# Characterisation of the vaginal microflora in health and disease

Bacterial vaginosis (BV) is an imbalance of the vaginal bacterial microbiota and its aetiology is still unknown. It is a common condition afflicting mostly women in reproductive age. BV is associated with increased risk of acquiring sexually transmitted infections, such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, genital herpes and HIV, and serious pregnancy outcomes such as preterm birth, miscarriage and post hysterectomy vaginal cuff infection.

BV is classically diagnosed by clinical criteria developed by Amsel and by microscopy. Different microscopy scores have been developed, but the most used is the Nugent classification, which is considered the gold standard for diagnosing BV.

The main purposes of this PhD thesis were to characterize the vaginal flora in women from Greenland by microscopy and quantitative PCRs (qPCR) for BV-associated bacteria, to investigate whether BV can be diagnosed from first void urine (FVU) and to evaluate qPCR as a tool for accurate diagnosis of BV. As some of the bacteria are present both in BV and in healthy women, determining a cut-off level that optimally predicted BV has been essential. For that purpose the statistical method of Receiver Operating Characteristic (ROC) analysis has been indispensable as it provides a method for the unbiased determination of a cut-off level.

The thesis comprises three studies:

STUDY I:

The purpose of this cross-sectional, observational study was to give a detailed quantitative characterization of vaginal bacterial composition in a cohort of women from Greenland. Participants were women who agreed to participate in the study, to answer an interviewer-administered structured survey and to deliver vaginal smears, swabs and first void urines. Vaginal smears were evaluated by Nugent's and Claeys' scores and also for yeasts and inflammation. PCR assays for four sexually transmitted infections (STIs) were performed both on vaginal swabs and urines. The vaginal swabs were analysed by 19 qPCR for selected vaginal bacteria. Seven of these 19 vaginal bacteria were shown to have an area under the ROC curve > 85 %, according to Nugent, suggesting a good prediction of BV. All non-*Lactobacillus* species, except for *Ureaplasma parvum* and *Ureaplasma urealyticum* were significantly associated with BV by quantitative detection and univariate analysis. Two of the

seven key species (*Prevotella* spp. and *Atopobium vaginae*) remained significantly associated with BV in a multivariate logistic regression analysis after adjusting for the others. BV was further subdivided into clusters with *Gardnerella vaginalis* and *Prevotella* spp. playing a major part. BV and STIs were highly prevalent in this population. BV could be diagnosed by molecular methods with high accuracy by performing and combining qPCRs for *A. vaginae* and/or *Prevotella* spp.

Further, the vaginal smears were evaluated by Claeys' criteria and a good agreement was found between Nugent's and Claeys' scoring systems with a Cohen's kappa of 0.90, but these results were discussed in the PhD thesis separately from STUDY I.

STUDY II:

The aim was to study the composition of the vaginal microbiota in a cohort of Swedish women by using 454 pyroseqencing and 16 qPCR assays that were the same as those used in STUDY I except for the *Prevotella* PCR, which was less broadly reactive, capturing only three *Prevotella* species. BV was diagnosed by Amsel's criteria. Eight BV-associated bacteria (*A. vaginae, G. vaginalis*, *Eggerthella*-like bacterium, *Megasphaera* type 1, *Prevotella,* BVAB2, *L. amnionii* and *S. sanguinegens*) were highly predictive for BV with areas under the ROC above 85 % and with the best diagnostic accuracy for *A. vaginae*. A kappa value was calculated in order to measure the agreement between the presence of an individual species / genus determined by 454 pyrosequencing versus quantitative real-time PCR and for the majority of species / genera the kappa values indicated fair to good agreement. The depletion of *Lactobacillus* species combined with the presence of either *G. vaginalis* or *A. vaginae* at diagnostic levels was a highly accurate BV predictor.

STUDY III:

The aim was to investigate whether BV can be diagnosed from first void urine (FVU). The study was based on the same population as used in STUDY I. qPCR for seven BV-associated bacteria were selected, as it was demonstrated in STUDY I that they had an area under the ROC curve > 85 %. When areas under the ROC curves were calculated for the quantitative detection of these bacteria in urine samples, it was found that all chosen BV-associated bacteria maintained their areas under ROC curve > 85 %, according to Nugent. All seven selected bacteria were significantly associated with BV in univariate analysis before and after applying cut-offs, as determined by ROC curve analysis.

*Megasphaera* type 1 and *Prevotella* spp. from urine samples were significantly associated with BV in multivariate analysis and a combination of the two qPCRs for *Megasphaera* type 1 or *Prevotella* spp. could diagnose BV from FVU with high accuracy (sensitivity 99 % and specificity 95 %).

In conclusion, the studies forming the basis of this PhD thesis have provided for the first time a detailed description of the vaginal flora in women from Greenland, stratifying BV in clusters dominated by single bacteria such as *G. vaginalis*, *Prevotella* spp., BVAB1 or bacteria in pairs such as *G. vaginalis/ Prevotella* spp. or BVAB 1/ *G. vaginalis*. These refined diagnostic methods may prove useful both in stratifying the treatment of BV and in targeting treatment for prevention of complications to relevant subgroups. We have further chosen seven BV-associated bacteria that predicted BV by qPCR in a population from Greenland and reproduced the findings in a cohort of Swedish women with *A. vaginae* having the best diagnostic accuracy in both studies.

Finally, we have demonstrated that BV can be diagnosed accurately by qPCR performed on FVU. This finding may prove useful as pregnant women with BV may be at increased risk for urinary tract infections (UTIs). The possibility that some BV-associated bacteria could cause UTIs should be further investigated and qPCR may become a valuable diagnostic tool, as many of these bacteria are not cultivable. The finding will also allow analysis of stored FVU specimens collected as part of studies on classical STI pathogens, where BV was not considered initially.