

# The female genital tract microbiota

Composition, relation to innate immune factors, and effects of contraceptives

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

- I. Nikolaitchouk N, Andersch B, Falsen E, Strömbeck L, Mattsby-Baltzer I.  
The lower genital tract microbiota in relation to cytokine-, SLPI- and endotoxin levels: application of checkerboard DNA-DNA hybridization (CDH).  
APMIS. 2008 Apr;116(4):263-77.
- II. Nikolaitchouk N, Hoyles L, Falsen E, Grainger JM, Collins MD.  
Characterization of *Actinomyces* isolates from samples from the human urogenital tract: description of *Actinomyces urogenitalis* sp. nov.  
Int J Syst Evol Microbiol. 2000 Jul;50 Pt 4:1649-54.
- III. Nikolaitchouk N, Wachter C, Falsen E, Andersch B, Collins MD, Lawson PA.  
*Lactobacillus coleohominis* sp. nov., isolated from human sources.  
Int J Syst Evol Microbiol. 2001 Nov;51(Pt 6):2081-5.
- IV. Nikolaitchouk N, Mattsby-Baltzer I, Andersch B.  
The influence of age and contraceptive methods on the prevalence of *Lactobacillus*-dominant vaginal microbiota in healthy fertile women.  
In manuscript.



GÖTEBORGS UNIVERSITET

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## Composition, relation to innate immune factors, and effects of contraceptives

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### ABSTRACT

Abnormal vaginal microbiota, as in bacterial vaginosis (BV), is associated with increased risk of obstetrical and gynaecologic complications and acquisition of sexually transmitted diseases. However, very little is known about the pathogenesis of BV. In BV, the normal vaginal *Lactobacillus*-dominated biota (LDB) is replaced by anaerobic bacteria. The diagnosis of BV is based on clinical symptoms (vaginal malodorous discharge) and/or microscopy of vaginal smears, methods that do not identify specific microorganisms.

The aim was to analyse the composition of the vaginal microbiota in healthy, asymptomatic women of reproductive age, and investigate the relationship between the bacterial species and locally secreted proinflammatory cytokines and the antimicrobial secretory leucoprotease inhibitor (SLPI).

In the study of 37 women, a total of 42 bacterial species were found in vaginal secretions, by cultivation. In the women with asymptomatic BV, particularly, high numbers of the lesser-known *Atopobium vaginae*, *Peptoniphilus harei*, and *Actinomyces urogenitalis* were noted (exceeding  $10^{11}$  bacteria per ml). The latter bacterium, together with *Lactobacillus coleohominis*, were both isolated from vaginal secretions and have been proposed as new species, based on phenotypic (biochemical testing, SDS-PAGE analysis of whole cell proteins) and phylogenetic results (16S rRNA gene sequencing).

The frequency of LDB in healthy asymptomatic women ( $n=313$ ) was found to decrease with age, analysing age (years) cohorts 20-29, 30-39, and 40-49. Furthermore, the contraceptive methods used (oral hormone pills, copper- or hormone intrauterine device) were found to affect the frequency of LDB.

A non-cultivation-based, semi-quantitative, checkerboard DNA-DNA hybridisation technique (CDH), based on genomic probes from 13 selected bacterial species, was applied for analysis of vaginal and cervical secretions of 26 women. It was found that the anaerobic bacteria were more frequently detected by CDH, compared to cultivation. Correlations were found between specific bacterial species and cytokines or SLPI. For instance, the strict anaerobic species, *B. ureolyticus* and *F. nucleatum*, both correlated with vaginal IL-1 $\alpha$ .

By identification and quantification of bacterial species of the lower genital tract, and analysis of their relationships to host-derived innate immune factors, it will be possible to define various types of abnormal microbiota, to develop ways of assessing the risk of specific bacterial species or groups of bacteria in various clinical settings, and to treat them. CDH will be a suitable tool for the quantitative analysis of as many as 40 specific bacterial species, making it possible to investigate large numbers of women. Both age and contraceptive method need to be considered when investigating the compositions of abnormal vaginal microbiota.

**Key words:** bacterial vaginosis, checkerboard DNA-DNA hybridisation, cytokines, SLPI, *Lactobacillus coleohominis*, *Lactobacillus iners*, *Actinomyces urogenitalis*