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Title of protocol

Assessment of vulvar microbiome changes and its correlation with vulvar skin parameters after the use of the intimate hygiene product SaugellaActi3 in women with an history of recurrent vulvovaginal infections (candidiasis or vaginosis).

Principal Investigator: Prof. Filippo Murina

Site of research: Lower Genital Tract Unit-Obst. and Gyn, Dept. –V. Buzzi Hospital-University of the Study of Milan-Milan, Italy

1. INTRODUCTION

The vulva is a unique region of skin that serves as a transition between the cutaneous epithelium of the skin and the mucosa of the female urogenital tract. To date, relatively few studies have been performed to characterize the microbes that colonize the vulva of healthy females. Vulvar microbial communities are thought to be of clinical are thought to be of clinical importance because they regulate the proliferation of non-indigenous flora, including pathogens that can cause infection and may also affect the comfort of the urogenital area. Therefore, factors that modulate the vulvar microflora may affect comfort and health of the vulvar area. Furthermore, the microbial organisms present on the vulva may trigger various vulvo-vaginal diseases, such as vulvovaginitis and vulvar dermatitis. It was found that a wider range of populations inhabited the labia majora (including bacterial species known to be commensals of the skin) than the labia minora. Intimate hygiene products are widely used by women as part of their daily cleansing routine; however, there is a lack of scientific literature about the impact of intimate personal hygiene product use on the vulvar area and even less information is available on the impact on vulvar microbiome stability. A proper routine washing of the vulva prevents the accumulation of vaginal discharge, urine, and fecal contamination to avoid offensive body odor and help maintain healthy vulvar skin for defense against infection. Thus, ideal female intimate hygiene products that support intimate health should be specifically formulated and clinically tested for the vulvovaginal area and need to be mild and hypoallergenic, provide protection against dryness, and maintain the natural pH and microflora.

2. TRIAL OBJECTIVE

The Research Hypothesis for the present study is to prospectively document the impact of intimate cleanser Saugella Acti3 on the vulvar microbiome by measuring species richness and diversity of the microbial communities using genetic sequencing techniques. We hypothesize Saugella Acti3 that for its properties (antimicrobial, moisturizing and providing a long-lasting effect of the active ingredients due to the muco-adhesive system) respects vulvar epithelium preventing vulvovaginal infection and discomfort in vulnerable women with an history of recurrent vulvovaginal infections (candidiasis or vaginosis).

3. OUTCOMES

Primary outcome will include:

-Vulvar microbiome analysis trough a 16S rRNA gene sequences. Microbiological samples will be taken from the mid-labium majus (both sides) and peri vestibular area.

- Evaluation of vulvar skin parameters regarding the hydration level, pH, and sebum level of the skin surface. The measurements will be performed using the Corneometer® CM 825, Skin-pH-Meter PH 905® and Sebumeter SM815® devices, each with a probe connected to an MDD4 base unit (Courage-Khazaka). We will assess sequential measurements on the middle third of the left and right sides of the labia majora and labia minora. Three repeated measures were taken at each vulvar skin site, and an average value will be calculated.

Secondary objectives will include:

- Subjective evaluations of burning and itching after 30 days, using a 0-10 point intensity scale (0 = absent, 10= very high)

-Collection of Patients' Global Impression of Change (PGIC) Scale (Italian translation), a questionnaire regarding treatment satisfaction after 30 days

4. DESCRIPTION OF RESEARCH DESIGN

4.1 Overall Study Plan

This is an open, interventional uncontrolled clinical trial in one cohort of healthy women to be conducted at Lower Genital Tract Unit-Obst. and Gyn, Dept. –V. Buzzi Hospital-University of Milan-Milan, Italy.

4.2 Study Duration
It is expected this investigator-initiated research study will be completed approximately 4-6 months following initial approval by the Institutional Ethical Board.

4.3 Institutional Ethical Board Approval (IEB)
Prior to conducting any study-related procedures, the Principal Investigators will each obtain written approval from their respective IEB for the informed consent form, protocol, recruitment materials, and any written information provided to Subjects pertaining to the procedure.

5. Selection and Withdrawal of Subjects

The study population will include healthy women without any vulvovaginal condition.

5.1 Subject Inclusion Criteria
All criteria below must be met to be eligible for study participation.
- Asymptomatic women at least 18 years of age and before menopause (Absence of menstruation for 12 months)
- History of recurrent vulvovaginal candidiasis or vaginosis (at least 4 documented episodes in the previous 12 months)
- Absence of contraindications to the proposed intimate cleanser product
- Read and signed informed consent.

5.2 Subject Exclusion Criteria
Subjects who meet any of the following criteria shall be excluded:
- Active vulvo-vaginal infections at the time of their gynecological examination.
- Genital bleeding of unknown origin
- Current pregnancy or breastfeeding
- Active vulvo-vaginal infection at the time of their gynecological examination
- Vulvar dermatosis or other vulvar disease
- Lesions in the vulva that have not been evaluated
- Unwillingness to wash out one's usual intimate cleanser
- Patients concomitantly included in different interventional clinical trials.
- Unwillingness to provide the informed consent to the trial.

5.3 Subject Withdrawal Criteria
The Principal Investigator may discontinue a subject's participation in the study at any time if it is considered in the subject's best interest to do so. Such a decision

may be precipitated by adverse events, new onset illness, clinically important changes in vital signs, physical examinations, or laboratory tests. Subjects who are noncompliant with study procedures and visits may also be withdrawn by the Principal Investigator. Subjects may withdraw from participation in the study at any time for any reason. A subject's decision to withdraw will not cause the subject to lose any benefits to which she is entitled. A subject who withdraws prematurely from the study will return to the clinic as soon as possible to undergo the final visit evaluations. If a subject prematurely withdraws or is withdrawn from study participation, the reason for the withdrawal must be recorded on the case report form (CRF).
Record the primary reason for premature withdrawal according to the following categories:

- Adverse Event: Subject experiences an intolerable event, which may or may not be related to the study medication
- Withdrawn Consent: Subject withdraws from study participation for personal reasons (exclude adverse experience before indicating this category)
- Concomitant Medication Violation: Subject initiates, discontinues, or changes dosing regimen of concomitant medication in violation of the protocol, which, in the judgment of the Principal Investigator, may adversely affect evaluation of safety
- Lost-to-Follow-up: Subject does not return for evaluation and no further contact is made by the Subject after three documented phone or email attempts and a final attempt by certified mail.
- Other: Any reason that does not fit in the above 4 categories: the reason will also be recorded on the CRF.

6. Clinical Procedures

Clinical procedures throughout the study are described in the sections below.

6.1 Informed Consent

Each potential study Subject must provide written informed consent and authorize release of her protected health information before any study procedure is conducted.

6.2 Study Day 0: Subject Screening (Visit 1)

Before initiation of any study procedures, Subjects will be fully informed of the

study plan. Each potential Subject will be required to read and to indicate her understanding by signing and dating the ICF prior to initiation of any screening procedures. Subject meeting inclusion and exclusion criteria will be enrolled.
For all the participants, the previous clinical history, age, marital status, ethnic origin, the use of contraception, and history of medication and vaginal infections will be recorded.
Following consent to participate, all subjects will undergo an evaluation of vulvar skin parameters regarding the hydration level, pH, and sebum level of the skin surface.
Before these measurements, a vulvar and vestibular sample will be obtained through a rubbed swab against the vulvar and vestibular wall finalized to evaluation of bacterial community composition by sequencing the 16S rRNA genes amplified from total genomic DNA isolated from the samples.
All enrolled women subsequently will receive the intimate hygiene product Saugella Acti3, and the women will be asked to use the product as a normal detergent for feminine hygiene twice daily (morning and evening) for 30 days.

6.3 Study Day 30 ± 2 days: Follow-Up (Visit 2)

-Vulvar and vestibular samples to evaluation of bacterial community composition by sequencing the 16S rRNA genes, and vulvar skin parameters regarding the hydration level, pH, and sebum level of the skin surface will be repeated.
- Subjective evaluations of burning and itching after 30 days, using a 0-10 point intensity scale (0 = absent, 10= very high) and Patients' Global Impression of Change (PGIC) Scale (Italian translation), a questionnaire regarding treatment satisfaction, will be collected.

6. Statistical Methods

6.1 Determination of Sample Size

The sample size of 20 subjects was chosen for this study based on this being a pilot study, and this sample size will be enough to prospectively document changes in vulvar skin parameters in this cohort of women.

References.

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