**Title:**

**The global health impact of vaginal dysbiosis**

**Authors:**

Janneke H. H. M. van de Wijgert a\*, Vicky Jespers b

**Affiliations:**

a Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, UK

b HIV and Sexual Health Group, Department of Public Health, Institute of Tropical Medicine, Nationalestraat 155, Antwerp, Belgium

**E-mail addresses:**

Janneke van de Wijgert: [j.vandewijgert@liverpool.ac.uk](mailto:j.vandewijgert@liverpool.ac.uk) “\*correspondence and reprints”

Vicky Jespers: vjespers@itg.be

**Abstract**

The most common dysbiosis of the vaginal microbiome (defined here as a vaginal microbiome not dominated by lactobacilli) is bacterial vaginosis, an anaerobic polybacterial dysbiosis. Other dysbiotic states of importance to global health are vaginal microbiota with a high abundance of streptococci, staphylococci or *Enterobacteriaceae*, vaginal candidiasis, and trichomoniasis. Knowledge about the different types of dysbiosis and their relationship to urogenital and reproductive disease burden has increased in recent years by applying non-culture based techniques but is far from complete. The burden of bacterial vaginosis is highest in sub-Saharan Africa and in women of sub-Saharan African descent living elsewhere. Vaginal dysbiosis has been associated with increased susceptibility to and transmission of HIV and other sexually transmitted infections, and increased risk of pelvic inflammatory disease, preterm birth, and maternal and neonatal infections. In this review we summarize the contribution of vaginal dysbiosis to the global burden of each of these and highlight areas that require more research.

**Keywords**

Vaginal dysbiosis; bacterial vaginosis; HIV; sexually transmitted infections; pelvic inflammatory disease; preterm birth.

**1. Introduction**

We have known for some time that most women have a vaginal microbiome (VMB) that consists predominantly of lactobacilli, and that vaginal dysbiosis (defined here as a VMB that is not dominated by lactobacilli) occasionally causes symptomatic conditions [1]. The most common and best studied clinical condition characterized by vaginal dysbiosis is bacterial vaginosis (BV), which is associated with subclinical vaginal inflammation [1]. Vaginal conditions associated with clinically overt inflammation have also long been recognized: these include desquamative inflammatory vaginitis, atrophic vaginitis, vaginal candidiasis, and trichomoniasis [1]. Women with vaginal symptoms, such as unusual vaginal discharge, unusual odor and/or vaginal itching, seeking clinical care will either receive antibiotic or antifungal treatment empirically, or might be offered diagnostic testing prior to treatment. This diagnostic testing is usually only offered in specialized clinics, and is usually limited to microscopic evaluation of vaginal secretions (referred to as a wet mount) and/or vaginal pH determination. In research settings, BV is typically diagnosed by the Amsel criteria, which rely on wet mount microscopy and the presence of clinical criteria [2], or by Gram stain Nugent scoring [3], which relies on microscopy after Gram staining of a vaginal smear (Table 1).

Since the beginning of the new century, molecular laboratory techniques to identify bacteria at the genus and species level have gradually become more available and affordable, and are increasingly being employed as a tool in molecular epidemiological studies [4, 5]. These molecular studies have now conclusively shown that lactobacilli-dominated VMB are indeed associated with a balanced immune-tolerant vaginal micro-environment and that BV is best described as an anaerobic polybacterial dysbiosis (reviewed in [4]). However, these studies have also shown that not all lactobacilli are equal from a clinical point of view: *Lactobacillus crispatus* has consistently been associated with lack of vaginal mucosal inflammation and protection from adverse outcomes, whereas *L. iners* is much more easily displaced and often co-occurs with dysbiosis-associated anaerobes, pathobionts and pathogens [4]. The picture is less clear for *L. gasseri*, *L. jensenii*, and *L. vaginalis*, but VMB containing a large abundance of those lactobacilli are less common.

Molecular studies are also beginning to shed light on different types of dysbiosis. In a systematic review of 63 molecular studies conducted between 2008 and 2013, all 17 studies that employed hierarchical clustering identified at least one anaerobic polybacterial cluster consistent with BV, and three of the 17 studies also identified clusters that were dominated by, or had high abundance of, a pathobiont (streptococci, staphylococci, or species of the *Enterobacteriaceae* family such as *Escherichia coli*, *Shigella* sp. or *Proteus* sp.) [4]. Some clinicians believe that these VMBs with high abundance of pathobionts are associated with ‘aerobic vaginitis’ ([6], and discussed by Donders in this journal issue). While the roles of vaginal pathobionts (and particularly *Streptococcus agalactiae* and *E. coli*) in invasive maternal and neonatal infections has been well-documented ([7], and discussed by Cools et al in this journal issue), their potential roles in causing a vaginitis syndrome distinct from BV has not yet been universally accepted. However, these vaginal pathobionts are thought to have higher pathogenicity indexes than BV-associated anaerobes, and they might therefore be clinically relevant even when present in relatively low abundance.

**2. Current limitations in assessing the global burden of vaginal dysbiosis**

Most of the epidemiological data that are available to assess the global burden of vaginal dysbiosis, and the clinical conditions that are associated with it, are based on the Amsel criteria and/or Nugent scoring of vaginal smears (Table 1). Molecular studies have shown that the extent of dysbiosis (no or low abundance of lactobacilli; increased bacterial diversity) correlates well with Nugent score and with vaginal pH but not with the other Amsel criteria [4]. We therefore trust that epidemiological studies that have employed Nugent scoring of vaginal smears can still be considered reliable, whereas studies based on Amsel criteria should be interpreted with more caution. However, it is important to keep in mind that Nugent scoring of vaginal smears cannot differentiate between different types of lactobacilli or different types of dysbiosis. Furthermore, our current knowledge about different types of dysbiosis is limited. In recent years, the field has adopted bacterial sequencing as the method of choice to characterize the VMB, but it is likely that additional laboratory methods will have to be employed to enable further clinically-relevant dysbiosis differentiation. For example, studies have consistently shown that *Candida* sp. and relatively low abundant pathobionts co-occur more often with lactobacilli than with BV-associated anaerobes [4, 8]. This means that not all women with a *Lactobacillus*-dominated VMB are at low risk for developing adverse outcomes. We hypothesize that women with high abundance of *L. iners* are more likely to harbor *Candida* sp. or relatively low abundant pathobionts than women with high abundance of *L. crispatus*, but well-powered molecular epidemiological studies are needed to prove this. Furthermore, data from recent vaginal biofilm studies have suggested that BV-associated dysbiosis could be subdivided into dysbiosis with or without biofilm, and that the former could be further subdivided into biofilm including both *Gardnerella vaginalis* and *Atopobium vaginae* (as well as potentially other anaerobes) or biofilm consisting predominantly of *G. vaginalis* but lacking *A. vaginae* [9]. In addition, some pathobionts might form a vaginal biofilm that is distinct from *G. vaginalis*-containing biofilms [10]. Much more research is needed to improve our understanding of these different types of dysbiosis and their relationships to urogenital and reproductive disease burden.

**3. Global burden of symptomatic and asymptomatic vaginal dysbiosis**

One of the first population-based studies to estimate BV prevalence using Nugent scoring of vaginal smears was the 2001-2004 National Health and Nutrition Examination Survey in the United States [11]. Among women aged 14-49 years, the BV prevalence (defined as a Nugent score of 7-10) was estimated to be 29.2%, but only 15.7% of the women with a Nugent score of 7-10 reported vaginal symptoms. The BV prevalence was 23.2% among non-Hispanic white women, 31.9% among Mexican American women, and 51.4% among non-Hispanic black women. We recently conducted a population-based study among women aged 18-30 years representing the six largest ethnic groups (Dutch, South-Asian Surinamese, African Surinamese, Ghanaian, Moroccan and Turkish) residing in Amsterdam, the Netherlands, and employed 16S rRNA gene sequencing to identify vaginal dysbiosis [12]. The overall prevalence of vaginal dysbiosis was 38.5%, of which 32.2% resembled BV and 6.2% represented other dysbiosis types (mostly VMBs containing a high abundance of gut bacteria or urogenital pathobionts). Women of sub-Saharan African descent (immigrants from Suriname or Ghana) were more likely to have vaginal dysbiosis than ethnically Dutch women after adjustment for sociodemographic, behavioral and clinical factors (unpublished data). Finally, we and others have conducted numerous studies in various sub-Saharan African countries in which BV was diagnosed by Nugent scoring and/or molecular methods. The BV prevalence typically ranged from 30 to 40% in women at average risk of HIV and sexually transmitted infections (STIs), and could be as high as 70% in female sex workers [13, 14]. It should be noted, however, that recruitment into these studies was not based on random sampling.

BV prevalence has consistently been shown to be higher in sub-Saharan African women, and in women residing in the United States and Europe who are of sub-Saharan African descent, compared to Caucasian or Asian women. Molecular studies found that women of sub-Saharan African descent are also more likely to carry *L. iners* and less likely to carry *L. crispatus* [4, 12]. Many potential explanations have been debated, and these include lower socioeconomic status, poorer access to healthcare services, differences in diets and vaginal hygiene practices, differences in sexual networks and local epidemics of STIs, and genetics. Thus far, none of these hypotheses provided a satisfactory explanation.

Epidemiological research in pregnant women has focused on (recto)-vaginal carriage of *S. agalactiae* because of its well-known association with pregnancy and neonatal complications. A systemic review and meta-analysis of the published literature between 1997 and 2015 found that rectovaginal *S. agalactiae* carriage (determined by selective culture methods) was highest in Africa (22.4% of women), followed by the Americas (19.7%), Europe (19.0%) and Asia (11.1%) [15]. We recently conducted a study that employed quantitative PCR to determine vaginal carriage in women in Kenya and South Africa at average risk of HIV/STIs, and found a vaginal carriage rate of 20.2% and 23.1%, respectively, for *S. agalactiae*, and 25.0% and 27.1%, respectively, for *E. coli* ([16], and discussed by Cools and Melin, and Cools and Fichorova, in this journal issue).

**4. Global burden of vaginal dysbiosis sequelae**

Vaginal dysbiosis has been associated with HIV and other STIs [14], pelvic inflammatory disease (PID) [17], and adverse pregnancy outcomes such as preterm birth [18], and maternal and neonatal infections [19]. Here, we briefly describe the contribution of vaginal dysbiosis to the global burden of each of these and highlight areas that require more research.

*4.1 HIV and STIs*

In 2015, about 37 million people worldwide were living with HIV, 500 million with herpes simplex virus type 2 (HSV-2), and 290 million with human papillomavirus (HPV) [20, 21]. In addition, the World Health Organization (WHO) estimates that more than one million infections with any of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, or *Trichomonas vaginalis* are acquired every day worldwide [21]. Research has consistently shown that vaginal dysbiosis is associated with HIV and STIs in cross-sectional studies, and this has been confirmed more recently in cross-sectional studies employing molecular methods [8, 22]. Research has also consistently shown that HIV-positive women with BV as assessed by Nugent scoring, or with increased vaginal bacterial diversity as assessed by molecular methods, have a higher HIV viral load in cervicovaginal lavages, suggesting an increased risk for female to male HIV transmission [22, 23]. Longitudinal studies have shown that the associations between BV (diagnosed by Nugent scoring or Amsel criteria) and HIV/STIs are bidirectional, and it is a well-known fact that HIV, HSV-2 and other STIs also fuel one another [24,25]. In a cohort of HIV-uninfected women in Uganda and Zimbabwe, it was estimated that 50% of incident HIV infections were attributable to HSV-2 infection, 17% to BV (Nugent score 7-10), 12% to intermediate microbiota (Nugent score 4-6], and 3% to vaginal candidiasis [25].

*4.2 Pelvic inflammatory disease*

PID is an infection-induced inflammation of the upper female genital tract: micro-organisms usually ascend from the vagina and endocervix to the endometrium and beyond, and may cause endometritis, salpingitis, parametritis, oophoritis, tubo-ovarian abscess, pelvic peritonitis and/or perihepatitis (Fitz-Hugh-Curtis syndrome) [1]. Traditionally, PID was thought to be caused predominantly by *C. trachomatis* and *N. gonorrhoeae*, and in some cases by BV-associated organisms [1]. More recently, the STI pathogen *Mycoplasma genitalium* has also been identified as a cause of PID [26]. In-depth molecular research on PID has been hampered by an imprecise and inconsistent PID case definition, and difficulty sampling the upper genital tract. However, studies are now beginning to accumulate, and these suggest that *N. gonorrhoeae*, *C. trachomatis* and/or *M. genitalium* are present in about 30% of PID cases and BV-associated bacteria or urogenital pathobionts (*S. agalactiae*, *Staphylococcus aureus* and *Enterobacteriaceae*) in about 70% of cases [27–30].

While PID is usually only diagnosed when symptoms are present, it has been estimated that subclinical PID may be twice as common as symptomatic PID [1]. Both symptomatic and subclinical PID have been associated with long-term sequelae such as infertility, ectopic pregnancy and chronic pelvic pain [1]. The rates and severity of PID have declined in Europe and North America in the past two decades, most likely due to improved control of *N. gonorrhoeae* and, to a lesser extent, *C. trachomatis* [31]. However, PID remains a problem because the true extent of subclinical PID and its sequelae are unknown, symptomatic PID has not declined in much of the developing world, and reproductive outcomes in treated patients are still suboptimal.

*4.3 Preterm birth*

The WHO-UNICEF Child Health Epidemiology Reference Group estimated that 3.1 million neonates died in 2010 [32]. The leading causes of death in neonates are complications of preterm birth (1.1 million deaths), intrapartum-related complications (0.7 million deaths), and neonatal sepsis or meningitis (0.4 million deaths). Preterm birth, which affects 7-15% of pregnancies worldwide, is defined as delivery before 37 completed weeks of gestation, and accounts for 80% of neonatal morbidity and mortality [33]. Human parturition is a complex interplay between hormonal and inflammatory pathways. Infection of the amniotic cavity (as well as other pathologies such as uteroplacental infarction or hemorrhage or uterine overextension) could disrupt these pathways and culminate in preterm birth [34]. Infection-induced inflammatory pathways are thought to be the causal driver of around 40% of preterm births, and of as many as 80% of early preterm births before 28 weeks of gestation [34,35]. The primary sources of infection are thought to be ascending bacteria from the lower genital tract [34]. In the past, many studies in the preterm birth field have tried to identify biomarkers of preterm birth so that interventions can be targeted to women at high risk. Available interventions currently include cervical cerclage and vaginal progesterone. Two biomarkers that are currently being used to determine when to intervene are shortened cervical length at 24 weeks gestation in women who have previously experienced preterm birth, and detectable fetal fibronectin in the posterior fornix between 22-35 weeks of gestation [34]. However, infection-related biomarkers of preterm birth risk have proved elusive thus far.

Molecular VMB studies in pregnant women have consistently shown that pregnancy strongly promotes lactobacilli: this is particularly true for *L. crispatus* but also for *L. iners* [36, 37]. Molecular VMB studies comparing women who delivered term to women who delivered preterm, however, have shown inconsistent results [38–41]. Women who delivered preterm were more likely to have *L. iners* dominance [39-41], or increased vaginal bacterial diversity [38], but all studies were underpowered and did not take the presence of *Candida* sp. (which are very common in pregnancy) and relatively low abundant urogenital pathobionts into account. Interestingly, Kindinger *et al.* recently showed that use of braided suture material for cervical cerclage, which is the most common intervention in women at high risk of preterm birth in the UK, was associated with a higher incidence of vaginal dysbiosis in the weeks after the intervention compared to women who received a monofilament cerclage [42]. A clinical trial is currently ongoing in the UK to determine if the UK preterm birth prevention guidelines should be revised.

*4.4 Maternal and neonatal infections*

Maternal health refers to the health of women during pregnancy and in the postpartum or postabortion period. WHO estimates that 303,000 maternal deaths occurred in 2015, which is a 43% decline compared to 1990 [43]. The vast majority (99%) of these deaths occurred in resource-poor countries. An estimated 11% of maternal deaths were due to infections, most of them postpartum, and an additional 7.9% due to unsafe abortions (including infections associated with unsafe abortions) [44]. Unsafe abortions are known to be underreported [44], and the above percentage is therefore likely an underestimate. The relative contribution of vaginal bacteria to these invasive maternal infections versus bacteria introduced from the environment due to poor hygiene and/or lack of sterile conditions during procedures is unclear.

As mentioned earlier, approximately 0.4 million neonates died in 2010 due to neonatal sepsis or meningitis (collectively referred to as neonatal disease) [32]. Early-onset neonatal disease (EOD) occurs in the first week of life and is thought to be caused by bacteria that are transmitted from the genital tract of the mother before or during delivery. Late-onset neonatal disease (LOD) occurs between the first week and the third month of life and may be caused by bacteria that are acquired vertically or horizontally. The prevalence rates of EOD and LOD vary widely, but most cases occur in Africa and South Asia [19]. *S. agalactiae* is by far the most studied etiologic agent, and rectovaginal carriage rates in mothers were reported above. However, recent studies have shown that other common etiologic agents include *E. coli*, *S. aureus*, *Klebsiella* spp., and *Acinetobacter* sp. [19]. Intrapartum antibiotic prophylaxis in women who are colonized by *S. agalactiae* has now been introduced in several African countries, and this has led to a reduction (but not elimination) of the incidence of EOD, whereas the incidence of LOD has not changed [45]. A vaccine against *S. agalactiae* is being developed, but would by design not eliminate cases associated with the other common etiologic agents.

In our opinion, maternal and neonatal infection studies would benefit greatly from the incorporation of molecular methods to identify micro-organisms in women and their neonates in a more comprehensive manner.

**5. Conclusions**

The global burden of vaginal dysbiosis and associated sequelae is high and women in resource-poor settings, particularly in sub-Saharan Africa, are worst affected. Vaginal dysbiosis is associated with many common and sometimes severe or even life-threatening urogenital, maternal and neonatal diseases. The need for a better understanding of the different types of dysbiosis and their relationships to the burden of these diseases is therefore paramount. We recommend that sufficiently powered epidemiological studies, ideally prospective in nature, apply molecular methods to address the etiology and pathogenesis of vaginal dysbiosis in the context of the clinical conditions described in this review.

**Conflicts of interest**

The authors declare that they have no conflicts of interest. The authors’ salaries are supported by their respective institutions.

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**Table 1.** Description of diagnostic methods for bacterial vaginosis

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| **Diagnostic method** | **Clinical and microscopy criteria** | **Diagnosis** |
| Amsel criteria [2] | 1. pH of vaginal secretions > 4.5 2. Fishy odor after adding KOH to vaginal secretions 3. ≥ 20% clue cells on wet mount 4. White, skim milk-like vaginal discharge | Bacterial vaginosis: if at least 3 of these 4 criteria are fulfilled |
| Nugent scoring of Gram stained vaginal smears [3] | 1. Gram-positive rods: score 0-4 ranging from high quantity (0) to none (4) 2. Gram-negative coccobacilli forms: score 0-4 ranging from none (0) to high quantity (4) 3. Curved Gram-negative rods: score 0-2 ranging from none (0) to high quantity (2) | Overall Nugent score - add the 3 scores:  0-3 = Normal microbiome  4-6 = Intermediate microbiome  7-10 = Bacterial vaginosis |