Summary

The theme of this thesis is a clinical perspective on the vaginal microbiome in Bacterial Vaginosis (BV) and Human papillomavirus (HPV) infection. The health impact of an unhealthy vaginal microbiome is enormous, increasing the risk of adverse pregnancy outcomes, sexually transmitted diseases and HPV infection. The thesis consists of three sections. The first section describes our study of the vaginal microbiome in relation to BV. Several diagnostics for BV were used, including microscopic scoring, Whiff testing, microarray analysis and 454-sequencing. In the second section, our examination of the effectiveness of probiotics is reported, inter alia as treatment of BV. For the third section, HPV was further examined. HPV genotypes were established in relation to cytology results and the vaginal microbiome.

Chapter 2. The vaginal microbiome in relation to BV

In Chapter 2.1, BV in South African women was examined by the use of a tailor-made DNA microarray containing probes representing the vaginal microbiome. BV was associated with an increased microbial diversity. It could be concluded that it is no longer useful to base the diagnosis of BV on *Gardnerella* alone. Rather, its presence with *Leptotrichia* and *Prevotella* species, and especially *Atopobium* was more indicative of an aberrant state of the vaginal flora.

In Chapter 2.2, a study of the vaginal microbiome of HIV-positive women in Tanzania is shown. The objective of this study was to examine Whiff testing and microscopy in relation to 22 DNA probes published previously as being important for the vaginal microbiome associated with BV in HIV-positive Tanzanian women. This chapter showed the use of microarray-based identification for the validation of conventional microscopy in comparison to Whiff testing. The findings show that the Whiff test could be used in resource-poor countries to diagnose a BV-associated vaginal microbiome when other diagnostics are not available.

Chapter 2.3, was done to further establish the differences in bacterial composition of the vaginal microbiome in Dutch women screened for STDs with and without BV using 454-sequencing.
An increased diversity of species, especially in combination with a depletion of *Lactobacillus crispatus* and *iners* in combination with an overload of Coriobacteriaceae (including *Atopobium vaginae*), Leptotrichiaceae (*Sneathia sanguinegens*), and of Veillonellaceae could be suggested as alternative markers to diagnose BV.

**Chapter 3. Perspective on Probiotics**
A review is presented in Chapter 3.1 examining the current understanding of the vaginal microbiome and assessing how probiotic bacteria might reduce infectivity. The mechanisms whereby certain probiotic lactobacilli improve urogenital health include immune modulation, pathogen displacement, and creation of a niche less conducive to proliferation of pathogens and their virulence factors. Probiotics offer a potential new means to prevent urogenital infections and help maintain a healthy vaginal ecosystem.

In Chapter 3.2, a literature review investigates the application of probiotics and selected nutrients in the first 1,000 days of life, a critical time period in the development of young children which is particularly risky in resource-poor countries. An alternative community based kitchen model that produces probiotics can be established to empower local people, make them socially responsible, produce affordable products and deliver benefits to over 3,000 children and adults daily.

Chapter 3.3 shows the results of a randomized controlled trial done in a resource-poor African community of Tanzania, which tested the impact of daily yogurt intake on the vaginal microbiome and well-being of HIV-positive women. The Nugent scoring showed almost 40% women had an improved vaginal microbiome over the month, albeit that the addition of the probiotic did not differ from the standard yogurt group. Yogurt provides a safe nutritious food that can be made locally and taken daily by HIV-positive subjects receiving anti-retroviral therapy. It has the potential to transfer health benefits to the gut and vagina. The extent to which a probiotic can add to this through transfer from the intestinal tract to the vagina remains to be determined.

**Chapter 4. The vaginal microbiome in relation to HPV infection**
In Chapter 4.1, microarray analysis of the *Lactobacillus* spp. of the South African women in combination with HPV analysis was performed. A significant reduction of *Lactobacillus crispatus* among women with HPV infection was found. In both HIV and HPV infection, a similar (but not identical) shift in the composition of the *Lactobacillus* flora was observed.
In Chapter 4.2, a study to determine the prevalence of HPV genotypes among HIV-positive women in Africa with normal and abnormal cervical cytology scores was done. Regional differences in HPV genotypes among African women were found. These differences warrant the need to consider different monitoring programmes for cervical preneoplasia. Furthermore, HPV-based screening tests for cervical preneoplasia would be highly inefficient unless coupled with cytology screening of the HPV-positive sample, especially in HIV-positive women.

The objective of Chapter 4.3 was to test the Cellient™ system on HPV-positive cervical scrapes collected in Tanzania. In HIV-positive HPV-positive women, the Cellient system resulted in high quality histology sections with perfect p16 images of dyskeratocytes.

Conclusions
- There is a need to develop a new gold standard for diagnosing BV.
- Next-generation sequencing and other techniques showed no correlation with complaints reported by women. Women with BV at risk of STDs and hr-HPV or pregnant could be defined as “resilient”. A definition of resilience in these women could be based on the health impact of having a certain vaginal microbiome type, rather than on the microbiome itself.
- It is no longer useful to base the diagnoses of BV on the presence of Gardnerella alone. An increased diversity of species, especially in combination with a depletion of Lactobacillus crispatus and iners in combination with an overload of Coriobacteriaceae (including Atopobium vaginae), Leptotrichiaceae (Sneathia sanguinogens), and of Veillonellaceae could be suggested as alternative markers to diagnose BV.
- Especially in developing countries, many challenges exist to implement simple food-based interventions because of their sustainability, education of consumers, and delivery.
- More studies are needed to improve diagnosis and management of BV and HPV infection in women. Future studies can further establish the definition of a resilient BV microbiome type, possibilities of host-specific treatment, and the impact of the symptomatic and asymptomatic vaginal microbiome on STD’s like hr-HPV.