Vaginal Discharge: difficulties of diagnosis

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Vaginal Microbiota

‘Healthy’ reproductive aged women - increased estrogen levels

Dominated by *Lactobacillus* species
  - lactic acid production
  - hydrogen peroxide production
  - antimicrobial compounds

Discourages growth of invasive bacteria

Promotes vaginal health
Vaginal Dysbiosis

• A disturbance to the ‘normal’ vaginal microflora

• Caused by endogenous and exogenous factors

• Decrease in *Lactobacillus* concentration with an increase of other bacteria
  
  – Aerobic vaginitis AV (*Streptococcus, E. coli*)
  
  – Bacterial vaginosis BV (*Gardnerella, Prevotella*)
Bacterial Vaginosis [BV]

- Polymicrobial condition with no single cause or aetiology

- Overgrowth of anaerobes
  - Amine production
  - ‘fishy’ odour discharge
  - Increased pH

- Common cause of abnormal vaginal discharge in child-bearing age women

- Symptoms can be absent or non-specific

- Not a recognised STI → sexually enhanced infection
Anaerobic Overgrowth

- Mixed population consisting of *Gardnerella vaginalis*, *Mobiluncus spp*, *Prevotella spp*, *Sneathia spp*, *Fusobacterium*

- *G. vaginalis* dominates in BV

- Detected in both healthy and unhealthy women but increased load associated with BV

- Research but no confirmed pathogenesis between different *G. vaginalis* clades
Polymicrobial Biofilm in BV

Rukavina Z and Vanic Z 2016 Current Trends in Development of Liposomes for Targeting Bacterial Biofilms Pharmaceutics 8(2), 18
Clinical Implications

- Sexual Health
- Fertility
- Quality of Life
- Pregnancy
Women with recent BV have a 3.5 fold increase risk of acquiring *M. genitalium* infection.
Comparative Study on the Vaginal Flora and Incidence of Asymptomatic Vaginosis among Healthy Women and in Women with Infertility Problems of Reproductive Age

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ABSTRACT
Introduction: The normal vaginal flora is highly complex, dominated by lactobacilli of doderan that plays a vital role in maintaining the women's health and inhibits other pathogenic microorganisms. Fluctuation in local environment or exposure to any oxygenous and endogenous sources changes the vaginal flora over a period of time. Disruption of the vaginal ecosystem changes the microflora of the healthy vagina, altering the pH and predisposing to lower reproductive tract infections. The change in the microflora of the female genital tract by pathogenic organisms may ascend from vagina to upper genital tract and may cause infertility. Although several studies demonstrate a higher prevalence of bacterial vaginosis in infertile population. The role of vaginal microbiome in infertility is not clear and need to be explored further.

Aim: To compare the vaginal flora and analyze the incidence of asymptomatic vaginosis among healthy women and in women with infertility problems.

Materials and Methods: A cross-sectional study was conducted over a period of six months at Sri Ramakrishna Medical College and Hospital Puducherry, India. A total of 200 high vaginal swabs were collected from Group 1 which included 84 healthy women with regular menstrual cycles without any gynecological disorder and from Group 2, 116 women with infertility problems attending fertility clinic within the age group of 18 to 45 years. All swabs were subjected to routine aerobic, anaerobic and fungal culture. Saline wet mount was performed for the detection of clue cells and Trichomonas vaginalis, 10% KOH was performed for demonstration of budding yeast cells and pseudo hyphae. Gram’s staining to determine the presence of yeast cells, leucocytes and bacterial morphotypes. The smear was also graded using Nugent scoring system.

Results: The vaginal flora of Group 1 was dominated by Lactobacillus (40, 27.8%) followed by Monococcus (22, 15.3%), Enterococcus (16, 11.1%), Coagulate negative Staphylococcus spp. (12, 8.3%). Whereas in Group 2, the most dominant flora was Candida spp. (30, 20.5%), Enterococcus (26, 23%) followed by Gram-negative bacilli such as E. coli (16, 14.1%). The percentage of Lactobacillus in Group 2 women with infertility problems was relatively low (4.3%). Asymptomatic vaginosis was present in 32 (27.6%) of Group 2 women compared to Group 1 women were only 6 (7.1%) had asymptomatic vaginosis.

Conclusion: Women with infertility problems showed higher prevalence of asymptomatic vaginosis and abundance of Bacterial Vaginosis (BV) associated compared to healthy women. Hence, this study recommends the screening of vaginal flora as a routine for all women, especially in women undergoing infertility treatment and also suggests the importance of vaginal culture and sensitivity in routine practice.

Keywords: High vaginal swab, Lactobacilli, Nugent’s scoring system

84 healthy women
116 women with fertility problems

*Lactobacillus* abundance lower in fertility problem women (P=0.023)

BV rate lower in healthy women (7.1% vs 27.6%)
Multi-centred study in France

Molecular assay to screen and treat dysbiosis during pregnancy

Will determine if preterm rate is reduced

Add to the evidence base of a vaginal dysbiosis with preterm delivery
BV Treatment

• Metronidazole to treat the anaerobic overgrowth

• Clindamycin as an alternative treatment

• The use of probiotics are under investigation

• Polymicrobial biofilm resistant to treatment
Diagnosis

‘Gold standard’ laboratory test – Nugent score

• Gram strain technique which quantifies different bacterial morphotypes

• Women are categorised as normal [0-3], abnormal [7-10] or intermediate [4-6]

• Insensitive and highly subjective

• Other dysbiosis entities missed

http://araratnews.am/medical-bacterial-vaginosis/
Utility of Gram stain

• Vaginal samples were Gram stained and given a Nugent score

• Samples underwent next generation bacterial 16S sequencing

• Percentage of *Lactobacillus* species were calculated
  
  target reads/total number of 16S reads X 100

• Plotted against Nugent score results and $R^2$ calculated
Lactobacillus reads vs Nugent score

$R^2 = -0.53$

No correlation
Inter-observer Agreement

- 8 trained Biomedical Scientists (BMS) scored 9 Gram stained slides for BV

<table>
<thead>
<tr>
<th>Sample</th>
<th>BMS</th>
<th>Agreement</th>
<th>Original Score</th>
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<tbody>
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<td>0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
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<td>12.5</td>
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- There is no external quality assurance scheme for Nugent score
Next Generation Sequencing

AIM: To identify microbial markers to define either a ‘normal or ‘abnormal’ vaginal micro-flora

• Vaginal swabs from women attending GUM clinic
  – 21 Nugent BV positive women
  – 20 Nugent BV negative women

• DNA was extracted and sequenced at QUB

• Library preps were constructed – V4 region of 16s rRNA gene amplified

• Subjected to Illumina MiSeq and analysed using Illumina Basespace software
Microbial Profiles from NGS data

• Nugent BV positive samples

• Nugent BV negative samples
  – All top reads consisted of *Lactobacillus* species with *L. acidophilus* being most predominant
Molecular Diagnostic Tool

- **Two real-time qPCR assays targeting a specific gene from G. vaginalis and L. acidophilus**

- **Optimal load threshold in vaginal swabs**
  - *G. vaginalis*: $10^8$ gene copies per ml
  - *L. acidophilus*: $10^4$ gene copies per ml

- **A Log difference in load between vaginal and endocervical samples**

[Image of qPCR assay diagram]

http://dyes.gene-quantification.info/
GUM Clinic: Prospective Study

- One vaginal swab from 228 women: 160 BV negative and 68 BV positive
- Tested against *L. acidophilus* (LA) and *G. vaginalis* (GV) qPCR assays

<table>
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<th>GV and LA Load threshold</th>
<th>Nugent Score</th>
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<td>BV</td>
<td>58</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>NON-BV</td>
<td>10</td>
<td>137</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>68</td>
<td>160</td>
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- **Sensitivity**: 85.3%
- **Specificity**: 85.6%
- **PPV**: 71.6%
- **NPV**: 93.2%

Molecular BV = LA < $10^4$ gene copies/ml and GV $\geq 10^8$ gene copies/ml

Molecular non-BV = LA $\geq 10^4$ gene copies/ml and GV < or $\geq 10^8$ gene copies/ml

**OR**
LA < $10^4$ gene copies/ml and GV < $10^8$ gene copies/ml

85.5% Agreement
Cohen’s k: 0.67
Substantial agreement
Indeterminate Women

- GV and LA load thresholds were applied to women with intermediate Nugent score 4-6

- Amongst 53 women:
  - 26 (49.1%) had a flora in-keeping with a BV
  - 27 (50.9%) were non-BV

- Molecular assay could be used to re-classify indeterminate women as BV or non-BV
Common Molecular Profiles

A. Low or no *L. acidophilus* load with high *G. vaginalis* load => BV flora

B. High *L. acidophilus* load with low or high *G. vaginalis* load => non-BV flora

C. No *L. acidophilus* and no *G. vaginalis* ~30% Nugent normal
   ~35% Nugent intermediate
   ~6% Nugent abnormal

→ Separate dysbiosis entity

→ Aerobic Vaginitis
Aerobic vaginitis [AV]

- First described as a vaginal dysbiosis in 2002

- Poses similar risks in pregnancy, fertility and sexual health as BV

- Vaginal purulent discharge, inflammation and epithelial disruption

- AV microflora composed of enteric commensal organisms (\textit{E. coli}, \textit{Staphylococcus}, \textit{Streptococcus}, \textit{Enterococcus} spp.)

- Different scoring method but still subjective and insensitive

- BV treatment is not effective against AV
Summary

- Vaginal microbiota is complex and varies between women
- Vaginal dysbiosis is associated with adverse outcomes in pregnancy, fertility, sexual health
- Current diagnostic test is imperfect with high proportion of women being indeterminate and other dysbiosis entities missed
- Diagnostic tool with improved accuracy and reliability is required
Future Prospects

- To design a panel of real time PCR assays to detect bacteria associated with vaginal dysbiosis, which can:
  - Distinguish between bacterial vaginosis and aerobic vaginitis
  - Give accurate and reproducible results within a clinical laboratory
  - Ensure women receive correct antimicrobial treatment to improve adverse outcomes
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