Vaginal Discharge: difficulties of diagnosis

Ciara Cox PhD

Research Fellow

Microbiology dept. Royal Victoria Hospital and Queens University Belfast

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Vaginal Microbiota

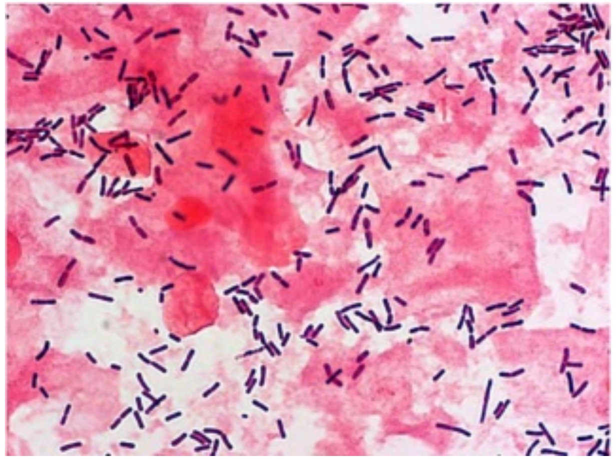
'Healthy' reproductive aged women - increased estrogen levels

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

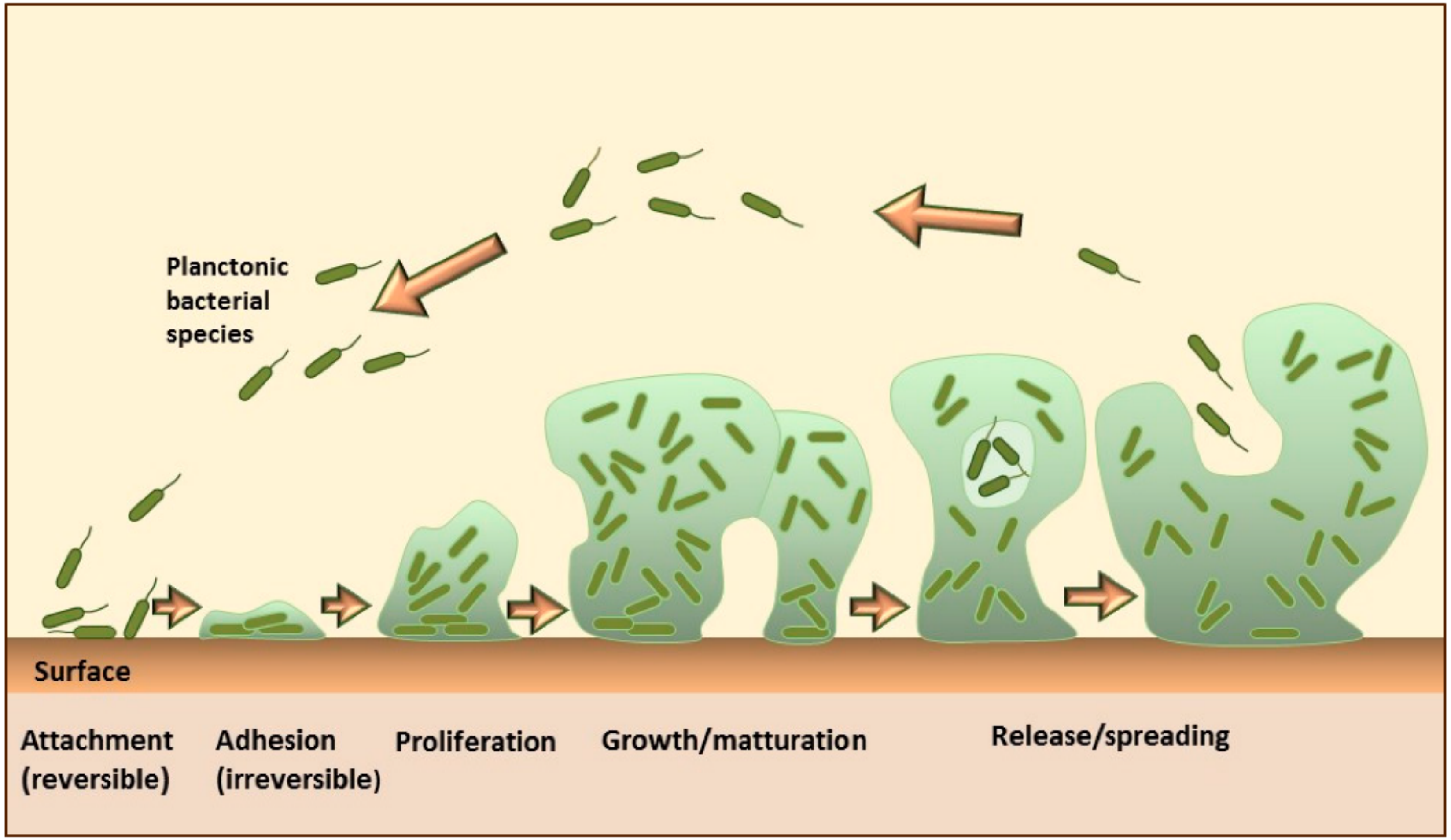
- Mixed population consisting of *Gardnerella vaginalis, Mobiluncus spp, Prevotella spp*, *Sneathia spp*, *Fusobacterium*

- *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

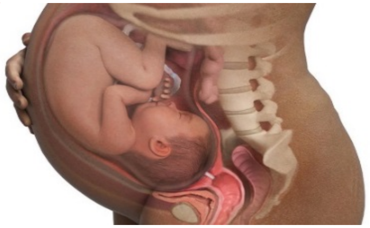
Clinical Implications

Sexual Health

Fertility

BV

Quality of Life



Pregnancy

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Original Contribution

Association of Recent Bacterial Vaginosis With Acquisition of *Mycoplasma genitalium*

Erica M. Lokken*, Jennifer E. Balkus, James Kiarie, James P. Hughes, Walter Jaoko, Patricia A. Totten, R. Scott McClelland, and Lisa E. Manhart

* Correspondence to Erica M. Lokken, Department of Epidemiology, School of Public Health, University of Washington, Box 359909, 325 9th Avenue, Seattle, WA 98104 (e-mail: elokken@uw.edu).

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We assessed the association between recent bacterial vaginosis (BV) and incident *Mycoplasma genitalium*, a sexually transmitted bacterium associated with adverse female reproductive health outcomes. Female sex workers in Mombasa, Kenya, completed a monthly sexual behavior interview and clinical examination. During February 2005–February 2006, vaginal fluid specimens collected from women every other month were tested for *M. genitalium* by nucleic acid amplification testing. Vaginal microbiota were assessed monthly and categorized by Nugent score (0–3 = normal microbiota, 4–6 = intermediate microbiota disruption, and 7–10 = BV). A discrete failure time analysis for multiple events using logistic regression was employed to estimate the odds of incident *M. genitalium* infection at follow-up visits among women with BV (vs. normal microbiota) at the preceding visit. Among the 280 women, 54.3% were positive for human immunodeficiency virus. At baseline, 16.1% had prevalent *M. genitalium* infection and 40.4% had prevalent BV. There were 59 incident *M. genitalium* infections among 50 women, for an incidence rate of 34.6 cases per 100 person-years. Following adjustment for age, human immunodeficiency virus status, and time, prior BV was associated with a 3.5-fold increase in odds of incident *M. genitalium* (adjusted odds ratio = 3.49, 95% confidence interval: 1.86, 6.56). This strong association suggests that BV may enhance susceptibility to *M. genitalium* infection.

bacterial vaginosis; *Mycoplasma genitalium*; Nugent score; vaginal microbiota

Abbreviations: aOR, adjusted odds ratio; BV, bacterial vaginosis; CI, confidence interval; FSW, female sex worker; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus type 2; IQR, interquartile range; STI, sexually transmitted infection.

Women with recent BV have a 3.5 fold increase risk of acquiring *M. genitalium* infection

Original Article

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Microbiology Section

Comparative Study on the Vaginal Flora and Incidence of Asymptomatic Vaginosis among Healthy Women and in Women with Infertility Problems of Reproductive Age

GEETHAVANI BABU[1], BALAMURU GANVELU SINGARAVELU[2], R SRIKUMAR[3], SREENIVASALU V REDDY[4], AFRAA KOKAN[5]

ABSTRACT

Introduction: The normal vaginal flora is highly complex, dominated by lactobacilli of doderlein that plays a vital role in maintaining the women's health and inhibits other pathogenic microorganisms. Fluctuation in local environment or exposure to any exogenous 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 analyse 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 Lakshmi Narayana 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 gynaecological 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 *Micrococcus* (22, 15.3 %), *Enterococcus* (16, 11.1%), Coagulase negative *Staphylococcus* spp. (12, 8.3%). Whereas in Group 2, the most dominant flora was *Candida* spp. (30, 26.5 %), *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, 3.5%). 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 bacteria 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%)

Bretelle *et al. Trials* (2015) 16:470
DOI 10.1186/s13063-015-1000-y

TRIALS

STUDY PROTOCOL Open Access

Screen-and-treat program by point-of-care of *Atopobium vaginae* and *Gardnerella vaginalis* in preventing preterm birth (AuTop trial): study protocol for a randomized controlled trial

Florence Bretelle[1,2], Florence Fenollar[2], Karine Baumstarck[3,4], Cécile Fortanier[5], Jean François Cocallemen[1], Valérie Serazin[6,7], Didier Raoult[2], Pascal Auquier[3,4] and Sandrine Loubière[3,4*]

Abstract

Background: International recommendations in favor of screening for vaginal infection in pregnancy are based on heterogeneous criteria. In most developed countries, the diagnosis of bacterial vaginosis is only recommended for women with high-risk of preterm birth. The Nugent score is currently used, but molecular quantification tools have recently been reported with a high sensitivity and specificity. Their value for reducing preterm birth rates and related complications remains unexplored. This trial was designed to assess the cost-effectiveness of a systematic screen-and-treat program based on a point-of-care technique for rapid molecular diagnosis, immediately followed by an appropriate antibiotic treatment, to detect the presence of abnormal vaginal flora (specifically, *Atopobium vaginae* and *Gardnerella vaginalis*) before 20 weeks of gestation in pregnant women in France. We hypothesized that this program would translate into significant reductions in both the rate of preterm births and the medical costs associated with preterm birth.

Methods/Design: A multicenter, open-label randomized controlled trial (RCT) will be conducted in which 20 French obstetrics and gynecology centers will recruit eligible pregnant women at less than 20 weeks gestation with singleton pregnancy and with a low-risk factor for preterm birth. Interventions will include a) an experimental group that will receive a systematic rapid screen-and-treat program from a point-of-care analysis using a molecular quantification method and b) a control group that will receive usual care management. Randomization will be in a 1:1 allocation ratio. The primary endpoint that will be assessed over a period of 12 months will be the incremental cost-effectiveness ratio (ICER) expressed as cost per avoided preterm birth before 37 weeks. Secondary endpoints will include ICER per avoided preterm birth before 24, 28 and 32 weeks, obstetrical outcomes, neonatal outcomes, rates of treatment failure and recurrence episodes for positive women. Uncertainty surrounding these estimates will be addressed using nonparametric bootstrapping and represented using cost-effectiveness acceptability curves. A total of 6,800 pregnant women will be included.

(Continued on next page)

- 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

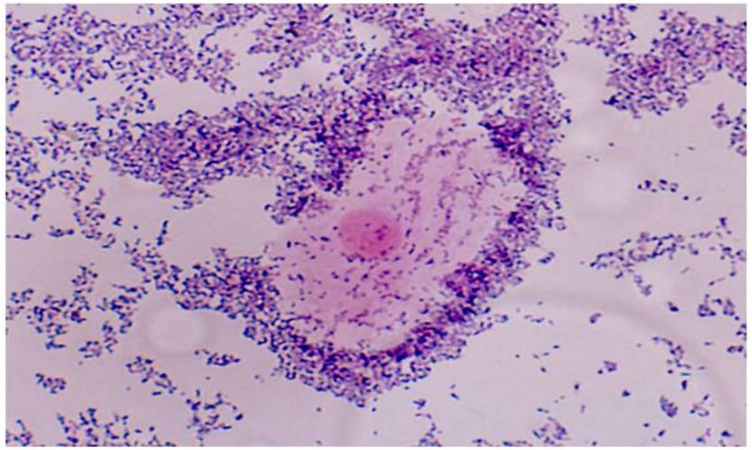
BV Treatment

- 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

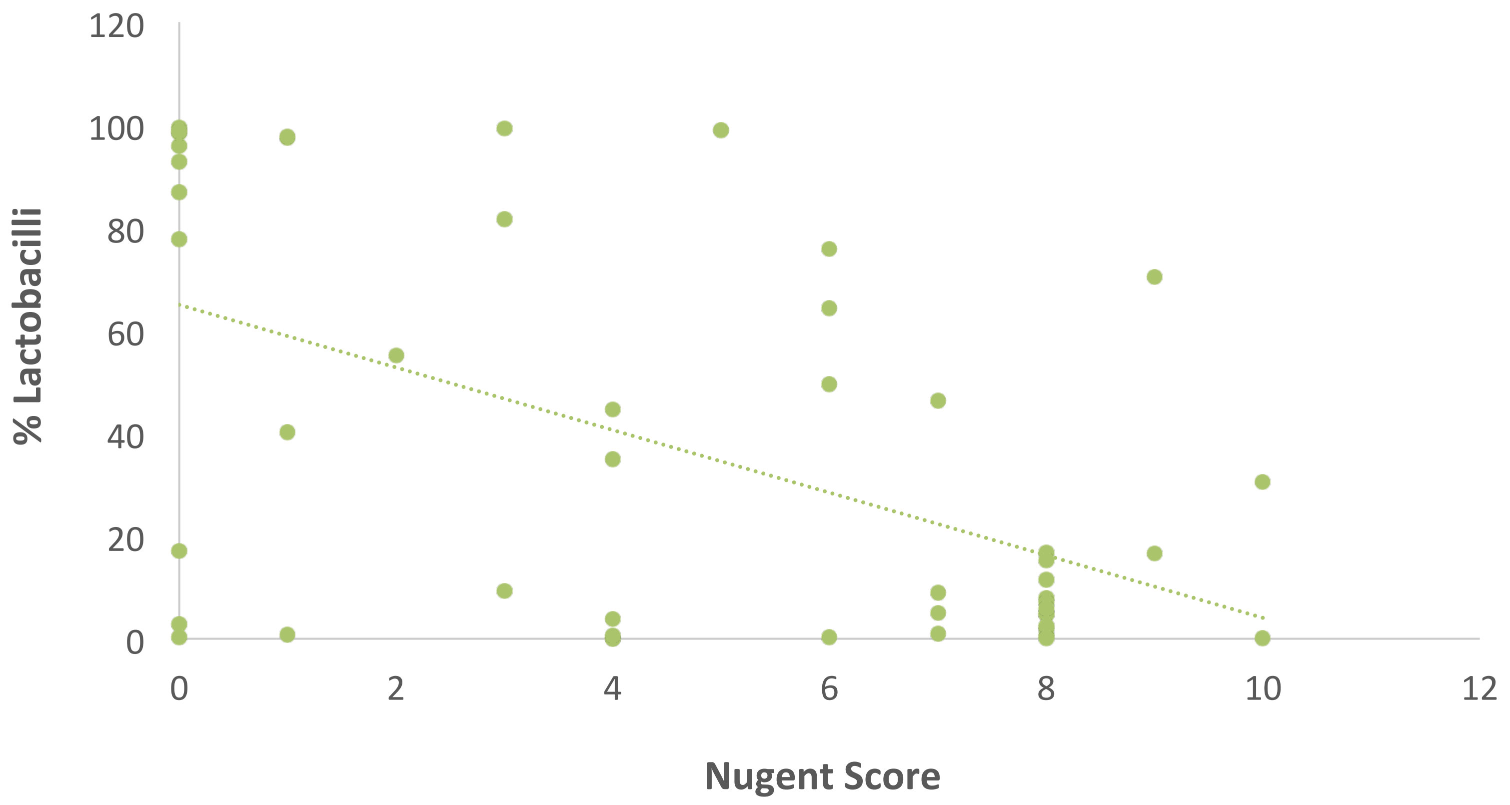
- Samples underwent next generation bacterial 16S sequencing

- 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

Nugent Score

Sample	**BMS 1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Agreement %**	**Original Score**
S1	0	0	0	1	1	2	0	0	100	1
S2	0	0	0	0	0	1	0	0	100	0
S3	0	0	0	1	1	2	0	0	100	1
S4	9	9	9	8	8	8	8	9	100	9
S5	6	8	8	6	7	6	8	8	62.5	8
S6	9	9	9	8	9	9	10	10	100	9
S7	4	4	4	4	4	4	4	4	100	4
S8	1	3	7	7	3	4	6	4	37.5	5
S9	3	3	2	3	2	3	3	4	12.5	4

Negative

Positive

Intermediate

- 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
  - Mixed populations: *G. vaginalis*, *Prevotella* spp, *Sneathia* spp, *Mycoplasma hominis, Ureaplasma parvum*

- Nugent BV negative samples
  - All top reads consisted of *Lactobacillus* species with *L. acidophilus* being most predominant

Molecular Diagnostic Tool

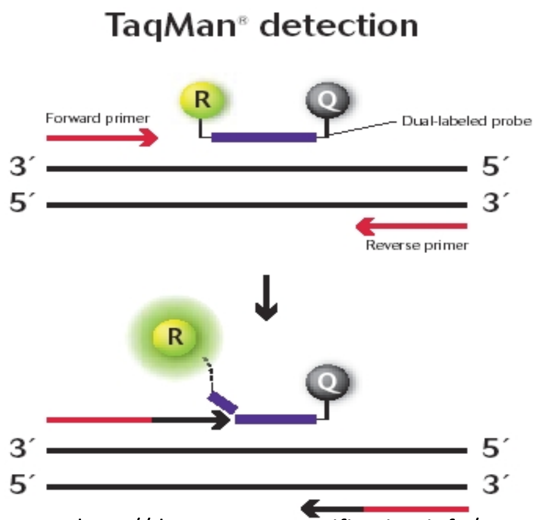
- *Two real-time qPCR assays targeting a specific gene from G. vaginalis and L. acidophilus*

- <u>Optimal load threshold in vaginal swabs</u>

  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



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

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

	GV and LA Load threshold	Nugent Score		TOTAL
		BV	Normal	
	BV	58	23	81
	NON-BV	10	137	147
	TOTAL	68	160	228
Sensitivity	85.3%			
Specificity	85.6%			
PPV	71.6%			
NPV	93.2%			

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* lo ith high *G. vaginalis* load => <u>BV flora</u>

B. High *L. acidop* *ginalis* load => <u>non-BV flora</u>

Separate dysbiosis entity

→ <u>Aerobic Vaginitis</u>

C. No *L. aci*

~30% Nugent normal
~35% Nugent intermediate
~6% Nugent abnormal

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, <u>inflammation</u> and epithelial disruption
- AV microflora composed of enteric commensal organisms (*E. coli*, *Staphylococcus*, *Streptococcus, Enterococcus* spp.)
- Different scoring method but still <u>subjective</u> and <u>insensitive</u>
- 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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