Microbiome Center

The vaginal microbiome

Vaginal complaints and urinary tract infections

12-05-2026

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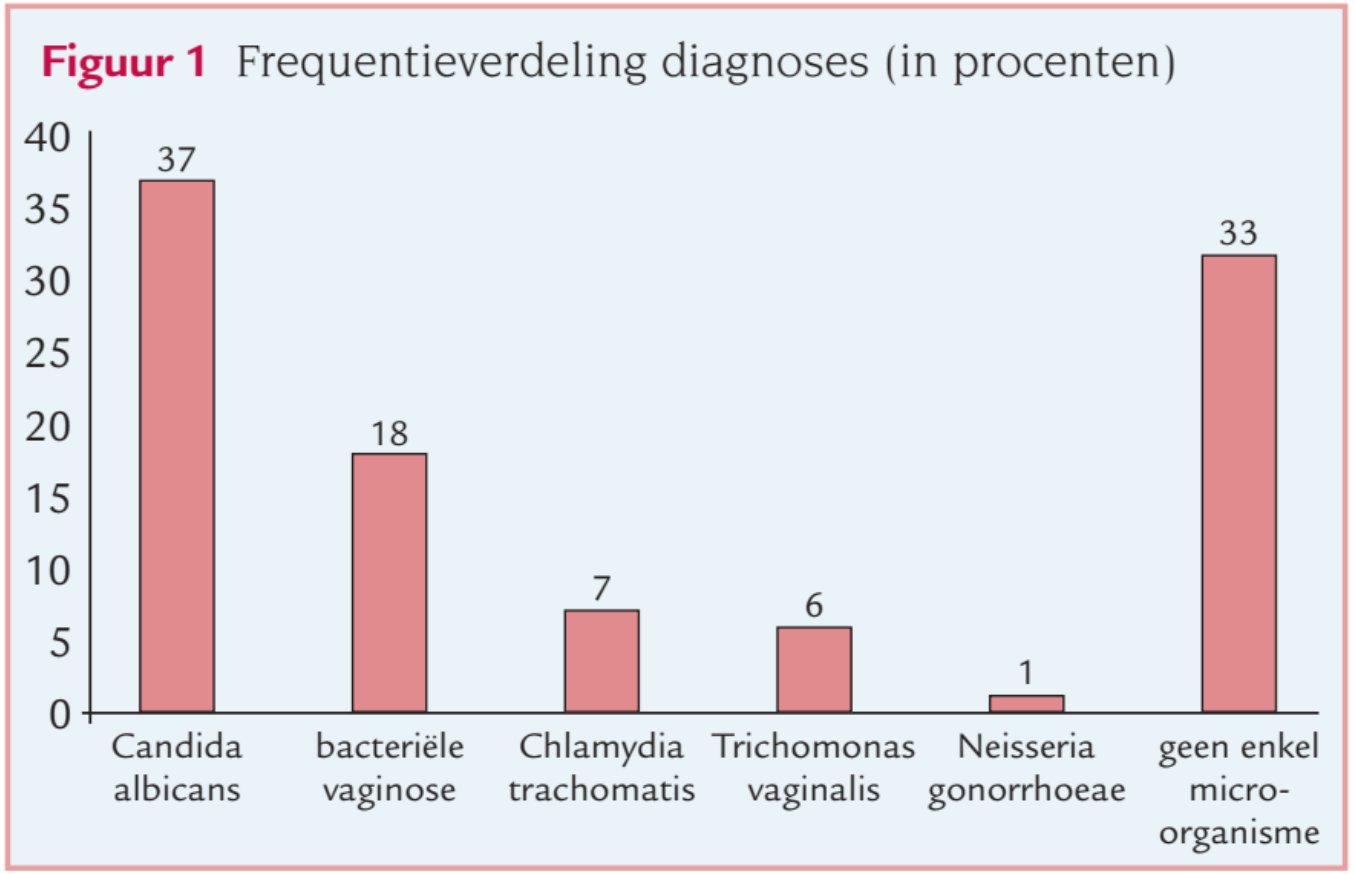
Introduction: vaginal complaints

Vaginal complaints

- According to the Dutch Family Doctor Association (NHG), the incidence of vaginal complaints is[1]:
  - 15.1 per 1000 per year for fluor vaginalis
    - Double for younger women
  - 24.6 per 1000 per year for urogenital candidiasis
- An older Dutch study among 682 women who visited the family doctor shows similar numbers (figure)[2].
- International figures of BV prevalence vary substantially (5-60%)
  - Finland and the US numbers are most representative for EU countries (5% and 30%)[3]



1. Fluor vaginalis | NHG-Richtlijnen, accessed 14-2-2023
2. Boeke, A. et al. Huisarts & Wetenschap 11, 616–620 (2002)
3. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)

Vaginal complaints

- Not only are vaginal complaint common, mental wellbeing is affected too[1]:
  - Fear (e.g. for severe disease or STD)
  - Shame
  - Problems in sexual relationship

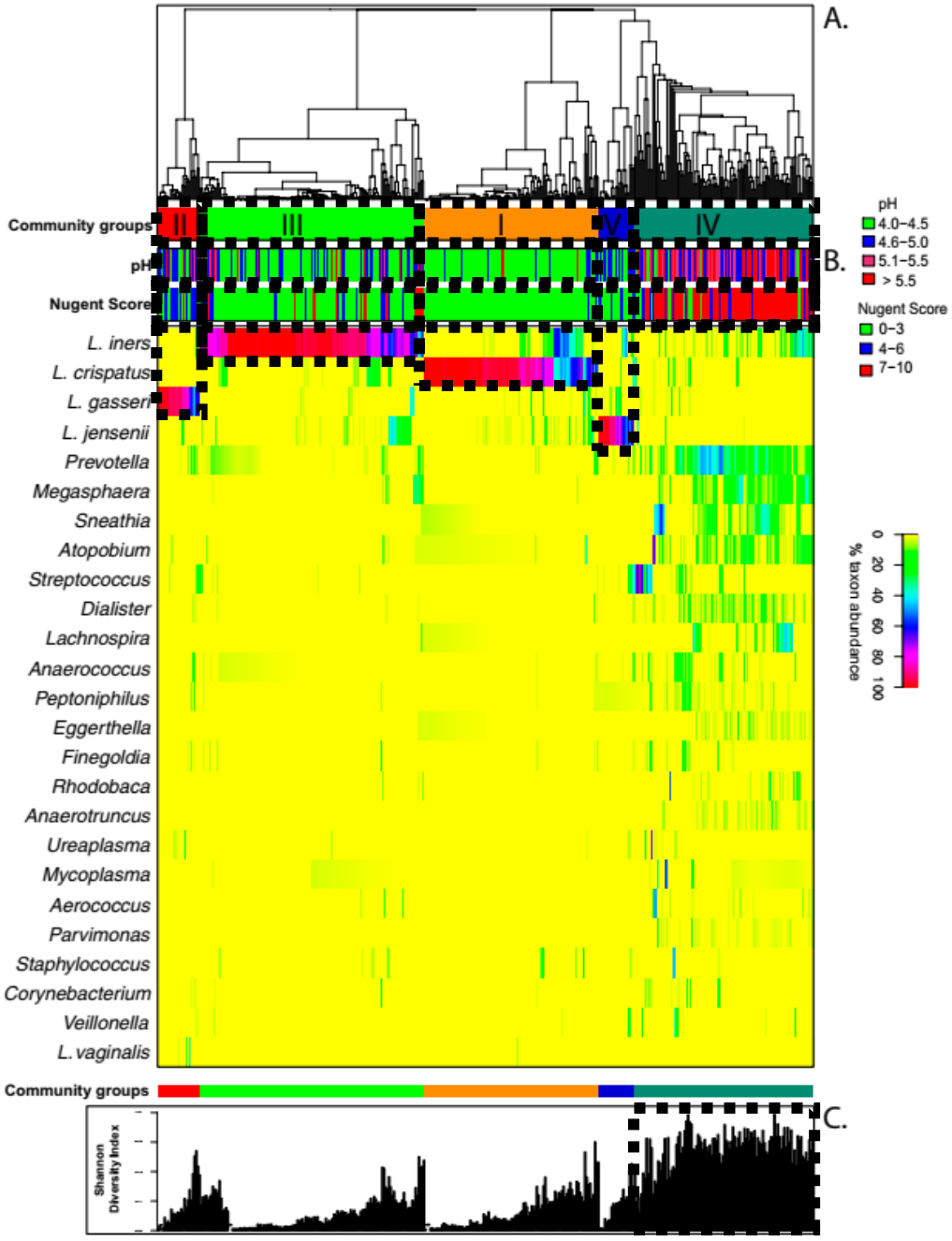
- Bacterial vaginosis (BV) is most common cause of abnormal vaginal discharge[2]:
  - Symptoms: malodor, increased pH, itching
  - 50% asymptomatic
  - Risk factors: sexual history, intravaginal practices, contraceptive use, antibiotic use.

1. Boeke, A. et al. Huisarts & Wetenschap 11, 616–620 (2002)
2. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)

The vaginal microbiome

Vaginal microbiome

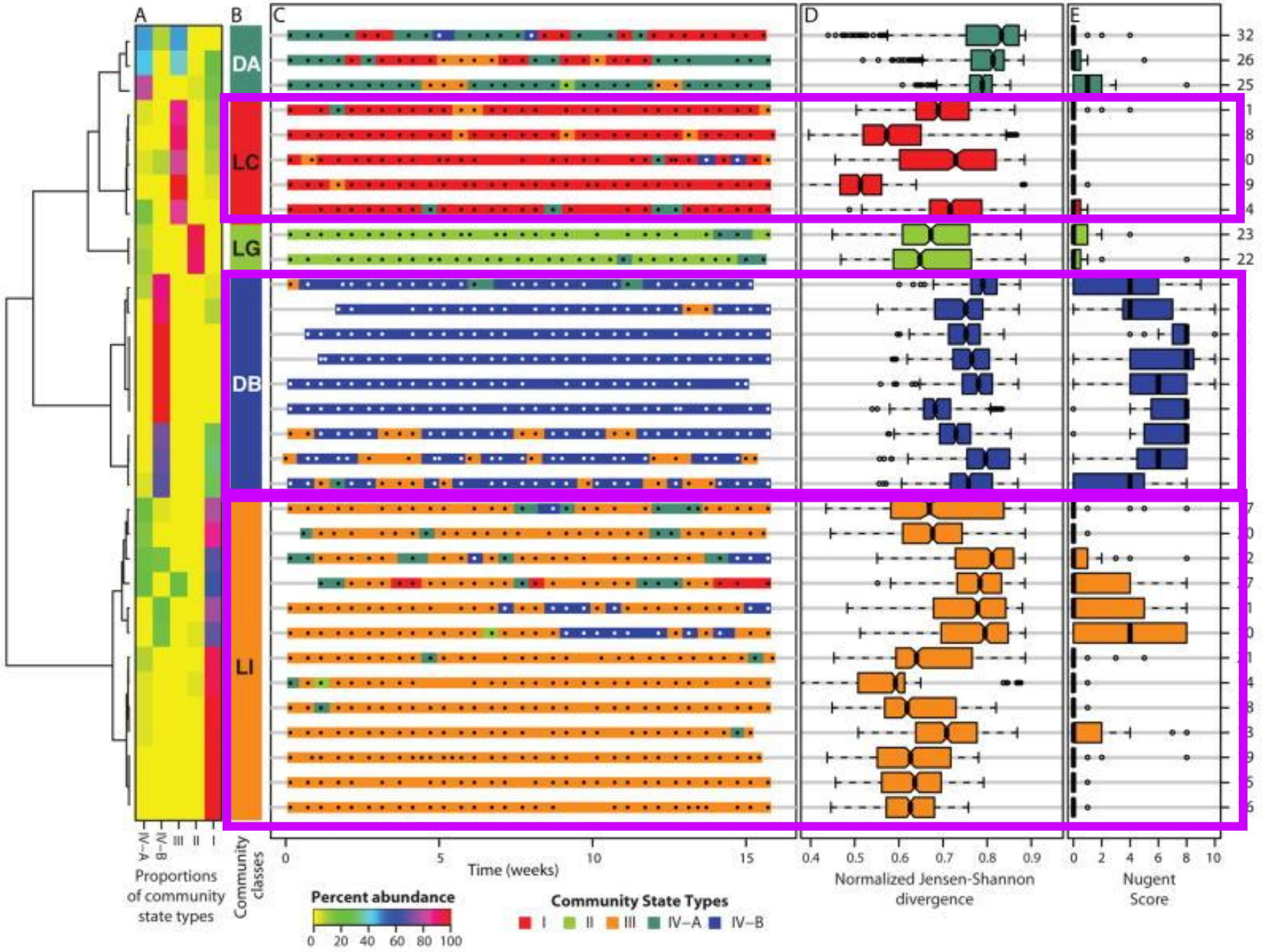
- The most cited study on the vaginal microbiome was published in 2011[1].
  - 5 groups were identified
  - In 4 of these the vaginal microbiome is dominated by a single Lactobacillus species: *L. iners, L. crispatus, L. gasseri, L. jensenii.*
  - The 5th group was characterized by high diversity. This 5th group has been regrouped into two subgroups[2,3].
    - One with high Nugent score.
- Although this grouping has been debated, conclusion remains that most women have a vaginal microbiome characterized by:
  - Single dominating Lactobacillus species (1 of 4)[2].
  - pH below 4.5



1. Ravel, J. et al. Proc Natl Acad Sci U S A 108, 4680–4687 (2011)
2. Verstraelen, H. et al. Journal of Lower Genital Tract Disease 26, 73 (2022)
3. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)

Vaginal microbiome

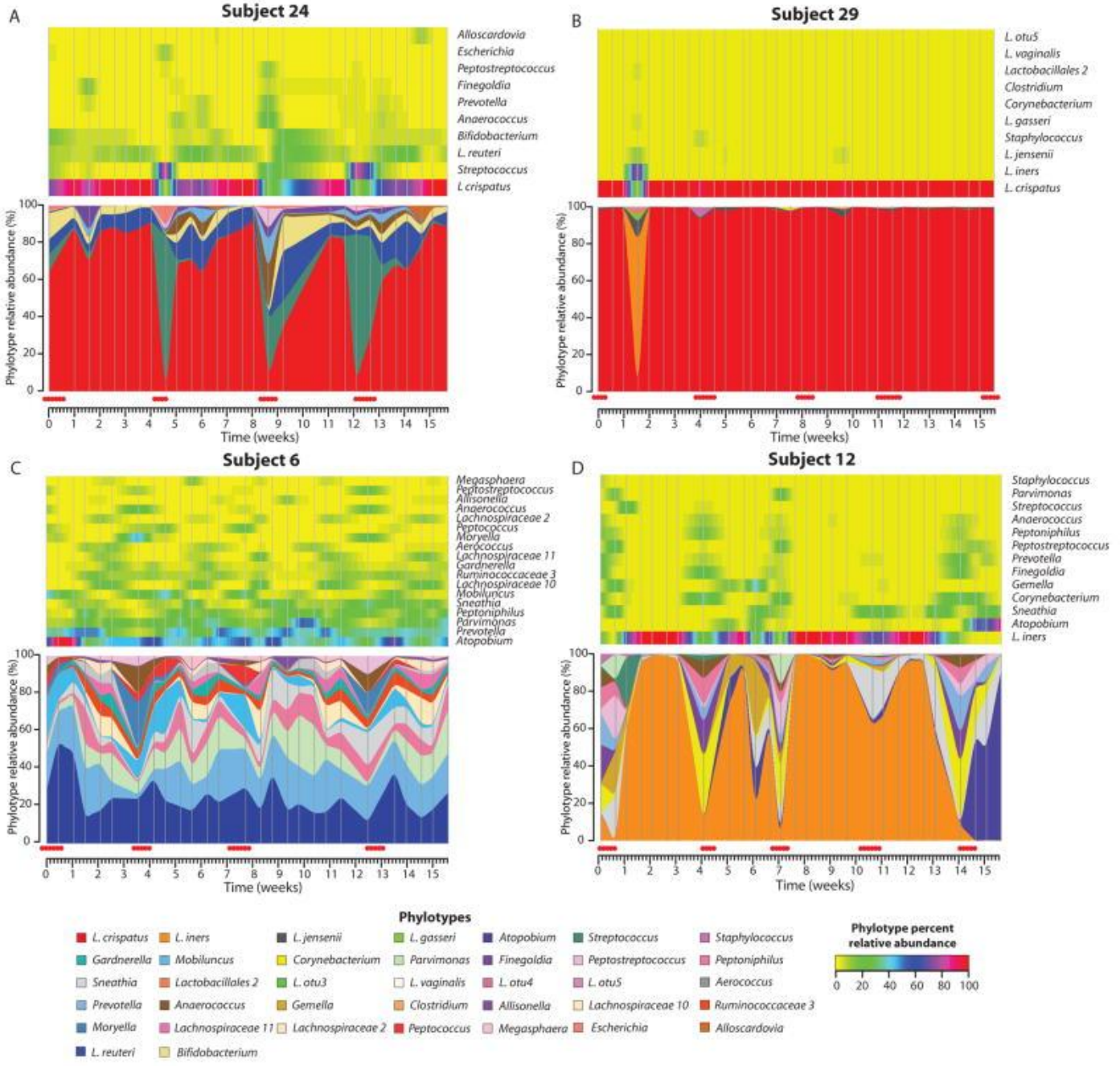
- Longitudinal research shows variation of the vaginal microbiome over time[1].
  - One of the high diversity subgroups was characterized by high Nugent scores (i.e. BV).
  - The *L. iners*-dominated vaginal microbiome is most prevalent in this population. The association of *L. iners* with health remains unclear, occurring in both dysbiotic and healthy states[2].
  - The *L. crispatus*-dominated and *L. gasseri*-dominated microbiomes are most stable and associated with lowest Nugent scores.



1. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)
2. Verstraelen, H. et al. Journal of Lower Genital Tract Disease 26: 73 (2022)

Vaginal microbiome

- Menses is major disturbing factor during the menstrual cycle[1,2]:
  - *L. crispatus* abundance declines during menses
  - *L. iners* concentrations increase along with *Gardnerella vaginalis* and is the dominant species during menses.
  - There is certain “dynamic stability”: most women retain their typical state or alternate between states.
  - Alternation is aligned with menses.



1. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)
2. Verstraelen, H. et al. Journal of Lower Genital Tract Disease 26: 73 (2022)

Vaginitis: various causes

Distinctions between causes of vaginitis

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Signs and Symptoms of Vaginitis

Diagnosis	Etiology	Symptoms	Signs	Other risks
Bacterial vaginosis	Anaerobic bacteria *(Prevotella, Mobiluncus, Gardnerella vaginalis, Ureaplasma, Mycoplasma)*	Fishy odor; thin, homogenous discharge that may worsen after intercourse; pelvic discomfort may be present	No inflammation	Increased risk of HIV, gonorrhea, chlamydia, and herpes infections
Vulvovaginal candidiasis	*Candida albicans,* can have other *Candida* species	White, thick, cheesy, or curdy discharge; vulvar itching or burning; no odor	Vulvar erythema and edema	—
Trichomoniasis	*Trichomonas vaginalis*	Green or yellow, frothy discharge; foul odor; vaginal pain or soreness	Inflammation; strawberry cervix	Increased risk of HIV infection Increased risk of preterm labor Should be screened for other sexually transmitted infections
Atrophic vaginitis	Estrogen deficiency	Thin, clear discharge; vaginal dryness; dyspareunia; itching	Inflammation; thin, friable vaginal mucosa	—
Irritant/allergic vaginitis	Contact irritation or allergic reaction	Burning, soreness	Vulvar erythema	—
Inflammatory vaginitis	Possibly autoimmune	Purulent vaginal discharge, burning, dyspareunia	Vaginal atrophy and inflammation	Associated with low estrogen levels

HIV = human immunodeficiency virus.

1. Paladine, H. L. et al. afp 97, 321–329 (2018)

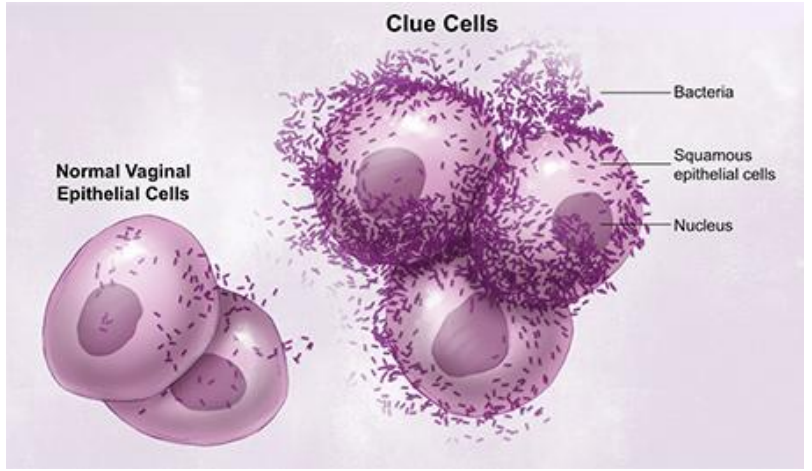
Bacterial vaginosis

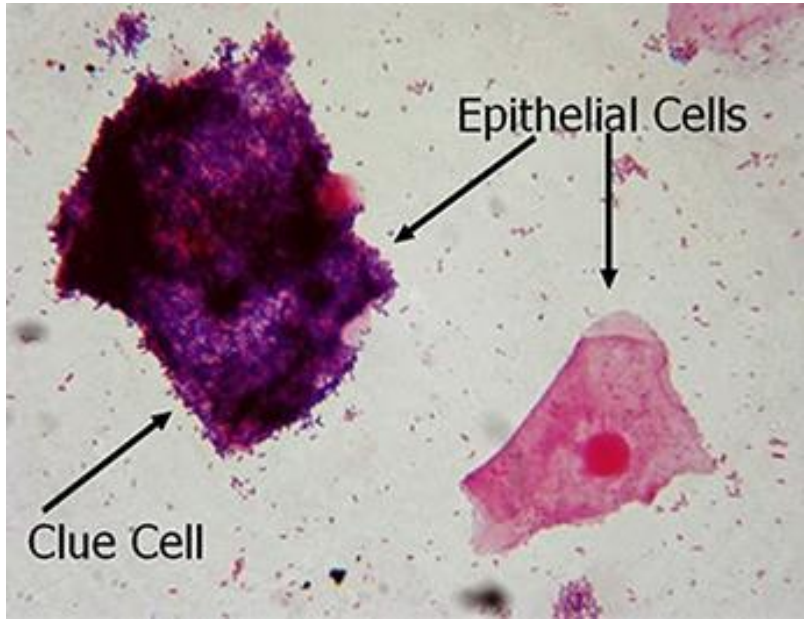
Diagnosis of bacterial vaginosis

- "Vaginosis" is vaginal form of dysbiosis but is not characterized by typical inflammatory changes generally implied by the suffix '-itis'[1].
- Diagnosis of BV can be done using various criteria[2]:
  - **Nugent score**. Vaginal smears plated. Minimum of 10 high power fields examined for three bacteria morphotypes: *Lactobacillus*, *Gardnerella*, and curved gram rods. Each category receives score for cell count. Scores added together and:
    - 0–3: negative for BV
    - 4–6: intermediate
    - 7+: positive for BV

    Method is cumbersome.
  - **Amsel criteria**. There are four parameters used to determine the presence or absence of BV. These are:
    - Thin, white, grayish, homogeneous discharge
    - Clue cells on wet mount microscopy
    - a vaginal fluid pH of over 4.5 when placing the discharge on litmus paper
    - Release fishy odor after adding 10% potassium hydroxide (KOH) solution to wet mount - also known as "whiff test."

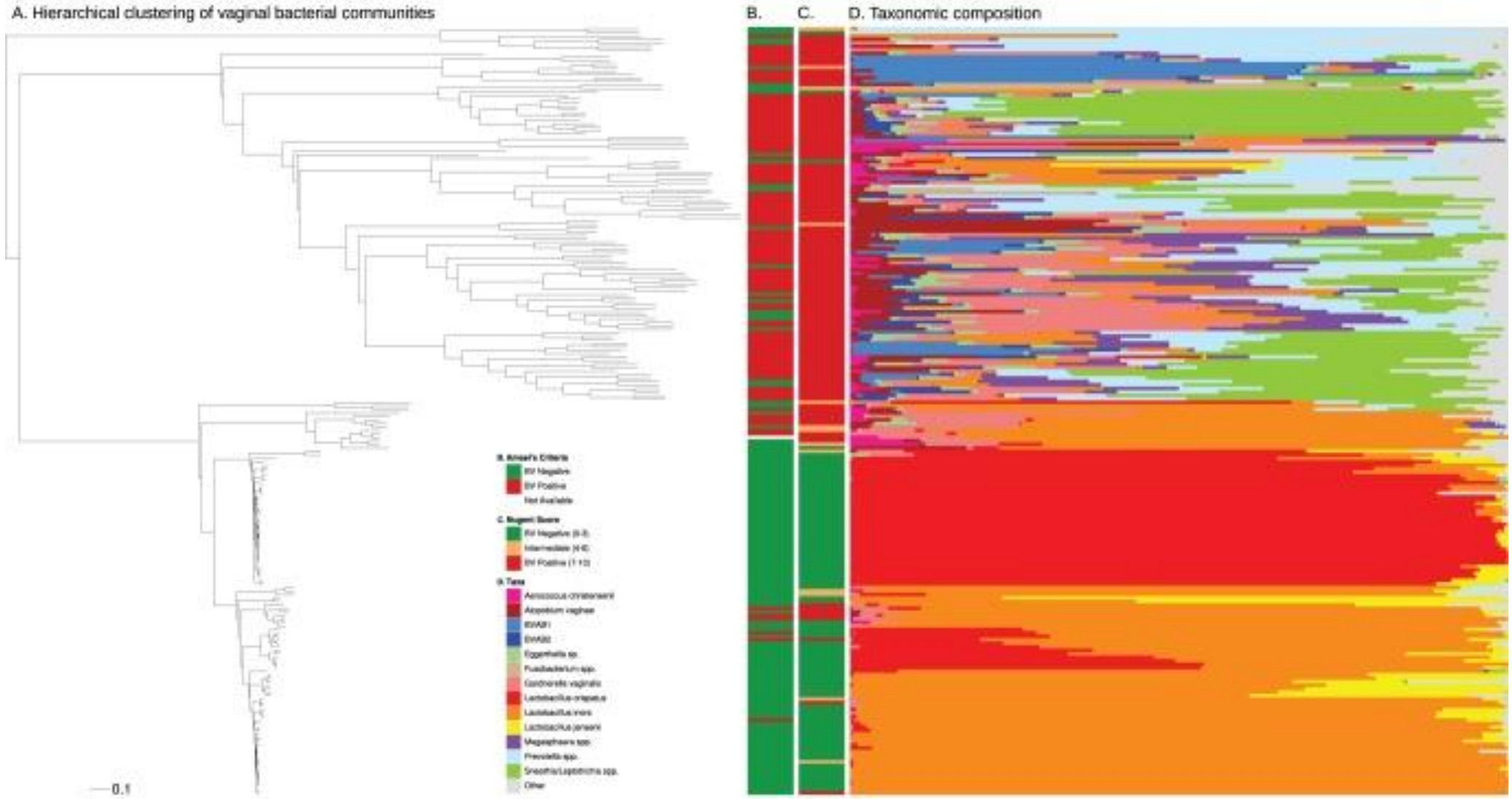
    More practical when there is limited diagnostician's microscopic experience, availability of microscope tools, or time. Meeting three (or two) criteria is considered positive for BV.
- Some women do not report symptoms even tough diagnostic criteria are met. Many women with BV may consider their discharge to be normal.





1. Schellenberg, J. J. et al. Research in Microbiology 168, 837–844 (2017)
2. Colonna, C. et al. in StatPearls (StatPearls Publishing, 2022)
3. Lev-Sagie, A. et al. Journal of Lower Genital Tract Disease 26, 79 (2022)

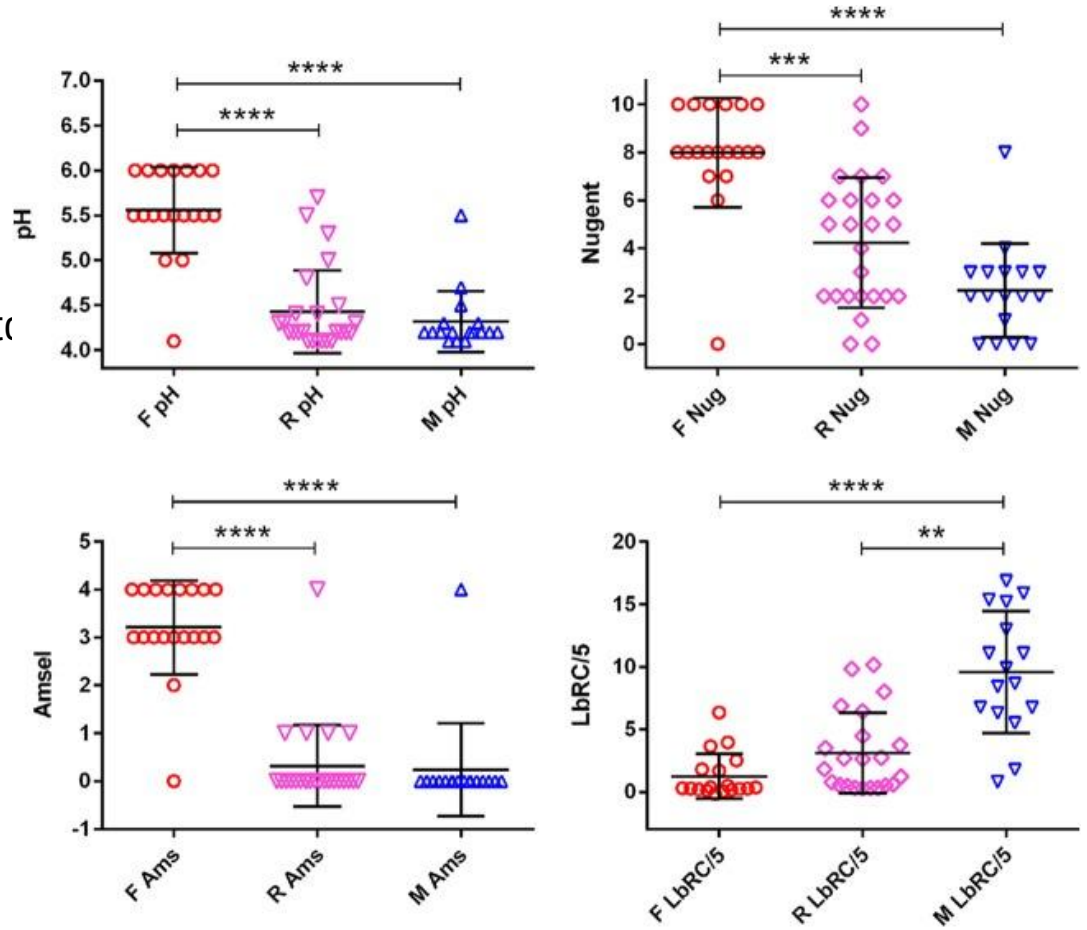
Vaginal microbiome: healthy vs bacterial vaginosis



1. Srinivasan, S. et al. PLoS ONE 7, (2012)

Traditional treatment of BV and recurrence

- According to guidelines BV is usually treated with antibiotics[1].
  - The reported initial cure rate in studies is high (80-90%)
  - The recurrence rate is high, ranging from 15-30% after treatment t 60-80% after 9 months
- One hypothesis for recurrence is reinfection with associated microbes via sexual partners[1]:
  - But: treatment of partners does not reduce recurrence rates.
  - Recurrence can also be due to relapse or ineffective treatment.
- **This is indirect evidence that susceptibility may be a more important factor. A disturbed vaginal microbiome plays a role**:
  - Indeed, the *Lactobacillus* relative composition is prognostic of cure rate and recurrence rate[2].

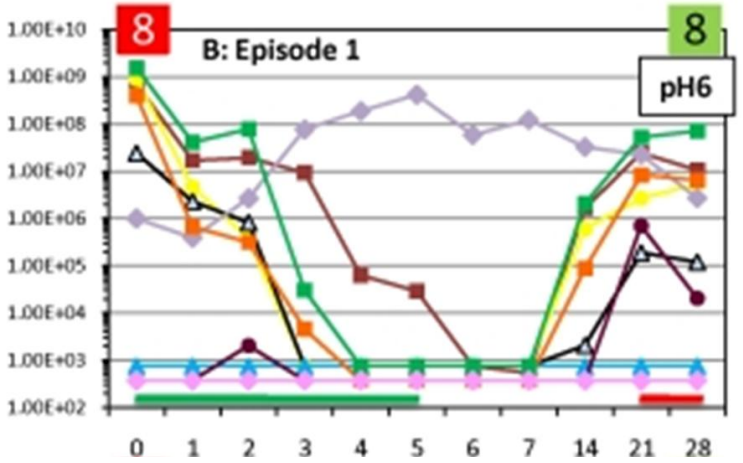


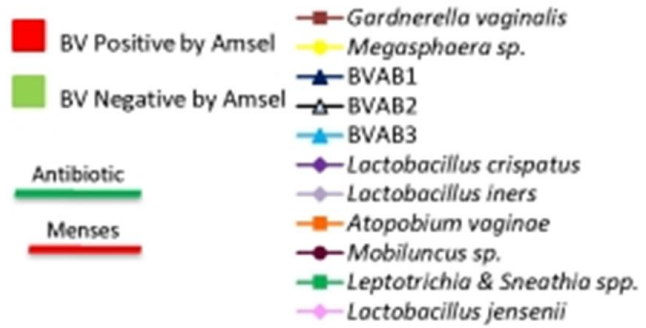
F = not cured after AB treatment
R = recurrent (initially cured, but relapse)
M = cured

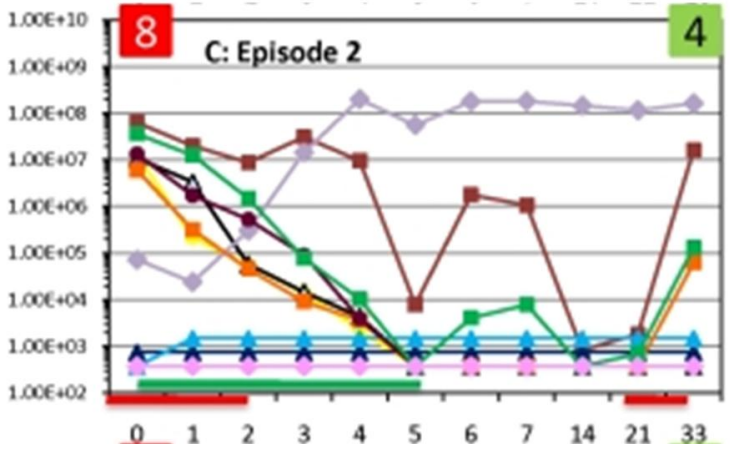
1. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)
2. Sobel, J. D. et al. J Clin Microbiol 57, e00227-19 (2019)

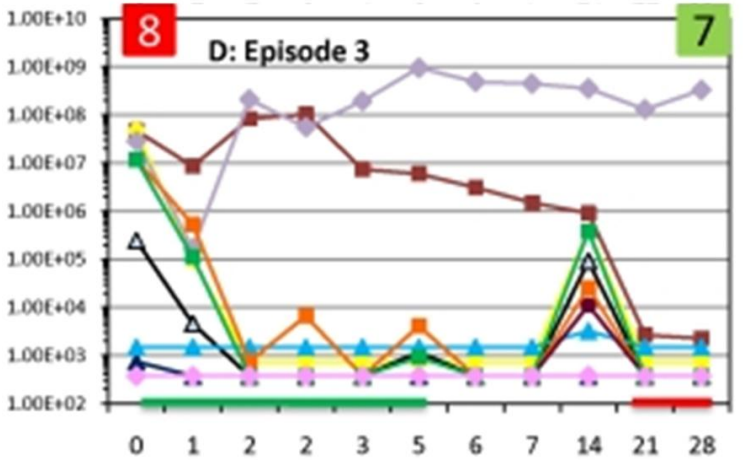
Vaginal microbiome: healthy vs bacterial vaginosis

- In women without BV[1]:
  - Vaginal microbiome is relatively stable
  - Disturbance occurs during menses
  - BV-associated *Gardnerella vaginalis* can be present and increase during menses
- In women with BV[1]:
  - Typically BV-associated species dominate (undefined species from *Clostridiales* order) and/or composition is diverse.









1. Srinivasan, S. et al. PLoS One 5, e10197 (2010)

Gut microbiome influence the vaginal microbiome. Treating gut dysbiosis may be needed

Microbiome Center

MAJOR ARTICLE

Colonization of the Rectum by *Lactobacillus* Species and Decreased Risk of Bacterial Vaginosis

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[1]Magee-Womens Research Institute, and [2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania

Lactobacilli colonizing the rectum may be a reservoir for vaginal lactobacilli. In a cross-sectional study of 531 females, vaginal and rectal colonization by lactobacilli were assessed by culture methods. A subset of isolates was identified to the species level by use of whole-chromosomal DNA probes. *Lactobacillus crispatus* (16%), *L. jensenii* (10%), and *L. gasseri* (10%) were the prevalent lactobacilli colonizing the rectums of 290 females. Only 13 (9%) of 147 females colonized by *L. crispatus* or *L. jensenii* vaginally and/or rectally had bacterial vaginosis (BV), compared with 12 (44%) of 27 females colonized by other H_2O_2-producing lactobacilli ($P < .001$). Cocolonization of the vagina and rectum by H_2O_2-producing lactobacilli was associated with the lowest prevalence of BV (5%), whereas females colonized only vaginally, only rectally, or at neither site had a successively increased risk of BV ($P < .001$). *Lactobacillus* species in the rectum may contribute to the maintenance of vaginal microflora.

1. Antonio, M. A. D. et al.The Journal of Infectious Diseases.(2005)
2. Fudaba, M. et al.Microorganisms.9,.1027.(2021)

Case report 1: VVC + BV

- Woman 22 years old
- Alternating vaginal complaints due to candida or due to bacterial vaginosis
- Was prescribed vaginal suppositories

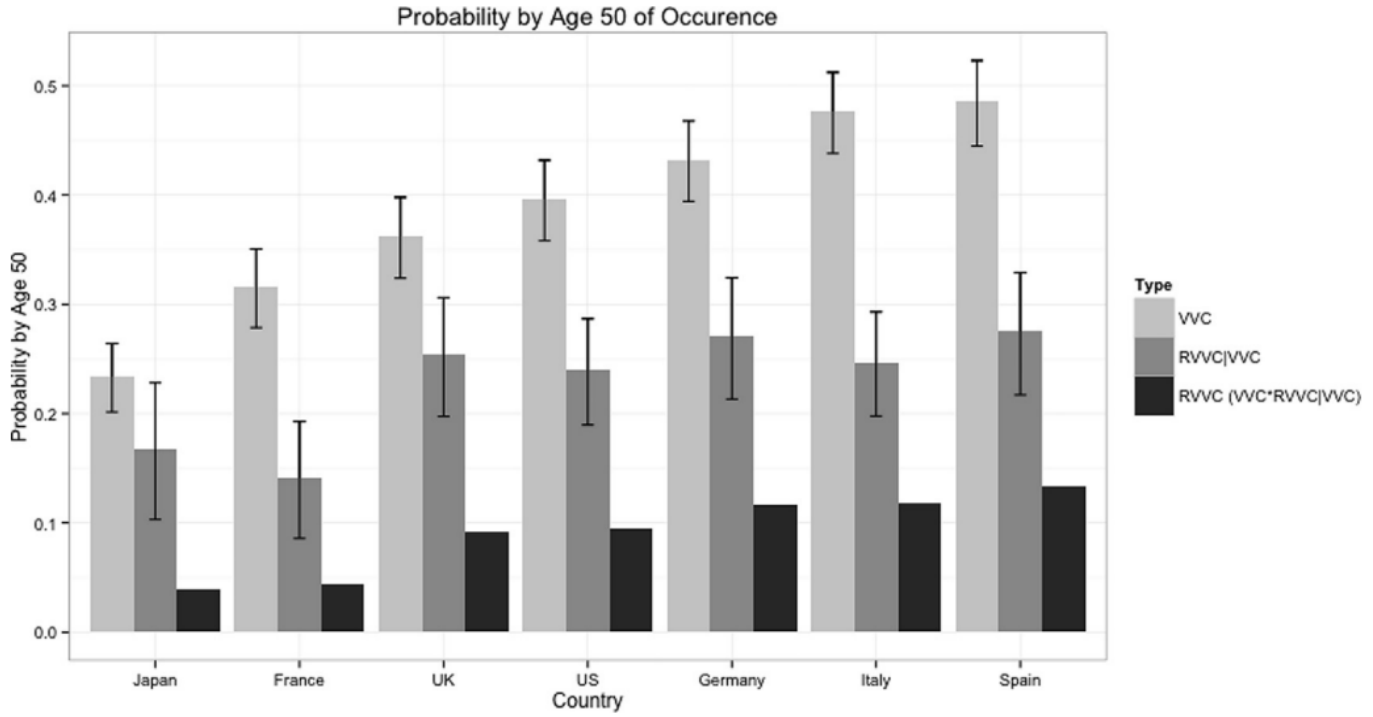
- Were they easy to use?
  - *“There seems to be some kind of coating over them that makes it easy. It doesn't hurt either.“*
- Did you experience any side effects?
  - *“I did not experience any side effects.”*
- Have they had the desired effect?
  - *“The suppositories often had the desired effect, especially with vaginal yeast infections. With bacterial infections, it seems to come back more quickly.”*

Vulvovaginal candidiasis

Vulvovaginal candidiasis

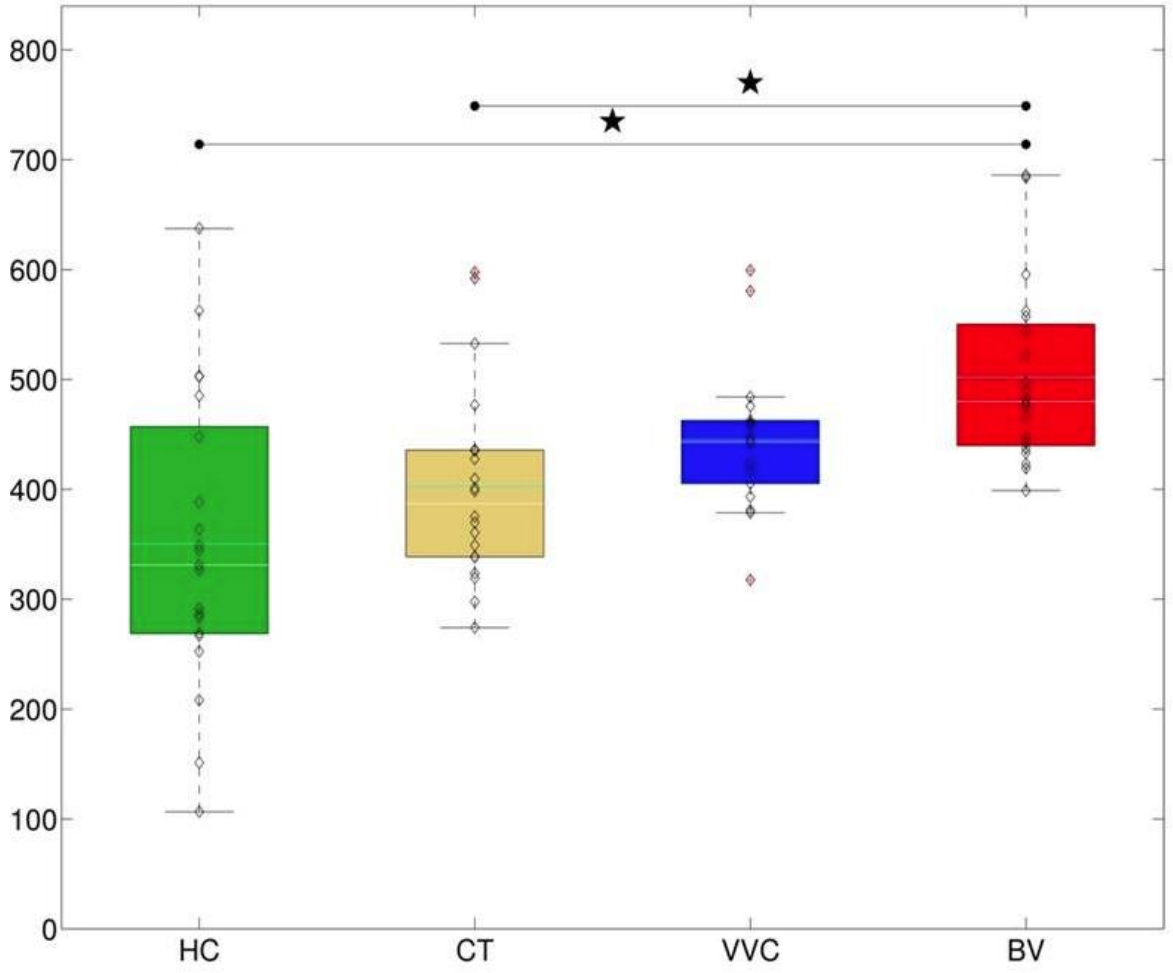
Vulvovaginal candidiasis[1]:

- Is most often caused by C. albicans (85-90%), sometimes by other candida species.
- Is characterized by vulvar erythema, excoriation, pruritus, and an abnormal "cheese-like" or watery vaginal discharge.
- Diagnosis is not straightforward: defined by combination of nonspecific vaginal symptoms and presence of yeast that is commensal.
    - Is often diagnosed and treated empirically, with much self-diagnosis and treatment.
- Has high cumulative incidence: 23-49% at age of 50.
- Is typically treated with vaginal azoles.
- Has often recurrence (50-60% within 1-2 years)

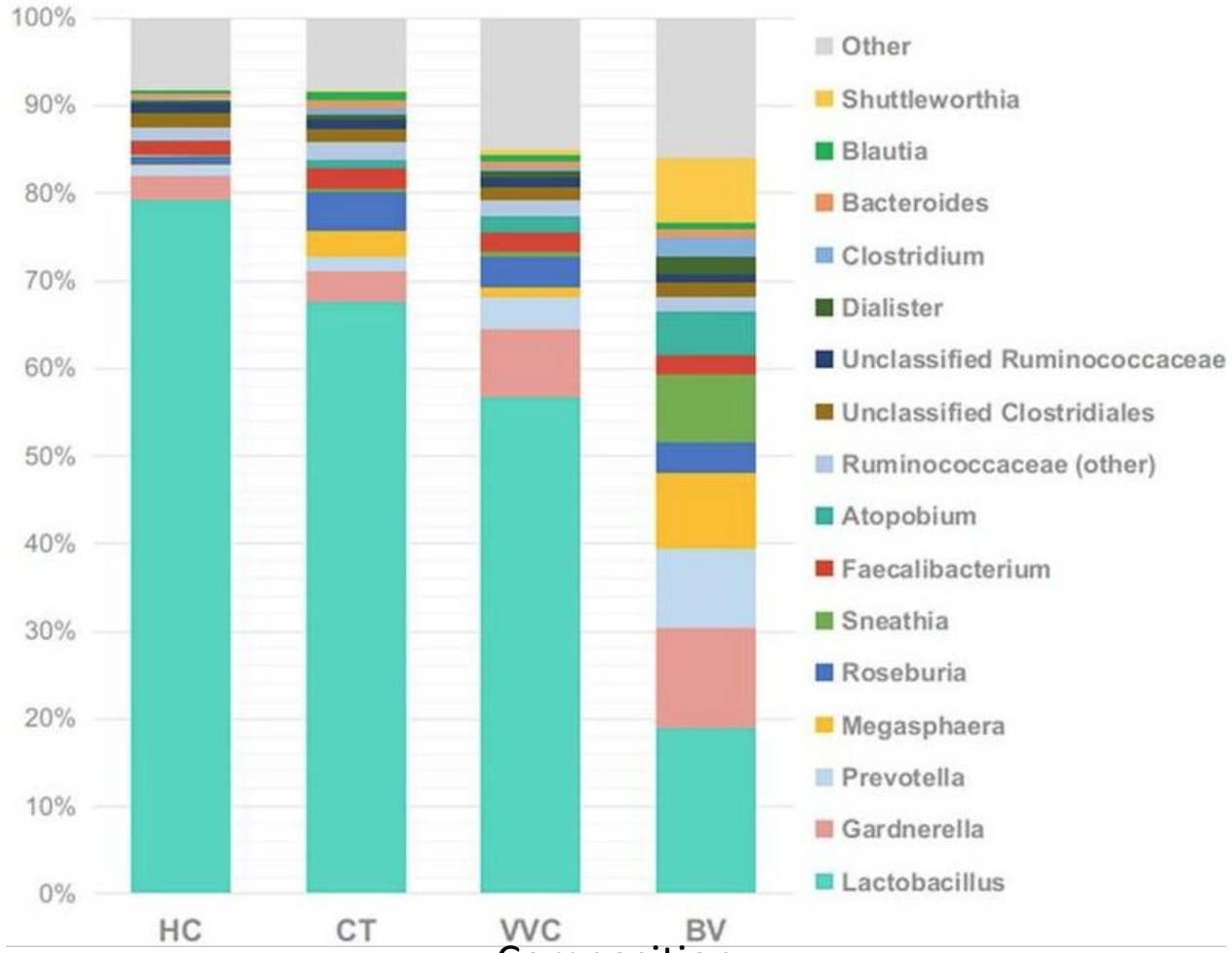


1. Blostein, F. et al. Annals of Epidemiology 27, 575-582.e3 (2017)

Vaginal microbiome: healthy vs VVC



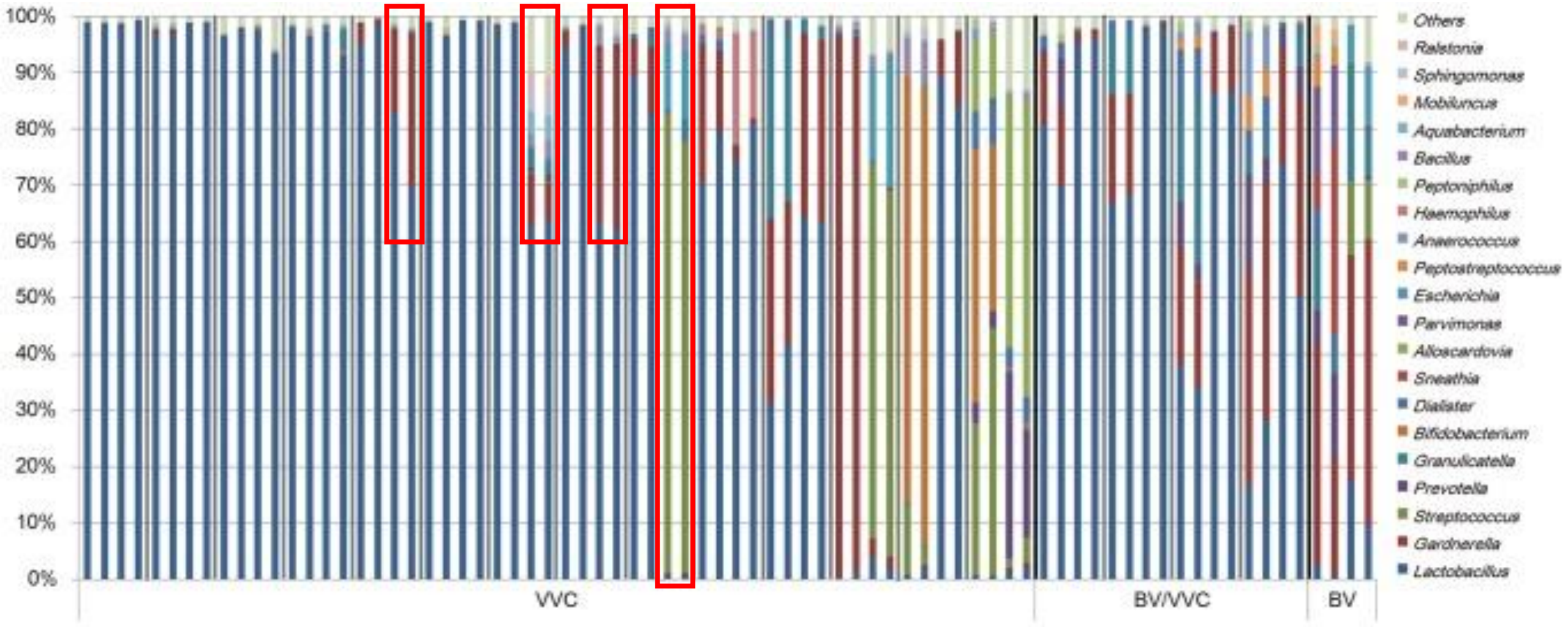
Shannon index



Composition

1. Ceccarani, C. et al. Sci Rep 9, 14095 (2019)

VVC: treatment may lead to less healthy microbiome



1. Liu, M.-B. et al. PLoS One 8, e79812 (2013)

Case report 2: VVC

- Women 40 years, doctor
- Recurring yeast infections, for which she preferred not to use medication.
- Prescribed vaginal suppositories to herself

- Her findings:

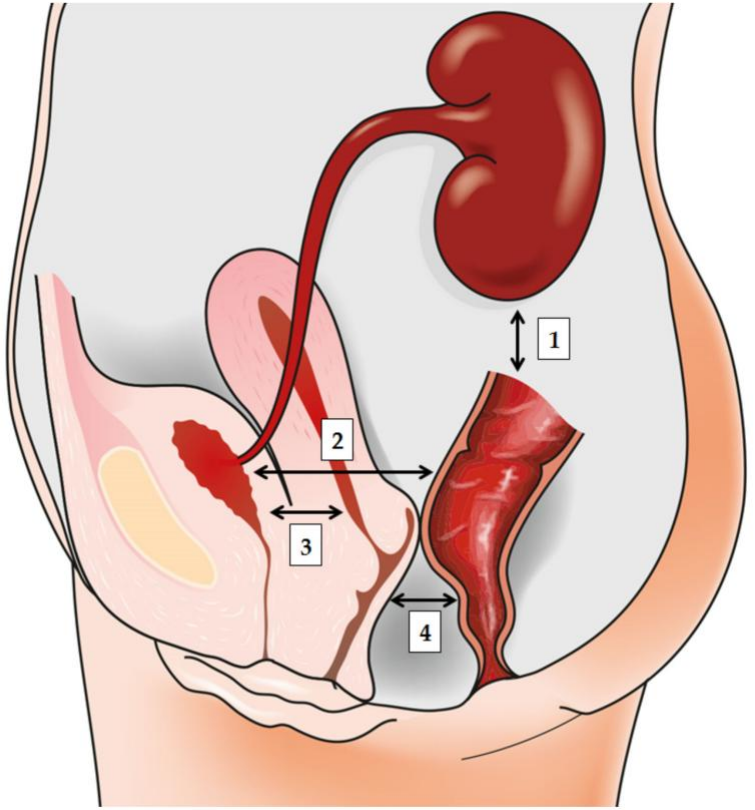
  “The symptoms of itching and crumbly discharge were over very quickly. I used the ovules for over a week, and then I got my period and didn't use them. Now I have used 1 more ovule twice on the day after my period, and that has ensured that to date both the Candida and an unpleasant odor stay away.

  So, I would definitely recommend the ovules”

Urinary tract infections

Role in urinary tract infections

- Historically, role of gut microbes in UTIs has been recognized:
  - Via contamination of the periurethral space by uropathogens residing in the gut[1]. In most cases UTI is caused by pathogenic *E. coli* strains.
- More recently, the role of the vaginal microbiome has been identified too.
  - In fact, various axes are identified[1].
- Lactobacilli (most notably *Lactobacillus crispatus*) of the vaginal microbiome can inhibit *E. coli* growth[1].
  - In women with UTIs, there is often no lactobacilli-dominance and/or a dysbiotic vaginal microbiome[2].
- Antibiotic use can increase risk of UTI[2].



1. Meštrović, T. et al. Diagnostics (Basel) 11, (2020)
2. Stapleton, A. E. Microbiol Spectr 4, (2016)

Case report 3: UTI

- 70-year-old woman
- Recurrent urinary tract infections; 2022 5 x AB, 2023 3 x AB
- Has been using vaginal estrogens for a while.
- No intestinal issues, therefore, no consideration of microbiome analysis or treatment.

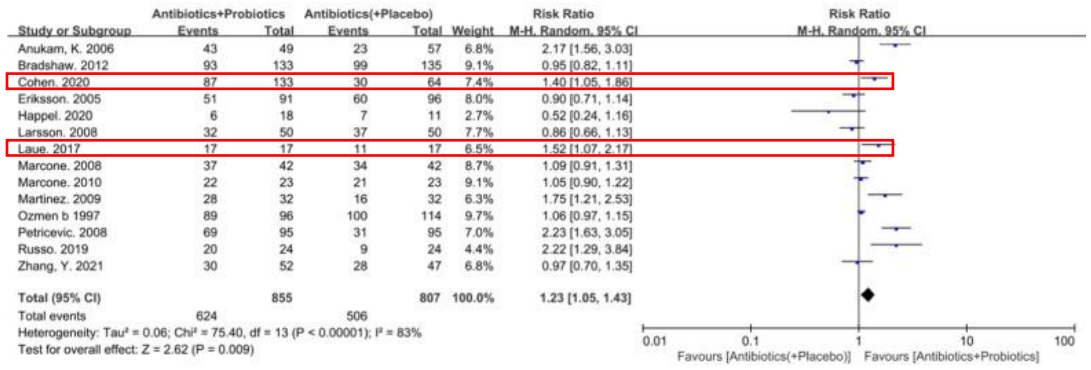
- Treatment with vaginal suppositories for one month, starting in March 2023.
- August 2023: 19 week free of UTI's

Treatment with probiotics

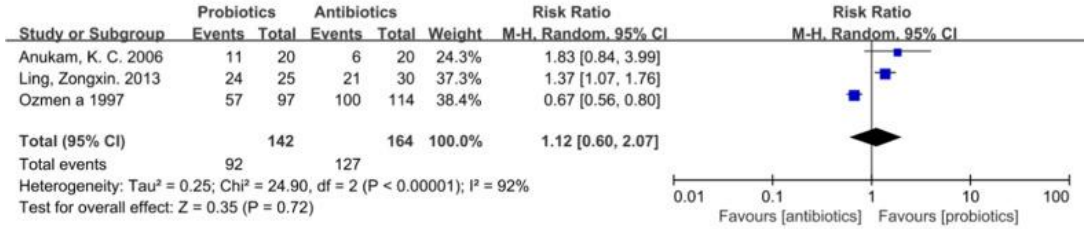
Probiotics for bacterial vaginosis

- Several meta-analyses on probiotics for treatment of bacterial vaginosis are published.
- One meta-analysis from 2022 includes 14 studies with 2093 participants[1]:
  - Antibiotics + probiotics are somewhat better than antibiotics alone (RR: 1,23)
  - Probiotics are much better than placebo (RR 15,2)
  - Half of studies used oral administration
  - Only 2 studies use a *L. crispatus* strain.

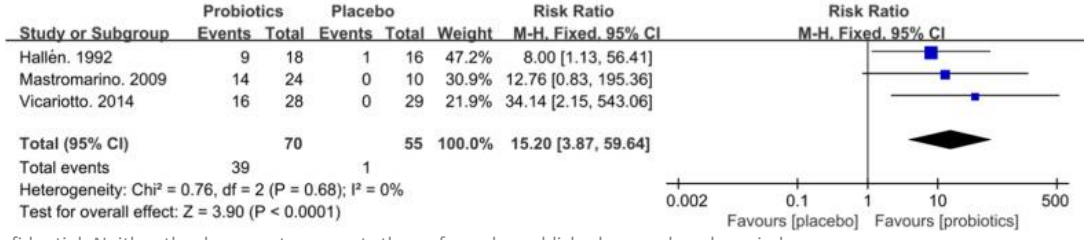
A. Antibiotics + Probiotics/Antibiotics (+Placebo)



B. Probiotics/Antibiotics.



C. Probiotics/Placebo.



1. Chen, R. et al. Reprod Health 19, 137 (2022)

Probiotics for vulvovaginal candidiasis

- A review from 2019 includes 14 studies using probiotics against VVC[1]:
  - The results are in general positive, although they are strain-specific.
- Two open label studies with vaginal product containing *L. acidophilus* LaO2 showing[2,3]:
  - LaO2 inhibits growth of various candida species
  - Administered 1dd for one week, then 1x per 3 days for three weeks, and finally once a week during second month.
  - Vaginal product cured vulvovaginal candidiasis in >86% of the women after 1 month and in >76% after two months[2].
  - Prevents recurrence of VVC in 72% of women with history of rVVC[3].

4.1 | Patients with acute VVC

Multiple studies have demonstrated that probiotics may be beneficial to patients with acute VVC treated with standard antifungals by improving vaginal symptoms. Kovachev et al. compared women with

TABLE 1. Clinical Outcome (Efficacy Analysis) of Study Population

Time	n/N (%) Cure	Recurrence
Prophylactic phase		
I phase—10 d	58/57 (98.3)	58/1 (1.7)
II phase—10 wk	57/49 (86.0)	57/8 (14.0)
Observation phase (mo)		
1	49/45 (91.8)	49/4 (8.2)
3	45/43 (95.6)	45/2 (4.4)
7	43/42 (97.7)	43/1 (2.3)
Cumulative rate	58/42 (72.4)	58/16 (27.6)

TABLE 3. Overview of Data and Statistical Analysis

Parameters	Time 0	Time 28	p (T28 vs. T0)	Time 56	p (T56 vs. T0)	p (T56 vs. T28)
Total women with infection	30	4	< 0.001	7	< 0.001	—
Total women without infection	0	26	—	23	—	—
Percentage of healing	—	86.67%	—	76.67%	—	—
Total women with recurrences	—	0	—	3	—	0.083
Percentage of recurrences	—	—	—	11.54%	—	—

1. Shenoy, A. et al. Dermatologic Therapy e12970 (2019)
2. Vicariotto, F. et al. Journal of Clinical Gastroenterology 46, S73–S80 (2012)
3. Murina, F. et al. Journal of Clinical Gastroenterology 48, S102–S105 (2014)

Probiotics for urinary tract infections

- A study with a *L. crispatus* strain shows reduced risk for recurring UTI after 10w, compared to placebo[1].
- Women who had high levels of *L. crispatus* colonization, had substantial lower risk of recurrent UTI than women with low levels of colonization.

Table 2. Urinary Tract Infection Rates by Intervention and *Lactobacillus crispatus* Colonization Pattern

Intervention	No. (%) of participants developing recurrent UTI	Relative risk (95% CI)
Lactin-V (n = 48)	7 (15)	.5 (.2–1.2)
Placebo (n = 48)	13 (27)	…
Intervention, *L. crispatus* colonization pattern		
Lactin-V, high level (n = 41)	2 (5)	.07 (.02–.3)
Lactin-V, low level (n = 7)	5 (71)	…
Placebo, high level (n = 32)	9 (28)	1.1 (.4–3.1)
Placebo, low level (n = 16)	4 (25)	…

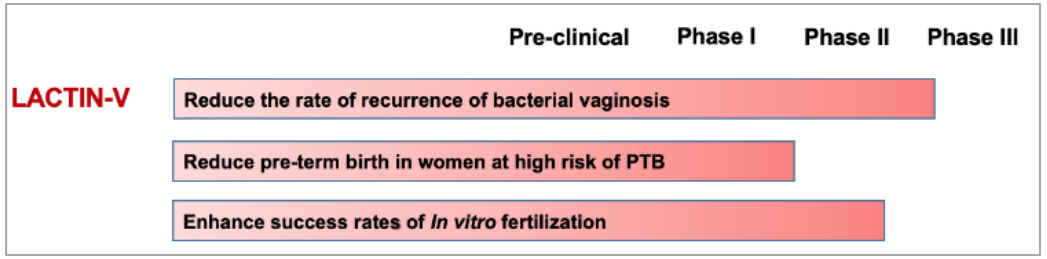
1. Stapleton, A. E. et al. Clinical Infectious Diseases 52, 1212–1217 (2011)

Vaginal suppositories

Vaginal suppositories

- Microbiome Center together with the pharmacist and small group of doctors developed vaginal suppositories.
  - Meant to replace over-the-counter options because:
    - Medical devices (i.e. vaginal applications) no longer allowed to contain live microorganisms[1]
    - Most commercial products do not contain *L. crispatus*
    - Often fecal strains being repurposed
    - The only medicine with a *L. crispatus* strain (CTV-05) is still under development and contains only a single strain[2].

Variable	Lactin-V (N = 152)	Placebo (N = 76)	Risk Ratio (95% CI)*	P Value
	no. (%)			
Status of recurrence by wk 12				
Recurrence†	46 (30)	34 (45)	0.66 (0.44–0.87)	0.01
No recurrence	87 (57)	30 (39)		
Unknown	19 (12)	12 (16)		
Status of recurrence by wk 24				
Recurrence	59 (39)	41 (54)	0.73 (0.54–0.92)	
No recurrence	63 (41)	21 (28)		
Unknown	30 (20)	14 (18)		



6. This Regulation does not apply to:

(h) products, other than those referred to in points (d), (f) and (g), that contain or consist of viable biological material or viable organisms, including living micro-organisms, bacteria, fungi or viruses in order to achieve or support the intended purpose of the product;

1. European Union. Official Journal of the European union L 117, (2017)
2. Cohen, C. R. et al. N Engl J Med 382, 1906–1915 (2020)

Vaginal suppositories

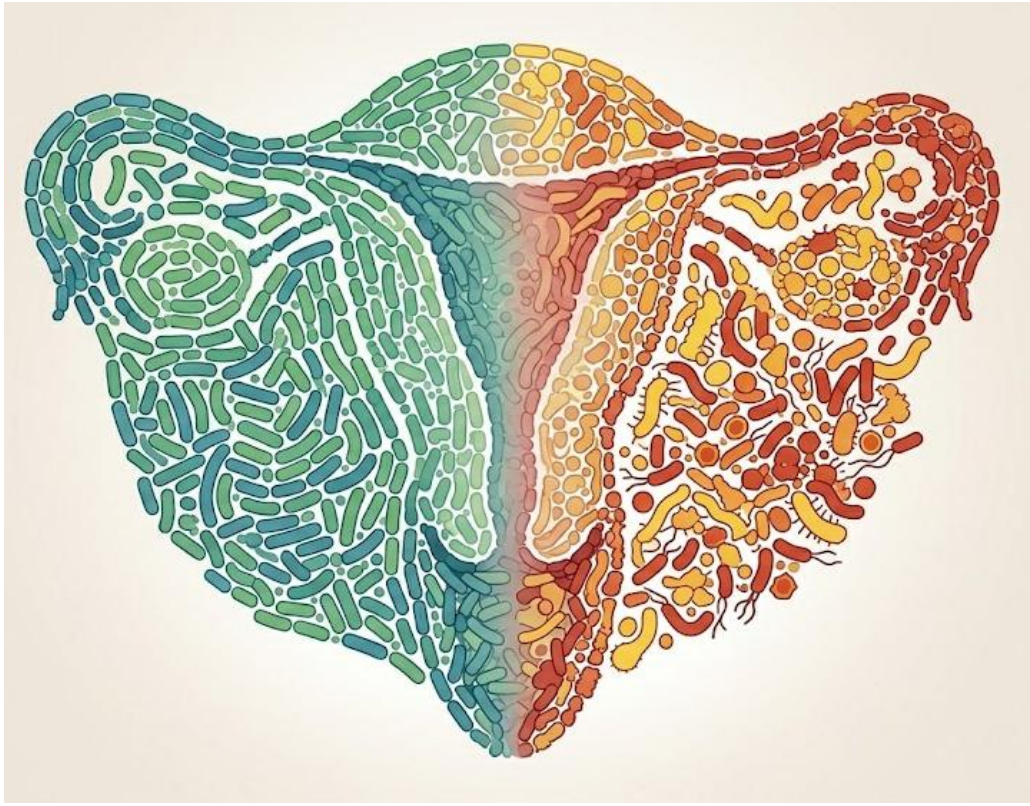
- Vaginal suppositories:
  - Contains three active strains:
    - *Lactobacillus crispatus* SP28 (2.5x10^9 cfu/ovule)
    - *Lactobacillus crispatus* LCR01 (2.5x10^9 cfu/ovule)
    - *Lactobacillus acidophilus* La02 (2.5x10^9 cfu/ovule)
  - Matrix from cacao butter with coconut butter.

- Available as 30 vaginal suppositories for EUR 88,-
- Includes leaflet with clear instructions

Conclusions

Conclusions

- Vaginal complaints are very prevalent
- A healthy vaginal microbiome:
  - Is dominated by *Lactobacillus* species
  - Has pH below 4,5
  - Has very low diversity
- Four dominating *Lactobacillus* species are found, with *L. crispatus* most consistently being associated with good health.
- Bacterial vaginosis, vulvovaginal candidiasis, and urinary tract infections are characterized by high diversity and elevated pH.
- Probiotics can be effective against BV, VVC and UTIs, depending on strains and route of administration.
- New vaginal suppositories fill gap in market due to regulatory changes, lack of products with *L. crispatus*, and slow developments of pharmaceutical products.



1. https://sqonline.ucsd.edu/2021/02/what-i-wish-i-learned-in-sex-ed/