Metabolic fingerprints of different clinical conditions affecting the female genital tract

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Background: In healthy pre-menopausal women, lactobacilli play a crucial role in maintaining the ecological equilibrium of the vaginal niche. In case of bacterial vaginosis (BV), a condition characterized by a depletion of vaginal lactobacilli and an increasing number of anaerobes, a higher risk of urogenital and sexually transmitted infections (STIs) acquisition is reported. The vaginal environment of healthy and BV-positive women has been extensively studied by molecular approaches, in order to identify the microbial species dominating these opposite conditions. Moreover, many studies have addressed the composition of vaginal metabolites produced by microbes and host cells, revealing BV-specific metabolic profiles. Besides that, less is known about the vaginal metabolome in case of STIs, as Chlamydia trachomatis (CT) infections. The aim of this study was therefore to characterize and compare the vaginal metabolic profiles in different conditions (healthy, BV, CT infections), by means of ¹H-NMR spectroscopy.

Material/methods: From July 2016, all the pre-menopausal women attending the STI Outpatients Clinic of Sant’Orsola-Malpighi Hospital in Bologna (Italy) and meeting one of the following criteria were
enrolled: presence of vulvo-vaginal symptoms or presence of risk factors for CT infection. Patients with vulvo-vaginal candidiasis were excluded from this study. For all the patients, after a clinical visit, a vaginal swab was collected for molecular CT detection (Versant CT/GC DNA 1.0 Assay; Siemens), whereas Amsel criteria were used for BV assessment. Moreover, for each woman, an additional vaginal swab stored in saline was collected for metabolomic analysis: after centrifugation, 1 ml of cells-free supernatant was added to 160 μL of a D_2O solution of 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt 6.25 mM. 1H-NMR spectra were recorded with an AVANCE spectrometer (Bruker). Similarities among the metabolic profiles of samples were investigated by means of a principal component analysis (PCA). Differences in metabolites concentrations were analysed using ANOVA test based on Tukey contrast. The study was approved by the Hospital Ethical Committee.

**Results:** Among all the women enrolled, 25 were considered healthy, 18 received a diagnosis of BV and 22 were positive for CT. PCA analysis confirmed that BV-metabolic profile was reasonably distinct from the one of healthy women. The metabolome of CT-positive women resembled more the one of healthy women rather than that of BV-positives. Comparing the metabolite composition of the three groups, statistically significant differences were found. As an example, higher concentrations of formate, trimethylamine, ethanolamine and methylamine were found in BV-patients, while tryptophan, lactate and phenyl-propionate were more present in healthy women. In CT-positive women, concentrations of these metabolites were similar to healthy women or intermediate between healthy and BV groups.

**Conclusions:** Specific metabolic signatures characterize different clinical conditions of the vaginal tract. At a metabolic level, CT-positive women are more similar to healthy than BV-subjects.