Efficiency of vaginal probiotic *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* versus oral metronidazole in the treatment of bacterial vaginosis: randomized double blind study

Thesis
Submitted for partial fulfillment of master degree in Obstetrics & Gynecology

By

Mahmoud Saber Mahmoud
M.B.B.Ch (2001)
Resident of Obstetrics & Gynecology
Kafre El-Sheikh General Hospital

Supervised by

Dr: Fekrya Ahmed Mohamed Salama
Assistant Professor of Obstetrics & Gynecology
Ain Shams University

Dr: Ahmed Adel Tharwat
Lecturer of Obstetrics & Gynecology
Ain Shams University

Dr: Iman Hussein Shehata
Assistant Professor of Medical Microbiology & Immunology
Ain Shams University

Faculty of Medicine
Ain Shams University

2009

Contents

List of Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
b.	Bacteroid
BLSs	Bacteriocin Like Substances
BV	Bacterial Vaginosis
C. albicans	*Candida albicans*
CDC	Center of disease control
CIN	Cervical intraepithelial neoplasia
cfu	Colony forming unit
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FOS	Fructo-oligosaccharides
G. vaginalis	*Gardnerella vaginalis*
GIT	Gastrointestinal Tract
GLC	Gas Liquid Chromatography
H_2O_2	Hydrogen peroxide
HDL	High Density Lipoproteins
HIV	Human Immune Deficiency Virus
HPF	High power field
hsp	Heat-shock protein
IgA	Immunoglobulin A
IUD	Intra Uterine Device
IVF	Intermediate vaginal flora
KOH	Potassium hydroxide
L.	*Lactobacillus*
M. curtisii	*Mobiluncus curtisii*
M. Mulieri	*Mobiluncus Mulieri*
M. hominis	*Mycoplasma hominis*
PAP	Proline amino peptidase
PAMPs	Pathogen associated molecular patterns
Pap smear	Papanicolaou smear
P. bivia	*Prevotella bivia*

PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
PROM	Premature rupture of the membranes
RCT	Randomized control study
RVVC	Recurrent vulvovaginal candidiasis
spp.	Species
T. vaginalis	*Trichomonas vaginalis*
UTI	Urinary tract infection
U. urealyticum	*Ureaplasma urealyticum*
VECs	Vaginal epithelial cells
VVC	Vulvo-vaginal candidiasis
WBCs	White blood cells
WHO	World Health Organization

Index of figures

Index of tables

Index of Graphs

Introduction

Bacterial vaginosis is a condition characterized by alteration of the vaginal ecology in which the normal flora, dominated by *lactobacilli*, is replaced by a mixed bacterial flora which includes *Gardnerella vaginalis, Mobiluncus spp., Mycoplasma hominis, Bacteroides spp.* and other anaerobes **(*Spiegel , 1991*).**

Its clinical presentation is typical, with an unpleasant fishy smelling discharge that is more noticeable after unprotected intercourse. The discharge is thin and homogeneous**.** Pruritus and inflammation are usually absent and most patients are symptom free **(*Sobel, 1997*).**

The diagnostic criteria established by ***Amsel and colleagues*** have proved to be remarkably simple and useful method in clinical practice. This requires three of the following four signs: (I) the presence of clue cells that are vaginal epithelial cells heavily coated with bacteria. (II) A homogeneous, non inflammatory discharge that adheres to the vaginal walls. (III) pH of vaginal fluid >4.5. and (IV)A fishy odor from vaginal discharge before or after addition of 10% KOH (***Whiff test***) **(*Amsel et al., 1983*).**

Women with Bacterial vaginosis are at increased risk for pelvic inflammatory disease (***Eschenbach et al., 1998***), postoperative cuff infections after hysterectomy **(*Soper et al., 1990*)** and abnormal cervical cytology **(*Platz-Christensen et al., 1994*).**

A significant association between infection with the human immunodeficiency virus 1 and the depletion of vaginal lactobacilli has been recently found, particularly among women with severe bacterial vaginosis **(*Martin et al., 1999*).**

Currently, metronidazole is the agent of choice for the treatment of bacterial vaginosis. This therapy is only moderately effective against *G vaginalis*

and Mobiluncus spp, and is inactive against *Mycoplasma hominis*, but its metabolites are highly active against anaerobes in general and the *Bacteroides spp*. Unfortunately, metronidazole is often poorly tolerated due to side effects, including gastrointestinal upset, alcohol intolerance, metallic taste, and infrequently neurological and/or hematological adverse reactions. In addition, standard antibiotic therapy for bacterial vaginosis with metronidazole is quite ineffective I that more than 50% get recurrent bacterial vaginosis infection within 3 to 6 months **(*Reid and Bocking, 2003*),** and there is a high incidence of overgrowth of pathogenic bacteria after treatment **(*Reid and Bruce, 2003*).**

The loss of vaginal *lactobacilli* appears to be the major factor in the cascade of changes leading to bacterial vaginosis **(*Sobel, 1997*)** and relapses are associated with failure to establish a healthy *lactobacilli* dominated vaginal flora **(*Reid and Bruce, 2003*).**

Probiotics are defined as live micro-organisms which, when administered in an adequate amount, confer a health benefit on the host **(*Andreu 2004*).** Currently, the best studied probiotics are lactic acid producing bacteria, particularly *Lactobacillus species* and *Bifidobacterium species* **(*Fooks and Gibson, 2002*).**

The actual mechanism of action of probiotics in the vagina is probably multifactorial. The production of lactic acid, bacteriocin, and hydrogen peroxide seems to be important, and modulation of immunity is another possible mechanism **(*Reid et al., 2004*).** The administration of these *Lactobacilli* by mouth, intravaginally or both has been shown to be safe and effective in reducing and/or treating urogenital infections **(*Reid et al., 2004*),** The recommended dose, by whatever route of administration, is 10^9 to 10^{11} air-dried or freeze-dried bacteria **(*Andreu 2004*).**

Lactobacilli are gram positive, catalase negative, non-sporing rods that dominate vaginal flora **(*Sieber 1998*).**The human vagina is normally lined by

multilayered stratified squamous non-keratinized epithelium. The middle and superficial layers contain glycogen, which is set free by the breakdown of superficial cells. Free glycogen is fermented by *Lactobacilli*, producing lactic acid and hydrogen peroxide **(*Andreu 2004*).**The presence and dominance of *Lactobacillus* in the vagina is associated with reduced risk of bacterial vaginosis and urinary tract infection **(*Reid and Burton, 2002*).** Women with hydrogen peroxide producing strains of *Lactobacilli* have 4% prevalence rate of bacterial vaginosis compared with 32% in women colonized by non-hydrogen peroxide producing strains and 56% in those without *Lactobacilli* **(*Ugwumadu 1999*).**

Although antimicrobial agents are quite effective at providing clinical cure for bacterial infections, urogenital pathogen drug resistance is on the increase. Also, drugs have local side effects including disruption of the protective vaginal flora, which create an increased risk of recurrent infections **(*Shennan 2006*)**,and antibiotics can cause general adverse effects including palpitations, flushes, nausea, vomiting, diarrhea, abdominal pain, rashes, headache and dizziness **(*Reid et al., 2004*).**

An efficient treatment of bacterial vaginosis should result in a long-term restoring of the ecological balance in vagina by increasing the natural bacterial flora while decreasing the growth of potentially pathogenic bacteria; this raises the question as to whether restoration of *Lactobacilli* by vaginal probiotic therapy can restore the normal flora **(*Reid and Bocking, 2003*).**

Patients and methods

This longitudinal study will be conducted on 60 lady attending the gynecology outpatient clinics at Ain Shams University Maternity Hospital and Kafer El Shiekh General Hospital in the period starting from April 2008 to April 2009.

The inclusion criteria in the study are:

- Age 20-40 years.
- Bacterial vaginosis diagnosed by presence of three out of the following four criteria:-
    - Thin, gray and homogeneous vaginal discharge.
    - Unpleasant fishy odor before or after addition of 10% KOH (Whiff test).
    - Vaginal pH>4.5.
    - The presence of clue cells (vaginal epithelial cells heavily coated with bacteria) in wet or stained smear.

The exclusion criteria in the study are:

- Pregnancy.
- Breast feeding.
- Condition that predispose to infection (diabetes mellitus, steroid therapy ...etc)
- Vaginal bleeding during the course of treatment.
- Recent vaginal douching or intercourse (within 24 hours).
- Evidence of other genital infection.
- Known hypersensitivity to metronidazole or probiotics.

Justification of sample size:

According to the clinical study carried out by Gregor Reid in (2006) comparing probiotic *lactobacillus GR-1* and *RC-14* with metronidazole in treatment of symptomatic bacterial vaginosis, sample size was calculated (to be 60 patients) using ***Ler***'s formula to the power of 80%. The level $p< 0.05$ was considered the cut off value of significance.

All patients will receive oral tablet three times daily for one week and vaginal capsules twice daily for five days as supplied by the candidate.

Oral tablet will be either 500mg metronidazole versus placebo while vaginal capsules will contain *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14*(10^9 each organism) versus placebo guided that no patient will receive combined therapy.

All patients will be interviewed and the attached questionnaire will be filled in by the investigator after a written consent before start of treatment protocol.

At the end of the study patients will be tabulated into two groups:

Group (1) including 30 patients who received oral metronidzole tablets and placebo vaginal capsules.

Group (II) including 30 patients who received oral placebo tablets and vaginal capsules contain Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14**.**

Follow up for all ladies will be done at end of treatment and after four Weeks by:

- History.
- Clinical examination.
- Laboratory investigations including vaginal pH and vaginal smear.

For cure (defined as less than 3 of the 4 clinical signs including normal flora on gram stain), persistence or relapse of bacterial vaginosis

Aim of the work

The aim of this work is to compare the efficiency of vaginally administered proboitic *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* with oral metronidazole therapy in treatment of symptomatic bacterial vaginosis.

Microbial ecology in the lower genital tract in Females

The vaginal flora was first reported by ***Albert Döderlein***, as early as 1892. ***Döderlein*** found that the microflora was homogenously colonized with Gram-positive rods, which were designated the name "*Döderlein's bacilli*". Over the years, these bacilli have been identified as *Lactobacillus* spp. and are still believed to be the dominant components of the genital microflora ***(Hyman et al., 2005***).

The last decade's increased global interest in women health has led to reports concerning the microbial ecology of the female genital tract. Furthermore; urogenital infections such as urinary tract infection (UTI), bacterial vaginosis (BV) and vulvo-vaginal candidiasis (VVC), most often include treatment with antibiotics and antimycotics, which have led to a raised concern regarding drug resistance among pathogenic species. In search for alternative treatments, therapies with cranberry juice, cranberry extract and vitamin A have been suggested for prevention of recurrent UTI (***Bailey et al., 2007***) polycarbophil gel has been suggested in treatment of BV(***Wu et al., 2007***) and boric acid has shown good effect on women with VVC (***Ray et al., 2007***). Additionally, it has been suggested that the improvement of the genital lactobacilli flora can play a key role in the prevention of these conditions (***Reid et al., 1990***). As a consequence of these alternative treatments, the expansion of antibiotic and antimycotic resistance could be reduced.

Normal vaginal microflora

The microbiological flora in the lower genital tract in women is very complex and unique for every woman, for that reason; there is no clear definition of what constitutes a normal genital flora (***Brown et al., 2007***).

The vagina of newborn is sterile and organisms appear in 12-14 hours after birth. *Staphylococci, enterococci* and *diphtheroids* appear at first, but are replaced in 2 to 3 days by an almost pure culture of *Döderlein's bacilli*, under the effect of

maternal estrogen, soon this is excreted in the urine, and the vaginal secretion becomes alkaline. Thereafter, until puberty, the secretion remains alkaline, and s*taphylococci*, *streptococci*, other than *strept. pyogenes*, *coliforms* and *diphtheroid bacilli* predominate (***Hurley, 1992***). At puberty, glycogen is again deposited in the vaginal wall, the secretion becomes acidic, and *Döderlein's bacilli* are re-established as the predominant organisms. The flora is mixed and *streptococci, diphtheroids* and fungi are present. In healthy women, the vagina contains 10^9 bacterial colony forming units (cfu)/ ml of secretions (***Sweet and Gibbs, 1995***).

Table (1) Classification of bacteria in the human vagina (***Onderdonk and Wissemann, 1993***).

Category	Aerobic	Anaerobic
Gram + ve cocci	*Staphylococcus epidermidids, staph aureus* *Streptococcus α-hemolytic Group B ,Group D Non-hemolytic* *Micrococcus*	*Peptostroptococcus* *Streptococcus*
Gram -ve cocci	*Neisseria*	*Veillonella*
Gram +ve bacilli	*Lactobacillus* *Corynebacterium*	*Lactobacillus* *Propionibacterium* *Clostridium* *Eubacterium* *Bifidobacterium* *Actinomyces*
Gram -ve bacilli	*Escherichia coli* *Klebsiella* *Other enterobacteriaceae*	*Bacteroids* *Mycoplasma homins* *M. fermentans* *Fusobacterium*

1-*Döderlein's Lactobacillus*

Lactobacilli have long been recognized as one of the dominant phenotypes in the vaginal environment. Lactobacilli are non-pathogenic, Gram-positive, rod-shaped, facultative anaerobic bacteria that, as well as colonizing the genital tract, are often present in the gut and the oral cavity (***Bannks, 1999***).