The Vaginal Microbiome and HIV/STIs

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Definitions: Types of organisms

IPI*	Which bacteria?	Colonised	Causes disease:
<0.1	Commensals E.g. Lactobacilli	100%	Rarely: Immuno-compromised, severe dysbiosis
0.1-0.3	Pathobionts E.g. Streptococci, Staphylococci, Enterococci, Enterobacteriaciae	20-80%	Sometimes: Specific circumstances / strains only
0.8-1.0	Pathogens E.g. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*	~0%	(Almost) always

* Intrinisic pathogenicity index = # diseased/# colonized = 0-1

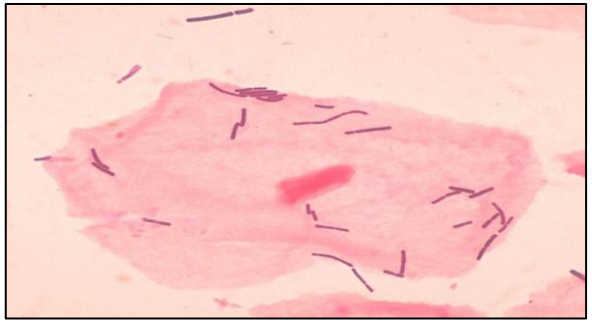
Vaginal microbiota < 2002

- Amsel criteria (Amsel, 1983)

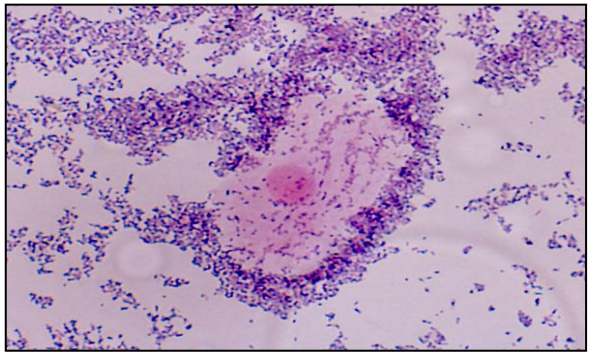
  3 of 4 present:
  - Vaginal pH > 4.5
  - Clue cells on wet mount
  - Amine smell after adding 10% KOH
  - Vaginal discharge

- Nugent score (Nugent, 1991):
  - Count 3 morphotypes on Gram stain slide
  - 0-3 normal, 4-6 intermediate, 7-10 BV

- Culture: Many missed.



Dispersed lactobacilli



Clue cells

Molecular studies 2002 onwards

- Many techniques but most commonly 16S sequencing:
  - 16S gene unique to bacteria
  - Some regions conserved → used for amplification
  - Some regions variable → used for taxonomic identification

- Important:
  - Does not identify viruses, yeasts, protozoa
  - Does not reliably identify bacterial pathogens/pathobionts (low relative abundance?)

van de Wijgert et al, PONE 2014; van de Wijgert and Jespers, Ann Epidemiol 2016

VMB compositions

Molecular data

Diversity

	CST (Ravel) Nugent
• No dysbiosis:	
– Dominated by *L. crispatus/iners* (most common)	I/III; 0-3
– Dominated by *L. gasseri/jensenii*	II/V; 0-3
– Mixed lactobacilli	0-3
– Lactobacilli with low abundance *G. vaginalis* or other taxa*	0-3
• Moderate dysbiosis:	
– Lactobacilli still present but increasing abundance of anaerobes** or pathobionts (streptococci, staphylococci, *Enterobacteriaceae*)	IV-A; 4-6
• Severe dysbiosis:	
– High abundance of pathobionts	4-6
– Dominated by *G. vaginalis* and/or *A. vaginae*	4-8
– Highly diverse mixture of anaerobes** with no/few lactobacilli	IV-B; 4-10

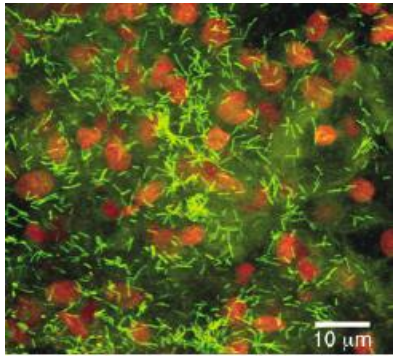
* Commonly includes *Bifidobacterium* and *Corynebacterium*

** Most commonly reported anaerobes: *Gardnerella vaginalis*, *Atopobium vaginae*, *Lachnospiraceae* (incl BVAB1, 2, *Mageebacillus indolicus*), *Prevotella*, *Megasphaera*, *Mobiluncus*, *Dialister*, *Eggerthella*, *Parvimonas*, *Veillonella*, *Gemella*, *Porphyromonas*, *Bacteroides*, *Sneathia*, *Leptotrichia*, *Mycoplasma*, *Ureaplasma*.

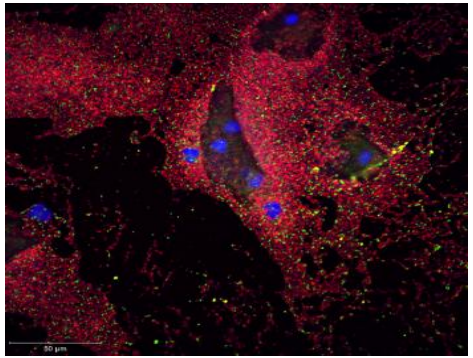
VMB biofilms

Lactobacilli: loose network

GV/AV: dense biofilm



Costerton et al, J Clin Invest 2003
Green = lactos
Orange = nuclei



Hardy et al, STI 2016
Red = *G. vaginalis*;
Green = *A. vaginae*;
Blue = nuclei

Also: Swidsinski 2005, 2010; Patterson 2010; Cerca 2013; others

RingPlus biofilm study, Kigali, Rwanda

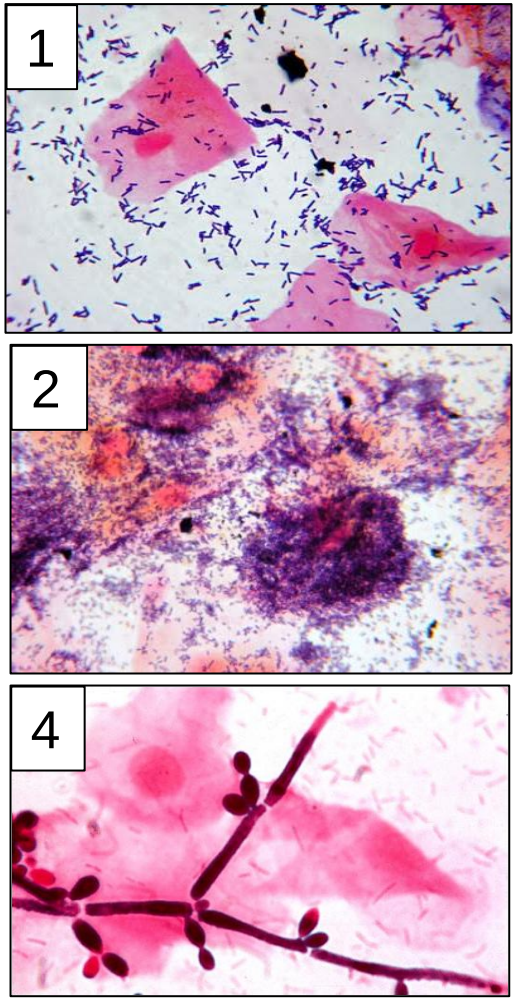
- 463 vaginal slides from 120 women
- FISH probes: universal bacterial probe (Bac-Uni1), GV (Gard162), AV (AtoITM1)
- 50.3% had biofilm: GV in 82%; AV in 54%; AV without GV in two slides.

Association with Nugent 4-10 (n=204) vs Nugent 0-3 (n=259)	aOR
GV and AV only dispersed	4.5
GV adherent+dispersed, no AV	49.2
GV adherent+dispersed, AV dispersed	75.6
GV and AV adherent+dispersed	119.0

Hardy et al, STI 2016; Hardy et al, PONE 2017

Current clinical perspectives

1. Healthy (= lactobacilli)
2. Anaerobic dysbiosis (= bacterial vaginosis) +/- biofilm
3. 'Aerobic' dysbiosis (= pathobionts)
4. Vulvovaginal candidiasis (= yeasts)
5. Sexually transmitted pathogens



Global burden

- High prevalences, esp in SSA [Torrone et al, PMED 2018]
- Most asymptomatic, most no sequelae

- Sequelae, even when asymptomatic:

  [van de Wijgert and Jespers, Res Microbiol 2017]:

  - HIV/STI acquisition and transmission
  - Pelvic inflammatory disease
  - Infertility, lower success rate of IVF
  - Miscarriage, preterm birth
  - Invasive post-abortion, maternal, neonatal infections

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HELIUS VMB Study, Amsterdam

Random (N=546), aged 18-35, 6 ethnicities, not seeking care

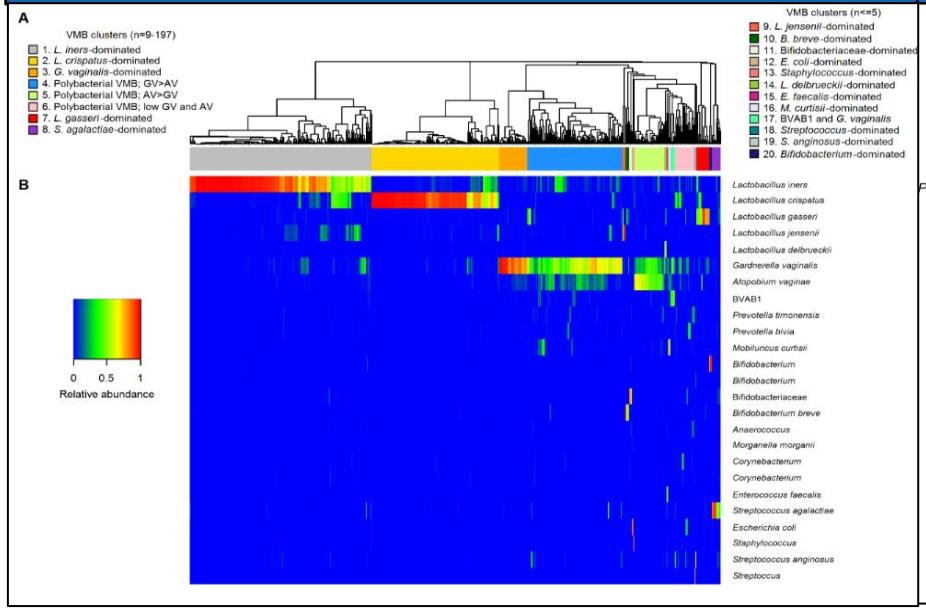
16S VMB groups	N	
L. crispatus dominated[1]	131	Normal (61.5%)
L. iners dominated[1]	187	
Other lactobacilli-dominated[1]	18	
Polybacterial GV-containing (high GV, AV, BVAB1)	134	BV (32.2%)
GV dominated[2]	29	
Other polybacterial (low GV, AV, BVAB1)	13	
Bifidobacteriaceae or Corynebacterium dominated[3]	14	Other dysbiosis (6.2%)
Pathobiont dominated (Strep, Staph, E. coli, …)[4]	20	

1. Most 50+% relative abundance with the remainder other lactos
2. 75-100% relative abundance with the remainder other anaerobes
3. Most 40+% relative abundance with the remainder lactos or anaerobes
4. Most 50+% relative abundance with the remainder lactos or anaerobes

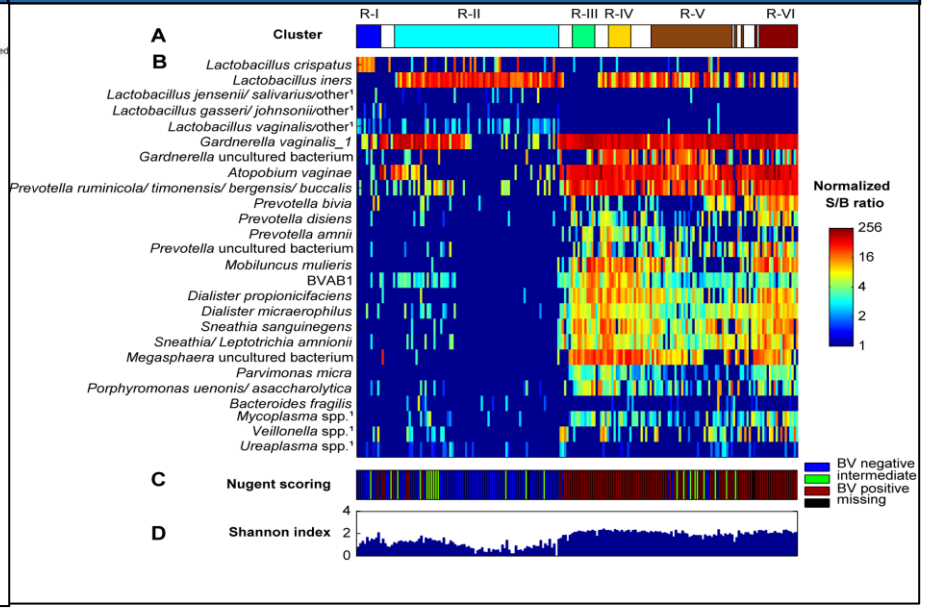
Borgdorff et al, PONE 2017

Variation by sexual risk [Borgdorff et al, PONE 2017 and ISME J 2014]

Amsterdam, NL, general population (HELIUS study; 16S sequencing)



Kigali, Rwanda, sex workers (KHIS study; DNA micro-array)



- Risk factors: condomless vaginal sex, new (uncircumcised) partner(s), STIs; also menses, smoking, vaginal douching
- Protective: hormonal contraception, pregnancy

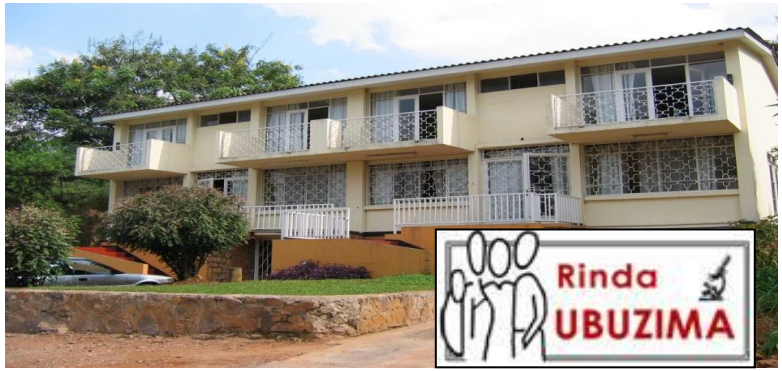
Cross-sectional associations with HIV/STIs

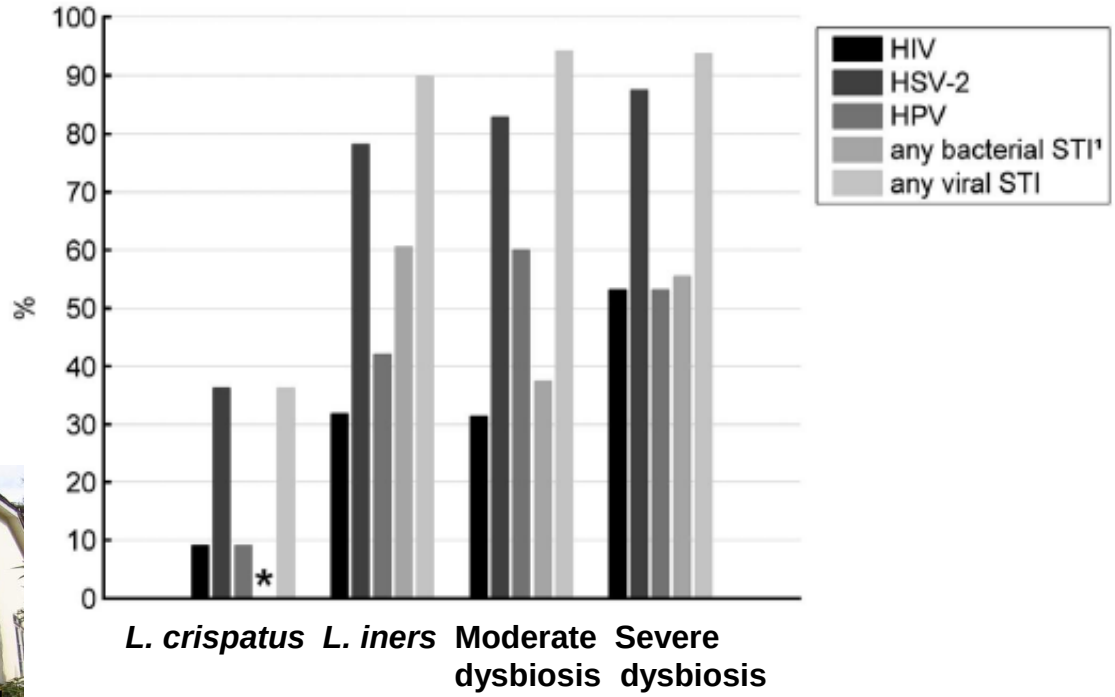
[Borgdorff et al ISME J 2014]

KHIS study Rwanda:
800 sex workers, 24% HIV+, high prevalence STIs

VMB by micro-array (n=202):

1. *L. crispatus*-dominated
2. *L. iners*-dominated
3. Moderate anaerobic dysbiosis
4. Severe anaerobic dysbiosis





* No bacterial infections

**In HIV-positive women, increasing HIV-1 RNA viral load

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Longitudinal associations with HIV

- **aHR=2.1** for Nugent 7-10 vs 0-3 [vd Wijgert et al, STI 2009]
- **aHR=1.7** for Nugent 7-10 vs 0-3 [Low et al, PMED 2011]
- **aHR=4** for high-diversity VMB vs *L. crispatus*-dominated VMB [Gossman et al, Cell 2017]
- *L. crispatus* ↓ and 7 anaerobic dysbiosis-associated taxa ↑ HIV acquisition risk in concentration-dependent manner [McClelland et al, Lancet ID 2018]

Mechanisms

KHIS study Rwanda:

Proteomics of cervicovaginal fluid (CVL; N=50) from 4 VMB groups:

1. *L. crispatus*-dominated
2. *L. iners*-dominated
3. Moderate anaerobic dysbiosis
4. Severe anaerobic dysbiosis

Borgdorff et al, Muc Immunol 2015; Borgdorff et al, ISME J 2014; Borgdorff et al, Sex Transm Dis 2015; Borgdorff et al, PONE 2016

Disruption cervicovaginal barrier

With increasing bacterial diversity:	
Mucus alterations	MUC 5AC, 5B ↑; MUC 6, 16 ==
Cell death	LDH-A, LDH-B ↑
Cytoskeleton alterations	Actin-organising proteins ↑ Keratins, cornified envelope proteins ↓
Altered antimicrobial peptide (AMP) balance	Psoriasin, calprotectin, histones ↑ Lysozyme C, ubiquitin ↓ Elafin, SLPI, LL-37, HNP-1, lactoferrin, BPI ==
Increasing proteolytic activity	Proteases/proteasomes ↑ Antiproteases, incl serpins, cystatin A↓ Elafin, SLPI ==
Immunoglobulins	Total IgG1, IgG2 ↓ Total IgA1, IgA2, IgG3, IgG4, IgM ==
Release of chemo/cytokines	Pro-inflammatory (IL-36α, C5, GPI, MIF) ↑

Mechanisms

Vaginal Biomarkers (VBM) Study:

- 430 women in Kenya, Rwanda, RSA selected by age (adolescents/ adults), HIV risk (average/high), HIV and pregnancy status
- VMB by qPCRs
- Immune mediators in CVLs by Bioplex and Meso Scale Discovery multiplex platforms or ELISAs

Anti-inflammatory

Pro-inflammatory

Kyongo et al, CVI 2015; Gautam et al, BMC-ID 2015; Jespers at al, Sci Report 2017

Inflammation/immune activation

By qPCR	Lower levels	Higher levels
L. crispatus	IL-1α/β, IL-8, IL-12p70, GM-CSF	IP-10
L. vaginalis	IL-1α/β, IL-12p70, MIP-1β	IP-10, SLPI
L. jensenii		IP-10, GM-CSF, SLPI
L. gasseri		IL-1RA, IL-12p70, GM-CSF
L. iners		IP-10, elafin
G. vaginalis	IP-10	IL-1α/β, IL-8, IL-12p70
A. vaginae	IP-10	IL-1α/β, IL-8, IL-12p70
P. bivia		IL-1β, IL-8
E. coli		IL-1RA, IL-1β, IL-6, IL-8, IL-12p70, GM-CSF, IP-10, MIP-1β
S. agalactiae		IL1β, IL-6, IL-8, IL-12p70, IP-10

VMB and PrEP drug metabolism

Tenofovir oral and vaginal PrEP trials in women have shown discrepant results → **exclusively due to poor adherence?**

Tenofovir PrEP efficacy by VMB status:

- CAPRISA 004 (vaginal gel): 61% if >50% vs 18% if ≤ 50% lactobacilli [Klatt et al, Science 2017]
- Partners PrEP (oral): no difference by Nugent score category - 77% vs 63% vs 73% [Heffron et al, Lancet HIV 2017]

→ **Dysbiosis-associated bacteria depleting tenofovir?**

van de Wijgert and McCormack, Lancet HIV 2017

Dysbiosis bacteria depleting tenofovir?

- More likely for vaginal than oral administration

But:

- Vaginal dysbiosis, HIV acquisition and poor PrEP adherence share risk factors → confounding?
- Minimum concentration required to prevent vaginal HIV acquisition not known and might not only differ by degree of dysbiosis, but also degree of vaginal immune activation for other reasons (VVC, STI, semen) and HIV exposure.

PrEP must continue → evaluate PK/PD of dosing schedules

Are some vaginal organisms worse than others?

- Pathogens, pathobionts, and *Candida* spp. activate immune system more than BV-associated anaerobes [many authors]
- Some BV-associated anaerobes associated with stronger inflammation?
  - *Fusobacterium* [Nikolaitchouk 2008; Anahtar 2015]
  - *Sneathia, Leptotrichia, Mobiluncus, Gemella* [Anahtar 2015; Gossman 2017]
  - *Prevotella bivia* [Zalenskaya 2015; Anahtar 2015; Gossman 2017]
- Likely also role for organism loads, not just types of organisms
- Currently unknown for drug metabolism

Interrelationships

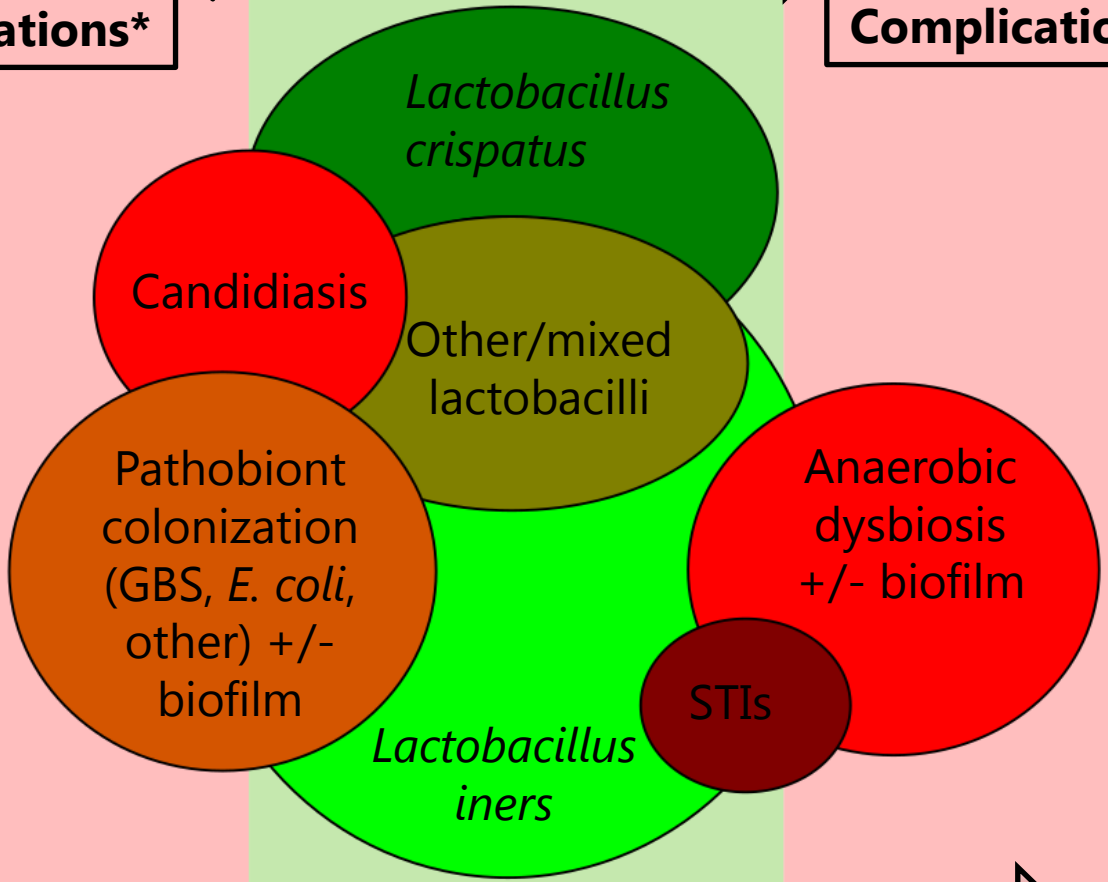
- Microbiota (vaginal dysbiosis, pathobiont carriage, VVC) and HIV/STIs are associated, many bidirectionally:
  - Shared risk factors related to sexual transmission
  - Biological links via cervicovaginal barrier disruption and inflammation

- Microbiota associations with HIV/STIs well documented, but also:
  - *Candida* spp. co-occur more often with lactobacilli than BV-anaerobes [van de Wijgert 2008, others]
  - *S. agalactiae* and/or *E. coli* carriage co-occur more often with lactobacilli and *Candida* spp. than with BV-anaerobes [Cools 2016]
  - Mechanisms: Individual bacterial preferences for vaginal pH, nutrients, microbial defense mechanisms, biofilms, attachment to *Candida* hyphae?

Inflammation Complications*

Inflammation Complications*

*HIV acquisition, pelvic inflammatory disease, adverse pregnancy outcomes, and maternal and neonatal infections.



Vaginal pH: 3.8-4.4; higher oestrogen → pH ≥ 4.5; lower oestrogen

PLOS MEDICINE

Citation: van de Wijgert JHHM (2017) The vaginal microbiome and sexually transmitted infections are interlinked: Consequences for treatment and prevention. PLoS Med 14(12): e1002478. https://doi.org/10.1371/journal.pmed.1002478

Diagnosis and treatment implications

Diagnosis:

- Differentiate dysbiosis types and whether biofilm present
- Differentiate from vaginal candidiasis, trichomoniasis, cervical STIs
- When to screen asymptomatic women?

Treatment:

- Effects of different antibiotics on VMB/pathobionts requires in-depth study on how entire vaginal niche is affected
- Vaginal probiotics and hormones (topical/systemic) as adjunct?
- Vaginal biofilm disruption?

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CHARM Combined Highly Active Anti-Retroviral Microbicides

EDCTP European and Developing Countries Clinical Trials Partnership

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