



**Karolinska
Institutet**

DEPARTMENT OF LABORATORY MEDICINE

Lactobacillus based treatment of vaginal infections

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras 4Z, Alfred Nobels Allé 8, plan 4, Huddinge.

Fredagen den 8 November 2013, kl 10:00

av

Sonal Pendharkar

Huvudhandledare:

Dr. Harold Marcotte
Karolinska Institutet
Institutionen för Laboratoriemedicin
Avdelningen för Klinisk Immunologi &
Transfusionsmedicin, Huddinge

Bihandledare:

Professor Lennart Hammarström
Karolinska Institutet
Institutionen för Laboratoriemedicin
Avdelningen för Klinisk Immunologi &
Transfusionsmedicin, Huddinge

Fakultetsopponent:

Professor Hans Verstraelen
Ghent University
Department of Obstetrics & Gynaecology
Belgium

Betygsnämnd:

Professor Eva Sverremark-Ekström
Stockholms Universitet
Department of Molecular Bioscience
The Wenner-Gren Institute, Immunology

Professor Anders Sönnernborg
Karolinska Institutet
Institutionen för Laboratoriemedicin

Professor Vinod Diwan
Karolinska Institutet
Institutionen för folkhälsovetenskap

Stockholm 2013

ABSTRACT

The vaginal microbiota (VMB) is a complex and delicate balance between different bacterial species and is normally dominated by *Lactobacillus* species. Under the effect of hormonal changes, behavioral or sexual activity VMB could lose this balance resulting in diseases. Bacterial vaginosis (BV) is one of the most common vaginitis affecting women and is defined as an increase in proportion of anaerobic bacteria and a reduction or absence of lactobacilli. BV has been linked with increased risk of acquiring sexually transmitted diseases (STDs) including infection by human immunodeficiency virus type-1 (HIV-1). Vulvovaginal candidiasis (VVC) is the second most common cause of vaginitis after BV, which is recognized by an overgrowth of yeast mainly belonging to the genus *Candida* and *albicans* species. The standard clinical treatment for these conditions prescribes antibiotics (clindamycin and/or metronidazole) and antifungal (fluconazole). This thesis focuses on investigating clinical interventions for the treatment of BV and VVC and attempting to express neutralizing antibody fragments in *Lactobacillus* against HIV-1 for future applications.

An extended antibiotic treatment with clindamycin and metronidazole together with adjuvant lactobacilli was tested in order to achieve long lasting cure and reduce relapse of BV (paper I). Five different combinations of *Lactobacillus* strains were tested which included newly characterized strains (paper I) and the commercial EcoVag[®] capsules (a mixture of *L. gasseri* DSM 14869 and *L. rhamnosus* DSM 14870). The cure rate was 74.6% after six months and 65.1% after 12 months. No significant difference was observed in cure rates depending on whether the women were colonized by any of the given strains. However, change of sexual partner was significantly associated with relapse of BV. The results were further confirmed in another clinical trial (paper II, trial I).

We subsequently assessed the efficacy of a prolonged treatment with EcoVag[®] lactobacilli in combination with similar antibiotic treatment in BV patients (paper II, trial II). The cure rate was 66.7% and 62.5% after six months and 12 months respectively and was comparable to that in paper I. Prolonged treatment with lactobacilli did not significantly improve the colonization by EcoVag[®] strains. Overall colonization by any lactobacilli and by EcoVag[®] strains was associated with cure of BV. Once again change of sexual partner was associated with relapse of BV. We also tested for the first time, a combination of EcoVag[®] and anti-fungal for treatment of recurrent VVC and evaluated if lactobacilli can colonize women when the microbiota is not disturbed by antibiotics. All the women were cured of VVC after six months and 87.5% remained cured after 12 months. EcoVag[®] strains colonized more in the presence of prior antibiotic treatment in BV patients and in women with VVC who were not colonized by lactobacilli in the beginning of the study.

The VMB has been extensively studied in European and American populations albeit less so in the African population. Therefore we have identified the vaginal *Lactobacillus* species in a group of 40 women in South Africa (paper III). We found that the vaginal *Lactobacillus* species identified were similar to those in other populations, suggesting that the strategies utilising probiotic and engineered lactobacilli could be applied in Africa. Finally llama-derived single domain antibody fragment (VHH) against HIV-1 glycoprotein gp140 was expressed in *L. paracasei* BL23 (our model strain) (paper IV). The VHH was expressed in secreted and cell surface anchored form, which were able to bind to recombinant HIV-1 glycoproteins. The VHH is currently being tested for *in vitro* neutralization of various HIV-1 viral strains. These results provide the basis for the use of lactobacilli and genetically modified lactobacilli for treatment of BV, yeast infection and HIV-1 in both developed and developing world.