enzymes present in the vaginal secretions determines the type of micro organisms which may cause infection. The knowledge of the commensal bacterial flora of vagina and cervical epithelium is important in pregnancy as many complications may arise due to infection like premature rupture of membranes, puerperal fever, intra or post partum neonatal infections via upper respiratory tract or the umbilical cord Bacterial vaginosis (BV) occurs in about 20% of the pregnant women and has been associated with adverse pregnancy out comes. The present retrospective study was undertaken with the primary aim of analyzing the common micro organisms causing vaginal infections in pregnant women coming to our microbiology department for vaginal swab cultures. *Materials and Methods:* High vaginal swabs were collected from 267 pregnant women coming to our Microbiology Department with vaginal

symptoms from June 2014 to May 2015. Samples were collected with sterile swabs and immediately inoculated on blood and MacConkey agar plates. After 24 hours, Gram's stain of pure colonies was done to indentify the organism and accordingly panel was selected for identification and sensitivity on Vitec II (Biomerieux). *Results:* A total of 159 / 267 (59.5%) cultures were sterile. 108 / 267 (40.5%) cultures showed growth of micro organisms out of which E.coli was isolated in 22 (20.3%), Candida (33.3%), Staph. aureus (20.3%), group B Streptococci (11.1%) and Klebsiella (2.7%). Candida species was the commonest organism isolated, followed by Staph aureus and E.coli, group B Streptococci and rarely Klebsiella . *Conclusion:* Lactobacilli constitute the major vaginal flora in women during child bearing age and is helpful in maintaining the acidic pH of vagina so that organisms do not invade and cause disease. The natural history of abnormal vaginal flora in pregnancy is still poorly understood but that it can cause perinatal morbidity and mortality is a well known fact. So, for a healthy perinatal outcome of pregnancy, a vaginal swab culture is required in all pregnant women.

Keywords: Lactobacilli; Bacterial Vaginosis; Premature Rupture of Membranes.

## Introduction

Colonisation of vagina and cervical epithelium by a variety of micro organisms can cause pelvic infection in pregnant women [1]. The pH and enzymes present in the vaginal secretions determines the type of micro organisms which may cause infection [2]. The knowledge of the commensal © Red Flower Publication Pvt. Ltd. bacterial flora of vagina and cervical epithelium is important in pregnancy as many complications may arise due to infection like premature rupture of membranes, puerperal fever, intra or post partum neonatal infections via upper respiratory tract or the umbilical cord [3, 4].

The vaginal flora of healthy asymptomatic women is determined by Lactobacillus which has a protective role to play in maintaining the natural healthy balance of vaginal flora especially during pregnancy. Lactobacilli are responsible for keeping the pH of the vagina below 4.5 by lactic acid production, which in turn inhibits the growth of non-acid tolerant micro organisms which are potentially pathogenic [5,6]. Lactobacillus species also produce hydrogen peroxide which is toxic to other micro organisms [7].

The normal vaginal bacterial flora is predominantly lactobacilliary type while abnormal flora consists of both aerobic and anaerobic micro organisms such as E.coli, group B streptococci, Enterococci, Candida species etc. Bacterial vaginosis occurs due to a change in the bacterial micro flora of vagina.

During physiological pregnancy, the high estrogen levels induce a better epithelial tropism and also an increased lactobacillary activity due to increased availability of glycogen, as the pregnancy advances, the bacterial flora of vagina changes. When lactobacilli decrease, the incidence of vaginal infection increases causing many adverse perinatal outcomes such as preterm labour, pre mature rupture of membranes, preterm birth and perinatal infection.

Bacterial vaginosis (BV) occurs in about 20 % of the pregnant women and has been associated with adverse pregnancy out comes [8].

The mechanism by which BV causes adverse pregnancy out comes is not yet well understood but may be due to alterations in the host defense mechanisms that can cause ascending intra uterine infection especially in immune compromised patients [9]. On the other hand, women with an exaggerated response to bacterial stimulus release large amount of cytokines at the maternal foetal interface and are at an increased risk for preterm labour if the bacteria gain access to the choriodecidual space. Micro organisms are recognized by Toll-like receptors which initiate release of inflammatory chemokines and cytokines. These trigger prostaglandin production in the amnion, chorion, decidua and myometrium leading to uterine contractions, cervical dilatation, and rupture of membranes, which in turn leads to bacterial entry into the uterine cavity. 40% of these peripartum febrile illnesses are caused by intra amniotic infections and early onset neonatal sepsis. The incidence increases with decreasing gestational age at delivery [10].

The present retrospective study was undertaken with the primary aim of analyzing the common micro organisms causing vaginal infections in pregnant women coming to our microbiology department for vaginal swab cultures.

#### Materials and Methods

High vaginal swabs were collected from 267 pregnant women coming to our microbiology department with vaginal symptoms from June 2014 to May 2015. Vaginal swabs were collected from pregnant women whether asymptomatic or with symptoms of abnormal vaginal discharge, itching, burning and lower abdominal pain.

Samples were collected with sterile swabs and immediately inoculated on blood and MacConkey agar plates to prevent drying. Simultaneously a wet smear and Gram's smear was examined for presence of pus cells clue cells, yeast cells, Trichomonas and other bacterial flora.

All plates were incubated at 37 degree Celsius for 24 hours. After 24 hours, Gram's stain of pure colonies was done to indentify the organism and accordingly panel was selected for identification and sensitivity on Vitec II (Biomerieux).

# Results

Total of 267 samples were analyzed during June 2014 to May 2015. The patients were divided into age groups of < 20 years (4 / 267, 1.4 %), 20 – 25 (46 / 267, 17.2 %), 26 – 30 (88 / 267, 32.4 %), 31 – 35 (65 / 267, 24.3 %), 36 – 40(29 – 267, 10.8 %), 41 – 45 (13 / 267, 4.86 %) and more than 45 years (22 / 267, 8.2 %). Table 1 shows the demographic data of these patients.

The organisms isolated in these groups are shown in table 2.

In < 20 years age group, no pathogenic micro organisms were isolated.

In 20 – 25 years age group 32 cultures were sterile, 1 each was E.coli, Staph. aureus and group B streptococci. Candida sps was isolated in 6 cases.

In 26 – 30 years age group, 40 cultures were sterile, 7 were E.coli, 18 were Candida sps, 8 were Staph. aureus and 4 were group B Streptococcus.

In 31 – 35 years age group, 40 cultures were sterile, 5 showed growth of E.coli, 6 were Candida sps, 4 were Staph. aureus, 2 each were group B Streptococcus and Klebsiella.

In 41 – 45 years age group, 10 cultures were sterile, 1 each was E.coli, Klebsiella and group B Streptococcus and 2 were Staph. aureus. In the age group above 45 years, 13 cultures were sterile, 4 showed growth of E.coli, 3 were Candida and 1 each were Staph. aureus and group B Streptococci.

A total of 159/267 (59.5%) cultures were sterile. 108 / 267 (40.5%) cultures showed growth of micro organisms out of which E.coli was isolated in 22 (20.3%), Candida (33.3%), Staph. aureus (20.3%), group B Streptococci (11.1%) and Klebsiella (2.7%). Candida species was the commonest organism isolated, followed by Staph. aureus and E.coli, group B Streptococci and rarely Klebsiella (table 3).

Maximum micro organisms were isolated in

26 - 30 years age group. No pathogenic organisms were isolated in age group below 20 years maybe because there were only 4 patients in this study group. Maximum patients were in 26 - 30 years age (88 / 267) group followed by 31 - 35 years age (65 / 267) group and 36 - 40 years of age (29/267).

The sensitivity pattern of the organisms isolates is shown in table 4.

The maximum growth in 26 – 30 years age group followed by 31 – 35 years age group is probably because this is the best child bearing age group and most pregnancies occur in these ages.

| Age group    | No. of cultures | Percentage |  |
|--------------|-----------------|------------|--|
| < 20         | 4               | 1.41%      |  |
| 20-25        | 46              | 17.20%     |  |
| 26-30        | 88              | 32.40%     |  |
| 31-35        | 65              | 24.30%     |  |
| 36-40        | 29              | 10.80%     |  |
| 41-45        | 13              | 4.86%      |  |
| >45          | 22              | 8.20%      |  |
| Total Sample | 267             |            |  |

|           | Organisms   |         |         |              |                |            |
|-----------|-------------|---------|---------|--------------|----------------|------------|
| Age group | Sterile     | E.coli  | Candida | Staph.aureus | Strepto coccus | Klebsiella |
| < 20      | 4           | 0       | 0       | 0            | 0              | 0          |
| 20-25     | 32          | 1       | 6       | 1            | 1              | 0          |
| 26-30     | 60          | 7       | 18      | 8            | 4              | 0          |
| 31-35     | 40          | 5       | 6       | 4            | 2              | 2          |
| 36-40     | 16          | 3       | 3       | 6            | 3              | 0          |
| 41-45     | 10          | 1       | 0       | 0            | 1              | 1          |
| >45       | 13          | 4       | 3       | 1            | 1              | 0          |
| No.o      | of cultures |         | 100 —   |              |                |            |
|           |             |         | 90 -    |              |                |            |
|           |             |         | 80 -    |              |                |            |
|           |             | ■ < 20  | 70 -    |              |                |            |
|           |             | 20-25   | 60 -    |              |                |            |
|           |             | 26-30   | 50      |              |                |            |
|           |             | ■ 31-35 |         |              |                |            |

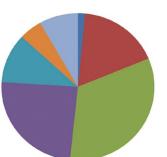
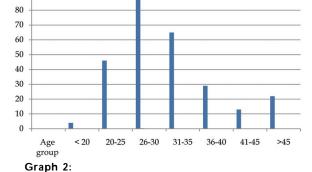


Table 3

Table 2: Isolates in different age groups





Graph 1:

|  | Organisms      | Total isolates | Percentage |  |  |  |
|--|----------------|----------------|------------|--|--|--|
|  | Sterile        | 159            | 59.50%     |  |  |  |
|  | E.coli         | 22             | 20.30%     |  |  |  |
|  | Staph aureus   | 22             | 20.30%     |  |  |  |
|  | Streptococci   | 19             | 11.11%     |  |  |  |
|  | Klebsiella     | 6              | 2.70%      |  |  |  |
|  | Candida        | 39             | 33.30%     |  |  |  |
|  | Total cultures | 267            |            |  |  |  |

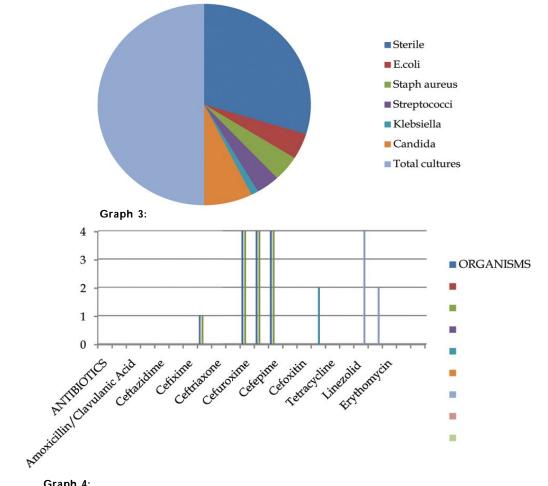
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|                        | Organisms         |   |       |                |       |     |               |   |  |
|------------------------|-------------------|---|-------|----------------|-------|-----|---------------|---|--|
| Antibiotics            | E.coli Klebsiella |   |       | Staphylococcus |       |     | Streptococcus |   |  |
| Ampicillin             | <=2               | S | <=2   | S              | <=8/4 | R   |               |   |  |
| Amoxicillin/Clavulanic |                   |   |       |                |       |     |               |   |  |
| Acid                   | <=2               | S | 16/8  | I              | <=4/2 | R   | <=4/2         |   |  |
| Amikacin               | <=2               | S | <=16  | S              | >32   | R   |               |   |  |
| Ceftazidime            | >=64              | R | >=64  | R              |       |     |               |   |  |
| Cefalotin              | >=64              | R | >=64  | R              | <=8   | R   |               |   |  |
| Cefixime               | >=64              | R | >2    | R              |       |     |               |   |  |
| Ciprofloxacin          | 1                 | S | 1     | S              | >2    | R   | <=1           |   |  |
| Ceftriaxo ne           | <=1               | S | <=1   | S              |       |     |               |   |  |
| Colistin               | <=0.5             | S | <=0.5 | S              |       |     |               |   |  |
| Cefuroxime             | 4                 | S | 4     | S              |       |     |               |   |  |
| Ertapenem              | 4                 | S | 4     | S              |       |     |               |   |  |
| Cefepime               | 4                 | I | 4     | I              |       |     |               |   |  |
| Fosfomycin             | <=16              | S | <=16  | S              |       |     |               |   |  |
| Cefoxitin              | >=64              | R | >=64  | R              | >4    | POS |               |   |  |
| Vancomycin             |                   |   |       |                | 2     | S   |               |   |  |
| Tetracycline           | >8                | R | >8    | R              | <=4   | S   | >8            | R |  |
| Teicoplanin            |                   |   |       |                | >8    | S   | <=8           |   |  |
| Linezolid              |                   |   |       |                | <=1   | S   | 4             |   |  |
| Levofloxacin           | <=2               | S | >4    | R              | >4    | R   | 2             | S |  |
| Erythomycin            |                   |   |       |                | >4    | R   |               |   |  |
| Clindamycin            |                   |   |       |                | <0.25 | R*  | <=0.25        | S |  |

Table 4: Sensitivity pattern of different isolates





Graph 4:

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# Discussion

Bacterial vaginosis is the commonest infection in women of reproductive age group and is an important cause of perinatal morbidity and mortality in pregnant women. The predominant micro organisms isolated from vaginal swabs in pregnant women include both gram positive and gram negative micro organisms like Staph. aureus, beta-hemolytic Streptococci, E.coli and Klebsiella pneumonia [11].

Bacterial vaginosis (40 - 45 %), vulvovaginal candidiasis (20 - 25 %) and other infections account for about 15 - 20 % of vaginal infections.Most vaginal infections are caused by commensal bacteria of the female genital tract [12]. The vaginal ecosystem changes from time to time due to a variety of endo and exogenous factors [13].

Highest incidence of vaginal infections in our study was found in 26 – 30 years age group, followed by 31 – 35 years age group probably because this is the sexually most active age group. The higher incidence can also be due to estrogen effect which increases the susceptibility of vaginal epithelium to micro organisms [14].

# Conclusion

Lactobacilli constitute the major vaginal flora in women during child bearing age and is helpful in maintaining the acidic pH of vagina so that organisms do not invade and cause disease. The natural history of abnormal vaginal flora in pregnancy is still poorly understood but that it can cause perinatal morbidity and mortality is a well known fact. So, for a healthy perinatal outcome of pregnancy, a vaginal swab culture is required in all pregnant women.

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